Apical versus Basal Neurogenesis Directs Cortical Interneuron Subclass Fate

Graphical Abstract

Highlights

- In utero electroporation can be used to fate map and manipulate MGE progenitors
- Somatostatin-expressing interneurons are derived from apical divisions
- Parvalbumin-expressing interneurons are generated from basal progenitors
- Driving progenitors toward apical or basal divisions can switch interneuron fate

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In Brief

Petros et al. reveal that the location of neurogenic divisions is a critical mechanism for determining interneuron fate. Somatostatin-expressing interneurons are derived predominantly from apical progenitors within the ventricular zone, whereas parvalbumin-expressing interneurons are generated from basal progenitor divisions in the subventricular zone.
Apical versus Basal Neurogenesis Directs Cortical Interneuron Subclass Fate

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http://dx.doi.org/10.1016/j.celrep.2015.09.079
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SUMMARY

Fate determination in the mammalian telencephalon, with its diversity of neuronal subtypes and relevance to neuropsychiatric disease, remains a critical area of study in neuroscience. Most studies investigating this topic focus on the diversity of neural progenitors within spatial and temporal domains along the lateral ventricles. Often overlooked is whether the location of neurogenesis within a fate-restricted domain is associated with, or instructive for, distinct neuronal fates. Here, we use in vivo fate mapping and the manipulation of neurogenic location to demonstrate that apical versus basal neurogenesis influences the fate determination of major subgroups of cortical interneurons derived from the subcortical telencephalon. Somatostatin-expressing interneurons arise mainly from apical divisions along the ventricular surface, whereas parvalbumin-expressing interneurons originate predominantly from basal divisions in the subventricular zone. As manipulations that shift neurogenic location alter interneuron subclass fate, these results add an additional dimension to the spatial-temporal determinants of neuronal fate determination.

INTRODUCTION

One of the most challenging areas in neuroscience is understanding the genetic and extrinsic mechanisms that direct cell fate decisions. While most studies investigating this topic focus on the spatiotemporal localization of neuronal subtype origins in distinct domains along the lateral ventricles, it is unclear whether the location of neurogenesis within a fate-restricted domain is not only associated with distinct fates but also instructive of those fates.

The telencephalon contains two main classes of neuronal progenitors. Apical progenitors (APs) divide along the ventricular surface, whereas basal progenitors (BPs) divide within the subventricular zone (SVZ). In the developing cerebral cortex, the BP population expands as neurogenesis proceeds, BP-derived neurons populate all cortical levels, and disrupting BP generation alters cortical size and lamination (Kowalczyk et al., 2009; Lui et al., 2011; Pilz et al., 2013; Postiglione et al., 2011; Sessa et al., 2008).

Similar types of APs, BPs, and modes of neurogenesis are observed in the subpallium (Hansen et al., 2013; Pilz et al., 2013; Sheth and Bhide, 1997), the source of all telencephalic GABAergic interneurons. Two of the largest neurochemically defined classes of cortical interneurons are the somatostatin-expressing (SST+) and parvalbumin-expressing (PV+) subclasses. Both PV+ and SST+ interneurons become fate committed around the time of cell-cycle exit in the medial ganglionic eminence (MGE), where their fate is predicted by both spatial and temporal factors. Specifically, SST+ interneurons, which are most plentiful in the deeper cortical layers, tend to be generated early in neurogenesis and arise predominantly from the dorsal MGE (dMGE). In contrast, a higher percentage of all neocortical PV+ interneurons are born later during neurogenesis, inhabit all cortical layers, and display a slight bias for arising from the ventral MGE (vMGE) (Butt et al., 2005; Flames et al., 2007; Inan et al., 2012; Wonders et al., 2008; Xu et al., 2010a). Thus, this temporal transition from predominantly apical divisions to basal divisions parallels the shift in generation of SST+ to PV+ interneurons, raising the question as to whether apical or basal neurogenic divisions preferentially give rise to SST+ and PV+ interneurons, respectively.

In this study, we utilize in utero electroporation (IUE) to conduct in vivo fate mapping of cortical interneurons originating from APs or BPs. Remarkably, MGE APs are strongly biased toward generating SST+ cortical interneurons. Moreover, genetic manipulations that bias MGE progenitors toward apical or basal neurogenesis strongly promote the generation of SST+ or PV+ subtype fates, respectively. Thus, in the subcortical telencephalon, neural fate diversity is a function of not only the spatiotemporal localization of progenitors along the lateral ventricles but also the apical-basal location of neurogenic divisions.

RESULTS

Strategy to Target MGE Progenitors via In Utero Electroporation

Previous studies have successfully labeled MGE-derived cortical interneurons in vivo via IUE (Gelman et al., 2009;
Targeted electroporation of a cre-dependent GFP-expression construct into the MGE of Nkx2.1Cre mice reliably labels MGE progenitors and their progeny, many of which become cortical interneurons (Figures 1A and 1B). Co-electroporation with a cre-independent pCAG-mCherry plasmid demonstrates the specificity of targeting GFP expression to MGE-derived cells. By postnatal day 21 (P21), MGE-derived GFP+ cells are observed in brain regions that contain Nkx2.1-lineage interneurons (Xu et al., 2008) (Figure 1C). We observe many cortical GFP+ cells that display the typical morphology of PV+ basket cells and SST+ Martinotti cells (Figure 1D).

**APs Are Biased toward Generating SST+ Interneurons**

To identify MGE progenitors that are biased toward AP divisions, we focused on cells driving reporter expression from the tubulin α-1 promoter (pTα1). pTα1 is active in neuronal-fate-committed progenitors and postmitotic neuronal precursors (Gloster et al., 1999; Mizutani et al., 2007; Sawamoto et al., 2001). In the neocortex, pTα1 primarily labels APs that have a short basal process (also known as short neural precursors [SNPs]) that generate relatively few BPs (Gal et al., 2006; Stancik et al., 2010). Electroporation of pTα1-GFP into the MGE revealed that pTα1-GFP+ progenitors in the ventricular zone (VZ) typically lack an elongated basal process compared to pNestin-GFP electroporated cells, and pTα1-GFP+ cells in the mantle possess a morphology that is generally indicative of migration (bipolar morphology with apparent leading and trailing processes) (Figure S1A). pTα1-GFP labels both cycling MGE progenitors (5-ethyl-2′-deoxyuridine [EdU]+) in the VZ and SVZ, and Lhx6+ postmitotic cells in the MGE mantle (Figure S1B). We found that a significantly higher percentage of pTα1-GFP-labeled MGE progenitors in the mantle expressed Lhx6 compared to control GFP electroporations (Figure S1C). Furthermore, pTα1-GFP+ MGE progenitors were less likely to be EdU+ cycling progenitors (Figure S2) or cyclin-D2+ (CCND2) BPs (Figure S3) compared to controls. These observations indicate that pTα1+ MGE progenitors are morphologically and phenotypically similar to cortical SNPs, in that they have short basal processes and preferentially bypass the BP phase to undergo direct neurogenic AP divisions.

In order to fate map pTα1+ progenitors and test the hypothesis that AP neurogenic divisions are biased toward generating SST+ interneurons, we electroporated Cre-inducible pTα1-FlpO recombinase (pTα1-LSL-FlpO) and Flp-inducible EGFP-expressing plasmids (pCAG-FSF-GFP) into Nkx2.1Cre embryos at embryonic day 12.5 (E12.5) (Figure 2A). For comparison, we used a nestin promoter-enhancer construct (pNes-LSL-FlpO + pCAG-FSF-GFP) that will fate map all MGE VZ cells and their progeny. 48 hr after IUE, GFP+ cells are restricted to the MGE and MGE-derived interneuron precursors migrating through the LGE into the cortex (Figure 2B). We counted the number of GFP+/PV+ and GFP+/SST+ cortical cells at P21 from pTα1- and pNes-electroporated brains to determine whether these progenitor classes produced different percentages of MGE-derived interneuron subgroups (Figure 2C). Similar to the overall percentages of PV+ and SST+ interneurons observed in the adult brain (Xu et al., 2010b), pNes-labeled cells resulted in a roughly 2:1 bias for PV+ over SST+ interneurons. In marked contrast, pTα1+ fate-mapped cells produced a roughly 2:1 bias for SST+ interneurons compared to PV+ interneurons (Figure 2D). These results indicate that pTα1+ APs are strongly biased toward generating SST+ interneurons over their PV+ counterparts.
Driving MGE Progenitors toward APs or BPs Can Alter Their Fate

Next, we tested whether directing MGE progenitors toward AP- or BP-mediated neurogenic divisions would bias their fate toward SST+ and PV+ interneurons, respectively. A dominant-negative version of the Mastermind-like-1 protein (dnMAML) blocks Notch signaling (Weng et al., 2003). When expressed in embryonic cortex, dnMAML promotes neurogenic divisions and greatly reduces the number of SVZ BPs (Bultje et al., 2009; Yoon et al., 2008). When expressed in the embryonic MGE via IUE of pCAG-LSL-dnMAML-IG, dnMAML greatly decreased the number of GFP+/EdU+ cells 24 hr after IUE (Figure S2), consistent with an effect of driving progenitors out of the cell cycle without allowing an additional BP division. To determine whether this reduced capacity to undergo basal divisions alters cortical interneuron fate, we electroporated Cre-inducible dnMAML or the control construct into Nkx2.1Cre embryos at E12.5 and compared the percentage of cortical GFP+ cells expressing SST+ or PV+ at P21 (Figure 3A). Relative to control, dnMAML-electroporated cells displayed a striking bias for generating SST+ interneurons (Figure 3B), and strongly suggests that directing MGE progenitors toward apical divisions promotes the generation of SST+ cortical interneurons at the expense of PV+ interneurons.

To test whether directing MGE progenitors toward basal divisions would have a complimentary bias for the generation of PV+ interneurons, we generated a Cre-inducible expression construct for Inscutable (pCAG-LSL-Insc-IG). Insc is an adaptor protein involved in establishing the apical-basal polarity of mitotic spindles that are required for proper cell division of neural progenitors (Culurgioni and Mapelli, 2013). Mice lacking Insc have fewer cortical BPs and overexpression of Insc converts radial progenitors into BPs (Postiglione et al., 2011). Electroporation of pCAG-LSL-Insc-IG into MGE progenitors at E12.5 produced a significant increase in BPs, as indicated by an increase in the number of GFP+/EdU+ cells 24 hr after IUE (Figure S2), consistent with an effect of driving progenitors out of the cell cycle without allowing an additional BP division. To determine whether this reduced capacity to undergo basal divisions alters cortical interneuron fate, we electroporated Cre-inducible dnMAML or the control construct into Nkx2.1Cre embryos at E12.5 and compared the percentage of cortical GFP+ cells expressing SST+ or PV+ at P21 (Figure 3A). Relative to control, dnMAML-electroporated cells displayed a striking bias for generating SST+ interneurons (Figure 3B), and strongly suggests that directing MGE progenitors toward apical divisions promotes the generation of SST+ cortical interneurons at the expense of PV+ interneurons.

DISCUSSION

Although recent studies have identified distinct neural progenitors in the VZ and SVZ of the mammalian forebrain (Lui et al.,...
BPs in the MGE correlates with changes in cortical PV+ interneurons in mice have demonstrated that alterations in the number of PV+ interneurons primarily originate from BPs (Figure 3C). Indeed, studies of transgenic mice show a bias for generating SST+ or PV+ neocortical interneurons originate from divisions in distinct neurogenic locations; SST+ interneurons are preferentially generated from APs, whereas PV+ interneurons are more likely from BPs (Anderson et al., 2010). Fate-mapped pTα1+ APs, whereas matrix neurons are born from subpallium-derived cell types. For example, in striatal medium spiny neurons derived from the lateral ganglionic eminence, it has been proposed that the striosomal compartment may be generated from APs whereas matrix neurons are born from BPs (Anderson et al., 1997a).

Interestingly, inhibition of notch signaling with dnMAML induces cell-cycle exit and strongly promoted SST+ fates. In contrast, overexpression of Insc enhances the number of CCND2+ BPs and strongly promotes PV+ interneuron fates. Thus, MGE progenitor fate appears to be malleable in terms of their capacity to generate interneuron subgroup fate simply by shifting their apical versus basal mode of neurogenesis. This plasticity of MGE progenitors giving rise to different interneuron subgroups is also consistent with the finding that both PV+ and SST+ cortical interneurons can originate from the same radial progenitor clone (Brown et al., 2011; Ciceri et al., 2013; Harwell et al., 2015; Mayer et al., 2015). Taken together, these results suggest that many cycling MGE progenitors are bipotential for interneuron subgroup fate and can generate either SST+ or PV+ interneurons in response to intrinsic or extrinsic factors that influence the decision to undergo apical or basal neurogenesis.

An alternative explanation for these results is that dnMAML or Insc expression could shift the balance of PV+ and SST+ cortical interneurons by altering their migration or postmigratory differentiation. However, both Notch signaling and Insc are predominantly active during cell proliferation and differentiation, so it is unclear how these permutations would effect later developmental aspects. In addition, we did not observe any obvious changes in the location (indicative of migration defects) or morphology of GFP+ interneurons in any experimental conditions. PV expression can be altered by activity, so reducing the connectivity of PV+ interneurons, for example by a notch-signaling influence on dendritogenesis (Redmond and Ghosh, 2001), could theoretically reduce PV levels and result in a...
perceived SST⁺ bias that would skew our results. However, we consistently observe that ~10% of all GFP⁺ interneurons are negative for both PV and SST in all IUE conditions (Figures 2D and 3B). This result indicates that neither dnMAML nor Insc expression increases the population of “unspecified” GFP⁺ interneurons. These observations, combined with the evidence that interneuron fate is committed around the time of cell-cycle exit (Butt et al., 2005; Flames et al., 2007; Inan et al., 2012; Xu et al., 2004), weigh against a postmitotic change in migration or connectivity impacting interneuron fate decisions in the Insc or dnMAML experiments.

The growing number of brain disorders associated with disruptions in neurogenesis (Barkovich et al., 2012) necessitates a more complete understanding of neurogenic mechanisms. The massive expansion of SVZ progenitors in primates, occurring in both the cortex and MGE of humans (Hansen et al., 2013; Lui et al., 2011), highlights the importance of understanding the implications of this expansion for neuronal fate determination. Additionally, these results have important implications for neuropsychiatric illnesses in which disruption in the development of distinct interneuron subtypes probably contributes to disease neuropathology (Marín, 2012; Rossignol, 2011). As the list of distinct subclasses of neural progenitors continues to expand, most notably in humans (Lui et al., 2011), it will be vital to determine how these different types of neural progenitors relate to each other and how they contribute to the wide diversity of neural types originating from the subpalium and other brain regions.

EXPERIMENTAL PROCEDURES

In Utero Electroporation
Timed-pregnant wild-type E12.5 C57/B6 females mated to homozygous Nkx2.1Cre males (Xu et al., 2008) were anesthetized with isoflurane, the uterine horn exposed, and ~0.5 μl DNA (at a concentration of ~1–3 μg/ml mixed with 1% Fast-Green dye [Sigma-Aldrich]) was injected through the uterine wall into the lateral ventricle of each embryo using a pulled glass micropipette (Drummond Scientific). For electroporation, five pulses (50 ms each, 950-ms intervals) of 35 mV were delivered ventrolaterally at an ~45° angle through the embryonic brain to target the MGE using 5-mm electrode paddles connected to a BTX ECM830 electroporator (Harvard Apparatus). After the procedure, the abdomen was filled with an antibiotic/analgesic-containing PBS solution and the wound was closed using a surgical suture and wound clips on the skin. All procedures were approved by the Research Animal Resource Center at Weill Cornell Medical College and the Department of Laboratory and Animal Research at New York University.

Embryonic Tissue Collection, Immunohistochemistry, and Analysis
Timed-pregnant C57/B6 mice containing electroporated embryos were anesthetized and embryonic brains (E13.5–E14.5) were drop fixed in 4% paraformaldehyde (PFA) overnight at 4°C. In some instances, dams were given an injection of EdU (100 μg/g) either 30 min or 4 hr prior to sac at E13.5. Embryonic brains were cryosectioned at 14 μm or 20 μm thickness and immunostained for combinations of GFP, dsRed, Nkx2.1, Lhx6, CCND2, and EdU depending on the experiment. For EdU counts, n = 8 embryos for pCAG-LSL-IG, n = 5 embryos for pCAG-DNMAML-IG, and n = 3 embryos for pT mex1-GFP, with a minimum of 50 GFP⁺ cells counted for each brain (average = 210 GFP⁺ cells/brain). For CCND2 counts, n = 9 embryos for pCAG-LSL-IG, n = 4 embryos for pCAG-InsC-IG, and n = 6 embryos for pT mex1-GFP, with a minimum of 80 GFP⁺ cells (average = 242 GFP⁺ cells/brain) counted from three or more different MGE sections for each brain. For Lhx6 analysis, n = 5 embryos for pCAG-LSL-IG and n = 7 embryos for pT mex1-GFP, with a minimum of 107 GFP⁺ cells (average = 244 GFP⁺ cells/brain) counted from two or more different MGE sections for each brain. All analysis was performed blind to which plasmid was electroporated.

Postnatal Tissue Collection, Immunohistochemistry, and Analysis
P21 animals that were electroporated in utero were anesthetized, perfused with 4% PFA, and post-fixed overnight in 4% PFA. P21 brains were vibromed at 50 μm and immunostained for GFP, PV, and SST. The numbers of GFP⁺/PV⁺ and GFP⁺/SST⁺ cells in cortical layers II–VI were counted blind to which plasmid was electroporated. The number of brains analyzed and cells counted for each condition were 12 for pCAG-LSL-IG (average 144 GFP⁺ cells/brain), 11 for pCAG-LSL-DNMAML-IG (average 68 GFP⁺ cells/brain), 11 for pCAG-LSL-InsC-IG (average 88 GFP⁺ cells/brain), 6 for pT mex1-LSL-FloP + pCAG-FS-FGFP (average 89 GFP⁺ cells/brain), and 4 for pNeso-LSL-FloP + pCAG-FS-FGFP (average 74 GFP⁺ cells/brain).

Data Analysis
All figures were composed in Adobe Illustrator or Photoshop, and quantitative data were collected in Microsoft Excel. All graphs depict average ± SEM. For statistics, we used either a student’s two-tailed t test or an ANOVA followed by post hoc analysis for multiple comparisons with Tukey’s HSD, using SPSS statistics software (IBM). Differences were considered statistically significant with a p value below 0.05.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures and three figures and can be found with this article online at http://dx.doi.org/10.1016/j.celrep.2015.09.079.

AUTHOR CONTRIBUTIONS


ACKNOWLEDGMENTS

We thank T. Haydar for constructs and reagents and T. Haydar and members of the Fishell and Anderson labs for critical reading of the manuscript. The NIH provided support to T.J.P. (F32NS074742, K99MH104595), M.E.R (P01NS048120), and S.A.A. (P01NS048120, R01MH070031).

Received: December 8, 2014
Revised: August 19, 2015
Accepted: September 25, 2015
Published: October 29, 2015

REFERENCES


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Supplemental Data

Figure S1
Figure S1. pTα1-GFP⁺ MGE progenitors are cycling progenitors that preferentially generate neurogenic divisions. Related to Figure 2. (A) The MGE of E12.5 mice were electroporated with pNestin-GFP (top) or pTα1-GFP (bottom) and fixed at E13.5. The number of GFP⁺ long radial fibers that extend basally from the pNes-labeled MGE cells is strongly reduced in the pTα1-labeled cells, which is most evident in the high magnification images (right). Scale bars = 50 and 10 µm. V = lateral ventricle. (B) The MGE of E12.5 Nkx2.1Cre mice were electroporated with pTα1-GFP and EdU pulses were administered 4 and 2 hrs prior to sacrifice at 24 hrs post IUE. Many GFP⁺ cells in the VZ/SVZ are EdU⁺ (indicating that pTα1⁺ cells are cycling progenitors) and many GFP⁺ cells in the mantle express Lhx6. Scale bars = 50 µm. (C) The percentage of GFP⁺ cells in the MGE mantle (outlined in white) co-expressing Lhx6 was compared between pTα1-GFP and pCAG-LSL-IG electroporated embryos. There is a significant increase in the percentage of GFP⁺/Lhx6⁺ cells in the pTα1-GFP condition, indicating that pTα1⁺ are biased towards neurogenic divisions compared to the general MGE progenitor population. N = 5 brains for pCAG-LSL-IG and 7 brains for pTα1-GFP, two tailed t-test, *p = .016. Scale bars = 50 µm.
Figure S2

(A) pCAG-LSL-IG vs. pCAG-LSL-dnMAML-IG

(B) pTα1-GFP

% of GFP⁺ cells that are Edu⁺

Percentages and error bars indicate statistical significance: *p = .056
Figure S2. DNMAML induces cell cycle exit of MGE progenitors and fewer pTα1\(^+\) progenitors are cycling compared to controls. Related to Figures 2 and 3.

(A) E12.5 embryos from Nkx2.1\(Cre\) mice were electroporated with pCAG-LSL-IG or pCAG-LSL-DNMAML-IG. One EdU pulse was administered 30 minutes prior to sacrifice 24 hours after IUE. Top, representative section through the MGE that was used to count the number of GFP\(^+\)/EdU\(^+\) (red dots) and GFP\(^+\)/EdU\(^-\) (green dots) cells for each condition, with the black line demarcating the SVZ-mantle boundary (based on the strong band of EdU labeling). Scale bar = 50 \(\mu m\). Bottom, there is a significant reduction in the number of GFP\(^+\)/EdU\(^+\) cells in the DNMAML condition, indicating that DNMAML induces cell cycle exit. N = 4 brains for pCAG-LSL-IG and 5 brains for pCAG-LSL-DNMAML-IG. Two tailed t-test, *p = .033.

(B) Same as above, but EdU was administered 4 hours and 2 hours prior to sacrifice 24 hours after IUE of pCAG-LSL-IG or pTα1-GFP. There is a strong trend for a reduction in the number of GFP\(^+\)/EdU\(^+\) cells in the pTα1-GFP. N = 5 brains for pCAG-LSL-IG and 7 brains for pTα1-GFP. Two tailed t-test, p = .0556.
Figure S3. Changes in the number of CCND2⁺ BPs in pTα1-GFP-labeled and Inscuteable-expressing MGE progenitors. Related to Figures 2 and 3.

E12.5 embryos were electroporated with pCAG-LSL-IG, pCAG-LSL-Insc-IG or pTα1-GFP and fixed 24 hrs after IUE. Sections through the MGE were immunostained for cyclin-D2 (CCND2), GFP and DAPI. The SVZ, as defined by the dense CCND2⁺ layer, is outlined in white (left). All GFP⁺ cells within the outlined region were scored as GFP⁺/CCND2⁻ or GFP⁺/CCND2⁺. There was a significant decrease in the number of GFP⁺/CCND2⁻ cells observed in pTα1-GFP brains and a significant increase in the number of GFP⁺/CCND2⁺ cells observed in Insc-expressing brains compared to controls. N = 6, 9 and 4 brains for pTα1-GFP, pCAG-LSL-IG and pCAG-LSL-Insc-IG conditions, respectively. ANOVA: $F_{(2,16)} = 29.51, p < .001$, with Tukey’s HSD post hoc test; *p ≤ .05, ***p ≤ .001.

Scale bar = 50 μm.
Supplemental Experimental Procedures

Construct generation

The plasmids pCAG-eGFP (Addgene plasmids 11150), pCAGIG (Addgene plasmids 11159), pBS302 (Addgene plasmid 11925), pCAG-Frt-NeoStop-Frt(FSF)-eGFP (Addgene plasmid 13772), pPGK-FlpO-bpA (Addgene plasmid 13793), MSCV-DNMAML-1-eGFP (Weng et al., 2003), and pTα1-DsRed2 (Mizutani et al., 2007) have been previously described. The eGFP cassette was replaced with mCherry to generate a pCAG-mCherry construct. We collected LoxP-Stop-LoxP (LSL) from pBS302 and ligated it into pCAGIG to generate pCAG-LSL-IG. DNMAML and Insc were PCR amplified from MSCV-DNMAML-1-eGFP and an Open Biosystems clone (MMM1013-202765359), respectively, and inserted into pCAG-LSL-IG to generate pCAG-LSL-DNMAML-IG and pCAG-LSL-Insc-IG. We replaced dsRed2 with eGFP to generate pTα1-eGFP. To generate pTα1-LSL-FlpO, we PCR amplified LSL from pBS302 and FlpO from pPGK-FlpO-bpA and inserted these cassettes after the Tα1 promoter. To generate pNes-LSL-FlpO, the LSL-FlpO cassette from pTα1-LSL-FlpO was PCR-amplified with and then ligated into the Nestin promoter-intron II expression vector (Zimmerman et al., 1994). A similar strategy was used to generate the pNes-eGFP construct.

Immunohistochemistry

Timed-pregnant C57/B6 mice containing electroporated embryos were anesthetized with Sleepaway® (sodium pentobarbital), and embryonic brains
(E13.5-E14.5) were fixed in 4% paraformaldehyde (PFA) in PBS overnight at 4°C. Brains were immersed in 30% sucrose in PBS, frozen in OCT (Tissue-Tek), sectioned on a Leica CM3050S cryostat at 14 µm or 20 µm thickness, and stored at -80°C. For immunofluorescence, slides were placed at room temperature (RT) to dry, immersed in PBS at RT for > 30 min before antigen retrieval (if necessary, 10 µM citrate at 95°C for 30 min or 1 µM EDTA at 65°C for 1 hr), blocked for ≥ 30 min at RT in blocking buffer (10% Normal Donkey Serum in PBS with 0.1% Triton X-100), then incubated with primary antibodies in blocking buffer at 4°C overnight. Slides were washed 3 x 5 min at RT in PBS, incubated for ≥ 2 hours at RT with secondary antibodies and DAPI in blocking buffer, washed 3 x 5 min at RT with PBS, and then mounted with coverslips.

P21 animals that were electroporated in utero were anesthetized with Sleepaway®, perfused with 4% PFA and fixed overnight in 4% PFA. Brains were sectioned on a Leica vibratome at 50 µm thickness and stored in cryoprotectant solution (40% PBS, 30% glycerol and 30% ethylene glycol) at -20°C. For immunofluorescence, floating sections were placed in 2 ml tubes for ≥ 30 min at RT in PBS, then blocked for ≥ 1 hr at RT in blocking buffer and incubated for 3 days at 4°C with primary antibodies in blocking buffer. Sections were washed 3 x 30 min at RT in PBS, incubated overnight at 4°C with secondary antibodies and DAPI in blocking buffer, washed 3 x 30 min at RT in PBST and once with PBS before being mounted on glass slides.

Analysis of E13.5 IUE sections
For EdU analysis, *Nkx2.1Cre* E12.5 embryos were electroporated with pCAG-LSL-IG or pCAG-LSL-DNMAML-IG. At E13.5, pregnant dams were injected with 100 µg/g of EdU dissolved in H2O and sacrificed 30 minutes later. For a separate set of pCAG-LSL-IG or pTα1-GFP electroporated animals, EdU was injected 4 hours and 2 hours prior to sacrifice at E13.5. Brains were harvested, fixed and cryosectioned as described above. Sections were stained for GFP, Nkx2.1, DAPI and EdU (using the Click-iT® EdU Imaging Kit, Life Technologies). For sections with GFP+ MGE cells, the SVZ/mantle boundary was determined by drawing a black line along the inner border of the EdU+ layer of SVZ cells prior to any analysis. The number of EdU+/GFP+ cells was divided by the total number of GFP+ cells within the VZ/SVZ to determine the percentage of GFP+ cells in the cell cycle.

For CCND2 analysis, *Nkx2.1Cre* E12.5 embryos were electroporated with pCAG-LSL-IG, pCAG-LSL-Insc-IG or pTα1-GFP and sacrificed 24 hours later. Cryosections through the MGE were immunostained for GFP, CCND2 and DAPI. The SVZ was outlined based on the CCND2 immunostaining prior to any analysis, and all GFP+ cells within this SVZ outline were scored either as GFP+/CCND2- or GFP+/CCND2+.

For Lhx6 analysis, *Nkx2.1Cre* E12.5 embryos were electroporated with pTα1-GFP or pCAG-LSL-Insc-IG and sacrificed 24 hours later. To demonstrate pTα1-GFP+ cells are cycling, EdU+ was injected at 4 hr and 2 hr timepoints prior to sacrifice in some mice. Cryosections through the MGE were immunostained for GFP, Lhx6 and DAPI. The MGE SVZ/mantle was outlined based on Lhx6
immunostaining prior to any analysis, and all GFP\(^+\) cells within this region were scored either as GFP\(^+\)/Lhx6\(^-\) or GFP\(^+\)/Lhx6\(^+\).

**Antibodies and microscopy**

We used the following primary antibodies and dilutions: rabbit \(\alpha\)-Nkx2.1 (1:2000; Epitomics), rabbit \(\alpha\)-CCND2 (1:2000; Santa Cruz #SC-593), rabbit \(\alpha\)-Parvalbumin (1:5000; Swant PV-28), rabbit \(\alpha\)-Lhx6 (1:200; generated by S. Anderson based on (Vogt et al., 2014)) rat \(\alpha\)-Somatostatin (1:400; Chemicon MAB354), rat \(\alpha\)-DsRed (1:1000; Chromotek 5F8), chicken \(\alpha\)-GFP (1:2000; Abcam ab13970), mouse \(\alpha\)-Nkx2.1 (1:100; NeoMarkers). Donkey \(\alpha\)-rabbit/-rat/-chick/-mouse secondary antibodies conjugated to Alexa-488, Alexa-594 or Alexa-647 (Invitrogen) were used at 1:500.

Images were acquired on a Nikon E800 microscope equipped with a Cool SNAP HQ (Photometrics) camera or a Zeiss Axio Imager A1 microscope with a cooled-CCD camera (Princeton Scientific Instruments), both with MetaMorph software (Universal Imaging). In some instances, z-stacks were compressed using ImageJ (NIH) software.
Supplemental References
