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Patterning of ventral telencephalon requires positive function of Gli transcription factors

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ABSTRACT

The ability of neuroepithelial cells to generate a diverse array of neurons is influenced by locally secreted signals. In the spinal cord, Sonic Hedgehog (Shh) is known to induce distinct cell fates in a concentration-dependent manner by regulating the activities of the three Gli transcription factors in neural precursors. However, whether Gli-mediated Shh signaling is also required to induce different cell types in the ventral telencephalon has been controversial. In particular, loss of *Shh* has little effect on dorsoventral patterning of the telencephalon when *Gli3* is also removed. Furthermore, no ventral telencephalic phenotypes have been found in individual *Gli* mutants. To address this issue, we first characterized Shh-responding ventral telencephalic progenitors between E9.5 and E12.5 and found that they produce neurons migrating to different layers of the cortex. We also discovered a loss of *Nkx2.1* and *Nkx6.2* expression in two subgroups of progenitors in embryos lacking major Gli activators. Finally, we analyzed the telencephalic phenotypes of embryos lacking all *Gli* genes and found that the ventral telencephalon was highly disorganized with intermingling of distinct neuronal cell types. Together, these studies unravel a role for Gli transcription factors in mediating Shh signaling to control specification, differentiation and positioning of ventral telencephalic neurons.

Introduction

The ventral telencephalon is the source of several types of neurons, including striatal, olfactory and cortical interneurons and is comprised of three proliferative zones: the lateral, medial and caudal ganglionic eminences (LGE, MGE and CGE). Following their generation, many MGE- and LGE-derived neurons undergo tangential migration to reach the cortex or olfactory bulb (Corbin et al., 2001; Marin and Rubenstein, 2001; Wonders and Anderson, 2006). Recent studies have further shown that the migration pattern and positioning of the transplanted cells are largely determined by donor tissues, suggesting that local signals within the ventral telencephalon determine cell fate and behavior of telencephalic neurons (Butt et al., 2005, 2008; Nery et al., 2002; Nobrega-Pereira et al., 2008; Valcanis and Tan, 2003; Wichterle et al., 2001).

Shh, a member of the Hedgehog (Hh) family of secreted proteins that specify many central nervous system (CNS) cell types (Dessaud et al., 2008), is expressed in early telencephalic tissues. The initial *Shh* expression appears in the prechordal plate (Echelard et al., 1993). By E9, a number of morphogenetic events transform the telencephalic anlage into a set of paired vesicles and *Shh* is expressed in the neural epithelium of the ventral telencephalon (Shimamura et al., 1995). By E12, *Shh* expression is shifted into the mantle area, and can no longer be detected in the neuroepithelium of the MGE (Nery et al., 2001). Both genetic and gain-of-function studies confirm the importance of Shh signaling in telencephalon development. Specifically, mutations in *Shh* or conditional loss of components of the *Shh* pathway at an early stage (~E8.5) using *FoxG1*-Cre lead to the loss of all ventral telencephalic tissues (Chiang et al., 1996; Fuccillo et al., 2004; Zhang et al., 2001), suggesting an early requirement for Shh in the dorso-ventral patterning of the telencephalon. On the other hand, disruption of Shh signaling pathway between E10 and E12 using *Nestin*-Cre has a more limited effect, as it only affects the dorsal-most expression of *Nkx2.1* (Balordi and Fishell, 2007; Machold et al., 2003; Xu et al., 2005). Conversely, ectopic dorsal expression of *Shh* leads to the induction of ventral telencephalic markers and repression of dorsal markers (Ericson et al., 1995; Gaiano et al., 1999; Kohtz et al., 1998; Rallu et al., 2002; Shimamura and Rubenstein, 1997). Shh signaling, therefore, could be a local signal linking progenitor cell specification to the fate of the migrating post-mitotic ventral neurons.

Although Shh is clearly required for telencephalic development, it is less clear whether and how Shh is involved in specifying different

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cell types in the ventral telencephalon. Shh signaling is mediated by Gli transcription factors, which include Gli1, Gli2 and Gli3 (Fuccillo et al., 2006; Hooper and Scott, 2005; Ingham and McMahon, 2001). Both Gli1 and Gli2 function primarily as transcriptional activator, as revealed by biochemical analysis and in vivo mouse knock-in genetic analysis (Bai and Joyner, 2001; Dai et al., 1999; Pan et al., 2006), and Gli3 functions primarily as a transcriptional repressor (Litingtung and Chiang, 2000; Wang et al., 2000). However, examination of the MGE marker *Nkx2.1* expression in mice mutated for individual *Gli* genes or of *Gli1/2* double mutants has not revealed obvious defects in patterning of ventral telencephalon (Park et al., 2000). On the other hand, *Gli3* mutant mice show a strong dorsal telencephalic phenotype (Aoto et al., 2002; Fotaki et al., 2006; Kuschel et al., 2003; Rallu et al., 2002; Tole et al., 2000), but ventral patterning appears to be largely normal. These analyses suggest that Gli3 repressor is required in the dorsal telencephalon. Interestingly, removal of *Gli3* from *Shh* mutant embryos largely rescues dorsoventral patterning defects in *Shh* mutants (Rallu et al., 2002), suggesting that the primary function of Shh signaling is to prevent the production of excessive Gli3 repressor. However, because Gli3 has also been shown to function as a weak activator in vivo (Bai et al., 2004; Wang et al., 2007), it remains to be determined whether there is any Gli activator function in the ventral telencephalon, and whether *Gli* genes have a compensatory function in mediating Shh signaling to influence specification and behavior of neurons derived from ventral telencephalon.

To address whether Gli activators are required for patterning of ventral telencephalon, we analyzed an array of domain-specific ventral telencephalic markers in *Gli2* and *Gli1/2* double mutants, and identified the loss of *Nkx2.1* and *Nkx6.2* expression in two subgroups of progenitors in the interganglionic sulcus. We also analyzed the ventral telencephalic phenotypes of pan-*Gli* mutant embryos and showed that *Gli* genes play multiple roles in telencephalon development, including specification, differentiation and positioning of ventral telencephalic neurons.

Material and methods

Mouse breeding

For fate-mapping experiments, *Gli1-CreER/+;R26R/R26R* (Ahn and Joyner, 2004) male mice were bred with 6–8-week-old CD-1 females (Charles River). Noon of the day of detection of a vaginal plug is considered E0.5. Tamoxifen (Sigma, T-5648) was dissolved in corn oil at a concentration of 20 mg/ml and was fed to pregnant female using a gavage-feeding needle (FST) at a dose of 4 mg/40 g body weight, at 5 PM on different gestational days. The genotyping of *Gli1-lacZ*, *Gli2-lacZ*, $Gli2^{zfd}$, and $Gli3^{xt}$ mice were as described (Bai and Joyner, 2001; Maynard et al., 2002; Mo et al., 1997). Double heterozygous mutant mice were maintained on an outbred background.

Histology, immunohistochemistry and RNA in situ hybridization

Mouse embryos were dissected in cold PBS and fixed in 4% paraformaldehyde (PFA) for 1 h at 4 °C. Adult mouse brains (8–10 weeks old) were dissected out after transcardiac perfusion and post-fixed with 4% PFA for 3 h. Embryos and brains were washed with PBS, cryo-protected in 30% sucrose and embedded in OCT (Tissue-Tek). Tissues were sectioned at 12 μm (embryos) or 20 μm (adult brain) on a Leica Cryostat. At least three brains were analyzed for each time point. Immunohistochemistry, X-gal staining, H&E staining and RNA in situ hybridization were performed essentially as described (Bai et al., 2002; Bai and Joyner, 2001). Subtype identity of labeled cells was determined by double immunofluorescence staining using antibodies against β-galactosidase and parvalbumin, somatostatin or calretinin. Antibodies used were: GABA (1:2000, Sigma), calretinin (1:2000, Chemicon), parvalbumin (1:2000, Sigma), somatostatin (1:250, Chemicon), β-galactosidase (1:500, Biogenesis; or 1:500, 5 prime->3 prime), Pax6 (1:20, Developmental Studies Hybridoma Bank), Gsh1/2 (1:200, Kenneth Campbell, Cincinnati Children's Hospital Medical Center, Cincinnati, OH, USA), BrdU (1:20, Becton-Dickinson), Ki67 (1:500, Abcam), phospho-Histone H3 (1:100, Upstate), cleaved Caspase-3 (1:200, Cell Signaling), and Nkx2.1 (TTF1, 1:500, Epitomics). Nuclear counter-staining was performed with Hoechst 33258 (Molecular Probes) or with 0.005% nuclear fast red (Polyscientific, Inc.). Probes for in situ hybridization have been described previously (Rallu et al., 2002). Sections were examined with a Leica DMLB epifluorescence microscope fitted with a SPOT camera in the Genetics Imaging Facility (supported by NIH-NCRR, RR-021228).

Fate-mapping analysis

Coronal sections of the brain were stained by nuclear fast red or Hoechst nuclear dye to reveal layer structures. To better quantify the position of labeled cells in the cortex, we divided the cortex into ten bins along the radial axis, with bin 1 closest to the white matter (WM) and bin 10 next to the pial surface. Only those cells located within primary motor cortex and somatosensory cortexes were used for quantification (as defined in Paxinos and Franklin, 2001). In order to map the location of the labeled cells, X-gal stained cells were assigned to one of the ten bins according to their location in the cortex. Three sections from one animal, located about 0.12 mm apart, were used for quantification. Three mouse brains were used for each time point. The numbers on the histograms represent the mean percentage of labeled cells in one bin over the total number of β-galactosidase labeled cells in the section ± S.E.M.

BrdU labeling, cell cycle and cell death analyses

E12.5 pregnant mice were injected intraperitoneally with BrdU (Sigma) at 100 μg/g body weight 1 h prior to dissection. The fraction of BrdU+ cells was calculated by counting the number of those positive cells in 75 μm wide areas spanning radially from the ventricular surface to the edge of VZ/SVZ (as judged by dense nuclear staining) and dividing the total number of nuclei in that area (each segment contains ~250 nuclei). To calculate the fraction of S-phase cells, the number of BrdU+ cells was divided by the total number of Ki67+ cells. To calculate the fraction of pH3+ cells, the number of those positive cells bound by a box (120 μm × 94 μm) in the VZ/SVZ was divided by the total number of nuclei. For cell death analysis, the total number of Caspase3+ cells in the MGE or LGE was divided by the

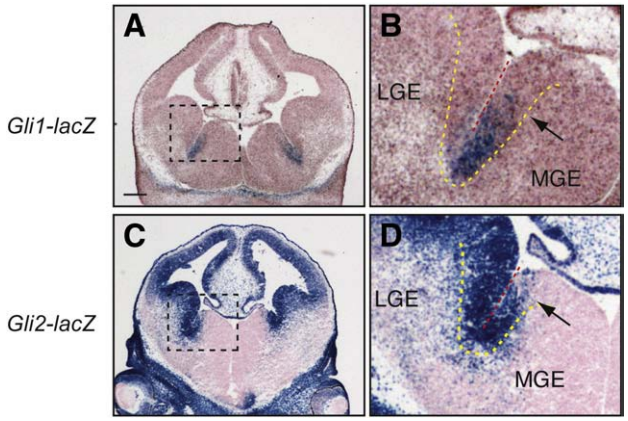

Fig. 1. X-gal staining of *Gli1-lacZ* and *Gli2-lacZ* on telencephalic sections of E12.5 mouse embryos. (B) and (D) are higher magnification images of the boxed regions in (A) and (C) respectively. Arrows indicate the ventral expression limits of *Gli1* and *Gli2*. Note the overlapping ventral expression limit of *Gli1* and *Gli2* in the ventral telencephalon. LGE, lateral ganglionic eminence; MGE, medial ganglionic eminence. Scale bar: 0.15 mm (A, C).

size of the area and converted to a percentage. Data from 3–5 embryos from at least two different litters were pooled together to determine average and S.E.M for each genotype. Student's *t*-test was used to calculate the *p* value and to determine whether the results were significantly different from each other.

Results

A subset of ventral telencephalic progenitors receives positive Hh signaling and expresses Gli1-lacZ

In order to visualize those cells that respond to Hh signaling, we examined the expression pattern of *Gli1-lacZ*, in which a *lacZ* gene is expressed from the *Gli1* genomic locus and thus provides a sensitive readout of Hh activator signaling (Bai et al., 2002). At E12.5, *Gli1-lacZ* was found in the interganglionic sulcus, with a higher level of expression on the MGE side (Figs. 1A, B), similar to previous reports (Tole et al., 2000; Wonders et al., 2008). The expression pattern suggests that cells in the interganglionic sulcus, in particular those on the side of the MGE, receive a high level of Hh signaling. We next examined the expression pattern of *Gli2-lacZ*, where a *lacZ* gene is expressed from the *Gli2* genomic locus (Bai and Joyner, 2001), and found that the ventral limit of *Gli2-lacZ* expression coincided with that of *Gli1-lacZ* on the MGE side of the interganglionic sulcus (Figs. 1C, D). The expression of these Hh-dependent transcriptional activators in the ventral telencephalon raises the intriguing possibility that different ventral telencephalic progenitors are generated in response to Hh signaling, as they are in the spinal cord.

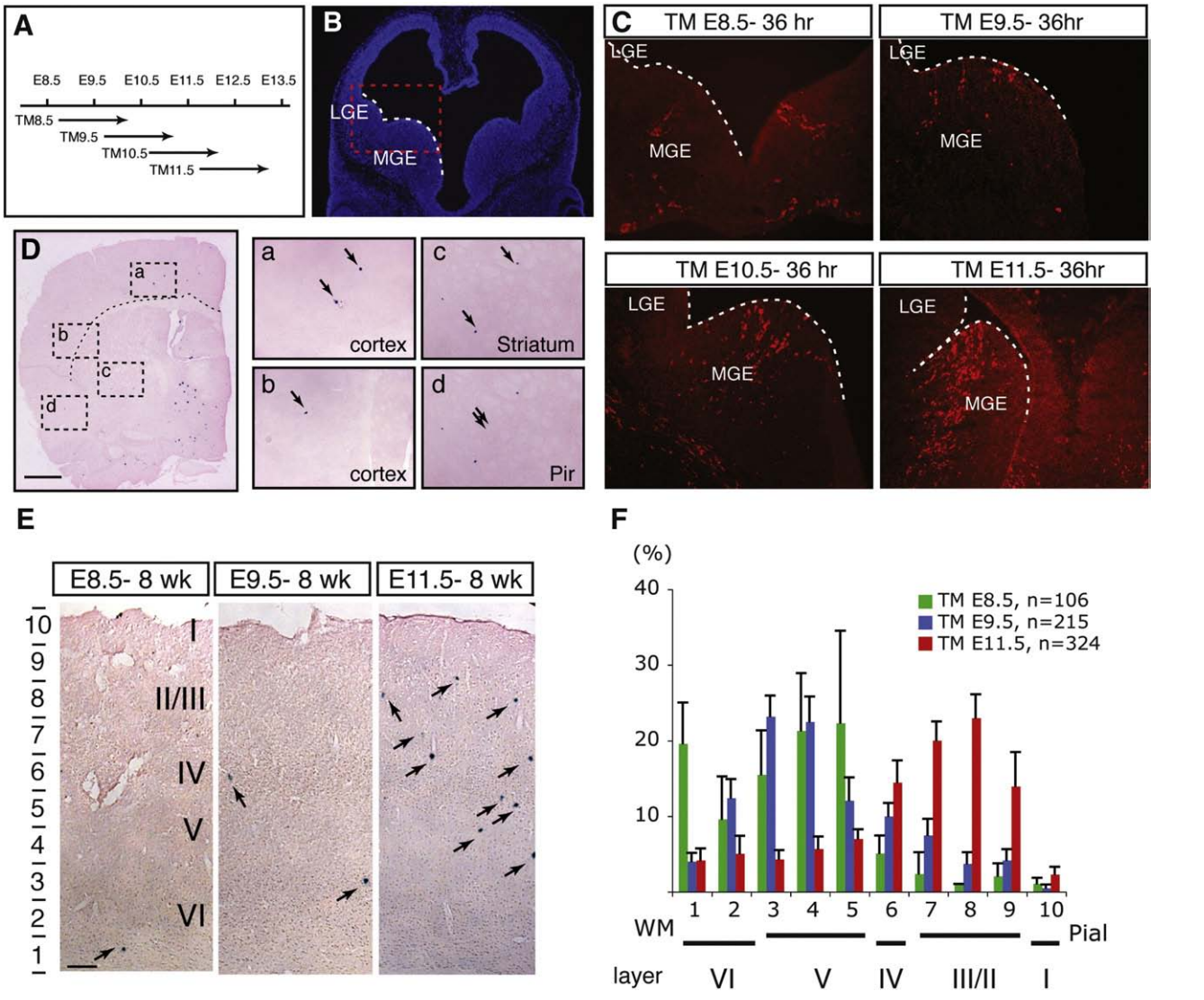

Fig. 2. Different cortical interneurons were generated from progenitors responding to Hh signaling from ~E9.5 to ~E12.5. (A) Schematic of Tamoxifen (TM) injection to analyze the initial populations of Hh-responding progenitors in the ventral telencephalon. TM was given at 5 PM on E8.5 to E11.5 to label Hh-responding cells from ~E9.5 to ~E12.5. (B) Coronal section of an E11.5 telencephalon showing the MGE and LGE. Red dotted box indicates the area shown in (C). (C) Immunofluorescence staining of β-galactosidase to reveal the initial population of cells responding to Hh signaling. Embryos were analyzed ~36 h after TM injection. White dotted lines indicate the edge of the ventral ganglionic eminence. (D) Coronal section of a 2-month-old brain section stained with X-gal to reveal the labeled cells. Cells expressing *Gli1* at ~E9.5 were permanently labeled with β-galactosidase from the *Rosa26* reporter. Labeled cells were found in somatosensory cortex (a, b), striatum (c), piriform cortex (Pir) (d) and septum. (E) Layer positioning of cortical interneurons correlates with the timing of exposure to Hh signaling. TM was given on E8.5, E9.5 and E11.5 to label progenitors responding to Hh signaling at ~E9.5, ~E10.5 and ~E12.5. The position of labeled cells in 2-month-old cortex was determined by X-gal staining, followed by nuclear fast red staining to reveal the different layer structure of the cortex (I to VI). Only labeled cells in the somatosensory and motor cortexes were counted. To facilitate calculation, the cortex was divided into 10 bins along the radial axis, with bin 1 closest to the white matter (WM) and bin 10 closest to the pial surface. Arrows indicate X-gal stained cells. (F) Layer distribution of labeled cells derived from progenitors responding to Shh signaling at different developmental stages. X-gal stained cells were placed into each bin and plotted in the graph. The approximate layer numbers are also indicated. The numbers are expressed as mean percentage ± S.E.M. Three coronal sections from each brain were examined, and three brains were used for calculation. Total numbers of label cell analyzed are: $n = 106$ (TM E8.5), $n = 215$ (TM E9.5) and $n = 324$ (TM E11.5). Scale bar: 0.1 mm (D).

Hh-responding progenitors produce progressively superficial cortical interneurons

To determine whether different cell types are generated from Hh-responding progenitors between E9.5 and E12.5, we fate mapped neurons produced from these *Gli1*-expressing progenitors using an inducible genetic approach (Joyner and Zervas, 2006), and examined cell fate 8 weeks after birth when telencephalic neurons have completed their migration and express mature cell type markers. This strategy involves using *Gli1-CreER*, in which an inducible *Cre* is inserted into the endogenous *Gli1* locus (Ahn and Joyner, 2004), and a *Rosa26* reporter (*R26R*) (Soriano, 1999). A single application of Tamoxifen (TM) allows for activation of Cre, recombination of the reporter allele and the labeling of distinct populations of *Gli1+* cells during a ~24 h time window beginning about 6 h after TM administration. Since dorsoventral patterning of the telencephalon has already been established by E12.5 (Balordi and Fishell, 2007; Machold et al., 2003; Xu et al., 2005), we sought to determine the influence of Hh signaling on telencephalic progenitors expressing *Gli1* prior to E12.5.

We first examined the expression of β-galactosidase ~36 h after TM delivery to determine the initial descendants of cells expressing *Gli1* (Figs. 2A, B). Very few β-galactosidase+ cells were found in the telencephalon when TM was given at 5 PM on E7.5 (data not shown). However, when TM was given on E8.5 or later, many β-galactosidase+ cells were detected in the ventricular layer of the dorsal MGE 36 h later (Fig. 2C). In particular, β-galactosidase+ cells labeled prior to E11.5 occupy a broader domain in the MGE than cells labeled on E11.5, as assessed by co-staining of Nkx2.1 (Supplemental Fig. 1), suggesting a mechanism that refines Hh-responding cells to the dorsal MGE during this time period. When analyzed at 2 months after birth, many β-galactosidase+ cells were found in the striatum, ventral striatum, ventral septum and the cortex (Fig. 2D). Because most of the cortical interneurons are known to derive from the ventral telencephalon, in particular from the MGE, we focused our analysis on these β-galactosidase+ cells in the somatosensory and motor cortexes of 8-week-old adult mouse brains.

To quantify the contribution of labeled cells to different cortical layers, we stained coronal sections of the brain with nuclear fast red to reveal the laminar structure, divided the radial axis of the cortex into ten bins along the pial surface and the white matter (WM), and assigned each labeled cell to one of these bins (Fig. 2E), as previously described (Hammond et al., 2006; Hevner et al., 2004; Valcanis and Tan, 2003). We found that the majority of labeled cells derived from ~E9.5 Hh-responding progenitors (TM injection at 5 PM of E8.5) were located close to the white matter (Fig. 2E), with about 88% of the progeny located within the inner half (bins 1–5, approximately layers V and VI) of the cortex (total labeled cells $n=106$, 3 adult brains) (Fig. 2F). In contrast, when progeny derived from ~E10.5 Hh-responding progenitors were analyzed, ~68% of the cells ($n=215$) occupied intermediate locations (bins 3–6, approximately layers V and IV) in the cortex. Lastly, we found ~72% of labeled cells ($n=324$) derived from ~E12.5 Hh-responding cells were located near the pial surface of the cortex (bins 6–9, or approximately layers IV, III and II). Our results thus show an 'inside-out' pattern of ventrally derived cortical interneurons derived from *Gli1* expressing progenitors.

To determine whether different subpopulations of cortical interneurons are generated at different stages, we examined the expression of three cortical interneuron markers, parvalbumin (PV), somatostatin (SST) and calretinin (CR) (Anderson et al., 2001; Butt et al., 2005; Valcanis and Tan, 2003; Wichterle et al., 2001; Xu et al., 2004) within the labeled cells (Supplementary Fig. 2A). We found similar numbers of PV+ neurons were generated from progenitors responding to Hh signaling from E9.5 to E11.5. In contrast, there was an increase in the number of SST+ and CR+ neurons generated from progenitors responding to Hh signaling from E9.5 to E11.5 (Supplementary Fig. 2B). Collectively, these analyses suggest that the temporal response of telencephalic progenitors to Shh, mediated primarily by Gli2, correlates with the generation of different waves of telencephalic neurons.

Gli activators are required for the specification of two progenitor groups in the telencephalic sulcus

Previous studies have shown that Gli activator is required for specification of different spinal cord progenitors but not for generation of different digits in the limb (Ahn and Joyner, 2004; Bai and Joyner, 2001; Ding et al., 1998; Matise et al., 1998). To determine whether Gli activator is required for specification of telencephalic progenitors, we analyzed the expression of critical transcription factors in E12.5 embryos lacking different Gli activators.

We first examined the expression of *Nkx2.1*, which normally marks the entire extent of the MGE, up to the point of the interganglionic sulcus (Figs. 3A, A'). In *Gli1* mutant embryos, the expression of *Nkx2.1* remained throughout the MGE, similar to wild-type (WT) embryos (Figs. 3B, B'). In contrast, in *Gli2* mutant embryos, although *Nkx2.1* was still expressed strongly in the MGE, the dorsal-most expression of *Nkx2.1* in the interganglionic sulcus was lost (Figs. 3C, C', arrow). Strikingly, the region where *Nkx2.1* expression was lost overlaps with the ventral expression of *Gli1* on the MGE side of the sulcus (Fig. 1B), suggesting that cells normally receiving the highest level of Shh signaling are either lost or transformed into a more dorsal identity. To further study the shift of the dorsal *Nkx2.1* border, we examined the expression of *Nkx6.2*, which encodes a homeobox transcription factor specifically expressed in the interganglionic sulcus between the MGE and LGE throughout prenatal stages and is dependent on Shh signaling (Stenman et al., 2003b; Xu et al., 2005). In *Gli1* mutants, like in WT embryos, *Nkx6.2* appeared to be expressed at the highest level in the sulcus, with a decreasing gradient towards the MGE and LGE (Figs. 3E, E', F, F'). However, in *Gli2* and *Gli1/2* double mutant embryos, the expression of *Nkx6.2* was lost in the sulcus (Figs. 3G, G', H, H'). The loss of *Nkx6.2* expression further confirms that when Shh signaling is disrupted in *Gli2* mutants, or in *Gli1/2* double mutants, specification of *Nkx6.2+* telencephalic progenitors is affected. The analyses of these *Gli* mutant embryos raise the possibility that Gli activator is required to specify two subgroups of telencephalic progenitors: *Nkx2.1+;Nkx6.2+;Gli1*^{high} progenitors located on the MGE side of the interganglionic sulcus and *Nkx2.1−;Nkx6.2+;Gli1*^{low} progenitors located on the LGE side of the sulcus (see summary in Fig. 8).

To determine whether the overall production of post-mitotic neurons is affected in the ventral telencephalon of different *Gli* mutants, we examined two additional neuronal markers at E12.5. *Lhx6* encodes a LIM homeodomain transcription factor that is expressed in all cells derived from the MGE (Lavdas et al., 1999). In *Gli1*, *Gli2* or *Gli1/2* double mutants, the expression of *Lhx6* appeared unchanged (Figs. 3I–L). We next examined the expression of *GAD67*, which encodes a rate-limiting enzyme in the synthesis of GABA, and is expressed in all ventral telencephalic-derived inhibitory GABAergic neurons. In *Gli1*, *Gli2* or *Gli1/2* mutants, *GAD67* was detected throughout the mantle area surrounding the MGE and the LGE, similar to that in WT embryos (Figs. 3M–P). The similar expression of these markers in *Gli* mutant and WT embryos shows that the loss of two subgroups of telencephalic progenitors does not dramatically alter the overall production of interneurons at E12.5.

By E14.5, we found that one out of four *Gli2* mutant brains had enlarged ventricles (Supplementary Fig. 3). Even in those *Gli2* mutant embryos with normal morphology, the reduction in the dorsal *Nkx2.1* domain was apparent (Figs. 3Q, Q'). Furthermore, the leading population of *Lhx6+* neurons migrating towards the cortex was reduced (Figs. 3R, R'), consistent with a requirement of *Nkx2.1* for the expression of *Lhx6* (Du et al., 2008). On the other hand, *Ebf1+*

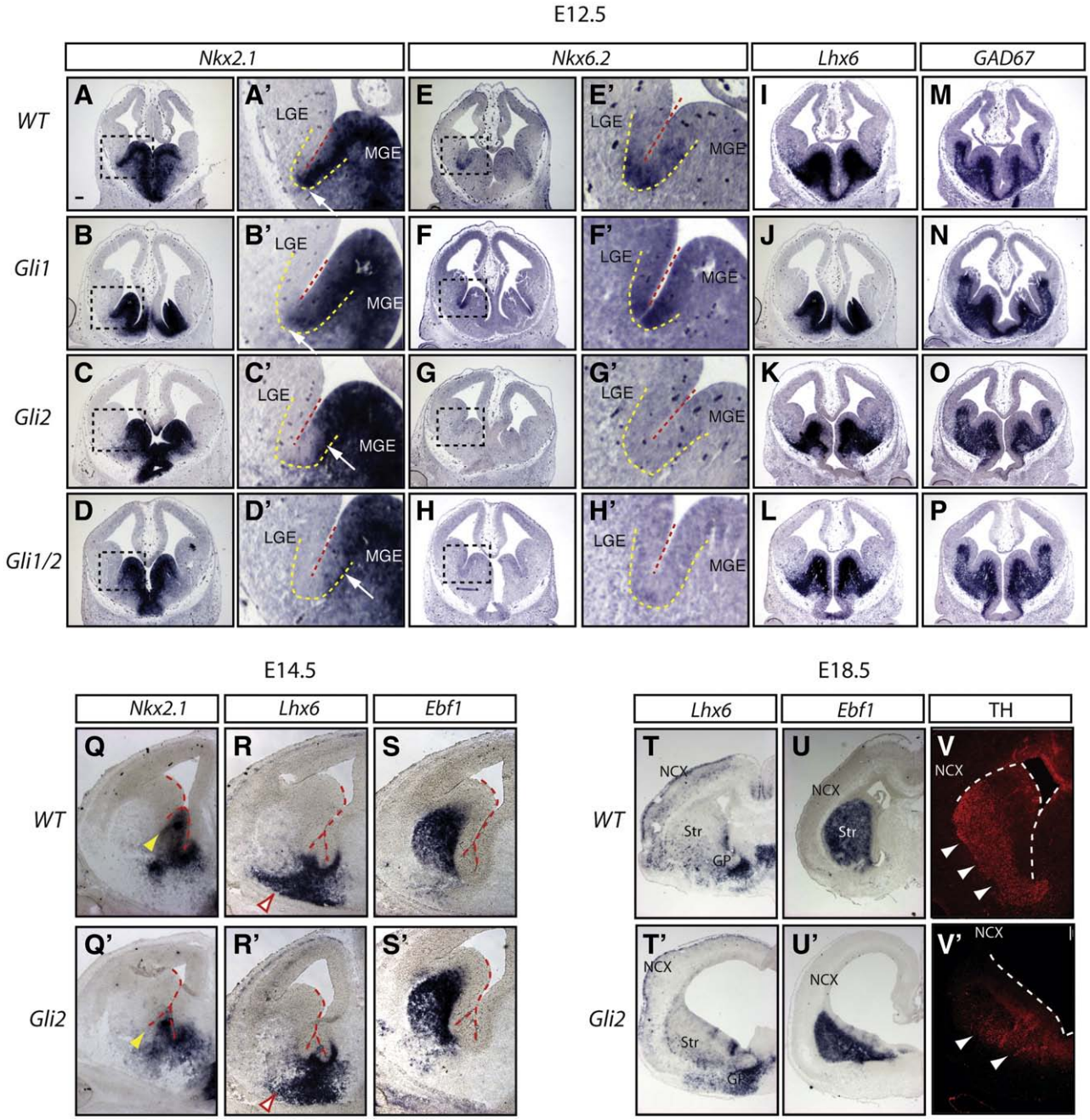

Fig. 3. Gli activator is required for the development of subgroups of ventral telencephalic progenitors. (A–D') The expression of *Nkx2.1* was affected in *Gli2* and *Gli1/2* double mutants. In WT and *Gli1* mutant, *Nkx2.1* was expressed in the interganglionic region (A, A', B, B'). However, in *Gli2* or *Gli1/2* mutants, the dorsal expression of *Nkx2.1* was lost (C, C', D, D', indicated by white arrows). (E–H') Loss of *Nkx6.2* expression in *Gli2* and *Gli1/2* mutants. *Nkx6.2* was expressed throughout the interganglionic region in WT and *Gli1* mutants (E, E', F, F'). However, the expression of *Nkx6.2* was lost in *Gli2* and *Gli1/2* mutants (G, G', H, H'). The overall production of *Lhx6*+ MGE neurons (I–L) and *GAD67*+ GABAergic neurons (M–P) did not appear to be dramatically altered. By E14.5, the reduction in the dorsal-most *Nkx2.1* domain (Q, Q', yellow arrowheads) persisted in *Gli2* mutants. Furthermore, the leading edge of migrating *Lhx6*+ neurons was reduced (R, R', red arrowheads). The expression of *Ebf1*, which labels LGE-derived neurons, did not appear to be altered in *Gli2* mutants (S, S'). By E18.5, all *Gli2* mutants (4/4) showed enlarged telencephalic ventricles, reduced numbers of *Lhx6*+ cortical interneurons (T, T') and tyrosine hydroxylase (TH) fibers (V, V', white arrowheads). The *Ebf1*+ striatum (Str) was largely normal (U, U'). NCX, neocortex; GP, globus pallidus. Scale bar: 75 μm (A–P).

population did not appear to be affected (Figs. 3S, S'). By E18.5, however, all four *Gli2* mutant brains were found to have enlarged ventricles, as previously noted (Palma and Ruiz i Altaba, 2004), and reduced ventral telencephalon (Supplementary Fig. 3). Other defects were also noted, including a reduction in *Lhx6*+ interneurons in the cortex (Figs. 3T, T') and reduced innervation of tyrosine hydroxylase fibers in the mutant ventral telencephalon (Figs. 3V, V'). The *Ebf1*+ striatum, however, does not appear to be significantly reduced (Figs. 3U, U').

Removal of all Gli genes disrupts production and proliferation of ventral telencephalic neurons

The three *Gli* genes are expressed in an overlapping manner in many embryonic tissues. Recent studies have shown that in addition to Gli1 and Gli2, Gli3 can also function as an activator in vivo to mediate Hh signaling (Bai et al., 2004; Wang et al., 2007). To determine whether Gli3 may play a role in ventral telencephalon development, we examined the expression of *Gli3* by RNA in situ

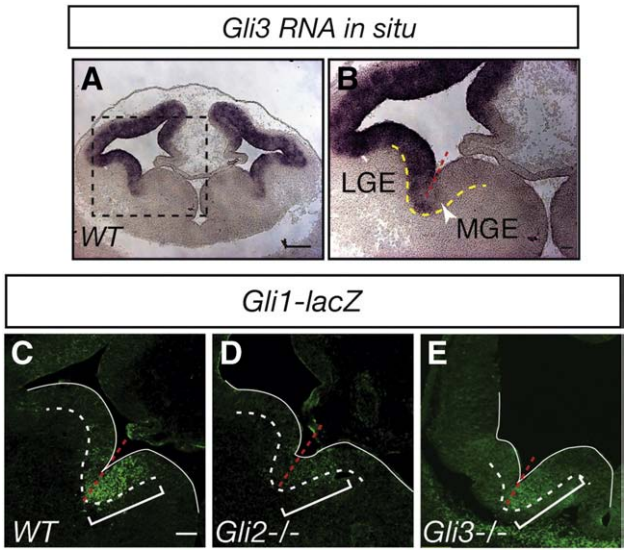

Fig. 4. *Gli2* and *Gli3* have overlapping function in regulating *Gli1* expression in the ventral telencephalon. (A, B) RNA in situ hybridization showing that *Gli3* is expressed in the interganglionic sulcus of E12.5 telencephalon. White arrowhead indicates the approximate area of *Gli1* expression. (C–E) Immunofluorescence staining of *Gli1-lacZ* in the telencephalon of E12.5 WT, *Gli2* and *Gli3* embryos. *Gli1* expression was dramatically reduced in *Gli2* mutants (D). In *Gli3* mutants, *Gli1* expression was reduced but remained restricted in the interganglionic sulcus (E). White lines indicate ganglionic eminences. White bracket lines indicate the expression of *Gli1-lacZ*. Scale bar: 0.1 mm.

hybridization. As previously reported (Tole et al., 2000), *Gli3* is strongly expressed in the dorsal telencephalon. In addition, *Gli3* was also detected in the interganglionic sulcus, with a ventral expression limit similar to that of *Gli2* (Figs. 4A, B), suggesting that Gli2 and Gli3 may promote *Gli1* expression in the ventral telencephalon. Indeed, we found a weakened *Gli1-lacZ* expression in the interganglionic sulcus of *Gli2* mutants (Figs. 4C, D). Furthermore, we found a slight reduction in *Gli1-lacZ* expression in the absence of *Gli3* (Fig. 4E), and a complete absence of *Gli1-lacZ* expression in *Gli2/3* double mutants (see Fig. 2 in Bai et al., 2004).

To determine whether ventral telencephalon patterning is normal in the absence of all *Gli* genes, we examined the expression of progenitor and neuronal markers in E12.5 *Gli2/3* mutant embryos, which lack the expression of all three *Gli* genes (hereafter referred to as pan-*Gli* mutants). We found that *Nkx2.1* expression was largely restricted in the MGE, although the dorsal expression was reduced and somewhat diffuse in the interganglionic sulcus (Figs. 5A, F, insert). The restricted expression of *Nkx2.1* suggests that specification of MGE progenitors is not greatly altered in *Gli2/3* mutants. Next we examined the expression of *Lhx6*, which is expressed in MGE-derived post-mitotic neurons, and found a severe reduction in *Gli2/3* mutants (Figs. 5B, G). To determine whether the production of LGE-derived post-mitotic neurons is affected, we examined the expression of *Ebf1* and also found a reduction in the number of *Ebf1*+ neurons (Figs. 5C, H). A similar reduction in *GAD67*, which is expressed in both MGE and LGE post-mitotic neurons, was also found in *Gli2/3* mutant embryos (Figs. 5D, I).

In addition to the reduced expression of markers for ventral telencephalic neurons, we also found a reduction in the expression of neuronal marker TuJ1 (Figs. 6A, E). To address whether disrupted neurogenesis is caused by defects in proliferation of MGE and/or LGE progenitors, we examined BrdU incorporation and Ki67 expression in telencephalic progenitors. BrdU was given to pregnant mice 1 h prior to dissection at E12.5 to label S-phase cells and Ki67 staining was used to reveal all proliferating cells (Figs. 6B, C, F, G). We found that the fractions of S-phase progenitors in MGE and LGE of *Gli2/3* double mutants were significantly lower ($p<0.01$, Student's *t*-test) (Fig. 6I). Moreover, the percentages of proliferating Ki67+ MGE and LGE progenitors were also significantly reduced in *Gli2/3* double mutants ($p<0.01$) (Fig. 6K). However, within the cycling population, there was a significant increase in the fraction of S-phase cells (BrdU+/KI67+) in both MGE and LGE progenitors (Fig. 6J), suggesting a faster cell cycle time. Indeed, the fraction of M phase cells, indicated by phospho-Histone H3 staining, was also increased (LGE: WT 4.01 ± 0.13, mutant 5.66 ± 0.41; MGE: WT 4.3 ± 0.3, mutant 5.11 ± 0.36). In addition to changes in cell proliferation, we also found a significant increase in the number of Caspase-3+ cells in both MGE and LGE progenitors in *Gli2/3* mutants ($p<0.01$) (Figs. 6D, H, L). Together, these results suggest that loss of *Gli* genes significantly affects the proliferation and survival of MGE and LGE progenitors.

The reduced number of post-mitotic neurons in the ventral telencephalon of *Gli2/3* mutants could also be caused either by defects in the differentiation of neuronal progenitors or by a delay in the generation of neurons. Because most *Gli2/3* double mutants die by E13, we cannot assess whether the number of post-mitotic neurons is restored at later developmental stages. However, if there is a developmental delay in *Gli2/3* double mutant embryos, then we would expect an absence of *PDGFRα*+ oligodendrocyte precursors from the ventral telencephalon, because these cells are produced in WT embryos around E12.5 (Nery et al., 2001; Olivier et al., 2001;

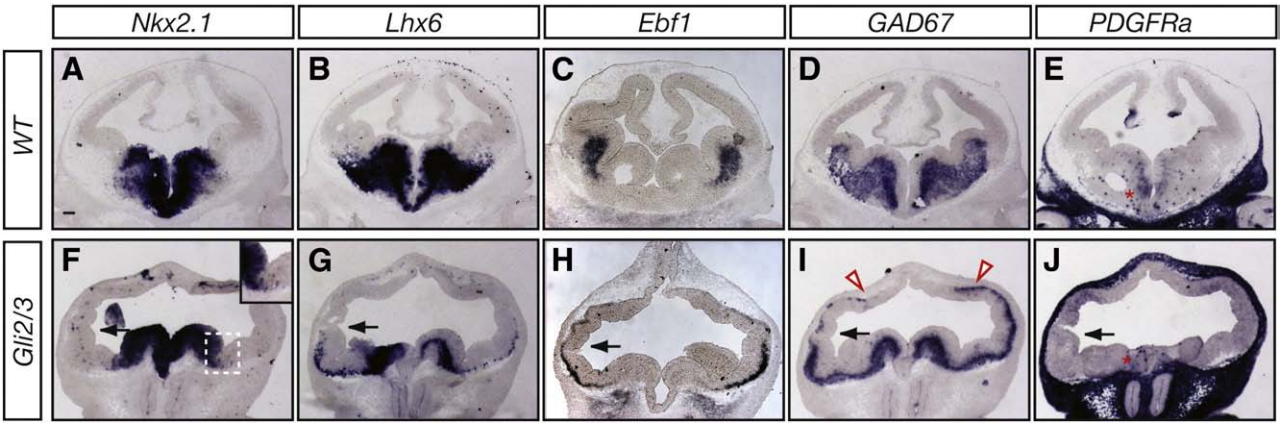

Fig. 5. Generation of ventral telencephalic neurons is affected in *Gli2/3* double mutants. (A, F) *Nkx2.1* expression, which is normal detected in MGE, did not appear to be significantly altered in *Gli2/3* mutant embryos. Note: the dorsal limit of *Nkx2.1* expression was reduced in *Gli2/3* double mutants (insert). (B, G) Expression of *Lhx6*, which marks MGE-derived neurons, was reduced in *Gli2/3* mutants. (C, H) Expression of *Ebf1*, which marks LGE-derived neurons, was also reduced in *Gli2/3* mutants. (D, I) *GAD67*, which is expressed in both the MGE and LGE-derived neurons, was found to expand into the dorsal telencephalon, as indicated by red arrowheads. (E, J) Oligodendrocyte precursors were generated in *Gli2/3* double mutants, although in a smaller number in the mutant telencephalon. Red asterisks indicate the *PDGFRα*+ cells. Note: *PDGFRα* is also expressed strongly outside of the neural tube. (F, G, I, J) were adjacent sections of the same embryo. Black arrows indicate the dorsoventral limit, as determined by Pax6 expression on an adjacent section. Scale bar: 90 μm.

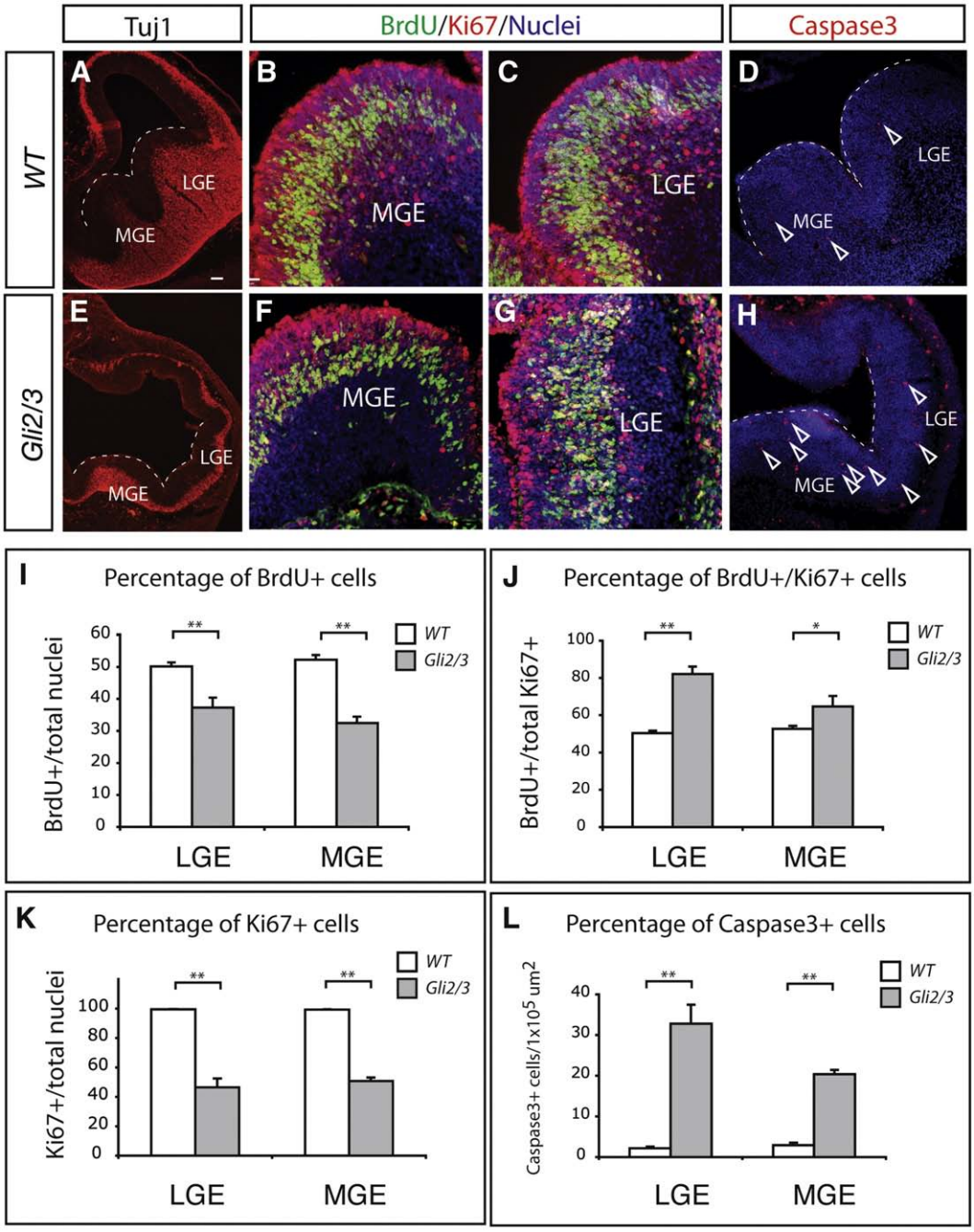

Fig. 6. The production of post-mitotic neurons is affected in *Gli2/3* double mutant telencephalon. (A, E) Tuj1 staining of coronal sections of WT and *Gli2/3* telencephalon. (B, C, F, G) Triple immunofluorescence staining of BrdU (green), Ki67 (red) and nuclei (blue) in the MGE and LGE region of WT and *Gli2/3* mutant embryos. (D, H) Immunostaining of cleaved Caspase-3 in WT and mutant embryos. There was a significant reduction in the numbers of BrdU+ cells (I) and Ki67+ cell (K) in *Gli2/3* mutants. However, the fraction of BrdU+ cells within proliferating Ki67+ cells was significantly increased in *Gli2/3* mutants (J). There was also a significant increase in the number of Caspase-3+ cells in *Gli2/3* mutants. $^{**}p<0.01$; $^{*}p<0.05$. Scale bar: 0.1 mm (A, E); 40 μm (B, C, F, G); 20 μm (D, H).

Tekki-Kessaris et al., 2001). In *Gli2/3* double mutants, as in WT embryos (Fig. 5E), *PDGFRα+* cells were detected in the telencephalic region and outside of the neural tube (Fig. 5J), although the number of positive cells was reduced compared with WT embryos. This result argues against a developmental delay in embryos that cannot respond to Shh, but instead suggests that *Gli* genes control the fate and differentiation of post-mitotic neurons in the ventral telencephalon.

Intermingling of different neuronal groups in Gli2/3 mutants

In addition to a reduced number of post-mitotic neurons, we noticed that some *Lhx6+* neurons occupied a more dorsal position in *Gli2/3* mutants (Fig. 5G). Similarly, some *GAD67+* ventral neurons were also found to occupy ectopic positions in the mutant dorsal telencephalon (Fig. 5I, red arrowheads). The ectopic expression of these markers suggests a disruption in the arrangement of distinct neuronal domains in the mutant telencephalon. Alternatively, the phenotype could be caused by a diencephalic displacement of dorsal telencephalic tissues, resulting in a joining of diencephalic and dorsal telencephalic tissues as has been demonstrated in *Gli3* mutants (Fotaki et al., 2006). To distinguish these two possibilities, we first performed RNA in situ hybridization of *FoxG1*, a forkhead box containing transcription factor that is normally expressed specifically in telencephalic, but not diencephalic tissues (Tao and Lai, 1992). In

WT, *Gli1*, *Gli2*, *Gli1/2* mutants, *FoxG1* is expressed throughout the telencephalon except in the dorsal cortical hem/choroid plexus anlage region (Figs. 7A–C, indicated by red arrowheads). As expected, *FoxG1* was not detected in most of the dorsal telencephalic region of *Gli3* mutants (Fig. 7D), since this region has been transformed into diencephalic fate (Fotaki et al., 2006). On the other hand, we found that in *Gli2/3* double mutants, *FoxG1* was expressed throughout the telencephalon except in the cortical hem/choroid plexus anlage region (Fig. 7E). Thus, unlike *Gli3*, the dorsal telencephalic region of *Gli2/3* mutants retains telencephalic characteristics.

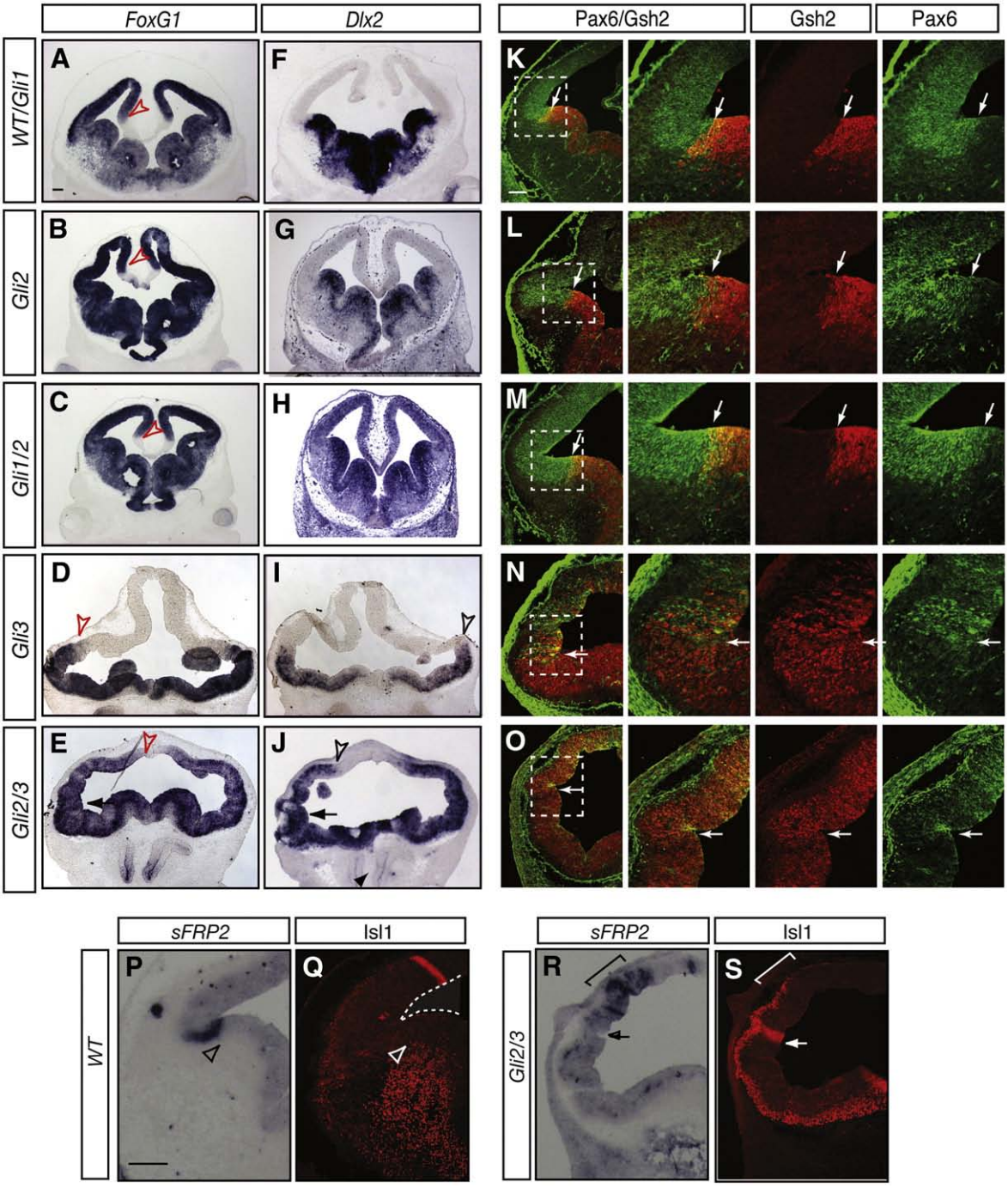

Fig. 7. Dorsoventral patterning in different *Gli* mutants. (A–E) RNA in situ hybridization of telencephalic marker *FoxG1*. *FoxG1* is normally expressed in telencephalic but not in diencephalic tissues (A). Red arrowheads indicate the expression limit of *FoxG1*. In *Gli2* (B), *Gli1/2* (C) and *Gli2/3* double mutants (E), *FoxG1* was detected throughout the telencephalon, suggesting that the dorsal tissues in these mutants are of telencephalic origin. On the other hand, in *Gli3* mutants, *FoxG1* was not detected in the dorsal region (D). (F–J) The expanded expression of *Dlx2* suggests a dorsal expansion of ventral markers in *Gli2/3* double mutants. Arrowheads indicate the dorsal expression limit of *Dlx2*. (K–O) Immunofluorescence staining of Pax6 and Gsh2 in different *Gli* mutant telencephalons, with higher magnification images shown to the right. Normally Pax6 is expressed in the dorsal telencephalon (K, green channel) and Gsh2 is expressed in the ventral telencephalon (K, red channel). White arrows indicate the ventral limit of Pax6 expression. In *Gli2* and *Gli1/2* double mutants, the expression of Pax6 and Gsh2 was similar to that in WT embryos. In *Gli3* mutants, Gsh2 was found to expand dorsally (N), reflecting a partial diencephalic transformation. In *Gli2/3* mutants, Pax6 remained expressed in the dorsal telencephalon (O, green channel). However, Gsh2 expression expanded into the dorsal telencephalon (O, red channel). (P, R) Expansion of *sFRP2* in WT and *Gli2/3* mutants. *sFRP2* is normally expressed in a small stripe in the corticostriatal area, as indicated by an arrowhead in (P). However, the domain of *sFRP2* was expanded in the *Gli2/3* mutants (indicated by a bracket in R). (Q, S) Expansion of Isl1+ neurons into the dorsal telencephalon. Isl1+ neurons are normally located ventrally to the *sFRP2* domain (white arrowhead in Q). In *Gli2/3* mutants, Isl1+ neurons were found to expand into the dorsal telencephalon to overlap with the *sFRP2* domain (S, white bracket indicates the *sFRP2* domain. R and S are adjacent sections). Scale bar: 0.1 mm.

The detection of ventral neurons in the *Gli2/3* dorsal telencephalon could be due to defective migratory pattern of the post-mitotic neurons or expansion of ventral progenitors to the dorsal domain. We therefore examined the expression of *Dlx2*, which labels all ventral telencephalic progenitors (Anderson et al., 1997). We found a normal expression of *Dlx2* in *Gli1*, *Gli2* and *Gli1/2* double mutants (Figs. 7F–H). In *Gli3* mutants, *Dlx2* expression was restricted in the tissues of telencephalic origin (Fig. 7I). Interestingly, in *Gli2/3* double mutants, the expression of *Dlx2* was detected in both ventral and dorsal regions of the telencephalon, suggesting that ventral progenitors expanded into the dorsal telencephalon (Fig. 7J).

To further determine whether different dorsal and ventral progenitors intermingle as a result of expansion of ventral progenitors, we performed double immunofluorescence staining of Pax6 and Gsh1/2. Pax6 and Gsh2 are normally expressed in a complementary pattern from E10.5 to E12.5, with Pax6 expressed in the dorsal telencephalic progenitors and Gsh2 expressed in the ventral telencephalic progenitors (Corbin et al., 2003). In WT embryos at E12.5, Pax6 and Gsh2 are co-expressed only in a few rows of cells in the corticostriatal region (Fig. 7K). The mutually exclusive expression pattern was maintained in *Gli1*, *Gli2* and *Gli1/2* double mutants (Figs. 7K, L, M). In *Gli3* mutants, Gsh2 was detected in the dorsal region, likely because of the diencephalic transformation (Fig. 7N). Interestingly, in *Gli2/3* double mutant embryos, the expression pattern of Pax6 was largely unperturbed (Fig. 7O, green channel). However, Gsh2 expression was expanded into the dorsal telencephalon and thus overlapped broadly with Pax6 expression (Fig. 7O, red channel).

To confirm that the expression domains of different markers intermingle as a result of loss of *Gli* function, we examined the expression of two additional makers, *sFRP2* and Isl1. *sFRP2* encodes an inhibitor of Wnt signaling and is expressed in the ventral-most region of the dorsal (pallial) telencephalon (Fig. 7P) (Stenman et al., 2003b) and Isl1 is expressed in neurons ventral to the *sFRP2* domain in WT embryos (Fig. 7Q). In *Gli2/3* double mutants, the expression domain of *sFRP2* was greatly expanded (Fig. 7R, indicated by a bracket). Moreover, Isl1+ neurons clearly expanded into the dorsal region, resulting in a partial overlap with *sFRP2* in *Gli2/3* double mutants (Fig. 7S). Together, these results suggest that both neural progenitors and neurons have lost their distinct spatial organization in the absence of *Gli* function.

Discussion

Previous studies have shown that *Gli1* and *Gli2* are expressed in the interganglionic sulcus, suggesting that this region receives a high level of Hh signaling (Tole et al., 2000; Wonders et al., 2008). We analyzed telencephalic patterning in embryos lacking major Gli activators in *Gli2* and *Gli1/2* double mutants, and found that the expression of three critical transcription factors, *Nkx2.1*, *Nkx6.2* and *Gli1*, was significantly reduced in progenitors receiving high levels of Shh signaling. Furthermore, we found that from E9.5 to E12.5, Shh-responding progenitors give rise to neurons occupying successively more superficial layers in the cortex. Finally, when all Gli transcription factors are removed, specification and production of ventral interneurons are severely perturbed. Together, our results uncover multiple roles for Gli proteins in specification, differentiation and survival of telencephalic progenitors.

Transcriptional activation mediated by Gli proteins is required to induce distinct progenitor populations in the ventral telencephalon

Shh signaling has been shown to specify distinct cell fates in a concentration-dependent manner in the developing spinal cord, with a higher concentration of Shh inducing floor plate cells and a progressively lower concentration of Shh inducing V3 interneurons, motor neurons and V2–V0 interneurons (reviewed in Jessell, 2000). The ability of Shh signaling to induce different cell types is dependent on the Gli family of transcription factors. In particular, it has been shown that a gradient of Gli activator is able to mimic Shh signaling to induce different cell types in the developing chick spinal cord (Stamataki et al., 2005).

It is known that different subtypes of interneuron are generated from different regions of the ventral telencephalon at different developmental stages in a predictable spatial–temporal sequence (Butt et al., 2005; Wichterle et al., 2001; Xu et al., 2004). The LGE contains at least two progenitor populations, with one population expressing *Dlx2* and *Er81* that generates olfactory bulb interneurons, and a second population expressing *Dlx2* and *Isl1* that contributes to striatal neurons (Stenman et al., 2003a). Furthermore, the dorsal-most LGE cells, located next to the Pax6 expressing pallium, have the distinct ability to contribute to the lateral cortical stream and migrate to the developing ventral telencephalon (Carney et al., 2006). A recent study has proposed to further divide the LGE into four progenitor groups (pLGE1–4) and the MGE into five progenitor groups (pMGE1–5), based on a combinatorial expression pattern of transcription factors in the ventral telencephalon (Flames et al., 2007).

Shh signaling has been postulated to inhibit the repressor function of Gli3 in the telencephalon (Rallu et al., 2002). Our analysis further suggests that Gli activator mediate Shh signaling to establish distinct progenitor populations in the ventral telencephalon. Based on the unique responses to Shh signaling, progenitors in the interganglionic sulcus can be divided into two subpopulations (Fig. 8), one located on the MGE side and express $Gli1^{high}$, *Nkx6.2* and *Nkx2.1* (group 1 in Fig. 8), and the second group located on the LGE side and express $Gli1^{low}$ and *Nkx6.2*, but not *Nkx2.1* (group 2). The two Shh responsive progenitor groups identified in our study appear to correspond to pLGE4 and pMGE1. In *Gli2* mutants, specification of both progenitor groups is disrupted. More dorsally, specification of two other progenitors (groups 3 and 4) does not appear to be affected in the absence of *Gli2* or *Gli1/2*. It is likely that a low level of Shh signaling, which is sufficient to attenuate the level of Gli3 repressor, is adequate for the specification of these two progenitor groups. Therefore, Shh signaling in the ventral telencephalon may have two different functions: to repress the function of Gli3 and at the same time, activate downstream transcriptional targets through Gli activators (Fig. 8), by a mechanism similar to that in the spinal cord (Bai et al., 2004; Jacob and Briscoe, 2003; Lei et al., 2004; Stamataki et al., 2005).

Consistent with the previous observation that dorsal MGE progenitors (pMGE1) have a bias for SST+ neurons (Flames et al., 2007; Fogarty et al., 2007; Wonders et al., 2008), we found that ~20% of progeny generated from E8.5 *Gli1*-expressing progenitors were SST+ neurons, and the number increased to ~25% at E11.5, suggesting that Hh signaling may have a role in the specification of this group of neurons. In addition, examination of β-galactosidase+ cells 36 h following TM injection revealed that cells labeled at E9.5 and E10.5 spread over a broader region in the MGE, compared with cells labeled at E11.5 that are limited to the interganglionic sulcus. Together, these results raise the possibility that Hh signaling may involve in specification and consolidation of SST+ progenitors in the dorsal MGE.

Proliferation and organization of ventral telencephalon require Gli-mediated Hh signaling

Several defects in ventral telencephalon development were observed in *Gli2/3* double mutant embryos, including dorsal expansion of ventral telencephalic markers, the intermingling of different progenitor domains and the reduced differentiation of ventral neurons. These phenotypes have never been observed in *Gli2* mutants (data not shown) and are distinct from the phenotypes observed in *Gli3* mutants.

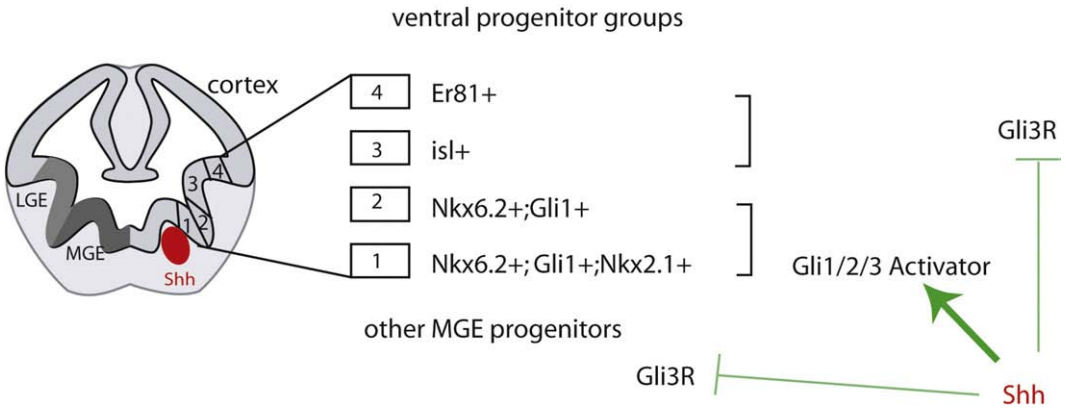

Fig. 8. Schematic of Shh/Gli signaling in the ventral telencephalon. Based on the expression of different transcription factors, ventral telencephalic progenitors responding to Shh signaling can be divided into four groups (1–4). The ventral-most progenitors (groups 1) express *Gli1*, *Nkx6.2* and *Nkx2.1*, and are located in the MGE side of the interganglionic sulcus. Group 2 progenitors express *Gli1*, *Nkx6.2* but not *Nkx2.1*, and are located on the LGE side of the interganglionic sulcus. These two groups of progenitors are not specified in the absence of Gli activator. More dorsally, another two groups of progenitors have been defined previously. One group produces *Er81+* neurons and the other group generates *isl1+* neurons. These four groups of progenitors are absent when Hh signaling is conditionally disrupted at E8.5, but are not affected in the absence of Gli activator. It is likely that groups 3 and 4 of progenitors require only a low level of Shh signaling to inhibit the formation of excess Gli3 repressor. Shh is also likely required to attenuate Gli3 repressor in most of the MGE cells, since conditional loss of Hh responsiveness eliminates all ventral telencephalic cells. See text for details. LGE, lateral ganglionic eminence. MGE, medial ganglionic eminence.

In *Gli3* mutant, *Gli1*, *Ptc1* and *Shh* were detected in the mutant ventral telencephalon, suggesting a normal ventral patterning (Theil et al., 1999; Tole et al., 2000). On the other hand, in the dorsal forebrain, there is a diencephalic displacement of telencephalic tissues, resulting in a joining of diencephalic and telencephalic tissues and the expression of several markers normally expressed in both ventral telencephalon and diencephalon (Fotaki et al., 2006; Kuschel et al., 2003; Rallu et al., 2002; Tole et al., 2000). As the loss of Gli3 repressor has been shown to increase the range of Shh signaling in the limb and to cause expansion of intermediate cell types in the ventral spinal cord (Persson et al., 2002), possibly through de-repression of Gli activator, an increase in Shh signaling from the zona limitans intrathalamica (Zli) is a likely reason for the diencephalic displacement of dorsal telencephalic tissues in *Gli3* mutants. Consistent with this, there appears to be an enlargement of the diencephalon based on a caudal shift of *Wnt1* expression in *Gli3* mutants (Theil et al., 1999).

The phenotypes of pan-*Gli* mutants are different from the phenotypes of *Gli3* in at least three aspects. First, *FoxG1* is expressed throughout pan-*Gli* mutant telencephalon, suggesting that the telencephalic characteristic is maintained in these embryos. Second, the expression of *Gli1* and *Nkx6.2* was not detected in the ventral telencephalon of pan-*Gli* mutants (Bai et al., 2004 and data not shown). The loss of these two markers thus suggests an additional loss of ventral telencephalic progenitors in pan-*Gli* mutant embryos. Third, different ventral telencephalic cell types intermingle in *Gli2/3* mutants, suggesting that positional information is lost. A similar phenotype has also been observed in the pan-*Gli* mutant spinal cord (Bai et al., 2004; Lei et al., 2004) or in the *Smo;Gli3* double mutant embryos (Wijgerde et al., 2002). Our analysis therefore provides further support that *Gli* genes are required for the establishment of separate neuronal domains throughout the ventral neural tube.

Finally we found that the production of post-mitotic neurons in the ventral telencephalon is severely reduced when all *Gli* function is removed. This phenotype is more severe than the phenotype of any single *Gli* mutant or *Gli1/2* double mutants. Most likely this phenotype is caused by an overlapping as well as unique functions between different Gli proteins. Indeed, it appears that different tissues have different requirements for the activator or repressor function. For example, only Gli repressor is needed for digit pattering in the limb (Ahn and Joyner, 2004; Litingtung et al., 2002; te Welscher et al., 2002) while Gli activator is required for cell fate specification in the spinal cord (Bai et al., 2004; Persson et al., 2002; Stamataki et al., 2005). Based on the analysis of the telencephalic phenotypes of different *Gli* mutants, we propose that only Gli3 repressor is required for the suppression of ventral telencephalic and/or diencephalic markers in the dorsal telencephalon, while the three Gli proteins are involved in controlling the specification of distinct progenitors, the separation of different progenitor domains and the differentiation of telencephalic neurons.

The fact that some aspects of dorsoventral patterning remain intact in embryos devoid of all *Gli* function also raises the question of what other signaling pathways, in addition to *Shh*, specify the remaining cell types. Our analysis of spinal cord development suggests that both Wnt and Shh signaling pathways are involved in controlling cell type generation (Yu et al., 2008), although one recent report excludes Wnt signaling in cell type specification in the ventral telencephalon (Gulacsi and Anderson, 2008). Another likely signal is FGF signaling. It is known that several of the FGF ligands and FGF receptors are expressed in the developing telencephalon (reviewed in Hebert, 2005). In particular, in *Fgfr1;Fgfr2* double mutant embryos, many ventral progenitor cells are not specified (Gutin et al., 2006). The loss of ventral progenitor phenotype is similar to that of conditional loss of *Smo* in the telencephalon (Fuccillo et al., 2004), raising the possibility that FGF signaling functions in a linear pathway with Shh signaling (Gutin et al., 2006). However, the phenotype of *Fgfr1;Fgfr2* double mutants is clearly more severe than that of the pan-*Gli* mutant embryos, because dorsal markers are not expanded into the ventral domains in pan-*Gli* mutants. The differences in phenotypes would therefore suggest that FGF signaling functions parallels that of Shh signaling, for example in lineage commitment, as has been recently shown in the ES cells (Kunath et al., 2007), although it is also possible that the *Gli* genes may have function independent of Shh signaling. Future challenges will be to delineate the relationship between Shh and FGF signaling in patterning and generation of telencephalic neurons.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ydbio.2009.07.026.

References

Ahn, S., Joyner, A.L., 2004. Dynamic changes in the response of cells to positive hedgehog signaling during mouse limb patterning. Cell 118, 505–516.

Anderson, S.A., Eisenstat, D.D., Shi, L., Rubenstein, J.L., 1997. Interneuron migration from basal forebrain to neocortex: dependence on Dlx genes. Science 278, 474–476.

Anderson, S.A., Marin, O., Horn, C., Jennings, K., Rubenstein, J.L., 2001. Distinct cortical migrations from the medial and lateral ganglionic eminences. Development 128, 353–363.

Aoto, K., Nishimura, T., Eto, K., Motoyama, J., 2002. Mouse GLI3 regulates Fgf8 expression and apoptosis in the developing neural tube, face, and limb bud. Dev. Biol. 251, 320–332.

Bai, C.B., Joyner, A.L., 2001. Gli1 can rescue the in vivo function of Gli2. Development 128, 5161–5172.

Bai, C.B., Auerbach, W., Lee, J.S., Stephen, D., Joyner, A.L., 2002. Gli2, but not Gli1, is required for initial Shh signaling and ectopic activation of the Shh pathway. Development 129, 4753–4761.

Bai, C.B., Stephen, D., Joyner, A.L., 2004. All mouse ventral spinal cord patterning by hedgehog is Gli dependent and involves an activator function of Gli3. Dev. Cell 6, 103–115.

Balordi, F., Fishell, G., 2007. Hedgehog signaling in the subventricular zone is required for both the maintenance of stem cells and the migration of newborn neurons. J. Neurosci. 27, 5936–5947.

Butt, S.J., Fuccillo, M., Nery, S., Noctor, S., Kriegstein, A., Corbin, J.G., Fishell, G., 2005. The temporal and spatial origins of cortical interneurons predict their physiological subtype. Neuron 48, 591–604.

Butt, S.J., Sousa, V.H., Fuccillo, M.V., Hjerling-Leffler, J., Miyoshi, G., Kimura, S., Fishell, G., 2008. The requirement of Nkx2-1 in the temporal specification of cortical interneuron subtypes. Neuron 59, 722–732.

Carney, R.S., Alfonso, T.B., Cohen, D., Dai, H., Nery, S., Stoica, B., Slotkin, J., Bregman, B.S., Fishell, G., Corbin, J.G., 2006. Cell migration along the lateral cortical stream to the developing basal telencephalic limbic system. J. Neurosci. 26, 11562–11574.

Chiang, C., Litingtung, Y., Lee, E., Young, K.E., Corden, J.L., Westphal, H., Beachy, P.A., 1996. Cyclopia and defective axial patterning in mice lacking Sonic hedgehog gene function. Nature 383, 407–413.

Corbin, J.G., Nery, S., Fishell, G., 2001. Telencephalic cells take a tangent: non-radial migration in the mammalian forebrain. Nat. Neurosci. 4 Suppl., 1177–1182.

Corbin, J.G., Rutlin, M., Gaiano, N., Fishell, G., 2003. Combinatorial function of the homeodomain proteins Nkx2.1 and Gsh2 in ventral telencephalic patterning. Development 130, 4895–4906.

Dai, P., Akimaru, H., Tanaka, Y., Maekawa, T., Nakafuku, M., Ishii, S., 1999. Sonic Hedgehog-induced activation of the Gli1 promoter is mediated by GLI3. J. Biol. Chem. 274, 8143–8152.

Dessaud, E., McMahon, A.P., Briscoe, J., 2008. Pattern formation in the vertebrate neural tube: a sonic hedgehog morphogen-regulated transcriptional network. Development 135, 2489–2503.

Ding, Q., Motoyama, J., Gasca, S., Mo, R., Sasaki, H., Rossant, J., Hui, C.C., 1998. Diminished Sonic hedgehog signaling and lack of floor plate differentiation in Gli2 mutant mice. Development 125, 2533–2543.

Du, T., Xu, Q., Ocbina, P.J., Anderson, S.A., 2008. NKX2.1 specifies cortical interneuron fate by activating Lhx6. Development 135, 1559–1567.

Echelard, Y., Epstein, D.J., St-Jacques, B., Shen, L., Mohler, J., McMahon, J.A., McMahon, A.P., 1993. Sonic hedgehog, a member of a family of putative signaling molecules, is implicated in the regulation of CNS polarity. Cell 75, 1417–1430.

Ericson, J., Muhr, J., Placzek, M., Lints, T., Jessell, T.M., Edlund, T., 1995. Sonic hedgehog induces the differentiation of ventral forebrain neurons: a common signal for ventral patterning within the neural tube. Cell 81, 747–756.

Flames, N., Pla, R., Gelman, D.M., Rubenstein, J.L., Puelles, L., Marin, O., 2007. Delineation of multiple subpallial progenitor domains by the combinatorial expression of transcriptional codes. J. Neurosci. 27, 9682–9695.

Fogarty, M., Grist, M., Gelman, D., Marin, O., Pachnis, V., Kessaris, N., 2007. Spatial genetic patterning of the embryonic neuroepithelium generates GABAergic interneuron diversity in the adult cortex. J. Neurosci. 27, 10935–10946.

Fotaki, V., Yu, T., Zaki, P.A., Mason, J.O., Price, D.J., 2006. Abnormal positioning of diencephalic cell types in neocortical tissue in the dorsal telencephalon of mice lacking functional Gli3. J. Neurosci. 26, 9282–9292.

Fuccillo, M., Joyner, A.L., Fishell, G., 2006. Morphogen to mitogen: the multiple roles of hedgehog signalling in vertebrate neural development. Nat. Rev., Neurosci. 7, 772–783.

Fuccillo, M., Rallu, M., McMahon, A.P., Fishell, G., 2004. Temporal requirement for hedgehog signaling in ventral telencephalic patterning. Development 131, 5031–5040.

Gaiano, N., Kohtz, J.D., Turnbull, D.H., Fishell, G., 1999. A method for rapid gain-of-function studies in the mouse embryonic nervous system. Nat. Neurosci. 2, 812–819.

Gulacsi, A.A., Anderson, S.A., 2008. Beta-catenin-mediated Wnt signaling regulates neurogenesis in the ventral telencephalon. Nat. Neurosci. 11, 1383–1391.

Gutin, G., Fernandes, M., Palazzolo, L., Paek, H., Yu, K., Ornitz, D.M., McConnell, S.K., Hebert, J.M., 2006. FGF signalling generates ventral telencephalic cells independently of SHH. Development 133, 2937–2946.

Hammond, V., So, E., Gunnersen, J., Valcanis, H., Kalloniatis, M., Tan, S.S., 2006. Layer positioning of late-born cortical interneurons is dependent on Reelin but not p35 signaling. J. Neurosci. 26, 1646–1655.

Hebert, J.M., 2005. Unraveling the molecular pathways that regulate early telencephalon development. Curr. Top. Dev. Biol. 69, 17–37.

Hevner, R.F., Daza, R.A., Englund, C., Kohtz, J., Fink, A., 2004. Postnatal shifts of interneuron position in the neocortex of normal and reeler mice: evidence for inward radial migration. Neuroscience 124, 605–618.

Hooper, J.E., Scott, M.P., 2005. Communicating with Hedgehogs. Nat. Rev., Mol. Cell Biol. 6, 306–317.

Ingham, P.W., McMahon, A.P., 2001. Hedgehog signaling in animal development: paradigms and principles. Genes Dev. 15, 3059–3087.

Jacob, J., Briscoe, J., 2003. Gli proteins and the control of spinal-cord patterning. EMBO Rep. 4, 761–765.

Jessell, T.M., 2000. Neuronal specification in the spinal cord: inductive signals and transcriptional codes. Nat. Rev., Genet. 1, 20–29.

Joyner, A.L., Zervas, M., 2006. Genetic inducible fate mapping in mouse: establishing genetic lineages and defining genetic neuroanatomy in the nervous system. Dev. Dyn. 235, 2376–2385.

Kohtz, J.D., Baker, D.P., Corte, G., Fishell, G., 1998. Regionalization within the mammalian telencephalon is mediated by changes in responsiveness to Sonic Hedgehog. Development 125, 5079–5089.

Kunath, T., Saba-El-Leil, M.K., Almousailleakh, M., Wray, J., Meloche, S., Smith, A., 2007. FGF stimulation of the Erk1/2 signalling cascade triggers transition of pluripotent embryonic stem cells from self-renewal to lineage commitment. Development 134, 2895–2902.

Kuschel, S., Ruther, U., Theil, T., 2003. A disrupted balance between Bmp/Wnt and Fgf signaling underlies the ventralization of the Gli3 mutant telencephalon. Dev. Biol. 260, 484–495.

Lavdas, A.A., Grigoriou, M., Pachnis, V., Parnavelas, J.G., 1999. The medial ganglionic eminence gives rise to a population of early neurons in the developing cerebral cortex. J. Neurosci. 19, 7881–7888.

Lei, Q., Zelman, A.K., Kuang, E., Li, S., Matise, M.P., 2004. Transduction of graded Hedgehog signaling by a combination of Gli2 and Gli3 activator functions in the developing spinal cord. Development 131, 3593–3604.

Litingtung, Y., Chiang, C., 2000. Specification of ventral neuron types is mediated by an antagonistic interaction between Shh and Gli3. Nat. Neurosci. 3, 979–985.

Litingtung, Y., Dahn, R.D., Li, Y., Fallon, J.F., Chiang, C., 2002. Shh and Gli3 are dispensable for limb skeleton formation but regulate digit number and identity. Nature 418, 979–983.

Machold, R., Hayashi, S., Rutlin, M., Muzumdar, M.D., Nery, S., Corbin, J.G., Gritli-Linde, A., Dellovade, T., Porter, J.A., Rubin, L.L., Dudek, H., McMahon, A.P., Fishell, G., 2003. Sonic hedgehog is required for progenitor cell maintenance in telencephalic stem cell niches. Neuron 39, 937–950.

Marin, O., Rubenstein, J.L., 2001. A long, remarkable journey: tangential migration in the telencephalon. Nat. Rev., Neurosci. 2, 780–790.

Matise, M.P., Epstein, D.J., Park, H.L., Platt, K.A., Joyner, A.L., 1998. Gli2 is required for induction of floor plate and adjacent cells, but not most ventral neurons in the mouse central nervous system. Development 125, 2759–2770.

Maynard, T.M., Jain, M.D., Balmer, C.W., LaMantia, A.S., 2002. High-resolution mapping of the Gli3 mutation extra-toes reveals a 51.5-kb deletion. Mamm. Genome 13, 58–61.

Mo, R., Freer, A.M., Zinyk, D.L., Crackower, M.A., Michaud, J., Heng, H.H., Chik, K.W., Shi, X.M., Tsui, L.C., Cheng, S.H., Joyner, A.L., Hui, C., 1997. Specific and redundant functions of Gli2 and Gli3 zinc finger genes in skeletal patterning and development. Development 124, 113–123.

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

Nery, S., Fishell, G., Corbin, J.G., 2002. The caudal ganglionic eminence is a source of distinct cortical and subcortical cell populations. Nat. Neurosci. 5, 1279–1287.

Nobrega-Pereira, S., Kessaris, N., Du, T., Kimura, S., Anderson, S.A., Marin, O., 2008. Postmitotic Nkx2-1 controls the migration of telencephalic interneurons by direct repression of guidance receptors. Neuron 59, 733–745.

Olivier, C., Cobos, I., Perez Villegas, E.M., Spassky, N., Zalc, B., Martinez, S., Thomas, J.L., 2001. Monofocal origin of telencephalic oligodendrocytes in the anterior entopeduncular area of the chick embryo. Development 128, 1757–1769.

Palma, V., Ruiz i Altaba, A., 2004. Hedgehog-GLI signaling regulates the behavior of cells with stem cell properties in the developing neocortex. Development 131, 337–345.

Pan, Y., Bai, C.B., Joyner, A.L., Wang, B., 2006. Sonic hedgehog signaling regulates Gli2 transcriptional activity by suppressing its processing and degradation. Mol. Cell. Biol. 26, 3365–3377.

Park, H.L., Bai, C., Platt, K.A., Matise, M.P., Beeghly, A., Hui, C.C., Nakashima, M., Joyner, A.L., 2000. Mouse Gli1 mutants are viable but have defects in SHH signaling in combination with a Gli2 mutation. Development 127, 1593–1605.

Paxinos, G., Franklin, K., 2001. The Mouse Brain. Academic Press.

Persson, M., Stamataki, D., te Welscher, P., Andersson, E., Bose, J., Ruther, U., Ericson, J., Briscoe, J., 2002. Dorsal–ventral patterning of the spinal cord requires Gli3 transcriptional repressor activity. Genes Dev. 16, 2865–2878.

Rallu, M., Machold, R., Gaiano, N., Corbin, J.G., McMahon, A.P., Fishell, G., 2002.

Dorsoventral patterning is established in the telencephalon of mutants lacking both Gli3 and Hedgehog signaling. Development 129, 4963–4974.
Shimamura, K., Rubenstein, J.L., 1997. Inductive interactions direct early regionalization of the mouse forebrain. Development 124, 2709–2718.
Shimamura, K., Hartigan, D.J., Martinez, S., Puelles, L., Rubenstein, J.L., 1995. Longitudinal organization of the anterior neural plate and neural tube. Development 121, 3923–3933.
Soriano, P., 1999. Generalized lacZ expression with the ROSA26 Cre reporter strain. Nat. Genet. 21, 70–71.
Stamataki, D., Ulloa, F., Tsoni, S.V., Mynett, A., Briscoe, J., 2005. A gradient of Gli activity mediates graded Sonic Hedgehog signaling in the neural tube. Genes Dev. 19, 626–641.
Stenman, J., Toresson, H., Campbell, K., 2003a. Identification of two distinct progenitor populations in the lateral ganglionic eminence: implications for striatal and olfactory bulb neurogenesis. J. Neurosci. 23, 167–174.
Stenman, J.M., Wang, B., Campbell, K., 2003b. Tlx controls proliferation and patterning of lateral telencephalic progenitor domains. J. Neurosci. 23, 10568–10576.
Tao, W., Lai, E., 1992. Telencephalon-restricted expression of BF-1, a new member of the HNF-3/fork head gene family, in the developing rat brain. Neuron 8, 957–966.
te Welscher, P., Zuniga, A., Kuijper, S., Drenth, T., Goedemans, H.J., Meijlink, F., Zeller, R., 2002. Progression of vertebrate limb development through SHH-mediated counteraction of GLI3. Science 298, 827–830.
Tekki-Kessaris, N., Woodruff, R., Hall, A.C., Gaffield, W., Kimura, S., Stiles, C.D., Rowitch, D.H., Richardson, W.D., 2001. Hedgehog-dependent oligodendrocyte lineage specification in the telencephalon. Development 128, 2545–2554.
Theil, T., Alvarez-Bolado, G., Walter, A., Ruther, U., 1999. Gli3 is required for Emx gene expression during dorsal telencephalon development. Development 126, 3561–3571.
Tole, S., Ragsdale, C.W., Grove, E.A., 2000. Dorsoventral patterning of the telencephalon is disrupted in the mouse mutant extra-toes(J). Dev. Biol. 217, 254–265.
Valcanis, H., Tan, S.S., 2003. Layer specification of transplanted interneurons in developing mouse neocortex. J. Neurosci. 23, 5113–5122.
Wang, B., Fallon, J.F., Beachy, P.A., 2000. Hedgehog-regulated processing of Gli3 produces an anterior/posterior repressor gradient in the developing vertebrate limb. Cell 100, 423–434.
Wang, C., Ruther, U., Wang, B., 2007. The Shh-independent activator function of the full-length Gli3 protein and its role in vertebrate limb digit patterning. Dev. Biol. 305, 460–469.
Wichterle, H., Turnbull, D.H., Nery, S., Fishell, G., Alvarez-Buylla, A., 2001. In utero fate mapping reveals distinct migratory pathways and fates of neurons born in the mammalian basal forebrain. Development 128, 3759–3771.
Wijgerde, M., McMahon, J.A., Rule, M., McMahon, A.P., 2002. A direct requirement for Hedgehog signaling for normal specification of all ventral progenitor domains in the presumptive mammalian spinal cord. Genes Dev. 16, 2849–2864.
Wonders, C.P., Anderson, S.A., 2006. The origin and specification of cortical interneurons. Nat. Rev., Neurosci. 7, 687–696.
Wonders, C.P., Taylor, L., Welagen, J., Mbata, I.C., Xiang, J.Z., Anderson, S.A., 2008. A spatial bias for the origins of interneuron subgroups within the medial ganglionic eminence. Dev. Biol. 314, 127–136.
Xu, Q., Cobos, I., De La Cruz, E., Rubenstein, J.L., Anderson, S.A., 2004. Origins of cortical interneuron subtypes. J. Neurosci. 24, 2612–2622.
Xu, Q., Wonders, C.P., Anderson, S.A., 2005. Sonic hedgehog maintains the identity of cortical interneuron progenitors in the ventral telencephalon. Development 132, 4987–4998.
Yu, W., McDonnell, K., Taketo, M.M., Bai, C.B., 2008. Wnt signaling determines ventral spinal cord cell fates in a time-dependent manner. Development 135, 3687–3696.
Zhang, X.M., Ramalho-Santos, M., McMahon, A.P., 2001. Smoothened mutants reveal redundant roles for Shh and Ihh signaling including regulation of L/R asymmetry by the mouse node. Cell 105, 781–792.