

# Competing waves of oligodendrocytes in the forebrain and postnatal elimination of an embryonic lineage

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The developmental origin of oligodendrocyte progenitors (OLPs) in the forebrain has been controversial. We now show, by *Cre-lox* fate mapping in transgenic mice, that the first OLPs originate in the medial ganglionic eminence (MGE) and anterior entopeduncular area (AEP) in the ventral forebrain. From there, they populate the entire embryonic telencephalon including the cerebral cortex before being joined by a second wave of OLPs from the lateral and/or caudal ganglionic eminences (LGE and CGE). Finally, a third wave arises within the postnatal cortex. When any one population is destroyed at source by the targeted expression of diphtheria toxin, the remaining cells take over and the mice survive and behave normally, with a normal complement of oligodendrocytes and myelin. Thus, functionally redundant populations of OLPs compete for space in the developing brain. Notably, the embryonic MGE- and AEP-derived population is eliminated during postnatal life, raising questions about the nature and purpose of the competition.

Different subclasses of neurons and glia are generated sequentially in different parts of the ventricular zones of the developing spinal cord and brain. For example, spinal motor neurons and oligodendrocytes (OLs, the myelin-forming cells) are derived from precursors that reside in a specialized domain of the ventral ventricular zone called motor neuron precursors (pMN), defined by expression of the transcription factor *Olig2* (refs. 1–4). From there, OLPs migrate all through the spinal cord before differentiating into myelin-forming OLs. Later, at least one additional source of OLPs arises in the dorsal spinal cord, contributing 10–15% of the final OL population<sup>5–7</sup>. OLP generation in the pMN depends on the signaling molecule Sonic hedgehog (Shh), whereas the dorsal source might be Shh independent<sup>5–9</sup>.

OL generation in more anterior parts of the neural tube—particularly the forebrain—is not as well understood. The neuroepithelium of the MGE and the AEP in the ventral forebrain expresses Shh and its receptor Patched (Ptc) as well as *Olig2*, suggesting that OLPs might be formed primarily in these regions<sup>10–12</sup>. Indeed, migratory OLPs, defined by the expression of platelet-derived growth factor receptor- $\alpha$  (*Pdgfra*), can be seen streaming away from the MGE and AEP in all directions after embryonic day 12 (E12) in the mouse, apparently entering the developing cerebral cortex (dorsal telencephalon) around E16 (refs. 11,13). The number of OLPs in the cortex increases markedly between E16 and birth (~E18), but it is not known whether this reflects continuing inward migration and proliferation of ventrally derived OLPs or a new source of OLPs within the cortex. There is some evidence on both sides. For example, some OLPs in the cortex express the transcription factors *Dlx1* and *Dlx2*, suggesting that they originate in the ventral telencephalon where *Dlx1* and *Dlx2* are first activated<sup>14,15</sup>. Moreover, in chick

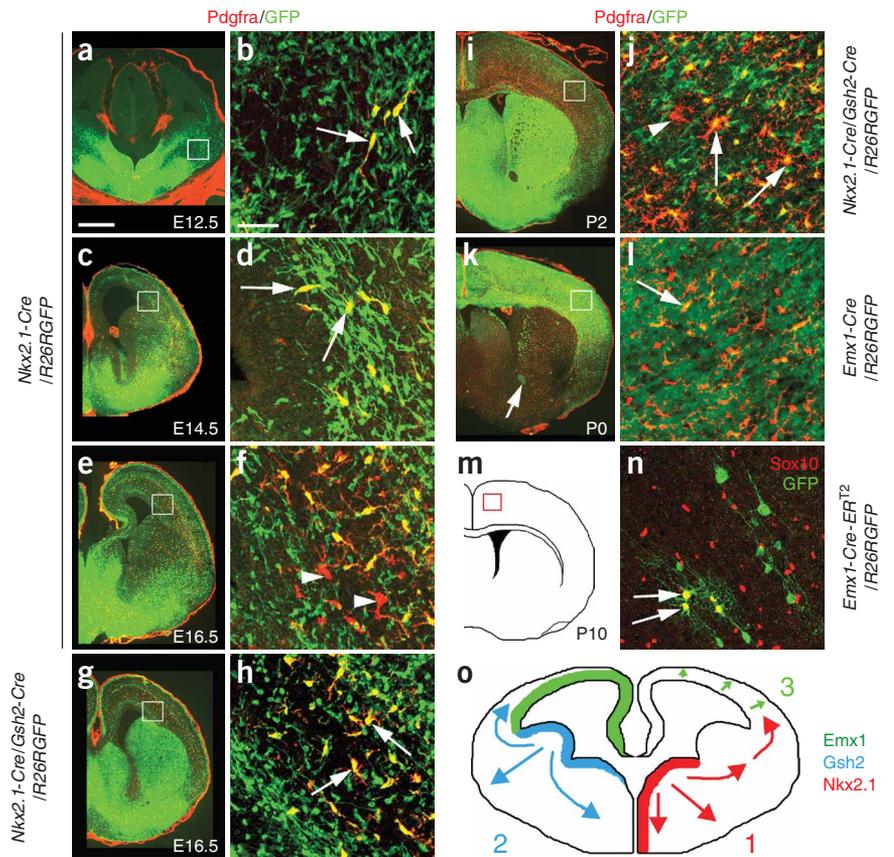
embryos, the expression of the oligodendrocyte-lineage markers myelin proteolipid protein (*Plp-Dm20*) and the O4 antigen is initially restricted to the AEP<sup>16</sup>, and chick-quail grafting experiments indicate that all cortical OLs are ventrally derived in birds<sup>17</sup>. In contrast, cell fate mapping using *Emx1-Cre* transgenic mice suggests that many OLs in the cortex are generated locally<sup>18</sup>. Furthermore, retroviral fate mapping demonstrates that cortical OLs continue to be formed in the postnatal period from precursors in the subventricular zones (SVZ) located at the tips of the lateral ventricles at the corticostriatal boundary<sup>19,20</sup>. It is important to resolve this uncertainty because OLs with different developmental origins, perhaps specified via different signaling pathways, might have distinct properties and functions in the mature brain.

To resolve the question of OL origins, we used the *Cre-lox* approach in transgenic mice to follow the development of distinct ventral or dorsal precursor populations in the telencephalon. We found that the development of OL lineage cells in the forebrain is unexpectedly complex and dynamic. There was an early wave of OLP generation from *Nkx2.1*-expressing precursors in the ventral forebrain; these first appeared in the ventricular zone of the ventral MGE and AEP around E12.5 and subsequently migrated widely into all parts of the telencephalon, entering the cerebral cortex after E16. At first they were the only OLPs in the forebrain, but soon their contribution to the total OLP population declined. By postnatal day 10 (P10), there were very few *Nkx2.1*-derived OLPs or OLs in the cortex, although they were still the major population in the ventral (subpallial) region. Instead, they were overtaken in the cortex by other populations of OLPs derived first from *Gsh2*-positive precursors in the LGE and CGE and later from endogenous *Emx1*-positive cortical precursors. Even in the ventral

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Received 7 September; accepted 30 November; published online 25 December 2005; doi:10.1038/nn1620

**Figure 1** Three successive waves of OLs generated from distinct precursor populations at different times during forebrain development. **(a,b)** Generation of the first wave of *Pdgfra*<sup>+</sup> OLs began at E12.5 from *Nkx2.1*-expressing precursors in the MGE-AEP. These OLs expressed GFP (green) in *Nkx2.1-Cre/Rosa26R-GFP* embryos (arrows in **b**). **(c,d)** By E14.5, *Pdgfra*<sup>+</sup> GFP<sup>+</sup> cells began to appear at the corticostriatal boundary in *Nkx2.1-Cre/Rosa26R-GFP* embryos (arrows in **d**), demonstrating migration of MGE-AEP-derived OLs into the developing cortex. **(e,f)** By E16.5, a new wave of (*Pdgfra*<sup>+</sup>, GFP<sup>+</sup>) OLs was observed in *Nkx2.1-Cre/R26R-GFP* cortex; these must have been generated from *Nkx2.1*-precursors (arrowheads in **f**). **(g,h)** All *Pdgfra*<sup>+</sup> cells in the telencephalon coexpressed GFP in *Nkx2.1-Cre/Gsh2-Cre/Rosa26R-GFP* triple transgenic embryos (arrows in **h**), indicating that all OLs were derived from the ventral forebrain (MGE-AEP and LGE-CGE) at this stage. **(i,j)** Another wave of (*Pdgfra*<sup>+</sup>, GFP<sup>+</sup>) OLs appeared in the telencephalon of early postnatal *Nkx2.1-Cre/Gsh2-Cre/Rosa26R-GFP* mice (arrowheads in **j**). **(k,l)** *Emx1*-expressing cortical precursors began to generate OLs and OLs after birth (arrows in **l**). **(m,n)** Tamoxifen administered once to pregnant *Emx1-Cre-ER<sup>T2</sup>* females at E9.5, before invasion of the cortex by ventrally derived OLs, activated Cre recombination in the embryos and resulted in the expression of the GFP reporter in a subset of Sox10<sup>+</sup> OLs (arrows in **n**)—which must therefore have been generated from endogenous cortical precursors. **(o)** Model illustrating our conclusion that three sequential waves of OLs were generated from different parts of the telencephalic ventricular zone: (i) from *Nkx2.1*-expressing precursors starting at E12.5; (ii) from *Gsh2*-expressing LGE-CGE precursors starting at E15.5; and (iii) from *Emx1*-expressing cortical precursors starting around birth (P0). Scale bars: **(a,c,e,g,i,k)**, 500 μm; **(b,d,f,h,j,l,n)**, 60 μm.



forebrain, close to their original source, the *Nkx2.1*-derived OLs and OLs were gradually lost and replaced by other populations and were almost completely eliminated from the adult forebrain. These findings help to integrate the plethora of previous studies that report either ventral, dorsal or multiple OL origins<sup>10–24</sup>.

Our results raise the question of whether the different OL lineages from MGE-AEP, LGE-CGE and cortex are functionally specialized. To address this, we devised a genetic ablation strategy to eliminate each of the three OL populations separately with diphtheria toxin A fragment (DTA; ref. 25). We found that when we killed any one of the populations, the effect was benign: adjacent populations spread into the vacant territory, a normal distribution of OLs was restored and the mice developed and survived normally. Therefore, we have not, so far, found evidence of functional heterogeneity among different OL lineages in the forebrain. Our data demonstrate that different regional subpopulations of OLs compete for space in the developing brain. This might contribute to the ultimate demise of the embryonic MGE-AEP (*Nkx2.1*)-derived OL population.

## RESULTS

### The first OLs are derived from the MGE-AEP

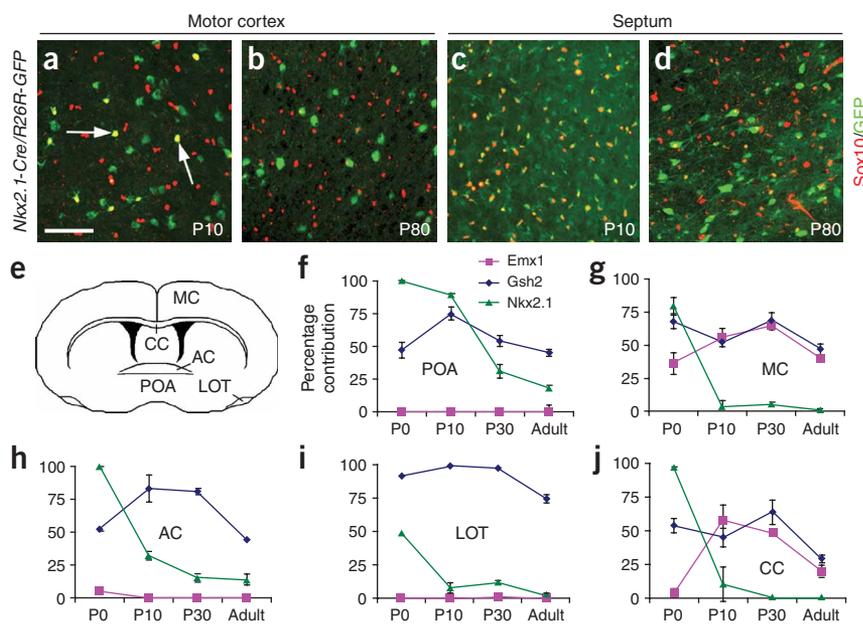
Previous immunohistochemical and transplant studies suggested that there is at least one source of OL lineage cells in the ventral forebrain (MGE and AEP)<sup>11,13–17,21,22</sup>. To directly test whether OLs are generated in the ventral telencephalon, we generated a P1-derived artificial chromosome (PAC) transgenic mouse that expressed Cre recombinase under the transcriptional control of *Nkx2.1*, which encodes a

homeodomain transcription factor expressed in the MGE, septum, AEP, preoptic area and other more posterior ventral forebrain regions. Expression of the Cre transgene faithfully recapitulated the endogenous *Nkx2.1* pattern (**Supplementary Fig. 1** online). We crossed the *Nkx2.1-Cre* mice to the Cre-dependent *Rosa26R-GFP* reporter line and examined the embryonic offspring for the presence of *Pdgfra*-positive (*Pdgfra*<sup>+</sup>) OLs that also expressed green fluorescent protein (GFP), indicating that they must have originated from *Nkx2.1*-expressing precursors.

In most parts of the telencephalon, the GFP<sup>+</sup> *Pdgfra*<sup>+</sup> OL population was only a small fraction of all GFP<sup>+</sup> cells. In the cortex, the majority of GFP<sup>+</sup> cells represent GABAergic interneurons that are known to originate in the MGE (refs. 26–28). The first GFP<sup>+</sup> *Pdgfra*<sup>+</sup> OLs appeared in the ventral MGE and AEP at E11.5–E12.5 (**Fig. 1a,b**) and gradually spread throughout the telencephalon in a ventral-to-dorsal manner. Until E14.5, the great majority of *Pdgfra*<sup>+</sup> OLs in the ventral telencephalon as well as in the cerebral cortex coexpressed GFP, indicating that the main source of OLs in the embryonic forebrain lay within the *Nkx2.1*-expressing neuroepithelium (**Fig. 1c,d**). However, by E16.5, a new population of GFP-negative (GFP<sup>-</sup>) *Pdgfra*<sup>+</sup> OLs appeared, mainly within the cortical intermediate zone (arrowheads, **Fig. 1e,f**). These GFP<sup>-</sup> OLs must have originated outside the MGE-AEP.

### Subsequent waves of OLs from the LGE-CGE and cortex

Another region of the telencephalon that expresses *Olig2* and seems likely to generate OLs is the LGE. To fate map the LGE, we generated PAC transgenics that expressed Cre under *Gsh2* control. *Gsh2* was



**Figure 2** The embryonic *Nkx2.1*-derived OL lineage is rapidly eliminated during postnatal life. (a,b) In *Nkx2.1-Cre/Rosa26R-GFP* mice, GFP<sup>+</sup> (green), Sox10<sup>+</sup> (red) double-positive OLs were already a minority in the P10 cortex (arrows in a), and their contribution dropped to zero by P80. (c,d) At P10, practically all OLs in ventral regions such as the septum were derived from *Nkx2.1*-expressing (MGE-AEP-derived) precursors (GFP, Sox10 double-positive; yellow nuclei in c), but even here they were replaced by other populations (GFP<sup>-</sup>) by P80. (e–j) The proportional contributions of the three different populations of Sox10<sup>+</sup> OL lineage cells are presented as percentage of the total number of Sox10<sup>+</sup> cells in each region (average  $\pm$  s.d.). OLs and OLPs derived from *Nkx2.1*-expressing precursors were largely eliminated between birth and adulthood from most of the regions examined. OLs and OLPs generated from cortical precursors (*Emx1* derived) remained within the cortex at all stages. *Gsh2*-expressing precursors gave rise to OLs and OLPs that spread throughout the telencephalon. POA, preoptic area; MC, motor cortex; AC, anterior commissure; LOT, lateral olfactory tract; CC, corpus callosum. Scale bar, 100  $\mu$ m.

expressed strongly in the LGE-CGE but partially overlapped with *Nkx2.1* in the MGE (Supplementary Fig. 1). To map the overall contribution of the ventral forebrain, we generated triple-transgenic *Gsh2-Cre/Nkx2.1-Cre/Rosa26R-GFP* mice. In E16.5 triple transgenics, practically all *Pdgfra*<sup>+</sup> OLs in the telencephalon, including the lateral cortex, were also GFP<sup>+</sup> (Fig. 1g,h). This shows that before birth, all OLs in the cortex were ventrally derived, migrating first from the MGE-AEP and subsequently from the LGE, CGE or both. By the day of birth, however, GFP<sup>-</sup> OLs had once again started to accumulate in the cortex of the triple transgenics (arrowhead, Fig. 1i,j), indicating the presence of at least one additional source of OLs outside either the MGE-AEP or the LGE-CGE—possibly within the cortex itself.

To examine the fates of endogenous cortical precursors, we generated *Emx1-Cre* transgenics (Supplementary Fig. 1). In these mice, Cre was expressed strongly in cortical precursors, and activation of the GFP reporter in *Emx1-Cre/Rosa26R-GFP* mice was widespread in the postnatal cortex (Supplementary Fig. 1). GFP was present throughout the cytoplasm of the cells and even along axons, so that fiber tracts projecting from the cortex to the ventral forebrain were GFP labeled (arrow, Fig. 1k). *Emx1*-derived (GFP<sup>+</sup> *Pdgfra*<sup>+</sup>) OLs first appeared in the cortex around birth (Fig. 1k,l) and rapidly increased in number thereafter. *Emx1*-derived OLs were never observed in the ventral telencephalon.

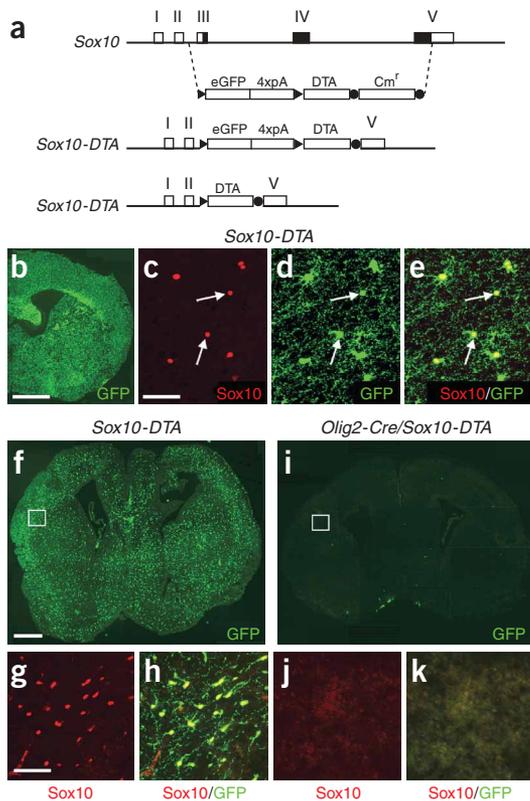
Our data suggested that cortical OLs are composed of two immigrant populations from the MGE-AEP and the LGE-CGE along with a resident cortical population. To exclude the possibility that the *Emx1*-derived OLs are ventrally derived cells that begin to express *Emx1* *de novo* after they migrate into the cortical field, we generated another transgenic line that expressed the tamoxifen-inducible form of Cre recombinase (CreER<sup>T2</sup>) under *Emx1* control (Supplementary Fig. 1). We induced Cre activity transiently by administering tamoxifen once at E9.5, before the onset of gliogenesis. Because tamoxifen was cleared within 48 h, Cre could not have been activated in OLs that migrated into the cortex from elsewhere after  $\sim$  E11.5. We analyzed the tamoxifen-treated mice at P9. *Pdgfra* expression in the CNS is restricted to early OLs and is downregulated as soon as the cells exit the cell cycle and begin to differentiate into OLs<sup>29,30</sup>. We therefore used an antibody

to Sox10, which begins to be expressed in OLs slightly later than *Pdgfra* but persists in mature OLs well after *Pdgfra* is downregulated (ref. 31). CreER<sup>T2</sup>-mediated recombination was relatively inefficient compared to regular Cre, so that well-separated GFP-labeled cells or clusters of cells were observed throughout the cortex. These included Sox10<sup>+</sup> OLs and OLPs, as well as cells with the morphology of neurons and astrocytes (Fig. 1m,n). The GFP<sup>+</sup> Sox10<sup>+</sup> cells comprised  $\sim$ 20% of all GFP<sup>+</sup> cells and were observed in all parts of the cortex (arrows, Fig. 1n). We concluded that they were formed from endogenous cortical precursors and not from inwardly migrating cells.

### Postnatal eradication of the *Nkx2.1*-derived lineage

Our data demonstrated that there were three successive waves of OL generation and migration in the embryonic telencephalon—from the MGE-AEP, the LGE-CGE and cortex, respectively (Fig. 1o). To determine how these three OL populations contribute to the postnatal and adult telencephalon and the extent to which they intermingle, we analyzed *Nkx2.1-Cre/Rosa26R-GFP*, *Gsh2-Cre/Rosa26R-GFP* and *Emx1-Cre/Rosa26R-GFP* mice from newborn (P0) to young adult (P80) (Fig. 2). The contribution of *Nkx2.1*-derived Sox10<sup>+</sup> OLs and OLPs throughout the forebrain was high at birth in dorsal regions such as the corpus callosum as well as in ventral regions such as the septum and preoptic area. We were surprised to find that by P10, the proportion of *Nkx2.1*-derived OLs and OLPs had plummeted to a very low level in the cortex (Fig. 2a,b,g,j). Most remarkably, the *Nkx2.1*-derived population declined to a very small fraction of all OL lineage cells in most parts of the adult forebrain, even in ventral regions close to their original source (Fig. 2c,d,f,h,i). Thus, the *Nkx2.1*-expressing region of the ventral forebrain gave rise to migratory OLs that populated the embryonic forebrain including the cortex, but most of these were eliminated after birth.

The majority of OLs in most regions of the postnatal telencephalon were generated by *Gsh2*-expressing precursors (Fig. 2e–j). The *Emx1* precursor contribution was almost entirely restricted to the cortex throughout life, although in the adult, a few *Emx1*-derived OLs were found in the septum. From P10 to P80, the cortex was populated by similar proportions of *Emx1*- and *Gsh2*-derived OLs and OLPs (*Emx1* contribution declined from  $\sim$ 70% to  $\sim$ 30%).



**Figure 3** Design and activity of a Cre-inducible *Dta* transgene under *Sox10* transcriptional control. **(a)** Intron and exon structure of the *Sox10* locus (top) and the *Sox10-DTA* transgene before (middle) and after (bottom) excision of the GFP-poly(A) cassette. **(b–e)** Coronal section through the telencephalon of a *Sox10-Dta* transgenic mouse at P3 and high-magnification images of the cortex from the same section. All *Sox10*<sup>+</sup> OLPs and OLs in the *Sox10-DTA* mouse coexpressed GFP, demonstrating the veracity of transgene expression. **(f–k)** The *Dta* transgene was activated by crossing *Sox10-DTA* to an *Olig2-Cre* mouse, excising the GFP cassette in all *Olig2*-expressing precursors and their descendants, hence permitting the expression of DTA and the highly specific killing of all *Sox10*<sup>+</sup> OL lineage cells. The effectiveness of this tool is demonstrated by the loss of all *Sox10*<sup>+</sup> GFP<sup>+</sup> OL lineage cells in the double transgenics (compare **f–h** with **i–k**). The *Olig2-Cre/Sox10-DTA* mice died shortly after birth, apparently from a motor deficit that prevented them from feeding. Scale bars: **(b)** 1 mm; **(c–e)** 50 μm; **(f,i)** 500 μm; **(g–h, j–k)** 80 μm.

of the GFP coding sequences and the activation of DTA (**Fig. 3f–k**). These embryos appeared normal at birth but died within 24–48 h. We do not yet know the cause of death but suspect a motor deficit.

To ask whether the region-specific telencephalic OL populations are functionally distinct, we generated a series of transgenics that combined *Sox10-DTA* with either *Emx1-Cre* or *Gsh2-Cre* (**Fig. 4**). In the presence of *Rosa26R-lacZ* as an independent reporter for Cre activity, cells from the region under study should have been positive for β-galactosidase (β-gal) (that is, labeled blue). Therefore, if region-specific ablation had been successful, there should have been an absence of blue OLs or OLPs whereas β-gal<sup>−</sup> GFP<sup>+</sup> OLs and OLPs that were generated outside the region of interest should have survived. This was illustrated most markedly in white matter tracts such as the corpus callosum and the anterior commissure, where the majority of cell bodies represented OLs and OLPs or other glia (**Fig. 4a–d**). For example, at P12 there were many β-gal<sup>+</sup> (blue) cells in the corpus callosum and anterior commissure of double-transgenic *Gsh2-Cre/Rosa26R-lacZ* mice (**Fig. 4a,c**) but practically none in *Gsh2-Cre/Rosa26R-lacZ/Sox10-DTA* triple transgenics (**Fig. 4b,d**). Nevertheless, there seemed to be just as many GFP<sup>+</sup> *Sox10*<sup>+</sup> cells in the corpus callosum and anterior commissure of ablated mice (*Gsh2-Cre/Sox10-DTA*) as in those of nonablated mice (*Sox10-DTA*) (**Fig. 4e–h**). For example, we counted  $(9.0 \pm 1.5) \times 10^4$  *Sox10*<sup>+</sup> cells per mm<sup>3</sup> in the corpus callosum of nonablated mice (**Fig. 4i**) compared to  $(9.1 \pm 1.2) \times 10^4$  such cells in *Gsh2-Cre/Sox10-DTA* mice (**Fig. 4j**). Similarly, we counted  $(9.0 \pm 1.4) \times 10^4$  *Sox10*<sup>+</sup> cells per mm<sup>3</sup> in the anterior commissure of control mice (**Fig. 4k**) compared to  $(9.0 \pm 0.8) \times 10^4$  such cells in *Gsh2-Cre/Sox10-DTA* mice (**Fig. 4l**). (Data are presented as mean ± s.d.) We concluded that the loss of *Gsh2*-derived OLs and OLPs was compensated for by one or more populations—in the corpus callosum presumably by *Emx1*-derived (cortical) cells, and in the anterior commissure presumably by *Nkx2.1*-derived cells, although there could also have been invasion from further afield. Compensation was also seen in the corpus callosum when we ablated the *Emx1*-derived OL and OLP population (data not shown). In none of these examples was there any obvious alteration in the amount of myelin in adult mice, as visualized by Sudan black histochemistry (**Fig. 4m–p**).

Although the number and distribution of GFP<sup>+</sup> *Sox10*<sup>+</sup> OLs and OLPs in *Emx1-Cre/Sox10-DTA*, *Gsh2-Cre/Sox10-DTA* and *Nkx2.1-Cre/Sox10-DTA* mice were ultimately indistinguishable from those in normal controls, the accumulation of OLPs was noticeably delayed in some regions (**Fig. 5**). For example, in *Sox10-DTA/Gsh2-Cre* mice, there was a slight delay in the arrival of OLPs in the outer layers of the cortex (compare **Fig. 5a,c**), consistent with our observation that *Emx1*-derived OLPs began to be generated slightly later than did the *Gsh2*-derived OLPs. Despite the loss of *Gsh2*-derived OLPs, the mice recovered fully by P10, by which time there was a normal number

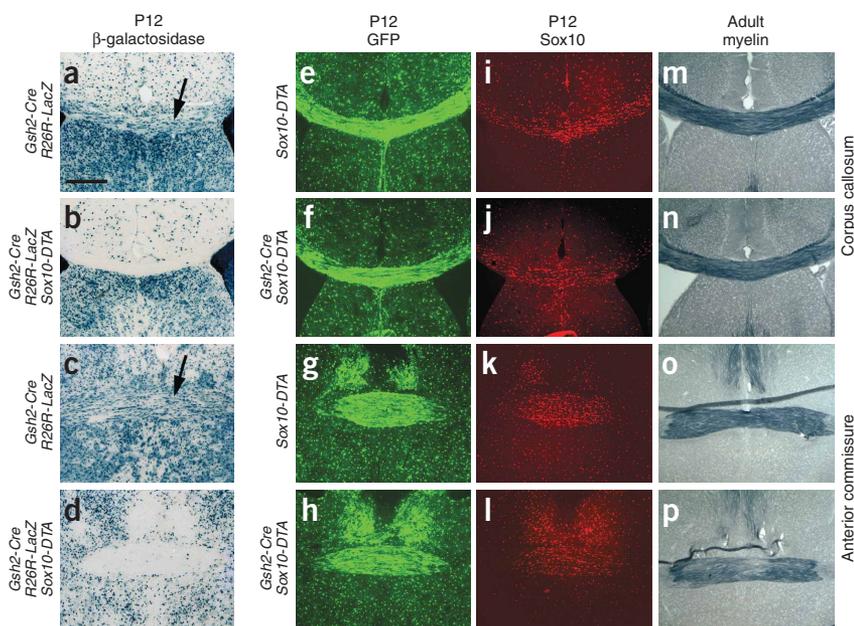
The cortical *Emx*-derived and ventral telencephalic *Nkx2.1*- and *Gsh2*-derived OLs and OLPs accounted for nearly all OL lineage cells in the early postnatal telencephalon. However, by adult stages, the total contribution of telencephalic OLs and OLPs dropped to 60–75%, especially in ventral territories such as the preoptic area (**Fig. 2f**) and in fiber tracts such as the corpus callosum, lateral olfactory tract and anterior commissure (**Fig. 2h,i,j**). Although we cannot exclude the possibility that this might have been due to the selective downregulation of the *Rosa26* promoter in some mature cells, we believe that it is more likely that this reflects a posterior to anterior migration of cells along fiber tracts. We observed such an influx of cells into the telencephalon from the diencephalon along axons of the internal capsule (data not shown).

### Functional equivalence of OLs with different origins

The presence of distinct OL populations within the telencephalon led us to question whether the different OL lineages were functionally specialized. To address this, we devised a transgenic approach to eliminate OL lineage cells according to their site of origin, by the targeted expression of DTA (ref. 32). A transgenic mouse line was produced using a PAC-based transgene with the structure *Sox10-lox-GFP-poly(A)-lox-DTA* (**Fig. 3a**). The transgene was faithfully regulated because *Sox10* and GFP were coexpressed in the vast majority of OL lineage cells throughout the CNS (>98% coexpression in the P3 cortex) (**Fig. 3b–e**). After Cre recombination, GFP should have been excised along with the poly(A) signal, thus activating DTA and killing the cells. Henceforth, we refer to this DTA line simply as *Sox10-DTA*.

To test our cell ablation strategy, we crossed *Sox10-DTA* to an *Olig2-Cre* line that expressed Cre recombinase in all OL lineage cells, regardless of their embryonic origin (N.K., D. Rowitch and W. R., unpublished data). *Olig2-Cre/Sox10-DTA* neonates had neither GFP<sup>+</sup> nor *Sox10*<sup>+</sup> cells anywhere in the brain or spinal cord, confirming efficient excision

**Figure 4** Genetic ablation of region-specific OL populations reveals functional redundancy and compensation among the different lineages. (a–d) *Gsh2*-derived white matter glia (mainly OL-lineage cells) were visualized in the corpus callosum (a,b) or anterior commissure (c,d) of P12 *Gsh2-Cre/Rosa26R-lacZ* (a,c) and *Gsh2-Cre/Sox10-DTA/Rosa26-R-lacZ* (b,d) mice (coronal sections, arrows). Many blue cells are visible in these tracts in *Gsh2-Cre/Rosa26R-lacZ* mice but are missing from the equivalent structures of *Gsh2-Cre/Sox10-DTA/Rosa26-R-lacZ* mice, showing that *Gsh2*-derived OLs have been effectively ablated in the latter through the action of DTA. (e–l) Despite the specific loss of *Gsh2*-derived OLs, there was no apparent reduction in the total complement of OLs in these white matter tracts as visualized by GFP staining (compare e with f, and g with h) or Sox10 staining (compare i with j, and k with l). There was no apparent reduction in the amount of myelin in adult white matter tracts of ablated *Gsh2-Cre/Sox10-DTA* mice as visualized by Sudan black histochemistry (compare m with n, o with p). Thus it seems that the loss of *Gsh2*-derived OLs was compensated for by the invasion of alternative OL lineages. Similar compensation was observed when the *Emx1*-derived OL lineage was ablated in *Emx1-Cre/Sox10-DTA* mice (data available on request). The mice resulting from these crosses survived, behaved and reproduced normally until at least P60. These experiments demonstrate that the different regional OL lineages competed for territory in the normal developing CNS and further suggest that the different OL populations were functionally equivalent. Scale bar, 400  $\mu$ m.



and distribution of OLPs in the cortex (Fig. 5b,d). Even in the olfactory tracts in the ventral forebrain—normally populated solely by *Gsh2*-derived OLs—there was complete replenishment of OLs from other sources. The mice all survived and reproduced normally, showing no obvious neurological symptoms. These and other data imply that the *Gsh2*-, *Emx1*- and *Nkx2.1*-derived OL lineages are functionally equivalent, in contrast to the different neuronal populations that are generated from the same germinal fields.

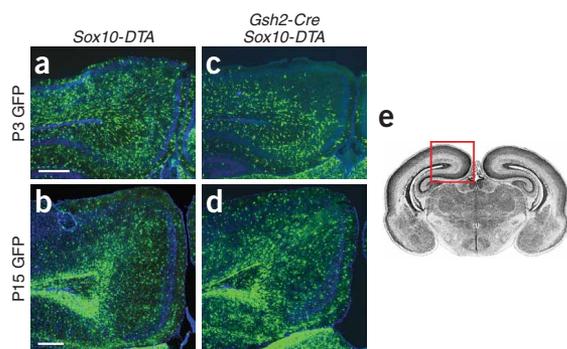
## DISCUSSION

We examined the developmental origins of OLPs in the forebrain by *Cre-lox* fate mapping in transgenic mice. We discovered that OLPs are generated in most parts of the telencephalic ventricular zone including the MGE-AEP, the LGE-CGE and cortex. There was a temporal ventral-to-dorsal gradient in production—OLPs were generated first from the MGE-AEP at E11.5, then from the LGE-CGE around E15 and finally from the cortex after birth. The earliest-formed OLPs, from the MGE-AEP, were later eliminated during postnatal development. These findings help resolve the confusion surrounding previous reports that emphasized ventral, dorsal or multiple origins, depending on the developmental stage examined, the experimental approach used or both<sup>10–24</sup>.

Although we have defined three region-specific OLP populations based on the expression of three regionally restricted transcription factors (*Nkx2.1*, *Gsh2*, *Emx1*), it seems possible that OLP generation is not itself regionally restricted but proceeds in a ‘Mexican wave’ from ventral to dorsal areas. This harks back to the early idea that the entire neuroepithelium undergoes a switch from neurogenesis to gliogenesis as part of a constitutive maturation program<sup>33</sup>. However, the pioneering early studies were necessarily speculative to a degree because, with the methods available at that time, it was difficult to identify glial cells unambiguously and it was not possible to detect long-range OLP migration or other dynamic cell behavior.

Our data seem to contradict previous fate mapping studies in chick-quail and chick-mouse chimeras, which pointed to a purely ventral source of cortical OLPs<sup>17</sup>. It seems possible that there is a real difference between birds and rodents in this respect—perhaps additional, more dorsal sources of OLPs evolved in mammals to cope with their increased cortical volume. Alternatively, late (dorsal) waves of OLP generation might have been missed in the avian transplant studies.

We were surprised to find that the earliest-forming population of OL lineage cells—from *Nkx2.1*-expressing precursors in the MGE-AEP—was almost completely eradicated by adulthood in most parts of the forebrain. This might be partly explained by a dilution effect caused by



**Figure 5** Transient delay in accumulation of OLPs after ablation of the LGE-CGE-derived population in *Gsh2-Cre/Sox10-DTA* mice. Coronal forebrain sections of P3 or P15 mice were immunolabeled for GFP (green) and Sox10 (data not shown). GFP<sup>+</sup> cells were Sox10-expressing OLPs that had not undergone Cre recombination to excise GFP and activate DTA. (a,b) Posterior, dorsomedial cortex of nonablated mice. GFP<sup>+</sup> cells were more or less evenly distributed through the outer cortex of control mice, both at P3 and P15. (c,d) Ablated mice. Accumulation of OLPs was delayed in the outer cortex after ablation of the LGE-CGE-derived population (compare a and c). The distribution was normalized by P15 (compare b and d). Because LGE-CGE-derived OLPs were absent from the ablated mice (c,d), neighboring populations had to make up the difference. (e) Orientation of sections. Scale bars, 300  $\mu$ m.

the ~20-fold increase in cerebral cortical volume between E16 and P10 (N.K., unpublished data). However, this cannot explain the removal of MGE-AEP-derived OLs from the ventral telencephalon. It is possible that MGE-AEP-derived OLs and OLs compete less effectively for survival factors in the postnatal CNS than do their counterparts, or that they have some other inherent disadvantage. Alternatively, there might be a steady rate of turnover and replacement of all OLs during postnatal life, and the *Nkx2.1*-derived OLs might be gradually substituted by other lineages. This is a plausible explanation: we observed in the course of our fate mapping experiments that the postnatal SVZ at the tips of the lateral ventricles—known to be a source of neurons and glia after birth<sup>19,20</sup>—was derived from the embryonic LGE-CGE and cortex, but not from the MGE-AEP (data not shown). Note that not all *Nkx2.1*-derived cells were eliminated in the adult; cortical interneurons and basal ganglia neurons persisted long term.

Specification of OLs in the ventral neural tube relies on morphogenetic signals, including Sonic hedgehog (Shh), from local organizing centers. For example, Shh is secreted from the notochord and floor plate at the ventral midline of the spinal cord<sup>34,35</sup>. In the telencephalon, it is synthesized by ventral neuroepithelial cells in the preoptic area, AEP and MGE<sup>11,36</sup>. In both the spinal cord and forebrain, Shh expression is required for the production of ventrally derived neurons and OLs<sup>10,11,22,36–40</sup>. Some OLs still form in the spinal cord and forebrain of *Shh*<sup>-/-</sup> mice<sup>5,22</sup> and there is clear *in vitro* evidence for the existence of a Hedgehog (Hh)-independent route to OL production<sup>8,9</sup>. Fibroblast growth factor (FGF) specifies OLs in cultures of embryonic dorsal spinal cord or cerebral cortex in the presence of cyclopamine, which blocks all hedgehog signaling by binding to the Hh coreceptor Smoothed (Smo)<sup>8,9</sup>. The existence of an Hh-independent pathway has also been demonstrated by the FGF-mediated induction of OL lineage markers in cultured *Smo*<sup>-/-</sup> embryonic stem cells<sup>5</sup>. It is possible that the postnatal wave of OLs that we observed within the cerebral cortex was triggered by this Hh-independent, FGF-dependent pathway, but it is also possible that late activation of Shh or another Hh family member within the cortex might have been responsible.

Different OL lineages have been described in the forebrain that either do or do not express *Pdgfra*<sup>10,21</sup>. However, there is currently no evidence for a *Sox10*-independent OL lineage, so our quantitative work, which is based on *Sox10* staining, describes the origin of all OLs and OLs regardless of their lineage as defined by the expression of other markers. Additional genetic studies will be required to resolve the issue of a *Pdgfra*-independent lineage because *Pdgfra*<sup>-</sup> OLs might represent cells that have begun to differentiate into OLs and have consequently downregulated *Pdgfra*, rather than a distinct type of progenitor cell *per se*. Morphological and biochemical subtypes of myelinating OLs have also been described<sup>41–43</sup>, but we do not yet know whether these are generated equally from all parts of the telencephalon. In any case, it seems that the different populations of OLs in the forebrain are functionally equivalent because when one population was substituted by another, the mice survived and behaved normally. We did not subject the mice to detailed behavioral analysis, so it remains possible that there might be subtle performance differences among the various OL populations or that differences will emerge as the mice age.

It is notable that when any one OL population was ablated, neighboring populations quickly expanded to fill the available space—demonstrating that OLs normally compete with one another for territory in the developing CNS. What is the nature of this competition? How do OLs sense the absence of their neighbors and invade the empty space? We previously presented evidence that OLs in the spinal cord compete for limiting quantities of PDGF ligand and proliferate until their rate of PDGF consumption (by receptor binding

and internalization) exactly balances the rate of supply from other cells (neurons and astrocytes)<sup>44,45</sup>. Because the rate and pattern of supply is fixed, so is the final number and distribution of OLs, no matter where they originate. It seems likely that similar principles apply in the developing forebrain.

The existence of both ventral and dorsal OL origins in the telencephalon mirrors the situation in the spinal cord. In the cord, there is a major source of OLs in the pMN domain of the ventral ventricular zone (refs. 1–4). These OLs begin to be produced around E12.5 in the mouse. However, a minority (~10–15%) of spinal cord OLs are generated from more dorsal territories that come into play later, around E16.5 (refs. 5–7). The dorsally derived OLs are normally held in check by their ventrally derived counterparts, because when the ventral source is suppressed in *Nkx6.1/Nkx6.2*-double knockout mice, the dorsal OLs proliferate more than they usually do and can generate up to 30% of the normal number of OLs before birth<sup>7</sup>. It is conceivable that dorsally derived OLs might even repopulate the cord completely, given enough time, but the *Nkx6*-null mice die at birth. Thus, dorsally and ventrally derived OLs compete for space in the spinal cord just as they do in the forebrain. Under normal circumstances, the ventrally derived population predominates in the spinal cord, whereas the more dorsally derived populations prevail in the forebrain. This compensatory redundancy might serve to ensure rapid, fail-safe myelination of the complex mammalian brain, a flexible response to demyelinating damage or disease, or both.

## METHODS

**Generation of transgenic mice.** PAC transgenic mice expressing Cre under control of *Nkx2.1*, *Gsh2* or *Emx1* were generated as previously described<sup>6</sup>. The genomic DNA fragments used to generate each of the three mice spanned approximately 180 kilobase (kb) of genomic DNA for *Nkx2.1* and *Emx1*, and 110 kb for *Gsh2*. Details of the genomic PACs are available on request. In all three cases, the codon-improved Cre recombinase (iCre) with a nuclear localization signal<sup>46</sup> was fused to the translation initiation codon using a polymerase chain reaction (PCR)-based approach. This was followed by an SV40 polyadenylation signal. The coding region of *Nkx2.9* was removed from the genomic *Nkx2.1* PAC by homologous recombination. To generate the *Sox10-lox-GFP-STOP-lox-DTA* mouse, we used a 120-kb *NotI* fragment from a *Sox10* genomic PAC. This region included 60 kb upstream and 50 kb downstream of *Sox10*. We replaced the entire *Sox10* open reading frame (ORF) with a *loxP*-flanked ('floxed') eGFP-poly(A) cassette, followed by the DTA fragment coding sequences, which encode an attenuated G383A version of DTA (gift from Ian Maxwell, University of Colorado, Denver)<sup>47</sup>. The genomic region spanning exons 3–5 was replaced with the cassette *lox-eGFP-polyA<sub>4</sub>-lox-DTA-frt-Cm<sup>r</sup>-frt* by homologous recombination in bacteria. The *Cm<sup>r</sup>* cassette was then removed by transient activation of *Flp* recombinase in bacteria, producing a latent *DTA* transgene that expresses GFP under *Sox10* transcriptional control (*Sox10-DTA*). In the presence of Cre recombinase, the GFP-poly(A) cassette is removed, thus activating the *DTA* cassette and killing the expressing cells (further details available on request).

All PACs were purchased from the UK Human Genome Mapping Project Resource center. PAC modification was carried out in a bacterial system<sup>48</sup>. Cre reporter mice used in this work were *Rosa26R-eGFP* (ref. 49) for GFP immunohistochemistry and *Rosa26R-lacZ* (ref. 50) for enzymatic detection of β-galactosidase activity. All animal experiments were approved by the University College London local ethical committee and conformed to the UK Animals (Scientific Procedures) Act 1986.

**In situ hybridization and immunohistochemistry.** Tissues were prepared for *in situ* hybridization and immunohistochemistry as described<sup>6</sup>. The probe used to detect expression of the Cre transgene was a 900-bp *iCre* fragment spanning the entire *iCre* ORF. Antibodies used in this study include rabbit antibody to GFP at 1:8,000 dilution (AbCam); guinea pig antibody to *Sox10* at 1:2,000 and rat antibody to *Pdgfra* at 1:500 dilution (BD Biosciences). Myelin was stained with Sudan black according to standard protocols.

**Cell counts.** For quantification of OLs and OLPs derived from different telencephalic regions (Fig. 2), we counted GFP<sup>-</sup> and GFP<sup>+</sup>, Sox10<sup>+</sup> OLs and OLPs in confocal microscope images taken with a 20× objective (at least three fields from three or more 20-μm sections taken from one or two mice). The average number of Sox10<sup>+</sup> cells per field ranged from ~50 (for example, in the preoptic area at P0) to over 450 (for example, in the adult anterior commissure). For each experiment, the data are presented as the proportion of total Sox10<sup>+</sup> cells that were also GFP<sup>+</sup>.

To quantify the total density of OLs and OLPs in control mice relative to mice in which one telencephalic OLP population had been ablated (for example, in *Gsh2-Cre/Sox10-DTA* mice) (Fig. 5), we counted GFP<sup>+</sup> Sox10<sup>+</sup> cells in white matter tracts (corpus callosum and anterior commissure) in micrographs taken with a 5× objective (five or six fields in five or six sections from each of two mice). The numbers were normalized and are quoted in the text as Sox10<sup>+</sup> cells per mm<sup>3</sup>.

**Induction of CreER<sup>T2</sup> by tamoxifen.** Tamoxifen was dissolved in corn oil by sonication for 30 min. A single dose of 4 mg (100 μl at 40 mg ml<sup>-1</sup>) was administered to pregnant females by oral gavage. At this dose, spontaneous abortion was usually not a problem. All pups were delivered by cesarean section at E18.5, reared by a foster mother and examined at P9.

Note: Supplementary information is available on the Nature Neuroscience website.

#### ACKNOWLEDGMENTS

We thank our colleagues at the Wolfson Institute for Biomedical Research and elsewhere for discussion, technical help and advice—especially M. Fruttiger, U. Dennehy and R. Taveira-Marques. We thank I. Maxwell for supplying the DTA plasmid. This work was funded by the UK Medical Research Council, the Wellcome Trust Functional Genomics Initiative and a Wellcome Trust Prize Studentship (M.F.).

#### COMPETING INTERESTS STATEMENT

The authors declare that they have no competing financial interests.

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