Development/Plasticity/Repair

Olig2 Directs Astrocyte and Oligodendrocyte Formation in Postnatal Subventricular Zone Cells

Christine A. G. Marshall,[1] Bennett G. Novitch,[1,2,4] and James E. Goldman[1,3]

[1]Center for Neurobiology and Behavior, [2]Department of Biochemistry and Molecular Biophysics, and [3]Department of Pathology, Columbia University, College of Physicians and Surgeons, New York, New York 10032, and [4]Department of Cell and Developmental Biology, University of Michigan Medical School, Ann Arbor, Michigan 48109

The subventricular zone (SVZ) in the neonatal mammalian forebrain simultaneously generates olfactory interneurons, astrocytes, and oligodendrocytes. The molecular cues that enable SVZ progenitors to generate three distinct cell lineages without a temporal switching mechanism are not known. Here, we demonstrate that the basic helix-loop-helix transcription factor Olig2 plays a central role in this process. Olig2 is specifically expressed in gliogenic progenitors in the postnatal SVZ and by all glial lineages derived from this structure. By expressing normal and dominant-interfering forms of Olig2 *in vivo*, we show that Olig2 repressor function is both sufficient and necessary to prevent neuronal differentiation and to direct SVZ progenitors toward astrocytic and oligodendrocytic fates. Although Olig2 activity has been associated previously with motor neuron and oligodendrocyte development, our findings establish a previously unappreciated role for Olig2 in the development of astrocytes. Furthermore, these results indicate that Olig2 serves a critical role in pan-glial versus neuronal fate decisions in SVZ progenitors, making it the first intrinsic fate determinant shown to operate in the early postnatal SVZ.

Key words: **SVZ; olig2; neurogenesis; gliogenesis; development; forebrain**

Introduction

In the developing telencephalon, the neuroepithelium gives rise to the subventricular zone (SVZ), a secondary proliferative layer of progenitors that simultaneously generates both neurons and glia (for review, see Marshall et al., 2003). The contemporaneous production of neurons and glia makes the SVZ a unique system in which to study the molecular mechanisms directing cell fate decisions. During the perinatal period, the SVZ expands to include an angular prominence between the developing striatum and subcortical white matter (see Fig. 1*A*,*B*) and forms a continuous structure with the rostral migratory stream (RMS) (see Fig. 1*A*,*C*). SVZ progenitors specified to a neuronal lineage migrate rostrally to the olfactory bulb and differentiate into granular and periglomerular interneurons (Altman, 1969; Luskin, 1993; Lois and Alvarez-Buylla, 1994). In contrast, cells specified to become glia migrate radially from the SVZ into the subcortical white matter, corpus callosum, striatum, and medial, dorsal, and lateral areas of the cerebral cortex, where they differentiate into astrocytes and oligodendrocytes (see Fig. 1*C*) (Marshall et al., 2003).

The point at which SVZ progenitors commit to neuronal versus glial lineages is not known. Studies of SVZ cell migration suggest that distinct glial and neuronal cell lineages are established within the SVZ before long-range migrations into the forebrain (Suzuki and Goldman, 2003). However, a delineation of committed neuronal and glial populations within the SVZ has not been possible thus far, and molecules directing SVZ cell fate decisions (see Fig. 1*D*) have not been identified.

Comprehensive work on neuronal and glial specification in the embryonic CNS has provided insight into molecular pathways involved in fate decisions. Basic helix-loop-helix (bHLH) transcription factors are important regulators of neuronal and glial cell fates in the embryo (for review, see Bertrand et al., 2002), but their involvement in postnatal fate decisions, while anticipated, has not been well described. One family of bHLH proteins, the Oligs, has received a great deal of attention for their essential involvement in both motor neuron and oligodendrocyte development in the embryonic spinal cord. Both Olig1 and Olig2 are expressed in the embryonic and postnatal forebrain, but their function in the development of this structure remains poorly understood. During embryogenesis, constitutive expression of Olig1 in forebrain progenitors has been shown to promote the formation of oligodendrocytes (Lu et al., 2001). The function of Olig genes in the postnatal forebrain, however, has not been evaluated, and progress in this area has been hampered by the failure of Olig mutant mice to survive embryonic development. Thus, other methods must be used to study the postnatal functions of Olig genes *in vivo*.

Received May 13, 2005; revised June 28, 2005; accepted June 29, 2005.

C.A.G.M. and J.E.G. were supported by National Institute of Neurological Disorders and Stroke Grant NS-17125. B.G.N. was a Marion Abbe Fellow of the Damon Runyon Cancer Research Fund (DRG-1569). We thank Tom Jessell and Fiona Doetsch for insightful discussions and comments on this manuscript. We are grateful to Carol Mason for graciously sharing imaging equipment, Rachel Ventura for help with Olig2 expression analysis, and Michael Hack and Magdalena Gotz for sharing unpublished work. Finally, we thank Satoshi Suzuki and Ana Milosevic for providing expert assistance with retroviral production and stereotaxic injections.

Correspondence should be addressed to Dr. James E. Goldman, Division of Neuropathology, Department of Pathology, Columbia University, College of Physicians and Surgeons, 630 West 168th Street, New York, NY 10032. E-mail: Jeg5@columbia.edu.

DOI:10.1523/JNEUROSCI.1924-05.2005

 0270-6474/05/257289-10$15.00/0

In this study, we use retroviral-mediated gene transduction to express normal and dominant-interfering forms of Olig2 in SVZ cells. We show that Olig2 repressor function is both sufficient and necessary to prevent neuronal differentiation and to direct SVZ progenitors toward astrocytic and oligodendrocytic fates. Together, our findings demonstrate a previously unappreciated role for Olig2 in astrocyte development and establish that Olig2 directs the primary specification of pan-glial versus neuronal lineages within SVZ progenitors in the neonatal forebrain.

Materials and Methods

Retroviral vectors and virus preparation. All fragments encoding Olig2 or mutant Olig2 and enhanced green fluorescent protein (eGFP) cDNAs were subcloned into the bicistronic replication-incompetent viral vector pQCXIX (Clontech, Cambridge, UK) 5′ and 3′ relative to an internal ribosomal entry site (IRES), respectively. The pQCXIX vector uses a cytomegalovirus immediate early promoter downstream of a 5′ long terminal repeat (LTR) to drive expression of both transcripts. Coding fragments (Novitch et al., 2001) were as follows: *mOlig2* (wild-type mouse Olig2, *Eco*RI fragment from pCS2+6MTmOlig2), *cOlig2bHLH* (chick Olig2 bHLH domains; amino acids 92–180; *Cla*I fragment from pCS2+MTcOlig2bHLH-VP16), *MTcOlig2bHLH-VP16* (chick Olig2 bHLH domains fused to the herpes simplex VP16 transactivation domain; *Cla*I fragment from pCS2+MTcOlig2bHLH-VP16), and *eGFP* [*Eco*RV fragment provided by Satoshi Suzuki (Kyushu University, Fukuoka, Japan)]. To generate virus, retroviral vectors were cotransfected with the vesicular stomatitis virus G (VSV-G) viral envelope into gp293 cells using Lipofectamine 2000 (Invitrogen, San Diego, CA). Growth medium was added to cells after 6 h, and supernatant was harvested 48 h posttransfection. Viral stocks were concentrated and titered to 1–3 $\times$ 10^5 cfu/ml. The pNIT vector (provided by T. Palmer and S. Suhr, The Salk Institute, La Jolla, CA) is a bicistronic replication-incompetent retrovirus that uses a 5′ LTR to drive expression of the tetracycline activator and the tetracycline operon enhancer/promoter to drive the expression of eGFP. pNIT virus was prepared as described previously (Kakita and Goldman, 1999).

Retroviral injections. Sprague Dawley rat pups at postnatal day 2 (P2) to P3 were deeply anesthetized by immersion in ice water and positioned in a stereotactic apparatus, and 0.5 μl of retrovirus solution was injected unilaterally at a rate of 0.2 μl/min using a 10 μl Hamilton (Reno, NV) syringe. Coordinates of the injection site used for all experiments are 1 mm anterior and 1.5 mm lateral to bregma at a depth of 2.0 mm.

Animals, histology, immunohistochemistry, and terminal deoxynucleotidyl transferase-mediated biotinylated UTP nick end labeling assay. Human glial fibrillary acidic protein (hGFAP)-GFP mice (The Jackson Laboratory, Bar Harbor, ME), C57BL/6J mice, and Sprague Dawley rats (Charles River, Quebec, Canada) were anesthetized by intraperitoneal injection of ketamine (75 mg/kg; Aveco, Fort Dodge, IA) and xylazine (5 mg/kg; Mobay, Shawnee, KS) and were transcardially perfused with 4% paraformaldehyde in PBS, pH 7.4. Mouse brains were postfixed after <6 h, rat brains were postfixed overnight, and all brains were cryoprotected with 30% sucrose/PBS. Olig2-GFP mice (B.G. Novitch and T. M. Jessell, unpublished observation) were generated by disruption of the coding region of Olig2 with an Ires-EGFP cassette and processed as above. Serial coronal or parasagittal sections (see Fig. 1*A*) were cut (16–30 μm) using a cryostat, air-dried, and stored at −80°C until use. Immunofluorescence was performed using the following antibodies: rabbit anti-GFP (1:500; Molecular Probes, Eugene, OR), mouse anti-GFP (1:200; Molecular Probes), rabbit anti-Dlx2 (1:200; provided by J. Rubinstein, University of California, San Francisco, San Francisco, CA), guinea pig anti-olig2 [1:20,000 (Wichterle et al., 2002); antibody specificity was confirmed by a complete lack of labeling in olig2 mutants (supplemental Fig. 1, available at www.jneurosci.org as supplemental material), an expression pattern similar to *in situ* hybridizations for olig2 transcript and a nonoverlap with olig1 immunoreactivity], mouse anti-CNPase (1:200; Sigma, St. Louis, MO), mouse anti-ZebrinII (1:100; provided by R. Hawkes, University of Calgary, Calgary, Alberta, Canada), rabbit anti-GFAP (1:1000; Dako, High Wycombe, UK), mouse anti-vimentin (1:100; Dako), NG2 (1:400; provided by W. Stallcup, The Burnham Institute, La Jolla, CA), mouse anti-CC1 (1:20; Oncogene Sciences, Uniondale, NY), mouse anti-neuronal-specific nuclear protein (NeuN; 1:250; Chemicon, Temecula, CA), mouse anti-Map2 (1:1000; Sternberger Monoclonals, Exeter, UK), mouse anti-calretinin (1:100; Chemicon), and rabbit anti-calbindin (1:1000; Swant, Bellinzona, Switzerland). Biotinylated secondary antibodies with avidin-conjugated fluorescein (1:300; Vector Laboratories, Burlingame, CA) were used to detect GFP in tissue sections from injected brains. Antigen retrieval was performed before immunolabeling with antibodies to Zebrin II and GFAP as described previously (Marshall and Goldman, 2002). For terminal deoxynucleotidyl transferase-mediated biotinylated UTP nick end labeling (TUNEL) assay, an *in situ* cell death detection kit (Roche Products, Hertfordshire, UK) was used.

SVZ cell cultures and immunocytochemistry. SVZs from P3 mice were dissected, dissociated with a 0.025%Trypsin-EDTA/0.25 mg/ml DNase solution, and filtered through a 35 μm Nitex mesh. Dissociated cells were grown in neurobasal medium with 0.1% fetal bovine serum (FBS) for 3 d. Cells were then cultured for one additional day in neurobasal medium with 3% FBS to generate cultures enriched for immature astrocytes and an additional 5 d for cultures enriched for mature astrocytes. Cell cultures were labeled using the following antibodies: mouse anti-vimentin (1:400; Dako), mouse anti-GFAP (1:600; Dako), and guinea pig anti-Olig2 (1:20,000) (Wichterle et al., 2002).

Results

Olig2 expression in the SVZ and RMS

We first examined the expression of Olig2 in the neonatal rodent forebrain SVZ and RMS using antibodies to Zebrin II (aldolase C) and the homeodomain transcription factor Dlx2 to define two major SVZ cell subpopulations. Zebrin II is specifically expressed by radial glia and astrocytes within the perinatal forebrain and is a useful marker for these cells (Staugaitis et al., 2001; Marshall and Goldman, 2002). Zebrin II expression in astrocytic "border cells" identifies the limits of the perinatal SVZ, whereas a reciprocal, Zebrin II negative, "central" cell population is defined by the expression of Dlx2 (Marshall and Goldman, 2002). Immunohistochemical analysis of mouse forebrain tissue at P0–P7 revealed that Olig2 was expressed in both SVZ border and central cells. Olig2 expression in Zebrin II+ border cells, which line the ventricles as well as the dorsolateral edges of the SVZ adjacent to the subcortical white matter and striatum, is shown in coronal sections (Fig. 1*E–G*). Olig2 expression in Dlx2+ central SVZ cells, which migrate radially into forebrain areas overlying the SVZ as well rostrally into the RMS, is best viewed in parasagittal sections (Fig. 1*H–J*). We found that only a minority of the Dlx+ cells also express olig2. The RMS was strikingly devoid of Olig2 expression (Fig. 1*K*), suggesting that Olig2 might be expressed specifically by glia or glial progenitors but not by interneurons or migratory neuronal progenitors in the perinatal forebrain. We also examined cells from acutely dissociated P1 SVZs and found that expression of neuronal β-tubulin (TuJ1) and Olig2 do not overlap but, in sum, can account for the large majority of cells (data not shown). If TuJ1 is a marker for immature cells of the neuronal lineage, then Olig2 may be expressed by glioblasts and also perhaps by multipotent cells, if such cells exist in the neonatal SVZ.

Developing astrocytes and oligodendrocytes express Olig2

We next used immunohistochemical labeling to examine the expression of Olig2 throughout the rest of the perinatal forebrain [embryonic day 18 (E18) to P10], with particular attention to developing glia. Olig2 was expressed in astrocytes as well as oligodendrocytes throughout the cortex, subcortical white matter, and striatum. Because astrocytes arise from at least two sources within the perinatal forebrain, ZebrinII+ radial glia, which trans-

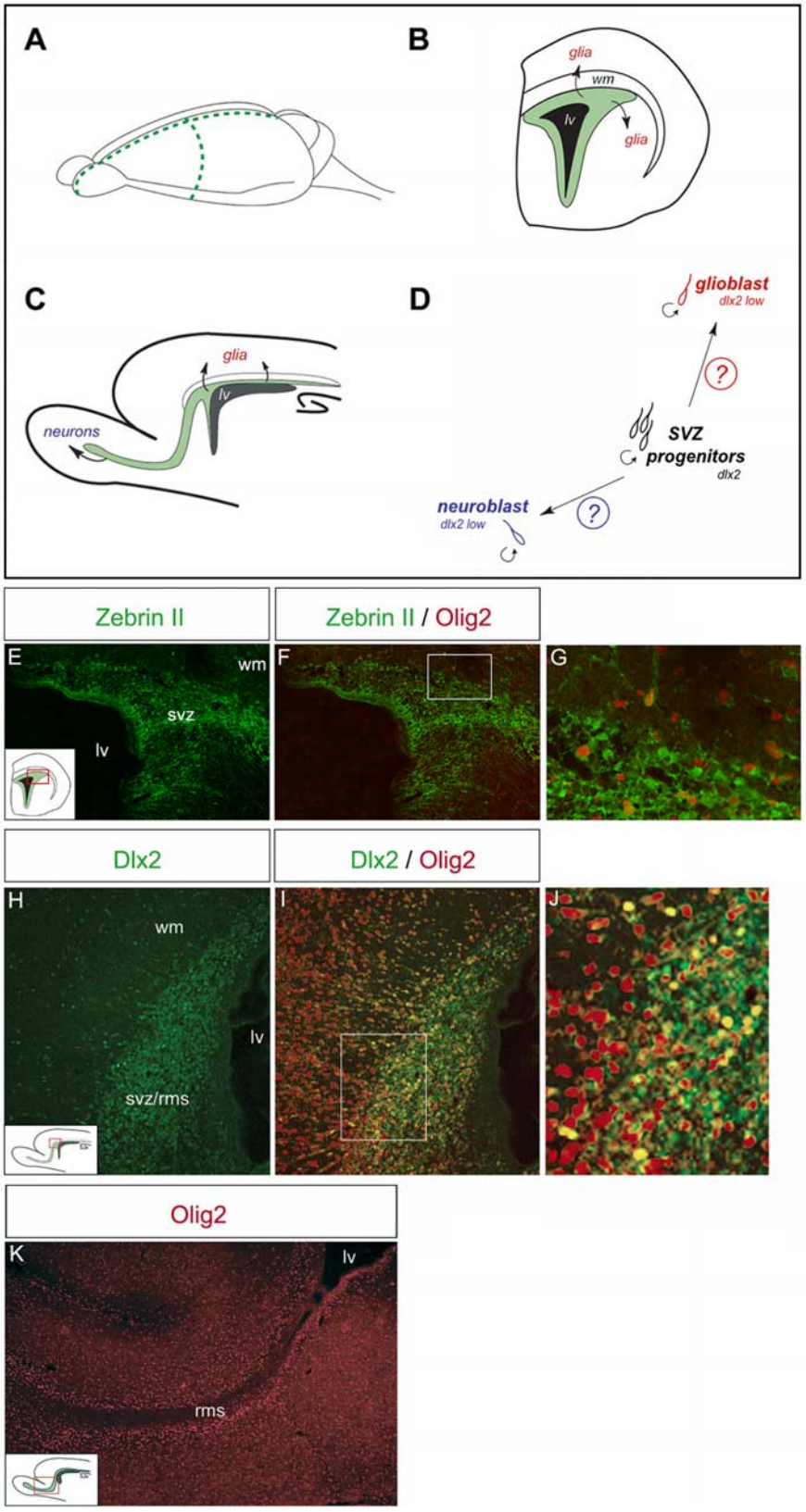

Figure 1. Olig2 expression in the SVZ. ***A***, Diagram of coronal and parasagittal planes highlighting the SVZ (***B***) and RMS (***C***) (both green), respectively. SVZ-derived glial precursors migrate radially within a coronal plane into adjacent forebrain areas, whereas SVZ-derived neuronal progenitors migrate rostrally in a parasagittal plane to the olfactory bulb. ***D***, Transcriptional regulators directing the formation of neuronal and glial lineages in SVZ cells are not known. ***E–G***, Olig2 expression in Zebrin II+ (border) and Zebrin II− (middle) SVZ cells, as indicated by immunostaining of P6 mouse forebrain. The inset in ***E*** depicts the area of photomicrographs shown in ***E*** and ***F***. The boxed area in ***F*** is magnified in ***G***. ***H–J***, Olig2 is expressed in Dlx2+ (central) SVZ progenitors. The inset in ***H*** depicts the area of the photomicrograph shown in ***H*** and ***I***. The boxed area in ***I*** is magnified in ***J***. ***K***, Cells within the RMS do not express Olig2. lv, Lateral ventricle; wm, white matter.

form directly into astrocytes, and Dlx2+ migratory SVZ progenitors, which colonize the forebrain and complete their differentiation in the white matter and gray matter, we examined Olig2 expression in both radial glia and immature astrocytes. Radial glia throughout the perinatal dorsal forebrain, including those with an intermediate morphology suggestive of an ongoing transformation into a mature astrocyte phenotype (Fig. 2*A*, arrowheads) (Voigt, 1989), expressed Zebrin II and Olig2. Immature astrocytes, defined by their relatively simple morphology (in contrast to the complex, bushy appearance of mature astrocytes) and their expression of Zebrin II but not GFAP, also expressed Olig2+ (Fig. 2*B*, arrowheads). In addition, astrocytes that were more differentiated and expressed detectable GFAP in their perinuclear region (Fig. 2*B–D*, asterisk) as well as astrocytes in late stages of maturation, which expressed GFAP in the perinuclear region as well as throughout their processes (Fig. 2*E–G*), were also Olig2+. Notably, the GFAP+/Olig2+ astrocytes that we identified were distributed widely throughout the forebrain and in the cerebral cortex, white matter, and striatum.

Although most, if not all, GFAP+ astrocytes in the neonatal forebrain expressed Olig2, mature and fully arborized astrocytes expressing the highest amounts of GFAP (Fig. 2*E*, arrowheads) consistently appeared to express slightly lower levels of Olig2, as judged by Olig2 immunoreactivity, than astrocytes with a more immature morphology and expressing less GFAP (Fig. 2*E*, asterisk), suggesting a downregulation of Olig2 in mature astrocytes. Immunohistochemical analysis of forebrain astrocytes at various developmental time points, from neonatal (P0–P7) to adult (P30), confirmed a gradual downregulation of Olig2 expression (R. Ventura, unpublished observations). Thus, Olig2 appears to be expressed transiently in forebrain astrocytes. Olig2 is expressed strongly as astrocytes become specified and differentiated, but it is subsequently downregulated after their development is mostly complete. In contrast, oligodendrocytes throughout the forebrain, defined here by their expression of CNPase (Fig. 2*H*,*I*), expressed Olig2 consistently from early developmental stages into adulthood (data not shown).

Because Olig2 expression in forebrain astrocytes has not yet been documented, we examined this expression further using three independent methods. First, we analyzed neonatal forebrains from *hGFAP-*

eGFP transgenic mice. The human GFAP promoter drives expression of GFAP at very early stages of astrocyte development relative to the mouse GFAP promoter. Hence, in these mice, the hGFAP promoter drives expression of GFP in immature astrocytes (Malatesta et al., 2000). Many developing astrocytes in the perinatal (P6) telencephalon of *hGFAP-eGFP* transgenics coexpressed GFP and Olig2 (Fig. 2*J*,*K*). Second, we performed a short-term lineage tracing analysis of cells that had expressed Olig2 by examining forebrain sections taken from Olig2-GFP knock-in mice. Olig2-GFP+ progenitors appeared to give rise to all glial lineages, including many GFAP+ astrocytes, in the white matter and cerebral cortex (Fig. 2*L*–*N*). Third, we analyzed the expression pattern of Olig2 at different stages of astrocyte development *in vitro*. Astrocytes express a predictable, sequential pattern of intermediate filaments during their differentiation. Immature astrocytes express vimentin (Dahl et al., 1981), whereas the additional expression of GFAP (Chiu et al., 1981) is often used as an indicator of maturation. SVZ progenitors, harvested at P3, were cultured for 4 d in astrocyte-supporting conditions to generate immature astrocytes. Most cells coexpressed vimentin and Olig2 after 4 d *in vitro* (div) (Fig. 2*O*). These cells did not express immunodetectable levels of GFAP. In contrast, older astrocyte cultures at 8 div were comprised of more mature, GFAP+ cells. However, few of these astrocytes coexpressed Olig2 strongly (Fig. 2*P*), pointing again to a downregulation of Olig2 in mature astrocytes. These findings support results from previous studies in which Olig2 expression was positively correlated with an immature astrocyte phenotype but negatively correlated with a mature astrocyte phenotype in neural progenitor cell cultures (Fukuda et al., 2003; Gabay et al., 2003). A summary of Olig2 expression with respect to glial cell lineages in the postnatal forebrain is depicted in Figure 2*Q*.

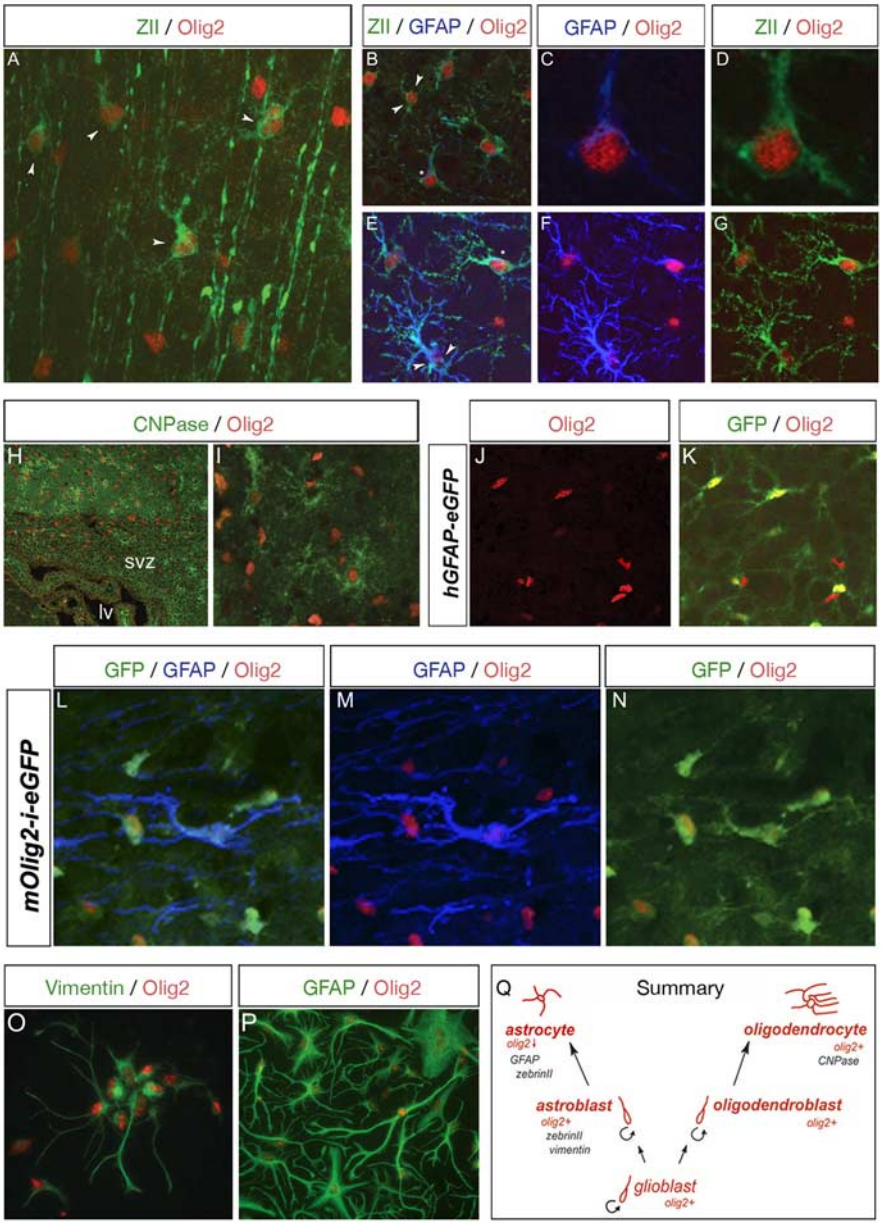

Figure 2. Developing astrocytes and oligodendrocytes express Olig2. ***A***, Cortical radial glia with an intermediate morphology (arrowheads), indicative of a transformation into astrocytes, express Zebrin II and Olig2 (P3, coronal section through murine cerebral cortex). ***B–D***, Olig2 and Zebrin II are expressed robustly in developing astrocytes (P6, murine cerebral cortex) at early stages of differentiation before GFAP expression (***B***, arrowheads) and at later stages as GFAP is upregulated (***B***, asterisk). The cell marked with an asterisk in ***B*** is magnified to show perinuclear GFAP (***C***) and Zebrin II (***D***) immunolabeling with respect to nuclear Olig2 expression. ***E–G***, Mature and fully arborized astrocytes (P6, murine cerebral cortex) expressing high amounts of GFAP and Zebrin II (***E***, arrowheads) consistently appeared to express lower levels of Olig2 than astrocytes with immature morphologies that express less GFAP (***E***, asterisk). ***H***, ***I***, CNPase+/Olig2+ oligodendrocytes in parasagittal sections of P6 mouse forebrain. ***J***, ***K***, Developing astrocytes in the *hGFAP-GFP* mouse (P7) forebrain coexpress GFP and Olig2. ***L***, ***M***, Astrocytes in the *mOlig2-I-eGFP* mouse forebrain (P6, cortex) coexpress GFAP, GFP, and Olig2. ***O***, Cell cultures generated from P3 mouse SVZ contained many vimentin+/Olig2+, immature astrocytes after 4 div. ***P***, After 8 div, cultures were mostly comprised of mature, GFAP+ astrocytes. Olig2 immunoreactivity in these mature astrocytes was markedly reduced. ***Q***, Summary of Olig2 expression with respect to glial cell lineages in the postnatal forebrain.

Olig2 is expressed exclusively by SVZ-derived glia

SVZ progenitors generate interneurons, astrocytes, and oligodendrocytes during the perinatal period. Those cells specified to become glia migrate radially into the cerebral cortex, subcortical white matter, and striatum, whereas those specified to become interneurons migrate rostrally, via the RMS, to the olfactory bulb. As noted above, astrocytes and oligodendrocytes in the forebrain expressed Olig2, whereas cells in the RMS did not. Furthermore, a short-term lineage analysis using Olig2-GFP knock-in mice revealed GFP expression in postnatal forebrain glia but not olfactory interneurons. To determine definitively which SVZ-derived cells express Olig2, we transduced SVZ cells *in vivo* with GFP using a replication-incompetent retrovirus encoding *GFP* (*X-ires-eGFP*) and analyzed infected cells at several time points. A virus was injected unilaterally into the SVZ of neonatal rat pups (P2/P3) at the rostral aspect of the SVZ, in which the RMS diverges from the SVZ (Fig. 3*F*, arrow), using a stereotax. Infected cells were examined at 2 and 4 d postinjection

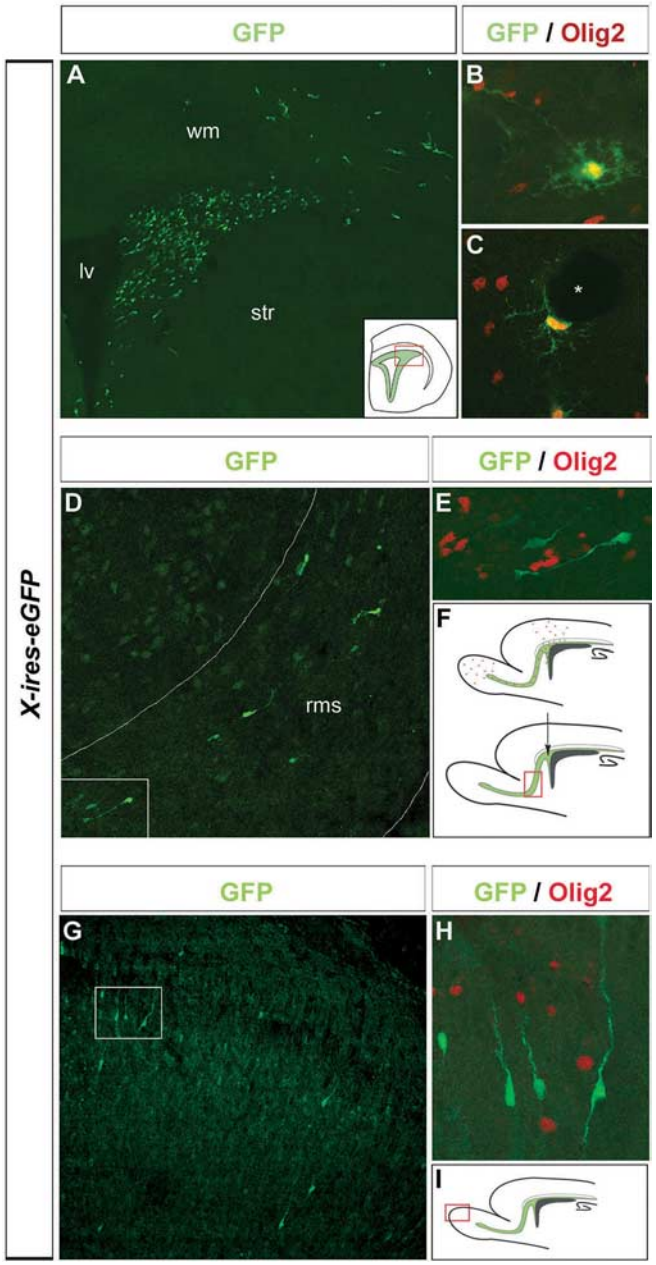

Figure 3. Olig2 is expressed exclusively by SVZ-derived glia. ***A***, GFP expression 2 d after stereotactic injection of replication-deficient retrovirus (*pNIT-eGFP*) into P2/P3 rat SVZ. See ***F*** for the site of injection and distribution of infected cells at 4 dpi. ***B***, ***C***, At 4 dpi, GFP+/Olig2+ cells with oligodendrocyte (***B***) and astrocyte (***C***) morphologies were observed in the parenchyma (the asterisk in ***C*** indicates a blood vessel enwrapped by an astrocyte). ***D***, ***E***, SVZ-derived neuroblasts do not express Olig2 as they migrate in the RMS, which is outlined for clarity. The boxed area is magnified in ***E***. ***F***, Distribution of SVZ cells transduced with GFP. The red box indicates the area of the photomicrograph in ***D***. ***G–I***, SVZ-derived interneuronal precursors remain Olig2− within the olfactory bulb. The boxed area in ***G*** is magnified in ***H***. ***I***, The red box indicates the area of the photomicrograph in ***G***. lv, Lateral ventricle; wm, white matter; str, striatum.

(dpi) in coronal (Fig. 3*A–C*) and parasagittal (Fig. 3*D–I*) sections. The distribution and phenotypes of GFP+ cells in the olfactory bulb, RMS, SVZ, white matter, and cortex (Fig. 3*F*) were similar to those observed previously (Suzuki and Goldman, 2003). At 2 dpi, most infected cells were found within the SVZ, whereas only a few had migrated into the overlying white matter (Fig. 2*A*). By 4 dpi, infected cells colonized the white matter, cortex, striatum, RMS, and olfactory bulb. Many of the infected SVZ cells that had migrated radially into the white matter, striatum, and cortex were GFP+/Olig2+ and exhibited morphologic characteristics of oligodendrocytes (Fig. 3*B*) as well as immature astrocytes (Fig. 3*C*; the asterisk marks a blood vessel associated with the astrocyte). In contrast, GFP+ cells migrating in the RMS (Fig. 3*D–F*) or colonizing the olfactory bulb (Fig. 3*G–I*) did not express Olig2. Interestingly, Olig2+ cells lining the RMS (Fig. 1*K*) are closely adjacent to Olig2- migratory neuroblasts (Fig. 3*E*) and perhaps comprise part of the astrocyte population that encapsulates neuroblasts within the RMS (Doetsch et al., 1997).

In summary, SVZ cells that became specified to a gliogenic lineage and migrated radially into the parenchyma expressed Olig2. In contrast, SVZ cells specified to an olfactory interneuron lineage that migrated rostrally via the RMS and differentiated in the olfactory bulb did not express Olig2. These findings demonstrated an intriguing correlation between Olig2 expression and glial fates, raising the possibility that Olig2 transcriptional regulatory function may direct the specification of glial versus neuronal lineages in SVZ progenitors.

SVZ progenitors transduced with Olig2 differentiate exclusively into glia

To examine the potential functions of Olig2 in directing SVZ progenitors to glial lineages, we transduced SVZ cells with a bicistronic retrovirus encoding *Olig2* and *eGFP* (*Olig2-ires-eGFP*) or a control virus encoding *eGFP* alone (*X-ires-eGFP*). All experiments were performed using the same retroviral vector (pQCXIX), protein coat (VSV-G), and stereotaxic coordinates.

Retrovirus was injected into neonatal rats as described above, and brains ($n = 8$) were harvested at 4 and 8 dpi. Olig2 expression in SVZ cells appeared to prevent the migration and differentiation of olfactory interneurons, because no infected cells were observed in the distal RMS or olfactory bulb (Fig. 4*C*). Immunohistochemistry using horseradish peroxidase and diaminobenzidine precipitation was performed to ensure that cells expressing low levels of GFP, and thus undetectable by immunofluorescence, were evaluated. This more sensitive analysis did not reveal any labeled cells in the RMS or olfactory bulb (data not shown). Instead, the large majority (~88%) of cells misexpressing Olig2 migrated radially from the SVZ into the white matter and cerebral cortex, areas typically colonized by glia (Fig. 4*C*). Others (~12%) differentiated within the SVZ/proximal RMS (Fig. 4*A–D*) before their migration. We did not observe differentiated cells within the SVZ or RMS in brains injected with our control virus; this premature differentiation is not a behavior typical of SVZ cells (Suzuki and Goldman, 2003).

To quantitate the relative distributions of infected cells, a sampling of three comparable parasagittal sections was taken from five brains misexpressing Olig2 and three brains expressing the control vector at 4 dpi. Selected sections contained the injection site and the entire RMS. Cells infected with the *X-ires-eGFP* control virus (Fig. 5*A*) migrated radially into the white matter, striatum, and all layers of the cerebral cortex as well as rostrally into the RMS and olfactory bulb (Fig. 5*B*). In these control parasagittal sections, 51% of the cells were found in the olfactory bulb, and 27% were found in the RMS. In contrast, the majority of cells misexpressing *Olig2-ires-eGFP* (Fig. 5*C*) migrated into the white matter (51%) and all layers of the cerebral cortex (37%); 12% were found in the proximal RMS, but no cells were identified in the distal RMS or olfactory bulb (Fig. 5*D*). Infected brains were cut along a parasagittal plane, enabling us to analyze the rostral versus radial migration of SVZ cells within each section. Our

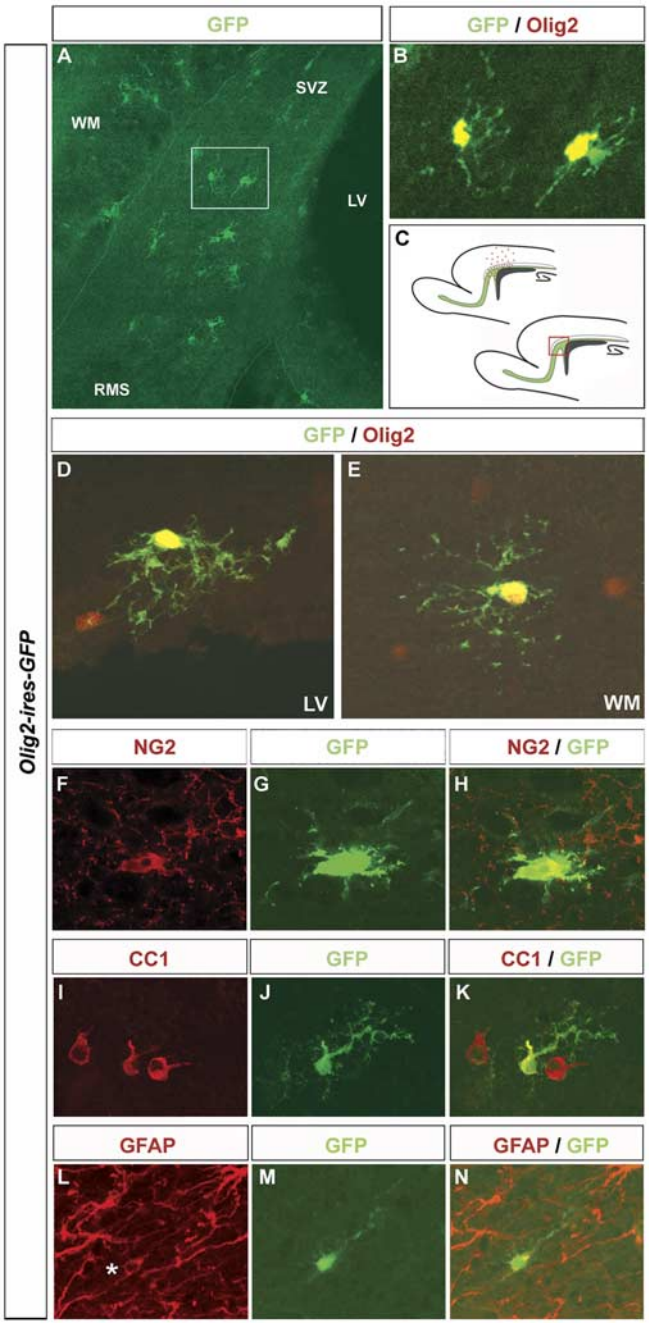

Figure 4. SVZ progenitors transduced with Olig2 differentiate exclusively into astrocytes and oligodendrocytes. ***A–C***, Parasagittal section of rat forebrain at 4 dpi, showing cells transduced with *Olig2-ires-eGFP*. The boxed area in ***A*** is magnified in ***B***, highlighting differentiated glial cells within the SVZ/RMS. ***C***, Distribution of cells expressing *Olig2-ires-eGFP* at 4 dpi. The red box indicates the area of SVZ and proximal RMS shown in ***A***. ***D***, Infected cells differentiated ectopically in the SVZ, adjacent to the lateral ventricles. ***E–K***, More than 70% of infected cells exhibited the morphology of oligodendrocytes (***E***) and expressed the oligodendrocyte-specific markers NG2 (***F–H***) and CC1 (***I–K***). ***L–N***, Approximately 20% of infected cells exhibited astrocyte morphologies and expressed GFAP (asterisk). lv, Lateral ventricle; wm, white matter; str, striatum.

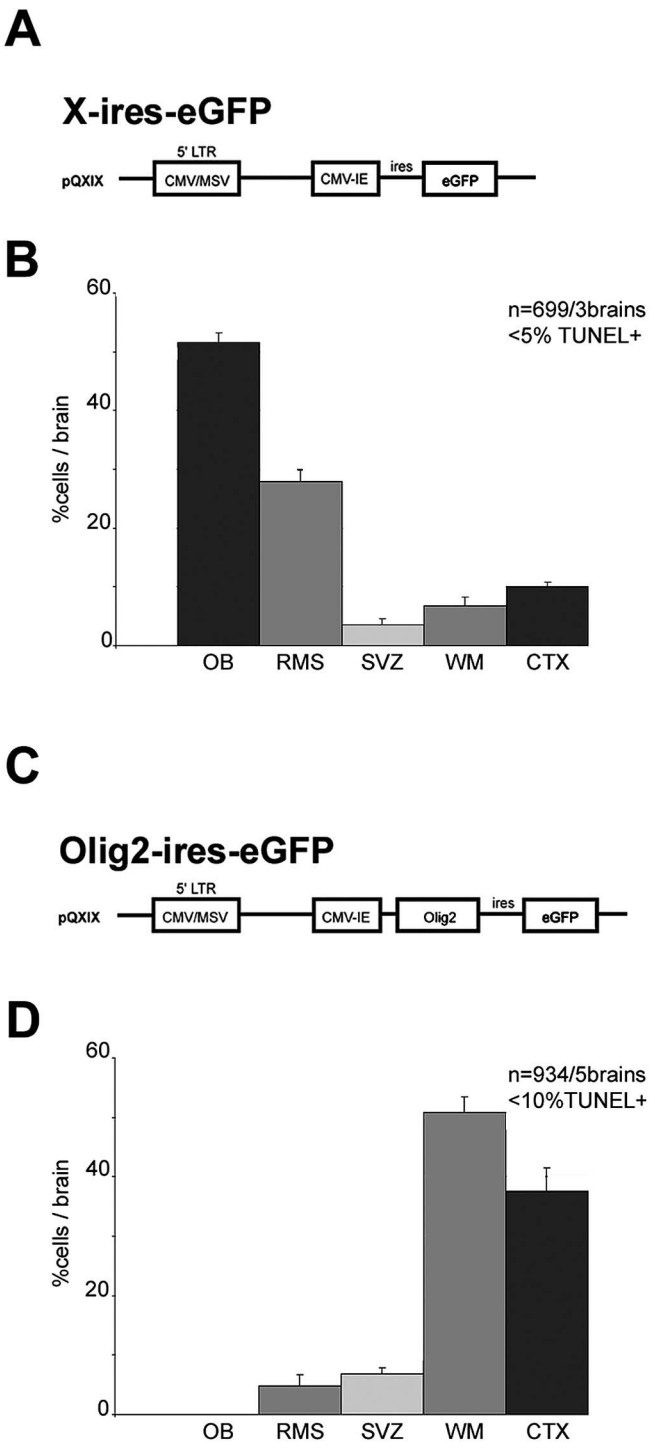

Figure 5. Distribution of SVZ cells expressing *X-ires-eGFP* and *Olig2-ires-eGFP*. ***A–D***, Retroviral constructs used to transduce SVZ cells with GFP (***A***) or Olig2-GFP (***C***) and quantified distributions of cells misexpressing GFP (***B***) or Olig2-ires-GFP (***D***), presented as a percentage of infected cells per brain located in each area (mean ± SEM). OB, Olfactory bulb; WM, white matter; CTX, cerebral cortex.

quantitative distributions (Fig. 5*B*,*D*) represent cells solely within this parasagittal plane, however, and do not predict the distribution of labeled cells throughout the entire brain. Because many SVZ-derived glia migrate to dorsolateral areas of the forebrain, these cells are not represented in our quantitations.

The skewed, radial distribution of cells misexpressing Olig2 suggests a redirection of cells migrating rostrally from the SVZ/RMS to areas normally colonized by SVZ-derived glia. It is unlikely that a difference in viral titer or apoptosis is responsible for the cellular distributions or phenotypes we observed. All viruses were titered to 1–3 $\times$ 10^5 cfu/ml. Furthermore, the numbers of infected cells were consistent across experiments. In brains harvested at 4 dpi, an average of 66.3 cells infected with *Olig2-ires-*

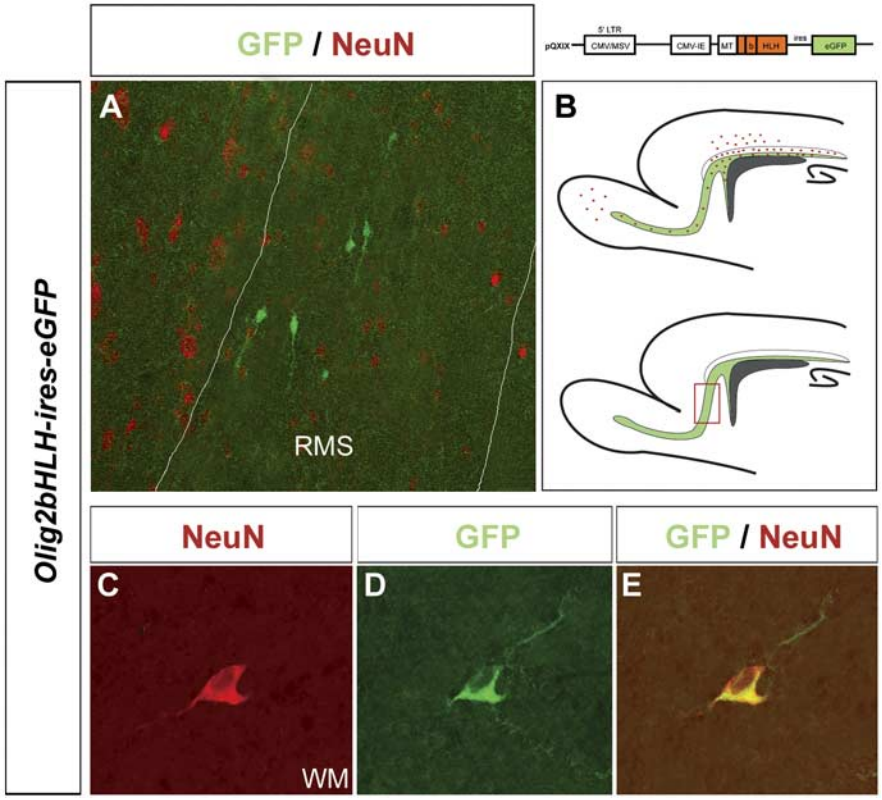

Figure 6. A dominant-interfering form of Olig2 blocks glial differentiation. ***A***, ***B***, SVZ cells misexpressing *Olig2 bHLH* exhibited normal migration to the olfactory bulb via the RMS (***A***), which is outlined for clarity. The distribution of total infected cells at 4 dpi is shown in ***B***. The red box indicates the area of RMS pictured (***A***). Infected cells also colonized the white matter and cerebral cortex, but glial differentiation was blocked in >90% of cells (data not shown). ***C–E***, However, ~25% of infected cells in the white matter and cortex ectopically expressed the neuronal marker NeuN, suggesting a potential respecification along a neuronal lineage. wm, White matter.

eGFP and 77 cells infected with the *X-ires-eGFP* control were observed per section. Furthermore, our findings do not appear to be the result of apoptosis. Few infected cells contained TUNEL+ nuclei in brains injected with either the *X-ires-eGFP* control (<5%) (Fig. 5*B*) or *Olig2-ires-eGFP* (<10%) (Fig. 5*D*); therefore, Olig2 misexpression did not selectively kill all SVZ cells destined to become olfactory interneurons. Rather, the neuroblasts, glioblasts, and progenitors that were infected with *Olig2-ires-GFP* appeared to differentiate unilaterally along a glial lineage. Over 70% of infected cells exhibited morphologies typical of lacy (Fig. 4*E*) or myelinating oligodendrocytes and expressed NG2 (Fig. 4*F–H*) or CC1 (Fig. 4*I–K*), whereas 20% had the morphology of astrocytes and expressed GFAP (Fig. 4*L–N*). Approximately 10% of the infected cells appeared to exhibit glial morphologies but did not express oligodendrocytic (NG2, CC1), astrocytic (GFAP), or neuronal [NeuN, microtubule-associated protein 2 (MAP2)] markers. In control experiments, the same relative percentage of oligodendrocytes (70%) and astrocytes (30%) were generated by perinatal SVZ cells that emigrated into the white matter and cortex (Levison and Goldman, 1993). Notably, *Olig2-ires-eGFP*+ cells that expressed GFAP (Fig. 4*L–N*) exhibited an immature astrocytic morphology with limited arborization, suggesting that constitutive Olig2 expression inhibited complete astrocyte maturation. In contrast, brains infected with control virus contained many GFP+/GFAP+ fully mature astrocytes and olfactory interneurons, as described previously (Levison et al., 1993; Luskin et al., 1993; Suzuki and Goldman, 2003).

In summary, the expression of Olig2 in neonatal SVZ cells is sufficient to direct glial versus neuronal identities, perhaps prematurely in some cells. Because no infected cells expressed either the neuronal marker NeuN or MAP2, our findings suggest that constitutive expression of Olig2 directs SVZ progenitors to migrate radially, away from the SVZ/RMS, and differentiate into glia at the expense of interneurons. Apoptotic indices do not appear to account for the differences in distributions of infected cells. However, rapid cell death of neuroblasts misexpressing Olig2 might not have been observed.

A dominant-interfering form of Olig2 blocks glial differentiation

Because Olig2 expression appeared to specify SVZ cells toward glial lineages, we examined the consequences of a loss of Olig2 activity. Olig2 influences cell identity through its ability to bind DNA and repress target gene expression (Novitch et al., 2001; Zhou et al., 2002). We reasoned that misexpression of the Olig2 bHLH DNA binding domain alone might interfere with the repressor function of endogenous Olig2. SVZ cells were therefore transduced with an Olig2 construct containing the bHLH domain alone (*Olig2bHLH-ires-eGFP*) (Fig. 6), as described above.

In all brains examined (4 and 8 dpi; $n = 8$), cells expressing *Olig2bHLH-ires-eGFP* migrated into the RMS, olfactory bulb, white matter, and cortex (Fig. 6*B*). However, glial differentiation was profoundly disrupted. Infected cells that migrated radially exhibited a normal but immature, unipolar or bipolar morphology. Few differentiated GFAP+ astrocytes (<5% per brain) and CC1+ oligodendrocytes (<5%) were observed, and those that were seen were found exclusively in the white matter (data not shown). Furthermore, ~5% of infected cells in the white matter and cortex retained Dlx2 expression, which was downregulated completely by SVZ-derived glia at the same time points in control experiments. In contrast, interneuron migration and differentiation did not appear to be affected by expression of the *Olig2bHLH-ires-eGFP* construct. Many infected cells (40%) migrated rostrally into the RMS (Fig. 6*A*,*B*) and olfactory bulb, where they differentiated normally (data not shown). We infer from these results that Olig2 serves a required function in both astrocyte as well as oligodendrocyte formation but is not necessary for interneuron development in SVZ cells.

Although *Olig2bHLH-ires-eGFP*+ cells colonized the white matter and cerebral cortex, ~25% of these infected cells ectopically expressed the neuronal marker NeuN (Fig. 6*C–E*). This was never observed in brains injected with the *X-ires-eGFP* control virus and is not a behavior typical of SVZ-derived cells (Suzuki and Goldman, 2003), suggesting that disruption of Olig2 function may cause cells in these normally gliogenic regions to adopt neuronal fates. We surmise that the appearance of cells with neuronal characteristics in the white matter and cerebral cortex most likely results from SVZ progenitors in which the onset of Olig2 bHLH domain expression had occurred at the time of or shortly

after these progenitors had begun their radial migration, and thus too late for these cells to enter the RMS. In addition to these aberrantly positioned neurons, we found that ~30% of *Olig2bHLH-ires-eGFP* infected cells in each brain lacked processes and had TUNEL+ nuclei. A likely possibility is that cells that become respecified to a neuronal fate ectopically may not receive appropriate environmental trophic support, leading to their premature death.

In summary, ectopic expression of a competitor form of Olig2 prevented both astrocyte and oligodendrocyte differentiation in >90% of infected SVZ cells. However, expression of the truncated Olig2 did not interfere with the specification of neuroblasts or their migration to the olfactory bulb. Cells that migrated radially into the developing forebrain either remained undifferentiated or began to differentiate ectopically along a neuronal lineage. Together, these results strongly support a requirement for Olig2 during SVZ progenitor specification to a glial fate.

An antimorphic activator form of Olig2 promotes neurogenesis in SVZ cells

To confirm that Olig2 repressor activity is required for SVZ progenitor specification to glial fates, we used a dominant-activator form of Olig2 to reverse endogenous Olig2 repressor function. A retrovirus encoding the Olig2 bHLH subdomains fused to a herpes simplex VP16 activator domain was generated (*Olig2bHLH/VP16-ires-eGFP*) (Fig. 7), and injections were performed as described.

Infected cells from brains harvested at 4 and 8 dpi ($n = 9$) were examined. Infected cells either remained within the SVZ or migrated a short distance into the overlying white matter, layer 6, or proximal RMS (Fig. 7*A–C*). No infected cells exhibited glial morphologies or expressed the glial markers vimentin, GFAP, CC1, or NG2. Rather, cells expressing the antimorph appeared to differentiate prematurely into interneurons. Strikingly, >75% of infected cells positioned within the SVZ, proximal descending arm of the RMS, white matter, and cortex, expressed the neuronal markers MAP2 (Fig. 7*D–F*) or NeuN (Fig. 7*G–I*). Furthermore, ~30% of infected cells also expressed markers specific to interneurons, such as calbindin (Fig. 7*J–L*) or calretinin (Fig. 7*M–O*) but exhibited relatively immature neuronal morphologies. Thus, expression of the dominant-activator form of Olig2 appeared to elicit an aberrant and premature program of neuronal differentiation. As in the case of the bHLH domain-alone construct, misexpression of the activator form of Olig2 led to a significant amount of apoptosis, ~50% of the infected cell population. Together, these findings provide evidence that Olig2 repressor function is required to prevent neuronal differentiation and to direct both astrocytic and oligodendrocytic fates in postnatal SVZ progenitors.

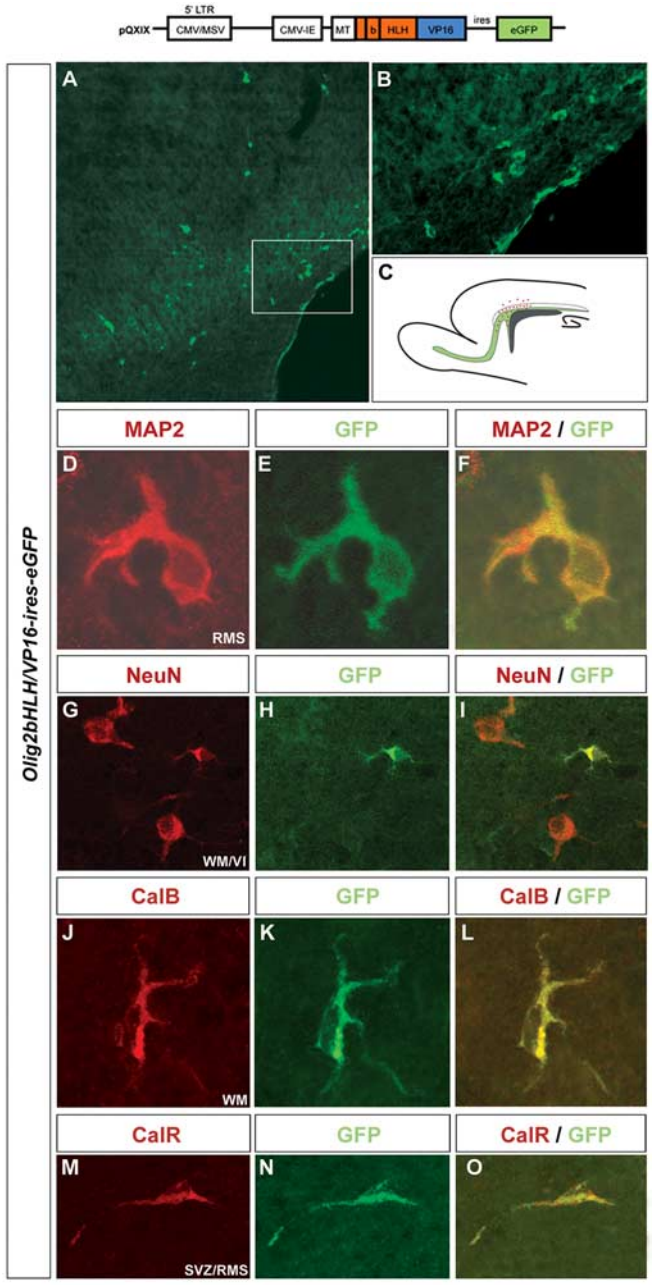

Figure 7. An antimorphic activator form of Olig2 promotes neurogenesis in SVZ cells. ***A–C***, Expression of an *Olig2bHLH-VP16* activator fusion construct limited the migration of SVZ cells. Cells migrated short distances into the proximal RMS, white matter, and layer 6 of the cerebral cortex. The boxed area in ***A***, containing infected SVZ cells, is magnified in ***B***. The distribution of infected cells is shown in ***C***. ***D–I***, More than 75% of infected cells expressed the neuronal markers Map2 (***D–F***) and NeuN (***G–I***). ***J–O***, Approximately 30% of cells in the white matter and SVZ/RMS expressed interneuron-specific markers such as calbindin (***J–L***) and calretinin (***M–O***). WM/VI, White matter/layer 6 border; LV, lateral ventricle.

Discussion

We provide evidence that Olig2 specifies SVZ cells to pan-glial versus neuronal lineages. By repressing a neuronal phenotype in SVZ progenitors, Olig2 expression permits the production of astrocyte and oligodendrocyte precursors. Our findings establish a previously unappreciated role for Olig2 in astrocyte development and suggest that Olig2 directs the primary specification of glial versus neuronal lineages in SVZ progenitors, making it the first intrinsic fate determinant shown to operate within the postnatal SVZ.

A role for Olig2 in astrocyte differentiation

Olig2 expression has not been reported previously in forebrain astrocytes. It is not surprising, however, that this is the case. GFAP is the most common molecular marker used for the identification of astrocytes, and it is expressed at relatively late stages of astrocyte development in rodents. Still, we observed many astrocytes throughout the forebrain that coexpressed Olig2 and GFAP. One possible explanation for the discrepancy between our findings and those of other studies is the method of detecting GFAP in tissue sections. Using basic immunofluorescence techniques, GFAP expression is readily detectable in a subset of the astrocytes populating the SVZ, white matter, and subpial area.

However, other astrocytes, located in gray matter, express lower levels of GFAP, which is less easily detectable. To visualize all forebrain astrocytes with GFAP immunofluorescence, we used an antigen retrieval technique. Many of these "unmasked" GFAP-expressing astrocytes were positive for Olig2. Thus, this difference in GFAP detection might account for differences between our data and those reporting no Olig2 expression in forebrain astrocytes (Zhou et al., 2002).

In addition, Olig2 expression is downregulated in mature astrocytes. Astrocytes in the adult forebrain express little, if any, immunodetectable Olig2 (R. Ventura, unpublished observations). Thus, studies focused on Olig2 expression in mature glia would not have seen expression in astrocytes. A transient expression in developing astrocytes supports *in vitro* studies in which Olig2 expression was positively correlated with an immature astrocyte phenotype but negatively correlated with a fully mature phenotype (Gabay et al., 2003) and partially inhibited GFAP transcription (Fukuda et al., 2003) in neural progenitor cultures. However, in these experiments, a significant proportion of cells misexpressing Olig2 remained GFAP+. Thus, Olig2 may not be sufficient to regulate GFAP expression. Together with our findings *in vivo*, these results point to two potential roles for Olig2 in astrocyte development (supplemental Fig. 2, available at www.jneurosci.org as supplemental material). First, Olig2 function is required for astrocyte formation in SVZ cells. Second, Olig2 may also serve to limit GFAP expression.

Olig1/2 mutants suffer a complete loss of oligodendrocytes, but no astrocyte phenotype was observed (Zhou et al., 2002). Several potential interpretations of the Olig1/2 mutants can be made with respect to the forebrain. First, there are at least two sources of astrocytes in the postnatal forebrain. One population arises directly from radial glia that transform during the perinatal period into astrocytes (Voigt, 1989), whereas another population arises from migratory progenitors within the SVZ (Levison and Goldman, 1993; Luskin et al., 1993). Therefore, even a complete deficit in one class of astrocyte may not be noticeable on a gross level of examination. Our findings only demonstrate a requirement for Olig2 in the development of SVZ-derived astrocytes. Importantly, our retroviral labeling technique does not infect postnatal radial glia, which proliferate infrequently at this time relative to SVZ progenitors. Thus, it is possible that astrocytes observed in the Olig1/2 mutants could be derived exclusively from radial glial origins but not from SVZ-derived astrocytes, which require Olig2 expression for their formation.

Could Olig1 compensate for Olig2? Olig1 and Olig2 expression is similar if not identical in the postnatal SVZ (C. A. G. Marshall, unpublished observations), and Olig1 misexpression increases astrocytes and oligodendrocytes in the developing forebrain (Lu et al., 2001); the two factors may work together in repressing neurogenesis and/or promoting gliogenesis. However, if Olig1 compensated fully for Olig2 in the Olig2 blocking experiments, we would have expected to see some gliogenesis in SVZ cells expressing the truncated Olig2 construct, but we did not. It is unlikely that Olig1 and Olig2 bind to identical DNA sequences, given the low homology these genes share in their bHLH domains. The Olig2 constructs used for functional experiments are specific (Novitch et al., 2001; Zhou and Anderson, 2002; Gabay et al., 2003).

Olig2 expression in radial glia

Although it is not clear whether Olig2 is required for the production of astrocytes from postmitotic radial glia, we found that cortical radial glia did express Olig2 during the perinatal period (we examined P0–P10). Unlike the spinal cord, the cerebral cortex does not express Olig2 during earlier, neurogenic stages of development (Nery et al., 2001; our unpublished data). Rather, Olig2 is expressed in radial glia later, at a time when they have finished generating projection neurons and have "switched" from a primarily neurogenic state to a primarily gliogenic state (Anthony et al., 2004). Therefore, another potential role for Olig2 in neonatal forebrain development is to repress neurogenesis in cortical radial glia. Radial glia express astrocytic molecules such as glutamate transporters and intermediate filaments and may be committed to their astrocytic fate, however, before expression of Olig2. Experiments aimed at understanding the function of Olig2 in these dynamic cells will be required to understand any part it might play in determining their fates or the fates of their progeny.

SVZ cell phenotype and migration

SVZ cells specified to become interneurons migrate rostrally via the RMS into the olfactory bulb (Altman, 1969; Luskin, 1993; Lois and Alvarez-Buylla, 1994), whereas SVZ cells specified to become glia migrate radially into the developing parenchyma (Fig. 1*A*) (Altman, 1966; Paterson et al., 1973; Imamoto et al., 1978; Levison and Goldman, 1993; Luskin et al., 1993; Suzuki and Goldman, 2003). The synchronization of SVZ cell phenotype and migration behavior is not understood. Do SVZ progenitors stochastically choose a rostral versus radial migration pathway before becoming committed to neuronal and glial lineages? Or, does the phenotype of a cell dictate the direction of its migration? One model that would explain this tight correlation between cell fate and migration behavior is one in which progenitors become specified to a glial lineage before their emigration from the SVZ. Our findings strongly support such a model. We demonstrate that SVZ cell fate decisions are likely to depend critically on the expression of transcriptional regulators such as Olig2. Olig2-directed glial specification may subsequently confer on these cells an ability to migrate across SVZ borders through transcriptional regulation of specific receptors or adhesion molecules involved in accessing the radial glial scaffold and migrating through the perinatal brain. Because Olig2 functions as a transcriptional repressor, it might serve to downregulate genes that otherwise prevent emigration from the SVZ. Thus, a knowledge of downstream targets for Olig2 may contribute to an understanding of phenotypic specification as well as cell distribution within the postnatal brain.

SVZ cell potential

Our model (supplemental Fig. 2, available at www.jneurosci.org as supplemental material) reconciles data from *in vitro* studies (Noble et al., 2004), which point to bipotent and tripotent cells in the developing forebrain (Levison and Goldman, 1997) and spinal cord (Gabay et al., 2003), with *in vivo* work (Levison and Goldman, 1993; Luskin et al., 1993; Parnavelas, 1999; Liu and Rao, 2004; Zerlin et al., 2004), in which a smaller population of cells demonstrate such broad potential. What intrinsic and extrinsic cues direct progenitors that are multipotent *in vitro* to a limited range of fates *in vivo*? Studies of progenitor specification have been performed predominantly in embryonic systems, such as the spinal cord, in which a sequential assignment of neuronal and glial phenotypes has been revealed and important molecular fate regulators have been identified (Lee and Jessell, 1999; Briscoe and Ericson, 2001). Although recent studies have indicated functions for some of these molecular regulators in forebrain development (Woodruff et al., 2001; Bertrand et al., 2002), the dynamics of neuronal and glial cell production in the subventricular

zone are distinct from those in the embryonic cord and are less clear.

The SVZ generates interneurons, astrocytes, and oligodendrocytes simultaneously during the perinatal period, but its output changes over time, so that the adult SVZ primarily generates neurons (Doetsch et al., 1999, 2002) and only limited numbers of glia (Hack et al., 2005). The molecular mechanisms regulating this switch to neurogenesis are not known, and a lack of temporospatial segregation among neuronal and glial progenitors has made this switch difficult to analyze. Even in the adult SVZ, however, constitutive expression of Olig2 via retrovirus leads to a substantial increase in gliogenesis (Hack et al., 2005), suggesting that perinatal mechanisms may persist into adulthood. Our data showing the sufficiency of Olig2 in directing primary, pan-glial versus neuronal fate decisions in the perinatal SVZ is a fundamental step toward understanding the molecular mechanisms that regulate SVZ cell fates. In the future, a detailed knowledge of SVZ cell fate regulation under normal conditions will be paramount for studying the responses of such cells to pathologic cues, as well as accessing this endogenous source of progenitors for potential therapeutic interventions in the diseased brain.

References

Altman J (1966) Proliferation and migration of undifferentiated precursor cells in the rat during postnatal gliogenesis. Exp Neurol 16:263–278.

Altman J (1969) Autoradiographic and histological studies of postnatal neurogenesis. IV. Cell proliferation and migration in the anterior forebrain, with special reference to persisting neurogenesis in the olfactory bulb. J Comp Neurol 137:433–458.

Anthony TE, Klein C, Fishell G, Heinz N (2004) Radial glia serve as neuronal progenitors in all regions of the central nervous system. Neuron 41:881–890.

Bertrand N, Castro DS, Guillemot F (2002) Proneural genes and the specification of neural cell types. Nat Neurosci Rev 3:517–530.

Briscoe J, Ericson J (2001) Specification of neuronal fates in the ventral neural tube. Curr Opin Neurobiol 11:43–49.

Chiu F-C, Norton WT, Fields KL (1981) The cytoskeleton of primary astrocytes in culture contains actin, glial fibrillary acidic protein, and the fibroblast-type filament protein, vimentin. J Neurochem 37:147–155.

Dahl D, Rueger DC, Bignami A (1981) Vimentin, the 57000 molecular weight protein of fibroblast filaments, is the major cytoskeletal component in immature glia. Eur J Cell Biol 24:191–196.

Doetsch F, Garcia-Verdugo JM, Alvarez-Buylla A (1997) Cellular composition and three-dimensional organization of the subventricular germinal zone in the adult mammalian brain. J Neurosci 17:5046–5061.

Doetsch F, Caille I, Lim DA, Garcia-Verdugo JM, Alvarez-Buylla A (1999) Subventricular zone astrocytes are neural stem cells in the adult mammalian brain. Cell 97:703–716.

Doetsch F, Petreanu L, Caille I, Garcia-Verdugo JM, Alvarez-Buylla A (2002) EGF converts transit-amplifying neurogenic precursors in the adult brain into multipotent stem cells. Neuron 36:1021–1034.

Fukuda S, Kondo T, Takebayashi H, Taga T (2003) Negative regulatory effect of an oligodendrocytic bHLH factor Olig2 on the astrocytic differentiation pathway. Cell Death Differ 11:196–202.

Gabay L, Lowell S, Rubin LL, Anderson DJ (2003) Deregulation of dorsoventral patterning by FGF confers trilineage differentiation capacity on CNS stem cells in vitro. Neuron 40:485–499.

Hack MA, Saghatelyan A, de Chevigny A, Pfeifer A, Ashery-Padan R, Lledo P-M, Götz M (2005) Neuronal fate determinants of adult olfactory bulb neurogenesis. Nat Neurosci 8:865–872.

Imamoto K, Paterson JA, Leblond CP (1978) Radioautographic investigation of gliogenesis in the corpus callosum of young rats. I. Sequential changes in oligodendrocytes. J Comp Neurol 180:115–138.

Kakita A, Goldman JE (1999) Patterns and dynamics of SVZ cell migration in the postnatal forebrain: monitoring living progenitors in slice preparations. Neuron 23:461–472.

Lee KJ, Jessell TM (1999) The specification of dorsal cell fates in the vertebrate central nervous system. Annu Rev Neurosci 22:261–294.

Levison SW, Goldman JE (1993) Both oligodendrocytes and astrocytes develop from progenitors in the subventricular zone of postnatal rat forebrain. Neuron 10:201–212.

Levison SW, Goldman JE (1997) Multipotential and lineage restricted precursors coexist in the mammalian perinatal subventricular zone. J Neurosci Res 48:83–94.

Liu Y, Rao MS (2004) Olig genes are expressed in a heterogeneous population of precursor cells in the developing spinal cord. Glia 45:67–74.

Lois C, Alvarez-Buylla A (1994) Long-distance neuronal migration in the adult mammalian brain. Science 264:1145–1148.

Lu QR, Cai L, Rowitch DH, Cepko CL, Stiles CD (2001) Ectopic expression of Olig1 promotes oligodendrocyte formation and reduces neuronal survival in developing mouse cortex. Nat Neurosci 4:973–974.

Luskin MB (1993) Restricted proliferation and migration of postnatally generated neurons derived from the forebrain subventricular zone. Neuron 11:173–189.

Luskin MB, Parnavelas JG, Barfield JA (1993) Neurons, astrocytes, and oligodendrocytes of the rat cerebral cortex originate from separate progenitor cells: an ultrastructural analysis of clonally related cells. J Neurosci 13:1730–1750.

Malatesta P, Hartfuss E, Gotz M (2000) Isolation of radial glial cells by fluorescent-activated cell sorting reveals a neuronal lineage. Development 127:5253–5263.

Marshall CAG, Goldman JE (2002) Subpallial Dlx2 expressing cells give rise to astrocytes and oligodendrocytes in the cerebral cortex and white matter. J Neurosci 22:9821–9830.

Marshall CAG, Suzuki SO, Goldman JE (2003) Gliogenic and neurogenic progenitors of the subventricular zone: who are they, where did they come from, and where are they going? Glia 43:52–61.

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

Noble M, Proschel C, Mayer-Proschel M (2004) Getting a GR(i)P on oligodendrocyte development. Dev Biol 265:33–52.

Novitch BG, Chen AI, Jessell TM (2001) Coordinate regulation of motor neuron subtype identity and pan-neuronal properties by the bHLH repressor Olig2. Neuron 31:773–789.

Parnavelas JG (1999) Glial cell lineages in the rat cerebral cortex. Exp Neurol 156:418–429.

Paterson JA, Privat A, Ling EA, Leblond CP (1973) Investigation of glial cells in semithin sections. III. Transformation of subependymal cells into glial cells as shown by radioautography after ^{3}H-thymidine injection into the lateral ventricle of the brain of young rats. J Comp Neurol 149:83–102.

Staugaitis SM, Zerlin M, Hawkes R, Levine JM, Goldman JE (2001) Aldolase C/zebrin II expression in the neonatal rat forebrain reveals cellular heterogeneity within the subventricular zone and early astrocytes differentiation. J Neurosci 21:6195–6205.

Suzuki SO, Goldman JE (2003) Multiple cell populations in the early postnatal subventricular zone take distinct migratory pathways: a dynamic study of glial and neuronal progenitor migration. J Neurosci 23:4240–4250.

Voigt T (1989) Development of glial cells in the cerebral wall of ferrets: direct tracing of their transformation from radial glia into astrocytes. J Comp Neurol 289:74–88.

Wichterle H, Lieberam I, Porter JA, Jessell TM (2002) Directed differentiation of embryonic stem cells into motor neurons. Cell 110:385–397.

Woodruff R, Tekki-Kessaris N, Stiles CD, Rowitch DH, Richardson WD (2001) Oligodendrocyte development in the spinal cord and telencephalon: common themes and new perspectives. Int J Dev Neurosci 19:379–385.

Zerlin M, Milosevic A, Goldman JE (2004) Glial progenitors of the neonatal subventricular zone differentiate asynchronously, leading to spatial dispersion of glial clones and to the persistence of immature glia in the adult mammalian CNS. Dev Biol 270:200–213.

Zhou Q, Anderson DJ (2002) The bHLH transcription factors Olig2 and Olig1 couple neuronal and glial subtype specification. Cell 109:61–73.