Development/Plasticity/Repair

Properties and Fate of Oligodendrocyte Progenitor Cells in the Corpus Callosum, Motor Cortex, and Piriform Cortex of the Mouse

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Oligodendrocyte progenitor cells (OPCs) in the postnatal mouse corpus callosum (CC) and motor cortex (Ctx) reportedly generate only oligodendrocytes (OLs), whereas those in the piriform cortex may also generate neurons. OPCs have also been subdivided based on their expression of voltage-gated ion channels, ability to respond to neuronal activity, and proliferative state. To determine whether OPCs in the piriform cortex have inherently different physiological properties from those in the CC and Ctx, we studied acute brain slices from postnatal transgenic mice in which GFP expression identifies OL lineage cells. We whole-cell patch clamped GFP-expressing (GFP^+) cells within the CC, Ctx, and anterior piriform cortex (aPC) and used prelabeling with 5-ethynyl-2 -deoxyuridine (EdU) to assess cell proliferation. After recording, slices were immunolabeled and OPCs were defined by strong expression of NG2. $NG2^+$ OPCs in the white and gray matter proliferated and coexpressed PDGFRα and voltage-gated Na^+ channels (I_{Na}). Approximately 70% of OPCs were capable of generating regenerative depolarizations. In addition to $OLIG2^+$ $NG2^+$ $I_{Na}{}^+$ OPCs and $OLIG2^+$ $NG2^{neg}$ $I_{Na}{}^{neg}$ OLs, we identified cells with low levels of NG2 limited to the soma or the base of some processes. These cells had a significantly reduced I_{Na} $_{Na}$) and potassium channels, which are downregulated when OPCs differentiate to form OLs (De Biase et al., 2010; Kukley et al., 2010). On the other hand, NG2-expressing ($NG2^+$) OPCs in the rat cerebellar white matter fall into two classes: one that receives synaptic input, expresses I_{Na}, and can generate action potentials and another that lacks these attributes (Káradóttir et al., 2008). Voltage-gated ion channel expression and spiking behavior also reportedly differ between white and gray matter OPCs (Chittajallu et al., 2004). Furthermore, perinatal OPCs have been shown to produce OLs and astrocytes *in vivo*, whereas postnatal OPCs generate only OLs, except in the piriform cortex in which they have been reported to also produce some neurons (Dayer et al., 2005; Tamura et al., 2007; Rivers et al., 2008; Guo et al., 2010; Zhu et al., 2011).

Despite reports of neurogenesis in the piriform cortex (Bernier et al., 2002; Pekcec et al., 2006; Shapiro et al., 2007; Arisi

2007, 2008, 2010; Ziskin et al., 20
regenerative action potential-like
al., 2004; Kaŕadoťtir et al., 2008; G
this signaling is uncertain, but
OPC migration and differentiatio
2006; Tong et al., 2009). Furthe

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et al., 2012), the source of the new neurons is contentious, with the main candidates being neural stem cells and OPCs. In many brain regions, OPCs express doublecortin (DCX) (Tamura et al., 2007; Guo et al., 2010; Ehninger et al., 2011), a marker of migratory neuroblasts, but those in the piriform cortex also express the neuroblast marker polysialylated neural cell adhesion molecule (PSA-NCAM) and the neural precursor markers Sox2 and Pax6 (Seki and Arai, 1999; Hayashi et al., 2001; Nacher et al., 2002; Pekcec et al., 2006; Shapiro et al., 2007; Bullmann et al., 2010; Guo et al., 2010), suggesting that they might be a functionally distinct cell population.

We report that OPCs in the mouse corpus callosum (CC), motor cortex (Ctx), and anterior piriform cortex (aPC) have similar membrane properties: they express I_{Na} but do not generate bona fide action potentials. By combining 5-ethynyl-2 -deoxyuridine (EdU) administration with transgenic lineage tracing, we demonstrate that OPCs within the postnatal forebrain proliferate and generate OLs but do not generate neurons at any time that we examined after postnatal day 25 (P25).

Transgenic mice. *Pdgfrα–H2BGFP* knock-in mice (Hamilton et al., 2003), referred to as *Pdgfrα–GFP* mice, were purchased from The Jackson Laboratory (line B6.129S4–*Pdgfrα*$^{tm11(EGFP)Sor}$/J). These mice have a histone–GFP fusion gene knocked into the *Pdgfrα* locus, resulting in nuclear labeling of PDGFRα-expressing cells, including OPCs. *Pdgfr*

manufacturer. Slices were washed once with PBS before immunolabeling for NG2 (see above).

For floating cryosections, the EdU labeling was developed immediately after immunolabeling, because some antibodies failed if the order was reversed. Floating cryosections were incubated at 21°C for 15 min in PBS with 0.5% (v/v) Triton X-100, transferred to the EdU developing mixture, incubated in the dark at 21°C for 40 min, washed three times in PBS, post-stained with Hoechst 33258 (1:1000; Sigma) to visualize cell nuclei, and mounted under coverslips in fluorescence mounting medium (Dako). Unlike BrdU detection, EdU detection does not require antigen retrieval protocols.

Microscopy and cell counts. All images were collected on an Ultra-

fied after subtracting the linearly scaled capacity transient and ohmic leak current that was evoked by a hyperpolarizing pulse (Fig. 2, bottom traces). The resulting peak inward current represents a combination of I_{Na} and I_K. To assess how the overlap of these currents alters the apparent magnitude of the voltage-gated Na^+ current, TTX was applied to some cells to isolate I_K (Fig. 3*a*). Subtraction of I_K in these cells from the total inward current revealed a 2.5-fold increase in the amplitude of the net inward current ($n = 3$) (Fig. 3*a*).

It has been reported that some OPCs can fire action potentials in response to depolarization (Chittajallu et al., 2004; Káradóttir et al., 2008; Ge et al., 2009). In our present study, depolarizing current injection resulted in passive membrane responses in I_{Na}^{neg} GFP

Individual GFP$^+$ cells in the CC, Ctx, and aPC were whole-cell patch clamped to determine whether or not they exhibited I_{Na} and then dye filled with Alexa Fluor-568. Dye filling not only revealed the cellular morphology but also permitted identification of the recorded cell after NG2 immunolabeling. Three categories of dye-filled cells were identified: (1) cells expressing NG2 strongly (NG2$^+$ cells) that we define to be OPCs (Fig. 5*a*,*c*,*e*), (2) cells expressing low levels of NG2 limited to the soma or the base of some processes (NG2^{low} cells) that were presumed to be early differentiating OLs (Fig. 5*g*), and (3) cells with no detectable NG2 (NG2^{neg} cells) that were assumed to be OLs (Fig. 5*i*).

At both P9 and P33 and in all brain regions examined, we found that the NG2$^+$ GFP$^+$ cell population was the same as the eged brain (2j/F41Tf7006411.464888.7173311.9(filled)Tf9.5009.5419.517514

change in cell capacitance (a measure of cell size) could be detected with age (Fig. 5*m*). OPCs were consistently larger in gray matter than in white matter, regardless of age (Fig. 5*m*). The resting membrane potential of OPCs ranged from between approximately −85 and −94 mV: at P9, the resting potential of OPCs was −94 3.8, −93 2.6, and −92 1.6 mV in the CC ($n = 14$), Ctx ($n = 14$), and aPC ($n = 10$), respectively, whereas at P33, the resting potential of OPCs was −89 1.9, −

population and to differentiate over time, providing a source of new projection neurons (Rivers et al., 2008; Guo et al., 2010). To assess the electrophysiological properties of dividing oligodendrogenic OPCs and the nondividing putative neurogenic OPCs, we administered the thymidine analog EdU to *Pdgfrα–GFP* mice for 5 d before making patch-clamp recordings at P9 and for 12–13 d before making patch-clamp recordings at P33. In addition to NG2 immunolabeling, slices were processed to detect EdU. These dosing regimens were selected to label the proliferating OPCs in the white and gray matter of the brain, which were predicted from Psachoulia et al. (2009) to be approximately half of all OPCs. Unexpectedly, given this prediction, the vast majority of GFP^+, $NG2^+$, $I_{Na}{}^+$ OPCs that we analyzed were EdU^+, regardless of brain region or age. At P9, 90% of $NG2^+$ I

White matter OPCs have been reported to receive ~140 synapses (Kukley et al., 2007), for each of which the miniature EPSC at the resting potential is ~6 pA (Kukley et al., 2007, 2010), implying that 10 simultaneous EPSCs would be needed to trigger this response *in vivo* (60 pA/6 pA). This is likely to happen more frequently during development when synchronized neuronal firing occurs in the cortex (Mao et al., 2001), hippocampus (Mohns and Blumberg, 2008), cerebellum (Watt et al., 2009), and retina (Meister et al., 1991; Demas et al., 2003). However, whether or not full action potentials are produced by mouse OPCs *in vivo*,

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