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Vorstand: Prof. Dr. Magdalena Götz

Progenitors in the intact brain parenchyma and their reaction towards acute injury

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Eidesstattliche Versicherung

Ich versichere hiermit an Eides statt, dass die Dissertation von mir selbständig und ohne unerlaubte Hilfe angefertigt ist. Die hier vorgelegte Dissertation wurde nicht in ähnlicher Form bei einer anderen Stelle zur Erlangung eines akademischen Grades eingereicht.

München, den 13.12.2011 _____

(Christiane Simon)

For my family

in deep gratefulness
for their endless support

So eine Arbeit wird eigentlich nie fertig,
man muß sie für fertig erklären,
wenn man nach Zeit und Umständen
das Mögliche getan hat.
(Johann Wolfgang von Goethe)

I Summary

The adult central nervous system (CNS) is a highly plastic system that not only shows ultrastructural plasticity (e.g. the movement of processes of glia or the turnover of neuronal synapses), but it is also interspersed with proliferating cells that generate continuously new cells. Besides the proliferative-active stem cell niches, dividing NG2+ cells reside within the adult brain parenchyma. In particular, these NG2+ glia attracted attention as they also react to a variety of brain injuries by altered proliferation, e.g. after acute traumata, in brain tumors and in the case of neurodegenerative diseases and demyelinating diseases. However, little is known about the function and role of NG2+ cells in the adult brain in vivo. Therefore, the aim of this PhD study was to characterize the proliferative behavior of NG2+ glia in the intact and acutely lesioned brain parenchyma in order to determine regulatory mechanisms that control their proliferation. Here, it is shown that adult NG2+ cells are the major proliferative cell population in the adult cerebral cortex that is slowly dividing due to a long G1-phase. Interestingly, these cells can alter their cell cycle speed according to the environment: Increased neuronal activity resulted in a temporal reduction of NG2+ cell proliferation and simultaneously increased their differentiation. In contrast, after acute inflammatory conditions NG2+ cells acutely re-entered the cell cycle and proliferated faster than in the intact cortex. In order to follow these cells live and pursue their fate in the intact and injured adult CNS a novel transgenic mouse line was generated. Therefore, a bacterial artificial chromosome (BAC) was constructed, expressing an inducible fusion protein (iCreER^{T2}) under the Sox10 promoter that allows mediating high recombination in NG2+ cells upon tamoxifen administration. This Sox10-iCreER^{T2} mouse line is now a new tool to gain further insights in the role and biology of adult NG2+ glia in the intact and lesioned brain, e.g. by cell- and time-specific ablation of distinct genes.

II Zusammenfassung

Das erwachsene zentrale Nervensystem (ZNS) stellt ein plastisches System dar, das sich nicht nur durch ultrastrukturelle Plastizität (z.B. die Motilität der Fortsätze von Gliazellen oder Veränderungen neuronaler Synapsen) zeigt, sondern auch sich teilende Zellen enthält, die stetig neue Zellen produzieren. Neben den teilungsaktiven Stammzellnischen, befinden sich auch teilende NG2+ Zellen im umgebenden Gehirngewebe. Besondere Aufmerksamkeit haben diese NG2+ Gliazellen erhalten, nachdem gezeigt wurde, dass sie auch auf verschiedene Verletzungen des Gehirns mit einer veränderten Zellteilungsrate reagieren können, z.B. nach akuten Traumata, in Gehirntumoren, neurodegenerativen Erkrankungen und in demyelinisierenden Krankheiten. Jedoch ist bisher relativ wenig über die Funktionen und Aufgaben von NG2+ Zellen im erwachsenen Gehirn in vivo bekannt. Daher war es das Ziel dieser Doktorarbeit das Zellteilungsverhalten von NG2+ Zellen im gesunden und im akut geschädigten Gehirn zu untersuchen und dabei regulatorische Mechanismen zu klären, die das Teilungsverhalten dieser Zellen beeinflussen. In dieser Arbeit konnte gezeigt werden, dass NG2+ Gliazellen die größte sich teilende Zellpopulation in der erwachsenen zerebralen Kortex darstellen und sich langsam teilen, in dem sie länger in der G1-Phase des Zellzykluses verweilen. Interessanterweise können diese Zellen die Geschwindigkeit ihres Zellzykluses der Umgebung anpassen: Bei erhöhter neuronaler Aktivität teilen sich NG2+ Zellen zeitweise langsamer und differenzieren vorwiegend. Bei akuten Entzündungen im Gehirn können NG2+ Zellen jedoch sofort wieder den Zellzyklus beginnen und teilen sich damit schneller als in der gesunden Kortex. Um nun diese NG2+ Zellen live zu beobachten und ihre Nachkommen weiter zu verfolgen, wurde eine neue genetisch veränderte Mauslinie generiert. Ein künstliches Chromosom mit bakteriellen Ursprung (BAC) wurde konstruiert, in dem ein induzierbares Fusionsprotein (iCreER^{T2}) unter dem Sox10-Promoter gebildet wird, wodurch nach Tamoxifen-Gabe NG2+ Zellen rekombiniert werden können. Diese neue Sox10-iCreER^{T2}-Mauslinie bietet nun die Möglichkeit die Funktion und Biologie von NG2+ Gliazellen, z.B. durch die zell- und zeitspezifische Auslöschung von bestimmten Genen, zu untersuchen.

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1 Introduction

Glial cells constitute one of the major cell population in the adult mammalian brain and can be divided into macroglia and microglia. In particular cells belonging to the group of macroglia can be further subdivided into astrocytes and oligodendrocytes providing either e.g. trophic support to neurons (reviewed in Nave and Trapp, 2008; Wang and Bordey, 2008) or facilitating neuronal conduction by enwrapping axons with myelin sheaths (reviewed in Trotter *et al.*, 2010). These macroglial cells are not only morphologically heterogeneous in different areas of the central nervous system (CNS) but have also unique features, as for example a subpopulation of dividing macroglial cells persists in the adult intact brain (in the following referred to as "progenitors"). Besides the proliferative-active stem cell niches in the adult brain (Beckervordersandforth *et al.*, 2010; Kriegstein and Alvarez-Buylla, 2009), parenchymal progenitors are widespread in the adult brain and are named either NG2+ cells, based on their antigenic expression profile, or oligodendrocyte progenitor cells (OPCs) as their progenies are mainly myelin-producing oligodendrocytes (Dimou *et al.*, 2008; Kang *et al.*, 2010; Nishiyama *et al.*, 2009; Rivers *et al.*, 2008). Therefore, these progenitors are historically considered to belong to the oligodendrocyte lineage. Notably, parenchymal progenitors are recently considered to constitute a 4th glial population, besides microglia, astrocytes and oligodendrocytes, due to their widespread distribution and unique properties in the mammalian brain (summarized in Nishiyama *et al.*, 2009; Trotter *et al.*, 2010).

1.1 Origin and fate of parenchymal progenitors – the oligodendrocyte lineage

Macroglial cells in the mammalian forebrain arise from the neuroepithelium and are generated at distinct time points during development (Wang and Bordey, 2008). In particular, the origin of OPCs in the CNS is heterogeneous which was first shown for the spinal cord (reviewed by Richardson *et al.*, 2006)(Richardson *et al.*, 2006). Here, the majority of OPCs in the spinal cord are generated from ventral domains at embryonic day (E) 12.5 before they spread and populate the gray and white matter of the spinal cord (Richardson *et al.*, 2006). However, a smaller proportion of the spinal cord OPCs also derives from dorsal sources later during development (Cai *et al.*, 2005; Vallstedt *et al.*, 2005). In line with this data, Kessaris et al. (2006) could show that also telencephalic OPCs are generated by

different precursors in three waves (Figure 1). The first cortical OPCs derive from ventral precursors at the ventricular zone of the medial ganglionic eminence (MGE) and anterior entopeduncular area, appearing around embryonic day (E) 11.5 (in mice) and enter the cerebral cortex at E16 (Kessaris *et al.*, 2006). Although these OPCs are largely depleted postnatally, they are replaced by OPCs originating from dorsal precursors in the lateral ganglionic eminence (LGE) and from endogenous cortical precursors at the day of birth (Kessaris *et al.*, 2006; Richardson *et al.*, 2006). Interestingly, these precursors do not account for all OPCs in the adult telencephalon thereby indicating additional sources of adult OPCs (Ventura and Goldman, 2006) .

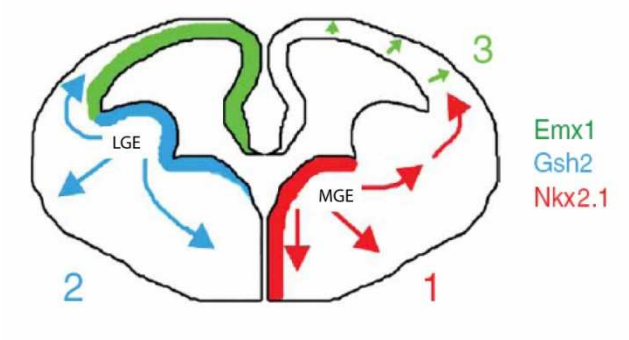

Figure 1 Origin of NG2+ cells (OPCs) during development. The first OPCs derive from Nkx2.1+ precursors of the MGE and arrive in the cortex during the mid-neurogenesis. Gsh2+ progenitors of the LGE give rise to a second wave of OPCs. Finally, OPCs also derive from Emx1+ cortical progenitors. Gsh2+ and Emx1+ derived OPCs compensate the loss of Nkx2.1 derived OPCs and account for the majority of cortical OPCs postnatally. However, the contribution of Emx1-derived OPCs declined later on. aOPCs – oligodendrocyte precursor cells; MGE – medial ganglionic eminence; LGE – lateral ganglionic eminence (adapted from Kessaris et al. 2006)

Postnatal OPCs differentiate into myelinating oligodendrocytes with a peak of myelination in the second postnatal week (Greenwood and Butt, 2003). However, not all postnatal OPCs differentiate but some also remain as progenitors in the adult CNS. These adult OPCs express similar antigens as their developmental counterparts (Figure 2), e.g. membrane proteins like neuron-glia antigen 2 (NG2; (Nishiyama *et al.*, 1997), platelet-derived growth factor receptor α (PDGFRα; (Dawson *et al.*, 2003), junctional adhesion molecule A (JAMA; (Stelzer *et al.*, 2010) and O4 (Nishiyama *et al.*, 2009; Polito and Reynolds, 2005; Reynolds and Hardy, 1997; Sommer and Schachner, 1982) or the transcription factors OLIG2 (Takebayashi *et al.*, 2002; Zhou *et al.*, 2000) and SOX10 (Kuhlbrodt *et al.*, 1998). Lately, it is proposed that adult OPCs differentiate via an intermediate state during which they express the G-protein coupled receptor 17 (GPR17; (Boda *et al.*, 2011)). This intermediate state can be clearly distinguished by its morphology and antigenic expression profile (Figure 2) from mature oligodendrocytes

that express cytoplasmatic proteins like glutathione-S-transferaseπ (GSTπ), adenomatosis polyposis coli (APC/CC1) and aspartoacylase (ASPA). Whether these mature oligodendrocytes could further differentiate to myelinating oligodendrocytes in adult mammals was controversially discussed in the field of research as myelination should be finished within the first postnatal weeks (Greenwood and Butt, 2003). Only recently, independent research groups showed a continuous generation of new myelinating oligodendrocytes in the adult CNS (Dimou *et al.*, 2008; Rivers *et al.*, 2008). These myelinating oligodendrocytes express typical antigens being present in the myelin sheath, e.g. myelin-associated glycoprotein (MAG), myelin oligodendrocyte glycoprotein (MOG), myelin basic protein (MBP) and myelin proteolipid protein (PLP/DM20; Figure 2; reviewed in Baumann and Pham-Dinh, 2001). One myelinating oligodendrocyte can ensheath several axons, thereby providing the basis for the fast saltatory conduction of action potentials between neurons (reviewed by Simons and Trotter, 2007), but also maintaining the axonal integrity (reviewed by Nave, 2010).

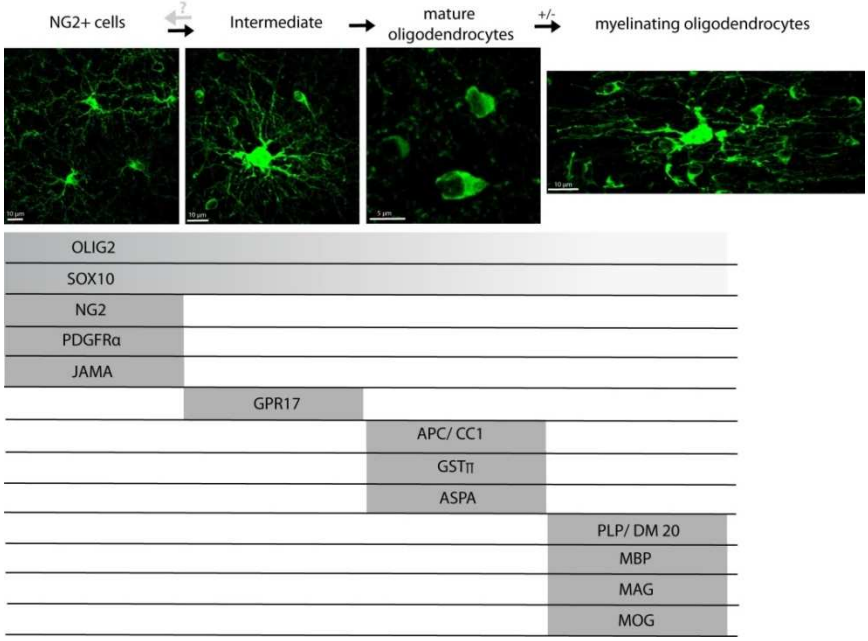

Figure 2 The oligodendrocyte lineage in the adult CNS. Pictures showing recombined cells of the Sox10-iCreER^{T2} mouse line that are stained for GFP. These GFP-expressing cells label the oligodendrocyte lineage and allow discriminating distinct differentiation states of this lineage by morphology. The oligodendrocyte lineage comprises some proliferating NG2+ cells that can differentiate into mature oligodendrocytes over a GPR17+ intermediate state. If these intermediate cells might also become again NG2+, remains to be open (gray interrogation mark). However, depending on the brain region some mature oligodendrocytes can differentiate to myelin-sheath producing oligodendrocytes. Examples of antigen expression are given for each differentiation state of the oligodendrocyte lineage.

1.2 NG2+ glia (OPCs) – a 4th glial cell population

Since OPCs are the major proliferating population in the adult brain parenchyma (Aguirre *et al.*, 2004; Buffo, 2007; Dawson *et al.*, 2000; Dawson *et al.*, 2003; Dimou *et al.*, 2008; Gensert and Goldman, 1997; Horner *et al.*, 2000), these cells attract attention as a possible intracranial source for cellular regeneration upon disease conditions. OPCs constitute 5-8 % of cells in the mammalian brain (Horner *et al.*, 2000) and remain constant in life (Dawson *et al.*, 2003; Rivers *et al.*, 2008). These glial cells are characterized by a stellate morphology with fine processes leaving from a central cell body and are uniformly distributed within the brain. Besides their fate to become mainly myelinating oligodendrocytes in higher vertebrates during development, these cells are already detectable in lower vertebrates with unmyelinated axons, suggesting additional functions of OPCs in the CNS (reviewed in Mangin and Gallo, 2011). Therefore, OPCs are recently considered to be a 4th glial population and are in the following referred to as "NG2+ cells" due to their diverse nature.

Cellular characteristics of NG2+ glia

NG2+ cells form a network with slightly overlapping domains in the CNS and are tightly integrated within the astrocytic and the neuronal network (Wigley and Butt, 2009). It is shown that they can form contacts with unmyelinated and myelinated axons, neurons, astrocytes and pericytes allowing probably a bidirectional communication between these cells and NG2+ glia (Hamilton *et al.*, 2010; Wigley and Butt, 2009). While the contact of NG2+ cells to pericytes might be involved in regulating the blood flow (Wigley and Butt, 2009), the synaptic contact of NG2+ glial processes to neurons, e.g. at the nodes of Ranvier, the neuronal soma and the dendrites, might have an impact on diverse cellular functions like migration and maturation of NG2+ glia (Mangin and Gallo, 2011). These synapses between neurons and NG2+ cells can be either glutamatergic or γ-aminobutyric acid (GABA)-ergic (reviewed in Mangin and Gallo, 2011) mediating excitatory (EPSC) or inhibitory postsynaptic currents (IPSC) in NG2+ cells via α–amino-3-hydroxyl-5-methyl-4-isoxazole-propionate (AMPA) receptors (Figure 3) or $GABA_A$ receptors, respectively (reviewed in Mangin and Gallo, 2011). The resulting currents are transduced in a locally restricted increase in Ca^{2+} in NG2+ glial processes (Figure 3; (Bergles *et al.*, 2000; Blaustein and Lederer, 1999; De Biase *et al.*, 2010; Hamilton *et al.*, 2010; Lin *et al.*, 2005; Mangin *et al.*, 2008; Tong *et al.*, 2009)). In addition, the EPSC/ IPSC induced opening of voltage-dependent Na+ channels might even

result in the generation of action potentials in NG2+ cells (Karadottir *et al.*, 2008), although this is still controversially discussed (reviewed by Frohlich *et al.*, 2011).

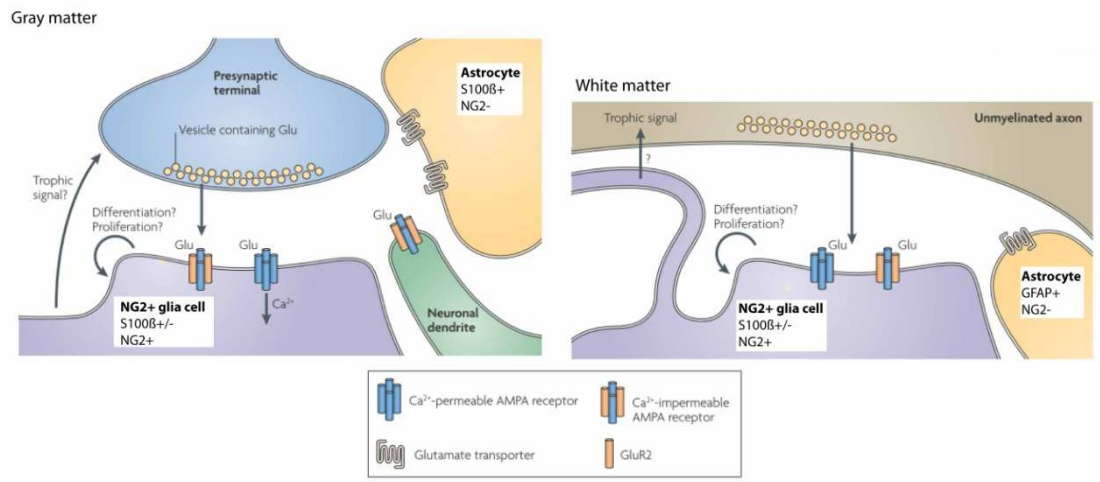

Figure 3 Scheme depicting the formation of a glutamate-mediated (Glu) tripartite contact between neurons, astrocytes and NG2+ cells in the gray and white matter. In the cortical gray matter, NG2+ cells form a synaptic contact to neurons. Glutamate, released by neurons, results in an intracellular increase of Ca^{2+}in NG2+ cells by Ca^{2+}-permeable AMPA receptors. In contrast, the neurotransmitter transmission between NG2+ cells and axons in the white matter is probably mediated via ectopic transmission, spillover or diffuse volume transmission (reviewed in Maldonado, 2011). *(adapted from Nishiyama et al. 2009)*

In contrast, Hamilton et al. (2010) suggests that ATP, released by neurons and astrocytes upon neuronal activity under physiological conditions, is a key signaling molecule that results in a widespread intracellular Ca^{2+} increase in NG2+ glia. ATP and its metabolites are well-known as sensors in energy metabolism and cellular homeostasis (reviewed by Butt, 2011) and are shown to bind to metabotropic P2Y and ionotropic P2X receptors on NG2+ cells (Hamilton *et al.*, 2010). Besides glutamate and ATP, the activation of muscarinic and nicotinic acetylcholine receptors (AChR; (Cui *et al.*, 2006; Velez-Fort *et al.*, 2009)) and cannabinoid receptors (Mato *et al.*, 2009) on NG2+ glia are also shown to alter their intracellular Ca^{2+} level. This signaling onto NG2+ glia, via all these different released transmitters, has pronounced roles in the proliferation, differentiation and migration ability of NG2+ cells in vitro (Agresti *et al.*, 2005a; Chen *et al.*, 2009; Gallo *et al.*, 1996; Ghiani *et al.*, 1999; Gudz *et al.*, 2006; Tong *et al.*, 2009; Yuan *et al.*, 1998). However, the impact of transmitters onto NG2+ cells in vivo under physiological conditions in the intact CNS is unknown. Therefore, a fate mapping approach was established during my PhD thesis in order to follow proliferating NG2+ cells and to discriminate environmental influences onto them in vivo.

The fate of NG2+ glia

Although NG2+ cells belong to the oligodendrocyte lineage (as described above) and seem to have a restricted fate, several publications challenged this point of view over the last decades of research. Early *in vitro* data of white matter (WM)-derived, postnatal NG2+ cells proposed a broader potential of these cells as they can give rise to type-2 astrocytes as well as oligodendrocytes (Raff *et al.*, 1983; Shi *et al.*, 1998; Wolswijk and Noble, 1989; Wren *et al.*, 1992). Therefore, NG2+ glia are also termed "O-2a" precursor cells in literature. In addition, postnatal NG2+ cells are shown to grow as neurospheres (a generally accepted in vitro method to test cells for their stem cell potential (Jensen and Parmar, 2006)) and can be differentiated into oligodendrocytes, astrocytes and neurons *in vitro* (Aguirre and Gallo, 2004; Aguirre *et al.*, 2004; Belachew *et al.*, 2003; Kondo and Raff, 2000). Interestingly, WM-derived NG2+ cells seem to keep this multipotency also until adulthood (Nunes *et al.*, 2003) whereas NG2+ cells of other brain regions seem to lose this property (Buffo *et al.*, 2008). In contrast, *in vivo* data suggest a restriction of the multilineage-potential of NG2+ glia to early embryonic stages, shown by giving rise to neurons, astrocytes and oligodendrocytes, (Masahira *et al.*, 2006). At later stages the fate of NG2+ cells is successively restricted to generate only astrocytes and oligodendrocytes (E16.5; (Zhu *et al.*, 2008)) or solely oligodendrocytes in the postnatal and adult brain (Dimou *et al.*, 2008; Kang *et al.*, 2010; Zhu *et al.*, 2011). There is also now a growing body of evidence that adult NG2+ cells can generate astrocytes after several types of injury (Busch *et al.*, 2010; Komitova *et al.*, 2011; Sellers *et al.*, 2009; Tatsumi *et al.*, 2008). Although so far other studies failed to confirm this so far (Barnabe-Heider *et al.*, 2010; Dimou *et al.*, 2008; Kang *et al.*, 2010; Zawadzka *et al.*, 2010), suggesting that this lineage plasticity may depend on the injury site and condition. In addition to the controversy about the astrocytic potential of adult NG2+ glia, the potential of these cells to generate neurons *in vivo* is highly debated (reviewed by Richardson *et al.*, 2011). There are publications suggesting the generation of neurons by NG2+ glia at distinct regions in the brain at postnatal stages (Aguirre and Gallo, 2004; Aguirre *et al.*, 2004; Belachew *et al.*, 2003) and adult stages (Guo *et al.*, 2010; Rivers *et al.*, 2008). However, these data could not be confirmed by others (Dimou *et al.*, 2008; Kang *et al.*, 2010; Zhu *et al.*, 2011). Thus, one explanation may be leakiness of the used transgenic lines (Kang *et al.*, 2010). On the other hand, other mouse lines (e.g. Olig2CreER^{TM}) showed a very low recombination rate raising concerns about the detection of only a subpopulation of NG2+

cells (Dimou *et al.*, 2008; Richardson *et al.*, 2011). In order to unravel the fate of NG2+ cells in the adult cortex, a mouse line independent approach was chosen in my PhD thesis to circumvent the described difficulties above.

1.3 Acute cortical injury as a model for glial scar formation

Traumatic brain injury, e.g. mechanical lesions or ischemia, causes stressed or dying cells at the site of injury as well as in retrograde connected cortical areas (reviewed by Viscomi *et al.*, 2009)y to release molecules, e.g. heat shock proteins, nucleotides, glutamate and metalloproteinases (MMPs), that initiate an inflammatory response around the primary lesion site (reviewed by Pineau and Lacroix, 2009). Within this process of inflammation the composition of the extracellular matrix is altered and different cells are recruited within the lesion site in a time-dependent manner, e.g. microglia, astrocytes, NG2+ cells, blood cells and other vascular cells (if the blood-brain barrier (BBB) was disrupted) as well as connective tissue (fibroblasts; if the meninges were injured; (Fawcett and Asher, 1999; Kerschensteiner *et al.*, 2009; Rolls *et al.*, 2009)). These alterations in the tissue structure persist in the lesion side for weeks and thereby create a glial scar. The formation of a scar around the damaged area is an immediate response to seal off the lesion side from the intact brain areas and to restore homeostasis during the acute phase of the injury (Rolls *et al.*, 2009). However, at later time points (chronic phase) the glial scar can inhibit regenerative mechanism, e.g. replacement of damaged neurons, axonal growth and remyelination (Alilain *et al.*, 2011; Fawcett and Asher, 1999; Silver and Miller, 2004).

Reaction of NG2+ glia upon traumatic brain injury

NG2+ cells respond to variety of acute traumata in the CNS, e.g. viral infection (Levine *et al.*, 1998), demyelinating lesions (Di Bello *et al.*, 1999; Keirstead *et al.*, 1998; Levine and Reynolds, 1999; Zawadzka *et al.*, 2010) and mechanical injuries (Alonso, 2005; Buffo *et al.*, 2005; Hampton *et al.*, 2004; Levine, 1994; Lytle *et al.*, 2006; McTigue *et al.*, 2001; Zai and Wrathall, 2005). Upon acute injuries, there is an increased extracellular level of excitatory/inhibitory neurotransmitters (Karadottir *et al.*, 2008) and ATP/adenosine (Matute, 2011) that results in a (prolonged) activation of AMPA receptors as well as P1 and P2 receptors on NG2+ cells, respectively. Both mechanisms can cause an increased death of

glial cells (Karadottir *et al.*, 2008; McTigue and Tripathi, 2008). However, remaining NG2+ cells within the lesion site undergo morphological changes as shown by shortening and thickening of their processes and cellular hypertrophy (reviewed by Levine *et al.*, 2001). In addition, NG2 expression is increased after acute injury, not only in NG2+ glia but also in meningeal cells, pericytes and in the extracellular matrix (ECM) surrounding the injury (Fawcett and Asher, 1999; Jones *et al.*, 2002; Levine *et al.*, 2001; Tang *et al.*, 2003). In particular, the accumulation of NG2 in the ECM is due to the release of the extracellular glycosaminoglycans chains of NG2 by proteases (Nishiyama *et al.*, 2009; Trotter *et al.*, 2010). Otherwise, NG2 localized on NG2+ cells is shown to have a supportive role by stabilizing axons within the lesion side against inflammatory-associated dieback (Figure 4; (Busch *et al.*, 2010)). But at the same time they prevent axonal regeneration after lesion (Chen *et al.*, 2002; Tan *et al.*, 2006). Therefore, the number of NG2+ glia in the lesion site has a profound impact on the regenerative capacity of the CNS showing the importance to analyze the proliferative response of NG2+ cells upon acute injury and to determine its regulation.

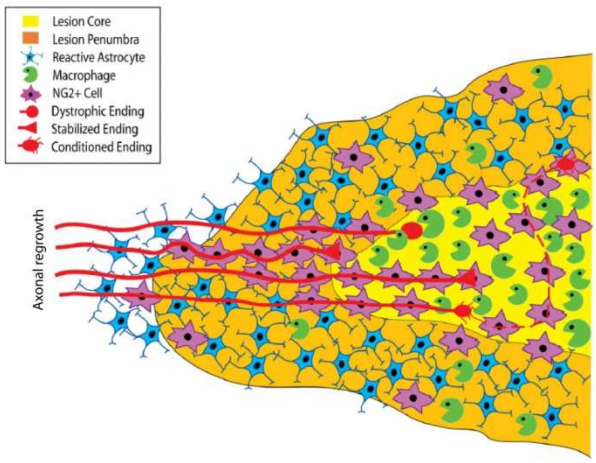

Figure 4 Scheme showing an acute lesion side. Although the lesion core contains mainly macrophages, the surrounding lesion area (penumbra) is composed of reactive astrocytes, reactive NG2+ glia and microglia (not depicted herein). Upon injury NG2+ cells support regrowing axons and help them to even pass the injury site by protecting them from macrophage associated axonal dieback. *(adapted from Busch et al. 2010)*

Reaction of microglia upon traumatic brain injury

However, NG2+ cells are not the only glial population reacting to injury. In order to gain further insights in the mechanism influencing the proliferation of NG2+ cells upon acute injury, one approach could be to correlate their response to the reactivity of other glial cells. Another cell type that responds immediately to lesions, e.g. brain structure disruption,

energy deprivation (e.g. during ischemia) or viral/bacterial infections, are microglia. They start to express and release proinflammatory chemokines and cytokines early after lesion (Pineau and Lacroix, 2007). However, the level of microglia reactivity depends on the severity of the injury. So traumatic lesions with disruption of the BBB can cause morphological alterations in microglia, e.g. retraction of their processes and hypertrophy (reviewed in (Kettenmann *et al.*, 2011). In addition, these cells increase in number around the lesion by migration (Davalos *et al.*, 2005; Nimmerjahn *et al.*, 2005) as well as by proliferation (Fawcett and Asher, 1999; Kettenmann *et al.*, 2011). In particular, upon activation microglia up-regulate the expression of Iba1, CD45 and CD11b (Kettenmann *et al.*, 2011). The latter protein is part of the innate immune system and involved in phagocytosis of cellular debris and dead cells (Ma *et al.*, 2003). At the same time activated microglia are able to secrete for example cytokines (that trigger the invasion of leukocytes into the brain parenchyma), MMPs, reactive oxygen species and nitric oxid that have detrimental roles and might augment the tissue destruction in the CNS (Figure 5;(Kerschensteiner *et al.*, 2009; Rolls *et al.*, 2009; Wang *et al.*, 2007)). In contrast, microglia also release factors that are neuroprotective, thereby supporting tissue remodeling and repair (Figure 5; reviewed in (Kerschensteiner *et al.*, 2009; Kreutzberg, 1996)). This two-sided role of microglia could depend on the severity of the lesion, in particular of the disruption of the BBB, and the state of the lesion (acute phase in contrast to chronic lesions; (Kerschensteiner *et al.*, 2009; Kreutzberg, 1996; Wang *et al.*, 2007)).

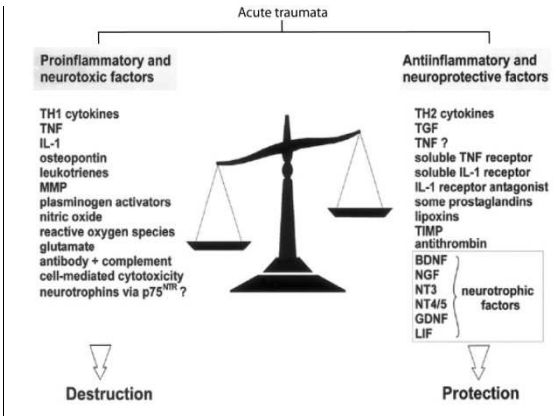

Figure 5 Scheme summarizing factors that are released by microglia amongst others upon acute inflammation and grouped according their effect on neurons into pro-inflammatory and anti-inflammatory cues. *(adapted from Kerschensteiner et al. 2009)*

Reaction of astrocytes upon traumatic brain injury

Besides NG2+ cells and microglia, also astrocytes respond to a variety of brain injuries including for example viral infections, acute traumata (e.g. stroke and ischemia) and neurodegenerative mechanism, in a process named astrogliosis (Sofroniew and Vinters, 2010). The degree of the gliotic reaction of astrocytes depends on the severity of the lesions, e.g. site of the lesion and BBB breakdown (Sofroniew and Vinters, 2010). The reaction of astrocytes comprises the up-regulation of distinct intermediate filaments (e.g. glial fibrillary acidic protein (GFAP), vimentin and nestin), cell body hypertrophy, thickening of their processes and an increased number around the injury site (Figure 6; (Buffo *et al.*, 2010; Robel *et al.*, 2011b; Sofroniew and Vinters, 2010)). In the intact brain parenchyma, astrocytes are quiescent whereas upon (severe) injury conditions reactive astrocytes re-enter the cell cycle and thereby increase in number around the lesion site. Due to these cellular alterations astrocytes form a tight barrier that restricts the area of inflammation and its associated neuronal cell death (Sofroniew, 2009; Sofroniew and Vinters, 2010). However, the long-term effect of astrogliosis is rather harmful. Reactive astrocytes are prone to secrete chondroitin sulphate proteoglycans, e.g. aggrecan, brevican, neurocan (Fawcett and Asher, 1999), after lesion that are all known to inhibit regeneration (Silver and Miller, 2004).

In addition, reactive astrocytes in the brain parenchyma mimic features, e.g. antigen expression profile and proliferation, of astrocytes in the stem cell niches of the intact CNS and therefore attracted attention as a possible *in vivo* source to regenerate neurons (Robel *et al.*, 2011b). In particular, Buffo et al. (2008) showed a stem cell potential for reactive astrocytes in the brain parenchyma upon acute injury by using the Glast::CreER^{T2} mouse line that labels astrocytes and their progeny in vivo. These recombined astrocytes can grow *in vitro* as multipotent neurospheres by giving rise to neurons, astrocytes and oligodendrocytes upon differentiation (Buffo *et al.*, 2008). However, there is so far no *in vivo* evidence that reactive astrocytes can generate new neurons upon acute injury without further manipulation (Barnabe-Heider *et al.*, 2010; Buffo *et al.*, 2008; Tatsumi *et al.*, 2008).

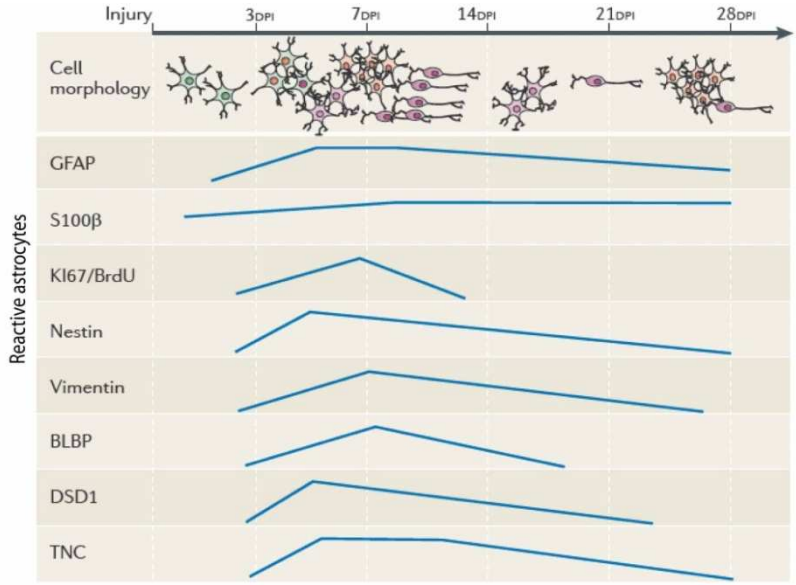

Figure 6 Scheme summarizing the reaction of gray matter astrocytes upon acute injury. Astrocytes react to traumata by morphological changes and the up-regulation of certain antigens, for example GFAP, nestin, vimentin and BLBP. Depending on the severity of the lesion reactive astrocytes start to proliferate, therefore being Ki67+ and incorporating BrdU. BrdU – 5'bromo-desoxyuridine, GFAP – glial fibrillary acidic protein, BLBP – brain lipid-binding protein, DSD1 – DSD1 proteglycan, TNC – tenascin*(adapted from Robel et al. 2011)*

Nevertheless, these data also show the importance of genetically modified mouse lines in order to gain further insights in the reactivity and potential of astrocytes upon acute injury *in vivo*. Besides the fate-mapping, such modified mouse lines are essential tools (e.g. Glast::CreER^{T2} line) to determine new molecular mechanism. For example the deletion of the RhoGTPase Cdc42 in astrocytes *in vivo* resulted in an increased number of microglia upon acute cortical injury (Robel *et al.*, 2011a). In order to understand now the role and function of proliferating NG2+ cells and their interaction with other cell types after lesion, genetically modified mouse lines are engineered to specifically target NG2+ cells.

1.4 Transgenic animal models to fate-map and to manipulate NG2+ glia

The most common approaches to target time and tissue-specific cells are the tetracycline/doxycycline system and the Cre/loxP system (reviewed by Bockamp *et al.*, 2002). In particular, the latter system contains the site-specific DNA recombinase Cre, being originally expressed in the P1 bacteriophage (Sauer, 1998). This recombinase recognizes distinct DNA sequences (loxP sites) and excises/ inverts the DNA sequence located between two loxP sites. Such loxP sites are most often used to (1) flank a stop cassette in front of a reporter gene, e.g. green fluorescent protein (GFP), to visualize successfully recombined cells

or (2) flank a gene of interest for loss-of-function experiments upon recombination. The efficiency of the Cre-mediated recombination is between 80-100%, but one recombination event requires at least four Cre molecules (Sauer, 1998). Temporal control can be acquired by fusing Cre to a mutated estrogen-receptor binding domain (ER) that has a higher affinity for synthetic estrogens (e.g. tamoxifen (Tam) and its metabolite 4-hydroxy-tamoxifen (OHT)) than for the endogenous estrogen (Jaisser, 2000). The advantage of the CreER system is its localization in the cytosol. Only after tamoxifen application the fusion protein is translocated in the nucleus (Figure 7).

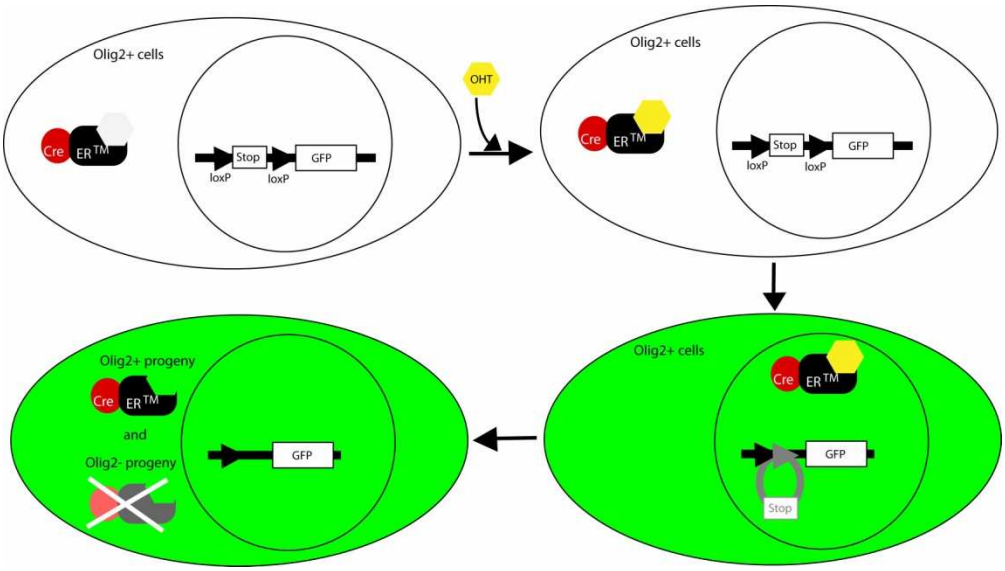

Figure 7 Cartoon summarizing the Cre/loxP system using the example of Olig2::CreER^{TM} mice crossed to a green fluorescent protein (GFP) reporter mouse line. Olig2CreER^{TM} mice express CreER^{TM} in the cytosol of Olig2 expressing cells. By administrating tamoxifen to the mice, tamoxifen and its metabolite 4-hydroxy-tamoxifen can pass the blood brain barrier and bind to the estrogen component of the CreER^{TM} protein. Thereby, CreER^{TM} will be translocated to the nucleus where the Cre recombinase can bind to the loxP sites within the DNA and removes the stop-cassette in front of the GFP. Thus, GFP will be expressed in Olig2+ cells and will remain expressed in Olig2+ progeny and Olig2- progeny, independently of the expression of Olig2.

Several ER constructs exist using either the mouse estrogen receptor (ER^{TM}; (Vasioukhin *et al.*, 1999)) or the human estrogen receptor (ER^{T} or ER^{T2} – an improved ER^{T}-version that is more sensitive to OHT (Indra *et al.*, 1999; Metzger *et al.*, 1995)). In addition to this temporal specificity, tissue-specificity can be achieved by expressing CreER under a gene-specific promoter, typically expressed within the tissue of interest. The most reliable cell-specific expression of CreER is achieved by knock-in of CreER into the endogenous gene locus. The disadvantage is however that only one CreER molecule is inserted in the endogenous locus, resulting probably in low recombination rate as it is the case in the Olig2::CreER^{TM} mouse

line (Masahira *et al.*, 2006). Although preserving the endogenous expression at a lower level in heterozygote knockin-mice, this can already alter the cellular homeostasis, causing cellular and behavioral abnormalities, e.g. in the Sox10-lacZ mice (Britsch *et al.*, 2001; Ludwig *et al.*, 2004). To overcome these limitations, transgenic mice can be generated by random integration of conventional bacterial vectors (e.g. pBluescript), expressing CreER, within the genome that would not influence endogenous expression levels. However, the integration side of these vectors can result in a cellular and tissue-dependent inaccurate expression of CreER. In addition, these bacterial vectors have a limited cloning capacity and contain usually a “minimal”-promoter in front of the gene of interest. Using parts of the promoter that are not well characterized or the lack of distal regulatory elements within these vectors might cause CreER-misexpression. In contrast, the use of bacterial artificial chromosomes (BACs) would circumvent such a limitation by inserting between 100-250 kilo basepairs (kb) of non-bacterial DNA into the BAC. In addition, it is shown that BACs can integrate multiple times within the genome which would result in a higher CreER expression and better recombination rate (Chandler *et al.*, 2007).

So far different inducible mouse lines are published targeting NG2+ cells and are summarized in Table 1. However, some of the available mouse lines either have a low recombination rate in NG2+ glia and thereby risk the analysis of a subpopulation of NG2+ cells (as mentioned above) or target different cell types besides the oligodendrocyte lineage.

Table 1 Summary of inducible transgenic mouse lines to target NG2+ glia

Mouse line	Type of transgenic mice	Recombination efficiency in adult NG2+ glia	Recombined cell types in the adult CNS	Literature reference
Olig2::CreER^{TM}	Knockin; Olig2 gene locus	low	NG2+ cells, mature oligodendrocytes, astrocytes, few neurons	(Dimou *et al.*, 2008; Tatsumi *et al.*, 2008)
NG2-CreER^{TM}	Bac-transgenic (RPCI 23-library)	low	NG2+ cells, mature oligodendrocytes	(Zhu *et al.*, 2008; Zhu *et al.*, 2011)
PDGFRα-CreER^{TM}	Bac-transgenic (RP24-148N4)	high	NG2+ cells, few neurons, pericytes, choroid plexus	(Kang *et al.*, 2010)
PDGFRα-CreER^{T2}	PAC-transgenic (PAC 546-M3)	high	NG2+ cells, mature oligodendrocytes, (neurons)	(Rivers *et al.*, 2008)
Plp1-CreER^{T2}	Transgenic (2.4 kb Plp1-promoter fragment)	low	NG2+ cells, mature and myelinating oligodendrocytes, neurons, astrocytes	(Doerflinger *et al.*, 2003; Guo *et al.*, 2010; Kang *et al.*, 2010)

2 Aim of the study

Given the importance of NG2+ glia the purpose of my study was to determine the proliferative behavior and the fate of NG2+ glia in the intact adult CNS and after acute injury. Therefore, the following questions were addressed:

1) How long is the cell cycle length of NG2+ cells in the intact cortical gray matter?
2) Do all NG2+ cells divide in the intact cortex?
3) Do NG2+ cells change their proliferative behavior under altered environmental conditions?
4) What is the fate of NG2+ glia in the intact and acutely injured cortex?

Thus, the cell cycle length of NG2+ cells in the intact cortical gray matter of rodents was determined first, in order to characterize then their proliferative response to two altered environmental conditions: (1) Acute cortical injury in the model of stab wound injury and (2) Physiologically increased neuronal activity by physical exercise (running wheel experiments). In addition, the fate of NG2+ cells was analyzed in two different ways either by characterizing the progeny of proliferating NG2+ cells with BrdU-retaining studies or by tracing the fate of randomly labeled NG2+ cells, comprising proliferating as well as non-proliferating NG2+ cells, by using the Olig2::CreER^{TM} mouse line (as described above). Given the low recombination rate in this genetically modified mouse line, I generated a new mouse line using a BAC-transgene that contains an improved Cre variant (iCre) fused to the ER^{T2}, as a basis to achieve high recombination rates. The transcription factor Sry-related high mobility group box protein 10 (Sox10) was chosen to drive the expression of iCreER^{T2}, having the advantage that the distal regulatory elements of Sox10 are well-described and Sox10 is exclusively expressed in the oligodendrocyte lineage in the adult CNS. Then the characterization of this new mouse line by fate-mapping analysis was intended.

3 Results

This chapter is split into three paragraphs, each of them representing an independent study that is already published or under revision in international peer-reviewed journals. The main results are summarized below and the contributions of the single authors to each study are provided.

3.1 Proliferative behavior of NG2+ glia and the regulation by acute injury and physical activity

This study herein is published as "Progenitors in the Adult Cerebral Cortex: Cell Cycle Properties and Regulation by Physiological stimuli and injury" by **Simon C,** Dimou L and Götz M in Glia 2011, 59: 869-881.

Here, I examined the cell cycle length of NG2+ glia in the adult cerebral cortex and the impact of environmental cues on the proliferative behavior of NG2+ glia. By administrating BrdU to wild type mice combined with immunohistochemical stainings for BrdU and Ki67 (an antigen highly expressed from the S- to M-phase), the cell cycle length of NG2+ cells in the adult cerebral GM was determined. NG2+ glia in the intact cortical GM had an impressively long cell cycle length. Interestingly, while the S- to M-phase took only five days these cells remained in the G1-phase for at least 32 days. Despite this, proliferating NG2+ cells can either re-enter the S- to M-phase and thereby self-renew, or enter the G0-phase and differentiate into mature oligodendrocytes. In addition, within a three day time window only 12% of the NG2+ cells proliferated whereas 80% of them divided within three months, thereby showing that NG2+ glia divide in an asynchronous manner. However, NG2+ cells can considerably shorten their cell cycle length in response to the environment. Stab wound injury resulted in an acute re-entry of the majority of NG2+ cells surrounding the injury site into the cell cycle. In contrast, increased physical exercise resulted in a temporarily increased exit of the cell cycle of NG2+ cells accompanied by fast differentiation. In summary, this study suggests a modulation of the cell cycle length of NG2+ glia by environmental influences. Thus, NG2+ glia proliferation and oligodendrocyte generation is profoundly influenced by environmental cues – a finding with implications for other pathologies, such as epilepsy and Multiple Sklerosis.

Contribution of the authors

The herein published experiments and analysis were done by me as well as the writing of the manuscript. L. Dimou and M. Götz thoroughly revised the manuscript, planned experiments and discussed implications of the results. The study was financed by M. Götz.

GLIA

GLIA 59:869–881 (2011)

Progenitors in the Adult Cerebral Cortex: Cell Cycle Properties and Regulation by Physiological Stimuli and Injury

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KEY WORDS
cell cycle regulation; cortical injury; running wheel; microglia; reactive astrocytes

ABSTRACT
The adult brain parenchyma contains a widespread population of progenitors generating different cells of the oligodendrocyte lineage such as NG2+ cells and some mature oligodendrocytes. However, it is still largely unknown how proliferation and lineage decisions of these progenitors are regulated. Here, we first characterized the cell cycle length, proliferative fraction, and progeny of dividing cells in the adult cerebral cortex and then compared these proliferation characteristics after two distinct stimuli, invasive acute brain injury and increased physiological activity by voluntary physical exercise. Our data show that adult parenchymal progenitors have a very long cell cycle due to an extended G1 phase, many of them can divide at least twice and only a limited proportion of the progeny differentiates into mature oligodendrocytes. After stab wound injury, however, many of these progenitors re-enter the cell cycle very fast, suggesting that the normally long G1 phase is subject to regulation and can be abruptly shortened. In striking contrast, voluntary physical exercise shows the opposite effect with increased exit of the cell cycle followed by an enhanced and fast differentiation into mature oligodendrocytes. Taken together, our data demonstrate that the endogenous population of adult brain parenchymal progenitors is subject to profound modulation by environmental stimuli in both directions, either faster proliferation or faster differentiation. © 2011 Wiley-Liss, Inc.

INTRODUCTION

The major pool of progenitors outside the neurogenic niches of the adult brain expresses the proteoglycan NG2. As these NG2+ cells also have antigens like PDGFRα, Olig2, and/or Sox10 they have been characterized as oligodendrocyte precursor cells (OPCs) (Dimou et al., 2008; Karram et al., 2008; Levine et al., 1993; Nishiyama et al., 2009). This concept was supported lately by fate mapping analysis demonstrating the oligodendrocyte lineage of NG2-, PDGFRα-, and Olig2-positive cells in the adult central nervous system (CNS; Dimou et al., 2008; Kang et al., 2010; Nishiyama et al., 2009; Rivers et al., 2008). Interestingly, NG2+ cells in the cortical white matter of the adult cerebral cortex generate mostly myelinating oligodendrocytes, while a notably higher proportion of the progeny remains NG2+ in the cortical gray matter (GM; Dimou et al., 2008; Kang et al., 2010). This raises the question whether GM NG2+ cells become postmitotic or remain as actively cycling progenitors. Moreover, it is still unresolved whether the different behavior of these cells may be subject to regulation by extrinsic cues. In particular, it is unclear to which degree neuronal activity may influence the proliferation and differentiation of NG2+ cells as they are closely associated with synapses and nodes of Ranvier (for review see Trotter et al., 2010).

In addition, NG2+ cells are also prone to react to injury and to increase in number after acute trauma (Alonso, 2005; Buffo et al., 2005; Hampton et al., 2004; Levine, 1994; McTigue et al., 2001; Rhodes et al., 2006; Zawadzka et al., 2010). However, it is not clear to which extent this increase is achieved by an increased rate of proliferation and if so whether this may be due to alterations in cell cycle length or activation of previously quiescent NG2+ cells.

Therefore, we first determined the proliferative properties of the progenitor pool in the GM of the adult cerebral cortex, in order to examine then their behavior after stab wound injury or altered neuronal activity upon voluntary exercise. These data revealed an intriguingly distinct control of proliferation and differentiation of these progenitors upon different stimuli.

MATERIALS AND METHODS

Animals

Adult wild-type mice (2–3 months old) received 5′-bromo-desoxyuridine (BrdU; Sigma Aldrich) either

Additional Supporting Information may be found in the online version of this article.

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intraperitoneally (0.5 mg BrdU/10 g mice) two times every 2 hours (in total 4 hours) or in the drinking water (1 mg/ml BrdU supplemented with 1% sucrose) for 12 hours, 3, 4, 5, 6, 7, 14, 28, 56, or 84 consecutive days (d) before sacrifice. The BrdU label-retaining experiments comprised of a 14-d application of BrdU in the drinking water followed by an additional period of 14, 28, 42, 56, 70, or 84 d of normal drinking water.

Stab wound injury was performed as a 2.0- to 2.5-mm-long and 0.5-mm-deep lesion with a 19-gauge-thick lancet in the somatosensory and visual cortex without injuring the cortical white matter. Operations were performed as described in Buffo et al. (2005) and in accordance with the policies of the state of Bavaria under license number 55.2-1-54-2531-144-07. Animals then received BrdU in the drinking water for 2, 3, 7, or 14 d before sacrifice. The BrdU label-retaining experiment comprised a 14-d BrdU pulse after the lesion followed by a 14-d retaining period (in total 28 d postinjury, dpi). To test whether proliferative-quiescent NG2+ cells react to acute injury, animals received for 14 d BrdU-water and underwent a 42-d retaining period before the stab wound was performed. Mice were then sacrificed at 3 dpi.

For voluntary physical exercise experiments mice were housed in a big cage equipped with several running wheels for 14 d and received during this period BrdU-water while control animals were housed in standard cages.

Histological Analysis

Mice were anesthetized and transcardially perfused with 4% paraformaldehyde (PFA). Brains were collected, shortly postfixed in 4% PFA and cryoprotected in 30% sucrose. Thirty-micrometer-thick brain sections were cut and stained according to standard protocols as described in Dimou et al. (2008) with the following primary antibodies: rat-BrdU (Biomol; 1:200), rat-CD45 (BD Pharmingen; 1:100), mouse (m)-GFAP (Sigma; 1:500), guinea pig (gp)-GLT1 (Millipore; 1:100), m-GSTπ (BD; 1:500), rabbit (rb)-Iba1 (Wako; 1:500), rb-Ki67 (ThermoScientific; 1:1,000), rb-NG2 (Millipore; 1:500), rb-Olig2 (Millipore; 1:500), rat-PDGFRα (BD Pharmingen; 1:200), m-S100β (Sigma; 1:500), and gp-Sox10 (kindly provided by Michael Wegner; 1:1000). Secondary antibodies were chosen according to the primary antibodies and were coupled to Alexa488, Cy2, Alexa555, Cy3, Alexa647, or Cy5 and sections were counterstained with 4′,6-diamidino-2-phenylindole (DAPI; Sigma Aldrich). For BrdU, Ki67, and Olig2 immunohistochemistry, staining of other antigens was performed first, then the sections were fixed with 4% PFA (10 min) before boiling in 0.01M citrate buffer (pH 6.0) for 20 min and then further stained.

Images were collected with an Olympus microscope (BX61TRF) or a Zeiss confocal microscope system (LSM710). The quantitative analysis was done with ImageJ counting confocal images of 15 μm thick z-stacks. At least two adjacent fields of view in the medial part of the intact motor and somatosensory cortical GM were analyzed in at least three animals and three different sections per animal for each time point unless stated otherwise. After stab wound an area of 0.12 mm^2 around the lesion was analyzed. All results are presented as average with the standard error of the mean. Unpaired Student's *t*-test was performed to test for significance.

RESULTS

Cell Cycle Characteristics of Progenitors in the Gray Matter of the Cerebral Cortex

In order to examine the cell cycle and proliferation characteristics of progenitors in the GM of the adult cerebral cortex, we used the thymidine analog BrdU that is incorporated into the DNA of cycling cells during S-phase. As BrdU would also be integrated into the DNA by DNA repair mechanisms, we first determined the proportion of BrdU-labeled cells that is actively cycling and thus immunopositive for Ki67, a protein expressed mainly during the active proliferation period, namely in S/G2/M-phase (Scholzen and Gerdes, 2000; Taupin, 2007). When BrdU was injected twice within 4 hours (see Materials and Methods) all BrdU labeled nuclei were also Ki67+, indicating that these cells were mitotically active (Fig. 1a–b′). Notably, these cells appeared to enter the G1-phase relatively fast, as after a 3-d BrdU pulse most BrdU+ cells had already down regulated Ki67 (Fig. 1a). Consistent with previous data (Dimou et al., 2008; Psachoulia et al., 2009), the vast majority of Ki67+ cells as well as virtually all BrdU+ nuclei labeled with different BrdU pulses (4 hours–14 d) colocalized with NG2, PDGFRα, and Olig2 (Fig. 1c–g). No BrdU-labeled cells were double stained for microglia, astroglia, or neuroblast markers (data not shown).

Given the antigenic homogeneity of the adult progenitors, we next assessed the proliferative behavior of these cells by determining the mitotic labeling index (LI) and their cell cycle length. BrdU labeled 44% of proliferating Ki67+ within 4 hours (LI = 0.44), indicating that 44% of all actively cycling cells were in the S-phase during this time window (Fig. 1h). Nearly all active proliferating cells (Ki67+) became BrdU+, when the duration of BrdU administration was extended up to 5 d, implying that the S/G2/M-phases last about 5 d (Fig. 1h). However, Ki67 is down regulated during the G1-phase and therefore does not cover the entire cell cycle. To calculate the total cell cycle length including the G1-phase, we determined the proportion of NG2+ cells that incorporated BrdU supplied for up to 84 d in the drinking water. The number of BrdU+/NG2+ cells amongst the NG2+ cell pool increased up to 80% within this time and reached this plateau after 37 d, suggesting that the total cell cycle length of NG2+ cells is 37 d as calculated by the equation proposed by Nowakowski et al. (1989) (Fig. 1i,j). Taken together, progenitors in the GM of the cerebral cortex have a long cell cycle length, mainly due to a prolonged G1-phase (Fig. 1j).

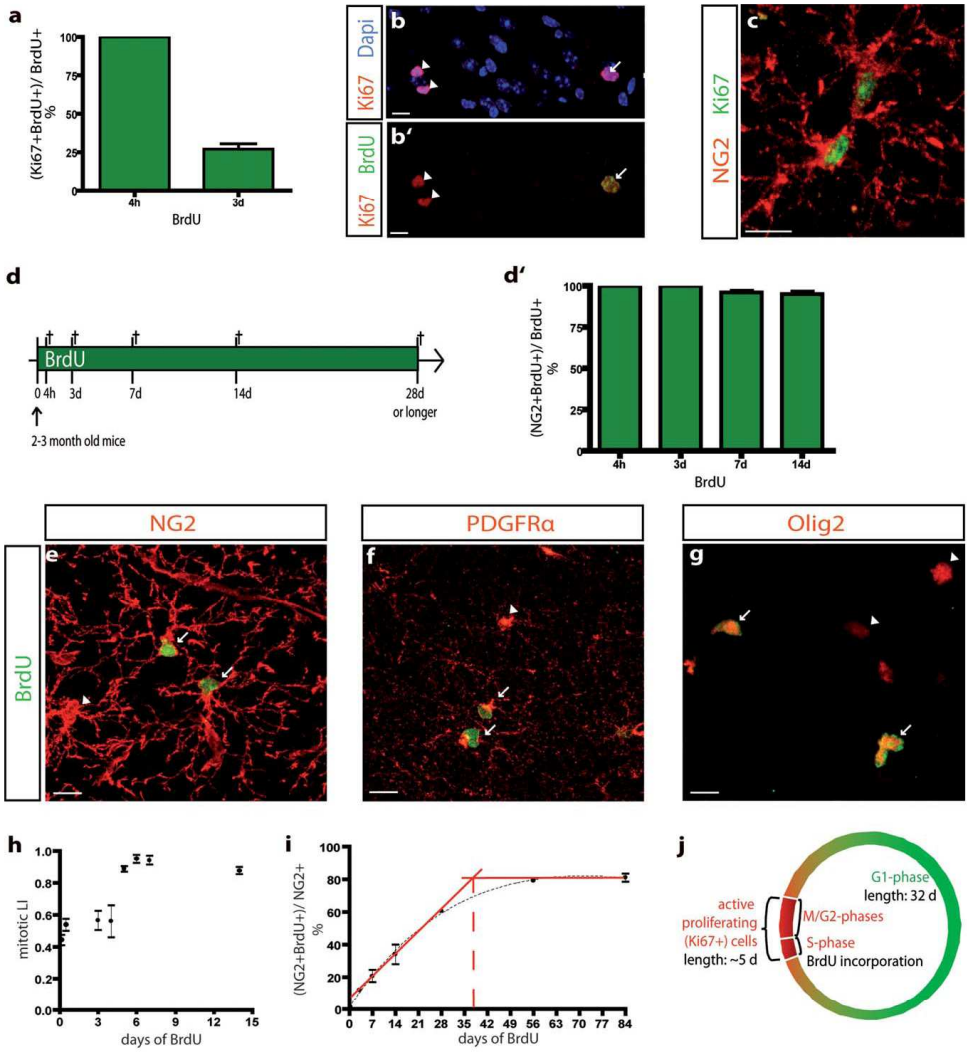

Fig. 1. Cortical NG2+ cells have a long cell cycle. (**a**) Quantification of the proportion of BrdU+ cells that are mitotically active (Ki67+). (**b**) Image of Ki67+ nuclei that (**b'**) either passed the S-phase being BrdU+ (arrow) or are in the G2/M-phases and therefore Ki67+/BrdU− (arrowheads) after double injection of BrdU within 4 hours. (**c**) Image of Ki67+/NG2+ cells. (**d**) Experimental approach: mice received BrdU for 4 hours or continuously up to 84 d. (**d'**) Bar graph depicting the proportion of NG2+/BrdU+ cells amongst the BrdU+ pool for different BrdU-pulses. (**e–g**) Confocal images showing BrdU+ nuclei colocalizing with NG2 (e), PDGFRα (f), or Olig2 (g) after a 3-d BrdU-pulse. (**h**) Quantification of Ki67+/BrdU+ cells amongst all Ki67+ cells for different BrdU-pulses (LI: labeling index). The time point when LI reaches its plateau reveals the length of the S/G2/M-phases (for 0.5 and 7 d BrdU, $n = 2$ mice). (**i**) Bar graph displaying the BrdU+/NG2+ growth fraction for different BrdU pulses. Black patterned line shows the regression curve whereas the red lines indicate schematically the calculation of the cell cycle length. (**j**) Scheme summarizing the cell cycle profile of adult NG2+ cells. (b–c, e–g): Maximum intensity projections of confocal images; arrows point to double immunopositive cells whereas arrowheads indicate single immunopositive cells; scale bars 10 μm.

Progeny of Proliferating Cells in the Gray Matter of the Cerebral Cortex

Although NG2+ cells are slow dividing, their number and distribution remains constant throughout life (Dawson et al., 2003; Rivers et al., 2008). This prompts the question about their progeny as these may mature into a different cell type and thereby be removed from the NG2+ progenitor pool. However, our previous fate mapping study of Olig2::CreER^{TM}-labeled cells observed only a minority of labeled cells differentiating into oligodendrocytes (Dimou et al., 2008), raising the question whether this is a hallmark of cells expressing the highest Olig2 levels (and hence mediating recombination). To determine the differentiation of all progenitors in the GM in an unbiased manner independent of expression levels of a specific gene, we gave BrdU for 14 d—a time point when nearly all BrdU+ cells are still NG2+ (Fig. 1d′)—and examined the progeny of these cells after removing BrdU and allowing differentiation of the newly generated cells for up to 70 d (Fig. 2a–a′).

Interestingly, while the number of BrdU+ cells remained stable independent of the retaining period, the identity of BrdU-labeled cells changed. After 70 d of retaining, almost two thirds of all BrdU labeled cells had differentiated into NG2-negative but GSTπ+ cells, indicating a maturation of proliferating NG2+ cells to oligodendrocytes (Fig. 2a′–c′).

Besides a considerable proportion (about 1/3) of BrdU-labeled cells that were still NG2+, we also detected some BrdU+ cells that were neither NG2+ nor GSTπ+ (Fig. 2b, Supp. Info. Fig. 1a–a‴). These BrdU+/NG2−/GSTπ− cells did not colocalize with PDGFRα (data not shown), astrocyte-specific markers (S100β) or neuronal markers, e.g. Dcx or NeuN (Supp. Info. Fig. 1b–c′ and data not shown). However, they could be stained for the transcription factor Sox10 (Supp. Info. Fig. 1d–d″) thereby identifying them also as part of the oligodendrocyte lineage, probably at a stage between the progenitor (NG2+) and the mature oligodendrocyte state (GSTπ+). Taken together, these data demonstrate that proliferating cells in the GM of the cerebral cortex give rise exclusively to cells of the oligodendrocyte lineage, mostly mature GSTπ+ oligodendrocytes and a considerable proportion of NG2+ cells.

Self-Renewal Capacity of Progenitors in the Adult Cerebral Cortex

As the above data revealed a considerable fraction of BrdU+ cells to be still NG2+ 70 d after the end of BrdU-labeling, this raised the question whether these cells remain cycling and hence self-renew or become quiescent serving as a reserve pool of progenitor cells in case of further demand or even become permanently postmitotic. To answer this, we examined the contribution of BrdU+ cells to the actively dividing and therefore Ki67+ cell pool. While 71 ± 10.9% of all Ki67+ cells were BrdU+ immediately after the end of a 14-d BrdU-labeling period, most labeled cells down-regulated Ki67 during the following 42 "BrdU-free" days (only 16% of the Ki67+ cells were BrdU+; Fig. 2d). This may be due to exit of these BrdU+ cells from the active cell cycle or to dilution of BrdU in further divisions, even though the long cell cycle length (Fig. 1i,j) would not allow a high degree of dilution as maximal one to two cell cycles would have been completed within this period. Interestingly, we observed a subsequent increase of BrdU-labeled cells amongst the Ki67+ cells at later time points (from 16 + 8.8% at 42 d to 52 + 10.7% at 84 d of retaining period; Fig. 2d) indicating that a considerable proportion of the NG2+ progenitors re-enter the cell cycle, and undergo at least two rounds of cell division *in vivo*.

Proliferative Reaction of Progenitors in the Adult Cerebral Cortex in Response to Acute Injury

Next, we examined how the above described progenitors react to injury. Previous genetic fate mapping analysis revealed that NG2+ cells increased in number after stab wound injury (Dimou et al., 2008) raising the question whether this is achieved by shortening of the cell cycle length or by increased recruitment of NG2+ progenitors. To answer this, we performed a stab wound injury in the somatosensory and visual cortex and gave BrdU in the drinking water for either 3, 7, or 14 d to monitor all proliferating cells (Fig. 3a). Already at 3 dpi, the number of BrdU+/NG2+ cells increased fivefold compared with a 3-d BrdU pulse at the noninjured site (Fig. 3a′). Notably, this number (188 ± 54.3 cells/mm^2) excludes NG2+ pericytes that we identified by their elongated shape and position—attached to the blood vessels (about 6.8 ± 3.1% of all BrdU+ cells are NG2+ pericytes at 3 dpi; arrowheads in Fig. 3b). At 7 dpi NG2+/BrdU+ cells (excluding pericytes) further increased in number to a maximum of 302 ± 17.5 cells/mm^2 (Fig. 3a′). Notably, at this time the total number of NG2+ cells increased and the BrdU+/NG2+ cells already accounted for 74% of the entire NG2+ pool (Supp. Info. Fig. 2a,b). To exclude any volume effects due to the injury we analyzed the cell density with and without any lesion and could not see any obvious difference in number of cells per area (data not shown).

BrdU incorporation in such a high proportion of NG2+ cells requires five times longer in the healthy brain (37 d, see Fig. 1i), suggesting that NG2+ cells shorten the G1 phase and enter the active stage of the cell cycle much faster upon injury than in the intact brain. This opens the question whether NG2+ cells that already had entered G1-phase and down-regulated Ki67, will resume proliferation faster after injury. Therefore, we examined BrdU-labeled cells at 42 d after the labeling period, the time point when only few of the previously BrdU labeled cells are still mitotically active (Fig. 2d). When mice were injured at 42 d after BrdU labeling and examined 3 d later, the number of BrdU+/NG2+

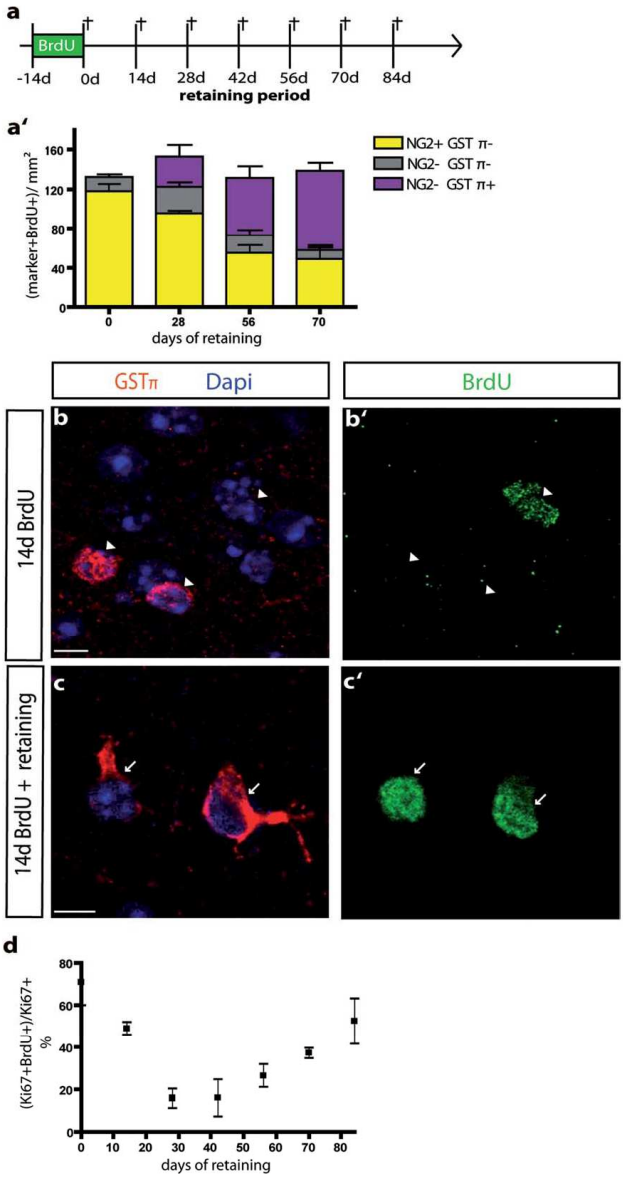

Fig. 2. A proportion of NG2+ cells differentiates to mature oligodendrocytes whereas some NG2+ cells can divide at least twice. (**a**) Experimental approach: Mice received BrdU for 14 d (day 0) and underwent different BrdU-retaining periods. (**a′**) Graph showing the composition of BrdU+ cells after different BrdU-retaining periods. (**b–c′**) Images depicting BrdU+/GSTπ− cells after a 14-d BrdU-pulse (b–b′) and BrdU+/GSTπ+ cells after a 42-d retaining period (c–c′). Note that the nuclear BrdU-signal in these cells shows sometimes an irregular distribution, especially with increased BrdU-retaining periods. (**d**) Graph depicting the proportion of Ki67+/BrdU+ cells amongst all Ki67+ cells with increased BrdU-retaining periods (for 84 d retaining period, $n = 2$ mice). (b–c′): Maximum intensity projections; arrows point to double immunopositive cells; arrowheads indicate single positive cells; scale bars 10 μm.

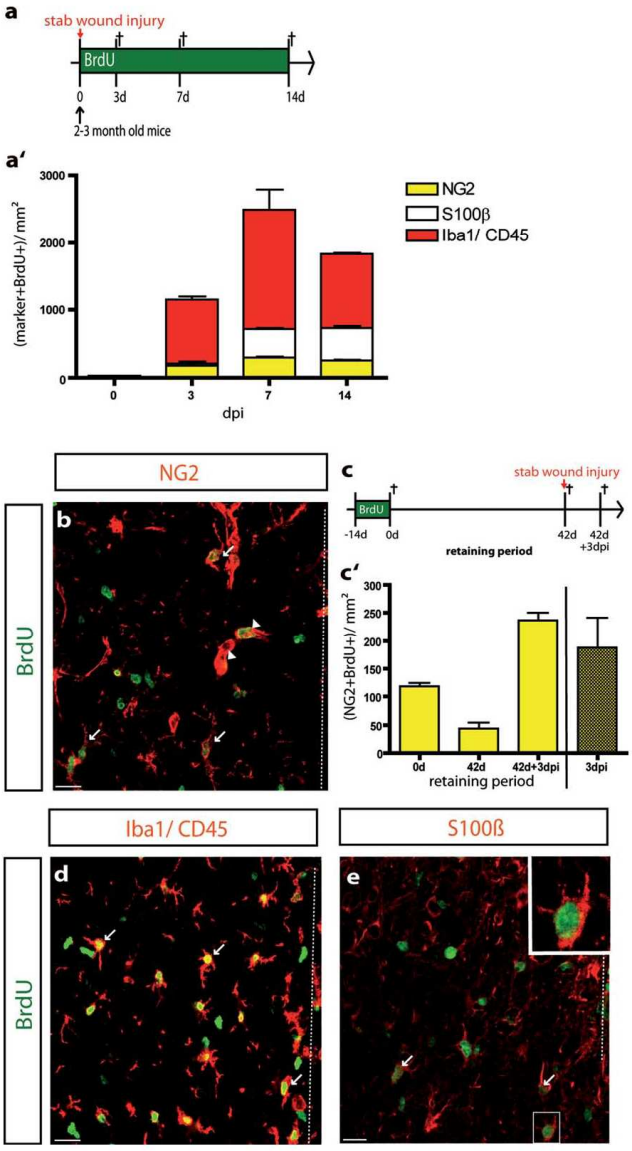

Fig. 3. NG2+ cells as well as microglia proliferate early after acute injury whereas astrocytes show a delayed response. (**a**) Experimental approach: Mice received BrdU for 3, 7, or 14 d after stab wound injury. (**a′**) Graph depicting the identity of BrdU+ cells at different days post-injury (dpi). (**b**) Image showing BrdU+ nuclei colocalizing with NG2. NG2+ cells (arrows) can be distinguished from NG2+ pericytes (arrowheads) based on their morphology and location. (**c**) Experimental approach: Mice received BrdU for 14 d (0 d), underwent a 42-d long retaining period (42 d) before the stab wound injury and were then sacrificed at 3 dpi (42 d + 3 dpi). (**c′**) Quantification of BrdU+/NG2+ cells of the experimental approach described in c (yellow bars). These numbers are opposed to the NG2+/BrdU+ cell number after a 3-d BrdU-pulse directly after injury (3 dpi, yellow-black patterned bar). (**d** and **e**) Micrographs depicting BrdU+ cells doublestained for Iba1/CD45 (d) and S100β (e). (b, d, e): Single confocal planes; the injury site is marked with a white dashed line. Double-positive cells are indicated by arrows. Arrowheads point to pericytes. (b, d) 3 dpi; (e) 7 dpi; scale bars 20 μm.

cells significantly increased fivefold within these 3 d (Fig. 3c–c′). This number is comparable to the number of BrdU+/NG2+ cells detected by a 3-d BrdU-pulse started directly after lesion (yellow-black patterned bar in Fig. 3c′). Thus, these results support the fast recruitment of quiescent NG2+ cells into the cell cycle after injury.

Proliferative Reaction of Other Glial Cells to Acute Injury

The temporal proliferation profile of NG2+ cells parallels the microglia response (Fig. 3a′). CD45+ blood-derived cells and Iba1+ microglia also increased considerably in number at 3 dpi (Fig. 3a′) and microglia became reactive, i.e. hypertrophic with few short processes (Fig. 3d). The number of these proliferating cells further increased at 7 dpi, and thereafter decreased (Fig. 3a′). In contrast to these populations, S100β+ astrocytes exhibited a delayed proliferative reaction. At 3 dpi, proliferating astrocytes were still rare (about 26 ± 5.3 cells/mm^2; Fig. 3a′) while their number increased about 16-fold at 7 dpi (Fig. 3a′,e; Supp. Info. Fig. 2c–f). By this time, 55% of the entire astrocyte pool had entered the cell cycle (Supp. Info. Fig. 2d). This proportion remained relatively constant until 14 dpi and contributed to the increase in astrocyte number around the injury site (Supp. Info. Fig. 2c). Notably, BrdU-labeled cells that divided within 14 dpi stayed around the injury site for at least the next 14 d (Supp. Info. Fig. 2g) and thereby contributed to the glial scar formation. Moreover, the markers used above stain virtually all BrdU+ cells indicating that these glial populations cover the entire pool of proliferating cells after injury.

Reaction Profile of Mitotically Active Cells to Acute Injury

The above experiments allowed to monitor all dividing cells within a period of 14 d by continuous supply with BrdU and showed a faster recruitment of NG2+ cells into the active proliferation phase of the cell cycle as response to the stab wound lesion. To examine whether this is due to a shortening of the cell cycle length, we examined the actively proliferating (Ki67+) cells and determined the labeling fraction of them incorporating BrdU amongst the NG2+ population at different time points after injury. The number of Ki67+ cells increased over 60-fold at 3 dpi while their number decreased again thereafter and reached almost basal levels again at 28 dpi (no lesion: 16 ± 2.2 *vs.* 20 ± 4.3 Ki67+ cells/mm^2 at 28 dpi; Fig. 4a). The massive increase in proliferating cells at 3 dpi was mostly due to Iba1+ microglia and CD45+ cells which constitute about 70% of the Ki67+ pool at this stage (Fig. 4a,c). The number of actively proliferating (Ki67+) NG2+ cells also strongly increased (>15-fold) compared to the noninjured site (Fig. 4a,b). However, the contribution of both microglia and NG2+ cells to the proliferating cell pool decreased rather fast at 7 dpi and astrocytes became the major fraction of proliferating cells (Fig. 4a,d). Already at 14 dpi the only mitotically active cells within this glial scar were NG2+ cells with proliferation levels comparable to the intact GM as described above (Fig. 4a).

Next, we determined the proportion of mitotically active (Ki67+) NG2+ cells that entered the S-phase up to 3 d after stab wound. While in the intact cortex only 56 + 6.0% of NG2+/Ki67+ cells had incorporated BrdU+ within 3 d (Fig. 1h), after injury virtually all cycling Ki67+/NG2+ cells (92.8 ± 4%) incorporate BrdU within 2 d (Fig. 4e–e′). Thus, upon injury slow dividing or quiescent NG2+ cells re-enter the cell cycle very fast and decrease the length of their S/G2/M-phases from 5 to maximum 2 d.

Voluntary Physical Exercise Promotes Differentiation of NG2+ Cells

Given the profound regulation of cell cycle length and cell cycle recruitment by stab wound injury, we wondered whether more mild physiological stimuli may have similar effects. Especially physical activity in a running wheel has been observed as a stimulus resulting in pronounced increase of proliferation in the adult neurogenic zones (Bednarczyk et al., 2009; Holmes et al., 2004; Krityakiarana et al., 2010; van Praag et al., 1999). We therefore examined the influence of motor activity on the proliferation of NG2+ cells in the GM of the cerebral cortex. However, contrary to the expected increase, the number of active proliferating cells (Ki67+) was dramatically decreased when mice had access to running wheels for 14 d (Fig. 5a). Consistently, cells labeled with BrdU during this period also decreased in number (Fig. 5b–d). As this result may be explained by an increased exit from the cell cycle due to differentiation, we examined the identity of BrdU-labeled cells after 14 d of access to running wheels. Indeed, we observed that almost half of all BrdU-labeled cells already expressed the mature oligodendrocyte marker GSTπ, while none of such differentiated oligodendrocytes was observed in the control animals after 14 d of BrdU exposure (Fig. 5e–g). Notably, also after voluntary exercise no fate switch of NG2+ cells to the neuronal or astrocyte lineage could be observed (data not shown). Thus, these data demonstrate profound and differential regulation of proliferation and differentiation of NG2+ cells by physical activity and injury.

DISCUSSION

NG2+ cells represent the majority of proliferating cells in the intact adult brain parenchyma (Dawson et al., 2003; Gensert and Goldman, 2001; Horner et al., 2000). However, little is known about the signals influencing these progenitors. Here we showed the profound

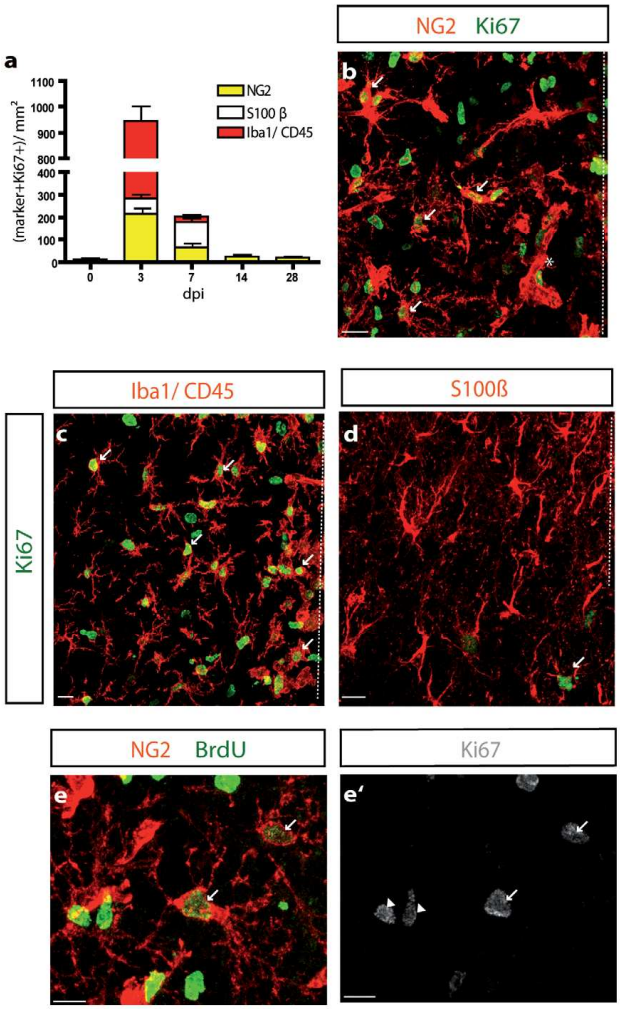

Fig. 1. NG2+ cells respond with strong proliferation to lesion by shortening their cell cycle length. (**a**) The composition of mitotically active cells is shown at different time points after lesion. (**b–d**) Images showing the colabeling of Ki67+ cells with NG2 (b) and Iba1/ CD45 (c) at 3 dpi and S100β at 7 dpi (d). (**e–e′**) High-power images depicting BrdU+/NG2+ cells (e) that are colabeled with Ki67 (e′) at 3 dpi. (b–e′): Maximum intensity projections; the injury site is marked with a white dashed line. Arrows point to double or triple immunopositive cells; Arrowheads indicate NG2- but BrdU+/Ki67+ cells; scale bars 10 μm.

impact of environmental cues on these progenitors. While voluntary physical exercise promoted their premature differentiation, invasive injury resulted in a pronounced shortening of cell cycle length and recruitment of quiescent NG2+ cells into the cell cycle.

Cell Cycle Properties of Progenitors in the Intact Cortex

Our study shows that all proliferating cells in the adult cerebral GM are NG2+ and generate exclusively

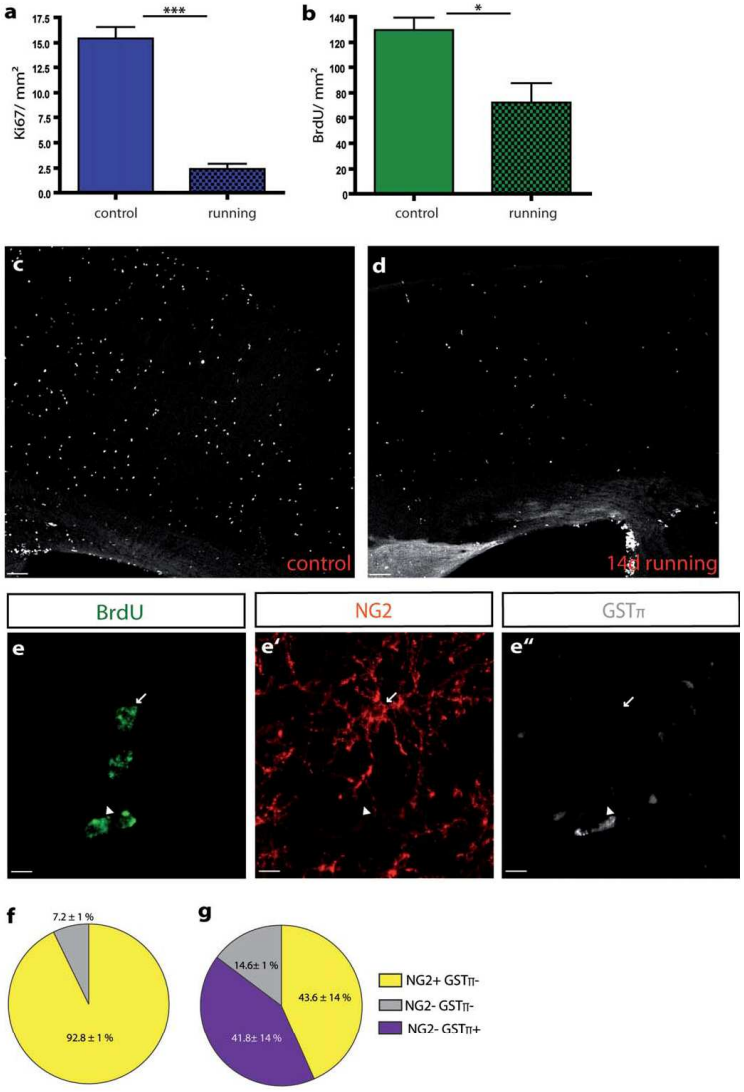

Fig. 5. Physical exercise results in reduced proliferation and early differentiation of NG2+ cells. (**a**, **b**) Quantifications of Ki67+ (a) and BrdU+ cells (b) after 14 d of BrdU application in running or control mice. (**c** and **d**) Images depicting BrdU+ cells in the cortical gray matter of control mice without running (c) and age-matched mice after physical exercise (d). (**e**–**e**″) Stainings depicting BrdU+ nuclei (e) colocalizing with NG2 (e′; arrow) and GSTπ (e″; arrowhead) at 14 d after physical exercise. (**f**, **g**) The composition of the BrdU+ cell pool of control animals (f) is compared with mice after 14 d of physical exercise (g). (c–e″): Maximum intensity projections; scale bars 100 μm (for c, d) and 10 μm (for e–e″).

cells of the oligodendrocyte lineage. These progenitors display a rather short active proliferation phase with a prolonged G1-phase. These data are consistent with data obtained in samples of the human cerebral cortex, where most of the NG2+/Olig2+ cycling cells were in the early G1-phase (Geha et al., 2010). Moreover, a con-

stant increase in cell cycle length of these progenitors with age was previously reported, data that correlate well with our data and especially the addendum, where the authors suggest due to new results that the actual cell cycle length at all ages might be longer than estimated (Psachoulia et al., 2010 and their addendum). However, the important consensus of all these studies is the long cell cycle of adult cerebral cortex progenitors. Indeed, this property seems to exist also in other regions of the adult CNS as Keirstead et al. (1998) found about half of the NG2+ cells in the adult rat spinal cord to be resistant to cell death upon irradiation, implicating that these cells are slow or not dividing.

Moreover, we show here for the first time directly that adult progenitors can re-enter the cell cycle, i.e. NG2+ cells which incorporated BrdU more than a month before re-enter the cell cycle and become Ki67+ again. These results elucidate previous observations of the capacity of NG2+ cells to self-renew, e.g. after depletion of NG2+ cells in irradiation experiments and in lesion paradigms of de/remyelination (Irvine and Blakemore, 2007; Levine and Reynolds, 1999; Panagiotakos et al., 2007). These data are best explained by re-entry in the cell cycle of a previously quiescent or very slow proliferating fraction of NG2+ cells as also observed in our study after injury.

Progeny of NG2+ Progenitors

Interestingly, although NG2+ cells are constantly proliferating, their number and distribution does not change much throughout life (Dawson et al., 2003; Rivers et al., 2008), implying that half of the daughter cells must either differentiate, migrate away or die. Indeed, BrdU label-retaining experiments revealed that NG2+ progenitors maintain such a balance between self-renewal and differentiation with about 1/2 to 2/3 of these cells differentiating into mature oligodendrocytes and the remainder maintaining NG2-immunoreactivity. Notably, the differentiation rate in the present study was higher than observed by genetic fate mapping following the progeny of cells labeled in an inducible Olig2::CreER^{TM} mouse model (Dimou et al., 2008). Recombination induced by Tamoxifen in the adult cerebral cortex is of low efficiency in these mice, suggesting that only the cells with the highest Olig2 expression levels mediate recombination. In contrast, in another fate mapping analysis using PDGFRα-CreER^{TM} (Kang et al., 2010) the proportion of progenitors differentiating into oligodendrocytes corresponds to levels of the current study. Taken together, these three studies suggest that the different rate of differentiation of labeled NG2+ cells depends on the Olig2, and hence CreER^{TM}, expression levels. Thus, NG2+ cells with high levels of Olig2 (that are mainly followed by the genetic fate mapping in Dimou et al. 2008) largely remain in a progenitor fate, while NG2+ cells with lower Olig2 levels differentiate into mature oligodendrocytes at higher rates. Like fate mapping methods also BrdU-labeling has its limitations as long pulses with high doses of the thymidine analog could lead to an overdose and therefore to increased cell death. Despite the low dose of BrdU used in this study which is below doses previously described to have no toxic effects in the adult brain (Cameron and McKay, 2001; Hancock et al., 2009), we can not fully exclude minor effects on cell survival (not detected by Caspase-3 or DAPI stainings) for the long BrdU application.

Cortical NG2+ cells have a long cell cycle and show self-renewal capacity, defined as re-entry of the cell cycle by cells with the same antigenic profile (NG2, PDGFRα, Olig2). While these two hallmarks are shared with stem cells, the third characteristic of stem cells, multipotency, seems less fulfilled by NG2+ progenitors in the adult brain. Multipotency *in vitro* as monitored in the neurosphere assay has been observed only with postnatal (Nunes et al., 2003) but not adult NG2+ cells (Buffo et al., 2008). Similarly, while these progenitors generate also astrocytes and a small number of neurons in the embryonic and possibly postnatal brain (Aguirre and Gallo, 2004; Belachew et al., 2003; Zhu et al., 2008, see however Kang et al., 2010), the generation of astrocytes from adult NG2+ cells (in the absence of injury) could only be observed *in vitro* (Shi et al., 1998; Wolswijk and Noble, 1989). So far neither neurons nor glial cells other than those of the oligodendrocyte lineage were observed in three of four fate mapping studies (Barnabe-Heider et al., 2010; Dimou et al., 2008; Kang et al., 2010). Only Rivers et al. (2008) showed low level neurogenesis from adult PDGFRα+ progenitors, but careful analysis in the same mouse line refuted these findings as due to the Tamoxifen protocol (Kang et al., 2010). While these genetic fate mapping techniques all depend on the respective gene locus expressing the inducible form of Cre, our study utilized BrdU incorporation to label all dividing cells without any bias in regard to their marker gene expression. As we could describe exclusively cells of the oligodendrocyte lineage expressing either NG2 or Sox10 or GSTπ, these data conform to the majority of the fate mapping studies also observing only cells of the oligodendrocyte lineage being generated in the adult CNS.

Change of Cell Cycle Properties After Injury

Several lines of evidence suggest that NG2+ cells can become reactive after injury in the adult CNS (Hampton et al., 2004; Levine, 1994; Rhodes et al., 2006). Their response does not only imply changes in cell morphology and strong upregulation of the NG2 antigen (Levine, 1994) but also a reversion to a rapidly dividing population (Keirstead et al., 1998; Levine and Reynolds, 1999; Watanabe et al., 2002).

Similar to other lesion models also after stab wound injury NG2+ cells are the first cells to react, and this rapid reaction is transient in nature resembling the time-course of microglia reactivity. The functional significance of this reaction to injury is still not fully understood. However, inhibition of cell proliferation by the

continuous infusion of the antimitotic drug cytosine-D-arabinofuranoside (AraC) resulted in a transient enhanced axonal regeneration (Rhodes et al., 2003), data that are in accordance with the idea that the accumulation of NG2+ cells in the scar may contribute to the inhibition of axonal growth (Chen et al., 2002; Tan et al., 2005).

Numerous factors have been identified or proposed to regulate proliferation of NG2+ cells in different injury paradigms that can be released by injured axons or by neighboring cells. These molecules include factors like platelet-derived, fibroblast and insulin-like growth factor, ciliary neurotrophic factor, and sonic hedgehog that affect NG2+ progenitor proliferation and number during development and hence may do the same after injury (Amankulor et al., 2009; Barres and Raff, 1994; Fortin et al., 2005; Mason et al., 2003; Redwine and Armstrong, 1998; Tripathi and McTigue, 2008). Interestingly, the effects of growth factors on NG2+ cells may also depend on the developmental stage and/or location of cells within the CNS (Mason and Goldman, 2002; Pfeiffer et al., 1993). Most pathological conditions also induce local production of certain cytokines and chemokines (McTigue and Tripathi, 2008) that together with blood derived factors can influence proliferation of NG2+ cells (Kerstetter et al., 2009; Rhodes et al., 2006; Taylor et al., 2010).

Exit of Cell Cycle and Enhanced Differentiation After Exercise

One of the most surprising outcomes of the present study is the observation that voluntary physical exercise results in a marked decrease in NG2+ cell proliferation and a converse increase in differentiation. Interestingly, an increase in oligodendrogenesis from nestin-GFP positive neural precursor cells was also observed in the spinal cord after running (Krityakiarana et al., 2010). However, it remains unclear whether the effect was due to enhanced proliferation or differentiation. In contrast, Ehninger and Kempermann (2003) reported that exercise did not affect NG2+ cells, while the microglia number increased. This discrepancy may well be explained by the way of BrdU-administration labeling exclusively fast proliferating cells (Ehninger and Kempermann, 2003) while our protocol with BrdU application for 14 d detects both fast and slow proliferating cells. Given the long cell cycle of most NG2+ progenitors (Fig. 1i), Ehninger and Kempermann missed most of the progenitor population examined in our analysis. Moreover, the impact of the duration of exercise is still unknown and could have implications in the reaction of NG2+ cells. For example, longer exercise may result either in a recovery of the proliferating NG2+ cell pool or in a further increase in the proportion of newly differentiated oligodendrocytes. Indeed, these exciting findings prompt further analysis of modulators of neuronal activity and their influence on the NG2+ progenitor proliferation and differentiation to determine whether the effects of running are a specific paradigm or whether neuronal activity may affect the finely tuned balance between proliferation and differentiation of progenitors in the adult cerebral cortex.

Of note in this regard, NG2+ cells receive synapses from neurons (Bergles et al., 2000; Butt et al., 1999; Etxeberria et al., 2010; Karadottir et al., 2008; Lin and Bergles, 2004; Muller et al., 2009) and it has been shown that glutamate and ATP released by neurons can inhibit their proliferation and differentiation, suggesting that neurotransmitters might directly affect the NG2 cell cycle and maturation process (Stevens et al., 2002; Yuan et al., 1998).

Activity dependent regulation of NG2+ cell differentiation could contribute to an experience dependent modification of neural circuits. Indeed, it has been shown that physical exercise leads to increased cortical thickness which has been suggested to be in part due to enhanced myelination (Anderson et al., 2002; Gaser and Schlaug, 2003). Along the same line extensive piano playing has been associated with increased myelination in the white matter, suggesting that myelination is a mechanism to improve circuit functions (Bengtsson et al., 2005; Fields, 2005). Our data are consistent with models proposing that neuronal activity can indeed fine tune the ratio of NG2+ cell proliferation and differentiation, suggesting an important role for the continuous generation of oligodendrocytes in the adult brain.

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REFERENCES

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.

Alonso G. 2005. NG2 proteoglycan-expressing cells of the adult rat brain: Possible involvement in the formation of glial scar astrocytes following stab wound. Glia 49:318–338.

Amankulor NM, Hambardzumyan D, Pyonteck SM, Becher OJ, Joyce JA, Holland EC. 2009. Sonic hedgehog pathway activation is induced by acute brain injury and regulated by injury-related inflammation. J Neurosci 29:10299–10308.

Anderson BJ, Eckburg PB, Relucio KI. 2002. Alterations in the thickness of motor cortical subregions after motor-skill learning and exercise. Learn Mem 9:1–9.

Barnabe-Heider F, Goritz C, Sabelstrom H, Takebayashi H, Pfrieger FW, Meletis K, Frisen J. 2010. Origin of new glial cells in intact and injured adult spinal cord. Cell Stem Cell 7:470–482.

Barres BA, Raff MC. 1994. Control of oligodendrocyte number in the developing rat optic nerve. Neuron 12:935–942.

Bednarczyk MR, Aumont A, Decary S, Bergeron R, Fernandes KJ. 2009. Prolonged voluntary wheel-running stimulates neural precursors in the hippocampus and forebrain of adult CD1 mice. Hippocampus 19:913–927.
Belachew S, Chittajallu R, Aguirre AA, Yuan X, Kirby M, Anderson S, Gallo V. 2003. Postnatal NG2 proteoglycan-expressing progenitor cells are intrinsically multipotent and generate functional neurons. J Cell Biol 161:169–186.
Bengtsson SL, Nagy Z, Skare S, Forsman L, Forssberg H, Ullen F. 2005. Extensive piano practicing has regionally specific effects on white matter development. Nat Neurosci 8:1148–1150.
Bergles DE, Roberts JD, Somogyi P, Jahr CE. 2000. Glutamatergic synapses on oligodendrocyte precursor cells in the hippocampus. Nature 405:187–191.
Buffo A, Rite I, Tripathi P, Lepier A, Colak D, Horn AP, Mori T, Gotz M. 2008. Origin and progeny of reactive gliosis: A source of multipotent cells in the injured brain. Proc Natl Acad Sci USA 105:3581–3586.
Buffo A, Vosko MR, Erturk D, Hamann GF, Jucker M, Rowitch D, Gotz M. 2005. Expression pattern of the transcription factor Olig2 in response to brain injuries: Implications for neuronal repair. Proc Natl Acad Sci USA 102:18183–18188.
Butt AM, Duncan A, Hornby MF, Kirvell SL, Hunter A, Levine JM, Berry M. 1999. Cells expressing the NG2 antigen contact nodes of Ranvier in adult CNS white matter. Glia 26:84–91.
Cameron HA, McKay RD. 2001. Adult neurogenesis produces a large pool of new granule cells in the dentate gyrus. J Comp Neurol 435:406–417.
Chen ZJ, Ughrin Y, Levine JM. 2002. Inhibition of axon growth by oligodendrocyte precursor cells. Mol Cell Neurosci 20:125–139.
Dawson MR, Polito A, Levine JM, Reynolds R. 2003. NG2-expressing glial progenitor cells: An abundant and widespread population of cycling cells in the adult rat CNS. Mol Cell Neurosci 24:476–488.
Dimou L, Simon C, Kirchhoff F, Takebayashi H, Gotz M. 2008. Progeny of Olig2-expressing progenitors in the gray and white matter of the adult mouse cerebral cortex. J Neurosci 28:10434–10442.
Ehninger D, Kempermann G. 2003. Regional effects of wheel running and environmental enrichment on cell genesis and microglia proliferation in the adult murine neocortex. Cereb Cortex 13:845–851.
Etxeberria A, Mangin JM, Aguirre A, Gallo V. 2010. Adult-born SVZ progenitors receive transient synapses during remyelination in corpus callosum. Nat Neurosci 13:287–289.
Fields RD. 2005. Myelination: An overlooked mechanism of synaptic plasticity? Neuroscientist 11:528–531.
Fortin D, Rom E, Sun H, Yayon A, Bansal R. 2005. Distinct fibroblast growth factor (FGF)/FGF receptor signaling pairs initiate diverse cellular responses in the oligodendrocyte lineage. J Neurosci 25:7470–7479.
Gaser C, Schlaug G. 2003. Brain structures differ between musicians and non-musicians. J Neurosci 23:9240–9245.
Geha S, Pallud J, Junier MP, Devaux B, Leonard N, Chassoux F, Chneiweiss H, Daumas-Duport C, Varlet P. 2010. NG2+/Olig2+ cells are the major cycle-related cell population of the adult human normal brain. Brain Pathol 20:399–3411.
Gensert JM, Goldman JE. 2001. Heterogeneity of cycling glial progenitors in the adult mammalian cortex and white matter. J Neurobiol 48:75–86.
Hampton DW, Rhodes KE, Zhao C, Franklin RJ, Fawcett JW. 2004. The responses of oligodendrocyte precursor cells, astrocytes and microglia to a cortical stab injury, in the brain. Neuroscience 127:813–820.
Hancock A, Priester C, Kidder E, Keith JR. 2009. Does 5-bromo-2′-deoxyuridine (BrdU) disrupt cell proliferation and neuronal maturation in the adult rat hippocampus in vivo? Behav Brain Res 199:218–221.
Holmes MM, Galea LA, Mistlberger RE, Kempermann G. 2004. Adult hippocampal neurogenesis and voluntary running activity: Circadian and dose-dependent effects. J Neurosci Res 76:216–222.
Horner PJ, Power AE, Kempermann G, Kuhn HG, Palmer TD, Winkler J, Thal LJ, Gage FH. 2000. Proliferation and differentiation of progenitor cells throughout the intact adult rat spinal cord. J Neurosci 20:2218–2228.
Irvine KA, Blakemore WF. 2007. A different regional response by mouse oligodendrocyte progenitor cells (OPCs) to high-dose X-irradiation has consequences for repopulating OPC-depleted normal tissue. Eur J Neurosci 25:417–424.
Kang SH, Fukaya M, Yang JK, Rothstein JD, Bergles DE. 2010. NG2+ CNS glial progenitors remain committed to the oligodendrocyte lineage in postnatal life and following neurodegeneration. Neuron 68:668–681.
Karadottir R, Hamilton NB, Bakiri Y, Attwell D. 2008. Spiking and nonspiking classes of oligodendrocyte precursor glia in CNS white matter. Nat Neurosci 11:450–456.
Karram K, Goebbels S, Schwab M, Jennissen K, Seifert G, Steinhauser C, Nave KA, Trotter J. 2008. NG2-expressing cells in the nervous system revealed by the NG2-EYFP-knockin mouse. Genesis 46:743–757.
Keirstead HS, Levine JM, Blakemore WF. 1998. Response of the oligodendrocyte progenitor cell population (defined by NG2 labelling) to demyelination of the adult spinal cord. Glia 22:161–170.
Kerstetter AE, Padovani-Claudio DA, Bai L, Miller RH. 2009. Inhibition of CXCR2 signaling promotes recovery in models of multiple sclerosis. Exp Neurol 220:44–56.
Krityakiarana W, Espinosa-Jeffrey A, Ghiani CA, Zhao PM, Topaldjikian N, Gomez-Pinilla F, Yamaguchi M, Kotchabhakdi N, de Vellis J. 2010. Voluntary exercise increases oligodendrogenesis in spinal cord. Int J Neurosci 120:280–290.
Levine JM. 1994. Increased expression of the NG2 chondroitin-sulfate proteoglycan after brain injury. J Neurosci 14:4716–4730.
Levine JM, Reynolds R. 1999. Activation and proliferation of endogenous oligodendrocyte precursor cells during ethidium bromide-induced demyelination. Exp Neurol 160:333–347.
Levine JM, Stincone F, Lee YS. 1993. Development and differentiation of glial precursor cells in the rat cerebellum. Glia 7:307–321.
Lin SC, Bergles DE. 2004. Synaptic signaling between GABAergic interneurons and oligodendrocyte precursor cells in the hippocampus. Nat Neurosci 7:24–32.
Mason JL, Goldman JE. 2002. A2B5+ and O4+ cycling progenitors in the adult forebrain white matter respond differentially to PDGF-AA, FGF-2, and IGF-1. Mol Cell Neurosci 20:30–42.
Mason JL, Xuan S, Dragatsis I, Efstratiadis A, Goldman JE. 2003. Insulin-like growth factor (IGF) signaling through type 1 IGF receptor plays an important role in remyelination. J Neurosci 23:7710–7718.
McTigue DM, Tripathi RB. 2008. The life, death, and replacement of oligodendrocytes in the adult CNS. J Neurochem 107:1–19.
McTigue DM, Wei P, Stokes BT. 2001. Proliferation of NG2-positive cells and altered oligodendrocyte numbers in the contused rat spinal cord. J Neurosci 21:3392–3400.
Muller J, Reyes-Haro D, Pivneva T, Nolte C, Schaette R, Lubke J, Kettenmann H. 2009. The principal neurons of the medial nucleus of the trapezoid body and NG2(+) glial cells receive coordinated excitatory synaptic input. J Gen Physiol 134:115–127.
Nishiyama A, Komitova M, Suzuki R, Zhu X. 2009. Polydendrocytes (NG2 cells): Multifunctional cells with lineage plasticity. Nat Rev Neurosci 10:9–22.
Nowakowski RS, Lewin SB, Miller MW. 1989. Bromodeoxyuridine immunohistochemical determination of the lengths of the cell cycle and the DNA-synthetic phase for an anatomically defined population. J Neurocytol 18:311–318.
Nunes MC, Roy NS, Keyoung HM, Goodman RR, McKhann G, 2nd, Jiang L, Kang J, Nedergaard M, Goldman SA. 2003. Identification and isolation of multipotential neural progenitor cells from the subcortical white matter of the adult human brain. Nat Med 9:439–447.
Panagiotakos G, Alshamy G, Chan B, Abrams R, Greenberg E, Saxena A, Bradbury M, Edgar M, Gutin P, Tabar V. 2007. Long-term impact of radiation on the stem cell and oligodendrocyte precursors in the brain. PLoS One 2:e588
Pfeiffer SE, Warrington AE, Bansal R. 1993. The oligodendrocyte and its many cellular processes. Trends Cell Biol 3:191–197.
Psachoulia K, Jamen F, Young KM, Richardson WD. 2009. Cell cycle dynamics of NG2 cells in the postnatal and ageing brain. Neuron Glia Biol 5:57–67.
Redwine JM, Armstrong RC. 1998. In vivo proliferation of oligodendrocyte progenitors expressing PDGFalphaR during early remyelination. J Neurobiol 37:413–428.
Rhodes KE, Moon LD, Fawcett JW. 2003. Inhibiting cell proliferation during formation of the glial scar: Effects on axon regeneration in the CNS. Neuroscience 120:41–56.
Rhodes KE, Raivich G, Fawcett JW. 2006. The injury response of oligodendrocyte precursor cells is induced by platelets, macrophages and inflammation-associated cytokines. Neuroscience 140:87–100.
Rivers LE, Young KM, Rizzi M, Jamen F, Psachoulia K, Wade A, Kessaris N, Richardson WD. 2008. PDGFRA/NG2 glia generate myelinating oligodendrocytes and piriform projection neurons in adult mice. Nat Neurosci 11:1392–1401.
Scholzen T, Gerdes J. 2000. The Ki-67 protein: From the known and the unknown. J Cell Physiol 182:311–322.
Shi J, Marinovich A, Barres BA. 1998. Purification and characterization of adult oligodendrocyte precursor cells from the rat optic nerve. J Neurosci 18:4627–4636.
Stevens B, Porta S, Haak LL, Gallo V, Fields RD. 2002. Adenosine: A neuron-glial transmitter promoting myelination in the CNS in response to action potentials. Neuron 36:855–868.
Tan AM, Zhang W, Levine JM. 2005. NG2: A component of the glial scar that inhibits axon growth. J Anat 207:717–725.

Taupin P. 2007. BrdU immunohistochemistry for studying adult neurogenesis: Paradigms, pitfalls, limitations, and validation. Brain Res Rev 53:198–214.

Taylor DL, Pirianov G, Holland S, McGinnity CJ, Norman AL, Reali C, Diemel LT, Gveric D, Yeung D, Mehmet H. 2010. Attenuation of proliferation in oligodendrocyte precursor cells by activated microglia. J Neurosci Res 88:1632–1644.

Tripathi RB, McTigue DM. 2008. Chronically increased ciliary neurotrophic factor and fibroblast growth factor-2 expression after spinal contusion in rats. J Comp Neurol 510:129–144.

Trotter J, Karram K, Nishiyama A. 2010. NG2 cells: Properties, progeny and origin. Brain Res Rev 63:72–82.

van Praag H, Kempermann G, Gage FH. 1999. Running increases cell proliferation and neurogenesis in the adult mouse dentate gyrus. Nat Neurosci 2:266–270.

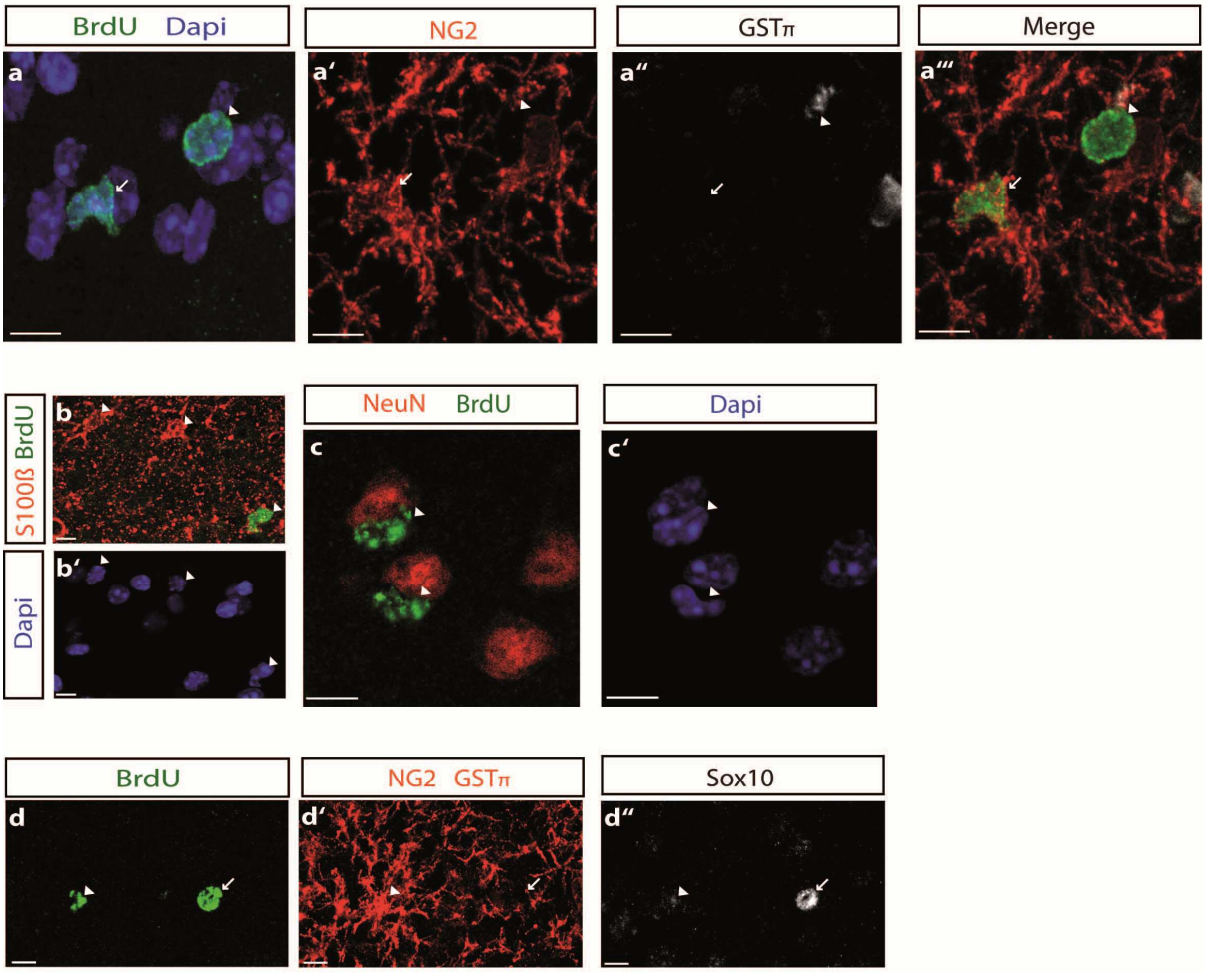
Watanabe M, Toyama Y, Nishiyama A. 2002. Differentiation of proliferated NG2-positive glial progenitor cells in a remyelinating lesion. J Neurosci Res 69:826–836.

Wolswijk G, Noble M. 1989. Identification of an adult-specific glial progenitor cell. Development 105:387–400.

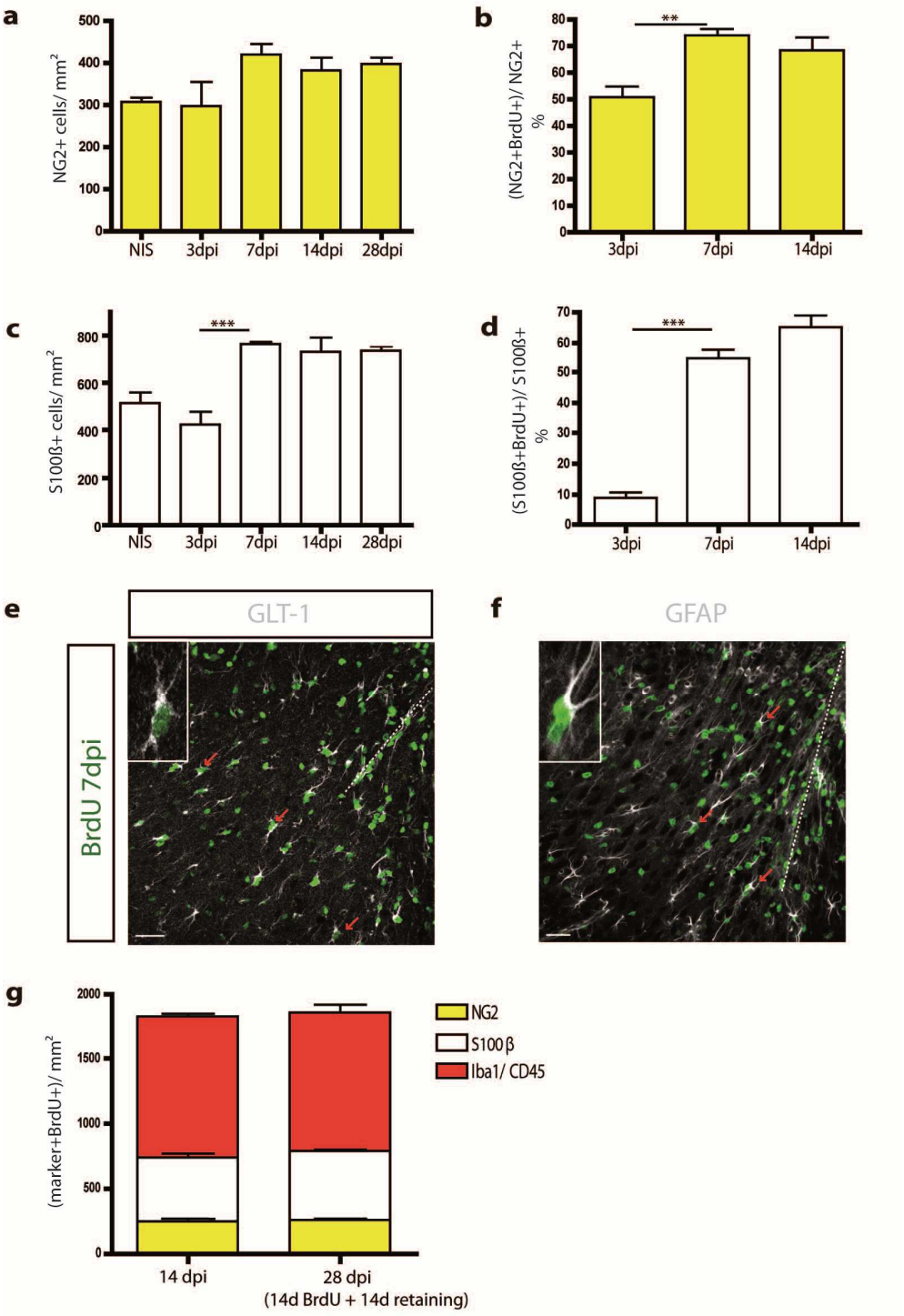
Yuan X, Eisen AM, McBain CJ, Gallo V. 1998. A role for glutamate and its receptors in the regulation of oligodendrocyte development in cerebellar tissue slices. Development 125:2901–2914.

Zawadzka M, Rivers LE, Fancy SP, Zhao C, Tripathi R, Jamen F, Young K, Goncharevich A, Pohl H, Rizzi M, Rowitch DH, Kessaris N, Suter U, Richardson WD, Franklin RJ. 2010. CNS-resident glial progenitor/stem cells produce Schwann cells as well as oligodendrocytes during repair of CNS demyelination. Cell Stem Cell 6:578–590.

Zhu X, Bergles DE, Nishiyama A. 2008. NG2 cells generate both oligodendrocytes and gray matter astrocytes. Development 135:145–157.



Supplemental figure 1: (a-a´´´) Images showing a BrdU+ cell that is NG2-/GSTπ- (arrowhead) after 42 days of BrdU-retaining. (b-c´) NG2-/GSTπ-/BrdU+ neither co-localize with S100β (b-b´) nor with NeuN (c-c´) after 42 days of BrdU-retaining period. (d-d´) Image depicting a NG2-/ GSTπ-/BrdU+ cell that is Sox10+ (arrow). Arrowhead points to a BrdU+ cell that expresses NG2 in its soma and processes.
(a-d´´): Maximum intensity projections; Arrows point to marker+/BrdU+ cells; Arrowheads indicate only single marker-positive cells; scale bars 10 µm.



Supplemental figure 2: (a) Bar graph showing the NG2+ cell number at the non-injured site (NIS) and at 3, 7, 14 or 28 dpi. (b) Quantification of NG2+/BrdU+ cells amongst the NG2+ cell pool at different time points after lesion. (c) The number of S100β+ cells is quantified before and at different time points after lesion. (d) Bar graph showing the number of BrdU+ astrocytes amongst all S100β+ cells. The proliferative response of S100β+ cells to the injury is confirmed by stainings for (e) GLT-1 or (f) GFAP –other markers for cortical or reactive astrocytes.
(g) BrdU+ cell pool is quantified 14 days BrdU after lesion (14 dpi) and after an additional 14 days of BrdU-retaining (28 dpi).
(e,f) Maximum intensity projections at 7 dpi; the stab wound lesion is indicated with a white dashed line. Arrows point to marker+/BrdU+ cells; scale bars 20 µm

3.2 Fate of NG2+ glia in the cortical gray and white matter

This study herein is published as "Progeny of Olig2-Expressing Progenitors in the Gray and White Matter of the Adult Mouse Cerebral Cortex" by Dimou L, **Simon C,** Kirchhoff F, Takebayashi H and Götz M in Journal of Neuroscience 2008, 28 (41): 10434-10442.

NG2+ cells are potent progenitors that give rise to myelinating oligodendrocytes postnatally. Although NG2+ cells are a widespread cell population in the adult CNS, the fate of these cells in adult mammals was largely unknown at the time of this study. In order to analyze the progeny of NG2+ cells in the adult mouse brain, the Olig2::CreER^{TM} mouse line, crossed to the Z/EG, GFP or R26R-reporter mouse line, was used as Olig2 is expressed in nearly all proliferating NG2+ cells in the brain parenchyma. However, Olig2 expression is not restricted to the oligodendrocyte lineage in Olig2::CreER^{TM} mice, but also recombines in some parenchymal astrocytes and in very few neurons. Despite the low reporter expression outside of the oligodendrocyte lineage, this study revealed intriguing regional differences in the progeny of NG2+ glia. NG2+ cells in the adult cerebral white matter (WM) mainly gave rise to new myelinating oligodendrocytes whereas NG2+ cells in the cerebral gray matter (GM) the majority remained as NG2+ glia and only gave rise only to few maturate (but not myelinating) oligodendrocytes. This regional heterogeneity can be either due to intrinsic differences in NG2+ cells, e.g. cell cycle differences as suggested within this publication, or be instructed by the environment. In summary, this study shows the generation of new myelinating and mature oligodendrocytes throughout life with an intriguing difference in gray and white matter regions.

Contribution of the authors

I performed the proliferation experiments in C57BL/6 mice by administrating 5'-bromo-desoxyuridine (BrdU) to the mice to label dividing cells. Following perfusion, I did the sectioning, staining and quantifications for Olig2+/ BrdU+ cells in the adult CNS (as shown in Suppl. Fig. 1). L. Dimou did all the experiments and analysis in the Olig2::CreER^{TM} mice that were kindly provided by H. Takebayashi. F. Kirchhoff kindly provided the Plp-DsRed mice. The experiments were planned and the manuscript was written by L. Dimou and M. Götz. M.Götz financed this work.

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Development/Plasticity/Repair

Progeny of Olig2-Expressing Progenitors in the Gray and White Matter of the Adult Mouse Cerebral Cortex

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Despite their abundance, still little is known about the rather frequent, constantly proliferating progenitors spread throughout the adult mouse brain parenchyma. The majority of these progenitors express the basic-helix-loop-helix transcription factor Olig2, and their number further increases after injury. Here, we examine the progeny of this progenitor population by genetic fate mapping using tamoxifen-inducible Cre-recombination in the Olig2 locus to turn on permanent reporter gene expression in the adult brain. Consistent with Olig2 expression in proliferating NG2$^+$ progenitors, most reporter$^+$ cells seen shortly after initiating recombination at adult stages incorporated BrdU and contained the proteoglycan NG2 in both the gray (GM) and the white matter (WM) of the cerebral cortex. However, at longer time points after induction, we observed profound differences in the identity of reporter$^+$ cells in the WM and GM. Whereas most of the Olig2$^+$ progenitors had generated mature, myelinating oligodendrocytes in the WM, hardly any reporter$^+$ cells showing mature oligodendrocyte characteristics were detectable even up to 6 months after recombination in the GM. In the GM, most reporter$^+$ cells remained NG2$^+$, even after injury, but stopped proliferating rather soon after recombination. Thus, our results demonstrate the continuous generation of mature, myelinating oligodendrocytes in the WM, whereas cells in the GM generated mostly postmitotic NG2$^+$ glia.

Key words: oligodendrocyte progenitors; NG2; myelination; stab wound injury; forebrain; lineage analysis

Introduction

Whereas progenitors in the adult neurogenic niches have attracted attention, still little is known about the fate of progenitors within the adult CNS parenchyma, despite their widespread nature (Horner and Gage, 2002). The vast majority of these dividing cells express markers found in oligodendrocyte precursors during development (NG2, PDGFRα, and/or Olig2) and have therefore been regarded as oligodendrocyte progenitors (Dawson et al., 2000; Miller, 2002; Ligon et al., 2006a). Indeed, after demyelination, remyelination occurs from dividing precursors (Gensert and Goldman, 1997; Chari and Blakemore, 2002), supporting the presence of oligodendrocyte progenitors in the adult white matter (WM). However, the progeny of these dividing cells in the absence of injury is not known. Indeed, few, if any, new oligodendrocytes were observed in the absence of a demyelinating injury after viral vector injection into the adult cortex WM (Gensert and Goldman, 1996; Menn et al., 2006). In addition, recent research has highlighted the distinct features of adult glial cells expressing the proteoglycan NG2. These cells possess unique physiological properties and are involved in neuron–glia communication (Butt et al., 2002; Matthias et al., 2003; Paukert and Bergles, 2006; Káradóttir et al., 2008). It is not known whether these represent a separate class of NG2$^+$ cells that are different from the dividing NG2$^+$ progenitors, and if so, whether their generation is restricted to developmental stages or continues into adulthood.

Although the progenitors characterized by Olig2 or NG2 are certainly involved in oligodendrogliogenesis during development, they have recently also been implicated in the generation of various other lineages during this time window. The transcription factor Olig2 is a necessary regulator of oligodendrocyte and motoneurons development, and Olig2-expressing cells also generate cholinergic neurons, ependymal cells, as well as some astrocytes during development (Takebayashi et al., 2002; Furusho et al., 2006; Ligon et al., 2006a; Masahira et al., 2006; Cai et al., 2007; Ono et al., 2008). Interestingly, Olig2 is also required for the specification of NG2$^+$ cells (Ligon et al., 2006b). Similar to Olig2, NG2 is also expressed in progenitors of various lineages, including oligodendrocytes and astrocytes during development (Cai et al., 2007; Zhu et al., 2008; Ono et al., 2008), as well as multipotent

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progenitors in the postnatal brain (Belachew et al., 2003; Aguirre et al., 2004) and possibly even neuronal progenitors in the adult cerebral cortex (Dawson et al., 2000; Dayer et al., 2005; Tamura et al., 2007).

The multitude of lineages suggested for Olig2$^+$ or NG2$^+$ progenitors during development highlights the need for appropriate fate-mapping techniques to elucidate their lineage in the adult brain. This has now become possible by tamoxifen-inducible Cre-mediated recombination targeted to adult progenitors (Olig2::CreER^{TM}) allowing to follow the progeny arising from Olig2$^+$ progenitors in the adult brain.

Materials and Methods

Mice. Heterozygous Olig2::CreER^{TM} (Takebayashi et al., 2002) were crossed with the PLP-dsRed (Hirrlinger et al., 2005) or the reporter mice Z/EG (Novak et al., 2000) or R26R (Soriano, 1999) to obtain double-heterozygous animals. Double-heterozygous mice older than 2.5 months received tamoxifen orally (10 mg; dissolved at 40 mg/ml in a 1:9 ethanol/corn oil mixture) three times (once a day every second day for 5 d). Brains were collected at 8 ($n = 8$), 12 ($n = 5$), 35 ($n = 10$), 65 ($n = 10$) d or 6 months ($n = 5$) after the end of tamoxifen treatment. BrdU (Sigma-Aldrich) was supplied in the drinking water at a concentration of 1 mg/ml for 1 or 3 constitutive days, and animals were examined immediately after BrdU treatment. Five days after tamoxifen induction, some mice underwent a stab wound as described in Buffo et al. (2005). Briefly, mice received Rimadyl (4 mg/kg carprofen, s.c.) as analgesic treatment and were anesthetized by ketamine (100 mg/kg; ketavet; GE Healthcare) and xylacine (5 mg/kg; rompun; Bayer). After trepanation, a 1–1.5 mm long/1 mm deep stab wound was made using a curved scalpel in the right sensorimotor cortex. Brains were examined 3 ($n = 8$), 7 ($n = 5$), and 30 ($n = 7$) d after lesion. For BrdU/CC1 ($n = 4$) analysis, 3-month-old wild-type C57BL/6 animals were supplied for 2 weeks with BrdU in the drinking water and were killed 2 weeks after the treatment. For activated caspase3 ($n = 9$) analysis, 3- and 6-month-old C57BL/6 mice were used. All animal procedures were performed in accordance with the policies of the use of Animals and Humans in Neuroscience Research, revised and approved by the Society of Neuroscience and the state of Bavaria under license number 55.2-1-54-2531-23/04.

Histological analysis. For histological analysis, animals were anesthetized and transcardially perfused with 4% paraformaldehyde. Brains were postfixed for 2–4 h, cryoprotected in 30% sucrose, and stained according to standard protocols. Immunohistochemistry was performed using antibodies described in Buffo et al. (2005) and Colak et al. (2008). For Olig2 immunostaining in Olig2::CreER^{TM} mice, pretreatment with citrate buffer at 95°C and amplification with biotinylated secondary antibody was performed. The images were collected with an Olympus microscope (BX61TRF; Olympus) and confocal systems from Olympus (Fluoview F1000) or Zeiss (LSM710) and digital camera system (FluoviewII). For quantitative analysis, the number of reporter$^+$ cells that were also immunopositive for a cell-type specific antigen was counted and expressed as a percentage of reporter$^+$ cells. Quantification was focused on the sensorimotor cortex in 3–5 sections per animal, in at least 3 animals for each time point and area [gray matter (GM) vs WM or lesion]. Thus, >250 reporter$^+$ cells per GM, >100 reporter$^+$ cells per WM, and >50 cells in the lesion site were counted per animal. All results are presented as averages with SEM.

Results

Cells labeled by Olig2::CreER^{TM}-mediated recombination are initially proliferating NG2$^+$ progenitors

Before monitoring the progeny of Olig2-expressing progenitors, we determined the exact proportion of proliferating cells in the adult brain that express Olig2. Toward this aim, we labeled dividing cells by administering the DNA base analog on BrdU for 3 or 14 continuous days in the drinking water and doublestained the BrdU-incorporating cells for Olig2. In most parenchymal regions of the forebrain (neocortex, piriform cortex, corpus callosum, and putamen), virtually all BrdU-labeled cells were Olig2-immunoreactive (supplemental Fig. 1, available at www.jneurosci.org as supplemental material). This was notably different in the regions of adult neurogenesis, such as the subgranular zone in the dentate gyrus, where only 13% of the proliferating cells were also Olig2$^+$ (supplemental Fig. 1*F*, available at www.jneurosci.org as supplemental material). Thus, almost all adult progenitors outside the neurogenic niches express Olig2.

Table 1. Absolute number of reporter cells per mm^2 at different time points after recombination in the different analyzed areas

Time after recombination (lesion)	GM	WM	Lesion
8 d (3 d)	10.3 ± 1.4	9 ± 2	22.5 ± 6
35 d (30 d)	9.5 ± 0.9	13.8 ± 1.6	15 ± 2.7
65 d (60 d)	9.9 ± 1.3	25.5 ± 1	14
6 months	11.1 ± 1.5	24.5 ± 4.5	

Time points in brackets refer to the time after lesion.

Table 2. Absolute number of reporter$^+$/CC1$^+$ double-positive cells per mm^2 (for GM and WM) and number of cells per lesion area (~0.4 mm^2 for lesion quantification) at different time points after recombination in the different analyzed areas

Time after recombination (lesion)	GM	WM	Lesion
8 d (3 d)	1.1 ± 0.04	1.6 ± 0.06	3.2 ± 0.1
65 d (60 d)	1.98 ± 0.01	20.7 ± 0.02	3.78 ± 0.2
6 months	1.99 ± 0.06	20.3 ± 0.1	

Time points in brackets refer to the time after lesion.

To follow the progeny of these Olig2$^+$ dividing cells in the adult cerebral cortex, we used tamoxifen-inducible Cre-mediated recombination in the Olig2 locus (Olig2::CreER^{TM}) of 2.5- to 6-month-old mice. Successful recombination was monitored by reporter gene expression in the brains of Olig2::CreER^{TM};Z/EG (GFP reporter) or Olig2::CreER^{TM};R26R (β-galactosidase reporter) mice 8, 12, 35, 65 d postrecombination (dpr) or 6 months postrecombination. As the results obtained with either reporter line were very similar, we pooled the data obtained with both lines. Although CreER^{TM} has been knocked into the endogenous Olig2 locus, thereby reducing the endogenous levels of Olig2 to about half (data not shown), we attempted to monitor the expression of Olig2 in the reporter$^+$ cells. Reporter$^+$ cells were observed earliest 5 d after tamoxifen application, and the majority of these cells were also Olig2-immunoreactive (supplemental Fig. 2, available at www.jneurosci.org as supplemental material). The reporter$^+$ population amounted to ~22% of the total Olig2-immunoreactive population with an average of 10.3 ± 1.4 recombined cells per mm^2 in the GM and 9 ± 2 cells per mm^2 in the WM (Table 1). Well consistent with the nature of Olig2-expresssing cells, the majority of these reporter$^+$ cells at 8 dpr in the forebrain were NG2$^+$ (Figs. 1*A–C,J*, 3*A–C,J*), whereas only a minor population was CC1$^+$ oligodendrocytes (Figs. 1*J*, 3*D–F,J*; for Table 2, numbers of reporter$^+$/CC1 double-positive cells). No reporter$^+$ astrocytes were detectable in the cortical WM, whereas some were found in the GM (see Fig. 3*G–I*) as well as in other parenchymal forebrain regions (data not shown). Furthermore, consistent with the majority of recombination occurring in Olig2$^+$/NG2$^+$ double-positive progenitors, many reporter$^+$ cells seen at 8 dpr had incorporated BrdU that was supplied for 3 d (59% in the GM and 46% in the WM) (Fig. 4) (see below). Notably, the proportion of reporter$^+$ cells incorporating BrdU at 8 dpr appears to be higher than this proportion among the total Olig2-immunoreactive pool (data not shown), suggesting that the cells expressing the

highest levels of CreER^{TM} in the Olig2 locus, and hence mediating recombination most efficiently, are biased toward proliferation.

Cells labeled by Olig2::CreER^{TM}-mediated recombination generate mature oligodendrocytes in the WM of the adult brain

To determine the further fate of these progenitors, we examined reporter$^+$ cells at later stages, from 35 d up to 6 months after recombination. In the WM, the composition of reporter$^+$ cells had changed profoundly at these later stages (compare Fig. 1*J*,*K*). The proportion of NG2$^+$ per reporter$^+$ cells was reduced to 19%, whereas the majority of recombined cells at 65 dpr expressed the mature oligodendrocyte marker CC1 (Fig. 1*D–F*,*K*). Thus, the proportion of GFP$^+$ per CC1$^+$ cells increased by 4.5-fold from 18 to 82% ($p < 0.001$). Notably, also the total number of recombined cells increased (Table 1), suggesting that the relative increase in CC1$^+$ cells is unlikely to result from selective cell death. Indeed, the total number of reporter$^+$ cells double-labeled with CC1 also increased by almost 13-fold in the WM (Table 2).

Interestingly, the absolute number of reporter$^+$ cells increased in the WM only during the first 65 dpr (from 9/mm^2 at 8 dpr over 13.8/mm^2 at 35 dpr to 25.5/mm^2 at 65 dpr) and remained constant thereafter until 6 months after recombination (24.5/mm^2) (Table 1). Indeed, this is also consistent with the decrease in the proportion of reporter$^+$ cells expressing Olig2 or incorporating BrdU (supplemental Fig. 2*B*, available at www.jneurosci.org as supplemental material; see Fig. 4*E*), suggesting that most labeled dividing progenitors in the WM leave the cell cycle within a few weeks and are mostly differentiated by 2 months after recombination. Only a small proportion of reporter$^+$ cells (14%), however, appears to continue to proliferate over the course of several months (see Fig. 4*E*). Nevertheless, no further increase in the number of reporter$^+$/CC1$^+$ double-positive cells or in the proportion of CC1$^+$ cells could be observed within 2–6 months after recombination (Tables 1, 2; compare Fig. 1*K* with supplemental Fig. 4*A*, available at www.jneurosci.org as supplemental material). As the same number and high proportion of CC1$^+$ cells among reporter$^+$ cells was still seen at 6 months after recombination (supplemental Fig. 4*A*, available at www.jneurosci.org as supplemental material), these cells seem to survive for some time, implying that they may further differentiate into myelinating oligodendrocytes, a hypothesis prompted by the intriguing morphology of many reporter$^+$ cells in the WM resembling myelinating oligodendrocytes (Fig. 1*G*,*I*).

We therefore used several antibodies directed against different myelin proteins, such as the myelin oligodendrocyte glycoprotein (MOG) (Figs. 1*G*,*H*, 2*D–F*), the myelin associated glycoprotein (MAG) (Fig. 2*A–C*), and the myelin basic protein (MBP) (Fig. 2*G–I*) as these proteins are targeted into oligodendrocyte processes when myelin sheaths can be detected by electron microscopy (Bartsch et al., 1989; Lindner et al., 2008; Shen et al., 2008). Specifically, MAG localization to processes is correlated to the presence of at least one and a half myelin wrappings around the axons (Bartsch et al., 1989), and the reappearance of MOG after demyelination is detectable only after remyelination is apparent at the electron-microscopic level (Lindner et al., 2008). Notably, all of these proteins could be colocalized in the elongated processes of reporter$^+$ cells in the WM, highly suggestive of their myelinating identity. Furthermore, we could observe spots immunoreactive for the paranodal marker Caspr in reporter$^+$ processes, indicative for some degree of nodal specification in the newly generated oligodendrocytes (supplemental Fig. 3, available at www.jneurosci.org as supplemental material).

As these proteins are mainly targeted to the processes of

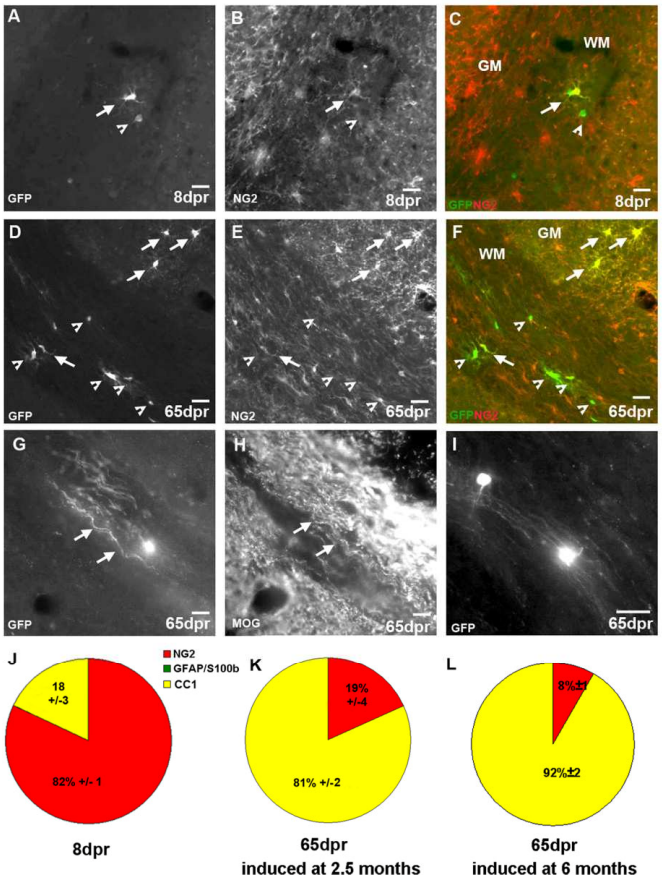

Figure 1. Identification of reporter$^+$ cells in the WM. ***A–I***, Micrographs of examples of GFP$^+$ cells 8 (***A–C***) or 65 (***D–I***) dpr double-stained for cell type-specific antigens. The pies in ***J*** and ***K*** depict the quantitative cell type analysis among reporter$^+$ cells 8 (***J***) or 65 (***K***) dpr recombined in 2.5-month-old animals. ***L***, Cell type analysis at 65 dpr in mice recombined at 6 months of age. Arrows point to double-stained cells; arrowheads point to single positive cells. Scale bars, 20 μm.

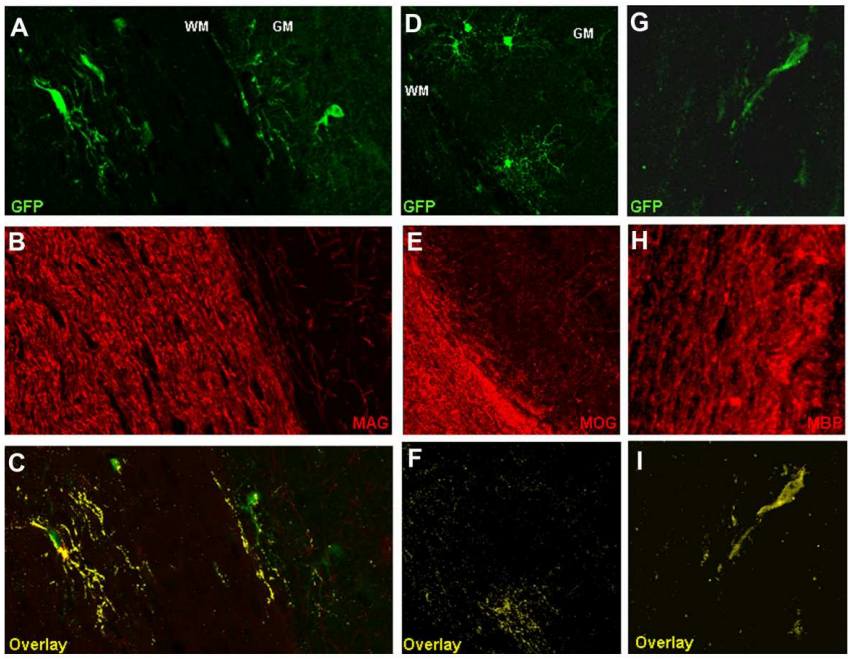

Figure 2. ***A–I***, Confocal microscope images showing the colabeling of reporter$^+$ cells and myelin sheaths with the myelin markers MAG (***A–C***), MOG (***D–F***), and MBP (***G–I***) in the white matter (corpus callosum) at 65 dpr. ***C***, ***F***, ***I***, Colocalizing pixels are in yellow.

reporter$^+$ cells, their colocalization cannot be used to quantify the number or proportion of myelinating oligodendrocytes among reporter$^+$ cells. Toward this end, we crossed the Olig2::CreER^{TM};Z/EG mice to PLP-dsRed animals (Hirrlinger et al., 2005), visualizing the expression from a myelin protein promoter by cytoplasmic localization of fluorescent protein (supplemental Fig. 5*A–C*, available at www.jneurosci.org as supplemental material). Consistent with the predominant progenitor identity of initially recombined cells, only 6% of the reporter$^+$ cells were expressing the PLP-transgene in the GM and the WM at 8 dpr (supplemental Fig. 5*D*, available at www.jneurosci.org as supplemental material). In pronounced contrast, however, 60% of reporter$^+$ cells in the WM were colocalizing with the PLP-transgene at 65 dpr (supplemental Fig. 5*D*, available at www.jneurosci.org as supplemental material), strongly supporting the synthesis of myelin proteins by the progeny of Olig2$^+$ progenitors in the WM. Thus, most (3 of 4) of the CC1$^+$/reporter$^+$ double-positive cells in the WM also express PLP, suggesting that more than half of the progeny of recombined Olig2$^+$ cells in the adult WM differentiated into myelinating oligodendrocytes. These newly generated myelin-forming cells were not restricted to a specific region but rather wide spread throughout the corpus callosum at all time points analyzed. As a similar change in the composition of reporter$^+$ cells was observed also when recombination was induced in 6-month-old mice (Fig. 1*L*), we conclude that Olig2$^+$ progenitors in the WM generate mature, myelinating oligodendrocytes throughout this period.

Cells labeled by Olig2::CreER^{TM}-mediated recombination generate mainly postmitotic NG2$^+$ glia in the GM of the adult brain

Strikingly and in pronounced contrast to the WM, the identity of recombined cells in the GM hardly changed over time with the NG2$^+$ cells still predominating at 65 dpr (Fig. 3*K*) and even 6 months after recombination (supplemental Fig. 4*B*, available at www.jneurosci.org as supplemental material). The same predominance of NG2$^+$ cells was also observed when older animals (6 months) were induced with TM and examined 65 d later (Fig. 3*L*). In both cases, we observed little changes in the proportion of reporter$^+$ astrocytes (5% initially, 7 or 6% at 65 dpr, and 11% at 6 months after recombination) (Fig. 3*J–L*; supplemental Fig. 4*B*, available at www.jneurosci.org as supplemental material). Similarly, only a small, nonsignificant ($p = 0.123$) increase in the proportion of reporter$^+$ cells colabeling with CC1 was observed in the GM (11% initially, 20% at 65 dpr, and 18% at 6 months after recombination) (Fig. 3*J*,*K*; supplemental Fig. 4*B*, available at www.jneurosci.org as supplemental material). Consistently, a similarly low proportion of reporter$^+$ cells in the GM became PLP$^+$ (6% at 8 dpr increasing to 11% at 65 dpr) (supplemental Fig. 5, available at www.jneurosci.org as supplemental material). Moreover, reporter$^+$ cells did not contain other myelin proteins (MOG, MAG, or MBP), and we could not observe recombined cells with a typical morphology of myelinating oligodendrocytes, even 6 months after TM application. Also, no reporter$^+$ cells were double-labeled with neuron-specific antigens (doublecortin or neuronal-specific nuclear protein; $n = 2125$), even in the ol-

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factory bulb and dentate gyrus (supplemental Fig. 6, available at www.jneurosci.org as supplemental material), suggesting that cells labeled by recombination mediated by CreER^{TM} expressed in the Olig2 locus do not contribute to adult neurogenesis. Notably, in a different reporter line, we observed some reporter$^+$ cells with neuronal morphology in the ventral thalamus already at 5 dpr, implying low-level Olig2 expression in some neurons of the adult brain.

As the composition of reporter$^+$ cells in the lines used here (Z/EG, Rosa) did not change over time in the GM (even 6 months after recombination) (supplemental Fig. 4*B*, available at www.jneurosci.org as supplemental material), we examined whether the predominant NG2$^+$ cells were still proliferating progenitors. When BrdU was given for 3 d at different time points after recombination, also in the GM, the proportion of BrdU-incorporating reporter$^+$ cells decreased over time to 18% at 27–30 dpr and furthermore to 8% at 117–120 dpr (Fig. 4). Thus, the overall decrease in proliferation is similar between GM and WM, with initially a majority of BrdU-incorporating cells giving way to cells that no longer incorporate BrdU and hence are likely to have either left the cell cycle or divide too slowly to be labeled by BrdU application for 3 d. This analysis also highlights the different nature of the reporter$^+$/NG2$^+$ double-positive cells most of which are BrdU-incorporating at short times after recombination but gradually lose this property within a few weeks. Thus, the main progeny of Olig2$^+$ progenitors in the GM is a slow or nonproliferating type of NG2$^+$ glia.

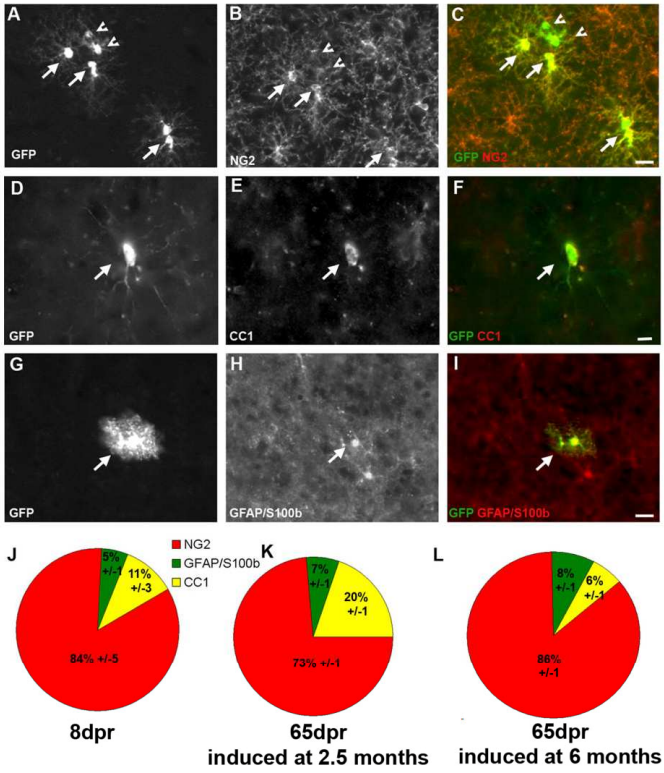

Figure 3. Identification of reporter$^+$ cells in the GM. ***A–I***, Micrographs of GFP$^+$ cells at 8 dpr. Arrows point to colabeled cells, arrowheads to reporter$^+$ cells negative for the cell type-specific antigen. Pies in *J* and *K* depict the quantitative cell type analysis of reporter$^+$ cells (*J*, 8 dpr; *K*, 65 dpr) recombined in 2.5-month-old animals. *L*, Cell type analysis at 65 dpr in mice recombined at 6 months of age. Scale bars, 20 μm.

The proliferation analysis of reporter$^+$ cells in the GM also provided further insights. Similar to the observations in the WM, also in the GM, a small proportion of reporter$^+$ cells (8%) continues to divide even up to 4 months after recombination, suggesting that Olig2$^+$ cells also comprise a long-term self-renewing progenitor population. However, this population was much smaller, almost half of the one in the WM (8 vs 14%) (Fig. 4). Indeed, we noted a faster decrease in the proportion of reporter$^+$ cells incorporating BrdU in the GM, compared with the WM (Fig. 4*D–F*), starting already shortly after recombination (8–9 dpr) (Fig. 4*F*). Comparing the proportion of reporter$^+$ cells incorporating BrdU given for either 1 or 3 d also revealed an interesting difference in the speed of cell proliferation between GM and WM reporter$^+$ cells. Although after 1 d BrdU administration at 5–6 dpr the proportion of BrdU$^+$ per reporter$^+$ cells was the same in the GM and WM, fewer reporter$^+$ cells were labeled by 1 d BrdU application at 8–9 dpr in the GM compared with those in the WM (Fig. 4*F*). However, when BrdU was given for 3 d a larger proportion of reporter$^+$ cells in the GM was labeled at 5–8 dpr, suggesting that GM Olig2$^+$ progenitors slow their cell cycle speed rather faster (see also Fig. 4*F*, decrease in cells labeled with 1 d BrdU at 5–6 and 8–9 dpr) than WM reporter$^+$ cells (Fig. 4*F*, no decrease in cells labeled with 1 d BrdU at 5–6 and 8–9 dpr). After further maturation, the proportion of cells labeled by 3 d BrdU application also declines faster among GM compared with WM reporter$^+$ cells reaching approximately half at 120 dpr (Fig. 4).

At least partially consistent with a slower proliferation of GM progenitors, the reporter$^+$ GM population hardly increased at all (~10% increase over 6 months) (Table 1). As such a profile could also be caused by cell death, we performed stainings for activated Caspase3 at 35 and 65 dpr, i.e., in the time interval when the number of reporter$^+$ cells increased most in the WM but failed to do so in the GM (Table 1). However, we could not detect any activated Caspase3$^+$/reporter$^+$ double-positive cells, either in the GM or in the WM. Conversely, many activated Caspase3-immunoreactive reporter$^+$ cells could be observed after stab wound injury (see below), indicating that the staining works reliably. As reporter$^+$ cells only label a small fraction of all Olig2$^+$ cells, we quantified the total number of activated Caspase3$^+$ cells in the GM and the WM based on the rationale that selective cell death in the GM should be detectable in these quantifications. However, quantification in nine animals at the age of 2.5 and 6 months revealed no differences in the number of activated Caspase3$^+$ cells between the GM (0.015 ± 0.005/mm^2) and the WM (0.034 ± 0.023/mm^2).

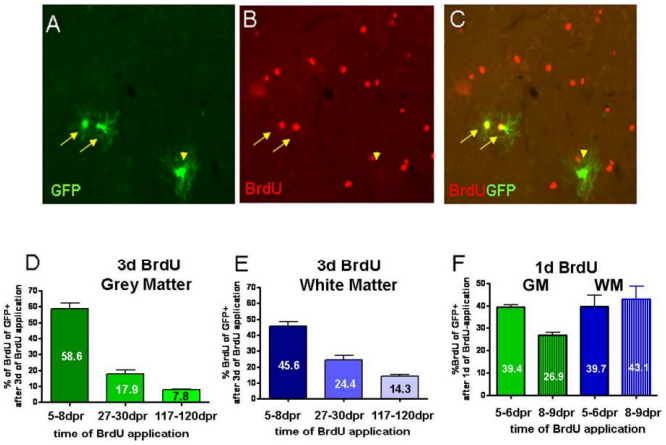

Figure 4. *A–C*, Identification of proliferating reporter$^+$ cells at 8 dpr. *D–F*, Graphs in *D* and *E* show the proportion of recombined cells that are proliferating at 8, 30, and 120 dpr in the gray (*D*) and white matter (*E*) and at 6 and 9 dpr in both areas (*F*). BrdU was applied for a period of 3 d (*D*, *E*) or 1 d (*F*) in the drinking water, directly before analysis of the animals.

Thus, the intriguing difference in the progeny of Olig2$^+$ progenitors in the GM and WM seems to be rather caused by a different fate and proliferation behavior than by selective cell death.

Cells labeled by Olig2::CreER^{TM}-mediated recombination still generate mostly NG2$^+$ glia after stab wound injury in the GM

To unravel whether GM progenitors have the potential to generate new oligodendrocytes after injury, when some of the previously existing oligodendrocytes degenerate, we performed stab wound lesions as described previously (Buffo et al., 2005) at 5 dpr and analyzed the recombined cells at 3, 5, 7, 30, and 60 d postlesion (dpl), corresponding to 8, 10, 12, 35, and 65 dpr, respectively (Fig. 5). Consistent with our previous data that Olig2$^+$ cells increase in number after injury (Buffo et al., 2005), here we also observed an increase in the number of reporter$^+$ cells around the lesion site to an average of 22.5 ± 6 cells at 3 dpl (Fig. 5*G*, 4.7× increase compared with contralateral side or control cortices). However, some of these cells are still subject to cell death, as the number of reporter$^+$ cells decreased between 3 and 5 dpl (Table 1) and plenty of activated Caspase3$^+$/reporter$^+$ double-positive cells were observed in the lesion site at this stage (6% ±3 reporter$^+$ cells per lesion area). Despite these dynamic changes, however, the cell type composition of reporter$^+$ cells at 3 or 30 dpl (Fig. 5*E*,*F*) did not change and closely resembled the composition in the noninjured cortex (Fig. 3*J*,*K*) with most reporter$^+$ cells colabeling with NG2$^+$ (Fig. 5*A*,*B*), some with CC1, few with S100β/GFAP (Fig. 5*C*,*D*), and none with neuroblast or neuronal antigens ($n = 569$). Similar to the unlesioned GM, the proportion and number of CC1$^+$ per reporter$^+$ cells did not significantly increase (Fig. 5, Table 2) ($p = 0.5476$), and no signs of reporter$^+$ cells expressing myelin proteins or exhibiting a morphology indicative of myelinating oligodendrocytes could be observed after stab wound injury. Thus, even after injury, the main progeny of Olig2-expressing progenitors remained NG2$^+$ glia in the GM, profoundly different from the maturating oligodendrocytes observed in the WM.

Progeny of BrdU-labeled cells in GM and WM

Given the surprising difference observed in the progeny of Olig2$^+$ cells in the GM and WM, we aimed to confirm this finding with an independent technique. In particular, it has to be kept in mind that the fate mapping experiments are performed on a heterozygous Olig2 background as one allele does not express Olig2 but rather CreER^{TM} (knock-in into the Olig2-locus) (Takebayashi et al., 2002). It is therefore conceivable that the lower dose of Olig2 may impair the full oligodendrocyte differentiation in the GM. Moreover, the recombination labels only a certain proportion of Olig2$^+$ cells (as mentioned above) that may perform differently from the total population of Olig2$^+$ cells. To ensure that the differences in progeny observed between the WM and GM may not be because of the low endogenous Olig2 levels or the nature of the labeled subtypes, we took advantage of the fact that virtually all proliferating, BrdU-incorporating cells express Olig2 in the adult brain parenchyma. We thus analyzed the progeny of all BrdU-labeled cells in wild-type animals 4 weeks after the onset of BrdU treatment. Whereas only 12% of the BrdU$^+$ cells acquired CC1-immunoreactivity in the GM, this proportion was profoundly higher in the WM with 41% (Fig. 6). Thus, within a month we could identify a 3.4× higher proportion of reporter$^+$ cells differentiating into CC1$^+$ oligodendrocytes in the WM compared with the GM, thereby confirming the results obtained by fate mapping.

Discussion

Progeny of Olig2-expressing cells in the adult versus developing brain

Consistent with the concept of so-called "adult oligodendrocyte progenitors" (Goldman, 2003), the Olig2$^+$ progenitors labeled by Cre-mediated recombination in the adult brain generated mainly NG2$^+$ glia or mature oligodendrocytes, but hardly any astrocytes and no neurons. This is in pronounced contrast to the multitude of lineages observed to arise from this progenitor pool during development using even the very same mouse line (Furusho et al., 2006; Masahira et al., 2006; Miyoshi et al., 2007; Ono et al., 2008) or the NG2::CreERTM^{T2} line (Zhu et al., 2008). Importantly, during development, the different cell types arise from distinct sets of Olig2$^+$ progenitors (e.g., Olig2$^+$ cells that generate motoneurons do not share their lineage with those that generate oligodendrocytes) (Mukouyama et al., 2006; Wu et al., 2006). Similarly, Mash1-expressing progenitors that generate neurons in the embryonic spinal cord do not share their lineage with those generating oligodendrocytes at later stages (Battiste et al., 2007). Similar to the Olig2-derived lineage, Mash1$^+$ progenitors in the adult also generate virtually exclusively cells of the oligodendrocyte fate with the exception that they continue to contribute to adult olfactory bulb and dentate gyrus interneuron generation (Kim et al., 2007). In contrast, Olig2-CreER^{TM}-labeled progenitors contribute to this lineage only during development (Miyoshi et al., 2007) but no longer in the adulthood. Thus, the change in progeny of both Mash1- and Olig2-

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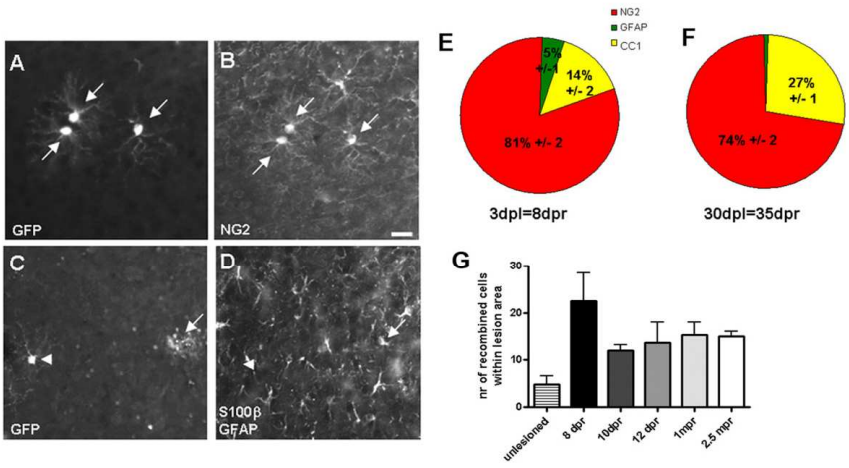


Figure 5. Identification of reporter+ cells after stab wound injury in the GM. ***A–D***, Micrographs of GFP+ cells in the lesion site at 3 dpl (8 dpr) double-stained for cell type-specific antigens as indicated in the panels. Pies in ***E*** and ***F*** depict the quantitative analysis of reporter+ cells with regard to cell identity (***E***, 3 dpl; ***F***, 30 dpl). Quantification of absolute numbers of recombined cells at different time points after the stab wound within the lesion area localized 150 μm away from the lesion track is shown in ***G***. Scale bar, 20 μm.

expressing progenitors over time seems to be caused by the selective persistence of only some of the lineages present at previous stages into adulthood.

Mechanisms mediating distinct progeny of Olig2+ progenitors

This raises the suggestion that also the different progeny in the WM and GM may be attributable to distinct lineages persisting into adulthood. The difference in the progeny of proliferating Olig2+ cells in the GM and WM of the adult cerebral cortex has been shown by both BrdU-labeling as well as Olig2::CreER^{TM}-mediated fate mapping. Also in this regard, our data are further corroborated by Mash1-CreER^{T2}-mediated fate mapping showing also a higher proportion of CC1+ oligodendrocytes in the WM compared with the GM (Kim et al., 2007). In addition to the possibility of intrinsic lineage differences of these progenitors in the GM and WM, differences in the local environment may be responsible. The previous suggestion that the local GM environment arrests oligodendrocyte differentiation at an immature state (Dawson et al., 2000; Levine et al., 2001) is indeed consistent with several of our observations. First, the GM reporter+ cells mostly maintain Olig2 (and NG2) expression, in contrast to those in the WM that downregulate Olig2 (and NG2) during differentiation into oligodendrocytes (supplemental Fig. 2, available at www.jneurosci.org as supplemental material). Second, Olig2+ progenitors proliferate slower in the GM compared with the WM and may even be subject to cell cycle arrest (see below). Third, even the few oligodendrocytes generated in the GM fail to acquire any further mature properties resembling myelinating oligodendrocytes in their morphology or expressing myelin proteins. It is therefore well conceivable that the Olig2-derived cells are arrested in an "immature," although postmitotic state. These NG2+ glial cells could then perform specific physiological functions (Butt et al., 2002; Greenwood and Butt, 2003; Paukert and Bergles, 2006; Nishiyama, 2007; Káradóttir et al., 2008), although it is not clear whether NG2+ progenitors also exhibit these properties. If it is the environment that inhibits the differentiation of mature oligodendrocytes in the GM, this inhibition is also not overcome by stab wound injury. It will be important, however, to further examine the fate of Olig2+ progenitors after demyelinating injuries where different local signaling pathways may be active.

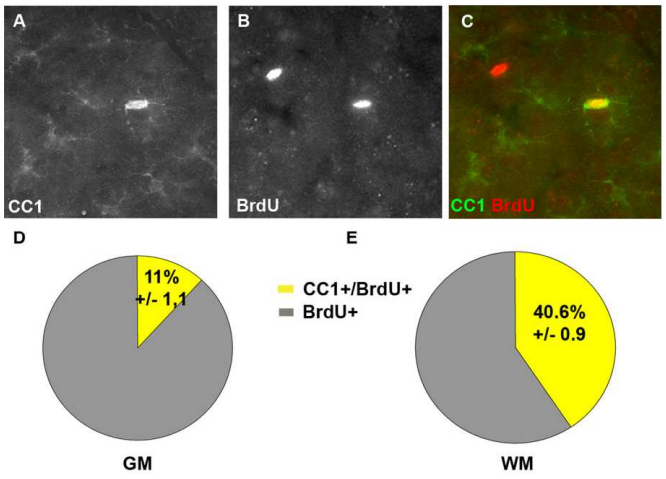


Figure 6. ***A–C***, Colabeling of BrdU with CC1 in the GM. Pies in ***D*** and ***E*** show the proportion of BrdU+ cells colabeling with CC1 (yellow) in the GM (***D***) and in the WM (***E***) after application of BrdU in adult wild-type mice.

A further difference between the progeny of Olig2$^+$ cells in the GM and WM was their population size, with reporter$^+$ cells increasing during the first 2 months after recombination in the WM, but remaining rather constant in the GM. Only after injury the total number of reporter$^+$ cells in the GM also increased, despite a transient phase of cell death. The differences in the number of reporter$^+$ cells in GM and WM without injury may result from differences in cell death, cell proliferation, or cell migration. Although staining for activated Caspase3 in the intact cortex provided no evidence for selective cell death in the GM compared with WM, it is possible that we missed a short window of cell death. However, such a mechanism would not explain the difference in "surviving" NG2$^+$ cells in the GM that also change their proliferation mode over time. In contrast, we could obtain direct evidence for different cell cycle kinetics that may contribute to the increase in number of reporter$^+$ cells in the WM as opposed to the GM. A third possibility to explain the difference in the number and identity of reporter$^+$ cells (in the WM and GM) may also be that cells recombined in the GM and differentiating into oligodendrocytes migrate into the WM. Whichever the exact mechanism may be, it may also be relevant for the impaired remyelination observed in the GM after demyelinating injuries (Levine et al., 2001).

Adult generation of mature oligodendrocytes in the WM

In pronounced difference to the predominance of an NG2$^+$ progeny of Olig2$^+$ progenitors in the GM, mature oligodendrocytes with the myelin proteins MAG, MOG, and MBP in their reporter$^+$ processes were seen in the WM. Although the generation of new myelinating oligodendrocytes has been frequently observed after demyelination (Gensert and Goldman, 1997; Chari and Blakemore, 2002), few newly generated oligodendrocytes were seen after viral vector injections into the intact WM (Gensert and Goldman, 1996; Menn et al., 2006). Our results now suggest that a considerable population of dividing Olig2-expressing progenitors in the intact WM give rise to mature oligodendrocytes. Viral vector tropism missing this progenitor population or partial silencing of virally driven reporters in specific cell types (Gaiano et al., 1999) may explain why this population has been missed in previous work. Moreover, previous studies have been performed in rat (Gensert and Goldman, 1996, 1997) or different strains of inbred mice (Menn et al., 2006). Given that different species and strains of mice show a high number of differentially expressed genes involved in myelination (Dimou et al., 2006), some of these differences may also be relevant with regard to the differentiation of oligodendrocytes. A third explanation for this discrepancy between previous data and our results is the rather focal labeling performed by viral vector injections as opposed to the more widespread labeling by Olig2::CreER^{TM}-mediated recombination or BrdU incorporation. If differentiating oligodendrocytes migrate from other regions toward the WM as discussed above, this may explain why fewer cells are labeled by focal injection into the WM. In addition to the suggestion proposed above that oligodendrocyte progenitors may migrate from the GM into the WM, it has been shown that a small proportion of progenitors originating in the adult subependymal zone give rise to oligodendrocytes in the cortical WM (Menn et al., 2006; Colak et al., 2008).

Whatever their origin might be, the observation of continuous generation of myelinating oligodendrocytes in the adult cortical WM raises intriguing questions about the identity of the axons that are newly myelinated. One possibility may be a turnover of adult oligodendrocytes such that the newly generated oligodendrocytes replace older ones. Indeed, this is the case for some specific neuronal populations, such as those in the olfactory bulb and dentate gyrus that are subject to constant turnover (Lagace et al., 2007; Ninkovic et al., 2007), whereas most neurons in other brain regions are not turned over. Alternatively or additionally, new oligodendrocytes may be added (rather than replacing preexisting oligodendrocytes). However, an increase in oligodendroglia was mostly found during the first months in rodents (Ling and Leblond, 1973; Levison et al., 1999) or years in humans (Peters et al., 1991), whereas the generation of myelinating oligodendrocytes observed by Olig2::CreER^{TM}-mediated fate mapping continues into 6 month old mice, an age when the growth of the brain should have come to an end. These data therefore propose that the generation of new oligodendrocytes is not limited to the early life but continues throughout.

The new findings are rather intriguing with regard to the continuous plasticity occurring in the adult cerebral cortex (Chang et al., 2005; Hofer et al., 2006) that may require changes in myelination also (Fields, 2008). It has been suggested that the end of the critical period coincides with the completion of myelin formation (Fields, 2008). However, our results now show a continuation of myelination into adult stages in line with the continued neuronal plasticity. Thus, our fate-mapping experiments identifying the adult generation of NG2 glia in the GM and mature oligodendrocytes in the WM prompts profound questions with regard to their function and why it is important to generate these cell types continuously in the adult brain. The new tools that allow inducing genetic recombination in the adult will help us to elucidate the function of this adult gliogenesis widespread in the brain parenchyma as opposed to the highly restricted adult neurogenesis.

Note added in proof. Similar results were obtained using a PDGFRα-CreER^{T2} line shown in a study by Rivers et al. (2008).

References

Aguirre AA, Chittajallu R, Belachew S, Gallo V (2004) NG2-expressing cells in the subventricular zone are type C-like cells and contribute to interneuron generation in the postnatal hippocampus. J Cell Biol 165:575–589.

Bartsch U, Kirchhoff F, Schachner M (1989) Immunohistological localization of the adhesion molecules L1, N-CAM, and MAG in the developing and adult optic nerve of mice. J Comp Neurol 284:451–462.

Battiste J, Helms AW, Kim EJ, Savage TK, Lagace DC, Mandyam CD, Eisch AJ, Miyoshi G, Johnson JE (2007) Ascl1 defines sequentially generated lineage-restricted neuronal and oligodendrocyte precursor cells in the spinal cord. Development 134:285–293.

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

Buffo A, Vosko MR, Ertürk D, Hamann GF, Jucker M, Rowitch D, Götz M (2005) Expression pattern of the transcription factor Olig2 in response to brain injuries: implications for neuronal repair. Proc Natl Acad Sci U S A 102:18183–18188.

Butt AM, Kiff J, Hubbard P, Berry M (2002) Synantocytes: new functions for novel NG2 expressing glia. J Neurocytol 31:551–565.

Cai J, Chen Y, Cai WH, Hurlock EC, Wu H, Kernie SG, Parada LF, Lu QR (2007) A crucial role for Olig2 in white matter astrocyte development. Development 134:1887–1899.

Chang EF, Bao S, Imaizumi K, Schreiner CE, Merzenich MM (2005) Development of spectral and temporal response selectivity in the auditory cortex. Proc Natl Acad Sci U S A 102:16460–16465.

Chari DM, Blakemore WF (2002) New insights into remyelination failure in multiple sclerosis: implications for glial cell transplantation. Mult Scler 8:271–277.

Colak D, Mori T, Brill MS, Pfeifer A, Falk S, Deng C, Monteiro R, Mummery C, Sommer L, Götz M (2008) Adult neurogenesis requires Smad4-

mediated bone morphogenic protein signaling in stem cells. J Neurosci 28:434–446.
Dawson MR, Levine JM, Reynolds R (2000) NG2-expressing cells in the central nervous system: are they oligodendroglial progenitors? J Neurosci Res 61:471–479.
Dayer AG, Cleaver KM, Abouantoun T, Cameron HA (2005) New GABAergic interneurons in the adult neocortex and striatum are generated from different precursors. J Cell Biol 168:415–427.
Dimou L, Schnell L, Montani L, Duncan C, Simonen M, Schneider R, Liebscher T, Gullo M, Schwab ME (2006) Nogo-A-deficient mice reveal strain-dependent differences in axonal regeneration. J Neurosci 26:5591–5603.
Fields RD (2008) White matter in learning, cognition and psychiatric disorders. Trends Neurosci 31:361–370.
Furusho M, Ono K, Takebayashi H, Masahira N, Kagawa T, Ikeda K, Ikenaka K (2006) Involvement of the Olig2 transcription factor in cholinergic neuron development of the basal forebrain. Dev Biol 293:348–357.
Gaiano N, Kohtz JD, Turnbull DH, Fishell G (1999) A method for rapid gain-of-function studies in the mouse embryonic nervous system. Nat Neurosci 2:812–819.
Gensert JM, Goldman JE (1996) In vivo characterization of endogenous proliferating cells in adult rat subcortical white matter. Glia 17:39–51.
Gensert JM, Goldman JE (1997) Endogenous progenitors remyelinate demyelinated axons in the adult CNS. Neuron 19:197–203.
Goldman S (2003) Glia as neural progenitor cells. Trends Neurosci 26:590–596.
Greenwood K, Butt AM (2003) Evidence that perinatal and adult NG2-glia are not conventional oligodendrocyte progenitors and do not depend on axons for their survival. Mol Cell Neurosci 23:544–558.
Hirrlinger PG, Scheller A, Braun C, Quintela-Schneider M, Fuss B, Hirrlinger J, Kirchhoff F (2005) Expression of reef coral fluorescent proteins in the central nervous system of transgenic mice. Mol Cell Neurosci 30:291–303.
Hofer SB, Mrsic-Flogel TD, Bonhoeffer T, Hübener M (2006) Prior experience enhances plasticity in adult visual cortex. Nat Neurosci 9:127–132.
Horner PJ, Gage FH (2002) Regeneration in the adult and aging brain. Arch Neurol 59:1717–1720.
Káradóttir R, R, Hamilton NB, Bakiri Y, Attwell D (2008) Spiking and nonspiking classes of oligodendrocyte precursor glia in CNS white matter. Nat Neurosci 11:450–456.
Kim EJ, Leung CT, Reed RR, Johnson JE (2007) *In vivo* analysis of Ascl1 defined progenitors reveals distinct developmental dynamics during adult neurogenesis and gliogenesis. J Neurosci 27:12764–12774.
Lagace DC, Whitman MC, Noonan MA, Ables JL, DeCarolis NA, Arguello AA, Donovan MH, Fischer SJ, Farnbauch LA, Beech RD, DiLeone RJ, Greer CA, Mandyam CD, Eisch AJ (2007) Dynamic contribution of nestin-expressing stem cells to adult neurogenesis. J Neurosci 27:12623–12629.
Levine JM, Reynolds R, Fawcett JW (2001) The oligodendrocyte precursor cell in health and disease. Trends Neurosci 24:39–47.
Levison SW, Young GM, Goldman JE (1999) Cycling cells in the adult rat neocortex preferentially generate oligodendroglia. J Neurosci Res 57:435–446.
Ligon KL, Fancy SP, Franklin RJ, Rowitch DH (2006a) Olig gene function in CNS development and disease. Glia 54:1–10.
Ligon KL, Kesari S, Kitada M, Sun T, Arnett HA, Alberta JA, Anderson DJ, Stiles CD, Rowitch DH (2006b) Development of NG2 neural progenitor cells requires Olig gene function. Proc Natl Acad Sci U S A 103:7853–7858.
Lindner M, Heine S, Haastert K, Garde N, Fokuhl J, Linsmeier F, Grothe C, Baumgärtner W, Stangel M (2008) Sequential myelin protein expression during remyelination reveals fast and efficient repair after central nervous system demyelination. Neuropathol Appl Neurobiol 34:105–114.
Ling EA, Leblond CP (1973) Investigation of glial cells in semithin sections. II. Variation with age in the numbers of the various glial cell types in rat cortex and corpus callosum. J Comp Neurol 149:73–81.
Masahira N, Takebayashi H, Ono K, Watanabe K, Ding L, Furusho M, Ogawa Y, Nabeshima Y, Alvarez-Buylla A, Shimizu K, Ikenaka K (2006) Olig2-positive progenitors in the embryonic spinal cord give rise not only to motoneurons and oligodendrocytes, but also to a subset of astrocytes and ependymal cells. Dev Biol 293:358–369.
Matthias K, Kirchhoff F, Seifert G, Hüttmann K, Matyash M, Kettenmann H, Steinhäuser C (2003) Segregated expression of AMPA-type glutamate receptors and glutamate transporters defines distinct astrocyte populations in the mouse hippocampus. J Neurosci 23:1750–1758.
Menn B, Garcia-Verdugo JM, Yaschine C, Gonzalez-Perez O, Rowitch D, Alvarez-Buylla A (2006) Origin of oligodendrocytes in the subventricular zone of the adult brain. J Neurosci 26:7907–7918.
Miller RH (2002) Regulation of oligodendrocyte development in the vertebrate CNS. Prog Neurobiol 67:451–467.
Miyoshi G, Butt SJ, Takebayashi H, Fishell G (2007) Physiologically distinct temporal cohorts of cortical interneurons arise from telencephalic Olig2-expressing precursors. J Neurosci 27:7786–7798.
Mukouyama YS, Deneen B, Lukaszewicz A, Novitch BG, Wichterle H, Jessell TM, Anderson DJ (2006) Olig2+ neuroepithelial motoneuron progenitors are not multipotent stem cells in vivo. Proc Natl Acad Sci U S A 103:1551–1556.
Ninkovic J, Mori T, Götz M (2007) Distinct modes of neuron addition in adult mouse neurogenesis. J Neurosci 27:10906–10911.
Nishiyama A (2007) Polydendrocytes: NG2 cells with many roles in development and repair of the CNS. Neuroscientist 13:62–76.
Novak A, Guo C, Yang W, Nagy A, Lobe CG (2000) Z/EG, a double reporter mouse line that expresses enhanced green fluorescent protein upon Cre-mediated excision. Genesis 28:147–155.
Ono K, Takebayashi H, Ikeda K, Furusho M, Nishizawa T, Watanabe K, Ikenaka K (2008) Regional- and temporal-dependent changes in the differentiation of Olig2 progenitors in the forebrain, and the impact on astrocyte development in the dorsal pallium. Dev Biol 320:456–468.
Paukert M, Bergles DE (2006) Synaptic communication between neurons and NG2+ cells. Curr Opin Neurobiol 16:515–521.
Peters A, Josephson K, Vincent SL (1991) Effects of aging on the neuroglial cells and pericytes within area 17 of the rhesus monkey cerebral cortex. Anat Rec 229:384–398.
Shen S, Sandoval J, Swiss VA, Li J, Dupree J, Franklin RJ, Casaccia-Bonnefil P (2008) Age-dependent epigenetic control of differentiation inhibitors is critical for remyelination efficiency. Nat Neurosci 11:1024–1034.
Soriano P (1999) Generalized lacZ expression with the ROSA26 Cre reporter strain. Nat Genet 21:70–71.
Takebayashi H, Nabeshima Y, Yoshida S, Chisaka O, Ikenaka K, Nabeshima Y (2002) The basic helix-loop-helix factor olig2 is essential for the development of motoneuron and oligodendrocyte lineages. Curr Biol 12:1157–1163.
Tamura Y, Kataoka Y, Cui Y, Takamori Y, Watanabe Y, Yamada H (2007) Multi-directional differentiation of doublecortin- and NG2-immunopositive progenitor cells in the adult rat neocortex in vivo. Eur J Neurosci 25:3489–3498.
Wu S, Wu Y, Capecchi MR (2006) Motoneurons and oligodendrocytes are sequentially generated from neural stem cells but do not appear to share common lineage-restricted progenitors in vivo. Development 133:581–590.
Zhu X, Bergles DE, Nishiyama A (2008) NG2 cells generate both oligodendrocytes and gray matter astrocytes. Development 135:145–157.

Supplemental Figure 1: Identification of proliferating cells in the adult mouse brain. Micrographs are examples of BrdU+ cells in the GM (**A**) and in the WM (**B**) after a 2 week application of BrdU in the drinking water. Doublestainings for BrdU (**C**) and for the transcription factor Olig2 (**D**) reveal colabeling for the two antigens. (**E**) Quantification of Olig2+ cells in the adult cerebral cortex as percentage of BrdU+ cells, after application of BrdU for 3 days (left) or 2 weeks (right) in the drinking water. (**F**) Proportion of Olig2+/BrdU+ cells in different areas of the brain after a 3 day application of BrdU. Scale bars: 20μm.

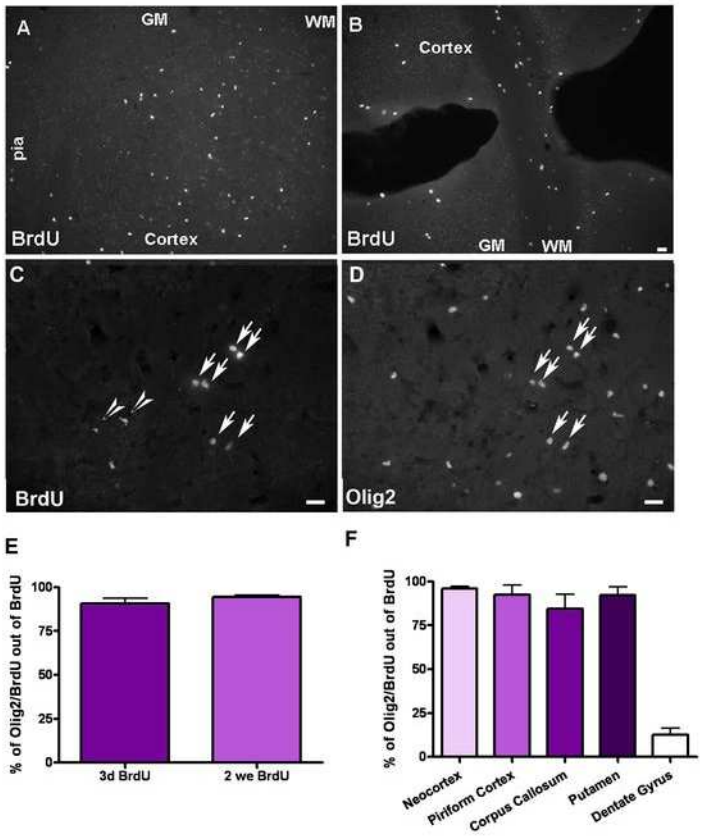
Supplemental Figure 2: Proportion of recombined cells coexpressing Olig2 at different timepoints after recombination in the GM (**A**) and the WM (**B**).

Supplemental Figure 3: (**A-C**) Confocal microscope images showing the colabeling of reporter+ cells (**A**) and the paranodal marker Caspr (**B**). 1μm optical sections were used. Arrows show some of the colocalizing spots.

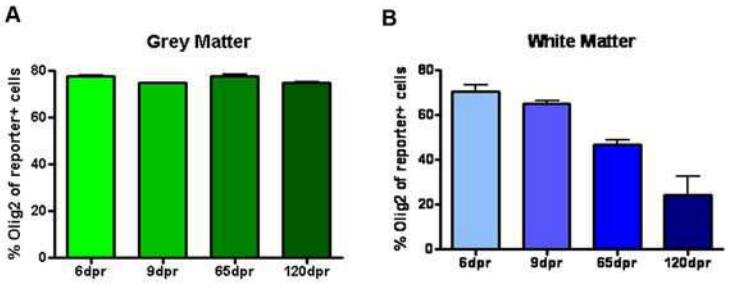
Supplemental Figure 4: Identification of reporter+ cells 6 months post recombination. Pies depict the quantitative analysis amongst reporter+ cells 6 months after recombination in the WM (**A**) or in the GM (**B**).

Supplemental Figure 5: (**A-C**) Micrographs of double labeled recombined cells with the PLP-dsRed transgene. (**D**) Quantification of the proportion of reporter+ cells expressing the PLP-dsRed transgene in the GM and WM at 8dpr and at 65dpr.

Supplemental Figure 6: Identification of reporter+ cells in the dentate gyrus and the olfactory bulb. (**A-C**) Micrographs of GFP+ cells at 30dpr in the dentate gyrus. No colocalization of GFP+ cells with the neuroblast marker dcx could be observed. (**D-I**) Micrographs of reporter+ cells at 30dpr in the olfactory bulb. No colocalization with dcx and often double-labeling with NG2 could be observed. ↑ point to double stained cells; ► point to single positive cells. Scale bars: (A-F) 60μm, (G-I) 20μm.



Supplemental Fig. 1



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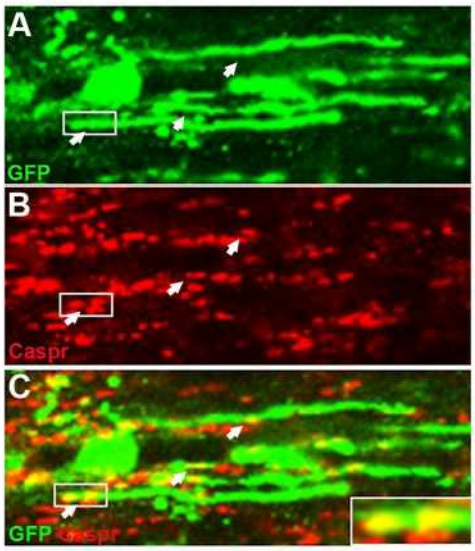
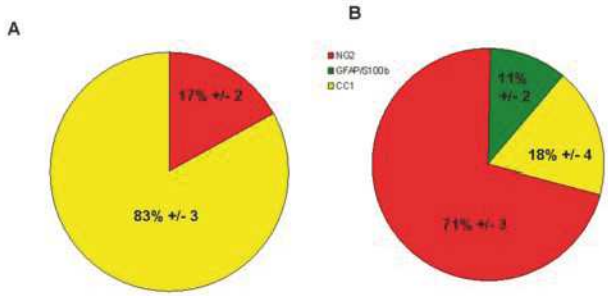
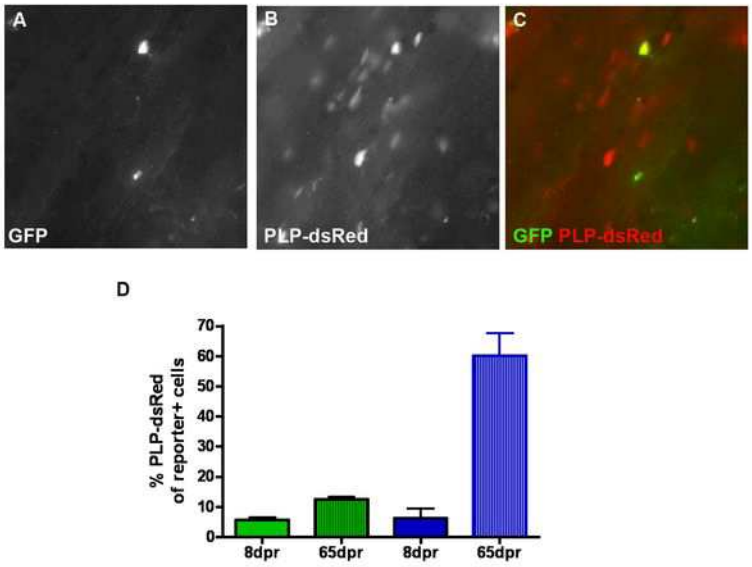


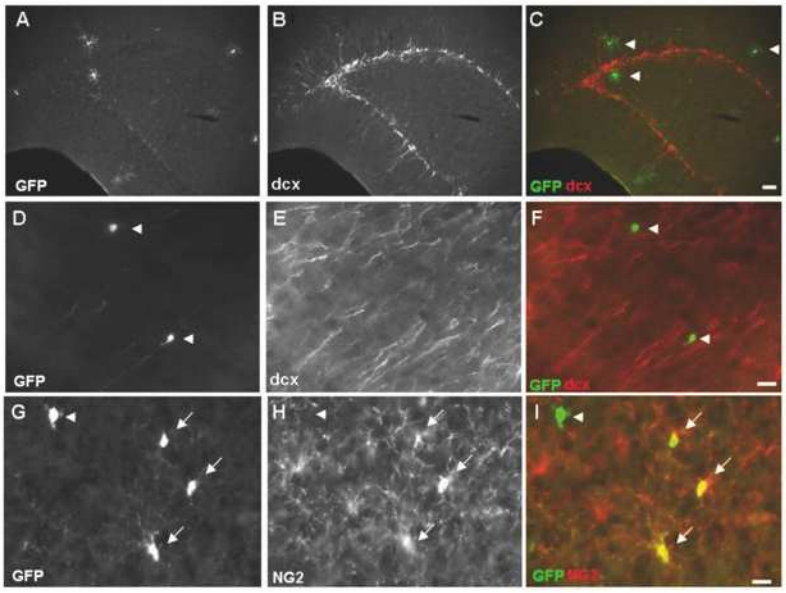
Supplemental Figure 3



Supplemental Figure 4



Supplemental Fig. 5



Supplemental Fig. 6

3.3 Generation and characterization of the Sox10-iCreER^{T2} mouse line

The revised manuscript of this study is submitted as "Sox10-iCreER^{T2}: a mouse line to inducibly trace the neural crest and oligodendrocyte lineage" by **Simon C,** Lickert H, Götz M and Dimou L (2011).

Here, the generation and use of a new transgenic mouse line to target exclusively the oligodendrocyte lineage in the adult CNS with high efficiency is described. We generated a BAC-transgenic mouse with the expression of an improved Cre (iCre) fused to the ER^{T2} under the Sox10 promoter. I characterized two BAC lines which both recombine in NG2+ cells with high efficiency and specificity. During development Sox10 is also expressed in neural crest and its derivatives and in the adult peripheral nervous system. Thus, the Sox10-iCreER^{T2} mouse line is also a new potent tool to study distinct progeny of the neural crest at different developmental stages. Indeed, by inducing recombination at early embryonic stages we showed that pericytes in the adult brain are the progeny of the neural crest. This result is in contrast to the notion that CNS pericytes belong to the hematopoietic system and therefore have a mesodermal origin. In summary, the Sox10-iCreER^{T2} mouse line is a powerful tool to gain further insights in the biology of neural crest cells and the oligodendrocyte lineage.

Contribution of the authors

The generation of the mouse line, comprising the cloning, sequencing and purification of the BAC-transgenic construct, and the characterization of this new mouse line was performed by me (except the fusion of the iCre to the ER^{T2} sequence). H. Lickert helped with his technical know-how and provided additionally several vector constructs for cloning. The manuscript was written by me and revised by H. Lickert, M. Götz and L. Dimou. This study was directed by H. Lickert, M. Götz and L. Dimou.

Technology Report

Sox10-iCreER^{T2}: a mouse line to inducibly trace the neural crest and oligodendrocyte lineage

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Abstract

SOX10 is a well-conserved and widely expressed transcription factor involved in the regulation of embryonic development and in the determination of cell fate. As it is expressed in neural crest cells, their derivatives and the oligodendrocyte lineage, mutations of the protein contribute to a variety of diseases like neurocristopathies, peripheral demyelinating neuropathies and melanoma. Here, we report the generation of an inducible Sox10-iCreER^{T2} BAC transgenic mouse line that labels, depending on the timepoint of induction, distinct derivatives of the otic placode and the neural crest as well as cells of the oligodendrocyte lineage. Surprisingly, we could show a neural crest origin of pericytes in the brain. Besides its use for fate-mapping, the Sox10-iCreER^{T2} mouse line is a powerful tool to conditionally inactivate genes in the neural crest cells, its progeny and/or the oligodendrocyte lineage in a time-dependent fashion to gain further insights into their function and contribution to diseases.

Text

The Sry (sex determining region Y)-related high mobility group box protein 10 (SOX 10) belongs to the group of SOX E proteins (SOX 8-10), a highly conserved family of transcription factors (reviewed in Lefebvre *et al.*, 2007). Sox10 mRNA can be detected from embryonic day (E) 8.5 in derivatives of the otic placode and in the neural crest (Kuhlbrodt *et al.*, 1998; Pusch *et al.*, 1998). At this developmental stage, SOX10 is critical for survival and maintenance of pluripotency in neural crest stem cells (Breuskin *et al.*, 2009; Britsch *et al.*, 2001; Paratore *et al.*, 2001; Reiprich *et al.*, 2008). Later, SOX10 expression is mainly restricted to glial cells in the peripheral (PNS) and central nervous system (CNS), such as Schwann cells and cells of the oligodendrocyte lineage. There, SOX10 is not only responsible for differentiation and myelination (reviewed in Stolt and Wegner, 2010) but also controls myelin protein expression as loss of Sox10 in the adult PNS results in neuropathy (Bremer *et al.*, 2011).

Several mouse lines have been created to label Sox10-expressing cells and their progeny either by fluorescent proteins (Shibata *et al.*, 2010) or by Cre-recombinase (Stine *et al.*, 2009). Studies using mouse lines that lead to Sox10-deletion revealed its potent role in development and in the adult (Bremer *et al.*, 2011; Ludwig *et al.*, 2004). However, none of these mouse lines allows analyzing the role of Sox10 at distinct developmental stages. Therefore, we generated a tamoxifen-inducible BAC-transgenic mouse line (Sox10-iCreER^{T2}) to target and fate-map Sox10+ cells in a temporal manner. Hence, we are able to detect distinct subpopulations of Sox10+ progeny depending on the timepoint of induction in contrast to the previous published S4F:Cre mice (for comparison see Table 1; Stine *et al.*, 2009). In addition, this mouse line shows no SOX10 dependent-heterozygote phenotype, as shown for Sox10-

knockin mouse lines (Britsch *et al.*, 2001; Ludwig *et al.*, 2004), and faithfully recapitulates endogenous SOX10 expression *in vivo*.

The Sox10-iCreER^{T2}-mouse line expresses a codon-improved Cre recombinase (iCre; Shimshek *et al.*, 2002) that is fused to a mutated ligand-binding domain of the human estrogen receptor (ER^{T2}; Feil et al., 1997) under the Sox10 promoter. Therefore, DNA recombination in Sox10+ cells can be temporally controlled after administration of tamoxifen.

To mimic the temporal and spatial expression pattern of Sox10, we used the bacterial artificial chromosome (BAC) RP 23-28O11 enclosing the Sox10-locus as well as its known transcriptional regulatory elements (Antonellis *et al.*, 2008; Deal *et al.*, 2006; Dutton *et al.*, 2008; Kuspert *et al.*, 2011; Werner *et al.*, 2007). Exon 3 of the Sox10 gene, encoding the start codon, was replaced by the targeting vector (Fig. 1a). The BAC-transgenic DNA was then injected into the pronucleus of fertilized C57Bl/6 oocytes to generate multiple transgenic lines (Fig. 1a-b). The resulting lines (1-Sox10 and 2-Sox10) integrated different copy numbers of the BAC as shown by real-time PCR with line 2-Sox10 revealing the highest expression of the transgene (1-Sox10: 2.2±0.4 transgene copies/ cell; 2-Sox10: 8.9±0.4 transgene copies/ cell). This result is confirmed by Southern Blot analysis (Fig. 1c).

Mice were crossed to the reporter mouse line CAG-eGFP (Nakamura *et al.*, 2006), thus Sox10+ cells and their progeny can be visualized by the enhanced green fluorescent protein (eGFP) after induction with tamoxifen at different developmental stages.

Mice of both transgenic lines are viable and fertile and show no obvious behavioral abnormalities. To examine Sox10 expressing cells in adult mice, we induced 1-3 month old transgenic mice with tamoxifen. Consistent with the expression of SOX10 in the adult brain, we observed reporter+ cells of both transgenic lines in the entire

brain (Fig. 2a-b), e.g. in the cerebral cortical white (WM, Fig. 2c) and gray matter (GM, Fig. 2d) and in the adult neurogenic niches (Fig. 2e-f). Quantification of reporter+ cells in the adult GM resulted in a high number of GFP+ cells (Fig. 2g), half of which costain with Sox10-immunoreactive nuclei (1-Sox10: 61±2.4 %; 2-Sox10: 46±11.9 % of GFP+ cells are SOX10+). The remaining GFP+/SOX10- cells are mature oligodendrocytes as assessed by their colocalization with GSTπ (Fig. 2h-h'', arrowhead) expressing low levels of SOX10 that might be below the detection threshold of immunohistochemistry maybe due to our perfusion and fixation protocol. Despite this, nearly all recombined cells in the cerebral GM colocalized either with NG2 or GSTπ (Fig. 2i) and thus belong to the oligodendrocyte lineage. Additionally, there are some GFP+/NG2-/GSTπ- cells detectable (arrowhead, Fig. 2j-j') that are probably the recently described transition state within the oligodendrocyte lineage, marked by GPR17 (Boda *et al.*, 2011). Note, that a reasonable number of reporter+ cells can also be induced by giving tamoxifen in a lower dose via food pellets in line 2-Sox10 but not in line 1-Sox10 (Fig. 2g, i, k). This difference in recombination efficiency can be explained by the stronger synthesis of iCreER^{T2} due to the higher number of integrated BAC-copies in line 2-Sox10 (Fig. 1c). Both lines also display an increased likelihood of very low number of GFP+ expression in neurons depending on the protocol/amount of tamoxifen administration (gavaging; line 1-Sox10 >40mg tamoxifen/mouse, line 2-Sox10 >30mg tamoxifen/mouse). Notably, both transgenic lines are characterized by high recombination efficiency as 75±1.1% and 93±0.6% of all SOX10+ cells are reporter+ in line 1-Sox10 and 2-Sox10, respectively (Fig. 2k).

Consistent with SOX10 expression not only in CNS but also in PNS glia (Kuhlbrodt *et al.*, 1998), we detected recombined Schwann cells in different organs in the Sox10-iCreER^{T2}-mouse line, like the intestine (Fig. 3a), stomach (Fig. 3b), adrenal gland (Fig. 3c) and sciatic nerve (Fig. 3d), as shown by the double-staining of reporter+

cells with either GFAP or SOX10 (Fig. 3a-d). Additionally, reporter+ cells were detected in other tissues like the skin (Fig. 3e) and the retinal pigment epithelium (Fig. 3f), suggesting an ongoing Sox10 promoter activity in adult melanoblasts.

Since Sox10 expression is not restricted to adult stages, we analyzed Sox10+ cells and their progeny also during development. Therefore, pregnant mice were induced at E10.5, a developmental stage when Sox10 is expressed in derivatives of the otic placode and the neural crest (Kuhlbrodt *et al.*, 1998; Pusch *et al.*, 1998). Embryos were analyzed at E17.5 showing reporter+ cells in different organs (Fig. 4a-f') like the inner ear where some GFP+ cells could be double-labeled with the neuronal marker NeuN (arrow, Fig. 4a-a''). These results suggest that Sox10+ cells can give rise not only to supporting cells but also to the mechanosensory hair cells of the organ of Corti, consistent with Sox10 expression in the inner ear (Breuskin *et al.*, 2009). Outside the auditory system, reporter+ cells were detected in different neural crest derivatives like the trigeminus (Fig. 4b) and the dorsal root ganglia (DRG; Fig. 4c-c'), where reporter+ cells partially colocalize with peripherin (arrows, Fig. 4c') - an intermediate filament expressed in a distinct subtype of sensory neurons (Black *et al.*, 2002; Goldstein *et al.*, 1991). Additionally, there are also GFP+ cells visible in the adrenal gland (Fig. 4d), the skin (Fig. 4e) and the enteric nervous system (Fig. 4f-f'), all being progeny of the trunk, sacral and vagal neural crest. In summary, the distribution of reporter+ cells in the Sox10-iCreER^{T2}-mouse line, recombined at E10.5 and analyzed at E17.5, confirms the well-established lineage of Sox10+ neural crest (Kuhlbrodt *et al.*, 1998).

One advantage of the Sox10-iCreER^{T2}-mouse line is the possibility of long-term fate-mapping studies, by recombining the Sox10-expressing cells e.g. during embryogenesis and analyzing their progeny in the adulthood. We therefore investigated reporter+ cells of mice, induced at E7.5, in different organs of adult

animals (Fig. 5a-f''). Notably, reporter+ cells were detected in the iris of the eye (Fig. 5a), the heart (Fig. 5b), the gut (Fig. 5c-c'') and the adrenal gland (Fig. 5d-d'') – peripheral tissues shown to be invaded by neural crest progeny (Burns and Delalande, 2005; Deal *et al.*, 2006; Montero *et al.*, 2002). While reporter+ cells of the gut are solely NeuN+ enteric neurons (arrows, Fig. 5c'-c''), recombined cells in the adrenal medulla comprise peripheral GFAP+ (arrow, Fig. 5d-d'') and GFAP- cells (arrowheads, Fig. 5d-d'') that probably belong to the sympathoadrenal lineage (Reiprich *et al.*, 2008). Thus, this mouse line allows the examination of neural crest progenitors that give rise to distinct cell populations at different time points and thereby allowing the analysis of Sox10 progeny that has not been examined so far. Interestingly, after embryonic induction there are also reporter+ cells detectable in the adult CNS (Fig. 5e-f''). These GFP+ cells colocalize with PDGFRβ (Fig. 5e-e'') and NG2 (Fig. 5f-f''), both antigens expressed by pericytes (Stallcup, 2002; Winkler *et al.*, 2010), thereby supporting the neural crest origin of CNS pericytes as suggested previously by quail chick transplantations (Korn *et al.*, 2002).

In summary, we generated a unique inducible mouse line that mimics successfully the *Sox*10 expression pattern during development and adulthood and allows studying the contribution of Sox10+ progeny to different organs. Due to its high recombination rate this mouse line is a powerful tool to conditionally inactivate genes to gain further insights in the biology of the neural crest derivatives and the oligodendrocyte lineage at different developmental stages.

Materials and methods

Generation of the mouse line

Vector pBluescript KS (-) containing a Kozak sequence (5'-GCCACC-3') together with a codon-improved Cre recombinase (iCre; Shimshek *et al.*, 2002) was digested with *Xho*I and *Kpn*I to remove the translational stop-codon of the iCre and to fuse it to the cDNA sequence of ER^{T2} (Feil *et al.*, 1997). $iCreER^{T2}$ was amplified by PCR with primers containing *Spe*I cutting sites (underlined; forward primer (Fw): 5'-NNACTAGTTATAGGGCGA ATTGGAGCTC-3', reverse primer (Rev): 5'-NNACTAGTTCAAGCTGTGGCAGG-3') and was cloned in the *Spe*I-site in front of an Simian virus 40 (SV40) intron-polyA sequence. A neomycin (neo) selection cassette (within a PL451 plasmid), composed of a phospho-glycerate kinase (PGK) promoter and the neomycin resistance gene sequence flanked by *FRT*-sites, was amplified by PCR (Fw: 5'-GGCCAGTGAATTGTAATACGA -3', Rev: 5'-NNNATCGATCCCGGGAAGTTCCTA-3') and digested with *Eco*RI and *Cla*I (underlined) and then cloned behind the SV40intron-polyA sequence. The 5' and 3' homology arm (HA) of Sox10 were amplified by PCR (5' HA Fw: 5'-NNNCCGCGG GAAAGAGAGGTGAGCGAAAAGG-3', 5' HA Rev: 5'-NNNGCGGCCGCCCTCGTG AAGAGCCCAA-3'; 3' HA Fw: 5'-NNNGTCGACAAAGCTAGCCGACCAGTACCCTCA CC-3', 3' HA Rev: 5'-NNNGGTACCCCACCCACAGAATTCCAACC-3') with the bacterial artificial chromosome (BAC) RP 23-28O11 (imaGenes) as template. PCR products contained respective restriction sites for *Sac*II, *Not*I, *Sal*I and *Kpn*I (underlined) and were cloned 5' of the iCre and 3' of the neo-selection marker resulting in the targeting vector as shown in Fig. 1a.

BAC RP 23-28O11 was transformed into the EL250 strain that contains an arabinose-inducible Flpe gene (Lee *et al.*, 2001). After homologous recombination of

the targeting vector within the BAC, the neo-selection marker was removed by the FlpE recombinase resulting in the final BAC-construct with the "targeted Sox10 ΔNeo-box" (Fig. 1a). The transgenic BAC-DNA was purified with a modified caesium chloride (CsCl) gradient (online protocol, www.med.umich.edu/tamc/BACDNA.html). The iCreER^{T2} insert was amplified by PCR and sequence confirmed. The BAC integrity was confirmed by amplification of the BAC 5'- and 3' ends by PCR. Used primers are:

Eif3eip Fw: 5'-ACCATGTACCCGATGCGGATCGACG-3',

Eif3eip Rev: 5'-TAGAAGTCCACCTCCGAGGCCGAC-3';

Micall1 Fw: 5'-TTCTGGCCAAGGTGAGAGGGGACCC-3',

Micall1 Rev: 5'-GCTGTGCCGACAGCCCAGAAGAG-3';

Pick1 Fw: 5'-GCAGCCTTGGATGGCACTGTGGCAG-3',

Pick1 Rev: 5'-GGCCAGTGAATTGTAATACGACTC-3';

Pla2g6 Fw: 5'-TGCACAGATCCAGATGGGCGGGC-3',

Pla2g6 reverse: 5'-CGGGAGCTCAGGGAGACAGCAGCAG-3').

Both, the BAC-transgenic clone and its CsCl-purified DNA were sent to PolyGene (Switzerland) for microinjections in the pronucleus of fertilized C57Bl/6 mouse oocytes. Two different founder lines (1-Sox10 and 2-Sox10) were generated and tested for germline transmission of the BAC-transgene. Mice of both founder lines were crossed to a CAG-GFP reporter mouse line (Nakamura *et al.*, 2006).

Southern Blot

20 µg genomic DNA of mouse tail biopsies of the lines 1-Sox10, 2-Sox10 and wildtypes as well as 1 µg of CsCl-purified targeted Sox10-Bac ΔNeo-box were extracted (Laird *et al.*, 1991) and digested with the restriction enzyme *Arv*I resulting in a 3095 bp fragment. Genomic fragments were separated in a 0.7% agarose gel by

electrophoresis. Gel was depurinated, denaturated and neutralized before blotting on a positively charged nitrocellulose membrane (Roche). The 308 bp long southern probe is located at the end of the iCre / beginning of the ER^{T2}, amplified by PCR (with the primers Fw: 5'-CAGTAAGCCCATCATCGAAG-3' and Rev: 5'-CCATCCCTGAAATCATGCAG-3') and purified (High Pure PCR Cleanup Micro Kit, Roche) before the digoxigenin (DIG)-labeling (according to the manufacturers protocol; Dig High Prime DNA labelling and Detection Starter Kit I; NBT/ BCIP detection - Roche). Hybridization and detection were done according to the manufacturers protocol (Roche).

Real-time PCR

Tail biopsies of mice of line 1-Sox10 (n=6), 2-Sox10 (n=6) and C57Bl/6 wildtype controls were prepared as described by Laird et al. (1991). Real-time PCR was performed on a Roche-LightCycler 480 system using LightCycler 480 Probes Master und Universal Probe Library (mouse) according to manufacturer's protocol. The following primers were used to detect BAC-DNA within the genome: Fw: 5'-GTCCCTGGGGGTTACCAA-3'; Rev: 5'-TCATCAGCCACACCAGACAC-3' together with universal probe number 22. The reference gene GAPDH was detected with the primers Fw: 5'-CTTGGGCTTCCTTTAGGGTAA-3'; Rev: 5'-GCGGTTCATTCATTT CCTTC-3' together with universal probe number 70.

Standard dilution series of CsCl-purified BAC DNA and C57Bl/6 genomic DNA were done in order to calculate the standard curve for iCre and GAPDH, respectively. For the transgene standard curve the appropriate number of BAC molecules were spiked into 40 ng of C57Bl/6 genomic DNA. Each PCR reaction contained 40 ng of genomic tail DNA (in total) and was performed in triplicates for each sample. The transgene

copy numbers of line 1-Sox10 and 2-Sox10 were calculated based on the iCre and GAPDH standard curve and were multiplied by two to account for a diploid genome.

Genotyping

Tail biopsies of mice were processed as described by Laird et al. (1991). DNA was amplified by PCR with primers (Fw: 5'-AAACACCCACACCTAGAGAC-3', Rev: 5'-ACCATTTCCTGTTGTTCAGC-3') binding in the 5' UTR (intron 1) of the Sox10 gene and in the iCre (as shown in Fig. 1a), respectively. 27 cycles at an annealing temperature of 52°C results in a PCR product of 452 bp.

Animals

Tamoxifen (Sigma-Aldrich) was diluted in ethanol (final concentration: 10%) and corn oil. For embryonic induction the mother received 3 mg tamoxifen suspension by gavaging. Adult (2-3 month old) mice received either three times every second day 10mg tamoxifen suspension by gavaging or for 14 days tamoxifen food (400 mg/kg tamoxifen; LASvendi) and were analyzed 5 days after the last induction day.

Histological analysis

Embryos were fixed for 4 to 12 hours in 4% paraformaldehyde (PFA) before cryoprotection in 30% sucrose. Whole embryos were frozen in OTC medium (TissueTek) on dry ice, cut in 14 µm thick sections and mounted on positively charged glass slides. Postnatal/ adult mice were anaesthetized and transcardially perfused with 4% PFA. Brains were collected and either postfixed in 4% PFA overnight (required for the Sox10 immunohistochemistry) or only for 10 min before being cryoprotected in 30% sucrose. 30 µm thick brain and 14 µm thick organ sections were cut and stained according to standard protocols as described

previously (Dimou *et al.*, 2008) with the following primary antibodies: rabbit (rb)-GFAP (Dako; 1:500), mouse (m)-GFAP (Sigma; 1:500), chick-GFP (Aves Lab; 1:500), m-GSTπ (BD; 1:500), m-NeuN (Millipore; 1:500), rb-NG2 (Millipore; 1:500), guinea pig-Sox10 (kindly provided by Michael Wegner; 1:1000). Secondary antibodies were chosen according to the primary antibodies and were coupled to Alexa488, Cy2, Alexa555, Cy3, Alexa647 or Cy5 and sections were counterstained with 4',6-diamidino-2-phenylindole (DAPI; Sigma Aldrich).

Images were collected with an Olympus microscope (BX61TRF) or a Zeiss confocal microscope system (LSM710). The quantitative analysis was done with ImageJ counting confocal images of 15 µm thick z-stacks. At least two adjacent fields of view in the medial part of the intact motor and somatosensory cortical GM were quantified in at least 3 animals and 3 different sections per animal. All results are presented as average with the standard error of the mean.

References

Antonellis A, Huynh JL, Lee-Lin SQ, Vinton RM, Renaud G, Loftus SK, Elliot G, Wolfsberg TG, Green ED, McCallion AS, Pavan WJ. 2008. Identification of neural crest and glial enhancers at the mouse Sox10 locus through transgenesis in zebrafish. PLoS Genet 4: e1000174.

Black JA, Renganathan M, Waxman SG. 2002. Sodium channel Na(v)1.6 is expressed along nonmyelinated axons and it contributes to conduction. Brain Res Mol Brain Res 105: 19-28.

Boda E, Vigano F, Rosa P, Fumagalli M, Labat-Gest V, Tempia F, Abbracchio MP, Dimou L, Buffo A. 2011. The GPR17 receptor in NG2 expressing cells: Focus on in vivocell maturation and participation in acute trauma and chronic damage. Glia.

Bremer M, Frob F, Kichko T, Reeh P, Tamm ER, Suter U, Wegner M. 2011. Sox10 is required for Schwann-cell homeostasis and myelin maintenance in the adult peripheral nerve. Glia 59: 1022-1032.

Breuskin I, Bodson M, Thelen N, Thiry M, Borgs L, Nguyen L, Lefebvre PP, Malgrange B. 2009. Sox10 promotes the survival of cochlear progenitors during the establishment of the organ of Corti. Dev Biol 335: 327-339.

Britsch S, Goerich DE, Riethmacher D, Peirano RI, Rossner M, Nave KA, Birchmeier C, Wegner M. 2001. The transcription factor Sox10 is a key regulator of peripheral glial development. Genes Dev 15: 66-78.

Burns AJ, Delalande JM. 2005. Neural crest cell origin for intrinsic ganglia of the developing chicken lung. Dev Biol 277: 63-79.

Deal KK, Cantrell VA, Chandler RL, Saunders TL, Mortlock DP, Southard-Smith EM. 2006. Distant regulatory elements in a Sox10-beta GEO BAC transgene are required for expression of Sox10 in the enteric nervous system and other neural crest-derived tissues. Dev Dyn 235: 1413-1432.

Dimou L, Simon C, Kirchhoff F, Takebayashi H, Gotz M. 2008. Progeny of Olig2-expressing progenitors in the gray and white matter of the adult mouse cerebral cortex. J Neurosci 28: 10434-10442.

Dutton JR, Antonellis A, Carney TJ, Rodrigues FS, Pavan WJ, Ward A, Kelsh RN. 2008. An evolutionarily conserved intronic region controls the spatiotemporal expression of the transcription factor Sox10. BMC Dev Biol 8: 105.

Feil R, Wagner J, Metzger D, Chambon P. 1997. Regulation of Cre recombinase activity by mutated estrogen receptor ligand-binding domains. Biochem Biophys Res Commun 237: 752-757.

Goldstein ME, House SB, Gainer H. 1991. NF-L and peripherin immunoreactivities define distinct classes of rat sensory ganglion cells. J Neurosci Res 30: 92-104.

Korn J, Christ B, Kurz H. 2002. Neuroectodermal origin of brain pericytes and vascular smooth muscle cells. J Comp Neurol 442: 78-88.

Kuhlbrodt K, Herbarth B, Sock E, Hermans-Borgmeyer I, Wegner M. 1998. Sox10, a novel transcriptional modulator in glial cells. J Neurosci 18: 237-250.

Kuspert M, Hammer A, Bosl MR, Wegner M. 2011. Olig2 regulates Sox10 expression in oligodendrocyte precursors through an evolutionary conserved distal enhancer. Nucleic Acids Res 39: 1280-1293.

Laird PW, Zijderveld A, Linders K, Rudnicki MA, Jaenisch R, Berns A. 1991. Simplified mammalian DNA isolation procedure. Nucleic Acids Res 19: 4293.

Lee EC, Yu D, Martinez de Velasco J, Tessarollo L, Swing DA, Court DL, Jenkins NA, Copeland NG. 2001. A highly efficient Escherichia coli-based chromosome engineering system adapted for recombinogenic targeting and subcloning of BAC DNA. Genomics 73: 56-65.

Lefebvre V, Dumitriu B, Penzo-Mendez A, Han Y, Pallavi B. 2007. Control of cell fate and differentiation by Sry-related high-mobility-group box (Sox) transcription factors. Int J Biochem Cell Biol 39: 2195-2214.

Ludwig A, Schlierf B, Schardt A, Nave KA, Wegner M. 2004. Sox10-rtTA mouse line for tetracycline-inducible expression of transgenes in neural crest cells and oligodendrocytes. Genesis 40: 171-175.

Montero JA, Giron B, Arrechedera H, Cheng YC, Scotting P, Chimal-Monroy J, Garcia-Porrero JA, Hurle JM. 2002. Expression of Sox8, Sox9 and Sox10 in the developing valves and autonomic nerves of the embryonic heart. Mech Dev 118: 199-202.

Nakamura T, Colbert MC, Robbins J. 2006. Neural crest cells retain multipotential characteristics in the developing valves and label the cardiac conduction system. Circ Res 98: 1547-1554.

Paratore C, Goerich DE, Suter U, Wegner M, Sommer L. 2001. Survival and glial fate acquisition of neural crest cells are regulated by an interplay between the transcription factor Sox10 and extrinsic combinatorial signaling. Development 128: 3949-3961.

Pusch C, Hustert E, Pfeifer D, Sudbeck P, Kist R, Roe B, Wang Z, Balling R, Blin N, Scherer G. 1998. The SOX10/Sox10 gene from human and mouse: sequence, expression, and transactivation by the encoded HMG domain transcription factor. Hum Genet 103: 115-123.

Reiprich S, Stolt CC, Schreiner S, Parlato R, Wegner M. 2008. SoxE proteins are differentially required in mouse adrenal gland development. Mol Biol Cell 19: 1575-1586.

Shibata S, Yasuda A, Renault-Mihara F, Suyama S, Katoh H, Inoue T, Inoue YU, Nagoshi N, Sato M, Nakamura M, Akazawa C, Okano H. 2010. Sox10-Venus mice: a new tool for real-time labeling of neural crest lineage cells and oligodendrocytes. Mol Brain 3: 31.

Shimshek DR, Kim J, Hubner MR, Spergel DJ, Buchholz F, Casanova E, Stewart AF, Seeburg PH, Sprengel R. 2002. Codon-improved Cre recombinase (iCre) expression in the mouse. Genesis 32: 19-26.

Stallcup WB. 2002. The NG2 proteoglycan: past insights and future prospects. J Neurocytol 31: 423-435.

Stine ZE, Huynh JL, Loftus SK, Gorkin DU, Salmasi AH, Novak T, Purves T, Miller RA, Antonellis A, Gearhart JP, Pavan WJ, McCallion AS. 2009. Oligodendroglial and pan-neural crest expression of Cre recombinase directed by Sox10 enhancer. Genesis 47: 765-770.

Stolt CC, Wegner M. 2010. SoxE function in vertebrate nervous system development. Int J Biochem Cell Biol 42: 437-440.

Werner T, Hammer A, Wahlbuhl M, Bosl MR, Wegner M. 2007. Multiple conserved regulatory elements with overlapping functions determine Sox10 expression in mouse embryogenesis. Nucleic Acids Res 35: 6526-6538.

Winkler EA, Bell RD, Zlokovic BV. 2010. Pericyte-specific expression of PDGF beta receptor in mouse models with normal and deficient PDGF beta receptor signaling. Mol Neurodegener 5: 32.

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Figure legends

Figure 1

Generation of the Sox10-iCreER^{T2} BAC-transgenic mouse line. a) Scheme showing the wildtype BAC used for cloning. Exon 3 of the Sox10 gene, encoding the start codon, is replaced by a targeting vector. This targeting vector comprises the 5' and 3' homology arm (HA), the iCre and ER^{T2} coding gene sequence and the neomycin-resistance box (Neo-box) flanked by Frt-sites. After homologous recombination of the targeting vector into the wildtype BAC, the Neo-box is excised by FlpE resulting in the transgenic BAC with the targeted Sox10 ΔNeo-box. Position of the forward (Fw) and reverse (Rev) primers for the genotyping PCR are indicated as well as the location of the southern probe. b) Genotyping PCR using primers Fw and Rev to distinguish transgenic mice from wildtype animals. c) Southern Blot analysis showing the DNA-ladder, ArvII-digested genomic DNA of tail biopsies from the two transgenic mouse lines and wildtype mice and positive control DNA.

Figure 2

Analysis of the brain of adult induced Sox10-iCreER^{T2}-mice. a-b) Overview of the distribution of reporter+ cells in the brain in line 1-Sox10 (a) and 2-Sox10 (b). c-f) Confocal images showing reporter+ cells in the cortical white matter (WM, c), gray matter (GM, d), dentate gyrus (e) and subependymal zone (SEZ, f) that colocalize with Sox10. g) Quantification of reporter+ cells in the adult cortical gray matter. h-h'') Confocal images of reporter+ cells in the adult gray matter (h) that colocalize with Sox10 (h') and/or GSTπ (h''). i) Graph showing the identity of recombined cells. j-j') Confocal images showing reporter+ cells (j) that are double-stained (arrow) or are immuno-negative (arrowhead) for NG2/GSTπ (j'). k) Graph depicting the

recombination efficiency of line 1- and 2-Sox10 in the adult cortical gray matter. c-f, h-h'', i-i': line 1-Sox10; a-f, h-h'', i-i': maximal intensity projections of confocal images; scale bars: 20 µm

Figure 3

Analysis of peripheral organs of adult induced Sox10-iCreER^{T2}-mice. a-f) Confocal images showing reporter+ cells in the intestine (a), stomach (b), adrenal gland (c), sciatic nerve (d), skin (arrowheads; e) and eye (f). Recombined GFP+ cells in the intestine, stomach, adrenal gland and sciatic nerve (a-d) are peripheral glial cells, co-localizing either with GFAP (a-c) or Sox10 (d). a-c, e: maximal intensity projections of confocal images; d, f: single confocal plain; scale bars: 10 µm

Figure 4

Embryonic induction (at E10.5) and analysis (at E17.5) of the Sox10-iCreER^{T2}-mouse line. a-f') Confocal images showing reporter+ cells in the inner ear (a-a''), trigeminus (b), dorsal root ganglia (c-c'), adrenal gland (d), skin (e) and gut (f-f'). Recombined GFP+ cells in the cochlea (a) colocalize either with NeuN+ hair cells (arrow, a') or are probably NeuN- supporting cells in the organ of Corti (arrowheads, a''). Reporter+ cells in the dorsal root ganglia (c) partially colocalize with peripherin (arrows, c'). c-e: single confocal plane; a-b, f-f': maximal intensity projections of confocal images; arrows point to double-immunopositive cells, arrowheads indicate single-immunopositive cells.

Figure 5

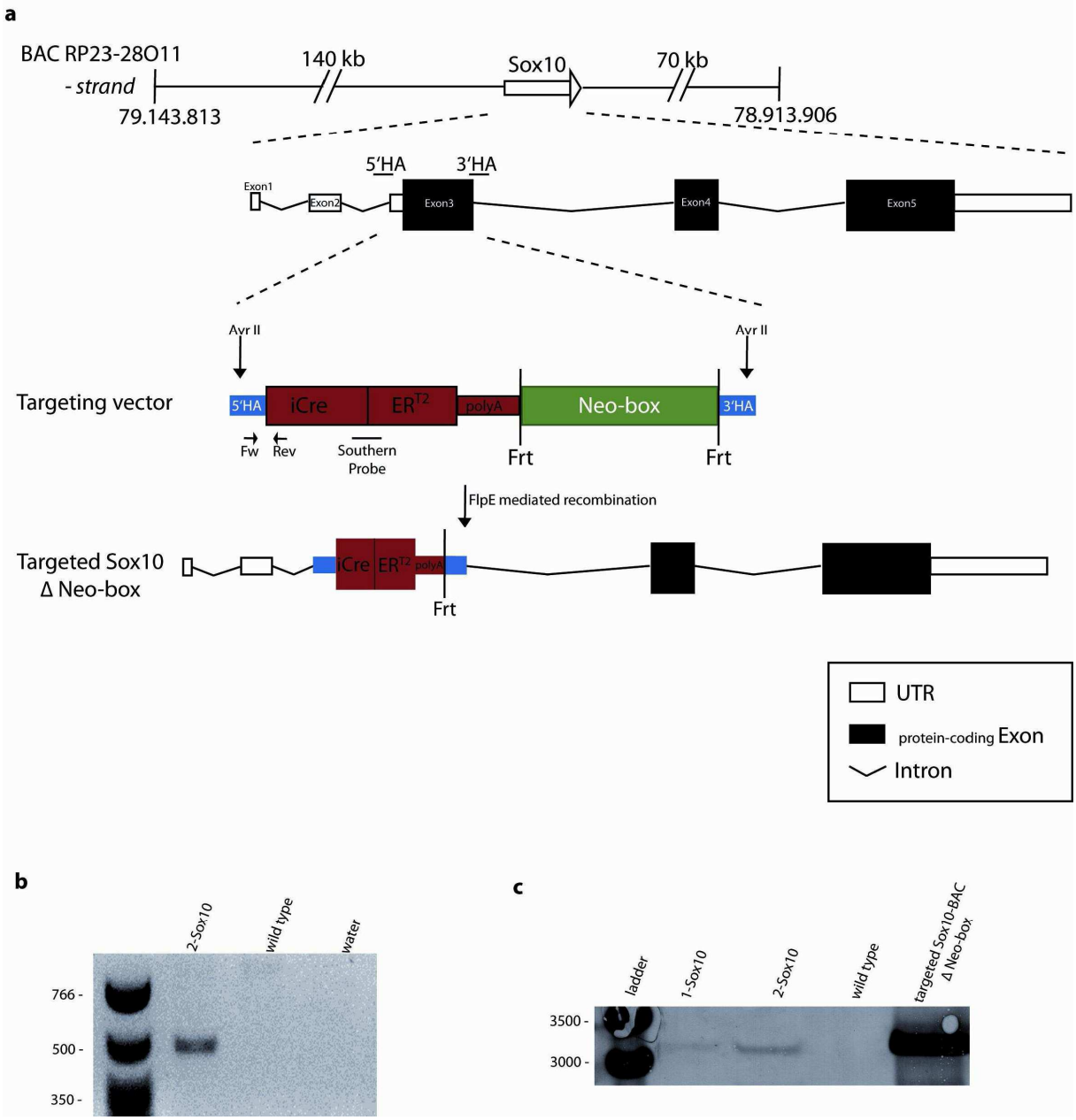
Analysis of adult Sox10-iCreER^{T2}-mice induced at E7.5. a-f'') Confocal images showing reporter+ cells in the eye (a), heart (b), gut (c-c''), adrenal gland (d-d'') and

the cortical gray matter (GM, e-f''). Recombined GFP+ cells in the intestine (c-c') are NeuN+ enteric neurons (c''). Instead, reporter+ cells in the adrenal gland (d) are either peripheral glial cells, colocalizing with GFAP (arrow, d'-d''), or are probably chromaffine cells (arrowheads, d-d''). Recombined GFP+ cells in the CNS (e, f) are pericytes, double-stained with PDGFRβ (arrows, e-e'') and NG2 (arrows, f-f''). b: single confocal plane; a, c-f'': maximal intensity projections of confocal images; arrows point to double-immunopositive cells, arrowheads indicate single-immunopositive cells; a,c, d-f'' scale bar: 10 µm; b, c'-c'' scale bar: 5 µm

Table1

Comparison of reporter+ cells in different tissues in two different Sox10 transgenic mouse lines, namely S4F:Cre (Stine et al. 2009) and the here described inducible Sox10-iCreER^{T2}. (n.a. – not analyzed)

Figure 1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Sox10
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Exon3
Exon4
Exon5
Ayr II
Ayr II
Targeting vector
5'HA
iCre
ERT2
polyA
Neo-box
3'HA
Fw
Rev
Southern Probe
Frt
Frt
FlpE mediated recombination
Targeted Sox10
Δ Neo-box
iCre
ERT2
polyA
Frt
UTR
protein-coding Exon
Intron
b
2-Sox10
wild type
water
766 -
500 -
350 -
c
ladder
1-Sox10
2-Sox10
wild type
targeted Sox10-BAC Δ Neo-box
3500 -
3000 -

Figure 2

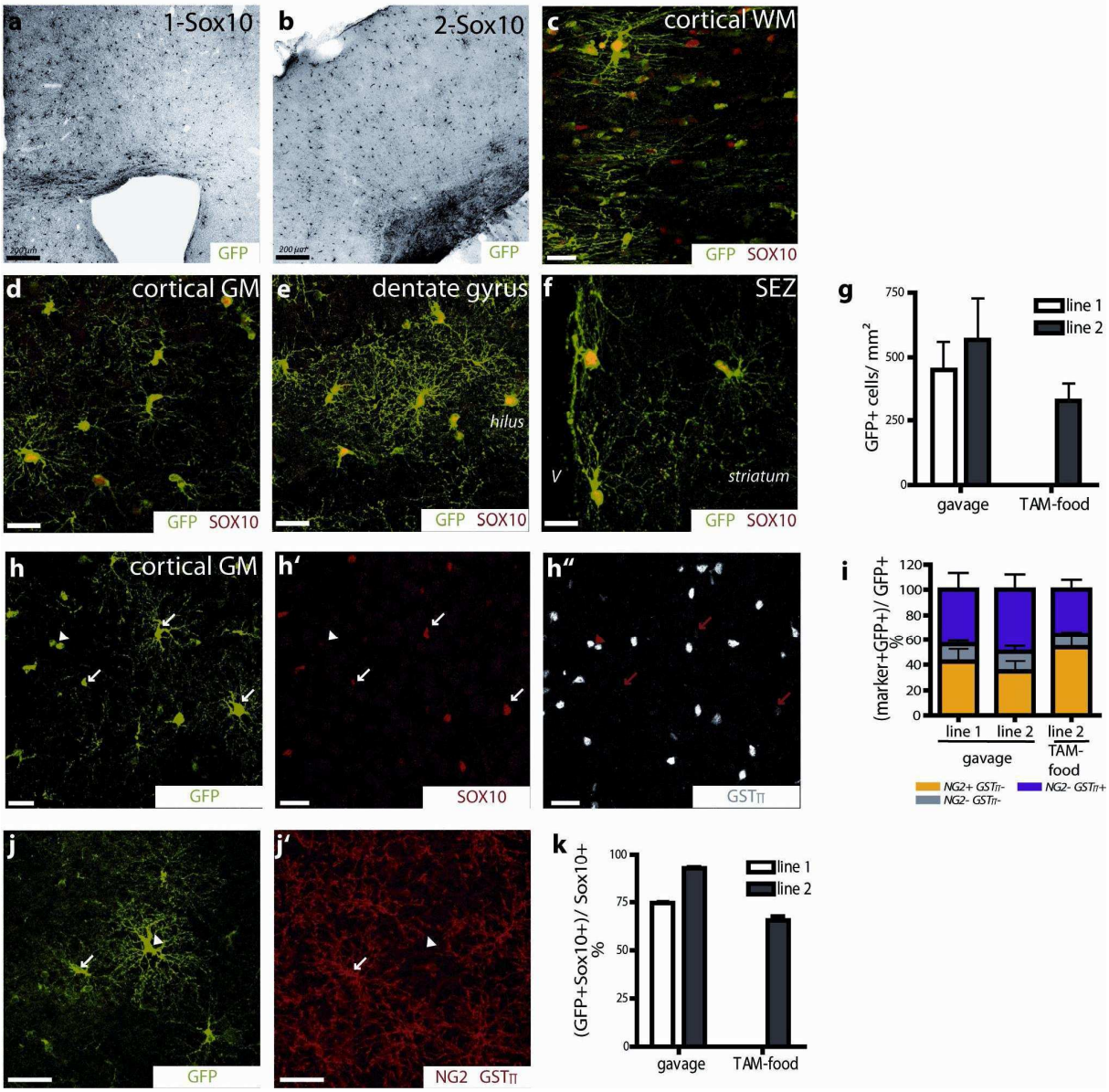

a
1-Sox10
GFP
b
2-Sox10
GFP
c
cortical WM
GFP SOX10
d
cortical GM
GFP SOX10
e
dentate gyrus
hilus
GFP SOX10
f
SEZ
V
striatum
GFP SOX10
g
GFP+ cells/ mm²
750
500
250
0
line 1
line 2
gavage
TAM-food
h
cortical GM
GFP
h'
SOX10
h''
GSTπ
i
(marker+GFP+)/ GFP+ %
120
100
80
60
40
20
0
line 1
line 2
line 2
gavage
TAM-food
NG2+ GSTπ-
NG2- GSTπ-
NG2- GSTπ+
j
GFP
j'
NG2 GSTπ
k
(GFP+Sox10+)/ Sox10+ %
100
75
50
25
0
line 1
line 2
gavage
TAM-food

Figure 3

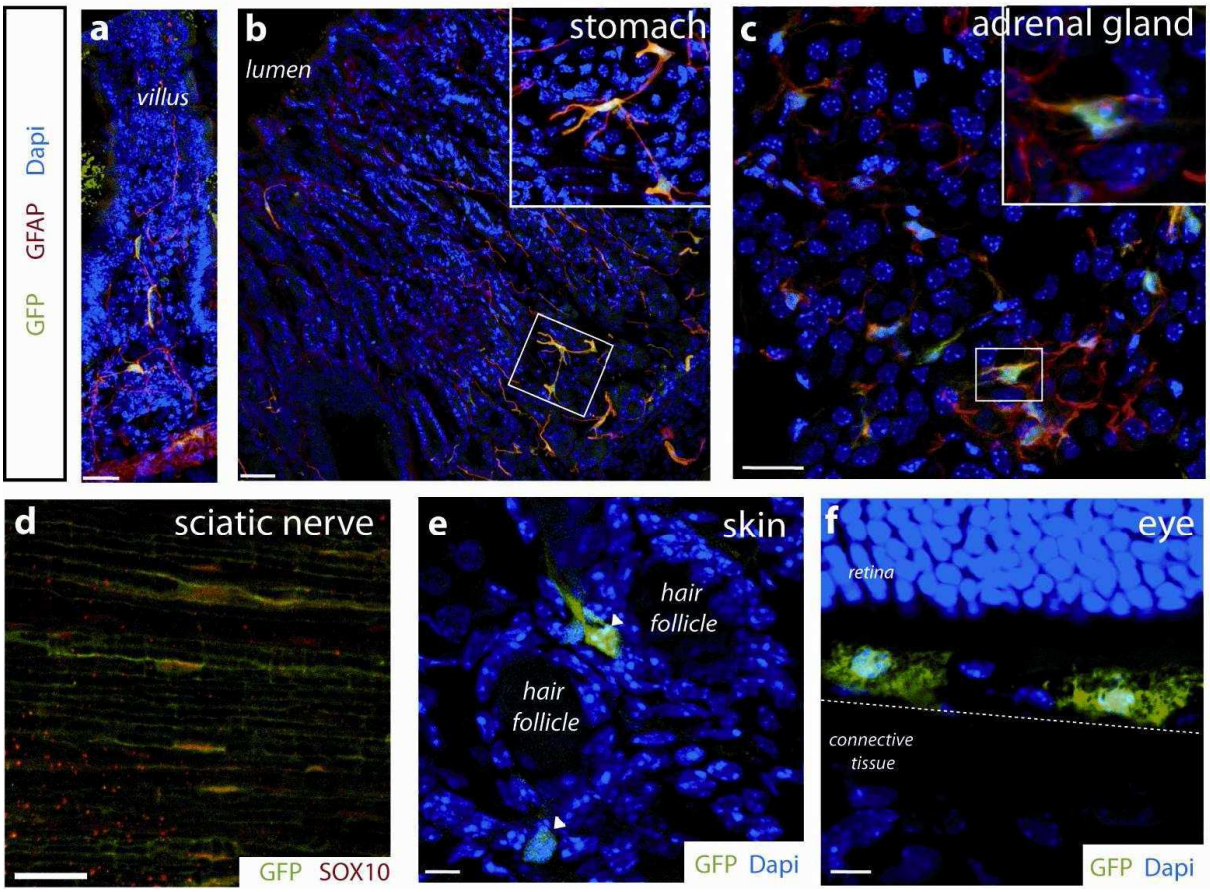

GFP GFAP Dapi
a
villus
b
lumen
stomach
c
adrenal gland
d
sciatic nerve
GFP SOX10
e
skin
hair follicle
hair follicle
GFP Dapi
f
eye
retina
connective tissue
GFP Dapi

Figure 4

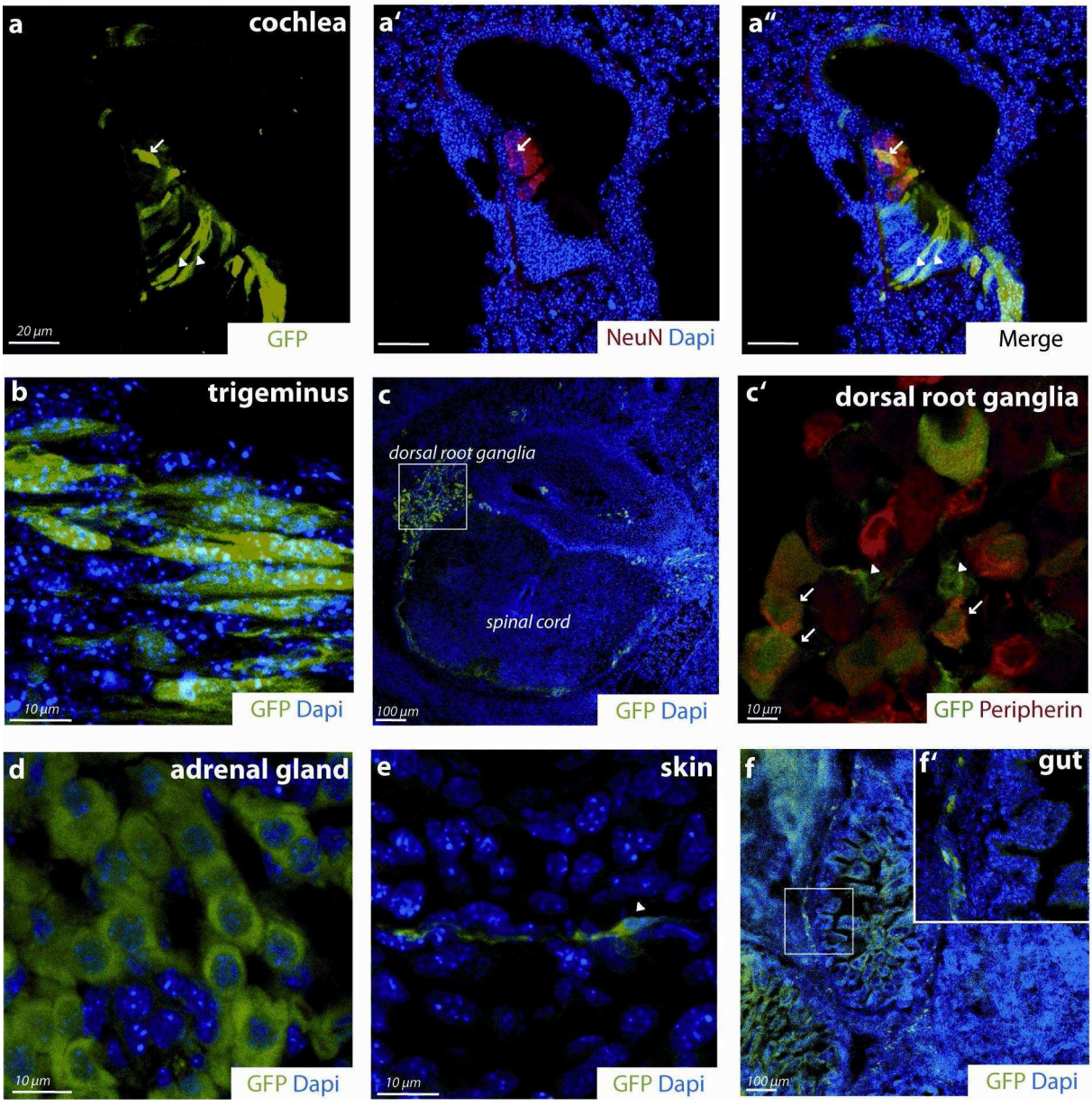
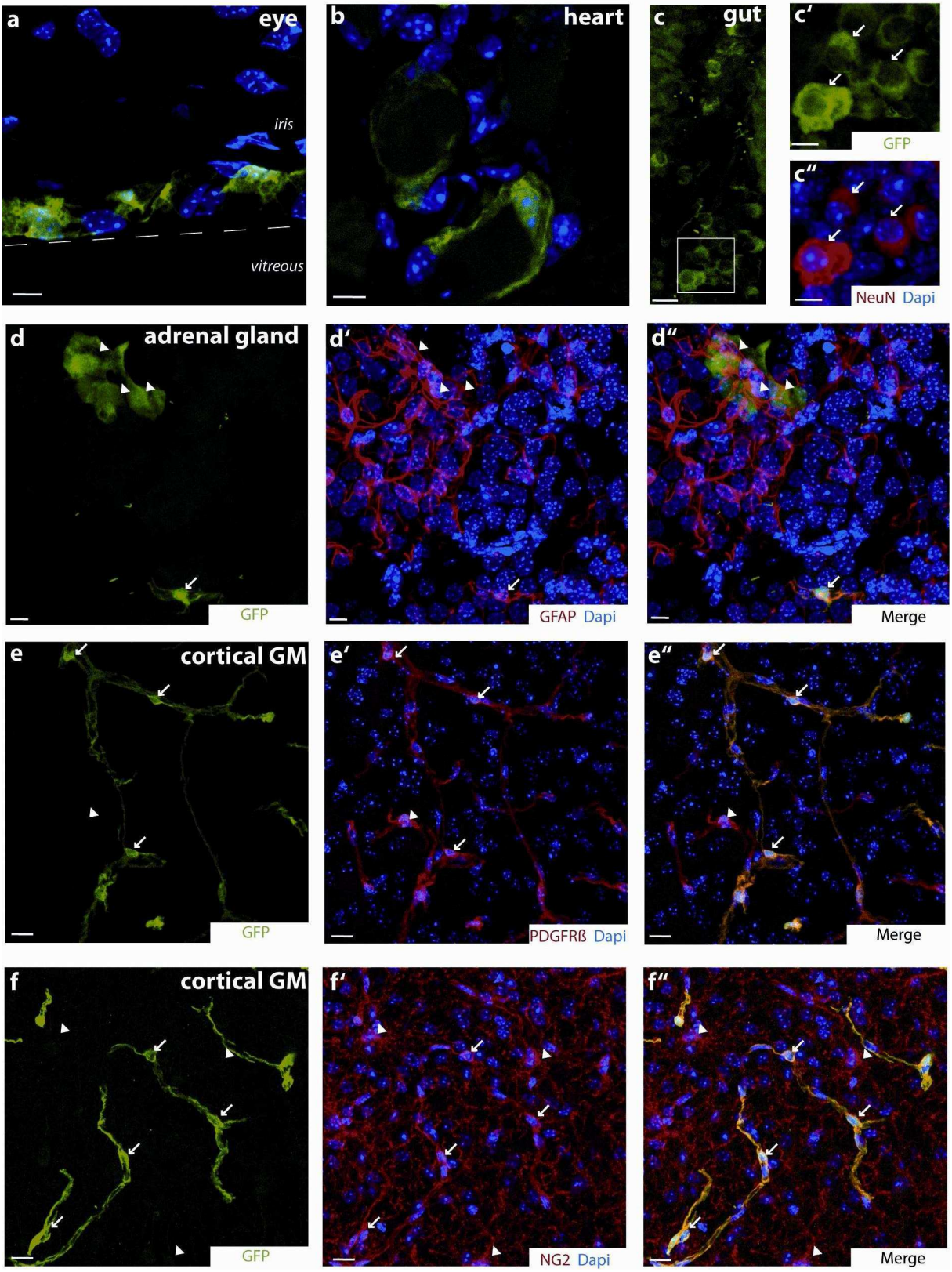

a
cochlea
20 µm
GFP
a'
NeuN Dapi
a''
Merge
b
trigeminus
10 µm
GFP Dapi
c
dorsal root ganglia
spinal cord
100 µm
GFP Dapi
c'
dorsal root ganglia
10 µm
GFP Peripherin
d
adrenal gland
10 µm
GFP Dapi
e
skin
10 µm
GFP Dapi
f
f'
gut
100 µm
GFP Dapi

Figure 5


a
eye
iris
vitreous
b
heart
c
gut
c'
GFP
c''
NeuN Dapi
d
adrenal gland
GFP
d'
GFAP Dapi
d''
Merge
e
cortical GM
GFP
e'
PDGFRß Dapi
e''
Merge
f
cortical GM
GFP
f'
NG2 Dapi
f''
Merge

Tissue	S4F:Cre	Sox10-iCreER^{T2} - Recombination	
		embryonic (E7.5-E10.5)	adult
Otic vesicle	Yes	Yes	n.a.
PNS	Yes	Yes	Yes
Melanocytes – Skin	Yes	Yes	Yes
Melanocytes - Eye	n.a.	Yes	Yes
Enteric Nervous System	Yes (cells not specified)	Yes (neurons and glial cells)	Yes (glial cells)
Adrenal Gland	n.a.	Yes	Yes
Oligodendrocyte lineage	Yes	No	Yes
Pericytes	n.a.	Yes	No
Limb	Yes	No	n.a.

4 Discussion

4.1 Adult NG2+ glia – a slow cycling and self-renewing cell population

NG2+ glia are the predominant proliferating cells outside the neurogenic niches in the adult intact CNS (Dawson *et al.*, 2003; Dimou *et al.*, 2008; Gensert and Goldman, 2001; Horner *et al.*, 2000; Simon *et al.*, 2011a). In the cortical GM these cells have a cell cycle length of about 37 days with a particular long G1-phase (Simon *et al.*, 2011a), being in line with other recent publications in humans and rodents (Geha *et al.*, 2010; Psachoulia *et al.*, 2009). Notably, the cell cycle length of NG2+ cells increases with age that might be due to a further prolongation of the G1-phase (Lasiene *et al.*, 2009; Psachoulia *et al.*, 2009) and results in a decrease in differentiation (Kang *et al.*, 2010; Zhu *et al.*, 2011). This age-dependent alteration of the cell cycle is not specific for parenchymal progenitors, but has already been described for neuroepithelial precursors, as for example during development (Takahashi *et al.*, 1995). Here, the length of the G1-phase of neuroepithelial progenitors increases during embryogenesis and can be even correlated to the fate of neuroepithelial progenitors and the mode of cell division (reviewed in Gotz and Huttner, 2005). In short, this cell cycle length hypothesis suggests that during early neurogenesis neuroepithelial progenitors (with a short G1-phase) divide symmetrically by expanding their pool. Later on, these progenitors have a prolonged G1-phase and will divide asymmetric by giving rise to a neuron and a neuroepithelial progenitor or symmetrically by generating two neurons (Gotz and Huttner, 2005). Then, at adult stages the stem cells in the neurogenic niches divide mainly asymmetric by giving rise to another stem cell and to a intermediate progenitor that can generate continuously new neurons via symmetric divisions *in vitro* (Costa *et al.*, 2011). However, adult NG2+ glia have been shown to divide mainly symmetrically *in vivo*, according to their morphology and receptor distribution (Ge *et al.*, 2009). In contrast, recent data now provide more evidence that a considerable proportion of NG2+ glia divides also asymmetric when analyzing NG2 and epidermal growth factor receptor (EGFR) expression (Sugiarto *et al.*, 2011) and thereby supporting previous publications *in vivo* (Polito and Reynolds, 2005; Zhu *et al.*, 2011). In summary, these *in vivo* data suggest heterogeneity amongst proliferating NG2+ cells: Some NG2+ cells divide symmetrically and either remain NG2+ or give rise to two mature oligodendrocytes or divide asymmetrically with one daughter cell to remain NG2+ and the other one to differentiate. However, it remains open whether there is a similar

relationship between the cell cycle length and the mode of division for NG2+ cells as it is shown for neural-glial progenitors. Instead, the age-dependent alterations of the cell cycle length of NG2+ glia and their differentiation seem to be rather complex. Their proliferation/ differentiation behavior might reflect intrinsic differences (Shi *et al.*, 1998), changes in the access to growth factors that are released by neighboring cells, e.g. neurons, astrocytes and endothelial cells (Arai and Lo, 2009a, 2009b; Kang *et al.*, 2010), or simply the reduced need of mature oligodendrocytes with age.

Despite the fact that adult NG2+ cells can differentiate into mature oligodendrocytes (Dimou *et al.*, 2008; Kang *et al.*, 2010; Rivers *et al.*, 2008; Zhu *et al.*, 2011), the number of NG2+ glia remains constant over life (Rivers *et al.*, 2008), thereby suggesting indirectly that these cells can self-renew. In my study I could provide for the first time direct evidence that NG2+ cells in the intact brain parenchyma can self-renew. In addition, radiation experiments of the adult cortex, causing a depletion of NG2+ glia, showed a subsequent restoration of the NG2+ glial network by the remaining NG2+ progenitors (Irvine and Blakemore, 2007; Panagiotakos *et al.*, 2007). Therefore, these data suggest a feedback mechanism between NG2+ cells to regulate their cellular density. However, less is known about it on a molecular basis (reviewed by Orentas and Miller, 1998). Results of primary oligodendrocytes cultures propose a regulatory system that is independent of other cell types, e.g. astrocytes and neurons (Zhang and Miller, 1996), and does not rely on the coupling of NG2+ glia to other cells by gap-junctions or electrical activity (Von Blankenfeld *et al.*, 1993). Therefore, to keep the NG2+ cell population constant a remaining model might consist of cell-cell contacts mediated by glycoproteins, as shown for fibroblasts (Wieser *et al.*, 1990), and/ or a locally restricted autocrine release of proliferative factors by NG2+ cells. In the future, it might be important to define the mechanisms that control NG2+ cell density as these cells are the basis to generate mature (myelinating) oligodendrocytes. Failure in repopulation of a functional NG2+ glial network could result in demyelination (Panagiotakos *et al.*, 2007), that contributes to neurodegeneration (Nave, 2010). Therefore, it would be interesting to distinguish the intrinsic properties of NG2+ glia from environmental influences in regard to their proliferation/ differentiation behavior. Such studies would certainly help to shed more light on the development of demyelinating diseases, e.g. Multiple Sclerosis.

4.2 Heterogeneity of NG2+ glia

Although NG2+ cells show mainly a homogenous antigen expression pattern (Figure 2), studies analyzing properties of NG2+ glia in different brain regions, e.g. cell cycle length, progeny and electrophysiological characteristics, suggest a region-dependent heterogeneity amongst the NG2+ cell population. Several publications could show that NG2+ cells in the cortical WM have an accelerated cell cycle speed compared to NG2+ progenitors in the cortical GM (Psachoulia *et al.*, 2009; Rivers *et al.*, 2008). This decrease in cell cycle length in the WM could be correlated to an increased differentiation of NG2+ progenitors to mature oligodendrocytes (Dimou *et al.*, 2008; Kang *et al.*, 2010; Psachoulia *et al.*, 2009; Rivers *et al.*, 2008). Indeed, fate-mapping analysis of the Olig2::CreER^{TM} mouse line extended this regional heterogeneity by showing that NG2+ cells in the WM generate more myelinating oligodendrocytes while NG2+ progeny in the cortical GM remain largely NG2+ and give rise to few mature oligodendrocytes (Dimou *et al.*, 2008). Kang et al. (2010) confirmed a cortical GM and WM difference, although they could demonstrate that NG2+ cells in both cortical areas are able to generate myelinating oligodendrocytes (Kang *et al.*, 2010). These contradicting results might be due to differences in the mouse lines, used in both studies. First, the recombination rate is higher in the PDGFRα-CreER^{TM} mice than in Olig2::CreER^{TM} mice (Dimou *et al.*, 2008; Kang *et al.*, 2010). Second, the Olig2::CreER^{TM} mouse line might restrict the analysis to a distinct subpopulation of NG2+ cells as mainly cells with high Olig2 levels were recombined (as discussed in Simon *et al.*, 2011a). Instead, Kang et al. (2010) labeled a broader NG2+ cell population independent of their Olig2 expression level. However, there seems to be a consistency about the diversity of NG2+ cells between cortical GM and WM that is not only reflected in their speed of proliferation and differentiation, but probably also in their receptor expression and structural relationship to neurons and astrocytes (reviewed by Maldonado *et al.*, 2011). How is this diversity of cortical NG2+ glia achieved? One assumption could be an intrinsic heterogeneity of cortical NG2+ cells. Therefore, NG2+ cells might be pre-primed based on their diverse developmental origin by being progeny of the lateral and dorsal wall of the subventricular zone and the diencephalon (Kessaris *et al.*, 2006; Psachoulia *et al.*, 2009). So far studies failed to show any correlation of the fate to distinct developmental origins (Psachoulia *et al.*, 2009). Therefore, regional diversity of NG2+ glia might be intrinsically instructed, independent of the developmental

origin, or rather being instructed by environmental cues, e.g. neuronal activity (as discussed below).

Nevertheless, all published studies can show likewise a continuous differentiation of NG2+ glia to mature and even to myelinating oligodendrocytes in the adult CNS (Barnabe-Heider *et al.*, 2010; Dimou *et al.*, 2008; Kang *et al.*, 2010; Psachoulia *et al.*, 2009; Rivers *et al.*, 2008; Simon *et al.*, 2011a). These data suggest de novo myelination, supported by results showing an increased number of myelinated axons during adulthood (Nunez *et al.*, 2000; Yates and Juraska, 2007). Also a life-long turnover of myelin with continuous de- and re-myelination could be possible as there is no obvious change in brain weight (before the onset of age-related decline) and only localized volume plasticity is detectable under physiological conditions (reviewed by Sowell *et al.*, 2004). However, the differentiation rate of mature oligodendrocytes towards myelinating oligodendrocytes in the adult cortical GM and WM is low compared to the number of mature oligodendrocytes being present and newly generated from NG2+ cells in the adult CNS (Dimou *et al.*, 2008; Simon *et al.*, 2011a). This raises questions about the biological relevance of the continuous generation of these mature oligodendrocytes *in vivo*. One possible function of mature oligodendrocytes is to alter the conduction velocity of neurons locally as a possible adaptation mechanism to neuronal firing (Nave, 2010; Sanchez *et al.*, 1996). In addition, oligodendrocytes might be relevant to support axonal integrity and survival (Nave, 2010). For example, loss of peroxisomes in oligodendrocytes can cause neurodegeneration (Kassmann *et al.*, 2007), whereas lack of peroxisomes in astrocytes and even in neurons has no impact on neuronal integrity (Bottelbergs *et al.*, 2010). Additionally, the direct cell-to-cell communication between mature oligodendrocytes and neurons might be important to maintain the axonal integrity as alterations in their signalling can cause progressive axonal loss as shown for non-myelinating Schwann cells – the cell type in the peripheral nervous system equivalent to oligodendrocytes (Chen *et al.*, 2003). Taken together, the precise biological impact of the diverse NG2+ progenitors and their progeny on neuronal survival and brain homeostasis in the adult CNS has just started to be elucidated and requires further analysis in regard to NG2+ glia heterogeneity.

4.3 Regulation of proliferation and differentiation of NG2+ glia by neuronal activity

Early *in vitro* data of primary oligodendrocyte cultures suggested a cell-intrinsic timer mechanism in NG2+ glia that measures the rounds of cell division (Raff, 2006) or time in general (Gao *et al.*, 1997) before these cells start to differentiate. However, co-cultures of NG2+ cells together with neurons pointed to additional regulatory mechanisms for their proliferation/differentiation, comprising environmental cues that might outweigh the intrinsic timer (Rosenberg *et al.*, 2008). In line with this, there are observations showing that NG2+ cells can keep their synaptic connections, e.g. to neurons, during cell division (Ge *et al.*, 2009; Tanaka *et al.*, 2009), whereas they receive less neuronal input upon differentiation (De Biase *et al.*, 2010; Kukley *et al.*, 2010). Not only the contact of NG2+ cells to neurons but also neuronal activity can influence the rate of proliferation/differentiation of NG2+ glia in the intact adult cortex. Upon increased physical activity the number of NG2+ progenitors is temporarily decreased while their differentiation is enhanced (Simon *et al.*, 2011a). The remaining proliferating cells are not randomly localized within the cortical GM compared to control mice. Instead, these NG2+ progenitors show a specific column-like distribution within the somatosensory and motor cortex upon increased physical activity (Simon *et al.*, 2011a). This proliferative pattern might be associated with neuronal activity as a synchronized firing of neurons in a column-like structure upon stimulation has been already described (Opris *et al.*, 2011; Zhang and Alloway, 2004). In addition, NG2+ progenitors prematurely leave the G1-phase and differentiate into oligodendrocytes, thereby supporting previous publications (Barres and Raff, 1993; Demerens *et al.*, 1996; Li *et al.*, 2010). Although direct stimulation of neurons resulted in an increase in proliferating cells in the spinal cord WM (Li *et al.*, 2010), NG2+ cells in the cortical GM decrease in proliferation temporarily upon a two weeks period of voluntary physical exercise (Simon *et al.*, 2011a). This difference could be due to different experimental approaches or the regional diversity of NG2+ glia (see discussion above about heterogeneity of NG2+ cells). However, NG2+ glia can compensate the decrease in proliferation during an additional two weeks period on the running wheel (in total: four weeks of increased physical activity; Simon et al. unpublished data). In addition, the generation and maintenance of new mature oligodendrocytes in the cortical GM during voluntary exercise seems to be dependent on increased neuronal activity: While the number of mature oligodendrocytes decreases during a subsequent period without running wheels,

BrdU+ oligodendrocytes are maintained during a prolonged period of voluntary exercise (Simon et al., unpublished data). Therefore, this neuronal activity-dependent differentiation mechanism of NG2+ cells might support distinct neuronal networks and may contribute to improved behavior. For example practicing playing the piano is related to structural changes in the human cortical GM and WM (Bengtsson *et al.*, 2005; Fields, 2005; Gaser and Schlaug, 2003). Along this line, Juraska et al. (1988) could show that an enriched environment results in an increased number of myelinated axons in the WM of adult rodents (Juraska, 1998). In contrast, reduced neuronal activity, e.g. by postnatal visual deprivation, resulted in a delay of myelination of the optic nerve and a delayed maturation of retinal neurons (Gyllensten and Malmfors, 1963). Overall this neuronal activity-dependent mechanism of proliferation/differentiation of NG2+ cells could be an additional form of plasticity in the adult brain whereby distinct neuronal circuits might be improved, newly generated or stabilized. However, it remains open whether neuronal stimulation not only results in the generation of mature oligodendrocytes, but also in myelinating oligodendrocytes and if this is directly correlated with a permanent improved behavior of adult mammals. In addition, it would be interesting to analyze the molecular correlation to this neuronal activity-dependent plasticity. *In vitro* data suggest that proliferation of NG2+ cells which, in turn, could be regulated for example (1) by a neuronal release of glutamate, adjacent activation of AMPA receptors on NG2+ cells that in turn could result in the activation of cAMP response binding element (CREB; (Redondo *et al.*, 2007)) or (2) by the secretion of PDGF (a known mitogenic signal for NG2+ glia) from astrocytes due to neuronal activity (Figure 8; (Barres and Raff, 1993; Calver *et al.*, 1998; Engel and Wolswijk, 1996)).

Little is known about mitogenic signals or even cell cycle inhibitors that are released by neurons and astrocytes upon neuronal activity *in vivo*. Instead, more data exist regarding the differentiation of NG2+ cells upon electrical stimulation of neurons. Pathways, that could be stimulated upon neuronal activity, are the expression of serum-response factor (SRF) in neurons (Knoll and Nordheim, 2009; Stritt *et al.*, 2009), resulting in the transcriptional inhibition of connective tissue growth factor (CTGF) – a neuronal paracrine factor, known to sequester extracellular insulin-like growth factor 1 (IGF 1; (Chong and Chan, 2010)). Therefore, IGF 1 could bind to its receptor on NG2+ cells that would result in differentiation, at least *in vitro* (Cui *et al.*, 2010; Galvin *et al.*, 2010). Besides neuronal SRF, the release of ATP by neurons could influence the proliferation and differentiation of NG2+ glia. Neuronal ATP

release can result for example in the secretaion of physiological concentrations of leukemia inhibitory factor (LIF) by astrocytes that in turn could promote maturation of NG2+ cells (Ishibashi *et al.*, 2006). In addition, adenosine (a metabolite of ATP) can inhibit the proliferation of NG2+ cells and promote their differentiation dependent on neuronal activity (Stevens *et al.*, 2002). Besides the above mentioned molecules, there is a wide range of other factors, being expressed by neurons (e.g. Jagged 1, Lingo and PSA-NCAM) or secreted by other cell types that can influence the differentiation of NG2+ glia (Figure 8; (Chong and Chan, 2010; Emery, 2010; Kremer *et al.*, 2011)). However, the majority of these data derived from *in vitro* experiments and the analysis of neonatal NG2+ cells

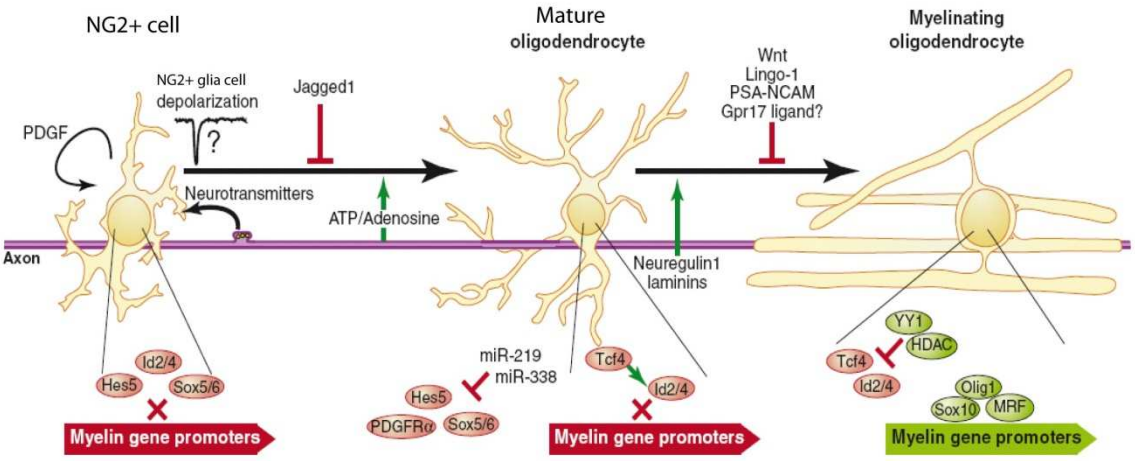


Figure 8 Scheme summarizing the neuronal influence on the differentiation of NG2+ cells. Possible neuronal cues are shown that can either enhance or block the differentiation of NG2+ glia towards myelinating oligodendrocytes. *(adapted from Emery 2010)*

The major intracellular pathways in NG2+ cells involved in the regulation proliferation/differentiation are the Notch and Wnt/BMP pathways that act as negative regulators, blocking the differentiation of NG2+ cells (Figure 8; (Emery, 2010; Feigenson *et al.*, 2011; Li and Richardson, 2009; Teo and Kahn, 2010; Wang *et al.*, 1998; Zhou and Armstrong, 2007)). Otherwise, sonic hedgehog (Shh; (Ulloa and Marti, 2010)), released by neurons in an activity-dependent way (Beug *et al.*, 2011), promotes the differentiation of NG2+ glia at least during development (Nery *et al.*, 2001). Although the differentiation of NG2+ cells seems to be tightly regulated by neuronal activity, it remains still open if neuronal stimulation has a direct impact on the proliferation rate of adult NG2+ glia. It could also be possible that the proliferation of NG2+ cells is a secondary consequence of the differentiation of NG2+ cells upon neuronal activity: Once a NG2+ cell differentiates, surrounding NG2+ cells could start to proliferate in order to maintain the NG2+ cell density

within the CNS as shown after four weeks of voluntary exercise (Simon et al., unpublished data). This would suggest that neuronal activity might largely influence NG2+ cell differentiation. Furthermore, it is still not clear whether the communication between neurons and NG2+ glia is uni- or bidirectional. Therefore, ablating NG2+ cells in the adult CNS might reveal new insights in the plasticity of neuronal networks and its behavioral impact. In addition, studying the reaction of NG2+ glia under pathological conditions, e.g. neuronal hyperexcitibility (epilepsy) or transmitter imbalance (autism), might shed new light on the disease mechanisms.

4.4 Reaction of NG2+ glia to acute injury

The response of NG2+ glia to trauma occurs with a similar temporal profile as microglia. Moreover, NG2+ glia react not only by cellular hypertrophy and up-regulation of NG2, but also by increased proliferation around the injury side (Simon *et al.*, 2011a). These data are consistent with other publications analyzing either acute lesion (stab wound as well as cryo injuries) in the cortical GM (Buffo *et al.*, 2005; Hampton *et al.*, 2004; Rhodes *et al.*, 2006; Tatsumi *et al.*, 2005) and in the cerebellum (Levine, 1994) or demyelinating lesion in the spinal cord (Watanabe *et al.*, 2002). The morphological and proliferative reaction of NG2+ cells is transient and decreases to basal levels already 14 days post injury (Simon *et al.*, 2011a). This decline in NG2+ glial reactivity again correlates again to the decrease in reactivity of microglia. Rhodes et al. (2006) showed that the response of NG2+ cells to traumata is dependent on the break-down of the BBB. Signals, being secreted by macrophages and platelets (e.g. tumor necrosis factor α, transforming growth factor β, interleukin 1α and interferon γ), can cause morphologically alterations of NG2+ cells towards a reactive phenotype. In addition, NG2+ cells achieve the fast proliferative response to acute traumata by re-entering the cell cycle and by shortening its length (Simon *et al.*, 2011a), consistent with data from spinal cord lesions (Watanabe *et al.*, 2002). This short cell cycle length after injury is comparable to postnatal stages (as determined by Psachoulia et al. (2009)), thereby supporting the hypothesis that NG2+ glia can recapitulate developmental stages upon pathological conditions (Chong and Chan, 2010). Indeed, *in vitro* data support the concept that adult NG2+ cells can accelerate their cell cycle speed in the presence of distinct mitogens, e.g. PDGF, neuregulin-1 and increased cAMP levels (Shi *et al.*, 1998), or decrease their proliferation by inhibitory signals, e.g. ADP/ ATP (Agresti *et al.*, 2005b) and

interferon-γ (Agresti *et al.*, 1996). In addition, numerous factors have been shown or proposed to regulate the proliferation of NG2+ cells after lesion *in vivo*, as for example factors that are released by injured axons and endothelial cells or by neighboring oligodendrocytes and astrocytes (reviewed in (Fawcett and Asher, 1999; Levine *et al.*, 2001). These molecules include growth factors that are up-regulated after injury, e.g. PDGF, FGF, IGF and CNTF (Barres and Raff, 1994; Fortin *et al.*, 2005; Liu *et al.*, 1994; Mason *et al.*, 2003; Redwine and Armstrong, 1998; Tripathi and McTigue, 2008). In particular, inflammatory lesions induce local production of several cytokines and chemokines (McTigue and Tripathi, 2008) which, together with other blood derived factors, might influence the proliferation of NG2+ cells (Kerstetter *et al.*, 2009; Rhodes *et al.*, 2006; Taylor *et al.*, 2010). In addition, other pathways might have an impact on the reactivity of NG2+ glia upon injury, e.g. Shh, Wnt, Notch and BMP signalling (Amankulor *et al.*, 2009; Boda and Buffo, 2010). However, the effects of mitogens on NG2+ glia might vary in different brain areas and injury paradigms and could also be dependent on the developmental stage (Mason and Goldman, 2002; Pfeiffer *et al.*, 1993).

The recapitulation of developmental stages of NG2+ cells upon acute injury might not only be limited to the cell cycle speed, but could also compromise a fate change towards astrocytes. Indeed, several studies showed that a small subpopulation of adult NG2+ glia can up-regulate intermediate filaments, e.g. GFAP, vimentin and nestin, upon acute injury (Alonso, 2005; Busch *et al.*, 2010; Komitova *et al.*, 2011; Sellers *et al.*, 2009; Tatsumi *et al.*, 2008). So far, these molecules are known to be expressed in reactive astrocytes after trauma in the adult brain parenchyma (Robel *et al.*, 2011b) and in adult stem cells (Kriegstein and Alvarez-Buylla, 2009). In addition, our fate-mapping data of the Sox10-iCreER^{T2} mouse line support the expression of astrocytic antigens (e.g. GFAP, S100β and vimentin) after acute injury in progeny of the oligodendrocyte lineage (Simon et al., unpublished data). This observation is supported by recent fate-mapping study, using NG2-CreER^{TM} mice (Komitova *et al.*, 2011) suggesting that some NG2+ cells could adopt at least partially astrocytic-like features. However, the generation of astrocytes from NG2+ cells is controversially discussed as others failed to confirm it, e.g. in fate-mapping analysis of Olig2+ progenitors in lesions in Olig2::CreER^{TM} mice (Barnabe-Heider *et al.*, 2010; Dimou *et al.*, 2008). The discrepancy between these studies could be due to differences between the mouse lines, e.g. particularly the different recombination rate (as already discussed herein) and the altered

recombination of distinct cell types. The Olig2::CreER^{TM} mouse line is characterized by recombining low numbers of parenchymal astrocytes already in the intact CNS (Dimou *et al.*, 2008) while recombined cells in Sox10-iCreER^{T2} mouse line are restricted to the oligodendrocyte lineage (Simon *et al.*, 2011b). In addition, the fate-mapping analysis of the Sox10-iCreER^{T2} mice after injury revealed that astrocytic-like NG2+ cells probably derive from non-dividing NG2+ cells as they do not incorporate BrdU after stab wound injury (Simon et al., unpublished data). Therefore, these cells also remained undetectable in other studies, focusing only on proliferating NG2+ glia (Simon *et al.*, 2011a). Additional data even suggest that these astrocytic-like subpopulation of NG2+ cells appear only transient (Komitova *et al.*, 2011; Sellers *et al.*, 2009) or these cells upregulate only transiently GFAP, S100β and vimentin. Furthermore, this subpopulation of NG2+ cells seems to be related to acute traumata while chronic/ degenerative lesion paradigms lack them (Kang *et al.*, 2010).

Nevertheless, less is known about the function of NG2+ cells around the injury site. Ablation of proliferating glial cells after acute trauma suggested an improved axonal recovery (Rhodes *et al.*, 2003). Whether this is due to a decrease in microglia, astrocytes or NG2+ glia, as all of them proliferate after acute injury, is still unkown. In addition, the astrocytic-like NG2+ cells are only detectable during the acute phase of lesions (Komitova *et al.*, 2011; Sellers *et al.*, 2009) and might be associated with phagocytic functions (Sellers *et al.*, 2009), e.g. by removing myelin debris that is another toxic component in the lesion site (Nave, 2010). To gain further insights in the contribution of NG2+ cells within the glial scar it would be important to define new molecules, allowing to distinguish “quiescent” NG2+ cells from reactive ones and to ablate NG2+ glia from the lesion.

4.5 Sox10-iCreER^{T2} mouse line – a new tool to fate-map neural crest cells and the oligodendrocyte lineage

In this study, I generated a mouse line to overcome the low recombination rate in NG2+ cells, as published for other mouse lines (Dimou *et al.*, 2008; Zhu *et al.*, 2011), and yet being specific to the neural crest and to the oligodendrocyte lineage in the adult CNS (Simon *et al.*, 2011b). This Sox10-iCreER^{T2} mouse line is indeed unique as the fate of Sox10+ cells can be analyzed at distinct stages. In contrast, previously published mouse lines, e.g. Sox10-Cre or Sox 10-Venus, label either Sox10+ cells already at embryonic stages or only transiently when the Sox10 promoter is active, respectively (Shibata *et al.*, 2010; Stine *et al.*, 2009). In

addition, the expression from both endogenous Sox10 alleles is spared in the BAC-transgenic Sox10-iCreER^{T2} mouse line and thereby circumventing a heterozygote phenotype, that is characterized e.g. by partial aganglionosis in the intestine of the Sox10::lacZ and Sox10::rtTA mice (Britsch *et al.*, 2001; Ludwig *et al.*, 2004; Paratore *et al.*, 2001). Taken together the results obtained so far, this new Sox10-iCreER^{T2} mouse line can reveal new insights in the neural crest lineage due to its unique features. We have now direct evidence that pericytes in the adult CNS are the progeny of neural crest. This data support similar observations in the thymus (Muller *et al.*, 2008) and are in line with results obtained from quail-chick transplantation experiments (Korn *et al.*, 2002) and in the human tissue plasminogen activator-Cre mice (Pietri *et al.*, 2003). Recently, neural crest derivatives gained in importance as these cells display stem cell capacity and can be reprogrammed to other cell types (Binder *et al.*, 2011; Dupin *et al.*, 2007; Zhao and Prather, 2011). In particular, adult cortical pericytes have been shown to be reprogrammed to neurons *in vitro* (Sanchez *et al.*, 2011, under revision) and might serve as a new endogenous source for brain repair upon lesion. In general, the Sox10-iCreER^{T2} mice recapitulate the endogenous Sox10 expression in the PNS and CNS during development and in the adult. For example, recombination in the adult CNS of Sox10-iCreER^{T2} mice will label exclusively the oligodendrocyte lineage with high efficiency in contrast to previous published mouse lines using the Olig2, NG2 and PDGFRα promoter (Dimou *et al.*, 2008; Kang *et al.*, 2010; Rivers *et al.*, 2008; Zhu *et al.*, 2011). This highly efficient recombination of Sox10+ cells is not only limited to the CNS but also detectable in the PNS. Therefore, the Sox10-iCreER^{T2} mouse line is a useful tool to conditionally ablate genes in Sox10+ cells to obtain more information about the Sox10+ lineage. This is of particular importance as Sox10+ cells can be involved in a variety of diseases in peripheral organs as well as in the CNS, e.g. melanoma, Wardenburg-Hirschsprung disease, glioma, acute traumata, MS, schizophrenia and autism (Agnarsdottir *et al.*, 2010; Britsch *et al.*, 2001; Carmody and Lewis, 2010; Iwamoto *et al.*, 2005; Lim *et al.*, 2011; Nishiyama *et al.*, 2009; Simon *et al.*, 2011a).

4.6 A translational perspective: NG2+ glial biology towards clinical application

NG2+ glia compose not only the major dividing cell population in the brain parenchyma of rodents, but also in humans (Geha *et al.*, 2010; Nunes *et al.*, 2003). Therefore, these cells are susceptible for oncogenic mutations that might result in tumor formation (Visvader, 2011). Indeed, NG2+ cells are the predominant proliferative population in different types of glioma (Chekenya and Pilkington, 2002; Liu *et al.*, 2011; Shoshan *et al.*, 1999) and are shown to be the origin of these tumors (Lindberg *et al.*, 2009; Liu *et al.*, 2011). Glioma are the most common brain tumors with a high rate of malignancy that goes along with poor survival probability (Norden and Wen, 2006). Common treatments besides operations are the administration of cytostatic drugs and the radiation of tumorigenic brain areas that are both strategies targeting proliferating cells. Liu et al. (2011) labeled a profound number of dividing tumorigenic NG2+ cells within one week BrdU pulse, suggesting that these cells might have an accelerated cell cycle speed compared to non-tumorigenic NG2+ progenitors (Simon *et al.*, 2011a). However, the cell cycle of tumorigenic NG2+ glia might be too slow to be efficiently targeted by cytostatica with short half-life times, e.g. Temozolomide (EssexPharma, 2009). In addition, brain-permeable cytostatica might also hit non-tumorigenic NG2+ cells that could result in an increased proliferation of surrounding NG2+ glia (as discussed above). In addition, radiation might, for example, cause neurotoxicity, disruption of BBB, inflammation and demyelination (Panagiotakos *et al.*, 2007; Perry and Schmidt, 2006; Zhao and Robbins, 2009) that are events associated with a high reactivity of NG2+ glia (as discussed above). Overall, cytostatic drugs and radiation might increase the likelihood of a tumorigenic transformation of NG2+ cells and could probably be one explanation for the high chemoresistance and recurrence rate of glioma (Giannopoulos and Kyritsis, 2010). Therefore, there is the need to distinguish non-tumorigenic NG2+ progenitors from their tumorigenic counterparts on a molecular basis to develop novel cellular-specific pharmacological approaches in glioma therapy.

References

Agnarsdottir M, Sooman L, Bolander A, Stromberg S, Rexhepaj E, Bergqvist M, Ponten F, Gallagher W, Lennartsson J, Ekman S, Uhlen M, Hedstrand H. 2010. SOX10 expression in superficial spreading and nodular malignant melanomas. Melanoma Res 20: 468-478.

Agresti C, D'Urso D, Levi G. 1996. Reversible inhibitory effects of interferon-gamma and tumour necrosis factor-alpha on oligodendroglial lineage cell proliferation and differentiation in vitro. Eur J Neurosci 8: 1106-1116.

Agresti C, Meomartini ME, Amadio S, Ambrosini E, Serafini B, Franchini L, Volonte C, Aloisi F, Visentin S. 2005a. Metabotropic P2 receptor activation regulates oligodendrocyte progenitor migration and development. Glia 50: 132-144.

Agresti C, Meomartini ME, Amadio S, Ambrosini E, Volonte C, Aloisi F, Visentin S. 2005b. ATP regulates oligodendrocyte progenitor migration, proliferation, and differentiation: involvement of metabotropic P2 receptors. Brain Res Brain Res Rev 48: 157-165.

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.

Aguirre AA, Chittajallu R, Belachew S, Gallo V. 2004. NG2-expressing cells in the subventricular zone are type C-like cells and contribute to interneuron generation in the postnatal hippocampus. J Cell Biol 165: 575-589.

Alilain WJ, Horn KP, Hu H, Dick TE, Silver J. 2011. Functional regeneration of respiratory pathways after spinal cord injury. Nature 475: 196-200.

Alonso G. 2005. NG2 proteoglycan-expressing cells of the adult rat brain: possible involvement in the formation of glial scar astrocytes following stab wound. Glia 49: 318-338.

Amankulor NM, Hambardzumyan D, Pyonteck SM, Becher OJ, Joyce JA, Holland EC. 2009. Sonic hedgehog pathway activation is induced by acute brain injury and regulated by injury-related inflammation. J Neurosci 29: 10299-10308.

Arai K, Lo EH. 2009a. An oligovascular niche: cerebral endothelial cells promote the survival and proliferation of oligodendrocyte precursor cells. J Neurosci 29: 4351-4355.

Arai K, Lo EH. 2009b. Oligovascular signaling in white matter stroke. Biol Pharm Bull 32: 1639-1644.

Barnabe-Heider F, Goritz C, Sabelstrom H, Takebayashi H, Pfrieger FW, Meletis K, Frisen J. 2010. Origin of new glial cells in intact and injured adult spinal cord. Cell Stem Cell 7: 470-482.

Barres BA, Raff MC. 1993. Proliferation of oligodendrocyte precursor cells depends on electrical activity in axons. Nature 361: 258-260.

Barres BA, Raff MC. 1994. Control of oligodendrocyte number in the developing rat optic nerve. Neuron 12: 935-942.

Baumann N, Pham-Dinh D. 2001. Biology of oligodendrocyte and myelin in the mammalian central nervous system. Physiol Rev 81: 871-927.

Beckervordersandforth R, Tripathi P, Ninkovic J, Bayam E, Lepier A, Stempfhuber B, Kirchhoff F, Hirrlinger J, Haslinger A, Lie DC, Beckers J, Yoder B, Irmler M, Gotz M. 2010. In vivo fate mapping and expression analysis reveals molecular hallmarks of prospectively isolated adult neural stem cells. Cell Stem Cell 7: 744-758.

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

Bengtsson SL, Nagy Z, Skare S, Forsman L, Forssberg H, Ullen F. 2005. Extensive piano practicing has regionally specific effects on white matter development. Nat Neurosci 8: 1148-1150.

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

Beug ST, Parks RJ, McBride HM, Wallace VA. 2011. Processing-dependent trafficking of Sonic hedgehog to the regulated secretory pathway in neurons. Mol Cell Neurosci 46: 583-596.

Binder E, Rukavina M, Hassani H, Weber M, Nakatani H, Reiff T, Parras C, Taylor V, Rohrer H. 2011. Peripheral nervous system progenitors can be reprogrammed to produce myelinating oligodendrocytes and repair brain lesions. J Neurosci 31: 6379-6391.

Blaustein MP, Lederer WJ. 1999. Sodium/calcium exchange: its physiological implications. Physiol Rev 79: 763-854.

Bockamp E, Maringer M, Spangenberg C, Fees S, Fraser S, Eshkind L, Oesch F, Zabel B. 2002. Of mice and models: improved animal models for biomedical research. Physiol Genomics 11: 115-132.

Boda E, Buffo A. 2010. Glial cells in non-germinal territories: insights into their stem/progenitor properties in the intact and injured nervous tissue. Arch Ital Biol 148: 119-136.

Boda E, Vigano F, Rosa P, Fumagalli M, Labat-Gest V, Tempia F, Abbracchio MP, Dimou L, Buffo A. 2011. The GPR17 receptor in NG2 expressing cells: Focus on in vivocell maturation and participation in acute trauma and chronic damage. Glia.

Bottelbergs A, Verheijden S, Hulshagen L, Gutmann DH, Goebbels S, Nave KA, Kassmann C, Baes M. 2010. Axonal integrity in the absence of functional peroxisomes from projection neurons and astrocytes. Glia 58: 1532-1543.

Britsch S, Goerich DE, Riethmacher D, Peirano RI, Rossner M, Nave KA, Birchmeier C, Wegner M. 2001. The transcription factor Sox10 is a key regulator of peripheral glial development. Genes Dev 15: 66-78.

Buffo A. 2007. Fate determinant expression in the lesioned brain: Olig2 induction and its implications for neuronal repair. Neurodegener Dis 4: 328-332.

Buffo A, Rite I, Tripathi P, Lepier A, Colak D, Horn AP, Mori T, Gotz M. 2008. Origin and progeny of reactive gliosis: A source of multipotent cells in the injured brain. Proc Natl Acad Sci U S A 105: 3581-3586.

Buffo A, Rolando C, Ceruti S. 2010. Astrocytes in the damaged brain: molecular and cellular insights into their reactive response and healing potential. Biochem Pharmacol 79: 77-89.

Buffo A, Vosko MR, Erturk D, Hamann GF, Jucker M, Rowitch D, Gotz M. 2005. Expression pattern of the transcription factor Olig2 in response to brain injuries: implications for neuronal repair. Proc Natl Acad Sci U S A 102: 18183-18188.

Busch SA, Horn KP, Cuascut FX, Hawthorne AL, Bai L, Miller RH, Silver J. 2010. Adult NG2+ cells are permissive to neurite outgrowth and stabilize sensory axons during macrophage-induced axonal dieback after spinal cord injury. J Neurosci 30: 255-265.

Butt AM. 2011. ATP: a ubiquitous gliotransmitter integrating neuron-glial networks. Semin Cell Dev Biol 22: 205-213.

Cai J, Qi Y, Hu X, Tan M, Liu Z, Zhang J, Li Q, Sander M, Qiu M. 2005. Generation of oligodendrocyte precursor cells from mouse dorsal spinal cord independent of Nkx6 regulation and Shh signaling. Neuron 45: 41-53.

Calver AR, Hall AC, Yu WP, Walsh FS, Heath JK, Betsholtz C, Richardson WD. 1998. Oligodendrocyte population dynamics and the role of PDGF in vivo. Neuron 20: 869-882.

Carmody DP, Lewis M. 2010. Regional white matter development in children with autism spectrum disorders. Dev Psychobiol 52: 755-763.

Chandler KJ, Chandler RL, Broeckelmann EM, Hou Y, Southard-Smith EM, Mortlock DP. 2007. Relevance of BAC transgene copy number in mice: transgene copy number variation across multiple transgenic lines and correlations with transgene integrity and expression. Mamm Genome 18: 693-708.

Chekenya M, Pilkington GJ. 2002. NG2 precursor cells in neoplasia: functional, histogenesis and therapeutic implications for malignant brain tumours. J Neurocytol 31: 507-521.

Chen S, Rio C, Ji RR, Dikkes P, Coggeshall RE, Woolf CJ, Corfas G. 2003. Disruption of ErbB receptor signaling in adult non-myelinating Schwann cells causes progressive sensory loss. Nat Neurosci 6: 1186-1193.

Chen Y, Wu H, Wang S, Koito H, Li J, Ye F, Hoang J, Escobar SS, Gow A, Arnett HA, Trapp BD, Karandikar NJ, Hsieh J, Lu QR. 2009. The oligodendrocyte-specific G protein-coupled receptor GPR17 is a cell-intrinsic timer of myelination. Nat Neurosci 12: 1398-1406.

Chen ZJ, Ughrin Y, Levine JM. 2002. Inhibition of axon growth by oligodendrocyte precursor cells. Mol Cell Neurosci 20: 125-139.

Chong SY, Chan JR. 2010. Tapping into the glial reservoir: cells committed to remaining uncommitted. J Cell Biol 188: 305-312.

Costa MR, Ortega F, Brill MS, Beckervordersandforth R, Petrone C, Schroeder T, Gotz M, Berninger B. 2011. Continuous live imaging of adult neural stem cell division and lineage progression in vitro. Development 138: 1057-1068.

Cui QL, 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.

Cui QL, Fragoso G, Miron VE, Darlington PJ, Mushynski WE, Antel J, Almazan G. 2010. Response of human oligodendrocyte progenitors to growth factors and axon signals. J Neuropathol Exp Neurol 69: 930-944.

Davalos D, Grutzendler J, Yang G, Kim JV, Zuo Y, Jung S, Littman DR, Dustin ML, Gan WB. 2005. ATP mediates rapid microglial response to local brain injury in vivo. Nat Neurosci 8: 752-758.

Dawson MR, Levine JM, Reynolds R. 2000. NG2-expressing cells in the central nervous system: are they oligodendroglial progenitors? J Neurosci Res 61: 471-479.

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

De Biase LM, Nishiyama A, Bergles DE. 2010. Excitability and synaptic communication within the oligodendrocyte lineage. J Neurosci 30: 3600-3611.

Demerens C, Stankoff B, Logak M, Anglade P, Allinquant B, Couraud F, Zalc B, Lubetzki C. 1996. Induction of myelination in the central nervous system by electrical activity. Proc Natl Acad Sci U S A 93: 9887-9892.

Di Bello IC, Dawson MR, Levine JM, Reynolds R. 1999. Generation of oligodendroglial progenitors in acute inflammatory demyelinating lesions of the rat brain stem is associated with demyelination rather than inflammation. J Neurocytol 28: 365-381.

Dimou L, Simon C, Kirchhoff F, Takebayashi H, Gotz M. 2008. Progeny of Olig2-expressing progenitors in the gray and white matter of the adult mouse cerebral cortex. J Neurosci 28: 10434-10442.

Doerflinger NH, Macklin WB, Popko B. 2003. Inducible site-specific recombination in myelinating cells. Genesis 35: 63-72.

Dupin E, Calloni G, Real C, Goncalves-Trentin A, Le Douarin NM. 2007. Neural crest progenitors and stem cells. C R Biol 330: 521-529.

Emery B. 2010. Regulation of oligodendrocyte differentiation and myelination. Science 330: 779-782.

Engel U, Wolswijk G. 1996. Oligodendrocyte-type-2 astrocyte (O-2A) progenitor cells derived from adult rat spinal cord: in vitro characteristics and response to PDGF, bFGF and NT-3. Glia 16: 16-26.

EssexPharma. 2009. Temodal Hartkapseln - Fachinformation. Rote Liste.

Fawcett JW, Asher RA. 1999. The glial scar and central nervous system repair. Brain Res Bull 49: 377-391.

Feigenson K, Reid M, See J, Crenshaw Iii EB, Grinspan JB. 2011. Canonical Wnt signalling requires the BMP pathway to inhibit oligodendrocyte maturation. ASN Neuro 3.

Fields RD. 2005. Myelination: an overlooked mechanism of synaptic plasticity? Neuroscientist 11: 528-531.

Fortin D, Rom E, Sun H, Yayon A, Bansal R. 2005. Distinct fibroblast growth factor (FGF)/FGF receptor signaling pairs initiate diverse cellular responses in the oligodendrocyte lineage. J Neurosci 25: 7470-7479.

Frohlich N, Nagy B, Hovhannisyan A, Kukley M. 2011. Fate of neuron-glia synapses during proliferation and differentiation of NG2 cells. J Anat 219: 18-32.

Gallo V, Zhou JM, McBain CJ, Wright P, Knutson PL, Armstrong RC. 1996. Oligodendrocyte progenitor cell proliferation and lineage progression are regulated by glutamate receptor-mediated K+ channel block. J Neurosci 16: 2659-2670.

Galvin J, Eyermann C, Colognato H. 2010. Dystroglycan modulates the ability of insulin-like growth factor-1 to promote oligodendrocyte differentiation. J Neurosci Res 88: 3295-3307.

Gao FB, Durand B, Raff M. 1997. Oligodendrocyte precursor cells count time but not cell divisions before differentiation. Curr Biol 7: 152-155.

Gaser C, Schlaug G. 2003. Brain structures differ between musicians and non-musicians. J Neurosci 23: 9240-9245.

Ge WP, Zhou W, Luo Q, Jan LY, Jan YN. 2009. Dividing glial cells maintain differentiated properties including complex morphology and functional synapses. Proc Natl Acad Sci U S A 106: 328-333.

Geha S, Pallud J, Junier MP, Devaux B, Leonard N, Chassoux F, Chneiweiss H, Daumas-Duport C, Varlet P. 2010. NG2+/Olig2+ cells are the major cycle-related cell population of the adult human normal brain. Brain Pathol 20: 399-411.

Gensert JM, Goldman JE. 1997. Endogenous progenitors remyelinate demyelinated axons in the adult CNS. Neuron 19: 197-203.

Gensert JM, Goldman JE. 2001. Heterogeneity of cycling glial progenitors in the adult mammalian cortex and white matter. J Neurobiol 48: 75-86.

Ghiani CA, Yuan X, Eisen AM, Knutson PL, DePinho RA, McBain CJ, Gallo V. 1999. Voltage-activated K+ channels and membrane depolarization regulate accumulation of the cyclin-dependent kinase inhibitors p27(Kip1) and p21(CIP1) in glial progenitor cells. J Neurosci 19: 5380-5392.

Giannopoulos S, Kyritsis AP. 2010. Diagnosis and management of multifocal gliomas. Oncology 79: 306-312.

Gotz M, Huttner WB. 2005. The cell biology of neurogenesis. Nat Rev Mol Cell Biol 6: 777-788.

Greenwood K, Butt AM. 2003. Evidence that perinatal and adult NG2-glia are not conventional oligodendrocyte progenitors and do not depend on axons for their survival. Mol Cell Neurosci 23: 544-558.

Gudz TI, Komuro H, Macklin WB. 2006. Glutamate stimulates oligodendrocyte progenitor migration mediated via an alphav integrin/myelin proteolipid protein complex. J Neurosci 26: 2458-2466.

Guo F, Maeda Y, Ma J, Xu J, Horiuchi M, Miers L, Vaccarino F, Pleasure D. 2010. Pyramidal neurons are generated from oligodendroglial progenitor cells in adult piriform cortex. J Neurosci 30: 12036-12049.

Gyllensten L, Malmfors T. 1963. Myelinization of the optic nerve and its dependence on visual function--a quantitative investigation in mice. J Embryol Exp Morphol 11: 255-266.

Hamilton N, Vayro S, Wigley R, Butt AM. 2010. Axons and astrocytes release ATP and glutamate to evoke calcium signals in NG2-glia. Glia 58: 66-79.

Hampton DW, Rhodes KE, Zhao C, Franklin RJ, Fawcett JW. 2004. The responses of oligodendrocyte precursor cells, astrocytes and microglia to a cortical stab injury, in the brain. Neuroscience 127: 813-820.

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

Indra AK, Warot X, Brocard J, Bornert JM, Xiao JH, Chambon P, Metzger D. 1999. Temporally-controlled site-specific mutagenesis in the basal layer of the epidermis: comparison of the recombinase activity of the tamoxifen-inducible Cre-ER(T) and Cre-ER(T2) recombinases. Nucleic Acids Res 27: 4324-4327.

Irvine KA, Blakemore WF. 2007. A different regional response by mouse oligodendrocyte progenitor cells (OPCs) to high-dose X-irradiation has consequences for repopulating OPC-depleted normal tissue. Eur J Neurosci 25: 417-424.

Ishibashi T, Dakin KA, Stevens B, Lee PR, Kozlov SV, Stewart CL, Fields RD. 2006. Astrocytes promote myelination in response to electrical impulses. Neuron 49: 823-832.

Iwamoto K, Bundo M, Yamada K, Takao H, Iwayama-Shigeno Y, Yoshikawa T, Kato T. 2005. DNA methylation status of SOX10 correlates with its downregulation and oligodendrocyte dysfunction in schizophrenia. J Neurosci 25: 5376-5381.

Jaisser F. 2000. Inducible gene expression and gene modification in transgenic mice. J Am Soc Nephrol 11 Suppl 16: S95-S100.

Jensen JB, Parmar M. 2006. Strengths and limitations of the neurosphere culture system. Mol Neurobiol 34: 153-161.

Jones LL, Yamaguchi Y, Stallcup WB, Tuszynski MH. 2002. NG2 is a major chondroitin sulfate proteoglycan produced after spinal cord injury and is expressed by macrophages and oligodendrocyte progenitors. J Neurosci 22: 2792-2803.

Juraska JM. 1998. Neural plasticity and the development of sex differences. Annu Rev Sex Res 9: 20-38.

Kang SH, Fukaya M, Yang JK, Rothstein JD, Bergles DE. 2010. NG2+ CNS glial progenitors remain committed to the oligodendrocyte lineage in postnatal life and following neurodegeneration. Neuron 68: 668-681.

Karadottir R, Hamilton NB, Bakiri Y, Attwell D. 2008. Spiking and nonspiking classes of oligodendrocyte precursor glia in CNS white matter. Nat Neurosci 11: 450-456.

Kassmann CM, Lappe-Siefke C, Baes M, Brugger B, Mildner A, Werner HB, Natt O, Michaelis T, Prinz M, Frahm J, Nave KA. 2007. Axonal loss and neuroinflammation caused by peroxisome-deficient oligodendrocytes. Nat Genet 39: 969-976.

Keirstead HS, Levine JM, Blakemore WF. 1998. Response of the oligodendrocyte progenitor cell population (defined by NG2 labelling) to demyelination of the adult spinal cord. Glia 22: 161-170.

Kerschensteiner M, Meinl E, Hohlfeld R. 2009. Neuro-immune crosstalk in CNS diseases. Neuroscience 158: 1122-1132.

Kerstetter AE, Padovani-Claudio DA, Bai L, Miller RH. 2009. Inhibition of CXCR2 signaling promotes recovery in models of multiple sclerosis. Exp Neurol 220: 44-56.

Kessaris N, Fogarty M, Iannarelli P, Grist M, Wegner M, Richardson WD. 2006. Competing waves of oligodendrocytes in the forebrain and postnatal elimination of an embryonic lineage. Nat Neurosci 9: 173-179.

Kettenmann H, Hanisch UK, Noda M, Verkhratsky A. 2011. Physiology of microglia. Physiol Rev 91: 461-553.

Knoll B, Nordheim A. 2009. Functional versatility of transcription factors in the nervous system: the SRF paradigm. Trends Neurosci 32: 432-442.

Komitova M, Serwanski DR, Lu QR, Nishiyama A. 2011. NG2 cells are not a major source of reactive astrocytes after neocortical stab wound injury. Glia 59: 800-809.

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

Korn J, Christ B, Kurz H. 2002. Neuroectodermal origin of brain pericytes and vascular smooth muscle cells. J Comp Neurol 442: 78-88.

Kremer D, Aktas O, Hartung HP, Kury P. 2011. The complex world of oligodendroglial differentiation inhibitors. Ann Neurol 69: 602-618.

Kreutzberg GW. 1996. Microglia: a sensor for pathological events in the CNS. Trends Neurosci 19: 312-318.

Kriegstein A, Alvarez-Buylla A. 2009. The glial nature of embryonic and adult neural stem cells. Annu Rev Neurosci 32: 149-184.

Kuhlbrodt K, Herbarth B, Sock E, Hermans-Borgmeyer I, Wegner M. 1998. Sox10, a novel transcriptional modulator in glial cells. J Neurosci 18: 237-250.

Kukley M, Nishiyama A, Dietrich D. 2010. The fate of synaptic input to NG2 glial cells: neurons specifically downregulate transmitter release onto differentiating oligodendroglial cells. J Neurosci 30: 8320-8331.

Lasiene J, Matsui A, Sawa Y, Wong F, Horner PJ. 2009. Age-related myelin dynamics revealed by increased oligodendrogenesis and short internodes. Aging Cell 8: 201-213.

Levine JM. 1994. Increased expression of the NG2 chondroitin-sulfate proteoglycan after brain injury. J Neurosci 14: 4716-4730.

Levine JM, Enquist LW, Card JP. 1998. Reactions of oligodendrocyte precursor cells to alpha herpesvirus infection of the central nervous system. Glia 23: 316-328.

Levine JM, Reynolds R. 1999. Activation and proliferation of endogenous oligodendrocyte precursor cells during ethidium bromide-induced demyelination. Exp Neurol 160: 333-347.

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

Li H, Richardson WD. 2009. Genetics meets epigenetics: HDACs and Wnt signaling in myelin development and regeneration. Nat Neurosci 12: 815-817.

Li Q, Brus-Ramer M, Martin JH, McDonald JW. 2010. Electrical stimulation of the medullary pyramid promotes proliferation and differentiation of oligodendrocyte progenitor cells in the corticospinal tract of the adult rat. Neurosci Lett 479: 128-133.

Lim SK, Llaguno SR, McKay RM, Parada LF. 2011. Glioblastoma multiforme: a perspective on recent findings in human cancer and mouse models. BMB Rep 44: 158-164.

Lin SC, Huck JH, Roberts JD, Macklin WB, Somogyi P, Bergles DE. 2005. Climbing fiber innervation of NG2-expressing glia in the mammalian cerebellum. Neuron 46: 773-785.

Lindberg N, Kastemar M, Olofsson T, Smits A, Uhrbom L. 2009. Oligodendrocyte progenitor cells can act as cell of origin for experimental glioma. Oncogene 28: 2266-2275.

Liu C, Sage JC, Miller MR, Verhaak RG, Hippenmeyer S, Vogel H, Foreman O, Bronson RT, Nishiyama A, Luo L, Zong H. 2011. Mosaic analysis with double markers reveals tumor cell of origin in glioma. Cell 146: 209-221.

Liu X, Yao DL, Bondy CA, Brenner M, Hudson LD, Zhou J, Webster HD. 1994. Astrocytes express insulin-like growth factor-I (IGF-I) and its binding protein, IGFBP-2, during demyelination induced by experimental autoimmune encephalomyelitis. Mol Cell Neurosci 5: 418-430.

Ludwig A, Schlierf B, Schardt A, Nave KA, Wegner M. 2004. Sox10-rtTA mouse line for tetracycline-inducible expression of transgenes in neural crest cells and oligodendrocytes. Genesis 40: 171-175.

Lytle JM, Vicini S, Wrathall JR. 2006. Phenotypic changes in NG2+ cells after spinal cord injury. J Neurotrauma 23: 1726-1738.

Ma J, Chen T, Mandelin J, Ceponis A, Miller NE, Hukkanen M, Ma GF, Konttinen YT. 2003. Regulation of macrophage activation. Cell Mol Life Sci 60: 2334-2346.

Maldonado PP, Velez-Fort M, Angulo MC. 2011. Is neuronal communication with NG2 cells synaptic or extrasynaptic? J Anat 219: 8-17.

Mangin JM, Gallo V. 2011. The curious case of NG2 cells: transient trend or game changer? ASN Neuro 3.

Mangin JM, Kunze A, Chittajallu R, Gallo V. 2008. Satellite NG2 progenitor cells share common glutamatergic inputs with associated interneurons in the mouse dentate gyrus. J Neurosci 28: 7610-7623.

Masahira N, Takebayashi H, Ono K, Watanabe K, Ding L, Furusho M, Ogawa Y, Nabeshima Y, Alvarez-Buylla A, Shimizu K, Ikenaka K. 2006. Olig2-positive progenitors in the embryonic spinal cord give rise not only to motoneurons and oligodendrocytes, but also to a subset of astrocytes and ependymal cells. Dev Biol 293: 358-369.

Mason JL, Goldman JE. 2002. A2B5+ and O4+ Cycling progenitors in the adult forebrain white matter respond differentially to PDGF-AA, FGF-2, and IGF-1. Mol Cell Neurosci 20: 30-42.

Mason JL, Xuan S, Dragatsis I, Efstratiadis A, Goldman JE. 2003. Insulin-like growth factor (IGF) signaling through type 1 IGF receptor plays an important role in remyelination. J Neurosci 23: 7710-7718.

Mato S, Alberdi E, Ledent C, Watanabe M, Matute C. 2009. CB1 cannabinoid receptor-dependent and -independent inhibition of depolarization-induced calcium influx in oligodendrocytes. Glia 57: 295-306.

Matute C. 2011. Glutamate and ATP signalling in white matter pathology. J Anat 219: 53-64.

McTigue DM, Tripathi RB. 2008. The life, death, and replacement of oligodendrocytes in the adult CNS. J Neurochem 107: 1-19.

McTigue DM, Wei P, Stokes BT. 2001. Proliferation of NG2-positive cells and altered oligodendrocyte numbers in the contused rat spinal cord. J Neurosci 21: 3392-3400.

Metzger D, Clifford J, Chiba H, Chambon P. 1995. Conditional site-specific recombination in mammalian cells using a ligand-dependent chimeric Cre recombinase. Proc Natl Acad Sci U S A 92: 6991-6995.

Muller SM, Stolt CC, Terszowski G, Blum C, Amagai T, Kessaris N, Iannarelli P, Richardson WD, Wegner M, Rodewald HR. 2008. Neural crest origin of perivascular mesenchyme in the adult thymus. J Immunol 180: 5344-5351.

Nave KA. 2010. Myelination and the trophic support of long axons. Nat Rev Neurosci 11: 275-283.

Nave KA, Trapp BD. 2008. Axon-glial signaling and the glial support of axon function. Annu Rev Neurosci 31: 535-561.

Nery S, Wichterle H, Fishell G. 2001. Sonic hedgehog contributes to oligodendrocyte specification in the mammalian forebrain. Development 128: 527-540.

Nimmerjahn A, Kirchhoff F, Helmchen F. 2005. Resting microglial cells are highly dynamic surveillants of brain parenchyma in vivo. Science 308: 1314-1318.

Nishiyama A, Komitova M, Suzuki R, Zhu X. 2009. Polydendrocytes (NG2 cells): multifunctional cells with lineage plasticity. Nat Rev Neurosci 10: 9-22.

Nishiyama A, Yu M, Drazba JA, Tuohy VK. 1997. Normal and reactive NG2+ glial cells are distinct from resting and activated microglia. J Neurosci Res 48: 299-312.

Norden AD, Wen PY. 2006. Glioma therapy in adults. Neurologist 12: 279-292.

Nunes MC, Roy NS, Keyoung HM, Goodman RR, McKhann G, 2nd, Jiang L, Kang J, Nedergaard M, Goldman SA. 2003. Identification and isolation of multipotential neural progenitor cells from the subcortical white matter of the adult human brain. Nat Med 9: 439-447.

Nunez JL, Nelson J, Pych JC, Kim JH, Juraska JM. 2000. Myelination in the splenium of the corpus callosum in adult male and female rats. Brain Res Dev Brain Res 120: 87-90.

Opris I, Hampson RE, Stanford TR, Gerhardt GA, Deadwyler SA. 2011. Neural activity in frontal cortical cell layers: evidence for columnar sensorimotor processing. J Cogn Neurosci 23: 1507-1521.

Orentas DM, Miller RH. 1998. Regulation of oligodendrocyte development. Mol Neurobiol 18: 247-259.

Panagiotakos G, Alshamy G, Chan B, Abrams R, Greenberg E, Saxena A, Bradbury M, Edgar M, Gutin P, Tabar V. 2007. Long-term impact of radiation on the stem cell and oligodendrocyte precursors in the brain. PLoS One 2: e588.

Paratore C, Goerich DE, Suter U, Wegner M, Sommer L. 2001. Survival and glial fate acquisition of neural crest cells are regulated by an interplay between the transcription factor Sox10 and extrinsic combinatorial signaling. Development 128: 3949-3961.

Perry A, Schmidt RE. 2006. Cancer therapy-associated CNS neuropathology: an update and review of the literature. Acta Neuropathol 111: 197-212.

Pfeiffer SE, Warrington AE, Bansal R. 1993. The oligodendrocyte and its many cellular processes. Trends Cell Biol 3: 191-197.

Pietri T, Eder O, Blanche M, Thiery JP, Dufour S. 2003. The human tissue plasminogen activator-Cre mouse: a new tool for targeting specifically neural crest cells and their derivatives in vivo. Dev Biol 259: 176-187.

Pineau I, Lacroix S. 2007. Proinflammatory cytokine synthesis in the injured mouse spinal cord: multiphasic expression pattern and identification of the cell types involved. J Comp Neurol 500: 267-285.

Pineau I, Lacroix S. 2009. Endogenous signals initiating inflammation in the injured nervous system. Glia 57: 351-361.

Polito A, Reynolds R. 2005. NG2-expressing cells as oligodendrocyte progenitors in the normal and demyelinated adult central nervous system. J Anat 207: 707-716.

Psachoulia K, Jamen F, Young KM, Richardson WD. 2009. Cell cycle dynamics of NG2 cells in the postnatal and ageing brain. Neuron Glia Biol 5: 57-67.

Raff M. 2006. The mystery of intracellular developmental programmes and timers. Biochem Soc Trans 34: 663-670.

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

Redondo C, Lopez-Toledano MA, Lobo MV, Gonzalo-Gobernado R, Reimers D, Herranz AS, Paino CL, Bazan E. 2007. Kainic acid triggers oligodendrocyte precursor cell proliferation and neuronal differentiation from striatal neural stem cells. J Neurosci Res 85: 1170-1182.

Redwine JM, Armstrong RC. 1998. In vivo proliferation of oligodendrocyte progenitors expressing PDGFalphaR during early remyelination. J Neurobiol 37: 413-428.

Reynolds R, Hardy R. 1997. Oligodendroglial progenitors labeled with the O4 antibody persist in the adult rat cerebral cortex in vivo. J Neurosci Res 47: 455-470.

Rhodes KE, Moon LD, Fawcett JW. 2003. Inhibiting cell proliferation during formation of the glial scar: effects on axon regeneration in the CNS. Neuroscience 120: 41-56.

Rhodes KE, Raivich G, Fawcett JW. 2006. The injury response of oligodendrocyte precursor cells is induced by platelets, macrophages and inflammation-associated cytokines. Neuroscience 140: 87-100.

Richardson WD, Kessaris N, Pringle N. 2006. Oligodendrocyte wars. Nat Rev Neurosci 7: 11-18.

Richardson WD, Young KM, Tripathi RB, McKenzie I. 2011. NG2-glia as multipotent neural stem cells: fact or fantasy? Neuron 70: 661-673.

Rivers LE, Young KM, Rizzi M, Jamen F, Psachoulia K, Wade A, Kessaris N, Richardson WD. 2008. PDGFRA/NG2 glia generate myelinating oligodendrocytes and piriform projection neurons in adult mice. Nat Neurosci 11: 1392-1401.

Robel S, Bardehle S, Lepier A, Brakebusch C, Gotz M. 2011a. Genetic deletion of cdc42 reveals a crucial role for astrocyte recruitment to the injury site in vitro and in vivo. J Neurosci 31: 12471-12482.

Robel S, Berninger B, Gotz M. 2011b. The stem cell potential of glia: lessons from reactive gliosis. Nat Rev Neurosci 12: 88-104.

Rolls A, Shechter R, Schwartz M. 2009. The bright side of the glial scar in CNS repair. Nat Rev Neurosci 10: 235-241.

Rosenberg SS, Kelland EE, Tokar E, De la Torre AR, Chan JR. 2008. The geometric and spatial constraints of the microenvironment induce oligodendrocyte differentiation. Proc Natl Acad Sci U S A 105: 14662-14667.

Sanchez I, Hassinger L, Paskevich PA, Shine HD, Nixon RA. 1996. Oligodendroglia regulate the regional expansion of axon caliber and local accumulation of neurofilaments during development independently of myelin formation. J Neurosci 16: 5095-5105.

Sanchez R, Karow M, Schichor C, Masserdotti G, Ortega F, Heinrich C, Gascón S, Kahn MA, Lie C, Dellavalle A, Cossus G, Goldbrunner R, Götz M, Berninger B. 2011. Direct conversion of pericyte-derived cells of the adult human brain into functional neurons. in revision.

Sanchez R, Karow M, Schichor C, Masserdotti G, Ortega F, Heinrich C, Gascón S, Kahn MA, Lie C, Dellavalle A, Cossus G, Goldbrunner R, Götz M, Berninger B. under revision. Direct conversion of pericyte-derived cells of the adult human brain into functional neurons. in revision.

Sauer B. 1998. Inducible gene targeting in mice using the Cre/lox system. Methods 14: 381-392.

Sellers DL, Maris DO, Horner PJ. 2009. Postinjury niches induce temporal shifts in progenitor fates to direct lesion repair after spinal cord injury. J Neurosci 29: 6722-6733.

Shi J, Marinovich A, Barres BA. 1998. Purification and characterization of adult oligodendrocyte precursor cells from the rat optic nerve. J Neurosci 18: 4627-4636.

Shibata S, Yasuda A, Renault-Mihara F, Suyama S, Katoh H, Inoue T, Inoue YU, Nagoshi N, Sato M, Nakamura M, Akazawa C, Okano H. 2010. Sox10-Venus mice: a new tool for real-time labeling of neural crest lineage cells and oligodendrocytes. Mol Brain 3: 31.

Shoshan Y, Nishiyama A, Chang A, Mork S, Barnett GH, Cowell JK, Trapp BD, Staugaitis SM. 1999. Expression of oligodendrocyte progenitor cell antigens by gliomas: implications for the histogenesis of brain tumors. Proc Natl Acad Sci U S A 96: 10361-10366.

Silver J, Miller JH. 2004. Regeneration beyond the glial scar. Nat Rev Neurosci 5: 146-156.

Simon C, Gotz M, Dimou L. 2011a. Progenitors in the adult cerebral cortex: Cell cycle properties and regulation by physiological stimuli and injury. Glia 59: 869-881.

Simon C, Lickert H, Götz M, Dimou L. 2011b. Sox10-iCreER^{T2}: a mouse line to inducibly trace the neural crest and oligodendrocyte lineage. in revision.

Simons M, Trotter J. 2007. Wrapping it up: the cell biology of myelination. Curr Opin Neurobiol 17: 533-540.

Sofroniew MV. 2009. Molecular dissection of reactive astrogliosis and glial scar formation. Trends Neurosci 32: 638-647.

Sofroniew MV, Vinters HV. 2010. Astrocytes: biology and pathology. Acta Neuropathol 119: 7-35.

Sommer I, Schachner M. 1982. Cell that are O4 antigen-positive and O1 antigen-negative differentiate into O1 antigen-positive oligodendrocytes. Neurosci Lett 29: 183-188.

Sowell ER, Thompson PM, Toga AW. 2004. Mapping changes in the human cortex throughout the span of life. Neuroscientist 10: 372-392.

Stelzer S, Ebnet K, Schwamborn JC. 2010. JAM-A is a novel surface marker for NG2-Glia in the adult mouse brain. BMC Neurosci 11: 27.

Stevens B, Porta S, Haak LL, Gallo V, Fields RD. 2002. Adenosine: a neuron-glial transmitter promoting myelination in the CNS in response to action potentials. Neuron 36: 855-868.

Stine ZE, Huynh JL, Loftus SK, Gorkin DU, Salmasi AH, Novak T, Purves T, Miller RA, Antonellis A, Gearhart JP, Pavan WJ, McCallion AS. 2009. Oligodendroglial and pan-neural crest expression of Cre recombinase directed by Sox10 enhancer. Genesis 47: 765-770.

Stritt C, Stern S, Harting K, Manke T, Sinske D, Schwarz H, Vingron M, Nordheim A, Knoll B. 2009. Paracrine control of oligodendrocyte differentiation by SRF-directed neuronal gene expression. Nat Neurosci 12: 418-427.

Sugiarto S, Persson AI, Munoz EG, Waldhuber M, Lamagna C, Andor N, Hanecker P, Ayers-Ringler J, Phillips J, Siu J, Lim DA, Vandenberg S, Stallcup W, Berger MS, Bergers G, Weiss WA, Petritsch C. 2011. Asymmetry-defective oligodendrocyte progenitors are glioma precursors. Cancer Cell 20: 328-340.

Takahashi T, Nowakowski RS, Caviness VS, Jr. 1995. The cell cycle of the pseudostratified ventricular epithelium of the embryonic murine cerebral wall. J Neurosci 15: 6046-6057.

Takebayashi H, Nabeshima Y, Yoshida S, Chisaka O, Ikenaka K. 2002. The basic helix-loop-helix factor olig2 is essential for the development of motoneuron and oligodendrocyte lineages. Curr Biol 12: 1157-1163.

Tan AM, Colletti M, Rorai AT, Skene JH, Levine JM. 2006. Antibodies against the NG2 proteoglycan promote the regeneration of sensory axons within the dorsal columns of the spinal cord. J Neurosci 26: 4729-4739.

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.

Tang X, Davies JE, Davies SJ. 2003. Changes in distribution, cell associations, and protein expression levels of NG2, neurocan, phosphacan, brevican, versican V2, and tenascin-C during acute to chronic maturation of spinal cord scar tissue. J Neurosci Res 71: 427-444.

Tatsumi K, Haga S, Matsuyoshi H, Inoue M, Manabe T, Makinodan M, Wanaka A. 2005. Characterization of cells with proliferative activity after a brain injury. Neurochem Int 46: 381-389.

Tatsumi K, Takebayashi H, Manabe T, Tanaka KF, Makinodan M, Yamauchi T, Makinodan E, Matsuyoshi H, Okuda H, Ikenaka K, Wanaka A. 2008. Genetic fate mapping of Olig2 progenitors in the injured adult cerebral cortex reveals preferential differentiation into astrocytes. J Neurosci Res 86: 3494-3502.

Taylor DL, Pirianov G, Holland S, McGinnity CJ, Norman AL, Reali C, Diemel LT, Gveric D, Yeung D, Mehmet H. 2010. Attenuation of proliferation in oligodendrocyte precursor cells by activated microglia. J Neurosci Res 88: 1632-1644.

Teo JL, Kahn M. 2010. The Wnt signaling pathway in cellular proliferation and differentiation: A tale of two coactivators. Adv Drug Deliv Rev 62: 1149-1155.

Tong XP, Li XY, Zhou B, Shen W, Zhang ZJ, Xu TL, Duan S. 2009. Ca(2+) signaling evoked by activation of Na(+) channels and Na(+)/Ca(2+) exchangers is required for GABA-induced NG2 cell migration. J Cell Biol 186: 113-128.

Tripathi RB, McTigue DM. 2008. Chronically increased ciliary neurotrophic factor and fibroblast growth factor-2 expression after spinal contusion in rats. J Comp Neurol 510: 129-144.

Trotter J, Karram K, Nishiyama A. 2010. NG2 cells: Properties, progeny and origin. Brain Res Rev 63: 72-82.

Ulloa F, Marti E. 2010. Wnt won the war: antagonistic role of Wnt over Shh controls dorso-ventral patterning of the vertebrate neural tube. Dev Dyn 239: 69-76.

Vallstedt A, Klos JM, Ericson J. 2005. Multiple dorsoventral origins of oligodendrocyte generation in the spinal cord and hindbrain. Neuron 45: 55-67.

Vasioukhin V, Degenstein L, Wise B, Fuchs E. 1999. The magical touch: genome targeting in epidermal stem cells induced by tamoxifen application to mouse skin. Proc Natl Acad Sci U S A 96: 8551-8556.

Velez-Fort M, Audinat E, Angulo MC. 2009. Functional alpha 7-containing nicotinic receptors of NG2-expressing cells in the hippocampus. Glia 57: 1104-1114.

Ventura RE, Goldman JE. 2006. Telencephalic oligodendrocytes battle it out. Nat Neurosci 9: 153-154.

Viscomi MT, Oddi S, Latini L, Pasquariello N, Florenzano F, Bernardi G, Molinari M, Maccarrone M. 2009. Selective CB2 receptor agonism protects central neurons from remote axotomy-induced apoptosis through the PI3K/Akt pathway. J Neurosci 29: 4564-4570.

Visvader JE. 2011. Cells of origin in cancer. Nature 469: 314-322.

Von Blankenfeld G, Ransom BR, Kettenmann H. 1993. Development of cell-cell coupling among cells of the oligodendrocyte lineage. Glia 7: 322-328.

Wang DD, Bordey A. 2008. The astrocyte odyssey. Prog Neurobiol 86: 342-367.

Wang Q, Tang XN, Yenari MA. 2007. The inflammatory response in stroke. J Neuroimmunol 184: 53-68.

Wang S, Sdrulla AD, diSibio G, Bush G, Nofziger D, Hicks C, Weinmaster G, Barres BA. 1998. Notch receptor activation inhibits oligodendrocyte differentiation. Neuron 21: 63-75.

Watanabe M, Toyama Y, Nishiyama A. 2002. Differentiation of proliferated NG2-positive glial progenitor cells in a remyelinating lesion. J Neurosci Res 69: 826-836.

Wieser RJ, Schutz S, Tschank G, Thomas H, Dienes HP, Oesch F. 1990. Isolation and characterization of a 60-70-kD plasma membrane glycoprotein involved in the contact-dependent inhibition of growth. J Cell Biol 111: 2681-2692.

Wigley R, Butt AM. 2009. Integration of NG2-glia (synantocytes) into the neuroglial network. Neuron Glia Biol 5: 21-28.

Wolswijk G, Noble M. 1989. Identification of an adult-specific glial progenitor cell. Development 105: 387-400.

Wren D, Wolswijk G, Noble M. 1992. In vitro analysis of the origin and maintenance of O-2Aadult progenitor cells. J Cell Biol 116: 167-176.

Yates MA, Juraska JM. 2007. Increases in size and myelination of the rat corpus callosum during adulthood are maintained into old age. Brain Res 1142: 13-18.

Yuan X, Eisen AM, McBain CJ, Gallo V. 1998. A role for glutamate and its receptors in the regulation of oligodendrocyte development in cerebellar tissue slices. Development 125: 2901-2914.

Zai LJ, Wrathall JR. 2005. Cell proliferation and replacement following contusive spinal cord injury. Glia 50: 247-257.

Zawadzka M, Rivers LE, Fancy SP, Zhao C, Tripathi R, Jamen F, Young K, Goncharevich A, Pohl H, Rizzi M, Rowitch DH, Kessaris N, Suter U, Richardson WD, Franklin RJ. 2010. CNS-resident glial progenitor/stem cells produce Schwann cells as well as oligodendrocytes during repair of CNS demyelination. Cell Stem Cell 6: 578-590.

Zhang H, Miller RH. 1996. Density-dependent feedback inhibition of oligodendrocyte precursor expansion. J Neurosci 16: 6886-6895.

Zhang M, Alloway KD. 2004. Stimulus-induced intercolumnar synchronization of neuronal activity in rat barrel cortex: a laminar analysis. J Neurophysiol 92: 1464-1478.

Zhao MT, Prather RS. 2011. The multi-potentiality of skin-derived stem cells in pigs. Theriogenology 75: 1372-1380.

Zhao W, Robbins ME. 2009. Inflammation and chronic oxidative stress in radiation-induced late normal tissue injury: therapeutic implications. Curr Med Chem 16: 130-143.

Zhou Q, Wang S, Anderson DJ. 2000. Identification of a novel family of oligodendrocyte lineage-specific basic helix-loop-helix transcription factors. Neuron 25: 331-343.

Zhou YX, Armstrong RC. 2007. Interaction of fibroblast growth factor 2 (FGF2) and notch signaling components in inhibition of oligodendrocyte progenitor (OP) differentiation. Neurosci Lett 421: 27-32.

Zhu X, Bergles DE, Nishiyama A. 2008. NG2 cells generate both oligodendrocytes and gray matter astrocytes. Development 135: 145-157.

Zhu X, Hill RA, Dietrich D, Komitova M, Suzuki R, Nishiyama A. 2011. Age-dependent fate and lineage restriction of single NG2 cells. Development 138: 745-753.

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Appendix

A1 List of abbreviations

µm	Micrometer
AChR	Acetylcholine receptor
AMPA	α-amino-3-hydroxyl-5-methyl-4-isoxazole-propionate
APC (CC1)	Adenomatosis polyposis coli
AraC	Cytosine-D-arabinofuranoside
ASPA	Aspartoacylase
ATP	Adenosine triphosphate
BAC	Bacterial artificial chromosome
BBB	Blood brain barrier
BDNF	Brain-derived neurotrophic factor
BLBP	Brain lipid binding protein
BMP	Bone morphogenic protein
bp	Base pairs
BrdU	5'-bromo-desoxyuridine
Ca^{2+}	Calcium ion
cAMP	Cyclic adenosine monophosphate
CC1	See APC
CD11b	Cluster of differentiation 11b
CD45	Cluster of differentiation 45
CNS	Central nervous system
CNTF	Ciliary neurotrophic factor
CsCl	Cesium chloride
CTGF	Connective tissue growth factor
d	Day
DAPI	4',6'-diamidino-2-phenylindole
Dcx	Doublecortin
DIG	Digoxigenin
dpi	Days post injury
dpr	Days post recombination
DRG	Dorsal root ganglia
E	Embryonic day
e.g.	Exempli gratia
ECM	Extracellular matrix
EGFR	Epidermal growth factor receptor
EPSC	Excitatory postsynaptic current
ER	Estrogen receptor
FGF	Fibroblast growth factor
G	gram
GABA	γ-aminobutyric acid
GDNF	Glial cell line derived neurotrophic factor
GFAP	Glial fibrillary acidic protein
GFP	Green fluorescent protein
GLAST	Alias Slc1a3 – solute carrier family 1 (glial high affinity glutamate transporter), member 3
Glu	Glutamate
GM	Gray matter
Gp	Guinea pig
GPR17	G-protein coupled receptor 17
GSTπ	Glutathione-S-transferase π
h	Hour
HDAC	Histon deacetylase
Hes	Hairy and enhancer of split
Iba1	Induction of brown adipocytes 1
$iCreER^{T2}$	Improved Cre recombinase fused to a truncated estrogen receptor
Id	Inhibitor of differentiation
IGF	Insulin-like growth factor
IL	interleukin
IPSC	Inhibitory postsynaptic current
JAMA	Junctional adhesion molecule A
kb	Kilo base pairs
kg	Kilogram

lacZ	β-galactosidase
LGE	Lateral ganglionic eminence
LI	Labeling index
LIF	Leukemia inhibitory factor
Lingo	Leucine rich repeat and Ig domain containing
m	Mouse
M	Molar
MAG	Myelin-associated glycoprotein
Mash	Ascl1 – achaete-scute complex homolog 1
MBP	Myelin basic protein
mg	Milligram
MGE	Medial ganglionic eminence
min	Minute
mm	Millimeter
MMP	Metalloproteinase
MOG	Myelin oligodendrocyte glycoprotein
MS	Multiple sklerosis
Neo	Neomycine
NeuN	Neuronal nuclear antigen
NG2	Neuron-glia antigen 2
NGF	Nerve growth factor
NT	Neurotrophin
OHT	4-hydroxy tamoxifen
OLIG2	Oligodendrocyte transcription factor 2
OPC	Oligodendrocyte precursor cell
PAC	P1-derived artificial chromosome
PDGF	Platelet-derived growth factor
PDGFRα	Platelet-derived growth factor α receptor
PFA	Paraformaldehyde
PhD	Doctor of Philosophy
PLP/ DM20	Myelin proteolipid protein
PNS	Peripheral nervous system
PSA-NCAM	Poly sialated-neural cell adhesion molecule
R26R	Rosa26 reporter mouse line, expressing β-galactosidase
Rb	Rabbit
Rt	Rat
rtTA	Tetracycline reverse transcription activator
s.c.	Subcutan
S100β	S100 calcium binding protein β
SEM	Standard error of the mean
Shh	Sonic hedgehog
Sox	SRY (sex determining region Y)-related high mobility group box protein
SRF	Serum response factor
TAM	Tamoxifen
Tcf	Transcription factor
TGF	T growth factor
TH1	T-helper cell 1
TH2	T-helper cell 2
TIMP	Tissue inhibitory of metalloproteinase
TM	Tamoxifen
TNC	Tenascin
TNF	Tumor necrosis factor
UTR	Untranslated region
WM	White matter
Wnt	Wingless-related MMTV integration site
Wt	Wildtype
YY	Ying and Yang
z.B.	Zum Beispiel
Z/EG	GFP reporter mouse line
ZNS	Zentrales Nervensystem

A2 List of figures

A3 List of tables