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Disruption of Early Events in Thalamocortical Tract Formation in Mice Lacking the Transcription Factors Pax6 or Foxg1

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Early events in the formation of the thalamocortical tract remain poorly understood. Recent work has suggested that thalamocortical axons follow a path pioneered by transient thalamic afferents originating from the medial part of the ventral telencephalon. We studied the development of these transient afferents and the thalamocortical tract in mutant mice lacking transcription factors normally expressed in the dorsal thalamus or ventral telencephalon. Pax6 is expressed in the dorsal thalamus, but not in the medial part of the ventral telencephalon, and the thalamocortical tract fails to form in Pax6−/− embryos. We found that transient thalamic afferents from the ventral telencephalon do not form in Pax6−/− embryos; this may contribute to the failure of their thalamocortical development. The distribution of Pax6−/− cells in Pax6−/− → Pax6+/+ chimeras supports conclusions drawn from forebrain marker gene expression that Pax6 is not required for the normal development of the medial part of the ventral telencephalon but is required in the dorsal thalamus. Failure of the transient afferent pathway to develop is therefore likely a cell nonautonomous defect reflecting primary defects in the thalamus. We then examined the formation of thalamic afferents and efferents in Foxg1−/− embryos, which lack recognizable ventral telencephalic structures. In these embryos thalamic efferents navigate correctly through the thalamus but fail to turn laterally into the telencephalon, whereas other axons are able to cross the diencephalic/telencephalic boundary. Our results support a role for the ventral telencephalon in guiding the early development of the thalamocortical tract and identify a new role for the transcription factor Pax6 in regulating the ability of the thalamus to attract ventral telencephalic afferents.

Key words: thalamocortical tract; diencephalon; ventral telencephalon; Pax6; Foxg1; transcription factor; axon guidance; chimera; optic tract; mouse; transient thalamic afferents; tract tracing

The thalamocortical tract supplies the cerebral cortex with innervation from the dorsal thalamus. In the mouse, thalamocortical axons emerge from the dorsal thalamus at around embryonic day 13 (E13) (Braisted et al., 1999; Tuttle et al., 1999; Auladell et al., 2000), approximately coincident with the cessation of neurogenesis in this structure (Angevine, 1970). The axons then navigate ventrally through the thalamus and make a sharp lateral turn, avoiding the hypothalamus and entering the ventral telencephalon to form the internal capsule. The first thalamic axons arrive in the cerebral cortex around E15 and form synaptic connections during subsequent development (Braisted et al., 1999; Tuttle et al., 1999; Auladell et al., 2000). Thalamic growth cones perform several maneuvers on their way to the cortex, and their behavior at each point is defined by the properties of the growth cones and the cells and secreted molecules that they encounter (Braisted et al., 1999, 2000; Garel et al., 1999; Tuttle et al., 1999; Pratt et al., 2000; Skaliora et al., 2000).

A model to explain how the thalamocortical tract forms postulates a role for transient axons projecting from cells in the medial part of the ventral telencephalon into the dorsal thalamus. According to this model, these early thalamic afferents guide thalamocortical efferents into the ventral telencephalon (Metin and Godement, 1996; Tuttle et al., 1999). This raises the possibility that although later in development the dorsal thalamus is the source of thalamocortical axons, one of its important early functions is to accept innervation from the ventral telencephalon. In this study we investigated how disturbances inflicted on these tissues by mutating the transcription factors Pax6 or Foxg1 affect the formation of this transient afferent tract and the subsequent trajectory of the thalamocortical tract.

Pax6 is expressed dynamically in the diencephalon, the lateral part of the ventral telencephalon, and the cerebral cortex during the formation of the thalamocortical tract (Stoykova et al., 1996, 2000; Warren and Price, 1997; Hirata et al., 2002). The thalamocortical tract does not form in Pax6−/− mouse (Pratt et al., 2000; Hevner et al., 2002) and rat (Kawano et al., 1999) embryos. We used tract tracing in Pax6−/− embryos, immunohistochemistry for Mash1 expression, and analysis of the distribution of Pax6−/− cells in Pax6−/− → Pax6+/+ chimeras to test the hypothesis that the failure of the thalamocortical tract to form is preceded by a failure of the dorsal thalamus to receive transient afferents from the medial part of the ventral telencephalon.

Foxg1 (formerly BF-1) is expressed in the telencephalon (with highest levels of expression ventrally), in the nasal retina of the eye, and not in the diencephalon. The phenotype of Foxg1−/− embryos reflects Foxg1 expression with the telencephalon reduced in size, the eye distorted, and the diencephalon appearing normal. Judging from the expression domains of forebrain marker genes (Foxg1, Pax6, Enx2, and Dlx2), the Foxg1−/− tel-
encephalon lacks recognizable ventral structures but possesses a structural correlate of the cerebral cortex, and the diencephalon is normal (Xuan et al., 1995; Dou et al., 1999; Huh et al., 1999). We examined the trajectories of thalamic and retinal axons in Foxg1 embryos to further test the hypothesis that a normal ventral telencephalon is required to guide thalamocortical axons laterally toward the cerebral cortex.

MATERIALS AND METHODS

Pax6 and Foxg1 alleles. Both Pax6 alleles (Pax6Sey and Pax6Sey-Neu) used in this study are predicted to cause loss of Pax6 function (Favor et al., 1988; Hill et al., 1991; Quinn et al., 1996). Pax6Sey-Neu, Pax6SeySey-Neu and Pax6Sey-Neu-Neu embryos have been reported to have the same phenotypic abnormalities (Quinn et al., 1996) and are denoted Pax6−/−.

Replacement of the coding sequences of Foxg1 with LacZ coding sequences generated the Foxg1tm1 allele, which causes loss of Foxg1 function (Xuan et al., 1995; Dou et al., 1999; Huh et al., 1999). The Foxg1tm1 allele used in this study was generated by targeted replacement of Foxg1 coding sequences with Cre recombinase coding sequences (using a targeting vector otherwise identical to that used by Xuan et al. (1995)) (Hebert and McConnell, 2000). The anatomical defects reported for Foxg1tm1Mtm1M embryos were recapitulated in the Foxg1tm1 allele used in this study, and these are denoted Foxg1−/−.

Animals for tract tracing. Pax6−/− and Foxg1−/− embryos were obtained from Pax6Sey-Neu × Pax6Sey-Neu and Foxg1tm1 × Foxg1tm1 timed matings and were identified by anatomical features described previously (Hill et al., 1991; Xuan et al., 1995; Quinn et al., 1997; Huh et al., 1999). The day of finding a vaginal plug was designated E0.5. Control embryos were obtained from wild-type timed matings or nonhomozygous littermates.

The numbers of embryos examined in this study were as follows: injections into dorsal thalamus: E12.5: control, n = 5; Pax6−/−, n = 6; Foxg1−/−, n = 5; E13.5: control, n = 3; Pax6−/−, n = 3; Foxg1−/−, n = 3; E14.5: control, n = 3; Pax6−/−, n = 3; Foxg1−/−, n = 3; E15.5: control, n = 3; Pax6−/−, n = 3; Foxg1−/−, n = 2; injections into E15.5 telencephalon: control, n = 9; Foxg1−/−, n = 9; injections into E15.5 optic cup: control, n = 3; Foxg1−/−, n = 3. For each embryo both left and right sides of the brain (or both eyes) were injected with dioctadecyltetramethylindocarbocyanine perchlorate (DiI) (Molecular Probes, Eugene, OR).

DiI labeling and imaging. Whole embryos (E12.5, E13.5, or heads (E14.5, E15.5) were fixed overnight at 4°C in 4% paraformaldehyde (PFA) in PBS. For injections into the dorsal thalamus, a coronal cut was made in the head to reveal the caudal end of the thalamus. For injections into the optic cup, the lens of the eye was removed to reveal the surface of the retina. For injections into the telencephalon, cuts were made in the head to reveal the surface of the telencephalon. DiI was applied to embryos at E12.5, E13.5, E14.5, and E15.5 optic cup: control, n = 3; Foxg1−/−, n = 3. For each embryo both left and right sides of the brain were injected with DiI.

Animals for tract tracing. Pax6−/− and Foxg1−/− embryos were obtained from Pax6Sey-Neu × Pax6Sey-Neu and Foxg1tm1 × Foxg1tm1 timed matings and were identified by anatomical features described previously (Hill et al., 1991; Xuan et al., 1995; Quinn et al., 1997; Huh et al., 1999). The day of finding a vaginal plug was designated E0.5. Control embryos were obtained from wild-type timed matings or nonhomozygous littermates.

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Analysis of chimeras. Two balanced chimeras (those in which the ratio of cells derived from the Pax6Sey-Neu × Pax6Sey-Neu crosses to those from the Pax6−/− × Pax6−/− crosses was ~1:1 as determined by GPIIIB analysis) from the Pax6Sey-NeuSey × Pax6−/− × Pax6−/− and Pax6SeyNeu-Neu × Pax6−/− crosses were selected for detailed analysis. Regions examined were the medial part of the ventral telencephalon and the dorsal thalamus. The brain was fixed in 4% paraformaldehyde (PFA) for 1 hour, and then embedded in 4% agarose. The agarose block was sectioned on a vibratome at 50 μm. Sections were stained with cresyl violet and examined with a fluorescent microscope. Cells were identified as being derived from the Pax6Sey-Neu × Pax6Sey-Neu or the Pax6−/− × Pax6−/− embryos by their size and shape. The Pax6−/− × Pax6−/− embryos were used as controls for the Pax6Sey-Neu × Pax6Sey-Neu experiments.

RESULTS

The ventral telencephalon does not project to the thalamus in Pax6−/− embryos

In control embryos at E12.5, DiI injections in the dorsal thalamus labeled a tract coursing through the thalamus in a dorsoventral plane and turning laterally into the ventral telencephalon. A population of retrogradely labeled cell bodies in the ventral telencephalon marked the lateral limit of DiI diffusion at this stage (Fig. 1a–d), indicating that thalamic afferents precede


Pratt et al. • Role of Pax6 and Foxg1 in Thalamocortical Tract
thalamocortical efferents. Ventral telencephalic cells projecting to the dorsal thalamus can be identified only by retrograde DiI labeling from the dorsal thalamus, and the number of cells labeled by injections that fill only a part of the dorsal thalamus will always be less than the total number actually projecting. This under-representation is likely to be compounded by the transient nature of the projections, which may mean that at a given time not all of the cells fated to project will be retrogradely labeled from the dorsal thalamus. Examination of our own material and previously published data (Tuttle et al., 1999) shows that retrogradely labeled cell bodies marking the lateral limit of DiI diffusion. A more rostral E12.5 section with higher magnification (d) of the area boxed in c showing retrogradely labeled cell bodies in the medial part of the ventral telencephalon. e–g: Caudal to rostral series of sections showing injection site and the trajectory of the thalamocortical tract at E14.5. Arrows mark the lateral limit of the tract in each section. h: By E15.5 the tract has reached the cerebral cortex (marked with arrow). All sections were cut in the coronal plane. c, Eye; cc, cerebral cortex; dt, dorsal thalamus; ht, hypothalamus; vtel, ventral telencephalon. Scale bars: a, c, e–h, 500 μm; b, d, 50 μm.

Mash1-expressing cells occupy their normal positions in the medial part of the Pax6−/− ventral telencephalon

Previous studies have shown that Pax6 is not expressed in the medial part of the ventral telencephalon (Stoykova et al., 1996; Hirata et al., 2002). In Pax6−/− embryos, the mRNA expression domains of genes including Nkx2.1 and Netrin1, which normally include the location of cells projecting to the dorsal thalamus (Tuttle et al., 1999), are not altered (Pratt et al., 2000; Stoykova et al., 2000). Expression of Mash1 mRNA, the function of which is required for the formation of these thalamic afferents (Tuttle et al., 1999), also appears unaffected in Pax6−/− embryos (Stoykova et al., 2000). Despite these findings, it remained possible that Pax6 is expressed by early progenitors that give rise to ventral telencephalic cells projecting to the thalamus. One consequence of this might be a defect of Mash1 protein expression, and we tested this using Mash1 immunohistochemistry. Cells expressing Mash1 protein occupy their normal positions in the ventricular zone of the ventral telencephalon of E12.5 Pax6−/− embryos (compare expression pattern of Mash1 protein in E12.5 Pax6−/− ventral telencephalon in Fig. 2c with that in Pax6−/− ventral telencephalon in Fig. 2e). We did not detect any defects in the pattern of Mash1 protein expression in Pax6−/− embryos throughout the rostrocaudal axis of the medial ventral telencephalon. This result implies that loss of Pax6 does not impair the ability of the medial part of the ventral telencephalon to develop molecular features associated with and essential for the formation of the transient tract to the dorsal thalamus. The primary defect seen in our DiI tracing experiments is therefore unlikely to be attributable to the disappearance of these cells.
Pax6−/− cells contribute normally to the medial part of the ventral telencephalon but are under-represented and segregate from Pax6+/+ cells in the dorsal thalamus of Pax6−/− ↔ Pax6+/+ chimera

It has recently become apparent that the developing telencephalon is a dynamic structure, with newly born cells participating in numerous migratory streams that carry them from their birthplace to their final destination (Wilson and Rubenstein, 2000). Although Pax6 is not expressed in the medial part of the ventral telencephalon, it is expressed more laterally (Stoykova et al., 1996; Hirata et al., 2002). It is conceivable that cells born in Pax6-expressing territory might migrate to the medial part of the ventral telencephalon before projecting transiently to the dorsal thalamus. To address this issue we turned to a Pax6−/− ↔ Pax6+/+ chimera assay that directly tests whether Pax6 is required autonomously by cells to occupy their correct positions at a given developmental stage. The principle of this approach is as follows. If there is an absolute requirement for Pax6 for a tissue to develop, then Pax6−/− cells will not contribute to that tissue in Pax6−/− ↔ Pax6+/+ chimera, as occurs in the lens of the eye and in the nasal epithelium (Quinn et al., 1996). Alternatively, the requirement may be more subtle, as is seen in the distal part of the optic cup (Collinson et al., 2000), and Pax6−/− cells either may make a reduced contribution to the tissue or be distributed abnormally within it. Pax6−/− cells would make a normal contribution if Pax6 is not required cell autonomously for the development of the tissue. Previous studies (Quinn et al., 1996; Collinson et al., 2000; J. C. Quinn and J. D. West, unpublished observations) have shown that the contribution of Tg+ cells to a particular tissue of a Pax6+/+, Tg− ↔ Pax6+/+ chimera approximates the global Tg+ contribution, although the two are seldom identical (reflecting stochastic events during tissue construction); large deviations from the global Tg+ contribution in Pax6−/+, Tg− ↔ Pax6+/+ chimera are likely to be a consequence of the mutation. To provide a measure of inherent variation between individual tissues in our chimeras, we performed counts in several tissues in the control (Pax6+/+, Tg− ↔ Pax6+/+) balanced chimeras and measured the difference in each case from the global percentage chimerism and found an SD of 7.5%. A Pax6−/− contribution to a Pax6−/− ↔ Pax6+/+ chimera of >15% (±2 SDs) above or below the global contribution measured by GPI isoform composition would therefore be evidence that Pax6−/− cells did not contribute normally to that tissue, indicating a cell autonomous requirement for Pax6 in that tissue.

We detect no abnormal distribution of Pax6−/−, Tg+ cells in the medial part of the ventral telencephalon of Pax6−/−, Tg− ↔ Pax6+/+ chimeras. Tg+, Pax6+/+ and Tg+, Pax6−/− cells were equally well mixed with unlabeled cells in Pax6+/+, Tg− ↔ Pax6+/+ (Fig. 3b) and Pax6−/−, Tg− ↔ Pax6+/+ (Fig. 3c) chimeras. Previous examination of Pax6−/− ↔ Pax6+/+ chimeras has shown that Pax6−/−, Tg+ cells exhibit abnormal patterns of distribution in relation to their wild-type counterparts in all forebrain tissues that express Pax6 (Quinn et al., 1996; Collinson et al., 2000) (data from diencephalon in this study, see below; Quinn, unpublished observations). Furthermore, cells were able to contribute to the medial part of the ventral telencephalon regardless of their Pax6 genotype. There was no significant reduction in Pax6−/− cell contribution to this region in the chimeras examined. In both Pax6−/−, Tg− ↔ Pax6+/+ and Pax6−/−, Tg− ↔ Pax6+/+ chimeras, the Tg− contribution to the medial part of the ventral telencephalon was similar to the global Tg− contribution values defined by GPI isoform composition for each chimera (Fig. 4). Therefore the loss of Pax6 function does not detectably impair the ability of cells to contribute to the medial part of the ventral telencephalon, implying that there is no cell-autonomous requirement for Pax6 for the development of this part of the brain. Taken together, the failure to detect abnormal distribution or reduced contribution of Pax6−/− cells in our chimeras provides compelling evidence that Pax6 does not participate directly in the genesis of the region of the ventral telencephalon that projects a transient tract to the dorsal thalamus.

In contrast, the ability of cells to contribute to the dorsal thalamus was affected dramatically by their Pax6 genotype. Tg+, Pax6+/+ cells were evenly distributed in the dorsal thalamic domain of Pax6+/+, Tg− → Pax6+/+ chimera (Fig. 3c), but Tg+, Pax6−/− cells were segregated from Pax6+/+ cells into radial stripes in Pax6−/−, Tg− ↔ Pax6+/+ chimera (Fig. 3f,g). These stripes could be followed in a dorsoventral direction through many sections and extended into the ventral thalamus (data not shown). When Tg+ signals and nuclei were counted in 25-μm-wide strips running perpendicular to the ventricular surface, we observed that either nearly all or virtually none of the cells in these stripes were Pax6−/− (Fig. 3f,g), suggesting that stripes of mutant cells form perpendicular to the ventricular surface. The stripes of mutant cells in the dorsal thalamus varied considerably in width from one or two cells to tens of cells (Fig. 3, compare f, g). These stripes were not a consequence of clonal expansion during normal development, because they were not seen in the dorsal thalamus of...
Pax6+/+ ↔ Pax6+/+ chimeras (Fig. 3c). Although the overall morphology of the dorsal thalamus in the balanced Pax6+/−↔Pax6+/+ chimeras that were examined did not appear disturbed and certainly did not recapitulate the distortions seen in Pax6−/− embryos (Stoykova et al., 1996; Warren and Price, 1997), the ventricular surface formed a dramatic kink coinciding with particularly large patches of mutant cells (Fig. 3g). This indicated that the normal expansion of the diencephalic wall was retarded where large clumps of Pax6−/− cells were present. Analysis of broad areas of the dorsal thalamus showed that, on average, Pax6−/− cells were under-represented in the dorsal thalamus of Pax6+/−↔Pax6+/+ chimeras. Although Pax6+/+ cells contributed to the dorsal thalamus of Pax6−/−↔Pax6+/+ chimeras at levels comparable to the global contribution, the contribution of Pax6−/− cells to the dorsal thalamus of Pax6−/−↔Pax6+/+ chimeras was less than half the global contribution (Fig. 4). Taken together, the abnormal distribution and under-representation of Pax6−/− cells in Pax6−/−↔Pax6+/+ chimeras provide strong evidence that Pax6 is required autonomously for cells to make a full contribution to the dorsal thalamus and to participate normally in its development.

**Thalamocortical axons do not turn laterally into the telencephalon in Foxg1−/− embryos**

In E12.5 Foxg1−/− embryos, axons labeled from the dorsal thalamus coursed dorsoventrally through the ventral thalamus (Fig. 5a), but although a few axons approached the diencephalic/diencephalic boundary, hardly any entered the telencephalon and those that did appeared disorganized (Fig. 5b–e). The telencephalon of Foxg1−/− embryos is highly abnormal, and no retrogradely labeled cell bodies were detected in any telencephalic tissues (Fig. 5d). At neither E14.5 nor E15.5 were thalamocortical axons seen turning laterally into telencephalic structures (Fig. 5e–h). At E14.5 and E15.5, the tract penetrated farther than normal into the lateral hypothalamus (Fig. 5f–h, compare with Fig. 1f–h).

Placement of DiI crystals in the telencephalon of Foxg1−/− embryos labeled a few axons that had crossed the diencephalic/telencephalic boundary (Fig. 5i,j), although these appeared disorganized and did not extend to the dorsal thalamus. Similar injections in control embryos labeled a much larger number of axons that followed the trajectory of the thalamocortical tract and terminated in the dorsal thalamus (data not shown).

**Most of the retinal axons navigate the thalamus appropriately in Foxg1−/− embryos but a subpopulation deviates into telencephalic structures**

The optic tract forms at about the same time as the thalamocortical tract. Retinal axons navigating toward the dorsal thalamus encounter diencephalic and telencephalic tissues (Mason and Wang, 1997) that are implicated in the formation of the thalamocortical tract (Braisted et al., 1999; Tuttle et al., 1999, Pratt et al., 2000). We were therefore interested to see how retinal axons responded to the disrupted forebrain environment present in
a primary defect in the projecting cells, in the environment through which their axons navigate, or both. Available evidence strongly suggests that the medial part of the ventral telencephalon develops normally in Pax6−/− embryos. First, Pax6 is not expressed in this region at the time when its thalamic connections form (Stoykova et al., 1996, 2000; Hirata et al., 2002). Second, the expression patterns of Mash1 mRNA and protein, Nkx2.1 mRNA, and Netrin1 mRNA are not altered in this region in Pax6−/− embryos (Pratt et al., 2000; Stoykova et al., 2000; the present study). Third, Pax6−/− cells in Pax6−/− → Pax6+/+ chimeras show no abnormal distribution and contribute normally to this region. In the forebrain of Pax6−/− → Pax6+/+ chimeras, abnormalities always manifest as segregation between Pax6−/− and Pax6+/+ cells rather than complete exclusion of Pax6−/− cells, even in areas with high persistent expression of Pax6 (Quinn et al., 1996; Collinson et al., 2000) (data from diencephalon in this study; Quinn, unpublished observations). We cannot rule out the formal possibility of a total exclusion of a subpopulation of Pax6−/− cells that would normally project to the thalamus if that subpopulation makes up <30% of the region analyzed (because this translates to a chimerism within 15% of the global chimerism of a balanced chimera, placing it within the limits of inherent variations between our control chimeric tissues; see Results). This is, however, unlikely because (1) the proportion of ventral telencephalic cells that project to the thalamus is likely to be high (see Results) and (2) a cell autonomous requirement for Pax6 is manifested as a segregation defect rather than exclusion in all other regions of the brain examined. The development of the thalamus is compromised in Pax6−/− embryos. First, Pax6−/− thalamic cells exhibit reduced proliferation (Warren and Price, 1997). Second, the Pax6−/− dorsal thalamus exhibits abnormal differentiation that is manifested as altered patterns of gene expression and projection of axons with altered navigation properties (Pratt et al., 2000). Third, Pax6−/− cells are abnormally distributed and under-represented in the dorsal thalamus of Pax6−/− → Pax6+/+ chimeras (the present study). Taken together these findings provide compelling evidence that a primary defect in Pax6−/− thalamus is responsible for its failure to receive transient afferents from the ventral telencephalon. The lack of this afferent tract may be one of several factors contributing to the failure of subsequent thalamocortical development in Pax6−/− embryos (Kawano et al., 1999; Hevner et al., 2002). Other factors are likely to include cell-autonomous defects of thalamic cells and axons (Pratt et al., 2000) (chimera results of the present study).

Axons forming the tract of the post-optic commissure (TOPC) fail to reach the dorsal thalamus in Pax6−/− embryos (Mastick et al., 1997). A recent preliminary report shows that TOPC axons can be rescued nonautonomously by transient expression of Pax6 within the thalamus at E10.5 (Mastick, 2001). This result is interesting in the context of this study because it suggests a more general Pax6-dependent role for the thalamus in attracting afferent axons.

Abnormal thalamic development in Pax6−/− embryos

Previous work has demonstrated that proliferation is reduced in the Pax6−/− dorsal thalamus at E10.5 (Warren and Price, 1997). The under-representation of Pax6−/− cells in the dorsal thalamus of E12.5 Pax6−/− → Pax6+/+ chimeras suggests a cell-autonomous proliferation defect that cannot be rescued by surrounding Pax6+/+ cells. Kinks in the ventricular zone coinciding with large areas consisting almost exclusively of Pax6−/− cells could reflect mechanical distortion imposed on the tissue by a

### DISCUSSION

#### Disruption to thalamic afferents in Pax6−/− embryos

Tract-tracing studies in several rodent species have identified a transient population of thalamic afferents that originate in the medial part of the ventral telencephalon before thalamocortical tract formation (Metin and Gomedt, 1996; Molnar et al., 1998; Braisted et al., 1999). The cell bodies of these projecting neurons map to ventral telencephalic domains expressing Nkx2.1 (transcription factor) and Netrin1 (secreted protein) mRNAs (Tuttle et al., 1999). In Mash1−/− embryos, these domains of gene expression are missing, the transient afferent tract does not form, and the thalamocortical tract does not penetrate the telencephalon. This led to the proposal that Mash1 is required in the medial part of the ventral telencephalon for the projection of this transient tract that guides thalamocortical afferents laterally into the ventral telencephalon (Tuttle et al., 1999).

Our observation that the ventral telencephalon does not project to the dorsal thalamus in Pax6−/− embryos could be explained by
patch of relatively slowly dividing mutant cells surrounded by more rapidly proliferating wild-type epithelium. That the tissue responds to these patches of mutant cells by forming a kink, rather than relieving the tension by allowing Pax6<sup>−/−</sup> and Pax6<sup>+/+</sup> cells to mix laterally, provides a measure of the strong forces opposing their mixing.

Misexpression of cell surface molecules or secreted proteins in the Pax6<sup>−/−</sup> thalamus is a likely mechanism for its failure to receive transient innervation from the ventral telencephalon. That Pax6<sup>−/−</sup> and Pax6<sup>+/−</sup> cells did not intermingle in the thalamus of E12.5 Pax6<sup>−/−</sup> → Pax6<sup>+/−</sup> chimeras suggests a role for Pax6 in influencing cell–cell interactions in the developing thalamus by regulating the expression of cell surface molecules or secreted signaling proteins. This hypothesis is appealing because,
in various developing systems, Pax6 directly or indirectly regulates the expression of many genes controlling cell–cell contact and signaling. These include genes encoding cell surface adhesion molecules L1, R-cadherin, β1 integrin, and trkB (Stoykova et al., 1997; Mechtler et al., 1999; Warren et al., 1999; Duncan et al., 2000) and secreted proteins Wnt7b, SFRP-2, and Netrin1 (Kim et al., 2001; Vitalis et al., 2001). We have previously made a preliminary report that, at E14.5, Pax6+/− and Pax6+/+ thalamic cells exhibit different abilities to adhere to and grow on slices of wild-type forebrain in culture (Pratt et al., 2001), suggesting that the control of thalamic cell surface properties by Pax6 spans the period of thalamocortical tract formation.

**Disruption of the ventral telencephalon prevents thalamocortical axons innervating the cerebral cortex in Foxg1+/− embryos**

The thalamocortical phenotype of Mash1−/− embryos provides evidence for the importance of transient thalamic afferents from the ventral telencephalon in guiding thalamocortical axons (Tuttle et al., 1999), but the thalamus itself is also disrupted, making it hard to determine where the primary defects causing thalamocortical axon misrouting lie. In these mutants, most of the axons stall at the border between the dorsal and ventral thalamus (Tuttle et al., 1999), raising the possibility that the ventral telencephalon might be required to guide axons through the thalamus. To test this we examined Foxg1+/− embryos in which development of the thalamus is normal but the telencephalon is severely disrupted and has no recognizable ventral structures (Xuan et al., 1995; Dou et al., 1999). Thalamic axons in Foxg1+/− embryos showed no clear pathfinding defects within the thalamus, indicating that a normal ventral telencephalon is not required for this segment of the thalamocortical tract to form. Thalamic axons did not, however, turn laterally into the telencephalon, indicating that this turn requires a normal ventral telencephalon. There is no evidence for a general physical barrier to axons at the diencephalic telencephalic boundary in Foxg1+/− embryos because our injections into the Foxg1+/− telencephalon or eye (see below) identified axons crossing this boundary. A striking feature of the lateral turn of thalamocortical axons into the ventral telencephalon is their avoidance of the hypothalamus. In vitro studies show that thalamocortical axons are repelled by molecules secreted by the hypothalamus (Braisted et al., 1999) and do not penetrate explants of hypothalamus (Pratt et al., 2000). Our observation that dorsal thalamic efferents in Foxg1+/− embryos penetrate farther ventrally than normal into the lateral part of the hypothalamus suggests that repulsion by the hypothalamus in vivo is not sufficient to propel thalamocortical axons laterally or to completely repel invasion by thalamocortical axons diverted from their normal course.

**Optic tract formation in Foxg1+/− embryos**

The optic tract grows from the retina to the dorsal thalamus and encounters tissues involved in guiding the thalamocortical tract (Mason and Wang, 1997). In Foxg1+/− embryos we labeled many axons that navigated from the retina to the lateral aspect of the dorsal thalamus along a similar trajectory to that seen in control embryos, indicating that diencephalic tissues are able to supply appropriate navigation cues to retinal axons in the absence of Foxg1. This result supports our premise, based on the absence of Foxg1 expression from the diencephalon and appropriate expression of diencephalic marker genes in Foxg1+/− embryos, that the Foxg1+/− diencephalon is normal (Xuan et al., 1995; Dou et al., 1999). We also observed a subset of axons that projected into the telencephalon, a feature never seen in control embryos. So although thalamocortical axons (which normally penetrate the telencephalon) are excluded in the Foxg1+/− mutant, some retinal axons (which are normally excluded from the ventral telencephalon) are able to penetrate in the Foxg1+/− mutant. It is tempting to speculate that the loss of Foxg1 from the developing telencephalon results in the simultaneous loss of attractive cues for thalamocytic axons and repulsive cues for some (but not all) retinal axons. Foxg1 is expressed in the nasal portion of the retina (Hatini et al., 1994; Xuan et al., 1995; Huh et al., 1999), so it is also possible that the pathfinding errors in the Foxg1+/− optic tract reflect an autonomous requirement for Foxg1 in a subset of retinal axons. These issues deserve examination in a future study.

REFERENCES


