Oligodendrocyte Progenitor Cells Become Regionally Diverse and Heterogeneous with Age

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In Brief
Spitzer et al. show that oligodendrocyte progenitor cells (OPCs) acquire ion channels and sensitivity to neuronal activity that differ between region and age. The onset and decline of ion channels follow developmental milestones. This heterogeneity indicates different functional states of OPCs.

Highlights
- Oligodendrocyte progenitor cells (OPCs) acquire ion channels with age
- OPCs become functionally heterogeneous both between brain regions and with age
- NMDARs disappear in non-myelinating regions but remain in actively myelinating areas
- Heterogeneity in OPC ion channel density indicates different functional states
Oligodendrocyte Progenitor Cells Become Regionally Diverse and Heterogeneous with Age

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SUMMARY

Oligodendrocyte progenitor cells (OPCs), which differentiate into myelinating oligodendrocytes during CNS development, are the main proliferative cells in the adult brain. OPCs are conventionally considered a homogeneous population, particularly with respect to their electrophysiological properties, but this has been debated. We show, by using single-cell electrophysiological recordings, that OPCs start out as a homogeneous population but become functionally heterogeneous, varying both within and between brain regions and with age. These electrophysiological changes in OPCs correlate with the differentiation potential of OPCs; thus, they may underlie the differential differences in OPCs between regions and, likewise, differentiation failure with age.

INTRODUCTION

Glial cells, astrocytes, oligodendrocytes, and microglia are conventionally thought of as homogeneous cell types; however, recent findings show that glial cells, like neurons, have a different transcriptome and proteome depending on the brain region and with age and that the glial cell transcriptome, but not the neuronal one, is mostly altered by aging (Soreq et al., 2017). How these changes relate to cellular function is unclear, and the functional implications of glial cell heterogeneity and age-related brain changes are yet to be understood.

The fourth type of glial cell, the oligodendrocyte progenitor cell (OPC), which expresses the proteoglycan NG2, is widely distributed throughout the adult brain and is the main proliferative cell present in the adult CNS (Dawson et al., 2003). Its function in the adult CNS is relatively unclear. During development, OPCs give rise to oligodendrocytes that generate myelin, which ensures fast signal transmission and provides metabolic support to axons (Nave, 2010; Saab et al., 2016). It is becoming evident that, in young adults, OPCs are needed for motor learning (McKenzie et al., 2014; Sampaio-Baptista et al., 2013; Xiao et al., 2016), myelin maintenance (Young et al., 2013), and myelin regeneration (Zawadzka et al., 2010). With age, however, motor learning and myelin regeneration decline (Huang et al., 2011; Ruckh et al., 2012), and white matter lesions accumulate (Habes et al., 2016), indicating a reduced potential of the OPCs for de novo myelination, maintenance, and regeneration.

Growing evidence, both in vitro and in vivo, shows that neuronal activity and glutamate signaling are regulatory signals for myelination and remyelination in young adults (de Faria et al., 2018; Gautier et al., 2015; Krasnow et al., 2018; Lundgaard et al., 2013; Mitew et al., 2018). OPCs have the capacity to sense changes in neuronal activity and glutamate release because OPCs express glutamate receptors and receive depolarizing synaptic inputs from axons in both white and gray matter (Bergles et al., 2000; Kárason et al., 2005, 2008; Kukley et al., 2007; Spitzer et al., 2016; Ziskin et al., 2007). These electrophysiological properties, along with the expression of voltage-gated ion channels such as tetrodotoxin (TTX)-sensitive voltage-gated sodium channels, have become a defining feature of OPCs (Clarke et al., 2012; De Biase et al., 2010). However, not all OPCs express these electrophysiological properties (Chittajallu et al., 2004; Kárason et al., 2008). Whether this heterogeneity has a functional significance or whether it is solely a transitional stage prior to differentiation is debated (Clarke et al., 2012; De Biase et al., 2010).

Whether OPCs are a heterogeneous cell population is controversial. On one hand, depletion and functional studies have indicated that forebrain OPCs, despite arising from three different origins in subsequent waves during early development, are functionally similar (Clarke et al., 2012; Tripathi et al., 2011). Similarly, electrophysiological studies of OPCs in the hippocampus and corpus callosum (CC) have reported OPCs to be homogeneous (Clarke et al., 2012; De Biase et al., 2010). On the other hand, OPCs have been shown to respond differently to growth factors (Mason and Goldman, 2002) and cytokines (Lentferink et al., 2018), and they have differential transcriptional profiles (Marques et al., 2018). Furthermore, depending on the region, OPCs differ in their capability to differentiate into myelinating oligodendrocytes. For example, OPCs from the CC differentiate into myelinating oligodendrocytes more efficiently than OPCs taken from the cortex (CTX; gray matter) (Viganò et al., 2013). In line with this, the electrophysiological properties of OPCs reportedly differ between gray and white matter OPCs (Chittajallu et al., 2004) despite having the same developmental origin.
Hence, we asked whether the electrophysiological properties of OPCs change with age and brain region as transcriptional studies have demonstrated for other types of glial cells, altering their capability to monitor and respond to neuronal activity. To address this, we used single-cell electrophysiological recordings in heterozygous NG2-EYFP (enhanced yellow fluorescent protein) knockin mice, where the expression of the reporter gene is regulated according to the endogenous NG2 gene, allowing unbiased sampling of this population. We report that OPCs start out as a homogeneous population but become functionally heterogeneous, varying both within and between brain regions and age. These electrophysiological changes correlate with the differentiation potential of OPCs.

RESULTS

OPCs Acquire Functional Voltage-Gated Ion Channels at Different Developmental Time Points

To investigate the electrophysiological membrane properties of OPCs from first appearance to old age, we used heterozygous NG2-EYFP knockin mice (NG2-EYFP); in these mice, all parenchymal EYFP-positive cells are Olig2- and NG2-positive throughout life, indicating that EYFP expression tightly follows expression of the NG2 protein (Karram et al., 2008; Figure 1B). We voltage-clamped OPCs at embryonic day 13 (E13) because mouse forebrain OPCs first appear at E12.5 (Kessaris et al., 2006); then at E18, when the second wave (E15.5) is established; at postnatal day 0 (P0), which coincides with the third wave (Kessaris et al., 2006); then weekly for the first month, when myelination rates are the highest (Hamano et al., 1998); and then every 2 to 3 months until P330, when myelination efficacy has declined (Huang et al., 2011; Tripathi et al., 2017; Figure 1A).

A supposed defining feature of OPCs is the presence of voltage-gated sodium channels (Nav), a stereotypic transient inward current that is evoked upon depolarization beyond −50 mV. This depolarization-evoked transient inward current was absent in OPCs at E13. In fact, at this time, no voltage-gated currents were detected in OPCs. We first detected a depolarization-evoked transient inward current, known to be TTX-sensitive Nav (De Biase et al., 2010; Káradóttir et al., 2008), and outward currents known to represent voltage-gated potassium channel (Kv) currents, in a subset of OPCs at E18. Notably, Kv currents were detected in 85% of cells, whereas Nav were detected in only 38% of OPCs at E18 (p = 0.04), indicating that Kv channel expression starts before Nav expression. Although both the proportion of OPCs with functional Nav channels and their density sharply increased after birth, the proportion of OPCs with Nav channel expression lagged behind Kv channels (Figure 1I). The peak Nav channel density coincided with the start of myelination (21.5 ± 1.7 pA/pF, p = 1.6 × 10⁻⁵) and was detected in over 90% of OPCs recorded from the second postnatal week (Hamano et al., 1998; Figures 1C, 1D, and 1I).

Despite the decline in Nav density with age, the majority, but not all, of postnatal OPCs had detectable Nav currents (Figures 1D and 1I). OPCs without Nav had a compact high membrane resistance, similar to that of OPCs with Nav (p = 0.54), but not a low membrane resistance, as is more reminiscent of OPCs that have started to differentiate (De Biase et al., 2010). Cell capacitance, a measure of membrane surface area, significantly changed with age (p = 4 × 10⁻⁷), starting low, peaking at the time when the myelination rate is highest in the forebrain, and then declining after the first month to a level similar to that of prenatal cells (Figure 1J). None of these properties differed between male and female animals (Figures S1A–S1C). These data show that newly formed OPCs acquire voltage-gated ion channels at different rates and that the density of voltage-gated ion channels, and thus OPC excitability, changes throughout the life of the animals. The timing of these changes,
intriguingly, aligns with key milestones of myelination and development.

**Glutamate Receptor Subtypes Appear at Different Developmental Time Points**

To address when OPCs acquire functional glutamate receptors, we voltage-clamped OPCs from E13–P330 as before. We first detected kainate-evoked currents (30 μM; activates both α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid [AMPAR] and kainate [KA] receptors) in a majority of OPCs at E18 (86%; Figures 1E, 1F, and 1I), coinciding with the appearance of Kv. At this time point, N-methyl-D-aspartate (NMDA; 60 μM)-evoked currents were undetectable (Figures 1G–1I). When myelination in the forebrain is at its highest rate (P6–P35; Hamano et al., 1998), NMDA receptor (NMDAR) density peaked, coinciding with peak Na+, density, and, at this time, the proportion of OPCs with NMDARs was the highest, at ~80% (Figures 1H and 1I). In contrast to NMDA, KA-evoked currents were detected in nearly all OPCs after birth (Figure 1E, 1F, and 1I). AMPA/KA receptor (KAR) densities gradually increased with age (p = 2.1 × 10−6; Figures 1E and 1F). Following sexual maturity, NMDAR density began to decrease, and NMDA-evoked currents were barely detectable by 6 months (0.006 ± 0.005 pA/pF; p = 3 × 10−2; Figures 1G–1I) and completely undetectable at 9 months, a time when remyelination potential has declined (Huang et al., 2011) and addition of new myelinating oligodendrocytes in the CC has ceased (Tripathi et al., 2017). The glutamate receptor density in OPCs did not, however, differ between female and male animals (Figures S1D–S1F). Spontaneous synaptic-like inputs became prominent at the end of the first week (p = 0.006) and remained comparable throughout life (0.02 ± 0.008 Hz, p = 0.2). These data show that OPCs acquire functional glutamate receptors at different rates and that their density peaks at different ages. In particular, NMDAR density and the proportion of OPCs with NMDARs peaked at the time of myelination. Importantly, although OPCs receive synaptic inputs at similar frequencies throughout life, their response to these inputs is likely to vary with age, in line with altered glutamate receptor expression.

**OPC Molecular Signatures Differ with Age**

The observed changes in ion channel and glutamate receptor densities in OPCs with age, intriguingly, align with milestones of myelination and development. Thus, to test whether the molecular signatures of key biological properties in OPCs also change with age, we performed bulk RNA sequencing (RNA-seq) of OPCs isolated by PDGFRα magnetic activated cell sorting (MACS; enriching for OPCs expressing PDGFRα) at different ages: E16, P12, P80, and P310. We observed that embryonic OPCs had molecular signatures of migrating cells (e.g., Dcc and Ephb2), significantly more so compared with OPCs from P12 or older mice. However, at P12, OPCs had stronger signatures of proliferating (e.g., Pdgfra, Ptc1, and Mki67) and committed OPCs (Myrf and Enpp6) and upregulated signal transduction but a reduced migratory molecular signature (Figures S2A, 2C, and 2D). Both molecular signatures of differentiation and proliferation decreased from P12 to P80 and further decreased in old OPCs. Between P12 and P80, there is a significant decrease in molecular signatures for cell cycle regulation (e.g., Mki67, 1.2F5, and Ccnb2), differentiation, and metabolism at the time when myelination starts to decline (Figure 2C). OPCs from older animals further downregulate cell cycle regulatory and differentiation genes, concomitant with upregulation of pro-quiescence genes and downregulation of genes regulating anti-quiescence, sensing neuronal activity, stem cell maintenance, and transcription (Figures 2B, 2C, and 2E).

The gene expression of Na+(α subunits Scn3a and Scn7a, and β auxiliary subunits Scn1b, Scn3b, and Scn4b) and NMDAR subunits (Grin1, Grin2a, Grin2C, and Grin3a) significantly increased from embryonic to postnatal OPCs, and subsequently all remained constant to old age, with the exception of Grin3a and Scn3b. However, AMPAR and KAR subunits, apart from Grik3, remained constant from E16 to old age (Figures S2A–S2C; Table S1). These results reflect that Na+, NMDAR, and AMPA/KAR protein expression is post-transcriptionally and/or post-translationally regulated in OPCs.

**OPC Proliferation Decreases with Age in Line with Na+ Density**

Given the clear change in cell cycle regulatory signatures in older OPCs, we investigated age-related cell cycle changes in OPCs with flow cytometry (Figures 3A, 3B, and S2E–S2G). We identified that the proportion of OPCs in G0/G1 and G2/M phase changed with age (p = 0.003 and p = 9 × 10−3; Figures 3C and 3E), but not the proportion of OPCs in S phase (p = 0.33; Figure 3D). The proportion of OPCs in G2/M phase peaked around P12 (Figure 3E), reflecting the high proliferative state at this time point, which coincided with the peak Na+ density (Figures 1C and 1D) and expression of proliferative genes (Figure 2C, 2E). The proportion of OPCs in G0/G1 phase gradually increased with age (Figure 3C), indicative of a gradual lengthening of G1 phase or an accumulation of OPCs entering G0 phase. The gradual increase in G0/G1 resembled the gradual increase in AMPA/KAR densities.

To test whether ion channel expression changes with the cell cycle, we crossed NG2-EYFP mice with either Fucci2a mice, in which mCherry is expressed during G0/G1 phase (Mort et al., 2014; Figures 3A and 3G), or Ki67-RFP mice, in which the reporter protein RFP is expressed during G2/M phase (Basak et al., 2014; Figures 3A and 3F). OPCs in an active cell cycle (cycling, Ki67-RFP+ or Fucci-mCherry+) have a 1.7-fold higher density of Na+ than OPCs in G0/G1phase (non-cycling, Ki67-RFP− or Fucci-mCherry−; Figure 3H; p = 4 × 10−3). There was no change detected in either NMDAR or AMPA/KAR densities between G2/M phase and G0/G1 phase of the cell cycle (NMDAR, p = 0.42; AMPA/KAR, p = 0.35; Figures 3I and 3J). In the proportion of OPCs with detectable evoked currents (NMDAR, p = 0.051; AMPA/KAR, p = 0.86). Neither mouse line can discriminate between OPCs in G1 and G0 phases of the cell cycle. However, if ion channel densities in the G1 phase differ from G0, then an increased heterogeneity would be expected in the recordings from non-cycling OPCs (G0/G1 phase) compared with cycling OPCs (G2/M phase). To test for heterogeneity, we analyzed the variance in the dataset between non-cycling...
OPCs and cycling OPCs. The variance in the density of Na\textsubscript{V} channels and NMDARs was identical between both states (Na\textsubscript{V}, \(p = 0.08\); NMDAR, \(p = 0.87\)), whereas the variance in AMPA/KAR densities differed greatly (\(p = 5 \times 10^{-3}\)), implying that AMPA/KAR densities in OPCs might differ between G1 and G\textsubscript{0} phase of the cell cycle.

OPC cell cycle time has been reported to differ between the CC and CTX and with age (Young et al., 2013). We investigated the proportion of OPCs labeled with the cell cycle protein Ki67 in brain slices from NG2-EYFP mice. In line with the RNA-seq and flow data, the proportion of proliferating OPCs both in the CC and CTX sharply decreased after the first postnatal weeks (Figures S2H–S2J). In the CC, the proportion of Ki67\textsuperscript{+} OPCs reached a constant level by the end of the first month, whereas in the CTX, the proportion of proliferating OPCs gradually decreased with age (Figures S2I and S2J). On average, the proportion of proliferating OPCs was higher in the CC compared with the CTX; however, at a very old age, the proportion of Ki67\textsuperscript{+} OPCs between the CC and CTX became nearly identical (Figures S2I and S2J).

**Gray and White Matter OPCs Diverge with Age**

OPCs have been reported to differ in both myelination potential (Viganò et al., 2013) and ion channel expression (Chittajallu et al., 2004; Káródotír et al., 2008) between the CTX and CC. Hence, we investigated when OPCs in these regions begin to differ. Recording from OPCs in both areas during the first postnatal week, in gray (CTX) and white (CC) matter, we found that OPCs are almost indistinguishable between regions with respect to Na\textsubscript{V} (\(p = 0.21\)), AMPA/KAR (\(p = 0.79\)), and NMDAR (\(p = 0.16\)).
Hence, at this time, OPCs have a similar sensitivity to neuronal activity. However, after the first postnatal week, OPCs diverged and varied significantly between gray and white matter, particularly in regard to AMPA/KAR (p = 4.3 x 10^-10/C0.4) and NMDAR (p = 0.02) densities (Figures 4 A–4D). Similarly, OPCs in the CTX diverged even further, as NMDAR (p = 0.02) densities differed between cortical layers 5/6, which is a myelinated area in the CTX, and layer 1, which is nearly unmyelinated; NaV channels (p = 0.29) and AMPA/KAR (p = 0.93) densities were unchanged between the two cortical layers (Figures 4E–4G). The white matter, on the other hand, is more homogeneous; no differences were identified in NaV channels (p = 0.88; AMPA/KAR, p = 0.08; NMDAR, p = 0.79) in OPCs located in either the anterior or posterior CC (Figures 4H–4J). These results indicate that the local environment affects OPC ion channel expression and, potentially, myelination potential.

To test whether the microenvironment can alter OPC electrophysiological properties, we cultured neonatal OPCs before their regional specification under different culture medium conditions. Glutamate receptor, NMDAR, and NaV densities were sensitive to the constituents of the medium (Figure S3). The effects of serum were reversible by neuronal conditioned medium in neonatal OPCs. These data show that altering the milieu around neonatal OPCs is sufficient to alter their functional ion channel expression.
The Age-Related Differentiation Potential of OPCs Is Not Reversed by an Altered Environment

Age-related changes in the systemic milieu have been shown to lead to a decrease in ion channel function in hippocampal neurons, similar to the changes we detect in OPCs, and are linked to declining cognitive function. Similarly, the differentiation and myelination potential of OPCs also decreases with age. Parabiosis of young blood can reverse both of these age-related changes (Ruckh et al., 2012; Villeda et al., 2011, 2014).

Therefore, we tested whether OPCs isolated from old mice, when the NMDARs had declined in vivo, could be reverted to have a similar myelination potential as neonatal OPCs when cultured in myelin-promoting medium for 3 weeks with neonatal dorsal root ganglion (DRG) neurons, mimicking youth (Figure 5A). The neonatal OPCs differentiated and myelinated the DRG axons, but only a small fraction of the adult OPCs differentiated, and only into non-myelinating MBP-positive cells (Figures 5B–5D; p = 6.4 \times 10^{-6}). Administration of growth and differentiation...
factor (GDF) 11 has been shown to recapitulate some of the rejuvenative effects of young blood in mice, although it has not yet been tested for myelination (Katsimpardi et al., 2014; Sinha et al., 2014). We thus tested whether the changes we detected in electrophysiological properties of OPCs could be reverted by *in vivo* administration of GDF11 for 4 weeks (intraperitoneally [i.p.], 0.1 mg/kg) from P150, when NaV and NMDAR density has declined. No differences were detected in NaV channel, AMPA/KAR, or NMDAR densities (Figures 5E–5H; NaV, p = 0.44; KA, p = 0.22; NMDA, p = 0.77) compared with saline administration. These data indicate that when OPC properties have changed, as occurs with maturation, ion channel expression and myelination potential do not easily revert to that of neonatal OPCs.

**OPCs Become Heterogeneous between and within Regions**

Next we addressed whether the changes in ion channel expression we identified differ between white matter (CC) and a gray matter region that has some myelination (CTX) or a gray matter region that is never myelinated (molecular layer of the cerebellum [ML]) and the subventricular zone (SVZ), an area that provides a continuous supply of myelinogenic OPCs throughout life (Menn et al., 2006; Figure 6A). At P7, OPCs in all regions tested had detectable NaV, AMPA/KAR, and NMDAR currents (Figures 6B–6E). There was a clear divergence in expression patterns between gray and white matter after the first postnatal weeks. NMDA-evoked currents rapidly disappeared in the ML OPCs after the first month, whereas NMDA-evoked currents in the CTX declined slower and became undetectable just after 3.5 months (p = 5 × 10⁻³; Figure 6E). In contrast, OPCs in the CC showed a slower decline in NMDAR density and slightly higher NMDAR densities than those in the CTX (Figure 6D), and a larger portion of CC OPCs, ~80% on average, had functional NMDARs compared with around half of the OPCs in the CTX (p = 3 × 10⁻⁵) and ML (p = 9 × 10⁻¹⁴; Figures 6D and 6E). In contrast to the parenchymal regions, NMDAR densities and the proportion of OPCs with NMDA-evoked currents remained unchanged throughout life in the SVZ (p = 0.43, p = 0.62) and were even detected in animals up to P503 (Figures 6D, 6E, 6H). The density of NMDARs in OPC in the SVZ.
was ~4 fold higher than in parenchymal OPCs \((p = 1.7 \times 10^{-4})\). Moreover, there was much greater variability \((p < 1 \times 10^{-15})\) in the NMDA-evoked currents in the SVZ compared with parenchymal OPCs, presumably indicating continuous cycles of early-born and old OPCs in the SVZ (Figure S4E).

KA evoked currents in nearly all postnatal OPCs regardless of age and region, except in the SVZ, where there was a higher proportion of OPCs with no detectable KA-evoked currents \((p = 0.024; \text{Figure 6C})\). Most OPCs without detectable KA-evoked currents also lacked detectable Na\(_V\), reminiscent of early embryonic OPCs (Figures 1C–1F). The AMPA/KA receptor densities tended to increase with the decline in NMDAR density, particularly evident in the CTX, where AMPA/KAR densities sharply increased at the same time as NMDAR density declined. Conversely, the density of KA receptors in OPCs in and around the SVZ only gradually increased and peaked late, at P270–P330 \((p = 2 \times 10^{-5})\), whereas in the CTX, the AMPA/KAR density peaked at P20–P35 \((p = 0.01; \text{Figure 6C})\).

These data show that OPCs are heterogeneous, varying mostly between regions and with age. The SVZ stands out as the area with the greatest heterogeneity within each time point but the most homogeneous across age. Intriguingly, NMDAR expression seems to be highly regulated in OPCs and may provide an explanation for why OPCs in the gray matter show less myelination potential than white matter OPCs (Vigano` et al., 2013).

**DISCUSSION**

We have identified that OPCs acquire Kv and Na\(_V\) channels, AMPA/KARs, and NMDARs during development. The time of onset, peak expression, and decline of these ion channels seems to be determined by environmental factors rather than being solely regulated by intrinsic mechanisms or developmental site of origin because the most significant changes are detected when OPCs from all developmental sites of origin are present at similar proportions (Kessaris et al., 2006). These findings are consistent with recent studies of origin-mapped OPCs (Marques et al., 2018; Tripathi et al., 2011). The most prominent age-related changes we observed were those related to Na\(_V\) channels (which determines OPC excitability) and NMDAR densities, which both peaked at a time when OPCs start to differentiate into myelinating oligodendrocytes and begin to express myelin genes (Figures 1 and 2; Marques et al., 2018). At this time, OPCs are highly sensitive to changes in neuronal activity, and this sensitivity changes differently with age depending on the region. As the myelination potential of OPCs declines, so do their NMDAR and Na\(_V\) densities and, thus, their excitability; intriguingly, NMDARs disappear earliest in a non-myelinating brain region but remained present throughout life in OPCs in a neurogenic region.

We have shown previously that NMDARs are important for activity-dependent myelination (Gautier et al., 2015; Lundgaard et al., 2013), but only when NMDARs are pre-activated in the presence of the growth factors neuregulin or brain-derived neurotrophic factor (BDNF), to switch OPCs to an activity-dependent myelination mode. In the absence of pre-activation, myelination occurs independent of neuronal activity but at a slower rate (Lundgaard et al., 2013). Accordingly, developmental myelination is slowed down (Kougioumtzidou et al., 2017; Saab et al., 2016) but relatively unaffected by knockout of NMDARs or AMPARs in oligodendrocyte-lineage cells (De Biase et al., 2011; Kougioumtzidou et al., 2017; Saab et al., 2016). This might be due to the fact that when receptors are knocked out of OPCs before they are expressed and activated, myelination occurs by default, independent of neuronal activity and glutamate receptor activation. However, presumably, when AMPA/KAR and NMDAR expression has started, and OPCs have been switched to depend on NMDAR activation for myelination, a loss of NMDARs, such as with age, may impede OPCs from terminal differentiation into myelinating oligodendrocytes. When culturing OPCs from this time point in myelination-promoting medium, they did not myelinate, similar to when NMDARs are blocked after they are expressed and activated, myelination is reduced (Gautier et al., 2015; Lundgaard et al., 2013).

The supposedly defining feature of OPCs is the expression of Na\(_V\), and OPCs that lack these channels have been suggested...
to be at the starting point of differentiation. However, we show here that OPCs are born without Na\textsubscript{V} and that the proportion of OPCs with these channels increases during the first postnatal weeks and then stays relatively constant throughout the ages tested. We identified that OPCs in an active cell cycle have a higher density of Na\textsubscript{V}. Concomitantly, Na\textsubscript{V} density peaks when OPC proliferation is highest and decreases in line with the decrease in the proportion of OPCs in G2/M phase and expression of proliferation genes as well as NMDAR density and myelin capacity. In contrast, AMPA/KAR densities increase with age and are expressed in all OPCs after birth. The tight control of AMPA/KAR, NMDA, and Na\textsubscript{V} densities we observed was unexpected, and so was the seemingly temporally opposing expression of AMPA/KARs and NMDARs in the parenchymal areas, similar to when NMDARs are knocked out of OPCs. AMPAR densities increase (De Biase et al., 2011). AMPARs have been reported to moderate OPC survival and promote more efficient myelination at a time when NMDARs are present (Kougioumtziou et al., 2017). In line with this, we have previously found that AMPARs are important for the early stages of the remyelination process, whereas NMDAR activation is important for the later stages (Gautier et al., 2015; Lundgaard et al., 2013).

It is important to note that, at all time points studied and in all regions, OPCs with embryonic electrophysiological properties were identified, but their proportion differed, with the highest proportion through all ages being found in the SVZ. The proportion of OPCs with different displays of ion channels and the density of each channel differed with age and region. The heterogeneity identified here may therefore reflect different cellular states, where densities of ion channels define a particular functional state of OPCs.

For example, when OPCs first appear, at E13, they have no detectable voltage-gated ion channels or glutamate receptors and may therefore be considered as being in a naive state. The first ion channels to appear are K\textsubscript{v} and AMPA/KARs, at E18. OPCs with these properties may indicate a migratory cell state because we identified a strong expression of migratory genes that then declined postnatally at this time point. The fraction of OPCs with detectable Na\textsubscript{V} and the Na\textsubscript{V} density increase sharply around birth. It is conceivable that OPCs with high Na\textsubscript{V} and K\textsubscript{v} and low AMPA/KAR densities reflect a high proliferation state of OPCs because (1) OPCs in S/G2/M phase have a higher density of Na\textsubscript{V} than OPCs in G\textsubscript{0}/G1 phase (Figure 3H), (2) the proportion of this type of OPC is the most prominent (Figure 1) at the time when the highest proportion of OPCs are in G2/M phase (Figure 3E), (3) EdU-OPCs show this pattern of ion channel expression (Clarke et al., 2012), and (4) this state of OPCs is the most prominent during the OPC recruitment phase (the period of highest proliferation) during myelin regeneration (Gautier et al., 2015). NMDARs are the last to appear at P0 and the only ion channel that disappears within the age range tested. OPCs expressing Na\textsubscript{V}, K\textsubscript{v}, AMPA/KARs, and NMDARs are most prominent at the time of OPC differentiation during development, when myelin gene expression starts (Figure 2; Marques et al., 2018), and at the start of the differentiation phase of myelin regeneration (Gautier et al., 2015) and so may reflect a “primed” OPC state for differentiation. K\textsubscript{v} and AMPA/KAR channels were expressed in nearly all recorded postnatal OPCs. Intriguingly, not all OPCs have Na\textsubscript{V} or NMDARs, but their channel density reaches a maximum after the first postnatal week, at a time when myelination starts and myelin-related genes start to be detected in OPCs, and declines again when the rate of myelination declines and both cell cycle and differentiation gene expression decline. The last state of OPCs represents low Na\textsubscript{V} density, lack of NMDARs, and high AMPA/KAR density and is observed at a time when OPC cell-cycle time lengths, cell-cycle and differentiation genes are downregulated and senescent molecular signature genes appear, and OPCs differentiation potential declines. Thus, perhaps this state represents a “quiescent” state. Although we could not differentiate between G1 and G\textsubscript{0}, the fact that there was a significant difference in variance in AMPA/KAR density (and not in Na\textsubscript{V} or NMDAR densities) in cells recorded in G\textsubscript{0}/G1 phase suggests that AMPA/KAR may differ between G1 and G\textsubscript{0}.

It is possible that these OPC states reflect prior identified subtypes of OPCs (Marques et al., 2016) or different epigenetic states (Liu et al., 2016). Currently, it is not clear whether the detected changes in Na\textsubscript{V} channel, AMPA/KAR, and NMDAR densities drive the age-related changes in OPC behavior nor whether maintaining high densities of NMDARs and Na\textsubscript{V}, and low AMPA/KAR densities in OPCs would counteract the age-dependent decline in myelination. What is clear is that these changes in Na\textsubscript{V} channel, AMPA/KAR, and NMDAR densities will have a profound effect on how OPCs sense neuronal activity and on the effect neuronal inputs will have on OPCs. With increasing understanding of the regulation of gene expression and generation of novel biological engineering technology, it may become possible to manipulate Na\textsubscript{V} channel, AMPA/KAR, and NMDAR densities in OPCs postnatually. However, as postnatal Na\textsubscript{V} channels, AMPA/KAR and NMDAR gene expression is relatively stable; this indicates more that the detected changes in the density of these channels are post-transcriptionally and/or post-translationally regulated. How these changes are regulated is currently unknown, but presumably they are environmentally regulated (Figure S3) although seemingly not easily reversible (Figure 5). How the environment regulates these changes is not known. G protein-coupled receptors such as metabotropic glutamate receptors (mGLuRs) and growth factors have been shown to regulate glutamate receptor expression in OPCs (Gallo et al., 1994; Lundgaard et al., 2013; Zonouz et al., 2011). Moreover, cytokines have been shown to alter AMPAR densities in hippocampal neurons (Stellwagen and Malenka, 2006), and it is possible that these OPC states reflect prior identified subtypes of OPCs (Marques et al., 2016) or different epigenetic states (Liu et al., 2016). Currently, it is not clear whether the detected changes in Na\textsubscript{V} channel, AMPA/KAR, and NMDAR densities drive the age-related changes in OPC behavior nor whether maintaining high densities of NMDARs and Na\textsubscript{V}, and low AMPA/KAR densities in OPCs would counteract the age-dependent decline in myelination. What is clear is that these changes in Na\textsubscript{V} channel, AMPA/KAR, and NMDAR densities will have a profound effect on how OPCs sense neuronal activity and on the effect neuronal inputs will have on OPCs. With increasing understanding of the regulation of gene expression and generation of novel biological engineering technology, it may become possible to manipulate Na\textsubscript{V} channel, AMPA/KAR, and NMDAR densities in OPCs postnatually. However, as postnatal Na\textsubscript{V} channels, AMPA/KAR and NMDAR gene expression is relatively stable; this indicates more that the detected changes in the density of these channels are post-transcriptionally and/or post-translationally regulated. How these changes are regulated is currently unknown, but presumably they are environmentally regulated (Figure S3) although seemingly not easily reversible (Figure 5). How the environment regulates these changes is not known. G protein-coupled receptors such as metabotropic glutamate receptors (mGLuRs) and growth factors have been shown to regulate glutamate receptor expression in OPCs (Gallo et al., 1994; Lundgaard et al., 2013; Zonouz et al., 2011). Moreover, cytokines have been shown to alter AMPAR densities in hippocampal neurons (Stellwagen and Malenka, 2006), and we identified that immune system genes, including cytokine receptors, were upregulated in old OPCs compared with young OPCs. Accordingly, a combination of G-protein-coupled receptors, growth factors, and cytokines may underlie these changes.

In summary, our data show that OPCs are functionally heterogeneous between regions and within regions at any given time point tested. These findings provide evidence of the functional relevance of heterogeneity identified at the transcriptional level. This heterogeneity in physiological properties may underlie the myelination potential of OPCs and implicate different functions or cell states.
STAR METHODS

Detailed methods are provided in the online version of this paper and include the following:

- **KEY RESOURCES TABLE**
- **CONTACT FOR REAGENT AND RESOURCE SHARING**
- **EXPERIMENTAL MODEL AND SUBJECT DETAILS**
- **METHOD DETAILS**
  - Brain slices
  - Solutions
  - Electrophysiology
  - Electrophysiological analysis
  - GDF11 administration
  - Cocultures
  - miRNA-seq libraries
  - Next-generation sequence data analysis
  - Flow cytometry
  - Immunohistochemistry
- **QUANTIFICATION AND STATISTICAL ANALYSIS**
- **DATA AND SOFTWARE AVAILABILITY**
  - Next-generation sequence data
  - Flow cytometry data
  - Electrophysiology analysis scripts

SUPPLEMENTAL INFORMATION

Supplemental Information includes four figures and one table and can be found with this article online at [https://doi.org/10.1016/j.neuron.2018.12.020](https://doi.org/10.1016/j.neuron.2018.12.020).

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AUTHOR CONTRIBUTIONS

Conceptualization, R.T.K. and S.O.S.; Investigation, S.O.S., S.S., Y.K., D.K.-V., O.d.F., K.A.E., and S.A.; Data Analysis, S.O.S., S.S., Y.K., S.D., D.K.-V., K.A.E., and R.T.K.; Writing, R.T.K., S.O.S., Y.K., and K.A.E.; Funding Acquisition, Resources, and Supervision, R.T.K.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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## STAR METHODS

### KEY RESOURCES TABLE

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| **Antibodies**       |        |            |
| Anti-NG2 Chondroitin Sulfate Proteoglycan Antibody | Millipore | Cat# MAB5384; RRID: AB_177646 |
| Anti-GFP antibody    | Abcam  | Cat# ab13970; RRID: AB_300798 |
| Anti-Ki67 antibody [SP6] | Abcam  | Cat# ab16667; RRID: AB_302459 |
| Anti-mCherry antibody | Abcam  | Cat# ab167453; RRID: AB_2571870 |
| CDP Antibody (M-222) (Cux1) | Santa Cruz Biotechnology | Cat# sc-13024; RRID: AB_2261231 |
| Anti-Clip2 antibody [25B6] - ChIP Grade | Abcam | Cat# ab18465; RRID: AB_2064130 |
| Doublecortin Antibody (C-18) | Santa Cruz Biotechnology | Cat# sc-8066; RRID: AB_2088494 |
| Rat anti MBP (aa82-87) | Bio-Rad | Cat# MCA409S; RRID: AB_325004 |
| Monoclonal Anti-Neurofilament 160/200 antibody produced in mouse | Sigma-Aldrich | Cat# N2912; RRID: AB_477262 |
| Alexa Fluor 647 Mouse anti-Ki-67 (Clone B56) | BD PharMingen | Cat# 558615; RRID: AB_647130 |
| Goat Anti-Chicken IgY H&L (Alexa Fluor 488) | Abcam | Cat# ab150169; RRID: AB_2636803 |
| Goat Anti-Chicken IgY H&L (Alexa Fluor 568) | Abcam | Cat# ab175477 |
| Alexa Fluor 647 AffiniPure Donkey Anti-Chicken IgY (IgG) (H+L) | Jackson ImmunoResearch Labs | Cat# 703-605-155; RRID: AB_2340379 |
| Invitrogen Goat anti-Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor 647 | ThermoFisher | Cat# A-21245; RRID: AB_2535813 |
| Goat anti-Rat IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor 647 | ThermoFisher | Cat# A-21247; RRID: AB_141778 |
| Goat anti-Rat IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor 488 | ThermoFisher | Cat# A-11006; RRID: AB_2534074 |
| Goat anti-Mouse IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor 555 | ThermoFisher | Cat# A-21424; RRID: AB_141780 |
| Anti-Goat IgG (H+L), highly cross-adsorbed, CF 568 antibody produced in donkey | Sigma-Aldrich | Cat# SAB4600074 |
| **Chemicals, Peptides, and Recombinant Proteins** |        |            |
| DAPI                 | Sigma-Aldrich | Cat# D9542 |
| Hoescht-33342        | Thermo Fisher | Cat# H3570 |
| Kainic Acid          | Tocris   | Cat# 0222  |
| NMDA                 | Tocris   | Cat# 0114  |
| Strychnine hydrochloride | Sigma-Aldrich | Cat# S8753 |
| Glycine              | Sigma-Aldrich | Cat# G8898 |
| Barium chloride dihydrate | Sigma-Aldrich | Cat# B0750 |
| NaCl                 | Sigma-Aldrich | Cat# S7653 |
| KCl                  | Sigma-Aldrich | Cat# P3911 |
| NaHCO₃               | Sigma-Aldrich | Cat# S761 |
| NaH₂PO₄              | Fisher Scientific | Cat# S/3760/53 |
| CaCl₂                | VWR      | Cat# 21114 |
| MgCl₂                | Fisher Scientific | Cat# 15656060 |
| D-glucose            | Sigma-Aldrich | Cat# G7528 |
| Kynurenic acid       | Sigma-Aldrich | Cat# K3375 |
| HEPES                | Sigma-Aldrich | Cat# H3375 |
| BAPTA                | Sigma-Aldrich | Cat# A4926 |
| D-gluconic acid      | Sigma-Aldrich | Cat# G1951 |

(Continued on next page)
| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| CsOH                | Sigma-Aldrich | Cat# 516988 |
| KOH                 | Sigma-Aldrich | Cat# P5958  |
| MgATP               | Sigma-Aldrich | Cat# A9187  |
| Na₂GTP              | Sigma-Aldrich | Cat# G8877  |
| K-Lucifer Yellow    | Sigma-Aldrich | Cat# L0144  |
| rGDF11              | PeproTech   | Cat# 120-11 |
| Fixation Buffer     | BioLegend   | Cat# 420801 |
| Intracellular Staining Permeabilization Wash Buffer | BioLegend | Cat# 421002 |
| Papain from papaya latex | Sigma-Aldrich | Cat# P3125 |
| L-cysteine          | Sigma-Aldrich | Cat# 30089 |
| Deoxyribonuclease I from bovine pancreas, Type IV | Sigma-Aldrich | Cat# D5025 |
| Albumine from bovine serum | Sigma-Aldrich | Cat# A4919 |
| Trypsin inhibitor from Glycine max (soybean) | Sigma-Aldrich | Cat# T9003 |
| Goat serum          | Sigma-Aldrich | Cat# G9023  |
| Donkey serum        | Sigma-Aldrich | Cat# D9663  |
| Triton X-100        | Fisher BioReagents | Cat# BP151 |
| Parafomaldehdy      | Fisher Scientific | Cat# P/0840/53 |

Critical Commercial Assays

| Myelin Removal Beads II, human, mouse, rat | Miltenyi Biotec | Cat# 130-096-733 |
| CD140a (PDGFRα) MicroBead Kit, mouse | Miltenyi Biotec | Cat# 130-101-502 |
| RNAeasy Micro Kit | QIAGEN | Cat# 74004 |
| SMARTer Stranded Total RNA-Seq Kit v2 – Pico Input | Takara Clontech | Cat# 634411 |

Deposited Data

| Raw data files for RNA sequencing | This manuscript | GEO: GSE121083 |
| Raw data files for Flow cytometry | This manuscript | https://flowrepository.org/id/7lFvFrXKqGrE6oSqbz3pUcrG4hhB4nIcmH5nBR0azMBaD8v7TJRxw08B6bjNgEFkr |

Experimental Models: Organisms/Strains

| Mouse: NG2-EYFP | Prof Jacqueline Trotter; Karram et al., 2008 | N/A |
| Mouse: Fucci2a | Mort et al., 2014 | IMSR Cat# RBRC06511, RRID: IMSR_RBRC06511 |
| Mouse: STOCK Mki67tm1.1Cle/J | Basak et al., 2014 | IMSR Cat# JAX 029802, RRID: IMSR_JAX:029802 |
| Mouse: C56BL/6 wild-type | Charles River Laboratories | C57BL/6N Crl, RRID: IMSR_CRL:27 |

Software and Algorithms

| ImageJ | NIH | https://ijl.sc/ or https://imagej.nih.gov/ij/ |
| LAS AF/LAS X | Leica | https://www.leica-microsystems.com/products/microscope-software/details/product/leica-las-x-ts/ |
| MATLAB | MathWorks | https://uk.mathworks.com/ |
| Cell capacitance analysis | Written in house | N/A |
| Na⁺ current analysis | Written in house | N/A |
| K⁺ conductance analysis | Written in house | N/A |
| SPSS | IBM | https://www.ibm.com/analytics/spss-statistics-software |
| GraphPad Prism | GraphPad Software | https://www.graphpad.com/scientific-software/prism/ |
CONTACT FOR REAGENT AND RESOURCE SHARING

Further information and requests for resources and reagents should be directed to, and will be fulfilled by, the Lead Contact, Ragnhildur T. Káradóttir (rk385@cam.ac.uk).

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Experiments were performed in accordance with EU guidelines for the care and use of laboratory animals, and with the guidelines of the UK Animals (Scientific Procedures) Act 1986 and subsequent amendments. Use of animals in this project was approved by the Animal Welfare and Ethical Review Body for the University of Cambridge and carried out under the terms of UK Home Office Licenses P9B1FBC4B and 70/7715. All mice were maintained under a 14 h light:10 h dark cycle with food and water supplied ad libitum. For electrophysiological studies and cell cycle studies, mice were heterozygous knock-in mice (Karram et al., 2008) expressing EYFP under the endogenous NG2 promoter, kindly provided by Jacqueline Trotter, allowing for identification of OPCs by EYFP expression. The ages of mice used were from E13 to P503 as stated in text and figures. These mice were bred on a C57BL/6 background. To assess electrophysiological properties of different cell cycle states in OPCs we crossed Fucci2a mice, which labels different cell cycle phases (Mort et al., 2014), or Ki67-RFP mice (Basak et al., 2014) to the NG2-EYFP mice to generate NG2-EYFP:FUCCI and NG2-EYFP:Ki67-RFP mouse lines. Fucci2a mice were bred on a mixed CBA/Ca and C57BL/6 background, while Ki67-RFP mice were bred on a C57BL/6 background. For all three lines, both male and female mice were used. For flow cytometry experiments, male and female C57BL/6 NG2-EYFP mice were used. For OPC transcriptome analysis C57BL/6 wild-type mice (Charles River Laboratories) were used; all mice were female, except for the embryonic and P12 time points, where full litters were used and equal number of animals per sex was assumed. The mice for the GDF11 infusion experiment were all male C57BL/6 NG2-EYFP mice.

METHOD DETAILS

Brain slices

Acute brain slices (225 μm thick) were prepared either from the cerebellum (parasagittally cut) or the forebrain (coronally cut) from transgenic mice in ice-cold (~1°C) oxygenated (95% O2–5% CO2) aCSF solution and kept at RT after a 1h recovery period. aCSF contained (in mM): 126 NaCl, 24 NaHCO3, 1 NaH2PO4, 2.5 KCl, 2.5 CaCl2, 2 MgCl2, 10 D-glucose, pH 7.4. 1mM Kynurenic acid was added to block glutamate receptors, which might be activated during the dissection procedure. For embryonic acute brain slices, the brains were embedded in agarose and sliced coronally in 225 μm thick sections as above.

Solutions

Slices were superfused at 22 ± 1°C with HEPES-buffered external solution containing (in mM): 144 NaCl, 2.5 KCl, 10 HEPES, 1 NaH2PO4, 2.5 CaCl2, 10 glucose, 0.1 glycine (to coactivate NMDARs), 0.005 strychnine (to block glycine receptors), 200μM BaCl2 (only in some instances to block potassium conductance), pH set to 7.4 with NaOH, continuously bubbled with 100% O2. OPCs were whole-cell clamped with electrodes containing a recording solution that comprised (in mM) of either 130 K-gluconate or 130 Cs-gluconate, 4 NaCl, 0.5 CaCl2, 10 HEPES, 10 BAPTA, 4 MgATP, 0.5 Na2GTP, 2 K-Lucifer yellow, pH set to 7.3 with KOH (or with CsOH). Final osmolarity was ~290mOsm. For activation of glutamate receptors, KA 30 μM (activates both AMPA and KA receptor; Tocris) or NMDA 60 μM (Tocris) was bath applied.
Electrophysiology
For whole-cell patch-clamp experiments parenchymal EYFP positive OPCs were selected in the brain area of interest (EYFP positive cells located close to or on blood vessels were excluded). In embryonic slices, OPCs were selected in the developing CTX at a sufficient distance from nearby blood vessels were excluded. When unspecified CTX OPCs were mainly recorded in cortical layers 5-2. When unspecified, OPCs in the CC were in and around the anterior CC. Patched cells were confirmed as OPCs by their post-recording dye-fill morphology, which overlaid EYFP, and by post hoc antibody labeling against the proteoglycan NG2 to identify oligodendrocyte progenitors (106/107 tested labeled for NG2 (Millipore) or GFP/EYFP (Abcam)). For identification of OPCs location: CTX Layer 1 was identified as the area between the edge of the slice and layer 2 marked with Cux1 antibody labeling; CTX layer 5/6 was marked with CTIP2 immunoreactivity; dorsal SVZ was identified with doublecortin labeling and the adjacent ventricle; anterior CC was identified at bregma 0 and posterior CC was identified overlying the hippocampus (Figures S4A–S4D). Recording electrodes had a resistance of 5–9 MΩ and the uncompensated series resistance was 40 ± 1 MΩ. Inclusion criteria was based on series resistance, leak current being lower than 500pA and a stable baseline. Electrode junction potential (~14 mV) was compensated. A Multiclamp 700B (Molecular Devices) or Axopatch 200 (Molecular Devices) was used for voltage clamp data acquisition. Data were sampled at 50 kHz and filtered at 10 kHz using pClamp10.3 (Molecular Devices).

Electrophysiological analysis
Synaptic events were detected based on threshold (amplitude > 2xSD) and included when decay time τ (10%–90%) was longer than its rise time. For event detection and analysis of frequency, amplitude and decay time τ pClamp 10.3 (Molecular Devices) and the Strathclyde Electrophysiology Software package WinEDR V3.3.7 WinWCP V4.6.2 were used. Voltage-gated ion channels, series resistance, and membrane capacitance was analyzed using custom-written MATLAB scripts (MathWorks).

GDF11 administration
EYFP-NG2 knock-in mice were implanted intraperitonealy with osmotic minipumps (Alzet Micro-Osmotic Pumps, model 1004, DURECT Corporation) at P150 for continuous i.p. delivery at a flow rate of 0.11 μl/h containing GDF11 (0.1mg/kg/d; rGDF11, catalog # 120-11, PeproTech) or saline. After 4-weeks delivery mice were used for electrophysiological recordings, only cells responding to an agonist were used for comparisons, the proportion of cells responding did not differ between conditions. The use of minipumps ensured reduced stress responses of the animals and consistent dosing compared to i.p. injections which require daily handling.

Cocultures
Mouse OPCs were isolated from EYFP-NG2 knock-in mice at different ages, as stated in manuscript, using magnetic cell sorting (MACS, Miltenyi Biotec). First myelin debris were removed from single-cell suspension by myelin removal beads (#130-096-733, Miltenyi), and subsequently, OPCs were isolated using microbeads conjugated to monoclonal PDGFRα (#130-101-502, Miltenyi). Myelinating co-cultures were made as previously described (Lundgaard et al., 2013). Briefly, dorsal root ganglion neurons were derived from E14–E16 rats and allowed to grow for 2 weeks before OPCs were plated on top. Myelination was analyzed after 3 weeks of co-culture in a non-blinded manner. Coverslips were imaged with an epifluorescence microscope and 3-4 images (depending on cell density) were randomly taken in each quadrant of the coverslip, with replicates of at least 4 coverslips. On coverslips where less than 5 MBP+ cells were detected (as in some coverslips carrying co-cultures with ‘old’ OPCs), each MBP+ cell was imaged individually. All MBP+ cells on the coverslip were counted and compared between ages (young: < P7; old: 8-24months). Cells were considered myelinating when there was a clear ensheathment of axons in an area around the cell soma. Cells merely attached to axons without elongated myelin sheaths in nearby axons or cells which produced sheaths without wrapping them around axons were considered as non-myelinating.

mRNA-seq libraries
All the mice used were wild-type C57BL/6 mice (Charles River Laboratories) at the ages detailed in the manuscript. OPCs were isolated as described above for co-cultures, except from the E15.5 and P12 time points, where the myelin removal step was omitted. For the E15.5 and P12 time-points, each sample (n = 3) was a pool of 2-3 brains, all other time-points were isolated from one brain per sample (n = 3, n = 4 for P306-308). After OPC enrichment RNA was extracted using RNeasy Micro Kit (QIAGEN) according to manufacturer’s instructions. The library was prepared with SMARTer Stranded Total RNA-Seq Kit v2 - Pico Input Mammalian (Takara Clontech) according to manufacturer’s instructions. Data was read on a HiSeq4000 a total of > 350M 150 bp strand-specific paired-end reads were generated.

Next-generation sequence data analysis
RNA-seq reads were pre-processed and quality-trimmed using Trim Galore! (https://www.bioinformatics.babraham.ac.uk/projects/trim_galore). Reads were aligned to the mouse reference genome GRCm38/mm10 using Tophat (version 2.1.1, https://ccb.jhu.edu/software/tophat/index.shtml) allowing for one mismatch and selecting uniquely mapping reads only. Normalization was performed and differential expression of genes was statistically evaluated by using the R Bioconductor DESeq2 package (https://bioconductor.org/packages/release/bioc/html/DESeq2.html).
Gene ontology (GO) term enrichment analysis for differentially expressed genes was performed using DAVID (https://david.ncifcrf.gov). GO terms were represented according to their semantic similarity using REVIGO (http://revigo.irb.hr).

**Flow cytometry**

NG2-EYFP mice were used for cell cycle analysis in the NG2 population, at the ages detailed in the manuscript. For animals older than three weeks, myelin debris were removed from single-cell suspensions with myelin removal beads (#130-096-733, Miltenyi Biotec) according to manufacturer’s instructions. Cells were fixed in Fixation Buffer (BioLegend) for 15 min, permeabilized with Intracellular Staining Permeabilization Wash Buffer (BioLegend) for 10 min. After washing, cells were stained with DAPI (1 μg/ml) for 10 min. Cells were washed again and acquired on a BD LSRFortessa flow cytometer (BD Bioscience) equipped with four lasers, a blue (488-nm), a red (640-nm), a violet (405-nm) and a yellow/green (561-nm) laser. The EYFP positive cells were detected by filtration through a 530/30 nm band pass (BP) filter and DAPI through a 450/50 nm BP filter. Analysis was performed with FCS Express 6 Flow (De Novo Software) (Figure S2).

**Immunohistochemistry**

225 μm thick slices were cut as described above, and fixed in 4% paraformaldehyde. Antibody labeling was performed as described previously (Káradoíttir and Attwell, 2006). Briefly, slices were incubated in permeabilization and blocking solution (10% goat or donkey serum, 0.5% Triton X-100, in PBS) for 4-6 hours. Slices were incubated with primary antibodies, in PBS, overnight. After washing, slices were incubated with secondary antibodies, in PBS, overnight at 4°C. Following washing and DAPI staining, slices were mounted and imaged on a Leica SP5 confocal microscope.

To measure proliferation with immunohistochemistry, 3-6 slices from an average of 2 animals were quantified for each time point. Images were taken on a confocal microscope. 6 z stacks (3 in the cortex and 3 in the CC) were taken randomly in each slice. Z stacks were taken in order to include all EYFP+ cells in a field of view. Analysis was performed manually on the maximum projection of each stack, with the number of EYFP+ Ki67+ cells divided by the number of EYFP+ cells. Experimenters were not blind to age during quantification. All data collected was included in the statistical analysis, which was performed with a one-way ANOVA for each brain region.

**QUANTIFICATION AND STATISTICAL ANALYSIS**

All statistical analysis was performed in GraphPad Prism or SPSS software or manually calculated. Data are shown as mean ± s.e.m. For electrophysiology results, numbers of cells are indicated on bars. For flow cytometry analysis (Figure 3), number of animals are indicated on bars. Individual coculture numbers are indicated on the bar graph for coculture experiments (Figure 5). When relevant, normality of data was assessed using Shapiro–Wilk tests. Non-parametric tests that do not assume data follow a normal distribution gave the same conclusions for significant and non-significant differences in all cases. Significance in the variance between dataset was assessed using F-test or Brown–Forsythe test. One-way ANOVA, or Welch’s ANOVA when variances were unequal, followed by Holm–Bonferroni-corrected post hoc t tests were used to compare multiple samples. Other P values for comparison of means are from Student’s two-tailed t tests, with Welch’s correction when variances were unequal. Differences in percentages between age groups were analyzed by Chi-square test with Yates’s correction for small n-numbers.

**DATA AND SOFTWARE AVAILABILITY**

**Next-generation sequence data**

All data have been deposited in NCBI’s Gene Expression Omnibus (Edgar et al., 2002) and are accessible through GEO Series accession number GSE121083 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE121083).

**Flow cytometry data**

All flow cytometry data has been deposited in Flowrepository.org (https://flowrepository.org/id/RvRxKqGrE6oSqb3pUcrGHhBsNLcmH5nBR0azMBaDS8vTjRXww0B6ibjNgEFlr)

**Electrophysiology analysis scripts**

Cell capacitance, Na \(_V\) current, K \(_V\) current and K\(^+\) conductance analysis scripts are available upon request.