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Genetic control of dorsal-ventral identity in the telencephalon: opposing roles for *Pax6* and *Gsh2*

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SUMMARY

We have examined the genetic mechanisms that regulate dorsal-ventral identity in the embryonic mouse telencephalon and, in particular, the specification of progenitors in the cerebral cortex and striatum. The respective roles of *Pax6* and *Gsh2* in cortical and striatal development were studied in single and double loss-of-function mouse mutants. *Gsh2* gene function was found to be essential to maintain the molecular identity of early striatal progenitors and in its absence the ventral telencephalic regulatory genes *Mash1* and *Dlx* are lost from most of the striatal germinal zone. In their place, the dorsal regulators, *Pax6*, neurogenin 1 and neurogenin 2 are found ectopically. Conversely, *Pax6* is required to maintain the correct molecular identity of cortical progenitors. In its absence, neurogenins are lost from the cortical germinal zone and *Gsh2*, *Mash1* and *Dlx* genes are found ectopically. These reciprocal alterations in cortical and striatal progenitor specification lead to the abnormal development of the cortex and striatum observed in *Pax6* (small eye) and *Gsh2* mutants, respectively. In support of this, double homozygous mutants for *Pax6* and *Gsh2* exhibit significant improvements in both cortical and striatal development compared with their respective single mutants. Taken together, these results demonstrate that *Pax6* and *Gsh2* govern cortical and striatal development by regulating genetically opposing programs that control the expression of each other as well as the regionally expressed developmental regulators *Mash1*, the neurogenins and *Dlx* genes in telencephalic progenitors.

Key words: Cerebral cortex, *Rbp1*, *Dlx*, *Emx1*, *Isl1*, Lateral ganglionic eminence, *Mash1*, Neurogenins, Striatum, Mouse

INTRODUCTION

In the embryonic telencephalon, the cerebral cortex and striatum develop largely from two adjacent neurogenic territories along the dorsal-ventral axis. The cortex derives from the pallium (Bayer and Altman, 1991), while the more ventrally situated lateral ganglionic eminence (LGE) gives rise to the majority of striatal neurons (Deacon et al., 1994; Olsson et al., 1995, 1998). A number of developmental control genes are specifically expressed by progenitors in either the cortex or LGE. For instance, progenitor cells in the developing cortex express the homeobox gene *Emx1* (Simeone et al., 1992; Briata et al., 1996) as well as the basic helix-loop-helix (bHLH) genes neurogenin 1 **(***Neurod3* – Mouse Genome Informatics**)** and neurogenin 2 (*Atoh4* – Mouse Genome Informatics) (Sommer et al., 1996). Progenitors in the LGE express homeobox genes of the *Dlx* family (Liu et al., 1997) and *Vax1* (Hallonet et al., 1998) as well as the bHLH gene *Mash1* (*Ascl1* – Mouse Genome Informatics) (Guillemot et al., 1993; Porteus et al., 1994). Each of these genes has been shown to be required for the normal development of their respective telencephalic compartments (Yoshida et al., 1997; Anderson et al., 1997b; Casarosa et al., 1999; Horton et al., 1999; Fode et al., 2000). Although the molecular and cellular mechanisms underlying the establishment of these expression domains along the dorsal-ventral axis of the embryonic telencephalon is not completely clear, it appears that there are many similarities with more caudal parts of the neural tube. Dorsal-ventral patterning in the developing spinal cord and hindbrain is known to be regulated by the actions of bone morphogenetic proteins (BMPs) and sonic hedgehog (SHH) emanating from the dorsal and ventral midline, respectively (Tanabe and Jessell, 1996). These signals establish domains of regulatory gene expression along the dorsal-ventral axis that subsequently control the generation of specific cell types (Ericson et al., 1997; Liem et al., 1997; Briscoe et al., 1999). SHH is also capable of inducing ventral gene expression in the telencephalon (Ericson et al., 1995; Kohtz et al., 1998; Gaiano et.al., 1999). BMPs, conversely, have been shown to regulate the expression of certain dorsal telencephalic markers in explant assays (Furuta et al., 1997). These signalling molecules, however, remain confined to the ventromedial (Shimamura et al., 1995) and dorsomedial

aspects (Furuta et al., 1997), respectively, despite a dramatic expansion of the telencephalic neuroepithelium. Thus, cell- and/or region-intrinsic factors must maintain the molecular specification of telencephalic progenitors located at different positions along the dorsal-ventral axis throughout neurogenesis.

In this respect, it is interesting to consider the homeobox genes *Pax6* and *Gsh2*, which show complementary expression in progenitors of the cortex and LGE, respectively. Small eye (*Sey*) mice, containing a point mutation in the *Pax6* gene (Hill et al., 1991), show severe disruptions in the development of the cortex (Schmahl et al., 1993; Caric et al., 1997). These abnormalities include a progressive dorsal spread of *Dlx1* and *Vax1* expression, in cortical cells (Stoykova et al., 1996; Hallonet et al., 1998). Conversely, *Gsh2* mutants show a reduction in the size of the LGE and a loss of *Dlx2* expression in the LGE but not the medial ganglionic eminence (MGE) (Szucsik et al., 1997). These findings implicate *Pax6* and *Gsh2* in the establishment and/or maintenance of dorsal-ventral identity. In this study we have examined the respective roles of *Pax6* and *Gsh2* in the specification of progenitors in the cortex and LGE. By studying single and double loss-of-function mouse mutants, we demonstrate that *Pax6* and *Gsh2* genetically oppose each other in the maintenance of *Mash1*, *Dlx* and neurogenin gene expression in telencephalic progenitors. Alterations in the expression domains of these genes in *Sey/Sey*, (i.e. *Pax6* mutant) and *Gsh2*$^{-/-}$ embryos, leads to abnormal development of the cortex and striatum, respectively.

MATERIALS AND METHODS

Genotyping of embryos

Sey/Sey mutants were identified by the lack of eyes. At early stages (e.g. E12.5), wild types were obtained from non-littermates, while in the later stage litters (E14 and E16.5) genotyping for wild types and heterozygotes was also carried out based on eye morphology as described by Hill et al. (1991). *Gsh2*$^{-/-}$ mutants were genotyped using PCR as previously described (Szucsik et al., 1997). Double *Sey/Gsh2* homozygotes were first identified by the absence of eyes and then with PCR for the mutant and wild type *Gsh2* alleles. All genotyping was confirmed by immunostaining for GSH2 or PAX6 (or both in the case of the *Sey/Gsh2* double mutants).

Histological analysis

All embryos were fixed overnight in 4% paraformaldehyde at 4°C and subsequently cryoprotected in 30% sucrose before sectioning at 10-12 μm on a cryostat. Immunohistochemistry was performed as described previously for DLX and RBP1 (Toresson et al., 1999). The other primary antibodies were used at the following concentration; rabbit anti-PAX6 at 1:400 (provided by S. Wilson), rabbit anti-ISL1/2 at 1:400 (provided by T. Edlund), mouse anti-MASH1 at 1:5 (provided by D. J. Anderson), rabbit anti-SCIP at 1:100 (provided by G. Lemke), rabbit anti-Ki67 at 1:100 (Dianova) and rabbit anti-EMX1 used at 1:500 (provided by G. Corti). The GSH2 antibody was raised in rabbits against the C-terminal peptide, ANEDKEISPL (Hsieh-Li et al., 1995). This antibody was used at a 1:5000 dilution. In situ hybridization was performed as described (Toresson et al., 1999), using digoxigenin-labeled cRNA probes for *Gsh2*, *Mash1*, neurogenin 1, neurogenin 2 and *Pax6*. TUNEL staining was carried out on embryos at E10.5, E11.5, E12.5, E14.5 and E16.5 according to the protocol provided by Boehringer Mannheim, and detection was made by fluorescence microscopy.

RESULTS

Complementary expression of PAX6 and GSH2 in telencephalic progenitors

The expression patterns of PAX6 and GSH2 in the embryonic telencephalon suggests a role for these molecules in the regulation of dorsal-ventral identity. Indeed, already at E10.5, when GSH2 is first detected in the ventral telencephalon (Fig. 1A), its expression was largely complementary to that of the cortically expressed PAX6 (Fig. 1B). By E12.5, when both the MGE and LGE were morphologically distinct, GSH2 was found in progenitors of both the MGE and LGE ventricular zone (VZ) (Fig. 1C). At this stage, PAX6 was expressed at high levels throughout the cortical VZ and into the lateral-most part of the LGE, forming a boundary with GSH2 (Fig. 1D). Beyond this limit, PAX6 was expressed at low levels in the LGE VZ (Fig. 1D and Sussel et al., 1999). The expression level of both PAX6 and GSH2 was graded along the dorsal-ventral axis, with the highest levels present in progenitor cells close to the boundary. In addition to the VZ expression, PAX6 was also observed in a stream of cells emanating from the boundary of PAX6 and GSH2 expression in the VZ (Fig. 1D). These cells have previously been suggested to form portions of the claustrum and amygdala (Puelles et al., 1999). The expression patterns of GSH2 and PAX6 were maintained throughout embryogenesis, marking the boundary between the LGE and cortical VZ (Fig. 1E,F).

Molecular disturbances in striatal progenitors of the *Gsh2*$^{-/-}$ LGE

In addition to the reported abnormalities in *Dlx2* expression (Szucsik et al., 1997), we show here that *Gsh2* mutants exhibit altered expression patterns of several developmental control genes in the LGE. At E11.5, when the LGE is first morphologically distinct, DLX proteins (including DLX1,2,5 and 6; Fig. 2A) and MASH1 (Fig. 2B), which are normally expressed at high levels in progenitors of the LGE were missing from a large portion of the *Gsh2* mutant LGE (Fig. 2G,H). Interestingly, however, the targeted *Gsh2* transcript could still be detected in its normal expression domain (compare Fig. 2I with 2C). The loss of DLX and MASH1 expression correlated well with ectopic expression of cortical markers in progenitors of the mutant LGE. Specifically, PAX6 (Fig. 2D) was dramatically upregulated in the mutant LGE (Fig. 2J), while the bHLH genes neurogenin 1 (not shown) and neurogenin 2 (Fig. 2E) were found ectopically in mutant LGE cells (Fig. 2K and data not shown).

Similar alterations in the expression of PAX6 (Fig. 3B), neurogenin 1 and neurogenin 2 (data not shown), DLX (data not shown), and MASH1 (Fig. 3D) were also observed in the E12.5 LGE of the *Gsh2* mutant. The mis-specification of striatal progenitors at this stage, however, is less severe than at E11.5 with almost half of the LGE expressing MASH1 and DLX. In addition to the mis-specification of progenitors in the LGE VZ, the size of the proliferative subventricular zone (SVZ) in the *Gsh2* mutant LGE, as marked by the cell cycle marker Ki67 (Schlüter et al., 1993), is reduced (Fig. 3F) when compared with the wild type (Fig. 3E). Furthermore, the stream of PAX6-positive cells in the lateral LGE, which normally emanates from the boundary between the cortical and striatal

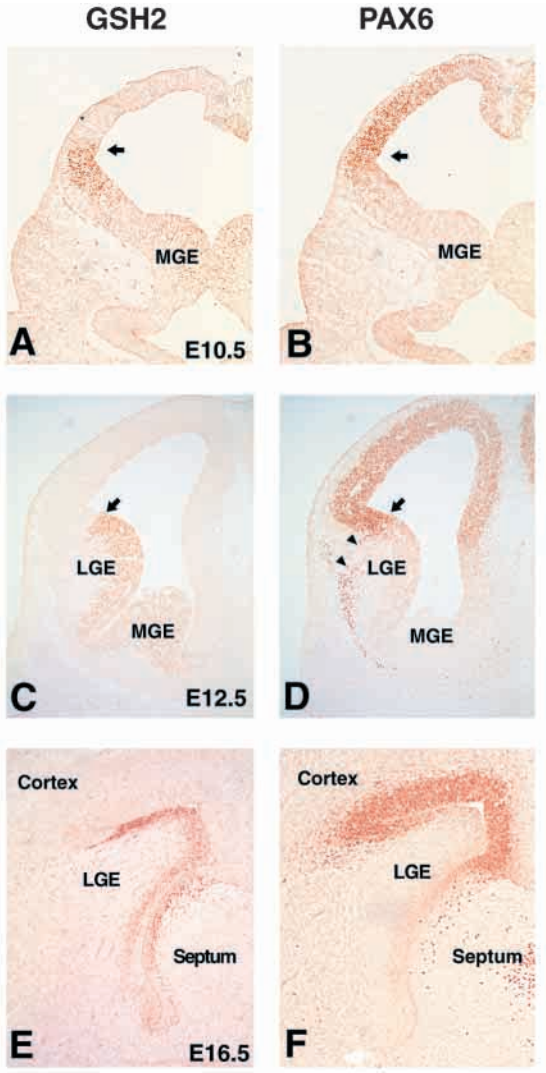

Fig. 1. Complementary expression of GSH2 and PAX6 in telencephalic progenitors. Coronal sections of E10.5 (A,B), E12.5 (C,D) and E16.5 (E,F) mouse telencephalon. (A) GSH2 expression in cells of the ventral telencephalic VZ. The highest level of GSH2 protein is detected in the lateral telencephalon. (B) Cells expressing high levels of PAX6 are largely found dorsal to the domain of GSH2 expression in the cortical VZ. Arrows in A and B point to the dorsal and ventral limits of GSH2 and PAX6 expression domains, respectively. (C,D) At E12.5, the boundary (indicated by arrow) between the GSH2 and PAX6 expression domains is more distinct. GSH2 expression is found in cells of both the LGE and MGE. Note that the expression level of both proteins is graded with the highest levels near the boundary. Arrowheads in D point to the stream of PAX6-positive cells in the lateral LGE. (E,F) The expression domains of GSH2 and PAX6 remain complementary also at later stages of neurogenesis.

VZ (Fig. 1D and 3A) was severely reduced in the *Gsh2* mutant (Fig. 3B).

As noted above, the targeted *Gsh2* transcript in the mutant LGE could still be detected in its normal expression domain (Fig. 2I), indicating that only a partial mis-specification of LGE progenitors has occurred. Indeed, cellular retinol binding protein 1 (RBP1), which is normally expressed in LGE VZ cells (Toresson et al., 1999 and Fig. 3C), was still expressed in its normal domain in the mutant LGE at E12.5 (Fig. 3D). Moreover, expression of the cortically restricted homeobox protein, EMX1 (Fig. 2F) was not altered in the *Gsh2* mutant (Fig. 2L). *Gsh2*, therefore, appears to act in parallel with other genetic pathways to control the molecular specification of early LGE progenitors.

The abnormalities observed in LGE development at earlier stages in *Gsh2* mutants were less pronounced by E16.5. Although the mutant LGE is notably smaller than the wild type, numerous Ki67-positive cells (i.e. proliferating progenitors) were observed in the SVZ of the mutant LGE (data not shown). Furthermore, MASH1 (Fig. 4B) and DLX proteins (data not shown) were expressed in progenitors throughout the LGE up to the LGE/cortex boundary, while PAX6 (Fig. 4B) and neurogenin 2 (data not shown) were no longer ectopically expressed in LGE progenitors.

Abnormal striatal development in *Gsh2*$^{-/-}$ mutants

Since the LGE is known to generate the vast majority of striatal neurons (Deacon et al., 1994; Olsson et al., 1995, 1998) the early mis-specification of VZ progenitors combined with the reduction in the proliferative SVZ in the *Gsh2* mutant is likely to have effects on striatal development. In fact, at E16.5, the size of the mutant striatal complex (including the dorsal striatum, nucleus accumbens and the olfactory tubercle), as marked by the LIM-homeodomain protein, islet 1 (ISL1) was notably reduced when compared with wild type (Fig. 4C-F). Moreover, ISL1 staining in the olfactory tubercle was missing in *Gsh2* mutants (Fig. 4D). Cell death, as detected by TUNEL staining, did not appear to contribute to this phenotype, since no differences in the number of apoptotic profiles were found between the mutant or wild-type LGE at any stage examined (data not shown). Interestingly, olfactory tubercle neurons, which are deficient in the *Gsh2* mutant, are largely generated at early time points in striatal neurogenesis (Bayer and Altman, 1995). Thus, these defects in striatal development correlate well with the early molecular mis-specification of striatal progenitors in the LGE.

Molecular mis-specification of cortical progenitors in *Sey/Sey* mice

The above results demonstrate that *Gsh2* is required to maintain the correct molecular identity of LGE precursor cells. In its absence, the dorsal genes, *Pax6*, neurogenin 1 and neurogenin 2 are ectopic, and ventral genes, like *Dlx*, and *Mash1* are lost. Interestingly, *Sey/Sey* mice (i.e. *Pax6* mutants (Hill et al., 1991) display ectopic ventral gene expression (*Dlx1* and *Vax1*) in cells of the developing cortex (Stoykova et al., 1996; Hallonet et al., 1998). We were therefore interested to determine if *Gsh2* is similarly deregulated in *Sey/Sey* cortical progenitors. Indeed, at E12.5, the expression of GSH2 was expanded throughout the lateral LGE and even into the ventrolateral cortical VZ of the *Sey/Sey* telencephalon (Fig. 5F). The expression level of the mutated *Pax6* transcript appears to be downregulated specifically within the domain of ectopic GSH2 (Fig. 5E). In addition, both *Mash1* (Fig. 5H) and DLX proteins (data not shown), whose correct LGE expression depends on *Gsh2* at this stage, were also ectopic in the germinal zone of the *Sey/Sey* cortex. The expression of the dorsally restricted neurogenin 1 and neurogenin 2 genes was

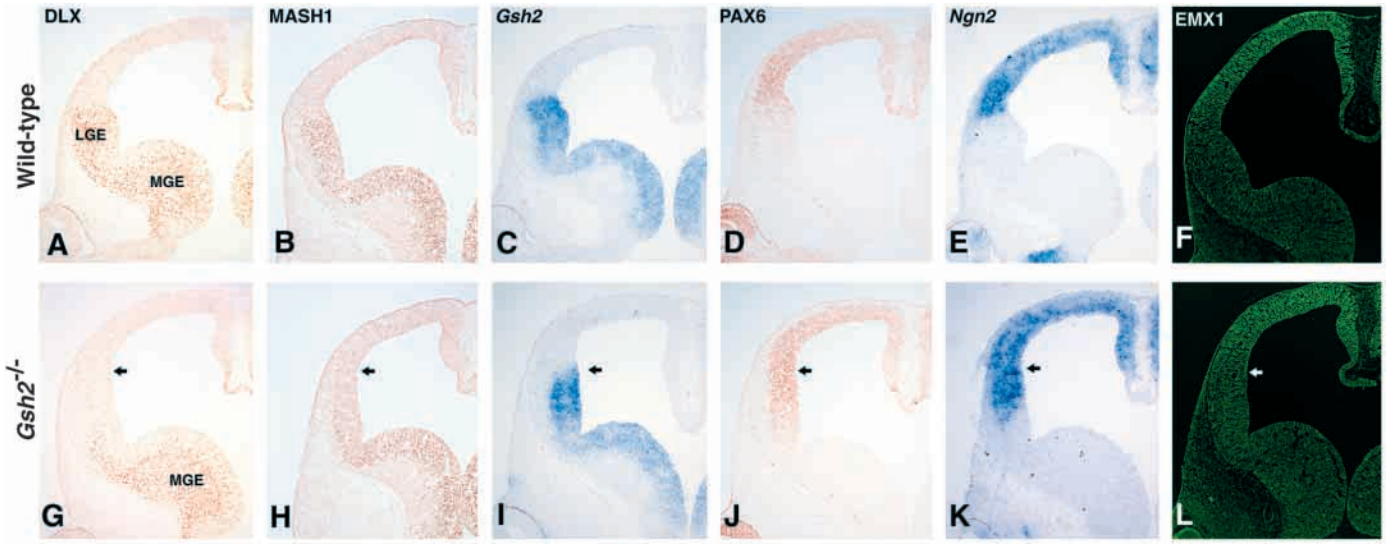

Fig. 2. Molecular mis-specification of early striatal progenitors in *Gsh2* mutants at E11.5. Coronal sections of E11.5 mouse telencephalon from wild-type (A-F) and *Gsh2*$^{-/-}$ (G-L) embryos. The ventrally expressed proteins of the DLX family (A) and MASH1 (B) show a reduced domain of expression in the *Gsh2* mutant (G,H), being expressed in only a few ventral LGE cells. In situ hybridization for *Gsh2* showing its transcript in the VZ of the LGE and MGE (C). The targeted *Gsh2* transcript is still detected in its normal domain in the *Gsh2* $^{-/-}$ telencephalon (I). PAX6 (D) and neurogenin 2 (E) are normally expressed in cortical progenitors. In the absence of *Gsh2* both are expressed ectopically in LGE progenitors (J,K). EMX1 (F), which is restricted to dorsal telencephalon in wild types, remains dorsally restricted in the *Gsh2* mutant (L). The arrows in G-L mark the dorsal extent of the mutant *Gsh2* transcript expression (i.e. the mutant LGE) in the adjacent sections.

significantly downregulated throughout the cortex and lost in the lateral-most edge of the LGE and ventrolateral cortex (Fig. 5G and data not shown).

The extent of ectopic GSH2 expression in *Sey/Sey* cortical progenitors progresses during development so that by E14 most of the lateral cortical VZ was covered (Fig. 6H). This progression was paralleled also by ectopic DLX and *Mash1* expression (Fig. 6F,G). While many of the cortical cells ectopically expressing DLX were found in the SVZ and intermediate zone, *Mash1* expression was confined to progenitor cells in the VZ. Again, despite the fact that the mutated *Pax6* transcript was expressed throughout its normal domain it was severely downregulated specifically within the domain of ectopic GSH2 expression (Fig. 6I). Furthermore, neurogenin 2 expression was completely extinguished in the dorsolateral cortical VZ where GSH2 is ectopic (Fig. 6J). These results indicate that *Pax6* is required for the maintenance of molecular identity within cortical progenitors throughout neurogenesis. Moreover, these findings implicate the ectopic expression of ventral genes (*Gsh2*, *Dlx* and *Mash1*) in the manifestation of cortical defects in *Sey/Sey* mutants.

As was the case in the *Gsh2* mutants, RBP1 (Fig. 6K) and EMX1 (Fig. 6L) expression was not altered in *Sey/Sey* mutants. These findings indicate that *Pax6* and *Gsh2* do not regulate all aspects of dorsal-ventral identity but rather a specific subset of developmental regulators, including *Mash1*, *Dlx* and neurogenin genes.

Restoration of molecular specification of LGE progenitors in *Sey/Gsh2* double homozygous mutants

The present results indicate that the loss of *Pax6* or *Gsh2* in their respective mutants leads to an imbalance in *Mash1*, *Dlx* and neurogenin gene expression and subsequent mis-specification of either cortical or striatal progenitor cells (Fig. 6M). Given this reciprocal regulation, we were interested to study telencephalic development in the absence of both *Pax6* and *Gsh2*. To do this, we generated *Sey/Gsh2* double homozygous mutants. The gross morphology of these mutants is similar to *Sey/Sey* embryos in that they lack eyes and nasal structures (data not shown). At E12.5 in the double mutant, expression of the mutated *Pax6* transcript was seen throughout the LGE (Fig. 7A), similar to that in the *Gsh2* mutant. Furthermore, the reduction in *Pax6* expression observed in the cortical VZ of *Sey/Sey* mice (Figs 5E, 6I and 7G), appeared restored to wild-type levels in the double mutants (Fig. 7A,H). These findings demonstrate that *Gsh2* is required to repress *Pax6* gene expression in the LGE and also when ectopic in the *Sey/Sey* cortical VZ. Expression of the targeted *Gsh2* allele in *Sey/Gsh2* double mutants appeared rather normal at E12.5 (Fig. 7B). At later stages, weak expression was found in the cortical VZ (data not shown), suggesting that functional GSH2 is required for the full ectopic expression of *Gsh2* in the *Sey/Sey* cortex.

Unlike the case in *Gsh2* mutants, at E12.5 in *Sey/Gsh2* double homozygous mutants, DLX (data not shown) and MASH1 (Fig. 7C) are expressed throughout the LGE and even into the ventrolateral cortex, similar to that seen in *Sey/Sey* embryos (Fig. 5H). Furthermore, at E16.5 DLX (Fig. 7F) and MASH1 (data not shown) remain ectopic in progenitors of the double mutant cortex but at a somewhat reduced level than in the *Sey/Sey* cortex (Fig. 7E). Moreover, proliferation in the double mutant SVZ (as marked by Ki67 expression) appeared to be improved over that in the *Gsh2* mutants but clearly not restored to wild-type levels (data not shown).

In addition to the improvements in DLX and MASH1

expression, neurogenin 2 was only found in a small number of LGE progenitors of the *Sey/Gsh2* double mutant (Fig. 7D). Furthermore, neurogenin 2 expression in the double mutants was similar to that in *Sey/Sey* (Figs 5F and 6J), being notably downregulated in the cortex at E12.5 (Fig. 7D) and lost in the dorsolateral cortex by E16.5 (data not shown).

Improved development of the striatum and cerebral cortex in *Sey/Gsh2* double homozygous mutants

The restoration of molecular identity in the LGE of *Sey/Gsh2* double homozygous mutants correlated with significant improvements in striatal development. In the double mutant embryos, the striatal complex was larger than in *Gsh2* mutants and the olfactory tubercle (as marked by ISL1) was present in each case (Fig. 8A,B). Thus, ectopic *Pax6* in the *Gsh2* mutant LGE appears to be largely responsible for the striatal phenotype observed in these mutants. Unlike the progenitors in the LGE, cortical progenitors in the *Sey/Gsh2* double mutant remain mis-specified with respect to *Mash1*, *Dlx*, neurogenin 1 and neurogenin 2 gene expression. Similar to that observed in the *Sey/Sey* cortex (Fig. 8E), the number of proliferating cells

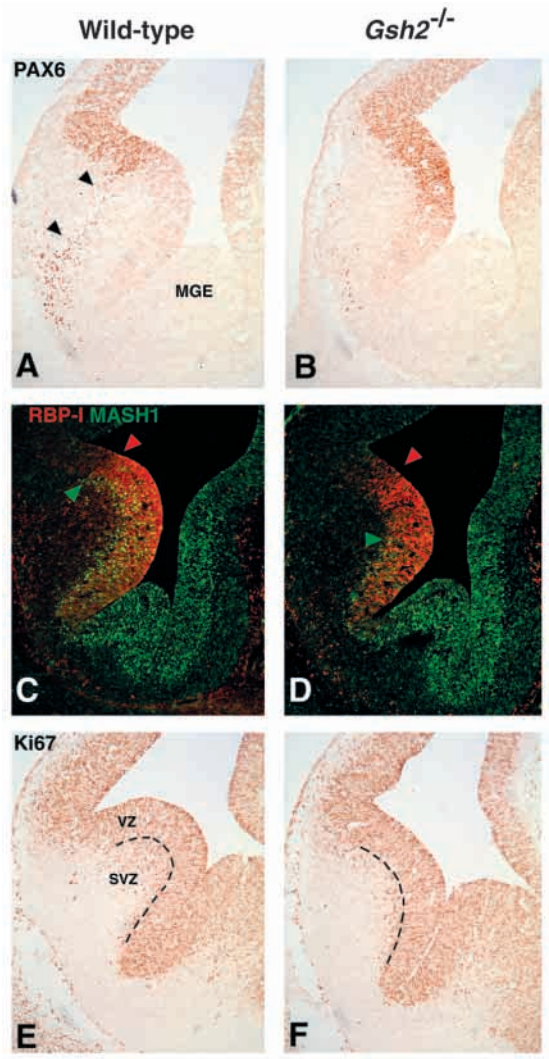

Fig. 3. Alterations in striatal progenitors in *Gsh2*$^{-/-}$ embryos at E12.5. **(**A,B) PAX6 expression at E12.5. PAX6 remains ectopically expressed in the *Gsh2* mutant LGE occupying more than half of the LGE VZ (B). Arrowheads in A point to the stream of PAX6-positive cells in the lateral LGE. These cells are severely reduced in the *Gsh2* mutant (B). (C,D) Confocal images showing the expression of RBP1 (red) and MASH1 (green). At E12.5, both of these molecules are detected in the wild-type LGE VZ with completely overlapping domains (C). In *Gsh2* mutants, RBP1 is still expressed in its normal domain, while MASH1-expressing cells are only detected in the ventral half of the LGE (D). Red and green arrowheads in C and D mark the dorsal limit of RBP1 and MASH1, respectively. (E,F) Ki67 staining of progenitor cells in the VZ and SVZ (separated by broken line) of wild-type (E) and *Gsh2* mutant LGE (F). Note the reduction in Ki67-positive cells in the mutant SVZ (F).

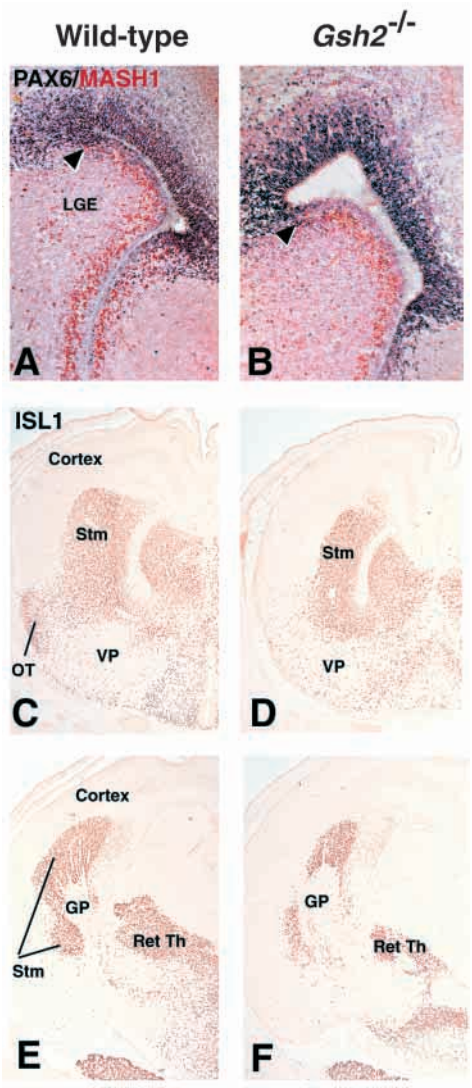

Fig. 4. Striatal development is disturbed in *Gsh2* $^{-/-}$ mice. **(**A,B) The mis-specification of LGE progenitors at earlier stages of striatal neurogenesis is not apparent at E16.5 where PAX6 (black nuclei) and MASH1 (red nuclei) share a boundary of expression (arrowhead) which corresponds to the LGE/cortex angle in both the wild type (A) and *Gsh2* mutant (B). (C-F) Immunohistochemistry for ISL1 reveals that at E16.5, the striatum (Stm) in *Gsh2*$^{-/-}$ embryos (D,F) is reduced in size when compared with wild types (C,E). At rostral levels, the olfactory tubercle (OT) is missing in *Gsh2* mutants (D) while the reduction in striatal size is even more apparent at caudal levels (F). GP, globus pallidus; Ret Th, reticular thalamic nucleus; VP, ventral pallidum.

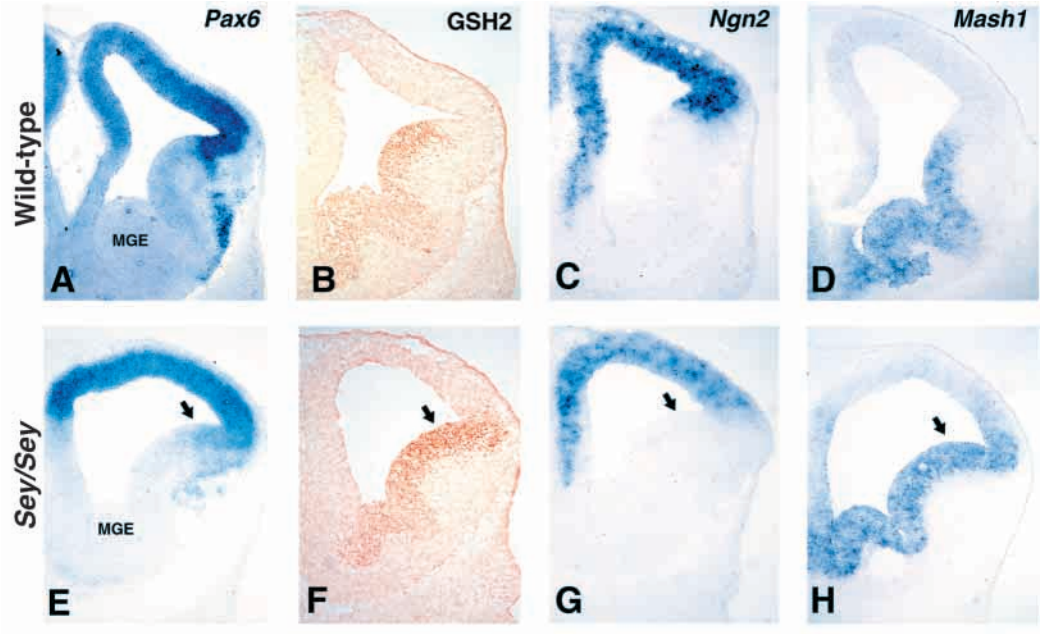

Fig. 5. Molecular mis-specification of cortical progenitors in the E12.5 *Sey/Sey* telencephalon. Coronal sections of E12.5 wild-type (A-D) and *Sey/Sey* (E-H) mouse telencephalon. (A) *Pax6* expression showing the highest levels close to the border of GSH2 expression (B). In *Sey/Sey*, the mutant *Pax6* transcript can be detected in its normal domain, however, the level in the lateral-most part of the LGE is reduced (E). This reduction correlates well with the extent of the expanded GSH2 domain (F) and the disappearance of neurogenin 2 transcripts (G). Already at this stage, the level of neurogenin 2 is reduced in its remaining expression domain (compare C with G). *Mash1* transcripts, normally present in the MGE and LGE VZ (D), are expanded into the ventrolateral cortical VZ in *Sey/Sey* (H). Arrows in E-H indicate the normal boundary between *Pax6* and *Gsh2* expression.

in the double mutant cortex, as marked by the expression of Ki67, was increased when compared with wild types (Fig. 8D,E). This increase in progenitors resulted in an enlargement of VZ/SVZ in the cortex of both *Sey/Gsh2* and *Sey/Sey* mutants (Fig. 8G,H). Despite these facts, however, development of the cortex in *Sey/Gsh2* double mutants was considerably improved over that in *Sey/Sey* embryos. The intermediate zone and cortical plate, which are severely deficient in *Sey/Sey* embryos (Fig. 8H and Schmahl et al., 1993; Caric et al., 1997), were restored in the double mutants, similar to wild types (Fig. 8F,G). Moreover, expression of the POU-domain transcription factor SCIP (POU3F1 – Mouse Genome Informatics), which marks migrating cortical neurons (Frantz et al., 1994), was more similar to that in wild types than in *Sey/Sey* mutants (Fig. 8I-K). Thus, ectopic *Gsh2* in the *Sey/Sey* cortex impedes the normal formation of the cortical plate.

DISCUSSION

Opposing roles for *Pax6* and *Gsh2* in the specification of telencephalic progenitors

The results of this study demonstrate that *Pax6* and *Gsh2* genetically oppose each other in the regulation of molecular identity within cortical and striatal progenitors, respectively. In this respect, *Gsh2* gene function is required to repress *Pax6* expression both in the wild-type LGE and when ectopic in the progenitors of the *Sey/Sey* cortex. Conversely, *Pax6* appears to be necessary for the repression of *Gsh2* in cortical progenitors but when ectopic in the LGE, as in the *Gsh2* mutant, no effect on the expression of the targeted *Gsh2* transcript is observed. This finding suggests that other factors, which are confined to the cortical VZ in the *Gsh2* mutants are required to co-operate with *Pax6* to repress *Gsh2* in cortical progenitors. In addition to their opposing regulation of each other, *Pax6* and *Gsh2* also reciprocally control the expression of *Mash1*, *Dlx* and neurogenin genes in telencephalic progenitors. *Pax6* appears to be required to maintain the expression of neurogenin 1 and neurogenin 2, as well as to repress *Mash1* and *Dlx* gene expression in cortical progenitors. Recently, Fode et al. (2000) demonstrated that neurogenins were required to repress *Mash1* and *Dlx* genes in the developing cerebral cortex. It is, therefore, likely that the apparent repression of these genes by *Pax6* is actually mediated via the neurogenins (Fig. 9). *Gsh2*, however, is not required for the expression of *Mash1* and *Dlx* genes or the repression of neurogenin 1 and neurogenin 2. Thus, an essential role for *Gsh2* in telencephalic development is to repress *Pax6* and its dorsalizing program in the LGE and thereby protect ventral identity (Fig. 9).

As is the case in *Sey/Sey* mutants, *Mash1* and *Dlx* genes are also found ectopically in the cortex of neurogenin 1 and neurogenin 2 mutants (Fode et al., 2000). The temporal profile of this phenotype, however, is different in the two mutants. While *Mash1* and *Dlx* genes are already ectopic throughout the cortex in the neurogenin 1 and neurogenin 2 mutants at E12.5 (Fode et al., 2000), their misexpression in the *Sey/Sey* cortex is only beginning at E12.5 but occupies most of the dorsolateral germinal zone by E16.5. This progressive phenotype is likely due, at least in part, to the fact that neurogenins are induced independently of *Pax6* and are thus present in the early *Sey/Sey* cortex but disappear at later stages. It should be mentioned that the progressive spread of ventral gene expression in the *Sey/Sey* cortex has previously been suggested to result from the increased tangential migration of neurons, taking origin from the ventral telencephalon (Stoykova et al., 1997). In fact, Chapouton et al. (1999) have recently demonstrated that increased numbers of ventrally generated neurons do cross the cortical-striatal boundary in *Sey/Sey* mutants and take up residence in the cortex, particularly in the ventrolateral regions. Our results, however, demonstrate that in addition to increased

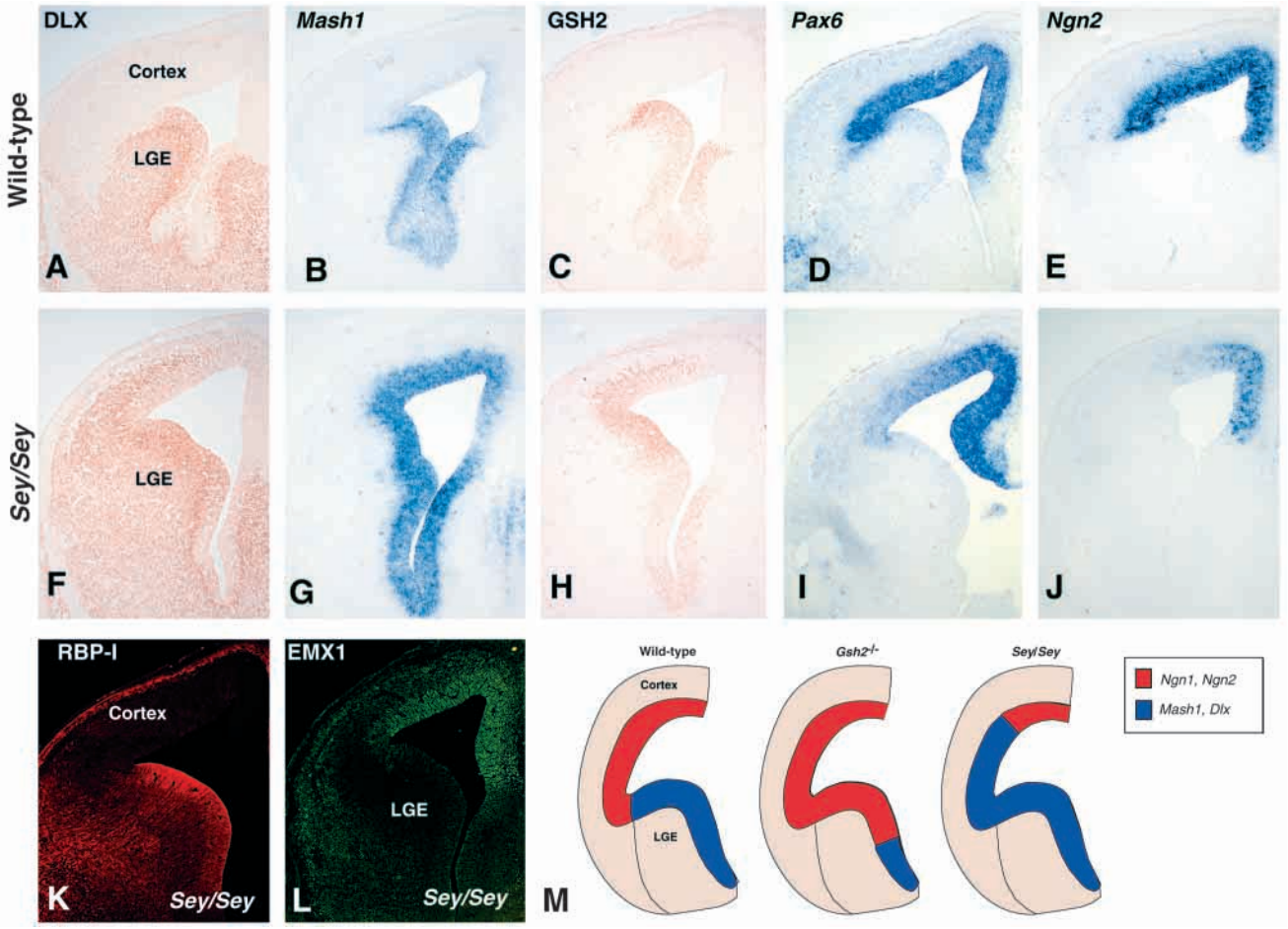

Fig. 6. Molecular mis-specification of cortical progenitors in the E14 *Sey/Sey* telencephalon. Coronal sections of E14 wild-type (A-E) and *Sey/Sey* (F-L) mouse telencephalon. By E14, the ventrally expressed DLX (A), *Mash1* (B) and GSH2 (C) are ectopic throughout much of the cortex (F-H). While *Mash1* and GSH2 are ectopic primarily within the VZ, DLX cells are mainly found deeper, in the SVZ and intermediate zone. The mutated *Pax6* transcript can still be detected in its normal domain but its expression level is significantly lower in the lateral cortex (compare I with D). Neurogenin 2 (E) is almost completely lost in precursors of the *Sey/Sey* lateral cortex (J). Note that the reductions in *Pax6* (I) and neurogenin (J) expression correlate well with the expansion of GSH2 (H). The expression domains of both RBP1 (K) and EMX1 (L) are unaltered in the *Sey/Sey* telencephalon marking the LGE and cortical VZ, respectively. (M) Model summarizing the results in *Gsh2* and *Sey/Sey* mutants. When *Gsh2* function is lost, the boundary between neurogenin 1, neurogenin 2 (red) and *Mash1*, *Dlx* genes (blue) shifts ventrally. The reverse is true in *Sey/Sey* mutants where this boundary shifts dorsally.

tangential migration of ventrally derived neurons, the loss of *Pax6* results in a progressive mis-specification of cortical progenitors. In support of this, Fode et al. (2000) showed that the ectopic expression of *Dlx1* in the cortex of neurogenin 2 mutants was a direct consequence of the mis-specification of cortical progenitors in the absence of neurogenin function and not due to increased migration of ventrally derived neurons.

The present findings indicate that the lateral telencephalon, including the LGE and dorsolateral cortex, is predisposed to express *Mash1* and *Dlx* genes. It is only through a *Pax6* and neurogenin-mediated repression that *Mash1* and *Dlx* genes are absent from most cortical progenitors during neurogenesis. Since *Mash1* and *Dlx* genes are known to be required for the generation of cortical interneurons (Anderson et al., 1997a; Casarosa et al., 1999), this mechanism may represent a way to generate cellular diversity in the cerebral cortex. Although many cortical interneurons are derived from the ventral telencephalon (for a review, see Parnavelas, 2000), at least some have been shown to derive from single progenitors of the cortical VZ that are capable of generating both glutamatergic projection neurons and GABAergic interneurons (Götz et al., 1995). In fact, wild-type cortical progenitors are known to express low but detectable levels of *Mash1* (Guillemot et al., 1993). Moreover, in the absence of neurogenin 2 function, ectopic expression of *Mash1* and *Dlx* genes is accompanied by an increase the number of neurons expressing the mRNA for the GABA-synthesizing enzyme glutamic acid decarboxylase, in the mutant cortex (Fode et al., 2000).

Radial migration defects in the *Sey/Sey* cortex are due to ectopic *Gsh2*

Sey/Sey mutants exhibit multiple defects in cortical development (Schmahl et al., 1993), including the disturbed migration of late-born cortical neurons into the developing cortical plate (Caric et al., 1997). This defect appears to be non-cell autonomous, since late-born mutant cells transplanted to

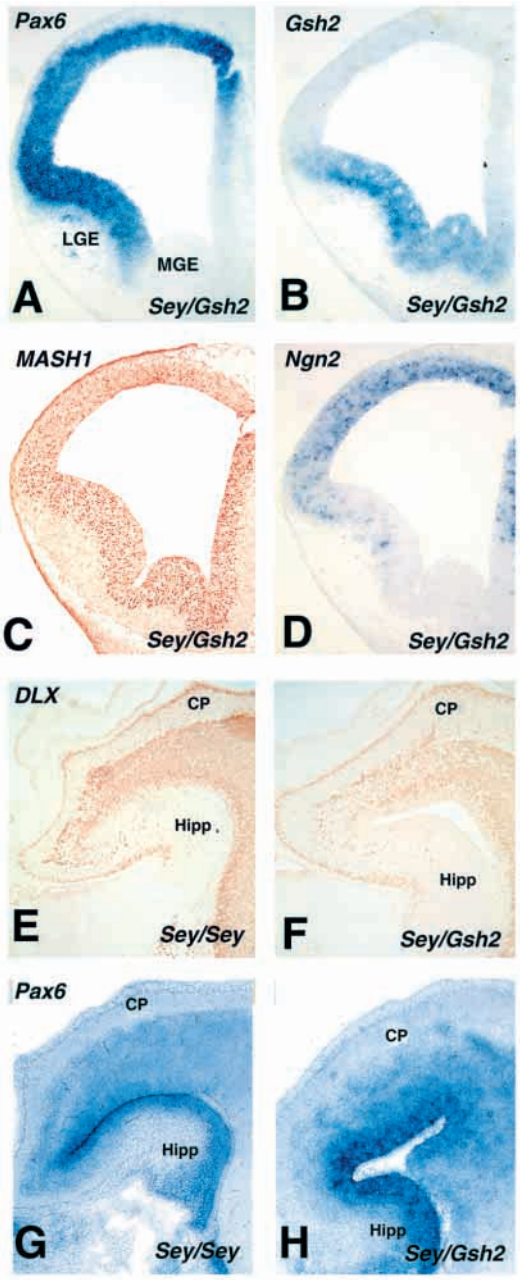

Fig. 7. Molecular identity of telencephalic progenitors in *Sey/Gsh2* double homozygous mutants. Coronal sections of *Sey/Gsh2* double mutant telencephalon, E12.5 (A-D), E16.5 (F,H) and *Sey/Sey* E16.5 telencephalon (E,G). At E12.5, the mutated *Pax6* (A) transcripts are found throughout the LGE while expression of the targeted *Gsh2* gene appears normal (B). At this stage, MASH1 expression in the *Sey/Gsh2* double mutants is found throughout the LGE and even into the cortex (C). Neurogenin 2 expression in the double mutants at E12.5 (D) is downregulated. A few cells, however, in the double mutant LGE do express neurogenin 2. At E16.5, a large number of cortical germinal zone cells in the *Sey/Sey* telencephalon express DLX (E). The domain of ectopic DLX expression is the same in the *Sey/Gsh2* double mutant cortex but fewer cells are ectopically expressing DLX (F). As is the case in earlier *Sey/Sey* embryos, *Pax6* mutant transcripts are downregulated in the cortical VZ (G) while cortical progenitors of the *Sey/Gsh2* double mutant maintain a high level of *Pax6* expression (H). CP, cortical plate; Hipp, hippocampus.

the wild-type cortex can migrate and differentiate normally (Caric et al., 1997). The temporal profile of this phenotype correlates well with that of ectopic *Gsh2*, *Mash1* and *Dlx* genes in the cortical VZ of *Sey/Sey* mutants, suggesting that the molecular mis-specification of cortical progenitors plays a central role in this. When *Gsh2* is removed from the *Sey/Sey* background (i.e. *Sey/Gsh2* double mutants), cortical progenitors remain mis-specified with respect to *Mash1*, *Dlx* genes and neurogenin genes, similar to what is seen in *Sey/Sey* cortical progenitors. Moreover, increased proliferation remains evident in the cortical germinal zone of the double mutants when compared with wild types, which is similar to that in *Sey/Sey* mutants (present results; Götz et al., 1998; Warren et al., 1999). Nevertheless, the morphology of the double mutant cortex is notably improved over that in the *Sey/Sey* mutant. The finding that the intermediate zone and cortical plate appear normalized in the *Sey/Gsh2* double mutant indicates a dramatic improvement in radial migration of cortical neurons. In support of this notion, cells in the double mutant cortex expressing the POU-domain transcription factor SCIP, which marks neurons migrating through the intermediate zone and into the forming cortical plate (Frantz et al., 1994), were distributed very similar to that in wild-type cortex. Since *Mash1* and *Dlx* genes remain ectopic in the double mutant cortex, this improvement can largely be ascribed to the loss of *Gsh2* on the *Sey/Sey* background. Interestingly, radial glia in the *Sey/Sey* cortex exhibit cell-autonomous defects in cellular morphology which has been suggested to be the cause of the cortical migration defects observed in the these mutants (Götz et al., 1998). In this respect, ectopic *Gsh2* may play a role in the altered differentiation of cortical radial glia observed in *Sey/Sey* mutants. Therefore, the cortical phenotype in *Sey/Sey* mutants can be divided into at least two distinct and independent components: one that is *Gsh2* independent, which includes the control of progenitor proliferation as well as *Mash1*, neurogenin and *Dlx* gene expression, and another that is *Gsh2* dependent and involves radial migration of cortical neurons.

Striatal defects in the *Gsh2* mutant are a result of ectopic *Pax6*

Our results demonstrate that the loss of *Gsh2* function results in abnormal development of the striatal complex including a reduction in the overall size and a loss of the olfactory tubercle. These defects correlate with the loss of *Mash1* and *Dlx* genes from most of the striatal progenitors at early stages of neurogenesis. *Mash1* mutants have recently been shown to exhibit alterations in LGE/striatal differentiation, which include a significant reduction in the size of the olfactory tubercle (Casarosa et al., 1999; Horton et al., 1999). Thus, the olfactory tubercle defect observed in the *Gsh2* mutants may, at least partly, be due to loss of *Mash1*. Double mutants for *Dlx1* and *Dlx2* also display abnormalities in striatal differentiation (Anderson et al., 1997b). These genes appear to be required for the correct development of the striatal neurons in the matrix compartment.

The mature striatum comprises two anatomically and neurochemically distinct compartments, termed the patch and matrix (for review see, Gerfen, 1990). Neurons generated at early stages of striatal neurogenesis preferentially occupy the patch compartment, while those arising later mainly join the matrix (van der Kooy and Fishell, 1987). Since the mis-

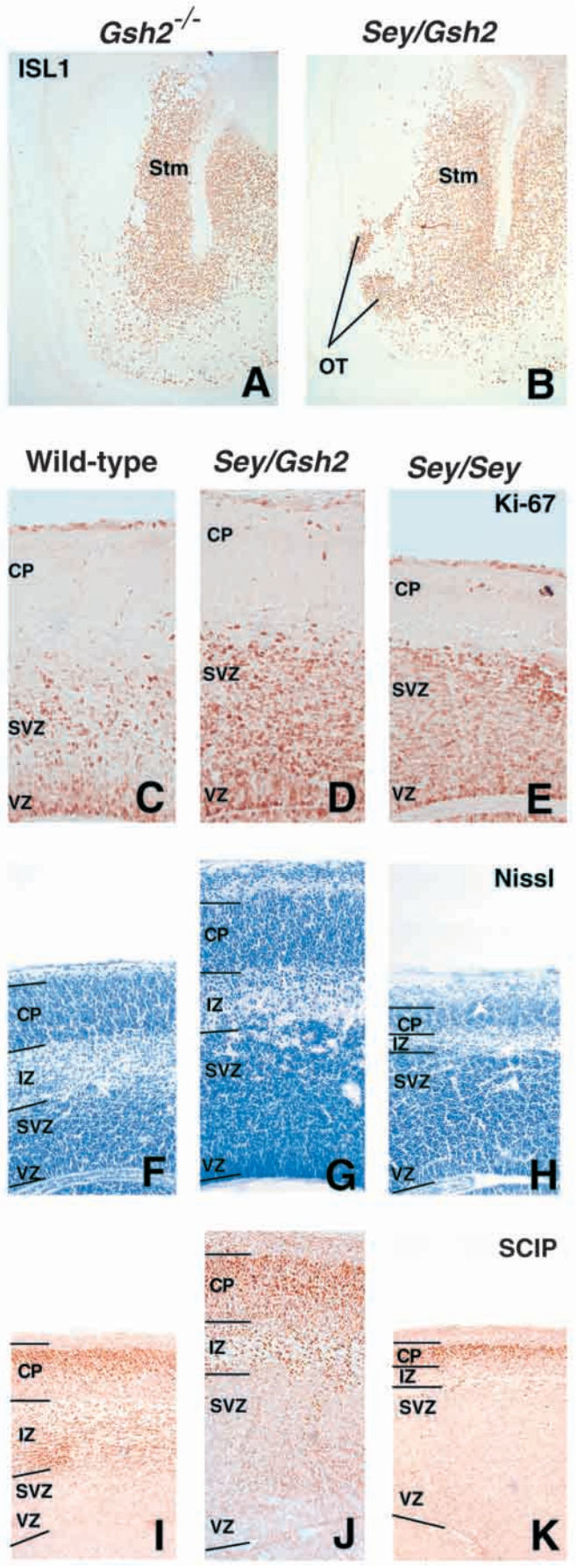

Fig. 8. Improvements in striatal and cortical morphology in *Sey/Gsh2* double homozygous mutants. Coronal sections of E16.5 mouse telencephalon. (A,B) The striatal complex of the *Sey/Gsh2* double mutant is larger than the $Gsh2^{-/-}$ striatum (compare B with A) and in each case (n=4), the olfactory tubercle is present (B). (C-E) Expression of the cell cycle marker, Ki-67 shows a similar pattern between the *Sey/Gsh2* double mutant (D) and the *Sey/Sey* mutant (E) indicating an increased number of proliferating cells compared with the wild type (C). (F-H) Nissl staining showing that the cortical plate (CP) and intermediate zone (IZ) of the double mutant (G) is similar in morphology to the wild type (F) and significantly larger than that in the *Sey/Sey* cortex (H). In addition, the VZ and SVZ of the *Sey/Gsh2* double mutant (G) is larger than in the wild type (F), similar to that in *Sey/Sey* embryos (H). (I-K) The pattern of SCIP-expressing cells in the double mutant (J) is similar to that in the wild-type cortex (I), while *Sey/Sey* mutants display a severely reduced number of SCIP-expressing cells (K). Note that the *Sey/Gsh2* and *Sey/Sey* embryos shown were littermates.

specification of striatal progenitors in the *Gsh2* mutant was most severe at early stages of neurogenesis, the patch compartment would be predicted to the most affected. The situation may, however, be more complex than this, since proliferation in the SVZ of the *Gsh2* mutant is also disrupted. It has been suggested that the VZ represents the source of neurons that will comprise the patch compartment, while the SVZ would provide the matrix neurons (van der Kooy and Fishell, 1987; Anderson et al., 1997b). Therefore, it seems likely that both striatal compartments are affected in the *Gsh2* mutant.

The fact that early striatal progenitors are most affected in the *Gsh2* mutant could be taken to indicate that *Gsh2* is only required for these stages of striatal neurogenesis and that other mechanisms regulate later stages. Alternatively, compensatory mechanisms may function in the absence of *Gsh2*. In this respect, we have observed an expansion in the expression domain of the *Gsh2*-related, *Gsh1* gene (Valerius et al., 1995) at late stages of striatal neurogenesis, from the

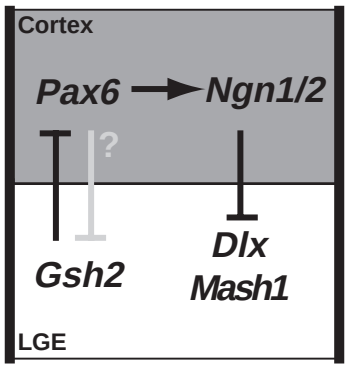

Fig. 9. Genetic model for the specification of cortical and striatal progenitors. The results from the present study demonstrate that *Gsh2* is required to repress *Pax6*, both in the LGE and lateral cortex. Furthermore, *Pax6* is shown to be required for maintaining neurogenin 1 and neurogenin 2 expression, which, in turn, is responsible for the repression of *Mash1* and *Dlx* genes in the cortex (Fode et al., 2000). The mechanism by which *Gsh2* expression is repressed from the cortex is presently unclear, however, it is possible that *Pax6* co-operates with another cortically restricted factor (indicated by ?) in this process.

ventromedial LGE in wild types to encompass the entire mutant LGE VZ (H. T. and K. C., unpublished). This could indicate that the restoration of the striatal germinal zone at late stages results from an expansion of the ventromedial domain of the LGE that is not mis-specified in the *Gsh2* mutant. However, the mis-specification of early LGE progenitors is only partial, since the mutated *Gsh2* gene and RBP1 are expressed throughout the mutant LGE VZ. Therefore, the restoration could be due to a respecification of the initially mis-specified dorsolateral (i.e. *Gsh2*-, *Rbp1*-positive, *Mash1*-, *Dlx*-negative) domain.

In addition to the loss of ventral genes in the *Gsh2* mutant LGE, the dorsal regulators, *Pax6* and the neurogenins are found ectopically. Given that *Pax6* and the neurogenins are required for the repression of *Mash1* and *Dlx* genes in cortical progenitors, it is likely that they are responsible for the loss of these ventral genes in the *Gsh2* mutant LGE and the resulting striatal phenotype. In support of this, removal of *Pax6* from the *Gsh2* mutant background (i.e. *Sey/Gsh2* double mutants) results in the restoration of correct molecular identity in LGE progenitors and concomitant improvements in the development of the striatal complex, including the reappearance of an olfactory tubercle. In the portion of the *Gsh2* mutant LGE that *Pax6* is ectopic, proliferation in the underlying SVZ is deficient, suggesting that the *Pax6*-induced mis-specification of early LGE progenitors not only represses *Mash1* and *Dlx* gene expression but also prevents the formation of a normal SVZ. This is interesting when compared with results from the *Sey/Sey* cortex, where the loss of *Pax6* leads to an increase in progenitor proliferation, particularly in the cortical SVZ (Götz et al., 1998; Warren et al., 1999 and the present results).

Evolutionary conservation of Gsh1/2 function in dorsal-ventral patterning

The *Gsh1/2* homolog, *intermediate neuroblasts defective* (*ind*), has recently been cloned in *Drosophila* and shown to be expressed in selected areas of the fly CNS including the intermediate region of the ventral nerve cord (Weiss et al., 1998). *ind* mutants show a reduction in the number of neuroblasts in this region of the ventral nerve cord. The remaining neuroblasts, by all markers tested, appear to have acquired a dorsal fate. This phenotype is reminiscent of the mouse *Gsh2* mutant phenotype where the progenitors in the LGE (i.e. the intermediate region of the telencephalon) have lost the expression of certain ventral genes and instead express the dorsal genes *Pax6*, neurogenin 1 and neurogenin 2. These findings therefore indicate that at least some aspects of *Gsh*/*ind* function in dorsal-ventral specification of the developing nervous system have been conserved throughout evolution.

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