Apical versus Basal Neurogenesis Directs Cortical Interneuron Subclass 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.

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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Apical versus Basal Neurogenesis Directs Cortical Interneuron Subclass Fate

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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 neural 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;
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-ethynyl-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-GFP+ 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.

Nóbrega-Pereira et al., 2010. 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),
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 \(\text{(Weng et al., } 2003)\). When expressed in embryonic cortex, dnMAML promotes neurogenic divisions and greatly reduces the number of SVZ BPs \(\text{(Bultje et al., } 2009; \text{Yoon et al., } 2008)\). 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 \(\text{(Figure S2)}\), 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 \(\text{(Figure 3A)}\). Relative to control, dnMAML-electroporated cells displayed a striking bias for generating SST⁺ interneurons \(\text{(Figure 3B)}\). This preference was similar to that observed with the pTα1⁺ fate-map (Figure 2), and strongly suggests that directing MGE progenitors toward apical divisions promotes the generation of SST⁺ interneurons at the expense of PV⁺ interneurons.

To test whether directing MGE progenitors toward basal divisions would have a complementary bias for the generation of PV⁺ interneurons, we generated a Cre-inducible expression construct for Inscutetable \(\text{(pCAG-LSL-Insc-IG)}\). 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 \(\text{(Culurgioni and Mapelli, } 2013)\). Mice lacking Insc have fewer cortical BPs and overexpression of Insc converts radial progenitors into BPs \(\text{(Postiglione et al., } 2011)\). 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 expressing the MGE BP marker cyclin-D2 \(\text{(CCND2)}\) \(\text{(Glickstein et al., } 2007a)\) \(\text{(Figure S3)}\). Fate mapping cells at P21 revealed a strong bias for Insc-elec-troporated cells to generate PV⁺ cortical interneurons compared to controls \(\text{(Figures 3A and 3B). In sum, dnMAML expression resulted in a } 2.5:1 \text{ bias in favor of SST⁺ interneurons whereas Insc expression generated a } 3.5:1 \text{ bias in favor of PV⁺ interneurons (Figure 3B).}

DISCUSSION

Although recent studies have identified distinct neural progenitors in the VZ and SVZ of the mammalian forebrain \(\text{(Lui et al., } 2015)\),
derived SST+ or PV+ neocortical interneurons originate from different progenitor locations and cortical interneuron fate indicate that MGE-derived neuron subclasses.

In vivo studies of the relationships between neural progenitor location and cortical interneuron fate indicate that MGE-derived SST+ or PV+ neocortical interneurons originate from divisions in distinct neurogenic locations; SST+ interneurons are preferentially generated from APs, whereas PV+ interneurons primarily originate from BPs (Figure 3C). Indeed, studies of transgenic mice have demonstrated that alterations in the number of BPs in the MGE correlates with changes in cortical PV+ interneuron number while SST+ interneuron numbers remain unchanged, which is consistent with this hypothesis (Glickstein et al., 2007b; Lodato et al., 2011). This finding is in contrast to studies of the neocortex, where cell fate is predominantly tied to birthdate and not known to be directed by apical versus basal locations of neurogenesis.

Our data indicate that different MGE progenitor populations have a bias for generating specific cortical interneuron subgroups. Fate-mapped pT\(\alpha\)1-expressing MGE progenitors (which are likely similar to cortical pT\(\alpha\)1 SNP; Gal et al., 2006; Stancik et al., 2010) are strongly biased toward generating SST+ cortical interneurons compared to the general population of pNestin-expressing MGE progenitors. This finding is consistent with the growing literature suggesting that the VZ and SVZ of the cortex and subpallium contain a heterogeneous population of progenitors (Lui et al., 2011; Pilz et al., 2013). In the cortex, some progenitors may be fate restricted to generate upper or lower layer projection neurons (Franco et al., 2012). As the subpallium produces a huge diversity of cell types that populate many different forebrain regions, it will be of interest to determine whether apical versus basal modes of neurogenesis regulate cell fates in other subpallial-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 dmMAML 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 dmMAML 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 (Marin, 2012; Rossignol, 2011). As the list of distinct subclasses of neural progenitors continues to expand, 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 subpallium 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 inter-pulse) of 35 mV were delivered ventrolaterally at an angle through the brain to target the MGE using 5-mm electrode paddles connected to a BTX ECM830 electroporator (Harvard Apparatus). After the procedure, the embryos were perfused with 4% PFA, and post-fixed overnight in 4% PFA. P21 brains were vibratome-sectioned 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-LSL-IG (average 88 GFP+ cells/brain), 6 for pTat-LSL-Floxed pCAG-EGFP (average 89 GFP+ cells/brain), and 4 for pNes-LSL-Floxed pCAG-EGFP (average 74 GFP+ cells/brain).

**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-DNAMAL-LSL-IG, and n = 3 embryos for pTat-LSL-IG, 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-LSL-IG, and n = 6 embryos for pTat-LSL-IG, with a minimum of 80 GFP+ cells (average = 242 GFP+ cells/brain) counted from three or more different MGE sections for each brain. For Hx6 analysis, n = 5 embryos for pCAG-LSL-IG and n = 7 embryos for pTat-LSL-IG, 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 vibratome-sectioned 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-LSL-IG (average 88 GFP+ cells/brain), 6 for pTat-LSL-Floxed pCAG-EGFP (average 89 GFP+ cells/brain), and 4 for pNes-LSL-Floxed pCAG-EGFP (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**


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