Development/Plasticity/Repair

Subventricular Zone Stem Cells Are Heterogeneous with Respect to Their Embryonic Origins and Neurogenic Fates in the Adult Olfactory Bulb

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We determined the embryonic origins of adult forebrain subventricular zone (SVZ) stem cells by Cre-lox fate mapping in transgenic mice. We found that all parts of the telencephalic neuroepithelium, including the medial ganglionic eminence and lateral ganglionic eminence (LGE) and the cerebral cortex, contribute multipotent, self-renewing stem cells to the adult SVZ. Descendants of the embryonic LGE and cortex settle in ventral and dorsal aspects of the dorsolateral SVZ, respectively. Both populations contribute new (5-bromo-2′-deoxyuridine-labeled) tyrosine hydroxylase- and calretinin-positive interneurons to the adult olfactory bulb. However, calbindin-positive interneurons in the olfactory glomeruli were generated exclusively by LGE-derived stem cells. Thus, different SVZ stem cells have different embryonic origins, colonize different parts of the SVZ, and generate different neuronal progeny, suggesting that some aspects of embryonic patterning are preserved in the adult SVZ. This could have important implications for the design of endogenous stem cell-based therapies in the future.

Key words: adult; mouse; subventricular zone; neural stem cells; olfactory bulb; interneurons; Cre-lox fate mapping; Gsh2; Emx1

Introduction

Since the identification of neural stem cells in the adult forebrain subventricular zone (SVZ) (Reynolds and Weiss, 1992; Richards et al., 1992), a lot of work has been done to characterize the phenotype of these stem cells, the SVZ cytoarchitecture, and factors involved in regulating stem cell maintenance and behavior (for review, see Gage, 2000; Doetsch, 2003; Alvarez-Buylla and Lim, 2004; Morshead and van der Kooy, 2004; Young et al., 2007). Adult neural stem cells have been described as a subpopulation of SVZ astrocytes because of their morphology and the fact that they express the astrocyte marker, glial fibrillary acidic protein (GFAP) (Doetsch et al., 1999a; Laywell et al., 2000). It is now well established that the slowly dividing SVZ stem cells generate rapidly dividing neuronal progenitors (transit amplifying cells) that in turn generate postmitotic neuroblasts. These migrate forwards along the rostral migratory stream (RMS) into the olfactory bulb, where they give rise to various subtypes of olfactory interneurons continuously throughout life (for review, see Lledo

et al., 2006). However, it is still unclear whether the SVZ contains a heterogeneous collection of neural stem cells with different neurogenic potentials or whether cell fate restriction occurs later, within the RMS or the olfactory bulb.

Neuroepithelial precursors in most parts of the embryonic telencephalon have a similar radial morphology and express antigenic markers characteristic of radial glia (Noctor et al., 2002; Malatesta et al., 2003; Anthony et al., 2004). However, there are also regional differences in gene expression, including regionally restricted expression of transcription factors (Kriegstein and Götz, 2003). For example, the medial ganglionic eminence (MGE) expresses Nkx2.1, the MGE and the lateral ganglionic eminence (LGE) express Gsh2, the corticostriatal sulcus expresses Dbx1, and the developing cortex expresses Emx1. As development proceeds, the neuroepithelial germinal zone [ventricular zone (VZ)] regresses and a relatively small proportion of neural stem cells persists in the SVZ of the adult forebrain. The relative contributions of different parts of the embryonic VZ to the adult SVZ have not yet been established, although the bulk of evidence suggests that adult stem cells are descendants of the LGE (embryonic striatum). For example, transcription factors that are expressed in the dorsal LGE of the embryo, such as Dlx1/2, Er81, Gsh2, and Pax6, are also present within the adult SVZ and RMS (Parmar et al., 2003; Stenman et al., 2003; Kohwi et al., 2005). In addition, viral lineage tracing of radial glia in the perinatal LGE has demonstrated that some of the progeny of these cells come to reside in the postnatal SVZ and contribute significantly to the adult neurosphere-forming activity (Merkle et al., 2004). Recent evidence supports the contribution of embryonic cortical (*Emx-1*-expressing) progenitors to generating the adult SVZ (Tamamaki, 2005; Willaime-Morawek et al., 2006; Ventura and Gold-

man, 2007). However, it is still unknown whether other parts of the embryonic VZ such as the MGE contribute cells to the adult SVZ or, if so, whether adult SVZ cells retain their previously established positional and functional identities into adulthood.

Here, we have examined the origins of adult SVZ stem cells by Cre-*lox* fate mapping of the embryonic telencephalic neuroepithelium, using *Nkx2.1-Cre*, *Gsh2-Cre*, *Dbx1-Cre*, *Emx1-Cre*, and *Emx1-CreER^{T2}* transgenic mice (Fogarty et al., 2005; Kessaris et al., 2006). We found that the MGE, LGE, and the embryonic cortex all generate cells that populate different parts of the adult SVZ and exhibit the *in vitro* characteristics of multipotent neural stem cells. Both the LGE- and cortex-derived stem cells generate RMS neuroblasts and are responsible for generating olfactory interneurons throughout life. However, these two stem cell populations make unequal contributions to adult neurogenesis. Cortex-derived stem cells, despite being the minority, generate the majority of calretinin-positive interneurons but none of the calbindin-positive interneurons. LGE-derived stem cells generate all of the adult-born calbindin-positive interneurons for the olfactory glomerulus.

Materials and Methods

Transgenic mice. The generation and genotyping of *Nkx2.1-Cre*, *Gsh2-Cre*, *Emx1-Cre*, *Dbx1-Cre*, and *Emx1-CreER^{T2}* mice were described previously (Fogarty et al., 2005; Kessaris et al., 2006). Each transgenic line was crossed with either of two Cre reporter lines: Rosa26-enhanced green fluorescent protein (*R26-GFP*) (Mao et al., 2001) (The Jackson Laboratory, Bar Harbor, ME) or Rosa26-yellow fluorescent protein (*R26-YFP*) (Srinivas et al., 2001) (kindly provided by S. Srinivas, Oxford, UK). To activate Cre recombinase activity in *Emx1-CreER^{T2}/R26-YFP* transgenics (Kessaris et al., 2006), we administered tamoxifen (Sigma, St. Louis, MO), dissolved by sonication at a concentration of 50 mg/ml in corn oil (Sigma). Induction at embryonic day 10.5 (E10.5) was performed using a single 4 mg dose of tamoxifen administered into the stomach of pregnant mothers by gavage. Induction in adult mice was achieved by administering 300 mg/kg tamoxifen per day by gavage on 5 consecutive days.

5-Bromo-2′-deoxyuridine labeling. To identify cells dividing *in vivo*, 5-bromo-2′-deoxyuridine (BrdU) (Sigma) was dissolved in PBS by sonication to a concentration of 20 mg/ml and was administered as four 2 mg intraperitoneal doses over 24 h (6:00 P.M., midnight, 6:00 A.M., noon).

Tissue preparation. Tissue was prepared as described previously (Young et al., 2007). Briefly, postnatal and adult mice [older than postnatal day 50 (P50)] were anesthetized with pentobarbitone and perfused through the ascending aorta, first with PBS and then with 4% (w/v) paraformaldehyde (PFA) (Sigma) in PBS. Brains were removed, immersed in fresh 4% PFA, and stored at 4°C overnight. Tissue was cryoprotected in 20% (w/v) sucrose in PBS for 24 h. All brains were embedded in OCT (optimal cutting temperature) compound, and coronal cryosections (15 or 30 μm nominal thickness) were collected onto Superfrost Plus slides (BDH Chemicals, Poole, UK) or floated in PBS.

Neurosphere culture. Brains were removed and examined under an inverted fluorescence microscope to confirm GFP expression. Neurosphere cultures were subsequently generated from the SVZ of 7- to 8-week-old (P50; young adult) mice by culturing in serum-free medium (Stem Cell Technologies, Vancouver, British Columbia, Canada) containing the mitogens epidermal growth factor (EGF) (Sigma) and basic fibroblast growth factor (bFGF) (Roche), as described previously (Reynolds and Weiss, 1992; Young et al., 2006, 2007). Primary cell suspensions were plated at clonal density (one cell per well) or at a density not exceeding 3500 cells/cm^2, and all cultures were maintained at 37°C in a 5% CO$_2$ atmosphere. Neurospheres were considered mature when they had reached an average diameter of 120 μm.

Expression of GFP in neurospheres was examined using an inverted fluorescence microscope at 6–7 d after plating. The number of GFP-positive neurospheres in each culture was scored and expressed as a percentage of the total. At day 7, a portion of the neurosphere culture was taken to test differentiation potential and the remainder was passaged.

Neurospheres were differentiated by plating onto glass coverslips pre-coated with poly-D-ornithine (Sigma) and cultured in DMEM/F12 medium (Invitrogen, Carlsbad, CA) with B27 supplement (Invitrogen) and 2% fetal calf serum (Invitrogen) for 5 d. Differentiated neurospheres were fixed for 10 min in 4% PFA at room temperature, washed three times with PBS, and stored at 4°C for immunostaining. The remainder of the culture was passaged by enzymatic dissociation with trypsin/EDTA (Invitrogen) to generate a single-cell suspension, replated at a density of 10^4 cells/cm^2, and serially passaged seven times or more.

Stem cell colony-forming assays. The proportion of neural stem cells originating from each region of the embryonic neuroepithelium was quantified using the Neurocult Neural Colony-Forming Cell Assay kit (Stem Cell Technologies) according to the manufacturer's instructions. In brief, a single-cell suspension was generated from the SVZ of each Cre-transgenic. The cells were mixed with NeuroCult NCFC Serum-Free Medium (Stem Cell Technologies), containing 20 ng/ml EGF (Sigma), 10 ng/ml bFGF (Roche), 3.5 μg/ml heparin (Sigma), and 30% (v/v) collagen solution (Stem Cell Technologies). The suspension was poured into three 35 mm plates per mouse and incubated for 3 weeks at 37°C in a 5% CO$_2$ atmosphere. Growth factors were replenished weekly. By the end of 3 weeks, ~5% of colonies were ≥2 mm in diameter

Each plate was fixed with 4% PFA for 45 min at room temperature, and then washed three times with PBS. The collagen matrix was removed from the 35 mm dishes and placed into a 100 mm Petri dish (three into one) containing 10 ml of blocking solution [PBS containing 0.1% (v/v) Triton X-100, 10% (v/v) normal sheep serum (Sigma)] and placed on an orbital shaker at room temperature for 3 h. The blocking solution was replaced with 10 ml of blocking solution containing rabbit anti-GFP (1:6000; Abcam, Cambridge, MA) and placed on a shaking platform overnight at 4°C. Matrices were washed in PBS during the day and stained with Alexa Fluor 488-conjugated goat anti-rabbit IgG (1:1000; Invitrogen) overnight at 4°C. Matrices were again washed in PBS for 4 h at room temperature before colony size was measured using a gridded Petri dish (Stem Cell Technologies). The neural stem cell colonies were characterized as ≥2 mm in diameter (B. Reynolds, unpublished data) (Bull and Bartlett, 2005) and scored as GFP positive or negative under a fluorescence dissecting microscope.

Immunocytochemistry. For GFP immunolabeling, sections were washed with PBS and permeabilized with blocking solution containing Triton X-100 and NGS (see above, Stem cell colony-forming assays) before incubating overnight with rabbit anti-GFP (1:6000) or rat anti-GFP IgG2a (1:1000; Nacalai Tesque, Kyoto, Japan). Sections were washed three times with PBS before adding Alexa Fluor 488-conjugated anti-rabbit IgG or Alexa Fluor 488-conjugated anti-rat IgG (1:1000; Invitrogen) in blocking solution. Sections were treated with Hoechst 33258 (1:10^4; Sigma) to detect cell nuclei and washed with PBS. Floating sections (30 μm) were transferred onto glass slides and mounted using Fluoromount (DakoCytomation, High Wycombe, UK). Sections were costained with Cy3-conjugated mouse anti-GFAP (1:3000; Sigma), rabbit anti-GFAP (1:500; DakoCytomation), mouse anti-neuronal-specific nuclear protein (NeuN) (1:500; Chemicon, Temecula, CA), rabbit anti-calbindin (1:1000; Swant, Bellinzona, Switzerland), rabbit anti-calretinin (1:1000; Swant), rabbit anti-parvalbumin (1:1000; Chemicon), rabbit anti-tyrosine hydroxylase (1:1000; Chemicon), guinea pig anti-doublecortin (1:3000; Chemicon), mouse anti-polysialylated neural cell adhesion molecule (PSA-NCAM) IgM (1:1000; Chemicon), or rabbit anti-Gsh2 polyclonal (1:2000; kind gift from Kenneth Campbell, University of Cincinnati, Cincinnati, OH) and detected with Alexa Fluor 568-conjugated anti-mouse IgG1, Alexa Fluor 568-conjugated anti-rabbit IgG, Alexa Fluor 568-conjugated anti-mouse IgM (Invitrogen; all used at 1:1000), or Cy3-conjugated anti-guinea pig (1:500; Pierce, Rockford, IL).

For BrdU labeling to determine the identity of newly born cells, 30 μm floating sections were first stained as above to detect GFP expression (or other listed antigens). The sections were subsequently collected onto glass slides and fixed with 70% ethanol/20% glacial acetic acid and treated for a further 20 min with 70% ethanol at −20°C. Slides were then washed with PBS/1% Triton X-100 (v/v) and acid treated with 6 M HCl/1% Triton X-100 in PBS for 15 min before washing in PBS and staining with anti-BrdU [1:15; American Type Culture Collection (Ma-

nassas, VA) mouse hybridoma], detected with Alexa Fluor 568-conjugated anti-mouse IgG1 or Alexa Fluor 647-conjugated anti-mouse IgG1 (1:1000; Invitrogen).

Protein colocalization (multiple fluorochromes) was assessed in the confocal microscope. Three mice were examined for each transgenic genotype. From each mouse, three 30 μm coronal brain sections (i.e., six olfactory bulb sections) were immunolabeled to count calretinin/BrdU/GFP triple-positive cells or tyrosine hydroxylase/BrdU/GFP-positive cells, or 10 30 μm coronal sections (20 olfactory bulb sections) for calbindin/BrdU/GFP. A minimum of 20 newly born (BrdU$^+$) neurons of each subtype (tyrosine hydroxylase, calretinin, or calbindin positive) were scored for GFP expression (see Fig. 7I). The proportions of BrdU- or calbindin-positive cells in the olfactory bulb were also examined solely for GFP coexpression (minimum of 300 positive cells per mouse) (Figs. 5H, 7J,K). Statistical comparisons were made by ANOVA (for multiple variables) or t test (for single variables).

To determine whether GFP-positive neurospheres could generate multipotent progeny, neurospheres were cultured on coverslips under differentiating conditions and were double labeled (Young et al., 2006, 2007) with antirabbit GFP and one of the following: mouse anti-βIII-tubulin (1:2000; Promega, Madison, WI), rabbit anti-GFAP-Cy3 (1:2500; Sigma), mouse anti-GFAP (1:1500; Sigma), monoclonal O4 IgM (1:5; American Type Culture Collection mouse hybridoma). Sections were poststained with Hoechst 33258 nuclear stain (1:10^4; Sigma). Fixed coverslips were treated with blocking solution (as above) for 30 min at 37°C, before the addition of the primary antibodies. The coverslips were washed three times with PBS before the addition of the appropriate secondary antibodies for 3 h at room temperature. Secondary antibodies included Alexa 568-conjugated goat anti-mouse IgG1 (1:1000; Invitrogen), Alexa 680-conjugated goat antimouse IgM (1:1000; Invitrogen), and Alexa 488-conjugated goat anti-rabbit IgG (1:1000; Invitrogen). Coverslips were washed and mounted as above.

Results

The embryonic LGE and cortex both contribute cells to the adult SVZ

To map the contributions of different regions of the embryonic telencephalic VZ to the adult forebrain SVZ, we made use of a battery of transgenic lines that express Cre-recombinase in defined neuroepithelial domains: *Nkx2.1*-Cre (to label the MGE), *Gsh2*-Cre (LGE and MGE), *Emx1*-Cre (cerebral cortex), and *Dbx1*-Cre (corticostriatal sulcus) (Fogarty et al., 2005; Kessaris et al., 2006) (Fig. 1O, E12). Each individual line (or combined *Emx1/Dbx1*) was crossed to the Cre-dependent *R26-GFP* reporter line to label specific subsets of neuropithelial cells and all of their progeny in the embryonic and postnatal brain.
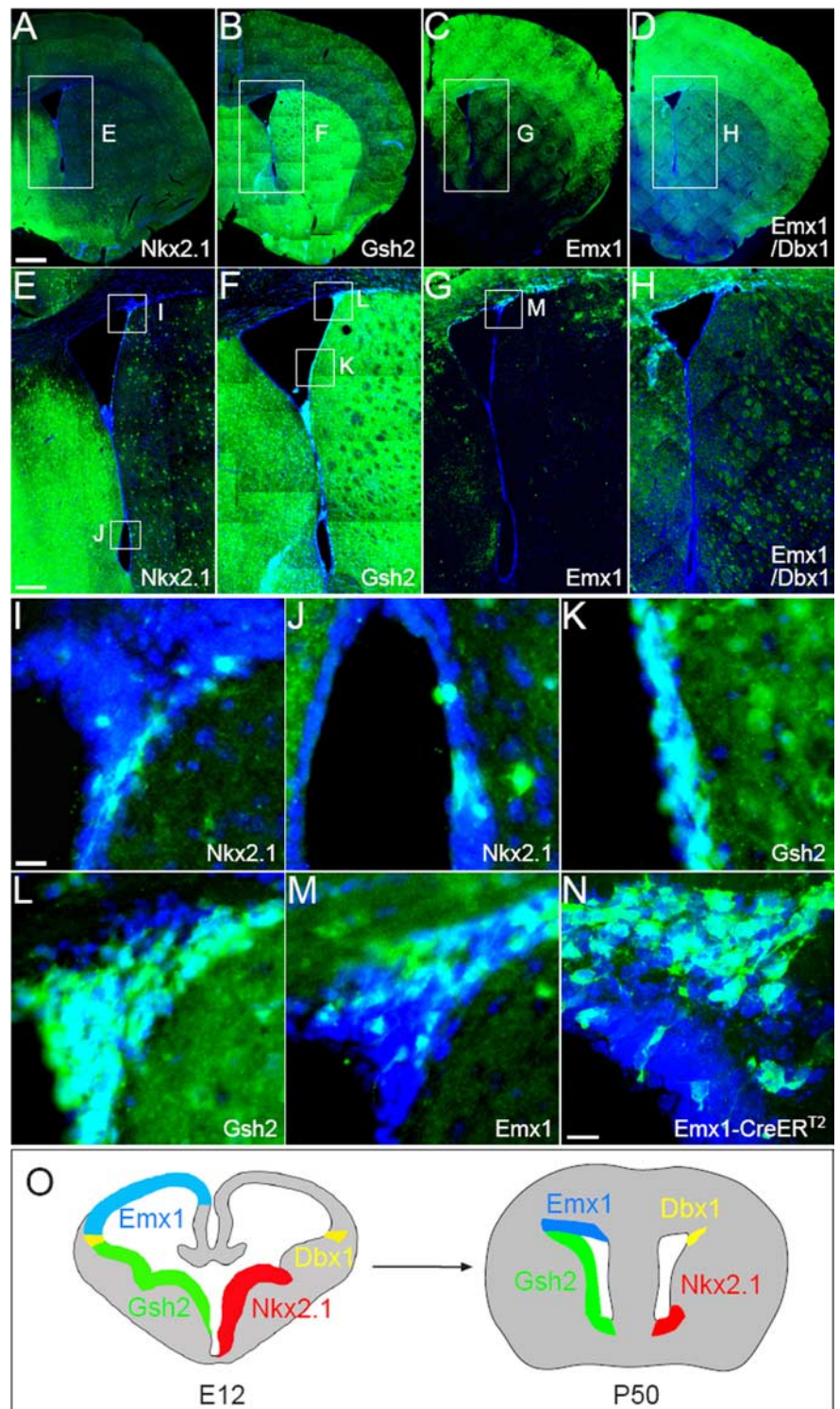
Figure 1. All regions of the telencephalic neuroepithelium contribute to the adult SVZ. Coronal sections (30 μm) of adult (P50) mouse brains were stained with rabbit anti-GFP (green) and Hoechst 33258 to visualize cell nuclei (blue). GFP-positive cells were detected by immunolabeling in sections from *R26-GFP* reporters carrying *Nkx2.1-Cre* (**A, E**), *Gsh2-Cre* (**B, F**), *Emx1-Cre* (**C, G**), or *Emx1/Dbx1-Cre* (**D, H**) transgenes. Each region of the embryonic neuroepithelium contributed preferentially to distinct regions of the adult SVZ. Images (**I–M**) are higher-magnification confocal images of the areas boxed in **E–H**. **I, J**, *Nkx2.1-Cre/R26-GFP* brain. Groups of GFP-positive cells were occasionally detected at the ventral edge of the dorsolateral corner of the SVZ (**I**), as well as the ventral region of the lateral wall (**J**). **K, L**, *Gsh2-Cre/R26-GFP* brain. Many GFP-positive cells were found in the lateral wall (**K**) and the dorsolateral corner (**L**) of the SVZ. **M**, *Emx1-Cre/R26-GFP* brain. Significant numbers of GFP-labeled cells are present, mainly in the dorsal half of the dorsolateral corner of the SVZ. **N**, Adult (P50) *Emx1-CreER^T2^/R26-YFP* brain, after tamoxifen induction at E10.5. The distribution of GFP-labeled cells was found to be similar to the *Emx1-Cre/R26-GFP* animals (i.e., in the dorsal part of the dorsolateral corner of the SVZ). **O**, Schematic depicting the different embryonic neuroepithelial domains targeted by our Cre mice and their relative contribution to generating the adult SVZ. Scale bars: **A–D**, 1 mm; **E–H**, 0.5 mm; **I–N**, 25 μm.
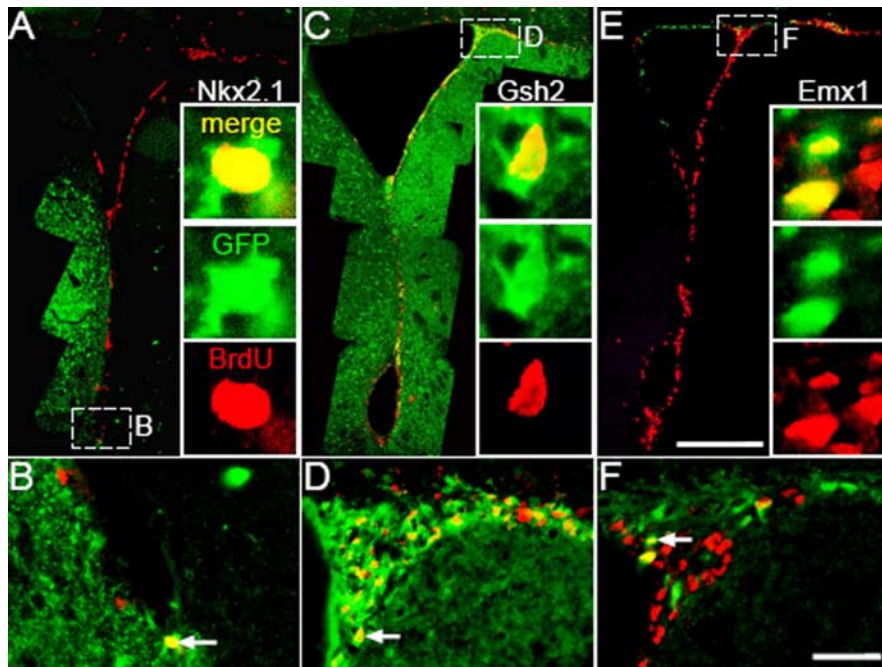
Figure 2. The embryonic striatum and cortex both contribute proliferative cells to the adult SVZ. BrdU was administered to adult *R26-GFP* reporter mice carrying *Nkx2.1-Cre* (*A*, *B*), *Gsh2-Cre* (*C*, *D*), or *Emx1-Cre* (*E*, *F*) transgenes, and the brains were analyzed 24 h later by immunolabeling with anti-BrdU (red) and anti-GFP (green) and confocal fluorescence microscopy. BrdU/GFP double-positive cells (yellow) were present in all animals, the greatest proportion being found in *Gsh2-Cre/R26-GFP* mice. The inset high-magnification images are of the cells indicated by arrows (*B*, *D*, *F*). All images are single confocal scans. Scale bars: *A–C*, 0.5 mm; *D–F*, 50 μm.

nal domains in the SVZ similar to those known to exist developmentally.

Multipotent, self-renewing SVZ stem cells are descended from both the LGE and cortex

To determine whether SVZ cells derived from both *Gsh2-* and *Emx1-*expressing embryonic territories continue to divide in the adult SVZ, as would be expected for stem or progenitor cells, we asked whether they can incorporate BrdU *in vivo*. Adult *Cre/R26-GFP* mice were given a dose of BrdU every 6 h for 24 h and analyzed 3 h after the final dose. Double immunolabeling for GFP and BrdU detected small numbers of *Nkx2.1* (MGE)-derived cells in the lateral wall of the SVZ that were BrdU positive (Fig. 2*A*,*B*). The majority of BrdU-positive cells in the SVZ were derived from *Gsh2*-expressing territories (Fig. 2*C*,*D*), so most proliferative SVZ cells have their origins within the LGE. A significant number of BrdU-labeled cells in the dorsolateral corner of the SVZ were *Emx1* derived (Fig. 2*E*,*F*), confirming that the embryonic cortex also contributes proliferative cells to the adult SVZ. These could be either multipotent stem cells or neural progenitor cells with more restricted potential.

To investigate the stem and/or progenitor cell nature of the *Emx1-* and *Gsh2-*derived SVZ cells, we turned to cell culture assays. The adult SVZ was dissected from *Cre/R26-GFP* mice and dissociated to generate neurosphere cultures (see Materials and Methods). Because each neurosphere was derived from a single proliferating cell in the starting culture, each sphere was either entirely positive or entirely negative for GFP (Fig. 3*A*). The proportions of GFP-positive and GFP-negative neurospheres were determined for each of our Cre lines. The majority of neurosphere-forming cells in the SVZ were of striatal origin; ~70% of all neurospheres were GFP positive in cultures from *Gsh2-Cre/R26-GFP* mice (Fig. 3*B*). In comparison, *Emx1*-derived SVZ cells accounted for ~20% of the neurosphere-forming activity (Fig. 3*B*). Some *Nkx2.1*-derived neurospheres were detected but their contribution was small, in keeping with the low contribution of the *Nkx2.1*-expressing VZ to the adult SVZ (see above). All SVZ neurosphere-forming activity could be accounted for by the combination of *Gsh2*-derived (LGE plus MGE) and *Emx1/Dbx1*-derived (cortex plus corticostriatal sulcus) SVZ cells (Fig. 3*B*). These data provide clear evidence that the neurosphere-forming SVZ cells are heterogeneous in origin.

Neurospheres derived from *Gsh2-Cre*, *Emx1-Cre*, and *Emx1/Dbx1-Cre* mice could be serially passaged more than seven times while remaining GFP positive, confirming the presence of self-renewing stem cells (data not shown). Moreover, cultures from each of these Cre mice contained GFP-positive neurospheres that were able to generate GFAP-positive astrocytes, O4-positive oligodendrocytes, and βIII-tubulin-positive neurons when transferred to differentiation conditions, confirming their multipotency *in vitro* (see Materials and Methods) (Fig. 3*C*). However, the *Nkx2.1*-derived neurospheres were less neurogenic than the

Immunolabeling for GFP identified a significant contribution of cells from all parts of the embryonic neuroepithelium to the adult brain (Fig. 1*A–H*). The *Nkx2.1* domain (MGE) made a minor contribution to the adult SVZ, with the greater number of GFP-positive cells being located near the ventral extremity of the lateral ventricle (Fig. 1*E*,*J*). In occasional sections, cells were also observed at the ventral edge of the dorsolateral corner of the SVZ (Fig. 1*I*). The majority of cells lining the lateral wall of the lateral ventricle, including the dorsolateral SVZ, were derived from the *Gsh2* domain (Fig. 1*F*,*K*,*L*). Together with the previous result, this indicates that most of the lateral wall cells are descendants of the LGE, as previously thought (see Introduction). However, we also observed a significant population of GFP-labeled SVZ cells in the *Emx1-Cre/R26-GFP* mice. These were found in the roof of the lateral ventricle and in the dorsolateral corner of the SVZ (Fig. 1*G*,*M*).

The *Emx1*-derived SVZ cells were found mainly in the dorsal half of the dorsolateral corner of the SVZ (Fig. 1*M*), suggesting that they were genuine descendants of the embryonic cortical VZ, not *Gsh2*-derived cells that had upregulated *Emx1* postnatally and consequently recombined *de novo* in the adult. We tested this directly by inducing transient recombination in *Emx1-CreER^{T2}*/*R26-YFP* mice with a single dose of tamoxifen at E10.5 and after the subsequent fates of labeled cells in the adult brain. The distribution of GFP-positive cells within the SVZ of pulse-labeled *Emx1-CreER^{T2}/R26-YFP* mice (Fig. 1*N*) was similar to the constitutive *Emx1-Cre/R26-YFP* mice (Fig. 1*M*). These data demonstrate that cells from the embryonic cortex do indeed give rise to the dorsal and dorsolateral edge of the adult SVZ. Therefore, we conclude that the adult SVZ is comprised of cells derived predominantly from the VZ of the embryonic LGE and cortex (Fig. 1*O*) and that the location of these cells potentially defines germi-
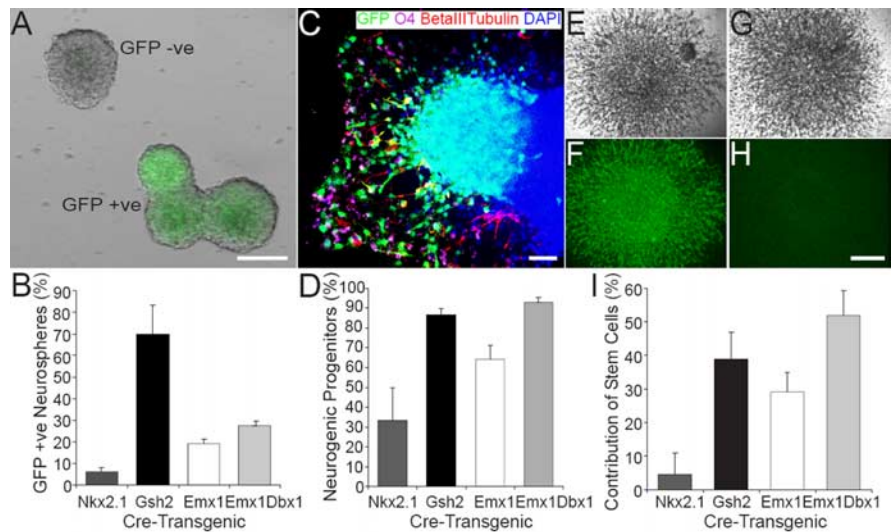
others, because a smaller proportion of GFP-positive neurospheres generated βIII-tubulin-positive neurons (Fig. 3D).

To quantify the proportions of true SVZ stem cells descended from different regions of the embryonic VZ, we dissociated SVZ cells from *R26-GFP* reporter mice carrying *Nkx2.1-Cre*, *Gsh2-Cre*, *Emx1-Cre*, or *Emx1/Dbx1-Cre* and plated them in a mitogen-containing collagen matrix. Neural progenitor cells with limited self-renewing capacity formed small colonies and stopped proliferating after 1–2 weeks, whereas self-renewing stem cells continued to proliferate for >3 weeks, forming colonies ≥2 mm in diameter. Stem cell colonies that were GFP positive (Fig. 3E,F) and others that were GFP negative (Fig. 3G,H) were identified in all cultures after 3 weeks (Fig. 3I). The proportions of self-renewing SVZ stem cells contributed from each embryonic territory, as judged by this collagen matrix assay, were not the same as the proportions of neurosphere-forming cells measured previously. The embryonic LGE (*Gsh2-Cre*) provided ~70% of the neurosphere-forming cells but only ~40% of the self-renewing stem cells, whereas the cortex (including corticostriatal sulcus, *Emx1/Dbx1-Cre*) provided only ~25% of the neurosphere-forming cells but ~50% of the self-renewing stem cells (Fig. 3B,I). This suggests that LGE-derived adult SVZ cells might contribute a greater proportion of the transit amplifying cells and RMS neuroblasts *in vivo*.

Figure 3. All regions of the embryonic neuroepithelium give rise to multipotent, self-renewing stem cells in the adult SVZ. *A*, Neurosphere cultures were generated from the SVZ of adult *R26-GFP* reporter mice carrying *Nkx2.1-Cre*, *Gsh2-Cre*, or *Emx1-Cre* transgenes, or both *Emx1-* and *Dbx1-Cre* together. Individual neurospheres were either uniformly GFP positive or negative, consistent with each being derived from a single cell in the starting culture. *B*, The proportions of GFP-positive neurospheres are shown graphically (mean ± SD; three independent cultures from three adult mice of each genotype). *C*, Neurospheres were replated under differentiation-inducing conditions for 5 d before being fixed and triple-immunolabeled with anti-GFP (green), anti-βIII-tubulin (red, to label neurons), and monoclonal O4 (magenta, to label oligodendrocyte lineage cells), and then counterstained with 4′,6′-diamidino-2-phenylindole (DAPI) nuclear stain (blue). The example shown in *C* depicts a GFP-positive and a GFP-negative neurosphere from an *Emx1-Cre/R26-GFP* culture differentiating side by side, illustrating the generation of neurons and oligodendrocytes from stem cells of differing embryonic origins. *D*, The proportions of GFP-positive neurospheres of each genotype that generated βIII-tubulin-positive neurons are presented graphically. *Nkx2.1*-derived neurospheres were significantly less neurogenic than the others (*p* < 0.05). All neurospheres regardless of genotype generated GFAP-positive astrocytes (data not shown). *E*, SVZ cells were dissected free of surrounding tissue, dissociated, and cultured in a semisolid matrix with mitogens for 3 weeks (see Materials and Methods). Long-term self-renewing stem cells gave rise to large colonies that distinguished them from transit-amplifying precursors, which have less proliferative potential. Individual colonies were either GFP positive (*E*, *F*) or GFP negative (*G*, *H*) depending on the origin of the founder stem cell. The examples shown are from an adult *Emx1-Cre/R26-GFP* culture. *I*, The proportions of GFP-positive stem cell colonies of each genotype are shown graphically. Scale bars: *A*, 100 μm; *C*, 25 μm; *E–H*, 0.5 mm.

LGE- and cortex-derived stem cells make neurons in the adult olfactory bulb

Adult SVZ stem cells have been identified as a subtype of subependymal astrocytes ("B cells"), because they express GFAP *in vivo*. We confirmed that a subset of GFP-labeled adult SVZ cells were also GFAP positive in *R26-GFP* reporter mice carrying either *Gsh2-Cre* or *Emx1-Cre* (Fig. 4A,B). There were also small numbers of cells colabeled for GFP and GFAP in *Nkx2.1-Cre/R26-GFP* mice (data not shown). To visualize migratory neuroblasts, we immunolabeled each transgenic to detect GFP expression and either Doublecortin (Dcx) or PSA-NCAM. Dcx- and PSA-NCAM-positive neuroblasts in the SVZ and RMS were detected only in the *Gsh2-Cre* and *Emx1-Cre* transgenics, indicating that the LGE- and cortex-derived stem cells are responsible for most or all olfactory neurogenesis in the adult (Fig. 4D,E; PSA-NCAM shown). Equivalent immunohistochemical analysis of the SVZ of adult *Emx1-creER^{T2}/R26-YFP* mice (Fig. 4C,F), induced at E10.5, produced a similar staining pattern to that of the adult *Emx1-cre/R26-GFP*, confirming that stem cells originating in the embryonic cortex and retained in the adult SVZ generate neuroblasts throughout life.

To determine directly the separate contributions of *Nkx2.1-*, *Gsh2-*, *Dbx1-*, and *Emx1*-derived stem cells to olfactory neurogenesis, we mapped GFP-labeled cells within the adult olfactory bulbs of our *Cre/R26-GFP* mice. GFP-positive cells were detected

in the granule cell layer (gcl), mitral cell layer (mcl), external plexiform layer (epl), and glomerular layer (gl) of *R26-GFP* reporters carrying the *Nkx2.1-Cre*, *Gsh2-Cre*, or *Emx1-Cre* transgenes (Fig. 5A–C) as well as the *Emx1/Dbx1-Cre/R26-GFP* triple-transgenic (data not shown). Most of the GFP-labeled cells represent olfactory bulb cells that were generated during embryonic or early postnatal development, as opposed to adult-born cells. To identify SVZ-derived cells that migrated into the olfactory bulb during adulthood, we administered BrdU at P50 and examined the olfactory bulbs 4 weeks later. Only ~1% of all BrdU-positive cells were *Nkx2.1* derived (Fig. 5D,H). The majority (~70%) were *Gsh2* derived (Fig. 5E,H), indicating that most adult-born cells in the olfactory bulb are generated from LGE-derived stem cells. Approximately 30% of BrdU-labeled olfactory bulb neurons were *Emx-1* derived (Fig. 5F,H). No additional contribution was observed in *Emx1/Dbx1-Cre/R26-GFP* mice (Fig. 5G,H), indicating that *Dbx1*-derived cells do not generate significant numbers of adult olfactory cells.

LGE- and cortex-derived adult SVZ stem cells have different properties

Gsh2- and *Emx1*-derived cells were found in all layers of the olfactory bulb, although there was a relatively higher contribution of *Emx1*-derived cells to the glomerular layer than the granule cell layer (Fig. 5K). To investigate whether this laminar bias might result from different cell fate specificities of *Gsh2-* and *Emx1-*
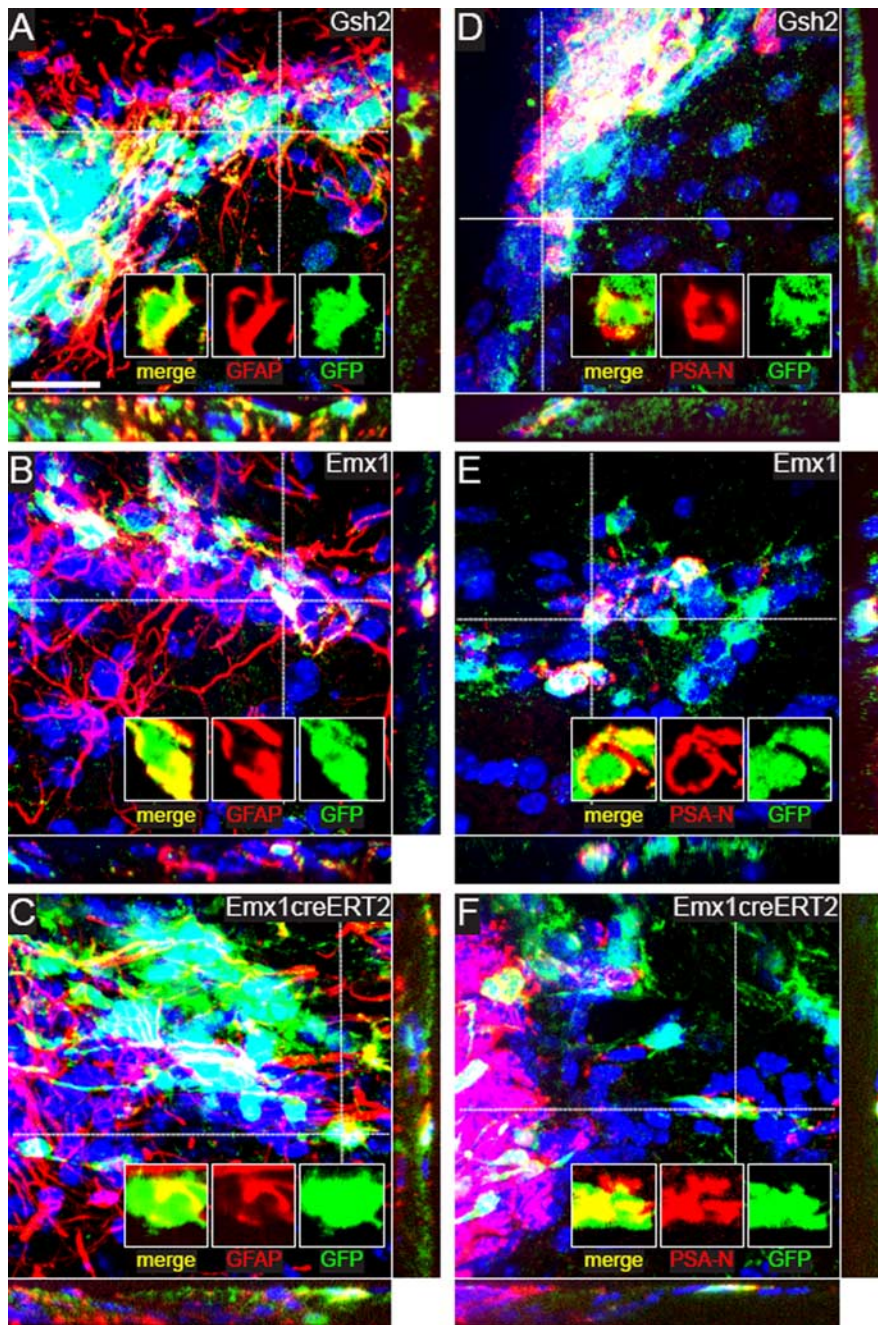
Figure 4. Cortex- and LGE-derived SVZ stem cells generate new neuroblasts *in vivo*. Coronal sections through the forebrain of *R26-GFP* reporters carrying either the *Gsh2-Cre* (**A**, **D**), *Emx1-Cre* (**B**, **E**), or *Emx1-CreER*[T2] (**C**, **F**) transgene were double immunolabeled for GFP (green) together with either GFAP (red; to identify multipotent stem cells) (**A–C**) or PSA-NCAM (red; to identify neuroblasts) (**D–F**). All sections were counterstained with Hoechst nuclear dye (blue). Images show the dorsolateral corner of the SVZ (compressed confocal series with orthogonal views taken at the level indicated by the dashed line). GFAP-positive stem cells and PSA-NCAM-positive neuroblasts descend from both the embryonic LGE and cortex (single confocal scan inset). Scale bar, 30 μm.

Cre backgrounds) suggesting that this population is not turned over significantly in the adult. Parvalbumin-positive neurons had dual developmental origins, with some cells coexpressing YFP in both the *Emx1-Cre* and *Gsh2-Cre* transgenics (Fig. 7*A*,*B*).

Tyrosine hydroxylase-positive, BrdU-positive neurons were detected in the glomerular layer. These adult-born tyrosine hydroxylase-positive interneurons were generated from both LGE- and cortex-derived SVZ stem cells (Fig. 7*C*,*D*,*I*) in numbers proportional to the overall contributions of these stem cell pools to the olfactory bulb (~70 and ~30%, respectively) (Figs. 5*H*, 7*I*) and similar to their neurosphere-forming activities *in vitro* (Fig. 3*B*). These data suggest that LGE- and cortex-derived neuroblasts are equally likely to generate tyrosine hydroxylase-positive (dopaminergic) interneurons.

The situation was different for calretinin-positive, adult-born neurons, the majority of which (~60%) were generated from cortex-derived (*Emx1*) stem cells (Fig. 7*E*,*F*,*I*), despite the fact that cortex-derived SVZ stem cells generate only ~30% of the total (Fig. 5*H*). This was the first indication that LGE-derived and cortex-derived stem cell progeny might have differential cell fates. This conclusion was strikingly reinforced when we immunolabeled for calbindin. We found that, although there were far fewer new adult-born calbindin-expressing interneurons generated in the olfactory glomeruli relative to calretinin (~10–15%), they were all produced by LGE-derived SVZ stem cells, and none from their cortex-derived counterparts (Fig. 7*G–I*). Interestingly 88 ± 6% of all calbindin-positive olfactory interneurons present at P80 were generated from striatum (*Gsh2*)-derived progeny (calbindin and GFP double-positive) (Fig. 7*J*). In comparison, only 1.5 ± 0.8% of calbindin-positive interneurons appeared to be GFP positive in *Emx1-Cre/ R26-GFP* mice (Fig. 7*K*) (for experimental details, see Materials and Methods, Immunocytochemistry). For further confirmation that embryonic cortex-derived cells fail to generate calbindin-positive olfactory interneurons, we again turned to the *Emx1-CreER*[T2]*/R26-YFP* transgenic line. Tamoxifen was administered at E10.5 and the olfactory bulbs were examined at P50. This experiment confirmed that descendants of embryonic cortical precursors included significant numbers of tyrosine hydroxylase- and calretinin-positive interneurons but very few calbindin-positive interneurons (Fig. 7*L–N*). Collectively, these data strongly support the idea that cortex-derived and striatum-derived precursor/stem cells remain functionally distinct throughout life, generating different subsets of olfactory neurons during development and even in the adult.

derived stem cells, we categorized their adult-born (BrdU-positive) neuronal progeny by immunolabeling for parvalbumin (PV), tyrosine hydroxylase (TH), calretinin (Crt), or calbindin (Cb). The locations and connections of olfactory bulb neurons that express these markers are illustrated in Figure 6.

BrdU was administered at 7 weeks of age, and the olfactory bulbs were analyzed 4 weeks later (P80) as before. Parvalbumin-positive neurons were detected in the external plexiform layer (EPL) but were never labeled with BrdU (>100 parvalbumin-labeled cells examined in three mice of both *Emx1-Cre* and *Gsh2-*

Tamoxifen was also administered to adult *Emx1-CreER^{T2}/R26-YFP* transgenic mice (~P50), allowing us to label and trace the descendants of any stem or progenitor cells that continue to express *Emx1* into adulthood. Animals were analyzed at different times after tamoxifen induction to follow the progress of YFP-labeled cells from the SVZ to the olfactory bulb. At 1 week after induction, the shortest time lag examined, YFP-positive cells were observed in the dorsal part of the adult SVZ and the proximal end of the RMS. The location of these cells within the SVZ was consistent with the restricted location of cortex-derived progeny reported previously (Fig. 1 *M,N*). The GFP-positive cells identified were also GFAP positive (Fig. 8 *A,B*). By 4 weeks after induction (YFP, GFAP) double-positive cells were still present in the SVZ (Fig. 8 *C*), and in addition, there were clusters of GFP-positive cells that were GFAP negative (Fig. 8 *D*). Some of these cells were Dcx-positive neuroblasts. Other YFP-positive, Dcx-negative cells were observed on the dorsal edge of the SVZ and in the corpus callosum (data not shown).

The olfactory bulb was devoid of YFP-positive cells at short times after tamoxifen induction, as expected if they migrate into the bulb from the SVZ via the RMS (Fig. 8 *E*). After 4 weeks, both (YFP, PSA-NCAM) double-positive neuroblasts and (YFP, NeuN) double-positive neurons could be found in the granule cell layer (Fig. 8 *F,G*) and the glomerular layer (data not shown). The number of YFP-labeled cells in the olfactory bulb remained small because of the inefficiency of tamoxifen induction in adulthood. However, there were some cells that colabeled for YFP and calretinin in the granule cell layer (Fig. 8 *H*) and the glomerular layer (data not shown). None of >250 YFP-positive cells that we examined coexpressed calbindin, reinforcing the results obtained with constitutive *Emx1-Cre/R26-YFP* mice and BrdU (Fig. 7 *H,K*). The fact that the *Emx1-CreER^{T2}* transgene remains active in the adult implies that cortex-derived SVZ stem cells continue to express *Emx1* into adulthood. In addition, the YFP-positive cells detected in the SVZ of *Emx1-Cre/R26-YFP* mice never immunolabeled for Gsh2 (supplemental Fig. S1, available at www.jneurosci.org as supplemental material). Therefore, we conclude that the different SVZ stem cell pools not only have different embryonic origins but also different molecular properties and different neurogenic fates in the adult olfactory bulb.

Discussion

The main findings of this study are as follows: (1) the adult SVZ contains stem cells derived from multiple regions of the embryonic neuroepithelium (MGE, LGE, and cortex), (2) stem cells
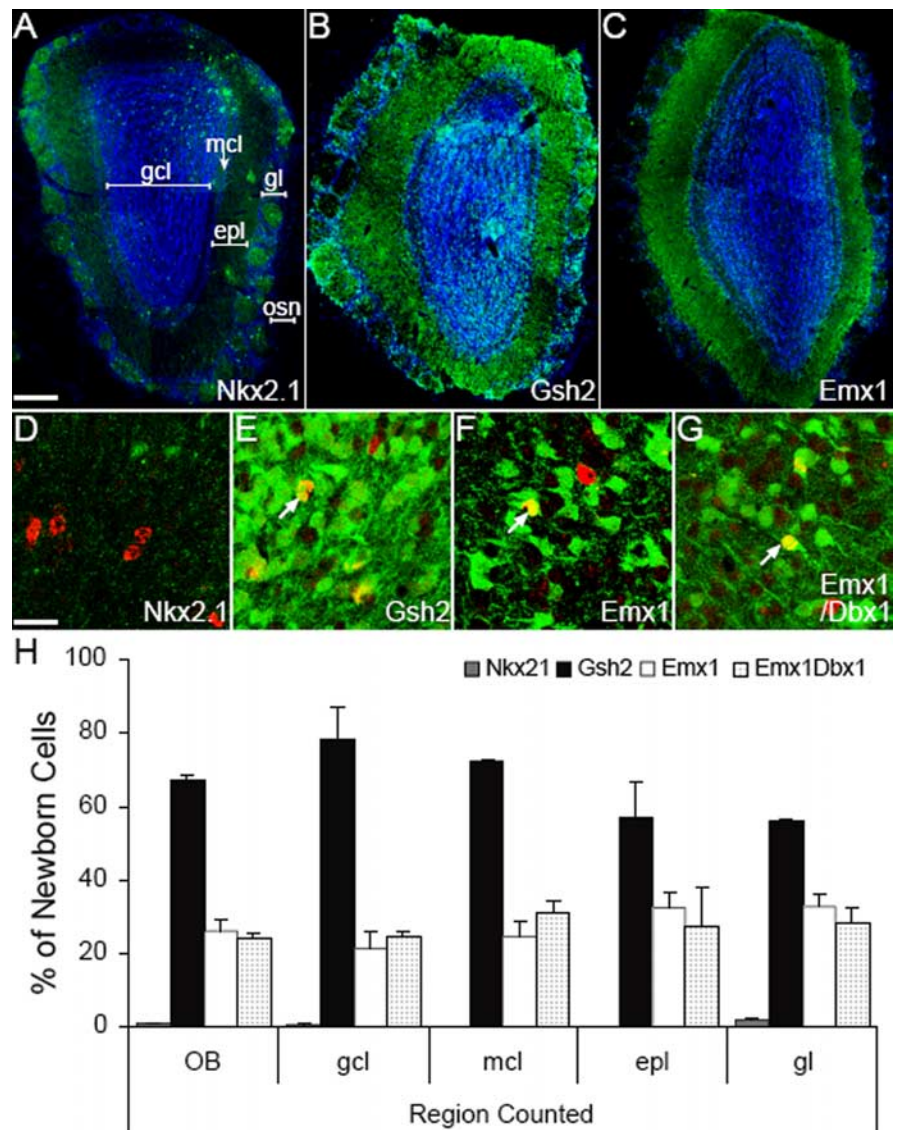

Figure 5. LGE- and cortex-derived stem cells give rise to new cells in the adult olfactory bulb. Thirty micrometer coronal sections through the olfactory bulb of adult *R26R-GFP* reporter mice carrying *Nkx2.1-Cre*, *Gsh2-Cre*, or *Emx1-Cre* transgenes were immunolabeled for GFP (*A–C*). To identify newly generated cells, we injected BrdU at P50 and analyzed the olfactory bulb 30 d later (P80) for the presence of (GFP, BrdU) double-positive cells. Colabeled cells were rarely detected in *Nkx2.1-Cre/R26-GFP* olfactory bulbs (*D, H*). However, many (GFP, BrdU) double-positive cells were present in *Gsh2-Cre/R26-GFP*, *Emx1-Cre/R26-GFP*, and *Emx1/Dbx1-Cre/R26-GFP* olfactory bulbs (*E–G*, arrows). The proportions of newly born adult cells were quantified according to genotype for the entire olfactory bulb (OB) and separately for the granule cell layer (gcl), mitral cell layer (mcl), external plexiform layer (epl), and glomerular layer (gl) (see *A*). There is laminar variation in the relative proportions of *Gsh2*- and *Emx1*-derived adult-born cells. For experimental details, see Materials and Methods, Immunocytochemistry. Scale bars: *A–C*, 200 μm; *D–G*, 20 μm. Error bars indicate SD. osn, Olfactory sensory neurons.

from both the LGE and cortex contribute to adult neurogenesis in the olfactory bulb, and (3) cortical and LGE-derived stem cells are distinct populations with regard to their distribution within the SVZ, their molecular properties, and their behavior, because they make nonproportional contributions to the genesis of olfactory interneuron subtypes.

SVZ stem cells are heterogeneous in origin and behavior

Previous evidence had suggested a purely striatal origin for adult SVZ stem cells (Stenman et al., 2003). However, we show here that the LGE contributes most but not all neurosphere-forming cells and less than one-half of the self-renewing stem cells, the remainder originating mainly from the embryonic cortex (*Emx1-*
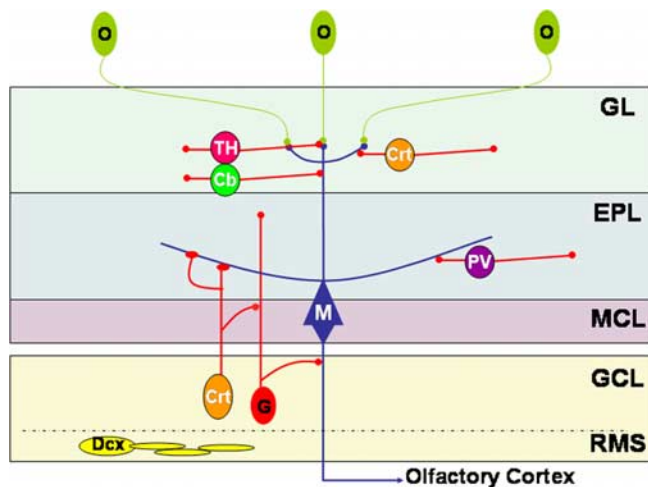
Figure 6. Locations and connections of interneurons in the olfactory bulb. The diagram represents a coronal section through an adult mouse olfactory bulb. New neuroblasts (Dcx positive) enter the bulb from the RMS, migrate radially and differentiate as interneurons in (1) the granule cell layer (GCL), (2) the mitral cell layer (MCL), (3) the EPL, and (4) the glomerular layer (GL). Immunolabeling for calretinin (Crt), calbindin (Cb), tyrosine hydroxylase (TH), or parvalbumin (PV) identifies subpopulations of olfactory interneurons distributed throughout the bulb as shown. The majority of granule neurons (G) are not identified by any of these markers. The MCL also contains mitral projection neurons (M), which are not replaced during adulthood from RMS neuroblasts. Olfactory sensory neurons (O) in the olfactory epithelium in the nasal cavity are also not replaced by RMS neuroblasts. Adapted from Lledo et al. (2006).

plus *Dbx1*-domains). Our findings confirm the recent report from Willaime-Morawek et al. (2006) that cells from the embryonic cortex contribute to the neurosphere-forming cells of the adult SVZ. Our findings are also consistent with the report from Ventura and Goldman (2007) that dorsal (cortical) radial glia are stem cells that contribute interneurons to the adult olfactory bulb, in addition to astrocytes and oligodendrocytes.

Because neurosphere cultures derived from our *Cre/R26-GFP* transgenic mice contained neurospheres that were entirely GFP positive and others that were entirely GFP negative, it is clear that the patterns of gene expression established early in the embryo remain stable in the adult, even when the cells are dissociated and cultured at low density (that is, cells from the embryonic cortex do not activate Gsh2 expression in culture). Stability of gene expression is also supported by the fact that SVZ cells that express YFP in the *Emx1-Cre/R26-YFP* transgenic do not exhibit Gsh2 immunoreactivity (supplemental Fig. S1, available at www.jneurosci.org as supplemental material) and that our *Emx1-CreER^{T2}* transgene made a similar contribution to the adult SVZ whether tamoxifen was administered in the embryo (E10.5) or in the adult (P50). We therefore presume that the adult SVZ contains distinct populations of Emx1-expressing and Gsh2-expressing stem cells (and possibly others) that have distinct and overlapping neurogenic properties.

It is known that at least three subtypes of GABAergic interneurons expressing the GABA-synthesizing enzyme glutamate decarboxylase can be identified in the periglomerular layer of the olfactory bulb. These three mostly nonoverlapping populations can be identified by their expression of calretinin, calbindin-D28K (two members of the EF-hand family of calcium-binding proteins), or tyrosine hydroxylase (dopaminergic subpopulation), and are all generated or regenerated continuously during adulthood (Kosaka et al., 1995; Dellovade et al., 1998; Hack et al., 2005; Kohwi et al., 2005). Adult-born calretinin-positive interneurons are also found in the granule neuron layer (Fig. 6). Our data

confirm that these neuronal subtypes continue to be generated during adulthood. However, when we examined the turnover of interneurons expressing parvalbumin, a third calcium-binding protein of the EF-hand family, we found that these neurons, located in the EPL, are not turned over significantly during adulthood. In this sense, they resemble projection neurons such as mitral cells but differ from most olfactory interneurons. Parvalbumin-positive neurons are also present in the human olfactory bulb and these were reported to be generated continuously in the adult (Bedard and Parent, 2004). However, human parvalbumin neurons are found in the glomerular layer, not the EPL, suggesting that parvalbumin marks different populations of neurons in humans and mice. Otherwise, adult-born neurons in the human olfactory bulb include GAD65-, tyrosine hydroxlyase-, and calretinin-positive interneurons, similar to those of the mouse (Kosaka et al., 1995; Bedard and Parent, 2004).

There are stem cells that reside in the RMS throughout life, in addition to those in the SVZ (Gritti et al., 2002). Hack et al. (2005) found that the RMS stem cells generate the majority of tyrosine hydroxylase-positive interneurons in the adult olfactory bulb, the remainder coming from the SVZ. Ventura and Goldman (2007) labeled dorsal radial glia directly using an adenovirus applied to the cortical surface and showed that they generate tyrosine hydroxylase-positive periglomerular neurons as well as astrocytes and oligodendrocytes. The RMS is contiguous with the SVZ and the cortical (*Emx1*-expressing) neuroepithelium contributes to the development of both structures, so our own fate-mapping experiments with *Emx1-Cre* cannot distinguish adult stem cells that reside in the RMS from those of the dorsal SVZ. Nevertheless, we too have confirmed that cortex-derived stem cells, presumably a combination of RMS and SVZ stem cells, generate tyrosine hydroxylase-positive (and calretinin-positive) olfactory interneurons in the adult.

Olfactory bulbectomy has provided evidence that interneuron cell type identity is established before neuroblasts reaching the olfactory bulb *in vivo* (Jankovski et al., 1998). Our data support this conclusion by demonstrating that the neurogenic properties of adult SVZ stem cells are determined very early, according to their sites of origin in the embryonic neuroepithelium. We found that LGE- and cortex-derived stem cells (whether from RMS or SVZ) generated all of the adult-born tyrosine hydroxylase interneurons in the olfactory bulb (Fig. 7*I*) and did so in proportion to the contributions of these stem cell populations to olfactory neurogenesis overall. However, this was not true for adult-born calretinin interneurons, most of which were generated from cortex-derived SVZ stem cells, despite their being less neurogenic overall than those from the LGE. Strikingly, all of the adult-born calbindin-D28K-positive interneurons originated from LGE-derived SVZ stem cells and none from cortex-derived stem cells. These data indicate that the fates of adult SVZ stem cells are already determined within the SVZ (or RMS). Together with the finding of Hack et al. (2005) that the neurogenic properties of the RMS and SVZ are distinguishable, our studies raise the possibility that some aspects of embryonic patterning along both dorsal–ventral and anterior–posterior axes are preserved in the adult SVZ.

**Different SVZ stem cells for different purposes?**

SVZ neural stem cells have been defined *in vitro* by their ability to proliferate, self-renew, and generate multipotent progeny (Richards et al., 1992; Reynolds and Weiss, 1992; Young et al., 2006, 2007). *In vivo*, the slowly dividing neural stem cells generate more rapidly dividing neural progenitor cells ("transit amplifying cells"), which in turn generate postmitotic neuroblasts destined

for the olfactory bulb (Doetsch et al., 1999a,b). Transit amplifying cells and neural stem cells are both capable of generating neurospheres (Doetsch et al., 2002), so, in addition to neurosphere assays, we cultured SVZ cells in a semisolid collagen matrix to identify the true stem cells, which proliferate longer and form larger colonies than transit-amplifying cells. We found that the numbers of stem cells and neurosphere-forming cells were not in proportion to one another. For example, the LGE contributed a greater proportion of neurosphere-forming cells than stem cells (~70 vs ~40% of the total), whereas for the cortex (*Emx1* plus *Dbx1* domains) the opposite was true (~30 vs ~50%). These data suggest that LGE-derived stem cells might be more active in generating transit-amplifying cells for the RMS than their cortex-derived counterparts. It is also worth noting that, whereas the embryonic *Dbx1*-expressing domain at the lateral edges of the cortex appears to contribute ~20% of the *in vitro* stem cell activity of the SVZ (Fig. 3*I*), this domain did not contribute significant numbers of adult born olfactory cells *in vivo* (Fig. 5*H*). Therefore, the *Dbx1*-derived stem cells might serve some function other than olfactory neurogenesis, perhaps contributing preferentially to adult gliogenesis, another known function of the postnatal SVZ (Levison and Goldman, 1993; Luskin and McDermott, 1994; Menn et al., 2006). Alternatively, they might lie dormant until activated after an ischemic insult or other CNS damage (Magavi et al., 2000; Picard-Riera et al., 2002; Sundholm-Peters et al., 2005) or in response to a physiological stimulus (Shingo et al., 2003).

Cell fate determinants in the SVZ and RMS

We have taken advantage of the spatially restricted embryonic expression of several transcription factors to show that embryonic origin is important for the future properties of adult stem cells and their progeny. This was highlighted by our examining the origin of calbindin-positive olfactory interneurons. The great majority of these cells are generated during early postnatal development (De-Marchis et al., 2007) from striatum-derived precursor cells (this study). Smaller numbers of calbindin-positive interneurons are generated continuously during adult life, and these are all derived from striatum-derived SVZ stem cells. In contrast, the cortex made an insignificant contribution to the genesis of calbindin-positive interneurons at any stage of development or in the adult. It therefore seems likely that the transcription factors we used to demarcate the embryonic stem cell domains (Gsh2 and Emx1) (and possibly others) remain active in adult stem cells and play a role in restricting their subsequent cell fates. It is known that Emx1 has an important function in olfactory behavior (Cao and
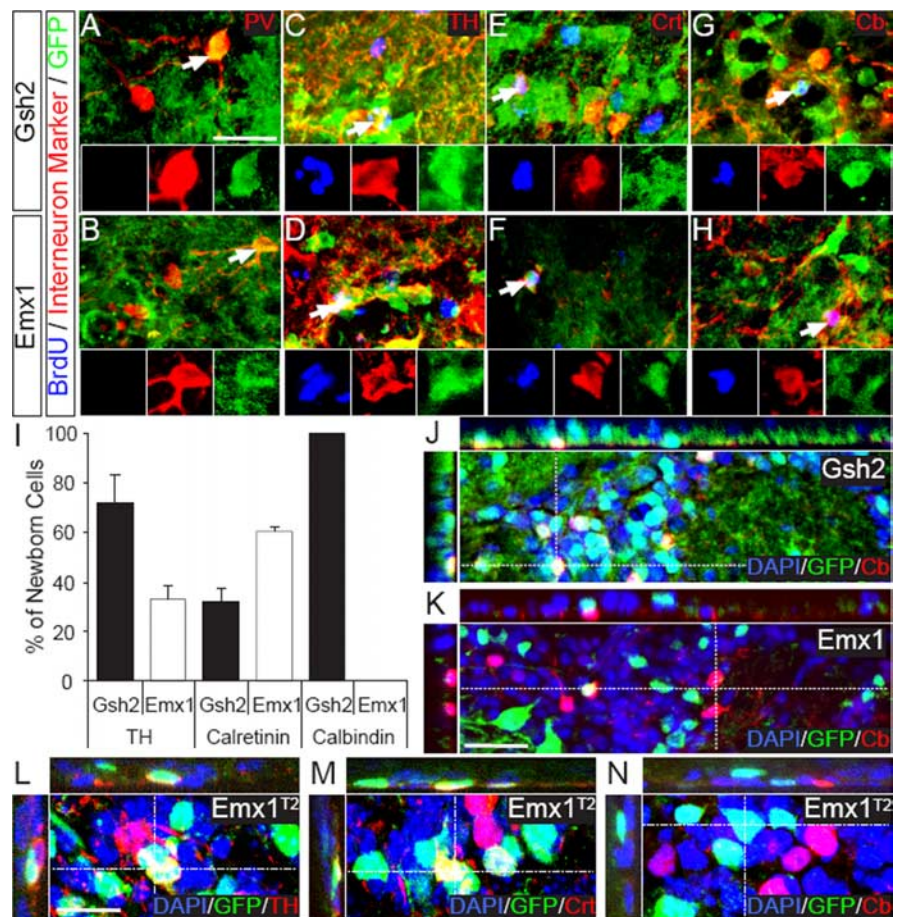

Figure 7. LGE- and cortex-derived SVZ stem cells generate different subpopulations of olfactory interneurons in the adult. BrdU was administered to adult (7 weeks of age) *R26-GFP* reporter mice carrying either *Gsh2-Cre* or *Emx1-Cre* transgenes. Four weeks later (~P80), coronal sections of olfactory bulbs were triple-immunolabeled for BrdU (blue), GFP (green), and parvalbumin (*A*, *B*), tyrosine hydroxylase (TH) (*C*, *D*), calretinin (Crt) (*E*, *F*), or calbindin (Cb) (*G*, *H*) (red). Cells indicated by arrows in the compressed confocal series are shown at high magnification in the insets (single confocal scans). The proportions of adult-born interneurons of each genotype are presented graphically in *I*. Error bars indicate SD. *Gsh2*- and *Emx1*-derived stem cells generate different proportions of TH-, Crt-, and Cb-positive neurons, the most striking example being Cb-positive neurons, which were exclusively *Gsh2* (LGE) derived. The vast majority of Cb-positive neurons (red) coexpress GFP (green) in olfactory bulb sections from *Gsh2-Cre/R26-GFP* (*J*) but not *Emx1-Cre/R26-GFP* mice (*K*), demonstrating that production of Cb-positive interneurons is the preserve of striatum-derived precursor/stem cells throughout development as well as in the adult. *L–N*, Tamoxifen was administered to *Emx1-CreER^{T2}/R26-GFP* transgenic mice at E10.5 and the progeny of these cells were traced into adulthood (P50). The olfactory bulbs were immunolabeled to detect GFP (green) and either TH (*L*), Crt (*M*), or Cb (*N*) (red). All nuclei were counterstained with Hoechst 33258 (blue). These data demonstrate that cells derived from the embryonic cortex make a significant contribution to the genesis of TH-positive and Crt-positive interneurons, but fail to generate Cb-positive interneurons. Compressed confocal *Z*-series are shown with orthogonal views taken at the levels indicated by the dashed lines. For experimental details, see Materials and Methods, Immunocytochemistry. Scale bars: *A–H*, 20 μm; *J–N*, 30 μm.

Li, 2002). Other transcription factors that have been detected in the SVZ and/or RMS include Pax6 (Hack et al., 2005; Kohwi et al., 2005), Mash1 (Kohwi et al., 2005), Dlx2 (Doetsch et al., 2002), Olig2 (Parras et al., 2004; Hack et al., 2005), Er81 (Stenman et al., 2003), and Sp8 (Waclaw et al., 2006). Sp8 is retained in calretinin-expressing and nondopaminergic GABAergic interneurons of the glomerular layer. It will be interesting to determine whether any of these transcription factors or specific combinations of them are expressed in subsets of neuroblasts that can be traced back specifically to either LGE- or cortex-derived SVZ stem cells.

Our demonstration that striatum-derived and cortex-derived stem cells generate distinct subsets of olfactory interneurons in the adult, together with evidence that they are spatially and developmentally distinct populations, indicates that adult SVZ
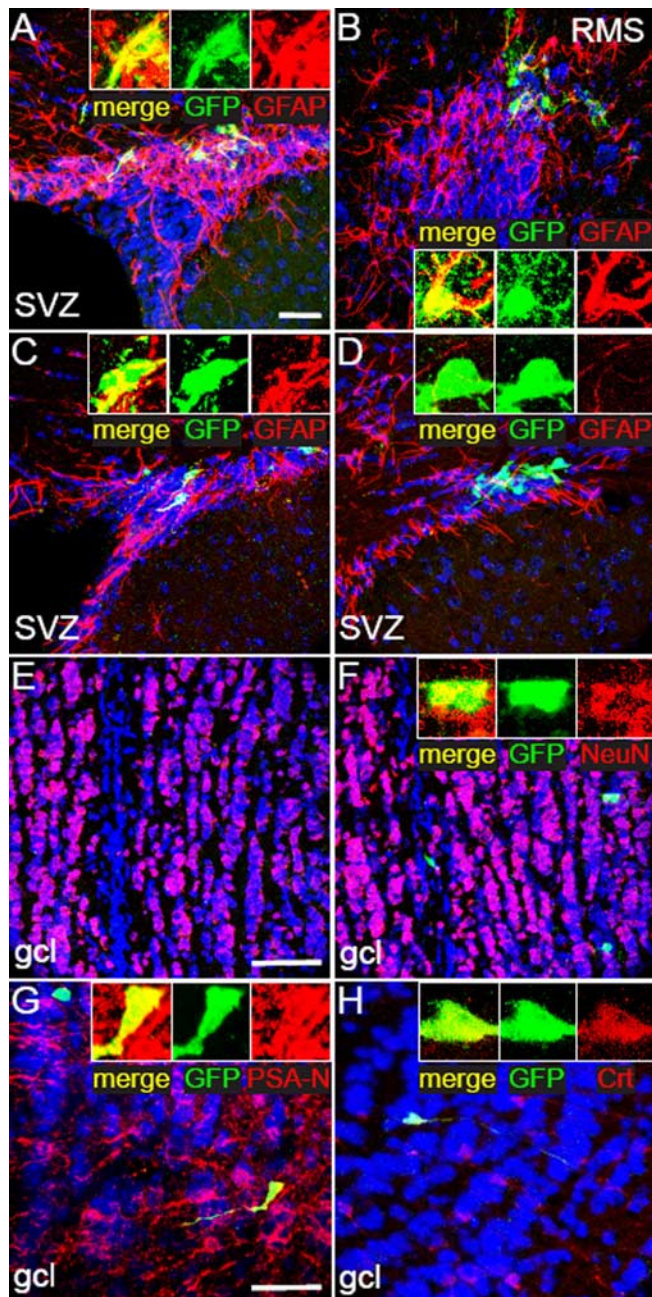
Figure 8. A population of adult SVZ stem cells expresses *Emx1* into adulthood. Tamoxifen was administered to young adult (7 weeks of age) *Emx1-CreER^{T2}/R26-YFP* transgenic mice. After 1 week (**A**, **B**) or 6 weeks (**C**, **D**), brain sections were double immunolabeled for YFP (green) together with GFAP (red) to label stem cells in the SVZ (**A**, **C**, **D**) or proximal RMS (**B**). Sections were counterlabeled with Hoechst nuclear stain (blue). (YFP, GFAP) Double-positive stem cells were present at both 1 and 6 weeks after tamoxifen. YFP-single-positive presumptive neuroblasts were also present at 6 weeks (**D**). No YFP-labeled neurons (NeuN-positive) were present in the olfactory bulb at 1 week after tamoxifen (**E**), but small numbers were found at 6 weeks (**F**). At 6 weeks after tamoxifen, (YFP, PSA-NCAM) double-positive neuroblasts and (YFP, calretinin) double-positive interneurons were also present (**G**, **H**). High-magnification images of representative cells are shown as insets (single confocal scans). These data are consistent with the interpretation that Emx1-positive stem cells in the SVZ generate neuroblasts that migrate via the RMS into the olfactory bulb and differentiate as olfactory interneurons. Scale bars, 50 μm.

stem cells are nonuniform with respect to their cellular properties and behavior. This is potentially an important consideration when attempting to harness endogenous neural stem cells for therapy or repair.

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