Neuronal Migration and Ventral Subtype Identity in the Telencephalon Depend on SOX1

Antigoni Ekonomou1✉, Ilias Kazanis1✉, Stavros Malas1✉, HEATHER WOOD1✉, Pavlos Alifragis1, Myrto Denaxa2, Domna Karagogeos2, Andrew Constanti9, Robin Lovell-Badge9, Vasso Episkopou1✉

1 Mammalian Neurogenesis Group, MRC Clinical Sciences Centre, Imperial College School of Medicine, Hammersmith Hospital Campus, London, United Kingdom, 2 Medical School and Institute of Molecular Biology and Biotechnology, University of Crete, Heraklion, Greece, 3 Department of Pharmacology, The School of Pharmacy, London, United Kingdom, 4 Division of Developmental Genetics, National Institute of Medical Research, London, United Kingdom

Little is known about the molecular mechanisms and intrinsic factors that are responsible for the emergence of neuronal subtype identity. Several transcription factors that are expressed mainly in precursors of the ventral telencephalon have been shown to control neuronal specification, but it has been unclear whether subtype identity is also specified in these precursors, or if this happens in postmitotic neurons, and whether it involves the same or different factors. SOX1, an HMG box transcription factor, is expressed widely in neural precursors along with the two other SOXB1 subfamily members, SOX2 and SOX3, and all three have been implicated in neurogenesis. SOX1 is also uniquely expressed at a high level in the majority of telencephalic neurons that constitute the ventral striatum (VS). These neurons are missing in Sox1-null mutant mice. In the present study, we have addressed the requirement for SOX1 at a cellular level, revealing both the nature and timing of the defect. By generating a novel Sox1-null allele expressing β-galactosidase, we found that the VS precursors and their early neuronal differentiation are unaffected in the absence of SOX1, but the prospective neurons fail to migrate to their appropriate position. Furthermore, the migration of non-Sox1-expressing VS neurons (such as those expressing Pax6) was also affected in the absence of SOX1, suggesting that Sox1-expressing neurons play a role in structuring the area of the VS. To test whether SOX1 is required in postmitotic cells for the emergence of VS neuronal identity, we generated mice in which Sox1 expression was directed to all ventral telencephalic precursors, but to only a very few VS neurons. These mice again lacked most of the VS, indicating that Sox1 expression in precursors is not sufficient for VS development. Conversely, the few neurons in which Sox1 expression was maintained were able to migrate to the VS. In conclusion, Sox1 expression in precursors is not sufficient for VS neuronal identity and migration, but this is accomplished in postmitotic cells, which require the continued presence of Sox1. Our data also suggest that other SOXB1 members showing expression in specific neuronal populations are likely to play continuous roles from the establishment of precursors to their final differentiation.

Introduction

The telencephalon is subdivided into dorsal (pallial) and ventral (subpallial) territories, which give rise to the cerebral cortex and the underlying basal ganglia, respectively. The embryonic subpallium consists of large protrusions—the ganglionic eminences. Several distinct types of neurons originate in the ganglionic eminences, and some migrate as far as the olfactory bulb, hippocampus, and neocortex [1–3], while others contribute more locally. The majority of neurons of the lateral ganglionic eminence (LGE) form the dorsal and ventral striatum (VS). The VS includes the caudate, putamen, nucleus accumbens, and olfactory tubercle (OT), which control various aspects of motor, cognitive, and emotional functions [4,5]. Little is known about the molecular mechanisms that control the emergence of various groups of neurons with distinct identities in this region.

Gene-expression studies and loss-of-function mutations in homeodomain transcription factors such as PAX6 [6,7] and GSH2/1 [8–13] confirm fate-mapping findings [14–16] that the majority of the VS neurons are specified within the progenitor domain of the LGE. The proneural basic helix-loop-helix (bHLH) factor Mash1 also marks the precursors of early-born neurons in the LGE progenitor domain, and its loss in the mouse leads to a deficit of both precursors and neurons of the telencephalon, including loss of VS neurons [17,18]. Therefore, GSH2 and MASH1 control VS precursor

*Correspondence: Ekonomou A, Kazanis I, Malas S, Wood H, Alifragis P, et al. (2005) Neuronal migration and ventral subtype identity in the telencephalon depend on SOX1. PLoS Biol 3(6): e186.
Neuronal Identity and Migration in the Forebrain

patterning and specification, but as they are not expressed in postmitotic cells it remained unknown to what extent they are involved in the emergence of neuronal subtypes in the ventral telencephalon, and whether different transcription factors with neuron-specific expression are required.

The SOX proteins constitute a family of transcription factors [19,20] that regulate transcription through their ability to bind to specific DNA sequences via their HMG box domains [21–24]. There are 20 Sox genes in mammals, and at least half are expressed in the developing nervous system [20,24]; however, their role in neural development is poorly understood. SOX1, SOX2, and SOX3 constitute the SOXB1 subfamily and share more than 95% identity within their HMG boxes and significant homology outside [25,26]. All three proteins are expressed in the neuroepithelium throughout central nervous system (CNS) development [25,27], and as they tend to be down-regulated upon neural differentiation they have been used as markers for neural stem cells and precursors [28,29]. Several studies suggest that SOXB1 factors function in stem cells and precursors to maintain broad developmental potential [30] and neural stem cell identity [30–32] by counteracting neurogenesis. Contradictory evidence, however, suggests that SOX1 promotes neurogenesis and cell cycle exit [33]. However, mice that are null for Sox1 [34] or Sox3 [35], or mice with one Sox2 allele deleted and the other hypomorphic [36], exhibit phenotypes associated with the loss of or functional deficit of only specific neuronal populations. As these SOXB1 factors are expressed in both precursors and neurons that are affected in these mutant mice, it was not known whether their function is required in precursors, postmitotic cells, or both.

We have previously shown that Sox1 is essential for the terminal differentiation of lens fibers and the activation of γ-crystallins [37], and for the development of VS neurons, the lack of which is associated with epilepsy [34]. Here, we show that absence of SOX1 has no effect on the generation, proliferation, and patterning of neuronal precursors. This is probably due to functional compensation by SOX2 and SOX3, which are co-expressed with SOX1 in precursors. Moreover, mice lacking only the neuron-specific expression of Sox1 in the ventral telencephalon still fail to develop VS neurons, revealing its requirement within these neurons. Consistent with this, maintenance of Sox1 expression in neurons of the ventral telencephalon is sufficient to direct them to the VS, confirming the adequacy of SOX1 function in postmitotic cells for their migration and identity. Therefore, VS-specific neuronal migration and subtype identity most likely is initiated in precursors but is completed in postmitotic cells by transcription factors such as SOX1.

Results

SOX1 Is Essential for the Histogenesis of the VS

To generate a detailed map of Sox1 expression in the developing and adult brain of mice, and to perform comparative studies between homozygotes and heterozygotes, we generated a novel targeted allele referred to as Sox1geo. This contains an insertion of β-galactosidase-neo (βgeo) fusion protein in-frame with the SOX1 open reading frame (Figure 1A). Mice homozygous for Sox1geo are null for Sox1 and exhibit the same phenotype as the previously described mice, which carry a deletion of the SOX1 coding region (Sox1M1) [34,37], namely, lens defects and epileptic seizures. Staining for β-galactosidase activity (X-gal staining) in Sox1geo heterozygous embryos matches that for the wild-type allele as revealed by whole-mount in situ hybridization and Sox1 antibody staining (Figure 1B–1E).

To elucidate the role of SOX1 in the formation of the VS, we compared the expression pattern of Sox1 in heterozygous (Figure 1F–1I) and homozygous (Figure 1J–1M) brains from embryonic day 14 (E14) to postnatal day 0 (P0) using X-gal staining. Throughout much of the CNS, X-gal staining in the Sox1geo homozygotes is double the intensity observed in heterozygotes (data not shown). To perform comparative histological studies, we equalized the levels of X-gal staining in homozygous mice with those of heterozygous animals by generating homozygous mice that harbor two different Sox1-null alleles: βgeo (Sox1geo) and the previously described M1-targeted allele (Sox1M1) [37], which does not express β-galactosidase.

Our analysis shows (via X-gal staining) that in the developing forebrain, Sox1 is expressed throughout the ventricular zone (VZ) and subventricular zone (SVZ) and in neurons around the anterior commissure region, where the prospective nucleus accumbens forms (red arrowheads in Figure 1K and 1M), and in the striatal bridges that link this intermediate cluster of cells with the prospective OT region toward the pial surface. X-gal-positive neurons start populating the OT area as early as E14 and continue to accumulate at least until birth (Figure 1F–1I). In the Sox1geo heterozygous mutants, the X-gal staining pattern of the VZ/SVZ is indistinguishable from that of the Sox1geo heterozygotes, and there is no obvious deficit of X-gal-positive cells around the anterior commissure. On the other hand, both the striatal bridges and the OT layers are absent in the Sox1-null brain at all developmental stages (compare Figure 1F–1I to 1J–1M). It is unlikely that neurons die en masse in this region, because an apoptosis assay did not reveal any evidence of increased cell death in the mutant (data not shown). In addition, X-gal staining is increased throughout the ventral telencephalon in the mutant postnatal brain (red arrowheads in Figure 2), suggesting that the Sox1-null cells are not correctly specified and contribute to other brain regions. Interestingly, although neurons that form the core of the nucleus accumbens express Sox1 highly, they form normally (Figure 2F and red arrowheads in Figure 1) and do not depend on Sox1 for their development. Therefore, SOX1 is required for histogenesis of the OT throughout its development.

Normal Precursor Proliferation and Neurogenesis but Loss of OT Neuronal Differentiation in the Absence of SOX1

Studies with conflicting results suggest that Sox1 either, like Sox2 and Sox3, counteracts neurogenesis [32] or, unlike Sox2 and Sox3, promotes neurogenesis [33]. To examine whether the loss of SOX1 affects general neuronal differentiation in the area of the striatum, we used an anti-βIII-tubulin (TuJ1) antibody, which is a marker for immature neurons [38], at E13, a critical time of differentiation in the LGE. TuJ1 immunocytochemistry did not reveal any obvious general differentiation problems in the Sox1 mutants (Figure 3A and 3B), suggesting that loss of SOX1 alone is not sufficient to compromise general neuron differentiation and maturity. The differentiation and distribution of specific mature neurons was examined in our previous study at adult
stages with the expression of striatal markers such as preproenkephalin and Gad65/67 [34], and in our current study, at embryonic stages with additional markers such as Brn4 [39] (Figure 3C and 3D) and Robo [40] (Figure 3E and 3F). This analysis revealed a differentiation defect restricted in the region of the nucleus accumbens/OT, and not the rest of the striatum.

As Sox1 expression is associated with dividing cells throughout the neuroepithelium, we examined whether a proliferation defect could partially account for the cell deficit in the ventral telencephalon. We used 5-bromo-2′-deoxyuridine (BrdU) to label all proliferating precursors in wild-type and Sox1-null embryos at E13–E15, and harvested their brains 1 h later. Most of the dividing cells were found in the VZ/SVZ, and there was no increase or ectopic proliferation (Figure 3G–3L; Table S1). Therefore, SOX1 is unlikely to be required for proliferation or the exit of precursors from the cell cycle in the LGE. Collectively, the above data show that SOX1 is not essential for the proliferation of precursors and general neuronal differentiation. However, it is required specifically for the differentiation and/or migration of VS neurons.

Early- and Late-Born OT Neurons Fail to Migrate in the Absence of SOX1

To investigate the possibility that neurons migrate in the Sox1-null OT regions, but are not visible because they do not express Sox1geo and other differentiation markers, we exposed embryos to BrdU. This way, we permanently marked all proliferating precursors independently of Sox1 or other striatal-marker gene expression and followed them at later embryonic stages and after birth (Figure 4). Labeling the precursors at different embryonic days also provided information on the birthdates of the OT neurons, which were previously unknown for the mouse. Specifically, birth of ventral striatal neurons commences early at E13 and continues until birth (Figure 4A, 4C, 4E, and 4G; data not shown) and is consistent with data from the rat [41,42]. Furthermore, in wild-type embryos, BrdU exposure between E13 and E16 with examination of embryos either 72 h later (Figure 4A; data not shown) or after birth (Figure 4C, 4E, and 4G) showed that early-born neurons migrate more laterally than those born later.

In the Sox1-null embryos, the presence and distribution of cells labeled with BrdU between E13 and E16 showed that the olfactory cortex is largely normal (bracket in Figure 4D), but in the VS area the number of labeled cells was found to be greatly reduced (Figure 4B, 4D, 4F, and 4H). Furthermore, in the Sox1-null postnatal brain the striatal mantle is more densely populated by BrdU-labeled cells (Figure 4D–4H), consistent with the general increase of X-gal staining.
Neuronal Identity and Migration in the Forebrain

Sox1forebrain viewed from the ventral surface, and (C–F) show 150-

The Generation and Patterning of LGE Precursors Is Normal in the Absence of SOX1

The LGE structure consists of neural progenitors with radial glial characteristics, having fibers that extend from the VZ to the pial surface. These cells also provide the substrate for the migration of neurons [45,46]. Staining with X-gal (see Figure 1) and β-galactosidase antibody in mice carrying the Sox1βgeo allele allowed visualization of the cytoplasmic compartment of the SOX1-expressing progenitors, which have radial glial morphology (Figures S2F and S3). We examined the morphology of radial glia in the Sox1-null LGE using the RC2 antibody [47], but we did not find any difference from wild-type (Figure S4). Therefore, we conclude that the abnormal distribution of Pax6-expressing neurons in the LGE of the Sox1-null mouse is unlikely to be caused by abnormal morphology of the radial glial fibers or substantial loss of radial glia-like precursors.

In the ventral telencephalon, the bHLH transcription factor MASH1 [18] and the homeodomain factor DLX1 mark LGE precursors [8]. Ablation of MASH1 in mice causes loss of specific subpopulations of precursors and striatal neurons that contribute to the OT and nucleus accumbens [18]. In addition, Gsh2-null mice, which also fail to develop the OT, exhibit reduced Dlx1 expression in LGE precursors [9]. We therefore examined the expression of these two genes in Sox1-null embryos, but found no difference (Figure 5E–5H), indicating that there is no deficit of early or late LGE precursors in the absence of SOX1. Collectively, the above data show that Sox1 is not required for patterning, generation, and maintenance of LGE precursors.

Sox1 Expression from the Endogenous Sox2 Promoter Can Be Tolerated In Vivo

It has been shown that all three SoxB1 genes are expressed [25,27] and share similar functions in neural precursors [31,32]. However, in the postmitotic cells of the mantle and the VS area, antibody staining for each of the genes at E15 -antibody immunohistochemistry in embryos at E12–E16, we found that loss of SOX1 has no effect on the expression of GSH2/PAX6 and the boundary in the dorsal LGE (Figures 5A, 5B, and S1). In addition, at this boundary, PAX6-expressing postmitotic cells form a stream (arrow in Figure 5A) that extends laterally to the VS (Figures 5A, 5C, S2E, and S2F) [6,7,44]. In the absence of SOX1, the stream of PAX6-positive postmitotic cells is normal (arrow in Figures 5B and S2F), but to characterize the PAX6- and SOX1-expressing neurons in the region of the OT, we used double antibody immunohistochemistry. Specifically, for the wild-type brain sections we used PAX6 and Sox1 antibodies, but to visualize the Sox1-expressing cells in the Sox1βgeo/M1-null brain sections we used an antibody for β-galactosidase.

Our data showed that Pax6 and Sox1 proteins were co-expressed in progenitors (Figures S2E and S2F), but in postmitotic cells of the LGE this expression became mutually exclusive (Figure 5C and 5D). Pax6-expressing neurons were clustered laterally to those expressing Sox1, at the border between the OT and olfactory cortex (Figures 5C, S2A, and S2C). In Sox1-null mice, the postmitotic Pax6-expressing cells were distributed throughout the VS area (Figures 5D, 5B, and S2D), suggesting structural disorganization. It is unlikely that these ectopically localized Pax6-expressing neurons are mis-specified Sox1-null neurons, because they should be expressing both Pax6 and βgeo from the mutant Sox1βgeo allele, but they do not (Figure 5D).

The generation and patterning of LGE precursors [8]. Ablation of MASH1 in mice causes loss of specific subpopulations of precursors and striatal neurons that contribute to the OT and nucleus accumbens [18]. In addition, Gsh2-null mice, which also fail to develop the OT, exhibit reduced Dlx1 expression in LGE precursors [9]. We therefore examined the expression of these two genes in Sox1-null embryos, but found no difference (Figure 5E–5H), indicating that there is no deficit of early or late LGE precursors in the absence of SOX1. Collectively, the above data show that Sox1 is not required for patterning, generation, and maintenance of LGE precursors.

Sox1 Expression from the Endogenous Sox2 Promoter Can Be Tolerated In Vivo

It has been shown that all three SoxB1 genes are expressed [25,27] and share similar functions in neural precursors [31,32]. However, in the postmitotic cells of the mantle and the VS area, antibody staining for each of the genes at E15

![Figure 2. Ectopic Distribution of Sox1-Null Neurons](https://www.plosbiology.org/file_attachments/186.png)

X-gal staining of mouse forebrains at P16. (A and B) show intact forebrain viewed from the ventral surface, and (C–F) show 150-μm coronal Vibratome sections for Sox1βgeo mice (A, C, and E) and Sox1βgeo/M1 mice (B, D, and E). Red arrows indicate the width of the OT. Red arrowheads indicate increased X-gal staining at more medial and posterior areas of the brain in (B), and in the striatum and septum in (D) and (F). White arrowheads indicate islands other than the medial islands of Calleja. an, accumbens nucleus; I, II, III, cell layers of the OT; lsn, lateral septal nucleus; ob, olfactory bulb; PC, olfactory piriform cortex; S, striatum; sb, striatal bridge Scale bar = 500 μm. DOI: 10.1371/journal.pbio.0030186.g002

observed throughout the striatum (see Figure 2). The above data suggest that in the absence of SOX1, early- and late-born neurons fail to migrate to the appropriate position to form the ventral areas of the striatum.

The Generation and Patterning of LGE Precursors Is Normal in the Absence of SOX1

It is known that the majority of VS neurons derive from precursors that are born in the LGE [10,15,43]. To investigate whether the defect is in the patterning of precursors, we examined the expression pattern of various transcription factors that mark LGE progenitors and are known to have a role in OT neuronal specification (Figures 5, S1, and S2).

The homeodomain transcription factors Pax6 and Gsh2 are expressed, respectively, in the pallial and subpallial precursor domains of the dorsal LGE. The boundary between them has been shown to be essential for the patterning of VS precursors [10,11]. Specifically, Gsh2-null mice do not form early-born OT neurons, and the precursors of the dorsal LGE are lost as Pax6 expands ventrally into the LGE. However, loss of both Pax6 and Gsh2 restores dorsoventral patterning and partially rescues OT formation [9–11]. Using double
indicated that SOX2- and SOX3-positive neurons represent a very small population compared to that expressing SOX1 (Figure 6A–6C). Therefore, it is likely that the other SoxB1 genes compensate for the loss of Sox1 in precursors, whereas they cannot do so in the LGE postmitotic cells. Nevertheless, it remained unknown whether SOX1 functions solely in precursors for VS fate specification or in postmitotic cells for maintaining this fate and the emergence of specific subtype identity and migration. To address this, we generated mice that express Sox1 mainly in precursors and not in LGE neurons. We took advantage of the fact that Sox2 is co-expressed with Sox1 in precursors but it is down-regulated in LGE neurons, and generated mice that express Sox1 from the endogenous Sox2 promoter. We confirmed the overlap of Sox1 and Sox2 expression in the VZ/SVZ of the LGE by staining serial coronal telencephalic sections with antibodies for each of the two genes and counter-staining with the nuclear stain TOTO at E14 (Figure S5), and by performing double anti-SOX1 and -SOX2 immunohistochemistry at E13 (Figure 6D–6L).

The replacement of the SOX2 open reading frame with that of SOX1 was achieved by targeting the Sox2 allele (Figure 7A). The new allele, Sox2R, was engineered to express not only Sox1 but also βgeo via an internal ribosomal entry site (IRES). Furthermore, the coding region of SOX1 in the Sox2R allele was flanked by LoxP sites, which can be deleted using Cre-mediated recombination [48]. In this way, we generated a mouse line carrying another allele, termed Sox2βgeo2 (but referred to hereafter as Sox2βgeo), which expresses only the βgeo reporter gene from the Sox2 promoter (Figure 7A) and not SOX1. Like the Sox2βgeo/+ heterozygotes, Sox2βgeo/+ mice were viable, fertile, and phenotypically normal, indicating that SOX1 over-expression in precursors, as well as ectopic expression in other locations where Sox2 is uniquely expressed, does not cause any obvious developmental abnormality.

X-gal staining of embryos showed that both targeted Sox2 alleles (Sox2βgeo and Sox2R) express βgeo in the CNS, but to verify that SOX1 protein was produced from the Sox2R allele, we used SOX1 antibody staining. We found that SOX1 protein was ectopically present at sites where SOX2 normally shows unique expression—for example, in the floor plate of the diencephalon (arrowheads in Figure 7B and 7C) and in the sensory placodes (arrows in Figure 7D and 7E).
intensity of the immunostaining at ectopic sites was comparable to the staining in areas with expression from two wild-type Sox1 alleles (VZ/SVZ), indicating that the level of expression was similar to the wild-type allele. Therefore, the Sox2R allele produces SOX1 ectopically in all neurons uniquely positive for SOX2 and increases the endogenous level of SOX1 in precursors and neurons that express both genes, without causing an obvious defect in mice. The fact that ectopic expression does not cause any obvious phenotype suggests either that the partner factors required for SOX1 target specificity [19] are absent in those cells uniquely expressing Sox2 or that the two proteins are interchangeable, sharing target genes. Further experiments are required to clarify this.

**Sox1 Over-Expression in Precursors Does Not Increase VS/OT Neuronal Fate Specification**

To investigate more subtle defects due to the over-expression of Sox1 in precursors of the Sox2R mice, we examined several litters \( (n > 10) \) of mice and visualized the...
Sox1 and Sox2 carrying endogenous wild-type alleles were compared with those expressing fgeo via the Sox2 promoter were found to be the same regardless of the number of endogenous Sox1 alleles or the ectopic presence of Sox1 (compare Figure 8A, 8B, and 8C).

To further investigate the differentiation of the VS neurons, we used the striatal-specific markers dopamine and cAMP-regulated phosphoprotein (DARP-32) at postnatal stages and found them to be unaffected in Sox2−/− mice (Figure 8E and 8F). The data therefore indicate that the over-expression of Sox1 in precursors does not increase OT neuronal specification.

**Sox1 Expression in Precursors Cannot Rescue OT Neuron Development**

To address directly whether Sox1 function is essential in precursors, we crossed Sox1M1/+; Sox2R/+ mice with Sox1fgeo/+; Sox2+/+ mice and examined whether offspring carrying Sox2R/+ without any wild-type Sox1 alleles (Sox1M1fgeo) could develop OT. In these Sox1R/+ embryos that carry no endogenous Sox1 functional allele (termed here HoHe), Sox1 is expected to be expressed only via the Sox2R allele in precursors and become down-regulated in postmitotic LGE cells. However, fgeo expression from the endogenous Sox1 mutant allele marks precursors and OT prospective neurons. We followed the Sox1M1fgeo prospective OT neurons with X-gal to determine whether they were capable of contributing to the OT in HoHe embryos (Figure 9). The Sox2R allele also expresses fgeo; however, in LGE postmitotic cells, Sox2-fgeo expression is much less than that of Sox1-fgeo and is not very visible by short (3 h) X-gal staining (only a slight increase of X-gal staining is seen in the VS; Figure 9C compared to Figure 9B).

Each brain was split into left and right hemispheres, and coronal sections of the left were used for short staining with X-gal (Figure 9A–9C) whereas sections of the right were stained with Sox1 antibody (Figure 9D and 9E). The hemispheres stained for Sox1-gal showed characteristic staining of OT neurons in heterozygous Sox1Bgeo/- mice (red arrowheads in Figure 9A), but the hemispheres of the Sox1-null embryos (Sox1BgeoM1) with Sox2R/+ (Figure 9B) or without (Figure 9C) did not. This indicates that Sox1-null embryos do not develop OT despite the presence of the Sox2R allele and Sox1 protein in progenitors. To verify the presence of Sox1 protein in the precursors of the HoHe mice, we examined the other hemisphere that was stained with Sox1 antibody. In the Sox1Bgeo/- embryos, we found Sox1 present in the VZ (yellow arrows in Figure 9D and 9E) and the OT neurons (red arrowheads in Figure 9D). In Sox1BgeoM1 (null) embryos, Sox1 expression was completely absent (data not shown); in the HoHe embryos, Sox1 protein was present in the VZ (yellow arrow in Figure 9E) and in very few neurons of the LGE (Figure 9E). HoHe mice, like the Sox1-nulls, are born with small eyes, and around weaning age develop seizures associated with lethality, which, if anything, is increased compared to that of Sox1-null mice (data not shown). In the

**Neuronal Identity and Migration in the Forebrain**

Immunofluorescence of coronal sections at LGE levels in (A–C) E15- and (D–L) E13-stage wild-type embryos visualized on a confocal microscope antibody staining for (A, D, G, and J) Sox1 (red), (B, E, H, and K) Sox2 (green), (C) SOX3 (green), (D–L) double Sox1 (red) and Sox2 (green), and (F, I, and L) merged. In the OT area and the LGE mantle, there are more neurons expressing Sox1 (A and J) than Sox2 (B and K) and Sox3 (C). Note the extensive co-expression of the SOX1 and SOX2 in precursors (D–I). (G–I) are higher magnifications of the areas within the rectangles. Scale bar = 300 μm. DOI: 10.1371/journal.pbio.0030186.g006

**Figure 6. SOX2 and SOX3 Down-Regulation in LGE Neurons and SOX1/ SOX2 Co-Expression in LGE Precursors**

migration of the Sox1/Sox2-positive neurons in the VS via X-gal staining. Newborn mice carrying Sox2Bgeo with two (Sox2R/+; Sox1M1/+; Figure 8A) or one (Sox2R/+; Sox1M1/+; Figure 8B) Sox1 endogenous wild-type alleles were compared with those carrying Sox2Bgeo, Sox1Bgeo that do not express Sox1 ectopically (Figure 8C). All the above mice have only one wild-type Sox2 allele. We also compared the Sox1-fgeo and Sox2-fgeo neurons of the ventral telencephalon in Sox1Bgeo (Figure 8C and 8D) and Sox2Bgeo (Figure 8A) mice, respectively. In this area of the brain, the Sox2-positive neurons are far fewer than those positive for Sox1. Therefore, for comparison purposes, we used thin tissue sections (80 μm) with short X-gal staining (3 h) for Sox1-fgeo, and thicker sections (100–150 μm) with a long staining period (48 h) for Sox2-fgeo. Consistent with the antibody staining data (see Figure 6B), Sox2-fgeo neurons contribute to the OT, indicating that they are a subset of the VS neuronal population. Therefore, the ectopic expression of Sox1 in LGE neurons is expected to be very limited. More importantly, the migration and the number of LGE neurons expressing fgeo via the Sox2 promoter were found to be the same regardless of the number of endogenous Sox1 alleles or the ectopic presence of Sox1 (compare Figure 8A, 8B, and 8C).
brain of P10 HoHe mice, we used staining with DARPP-32 antibody (which is a SOX1-independent striatal marker) to investigate the recovery of OT neurons, and found staining in the striatal mantle but not in the VS (Figure 9G compared to Figure 9F). We therefore concluded that SOX1 expression in precursors is not sufficient to rescue VS/OT neuron fate specification, and that the continued presence of SOX1 in postmitotic cells is required for their identity.

Sox1/Sox2 Expression in Neurons Is Sufficient for Their Migration to the VS

We have shown that in mice carrying two (Sox1<sup>+/+</sup>), or one (Sox1<sup>+/−</sup>), Sox1 wild-type alleles (see Figure 8A, 8B, and 8C), the migration of the Sox2-positive LGE neurons is not overly different from that observed in mice carrying the Sox2<sup>−/−</sup> allele. However, it remained unknown whether the Sox1/Sox2 double-positive LGE neurons migrated to the VS when both Sox1 endogenous alleles were missing (HoHe mice). We used X-gal staining to follow these neurons in several litters (n = 10), including HoHe mice, which have two Sox1<sup>M1</sup> alleles and thus βgeo expression exclusively driven by the Sox2<sup>R</sup> allele. We found that in the LGE of these mice, the double-positive neurons are generated and migrate to the OT area (compare Figure 10A and 10B), but this area is compacted in the absence of the majority of the OT/SOX1 neurons. The above data show that the continued expression of Sox1 in neurons of the LGE is sufficient to direct their migration to the OT in the absence of endogenous Sox1.

Discussion

The specification of neurons in the ventral telencephalon has been shown to depend on several transcription factors that are expressed mainly in proliferating precursors.
However, it was unknown to what degree specification in precursors included the emergence of neuronal subtype identity in the ventral telencephalon, and whether expression of additional transcription factors was required. We showed that the differentiation and migration of early- and late-born neurons that constitute the VS require SOX1 expression not only in precursors but also in postmitotic cells. Furthermore, in this region, the migration and organization of other neurons such as those expressing Pax6 also depend on the presence of SOX1-positive VS neurons. The finding that SOX1 functions in neurons to control migration and identity is novel and suggests that the other SOXB1 factors, in addition to their roles in precursors, have similar functions in neurons.

Identity and Migration of Neurons in the VS

The development of subtype identity and migration of neurons in the ventral telencephalon has not been well characterized. The expression of differentiation markers reveals neurons in both VS and dorsal striatum, but we showed that SOX1 specifically marks a large population of VS neurons that form the principal layer II of the OT, the islands of Calleja, and the nucleus accumbens (see Figures 1 and 2). In the absence of SOX1, the majority of neurons of the VS fail to develop. All Sox1-expressing neurons of the OT and the islands of Calleja require SOX1 for their development, but it is essential only for the shell of the nucleus accumbens, although the core also expresses it. While neurons of the adjacent striatal mantle and the olfactory cortex that do not express Sox1 develop normally in its absence, other groups of neurons within the VS appear to be disorganized. Specifically, we identified a distinct population of neurons located lateral to the OT that constitute the ventrolateral region of the telencephalon, with later-born neurons positioned progressively more medial positions (see Figure 4). In the absence of SOX1, these neurons migrate to ventrolateral positions (see Figure 5). These are not mis-specified Sox1-null neurons because they do not express βgeo. This indicates that Sox1-expressing OT neurons play a non-cell-autonomous role in the organization of other neurons in this region, including the production of essential signals for migration. Most likely, the disorganization of the VS in the absence of SOX1 results in abnormal local neuronal connectivity, which in turn leads to the abnormal (epileptiform) electrophysiological behavior observed in the SOX1-deficient animals [34].

SOX1 Function in Precursors

SOXB1 factors share considerable homology in both their DNA binding and C-terminal transcriptional activation domains, and they are co-expressed in precursors. It is therefore possible that in the LGE precursors, the role of
SOX1 in the specification of OT/VS neurons is redundant. However, as Sox1 genes have a broad expression in the neuroepithelium, we have to assume that their specific function at different areas of the VZ, and particularly the VZ of the LGE, is controlled by the presence of LGE-specific partner factors. SoxNeuro and Dichaetae, the two Drosophila orthologs of the vertebrate Sox1 group genes, also show overlapping functions during neural development [49]. Furthermore, in Drosophila, these two genes have been shown to genetically interact with the dorsoventral patterning genes ind (intermediate neuroblast defective) and vnd (ventral nerve chord defective) [50,51]. The vertebrate orthologs of ind and vnd are Gsh1/2 [52,53] and Nks2.2 [54], respectively. In the mouse, Gsh2 is expressed in the VZ/SVZ, and like Sox1, its loss results in a reduction of VS neurons. As target gene specificity of SOX proteins depends on partnering with other transcription factors [20], our work, along with the data from Drosophila, supports the hypothesis that in the LGE precursors GH1/2 may act as partners for SOXB1 factors to initiate ventral telencephalic neuronal identity.

The neurons of the VS area occupy approximately a quarter of the striatal mass [55], and migrate there over a period of at least 10 d (E13 to first postnatal week). The LGE precursors that generate the OT/VS in the LGE are expected to have an equivalent representation during this period of precursors that generate the OT/VS in the LGE are expected to have an equivalent representation during this period of at least 10 d (E13 to first postnatal week). The LGE contains any other selectable markers or promoters, and the targeting vector did not contain any other selectable markers or promoters, and the targeting vector did not show any deficit in precursors. The increase of X-gal-stained (Sox1-βgeo; see Figure 2) and BrdU-labeled neurons (see Figure 4D and 4F) in the area of the septum and the striatum supports the notion that the VS/OT neurons are born but lack VS subtype identity to migrate toward ventral positions. The normal expression of TuJ1, a marker of immature neurons, excluded the possibility that loss of SOX1 delays or enhances differentiation. Therefore, in the absence of SOX1, the precursors are there and generate neurons, but these fail to migrate to the VS because they assume different identity and position. The finding that Sox1-null neurons contribute widely to different areas argues that the presence of SOX1 provides neurons with ventral identity and the ability to migrate to ventral regions.

Emergence of VS Neuron Identity

To test the role of Sox1 expression in neurons and to determine whether ventral identity emerges in postmitotic cells, we limited expression of Sox1 largely to precursors of LGE neurons. Sox2βgeo mice express Sox1 from one of the Sox2 alleles. When the Sox2α allele is present in animals with no endogenous Sox1 wild-type alleles (HoHe), Sox1 expression mimics that of SOX2—being present in VS/OT precursors but largely absent from the neurons they give rise to. HoHe mice also fail to develop the majority of VS/OT neurons (see Figure 9) and exhibit an equally severe phenotype to that of Sox1-null mice in the OT. As these mice reproduce faithfully the Sox1-null phenotype without any evidence of a partial rescue, it is unlikely that this is the result of incomplete expression of Sox1 from the Sox2 promoter in the precursors. However, to exclude the possibility that the failure of OT/VS neuron development in HoHe mice was due to a low level of expression of SOX1 protein in precursors, we used one hemisphere of the brain to assay OT development and the other for SOX1 antibody staining, linking in each animal the phenotype with the presence of SOX1 protein in precursors. We found no difference in the extent and level of expression of SOX1 protein in postmitotic cells from one copy of Sox1, whether it is expressed from the Sox2 locus in HoHe (see Figure 9E) or the endogenous Sox1 allele in Sox1βgeo/βgeo heterozygotes (see Figure 9D). Therefore, the emergence of VS/OT identity requires Sox1 expression in postmitotic cells. Consistent with the above findings, the small population of LGE postmitotic cells in HoHe mice that maintain SOX1 expression from the Sox2α allele migrate to the VS. However, the number of these neurons is small and cannot rescue the deficit in the area of the VS. In conclusion, although specification of neuronal identity is initiated in precursors, emergence of neuronal subtype and ventral migration require the continued presence of SOX1. Our findings suggest that in other brain areas, subtype identity and migration may also be controlled by the expression of transcription factors in postmitotic cells.

The current study, along with our previous one showing that SOX1 expression in the lens of the mouse is responsible for terminal differentiation and the expression of γ-crystallin genes [37], has revealed that SOX1 has important functions in postmitotic cell differentiation at two distinct sites. It is possible that the other SOX1 factors have similar roles in postmitotic cells in which their expression is maintained.

Materials and Methods

Gene targeting. The βgeo gene was inserted into the Sox1 single exon as previously described [37]. The resulting targeted locus produces a fusion protein consisting of the first 50 amino acids from SOX1 (which excludes the HMG box) followed by ten amino acids that are encoded by a synthetic linker sequence, and the βgeo sequence (including a polyadenylation signal). Tissue culture was carried out as described before [37], omitting the addition of gancyclovir for negative selection. The targeting vector did not contain any other selectable markers or promoters, and the targeting frequency was 1/52. As Sox1βgeo is not normally expressed in embryonic stem (ES) cells, we used the minimum level of G418 for selection. Positive recombinants were identified by Southern blotting, using a 3′ single XhoI 2.2-kb external probe on an EcoRI digest. Three ES cell clones were obtained, and one was successfully passed through to the germ line. All anatomical investigations were performed on mice of mixed genetic background. Sox1βgeo mice were mated with mice that were heterozygous for the previously described [37] Sox1 deletion (Sox1Δgeo) and did not express β-galactosidase.

For the Sox2 replacement vector, the 5′ and 3′ homologies used were the same as described before [30]. A Smal-XhoI 2.2-kb Sox1 fragment containing the SOX1 open reading frame was flanked by LoxP cassettes followed by the NotI-Sall IRES–βgeo-polyA fragment (plasmid gift from Dr. A. Smith, University of Edinburgh). The replacement vector was linearized with Sall and electroporated into ES cells. Positive recombinants were identified by Southern blotting as described before [30]. Several targeted ES cells were isolated at a frequency of 1/20 and gave germ-line transmission of the mutation. Heterozygous animals carrying the Sox2sox2geo allele (referred to in the text as Sox2βgeo) expressed Sox1 and βgeo where Sox2 is normally expressed.

Deletion of the Sox1 coding region from the Sox1βgeo/βgeo allele was achieved via pronuclear injection of a supercoiled plasmid expressing Cre-recombinase (gift from Dr. K. Rajewsky, Harvard Medical School). Although in the text we refer to this new allele as Sox2geo,
it is officially named Sox2g<sup>geo</sup> to distinguish it from the one previously described [30].

**X-gal staining and in situ hybridization.** For β-galactosidase staining, fetal, newborn, or adult mouse brains were processed as previously described [30]. Detection of Sox1 mRNA was performed in whole embryos, as described previously [27,54]. The probes that were used in brain slices were generated by RT-PCR from embryonic brain cDNA. The position of the probes was Ddxl (nt 41–573), Bm<sup>nf</sup> (nt 199–541), and Robo (nt 8–779). All fragments were cloned into a suitable cloning vector (pGEM-easy, Promega, Madison, Wisconsin, United States), and were re-amplified using a sense oligonucleotide and an oligonucleotide upstream of either the T7 or SP6 sites. The resulting products were gel-purified, and 40 ng was used for probe synthesis. The brains were processed as described before [27,54].

**BrdU labeling.** A 25 mg/ml solution of BrdU (Sigma, St. Louis, Missouri, United States) was made in PBS warmed to 37°C. The solution was sterilized through a 0.2-μm syringe filter and injected into the peritoneal cavity of pregnant mice (0.1 ml per 25 g of body weight, to give a final dosage of 0.1 mg/g). Brains were harvested 1 or 72 h after the injection, or on P16. The brains were fixed in 4% PFA in PBS at 4°C overnight, embedded in paraffin wax, and cut into 5-μm thick sections. The sections were processed for immunohistochemistry as previously described [18].

**Immunohistochemistry.** The source of antibodies and the dilutions used are as follows: Pax6, 1:10 (gift from Dr. J. Briscoe); Gsh2, 1:5,000 (gift from Dr. K. Campbell); β-galactosidase 1:2,000 (Cappel); Tuj1, 1:1,000 (Novus Biologicals, Littleton, Colorado, United States); and Sox1, 1:500; Sox2, 1:500; and Sox3, 1:500 (gift from Dr. T. Edlund). MASH1, 1:2 (gift from Dr. F. Guillemot) [18]. RC2 [56], and DARPP-32 [57] were used as previously described. For single- or double-labeled immunofluorescence, embryonic tissue was fixed either for 1 h in 4% PFA in PBS at room temperature or for 15 min in MEMFA as described before [58]. The brains were then washed in PBS, cryoprotected overnight in 30% sucrose in PBS at 4°C, embedded in OCT (Raymond Lamb), and cut in 10-μm and 15-μm sections using a cryostat. Sections were rehydrated in PBS, blocked in 4% goat serum and 0.1% Triton X-100 in PBS, and incubated with anti-SOX2 antibody in blocking solution. After incubation, the slides were washed in PBS and incubated with fluorescent secondary antibodies (FITC- or TRITC-labeled, 1:200 in blocking solution) for 1 h at room temperature. Slides for double immunolabeling were first immunostained for SOX1 as described above and visualized with Alexa568 goat anti-rabbit antibody (1:500, Molecular Probes, Eugene, Oregon, United States), and then incubated with unlabeled anti-rabbit secondary antibody (1:100, Dako, Glostrup, Denmark) for 1 h at room temperature to block existing unlabeled anti-SOX1 antibody. Subsequently, the slides were incubated with anti-SOX2 antibody in blocking solution without Triton X-100 for 48 h at room temperature and visualized with Alexa647 goat anti-rabbit antibody (1:500, Molecular Probes). The cross-reactivity of the SOX1 and SOX2 antibodies when using immunohistochemistry was excluded by looking at tissues where SOX1 (lens [37]) or SOX2 (see Figure 7B–7E) are uniquely expressed. For single- or double-labeled immunofluorescence, embryonic tissue was fixed either for 1 h in 4% PFA in PBS at room temperature or for 15 min in MEMFA as described before [58]. The brains were then washed in PBS, cryoprotected overnight in 30% sucrose in PBS at 4°C, embedded in OCT (Raymond Lamb), and cut in 10-μm and 15-μm sections using a cryostat. Sections were rehydrated in PBS, blocked in 4% goat serum and 0.1% Triton X-100 in PBS, and incubated with 48 h at room temperature and visualized with Alexa647 goat anti-rabbit secondary antibody (1:500, Molecular Probes). The cross-reactivity of the SOX1 and SOX2 antibodies when using immunohistochemistry was excluded by looking at tissues where SOX1 (lens [37]) or SOX2 (see Figure 7B–7E) are uniquely present, and in Western blots where each antibody recognizes a different size band (data not shown). After incubation with the secondary antibodies, the slides were washed in PBS and the sections were mounted with Vectashield mounting medium (Vector Laboratories, Burlingame, California, United States) and observed under a fluorescence or a confocal microscope.

**Supporting Information**

**Figure S1.** The Gsh2/Pax6 boundary is unaffected throughout development in the absence of Sox1. Similar to earlier stages (E12; see Figure 5), the expression of Gsh2 (green) and Pax6 (red) protein in wild-type (A, C, and E) and mutant brains (B, D, and F) is the same at E14 and E15, as shown by DAPI (blue) nuclear stain. Cx, cortex; Ige, lateral ganglionic eminence; mge, medial ganglionic eminence. Scale bar = 200 μm for (C) and (D).

**Figure S2.** Abnormal distribution of VS Pax6-expressing neurons in the absence of Sox1. Ventral telencephalic region of coronal brain sections stained with antibodies: Pax6 (brown) at E16 (A and B) and adult (C and D); Sox1 (green) and Pax6 (red) at E14 (E); and Sox1 and β-galactosidase (green) at E15 (F). Note the absence of Sox1 expression and the ectopic expression of Pax6 in the absence of Sox1. Arrows indicate ectopically localized Pax6-expressing cells. Pax6 and Sox1 are co-expressed in precursors but not in postmitotic cells in both mutant and wild-type. Scale bar = 500 μm for (C) and (D).

**Figure S3.** Sox1-expressing VZ precursors have radial glial morphology. Detail from immunofluorescence with β-galactosidase antibody of an E15 coronal brain section from mice carrying the Sox1<sup>geo</sup> allele. In the cortical ventricular zone, this staining reveals the cytoplasmic compartment of the SOX1-expressing progenitors, which have radial glial morphology.

**Figure S4.** The distribution of radial glia in the LGE is unaffected in the absence of Sox1. Coronal brain sections of wild-type (+/+) and Sox1-null (−/−) mice at E16. Immuno-stained with RC2 antibody (a radial glia marker) showing no differences.

**Figure S5.** Widespread presence of Sox1 and Sox2 proteins in the nuclei of the LGE VZ. Immunofluorescence (green) of E14-stage wild-type coronal brain sections at the level of the LGE stained with Sox1 (C and G) and Sox2 (D and H) antibodies and visualized under a confocal microscope. (A, B, E–H) are stained with TOTO red-fluorescent nuclear stain. (C, E, and G) and (D, F, and H) show high magnification of the area indicated in the rectangle in (A) and (B), respectively. Scale bar = 200 μm.

**Table S1.** No difference in the number of LGE dividing precursors in the absence of Sox1. BrdU-positive cells were counted using the Openlab image analysis program (Improvis, Coventry, United Kingdom). Measurements were performed in the area of the LGE (VZ/SVZ) and of the pallial VZ. All data are represented as mean ± standard error of the mean. Cell counts were done in at least three different slides (sections) from each brain and in at least three separate optical fields in each slide (n = 4). To correct for tissue thickness and accounting for a better estimate of the proliferation within the LGE, VZ/SVZ, and of the pallial VZ. All data are represented as mean ± standard error of the mean. Cell counts were done in at least three different slides (sections) from each brain and in at least three separate optical fields in each slide (n = 4). To correct for tissue thickness and accounting for a better estimate of the proliferation within the LGE, VZ/SVZ, and of the pallial VZ. All data are represented as mean ± standard error of the mean. Cell counts were done in at least three different slides (sections) from each brain and in at least three separate optical fields in each slide (n = 4).

**Accession Numbers**

The GenBank (http://www.ncbi.nlm.nih.gov/Genbank/) accession numbers for the entities discussed in this paper are Brm<sup>−/−</sup> (NM_009891), Ddxl (NM_010053), Robo (MMU_17793), Sox1 (NM_009233), and Sox2 (NM_011443).

**Acknowledgments**

We thank for antibodies Drs. K. Campbell (Children’s Hospital Research Foundation, Cincinnati, Ohio), T. Edlund (University of Uppsala, Sweden), and F. Guillemot (National Institute for Medical Research Medical Research [NIMR MRC], London). For comments on the manuscript we thank J. Briscoe (NIMR MRC) and J. Corbin (Georgetown University, Washington, DC). We are grateful to Z. Webster for the generation of transgenic mice and M. Delahaye for technical support of E14 brains. This work was supported by the MRC, the Wellcome Trust (grant 062197 to AC), and a Marie Curie Fellowship of the European Community Program (contract QLGA-CT-2001–50880 to AE). We thank the Wellcome Trust (grant 062197 to AC), and a Marie Curie Fellowship of the European Community Program (contract QLGA-CT-2001–50880 to AE). We thank for antibodies Drs. K. Campbell (Children’s Hospital Research Foundation, Cincinnati, Ohio), T. Edlund (University of Uppsala, Sweden), and F. Guillemot (National Institute for Medical Research Medical Research [NIMR MRC], London). For comments on the manuscript we thank J. Briscoe (NIMR MRC) and J. Corbin (Georgetown University, Washington, DC). We are grateful to Z. Webster for the generation of transgenic mice and M. Delahaye for technical support of E14 brains. This work was supported by the MRC, the Wellcome Trust (grant 062197 to AC), and a Marie Curie Fellowship of the European Community Program (contract QLGA-CT-2001–50880 to AE).

**Competing Interests.** The authors have declared that no competing interests exist.

**Author Contributions.** AE, IK, SM, HW, and VE conceived and designed the experiments. AE, IK, SM, HW, PA, MD, and VE performed the experiments. AE, IK, SM, HW, and VE analyzed the data. AE, IK, SM, HW, DK, AC, RL, and VE contributed reagents/materials/analysis tools. VE wrote the paper.
References

1. Anderson S, Mione M, Yun K, Rubenstein JL (1999) Differential origins of neocortical projection and local circuit neurons: Role of Dlx genes in neocortical interneuronogenesis. Cereb Cortex 9: 646–654.

2. Anderson SA, Marin O, Horn C, Jennings K, Rubenstein JL (2001) Distinct cortical migrations from the medial and lateral ganglionic eminences. Development 128: 353–363.

3. Parnavelas JG (2000) The origin and migration of cortical neurones: New vistas. Trends Neurosci 23: 126–131.

4. Deacon TW, Pakzaban P, Fischman S, Isacson O (1994) The lateral ganglionic eminence is the origin of cells committed to striatal phenotypes: Neural transplantation and developmental evidence. Brain Res 608: 211–219.

5. Heimer L (2000) Basal forebrain in the context of schizophrenia. Brain Res Rev 31: 235–255.

6. Stoykova A, Fritsch R, Walther C, Gruss P (1996) Forebrain patterning defects in small eye mutant mice. Development 122: 3453–3465.

7. Fernandez AS, Pieau C, Reparant J, Boncini E, Wassef M (1999) Expression of the Enhancer of Split-1 and Dlx-2 homeobox genes in mouse embryonic molecularly distinct domains in the telencephalon of mouse, chick, turtle and frog embryos: Implications for the evolution of telencephalic subdivisions in amniotes. Development 125: 2099–2111.

8. Anderson SA, Quinones-Baldrich W, Eisenstat DD, Meenees J, et al. (1997) Mutations of the homeobox genes Dlx-1 and Dlx-2 disrupt the striatal subventricular zone and differentiation of late born striatal neurones. Neuron 19: 27–37.

9. Corbin JG, Gatti N, Marichal RP, Langston A, Fishell G (2000) The Gsh2 homeodomain gene controls multiple aspects of telencephalic development. Development 127: 5007–5020.

10. Toresson H, Potter SS, Campbell K (2000) Genetic control of dorsal-ventral identity in the telencephalon: Opposing roles for Pax6 and Gsh2. Development 127: 4363–4371.

11. Yun K, Potter S, Rubenstein JL (2001) Gsh2 and Pax6 play complementary roles in dorsal-ventral patterning of the mammalian telencephalon. Development 128: 195–204.

12. Yun K, Vareau S, Fischman S, Rubenstein JL (2003) Patterning of the lateral ganglionic eminence by the Gsh1 and Gsh2 homeobox genes regulates striatal and olfactory bulb histogenesis and the growth of axons through the basal ganglia. J Comp Neurol 461: 151–165.

13. Szucsk EC, Witt D, Li H, Piyatek SK, Small KM, et al. (1997) Altered forebrain and hindbrain development in mice mutant for the Gsh-2 homeobox gene. Dev Biol 191: 250–292.

14. Jimenez D, Lopez-Mascaraque LM, Valverde F, De Carlos JA (2002) Tracking of the Dlx-1 and Dlx-2 homeobox genes during the lateral ganglionic eminence of the developing dorsal telencephalon and their role in cell migration from the lateral ganglionic eminence into the striatum. J Neurosci 16: 6146–6156.

15. Bayer SA, Altman J, Russo RJ, Dalf DP, Simmons JA (1991) Cell migration in the rat embryonic neocortex. J Comp Neurol 307: 499–516.

16. Horton S, Meredith A, Richardson JA, Johnson JE (1999) Correct coordination of neuronal differentiation events in ventral forebrain requires the bHLH factor MASH1. Mol Cell Neurosci 14: 355–369.

17. Böhlen SH, Vickers JW, Wu J, Jones BK, Gao J, et al. (1999) Neurogenesis in the early mouse telencephalon: Localization of Sox-1 and Sox-2. Development 122: 509–520.

18. Casarosa S, Fode C, Guillemot F (1999) Mash1 regulates neurogenesis in the ventral forebrain and subpallial derivatives in the embryonic chick and mouse telencephalon, traced by the expression of the genes Dlx-2, Emx-1, Nkx-2.1, Pax-6, and Tbr-1. J Comp Neurol 419: 424–458.

19. Malatesta P, Hack MA, Hartfuss E, Kettenmann H, Klinkert W, et al. (2005) Neuronal or glial progeny: Regional differences in radial glia fate. Neuroreport 16: 751–754.

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

21. Pinos JM, Edwards DA, Yamamoto M, Caviness VS Jr (1998) Identification of radial glial cells within the developing murine central nervous system: Studies based upon a new immunohistochemical marker. Brain Res Dev Brain Res 45: 95–109.

22. Rajewsky K, Gu H, Kuhn R, Betz UA, Muller W, et al. (1996) Conditional gene targeting. J Clin Invest 98: 690–693.

23. Overton PM, Meadows LA, Urban J, Russell S (2002) Evidence for differential and redundant function of the Sox genes Dichaete and SoxN during CNS development in Drosophila. Development 129: 427–438.

24. Buescher M, Hing FS, Chia W (2002) Formation of neuroblasts in the embryonic central nervous system of Drosophila melanogaster is controlled by SoxNeuro. Development 129: 4193–4203.

25. Zhao G, Skeath JB (2003) The Sox2 domain containing gene Dichaete-fishhook acts in concert with vnd and ind to regulate cell fate in the Drosophila neuroectoderm. Development 129: 1165–1174.

26. Valerius MT, Li H, Stock JL, Weinstein M, Kaur S, et al. (1995) Gsh-1: A murine homeobox gene expressed in the developing central nervous system. Dev Dyn 203: 357–351.

27. Hsieh-Li HM, Witte DP, Szucsik JC, Weinstein M, Li H, et al. (1995) Gsh-2, a phosphoprotein enriched in dopamine-innervated brain regions. III. DARPP-32, a dopamine- and adenosine 3’,5’-monophosphate-regulated phosphoprotein enriched in dopamine- and adenosine 3’,5’-monophosphate-regulated phosphoprotein-enriched brain regions. III. Immunocytotoxicological localization. J Neurosci 4: 1114–1124.

28. Peynv LH, Sockanathan S, Placzek M, Lovell-Badge R (1998) A role for SOX1 in neural determination. Development 125: 1967–1978.