Disruption of early events in thalamocortical tract formation in mice lacking the transcription factors Pax6 or Foxg1

Citation for published version:

Link:
Link to publication record in Edinburgh Research Explorer

Document Version:
Publisher's PDF, also known as Version of record

Published in:
Journal of Neuroscience

General rights
Copyright for the publications made accessible via the Edinburgh Research Explorer is retained by the author(s) and / or other copyright owners and it is a condition of accessing these publications that users recognise and abide by the legal requirements associated with these rights.

Take down policy
The University of Edinburgh has made every reasonable effort to ensure that Edinburgh Research Explorer content complies with UK legislation. If you believe that the public display of this file breaches copyright please contact openaccess@ed.ac.uk providing details, and we will remove access to the work immediately and investigate your claim.
Disruption of Early Events in Thalamocortical Tract Formation in Mice Lacking the Transcription Factors Pax6 or Foxg1

Thomas Pratt,¹ Jane C. Quinn,¹ T. Ian Simpson,¹ John D. West,² John O. Mason,¹ and David J. Price¹

¹Biomedical Sciences and ²Department of Reproductive and Developmental Sciences, Genes and Development Group, University of Edinburgh, Edinburgh EH8 9XD, United Kingdom

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 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; Skaliara 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. 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. 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. 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.
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 (Pax6<sup>sey</sup> and Pax6<sup>sey-neu</sup>) used in this study are predicted to cause loss of Pax6 function (Favor et al., 1988; Hill et al., 1991; Quinn et al., 1996). Pax6<sup>sey-neu</sup>, Pax6<sup>sey-sey-neu</sup> and FoxG1<sup>tm1(cre)skm</sup> embryos have been reported to have the same phenotypic abnormalities (Quinn et al., 1996) and are denoted Pax6<sup>fs</sup> and FoxG1<sup>fs</sup>

Replacement of the coding sequences of FoxG1 with LacZ coding sequences generated the FoxG1<sup>LacZ</sup> allele, which causes loss of FoxG1 function (Xuan et al., 1995; Dou et al., 1999; Huh et al., 1999). The FoxG1<sup>LacZ</sup> 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 FoxG1<sup>LacZ</sup> embryos were recapitulated in the FoxG1<sup>tm1(cre)skm</sup> embryos used in this study, and these are denoted FoxG1<sup>fs</sup>.

**Animals for tract tracing.** Pax6<sup>fs</sup> and FoxG1<sup>fs</sup> embryos were obtained from Pax6<sup>sey-neu</sup> × Pax6<sup>sey-neu</sup> and FoxG1<sup>tm1(cre)skm</sup> × FoxG1<sup>tm1(cre)skm</sup> 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 littersates. The numbers of embryos examined in this study were as follows: injections into dorsal thalamus: E12.5: control, n = 5; injections into E15.5 optic cup: control, n = 5; injections into E15.5 telencephalon: control, n = 3; injections into E15.5 optic cup: control, n = 3; injections into E15.5 telencephalon: n = 6; FoxG1<sup>fs</sup>, n = 5; E13.5: control, n = 3; FoxG1<sup>fs</sup>, n = 3; E14.5: control, n = 3; FoxG1<sup>fs</sup>, n = 3; E15.5: control, n = 3; FoxG1<sup>fs</sup>, n = 3; E16.5: control, n = 3; FoxG1<sup>fs</sup>, n = 3. For each embryo both left and right sides of the brain (or both eyes) were injected with diocetyl-ditetramethylene-carbocyanine perchorlate (DiI) (Molecular Probes, Eugene, OR).

**DiI labeling and imaging.** Whole embryos (E12.5, E13.5) or heads 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 the dorsal thalamus. In each chimera, the number of Tg<sup>fl</sup>-globin transgene (<sup>fl</sup>tg) cells nuclei in a 250 μm × 250 μm region was counted. In each embryo both left and right sides of the brain (or both eyes) were injected with diocetyl-ditetramethylene-carbocyanine perchorlate (DiI) (Molecular Probes, Eugene, OR).

**Analysis of chimeras.** Two balanced chimeras (those in which the ratio of cells derived from the Pax6<sup>sey-neu</sup> × Pax6<sup>sey-neu</sup> crossed to those from the Pax6<sup>fs</sup> × Pax6<sup>fs</sup> crosses was ~1:1 as determined by GPI1B analysis) from the Pax6<sup>sey-neu</sup>/Pax6<sup>sey-neu</sup> × Pax6<sup>fs</sup>/Pax6<sup>fs</sup> and Pax6<sup>fs</sup>/Pax6<sup>fs</sup> genotype groups were selected for detailed analysis. Regions examined were the medial part of the ventral telencephalon and the dorsal thalamus. In each chimera, the number of Tg<sup>fl</sup>-globin transgene (<sup>fl</sup>tg) cell nuclei in a 250 μm × 250 μm region was counted in two nonconsecutive sections for both the left and right sides of the brain (see Fig. 3 for examples of sections used for analysis). A count of Tg<sup>fl</sup> signal detected to nuclei counted does not give a true estimate of the proportion of Tg<sup>fl</sup> cells in a tissue. This is because of tissue-specific differences in the efficiency of detecting Tg<sup>fl</sup>-globin and nuclei, caused by variations in nuclear morphologies and packing densities (Quinn et al., 1996). To correct for this effect we performed identical counts in nonchimeric embryos, in which all cells contained the β-globin transgene, to generate tissue-specific correction factors for those regions examined. These tissue-specific correction factors were as follows: dorsal thalamus, 1.07; medial ventral telencephalon, 1.16. A hybridization index was then calculated for each tissue [hybridization index = (~<sup>fl</sup>tg signal/nuclei)/tissue specific correction factor]. Because the corrected hybridization index gives the proportion of Tg<sup>fl</sup> cells in a particular tissue, this can be compared directly with the global contribution of Tg<sup>fl</sup> cells as determined by GPI1B genotyping.

**RESULTS**

The ventral telencephalon does not project to the thalamus in Pax6<sup>fs</sup> 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
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.

Figure 1. Labeling of thalamic afferents and efferents in control embryos by DiI placement in the dorsal thalamus at E12.5 (a–d), E14.5 (e–g), and E15.5 (h). a, A caudal E12.5 section showing the injection site and axons growing laterally at the diencephalic/telencephalic boundary (white box). b, A higher magnification of the area boxed in (a) showing retrogradely labeled cell bodies marking the lateral limit of DiI diffusion. c, 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−/−,GFP−/− → Pax6+/+ chimeras.

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, 2000; Hirata et al., 2002). It is conceivable that cells born in the ventral telencephalon, it is expressed more laterally (Stoykova et al., 1996, 2000; Hirata et al., 2002). It is conceivable that cells born in the ventral telencephalon before projecting transiently to the dorsal thalamus at E12.5 (a) and E14.5 (f), a. An E12.5 rostral section, at a level similar to that in Figure 1c, showing that no retrogradely labeled cells were seen in the ventral telencephalon. Boxed area indicates regions of ventral telencephalon shown in b–e. Mash1 immunohistochemistry in E12.5 Pax6+/+ (c) and Pax6−/− (e) ventral telencephalon. In both cases cells expressing Mash1 protein (green) are distributed in the ventricular zone (filled yellow arrows) and not in the central region. b, d, Phase-contrast images corresponding to fluorescent images in c and e. E14.5 caudal (f) and rostral (g) sections showing injection site and the absence of Dil labeling in the ventral telencephalon. h, Thalamic axons descending through the thalamus (indicated by arrow); boxed area in h is shown at higher magnification in i illustrating an axon tipped with a growth cone (arrow in i). dt, Pax6−/− correlate of the Pax6+/+ dorsal thalamus; ht, hypothalamus; vtel, ventral telencephalon. Pax6−/− embryos lack eyes altogether. All sections were cut in the coronal plane. Scale bars: a, f–h, 500 μm; b–e, 50 μm; i, 10 μm.

Figure 2. a, f–i, Labeling in Pax6−/− embryos after Dil placement in the dorsal thalamus at E12.5 (a) and E14.5 (f–i), a. An E12.5 rostral section, at a level similar to that in Figure 1c, showing that no retrogradely labeled cells were seen in the ventral telencephalon. Boxed area indicates regions of ventral telencephalon shown in b–e. Mash1 immunohistochemistry in E12.5 Pax6+/+ (c) and Pax6−/− (e) ventral telencephalon. In both cases cells expressing Mash1 protein (green) are distributed in the ventricular zone (filled yellow arrows) and not in the central region. b, d, Phase-contrast images corresponding to fluorescent images in c and e. E14.5 caudal (f) and rostral (g) sections showing injection site and the absence of Dil labeling in the ventral telencephalon. h, Thalamic axons descending through the thalamus (indicated by arrow); boxed area in h is shown at higher magnification in i illustrating an axon tipped with a growth cone (arrow in i). dt, Pax6−/− correlate of the Pax6+/+ dorsal thalamus; ht, hypothalamus; vtel, ventral telencephalon. Pax6−/− embryos lack eyes altogether. All sections were cut in the coronal plane. Scale bars: a, f–h, 500 μm; b–e, 50 μm; i, 10 μm.

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−/−,GFP−/− → Pax6+/+ chimeras.

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, 2000; Hirata et al., 2002). It is conceivable that cells born in 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+/+ chimeras, 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+/+,GFP−/− → 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+/+,GFP−/− → Pax6+/+ chimeras 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+/+,GFP−/− → Pax6+/+) balanced chimeras and measured the difference in each case from the global percentage chimera 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 isofrom 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−/−,GFP−/− → Pax6+/+ cells in the medial part of the ventral telencephalon of Pax6−/−,GFP−/− → Pax6+/+ chimeras, Tg−,Pax6+/+ and Tg−,Pax6+/− were equally well mixed with unlabeled cells in Pax6+/+,GFP−/− → Pax6+/+ (Fig. 3b) and Pax6+/−,GFP−/− → Pax6+/+ (Fig. 3c) chimeras. Previous examination of Pax6−/− → Pax6+/+ chimeras has shown that Pax6−/−,GFP−/− 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−/−,GFP−/− → Pax6+/+ and Pax6+/−,GFP−/− → Pax6+/+ chimeras, the Tg− contribution to the medial part of the ventral telencephalon was similar to the global Tg− contribution values defined by GPI isofrom 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 thalamus of Pax6+/+,GFP−/− → Pax6+/+ chimeras (Fig. 3c), but Tg−,Pax6−/− cells were segregated from Pax6+/+ cells into radial stripes in Pax6+/−,GFP−/− → Pax6+/+ chimeras (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 dorsally through the ventral thalamus (Fig. 5a), but although a few axons approached the diencephalic/
in the optic cup labeled a tract leaving the retina (Fig. 6).

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 (TPOC) fail to reach the dorsal thalamus in Pax6−/− embryos (Mastick et al., 1997). A recent preliminary report shows that TPOC 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 that the 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 a mechanical distortion imposed on the tissue by 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 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; Collignon 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 chimera within 15% of the global chimeraism 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 (TPOC) fail to reach the dorsal thalamus in Pax6−/− embryos (Mastick et al., 1997). A recent preliminary report shows that TPOC 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 that a more general Pax6-dependent role for the thalamus in attracting afferent axons.

**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 telencephalon and course over the ventral surface of the brain, and continue dorsally and caudally to the lateral dorsal thalamus (Fig. 6c). Unlike in the wild type, a subpopulation of axons deviated into telencephalic structures (Fig. 6j).

**Figure 4.** Histogram showing quantification of chimism in Pax6−/− → Pax6+/+ and Pax6−/+ → Pax6+/− chimeras. Global chimism and chimism for the medial part of the ventral telencephalon and the dorsal thalamus are shown for each chimera, with the numbers above each bar indicating the percentages of Tg+ cells. Primary Tg+ signal/nuclei counts for the medial ventral telencephalon and the dorsal thalamus were as follows: Pax6+/−, Tg− → Pax6+/−/medial vtel; JC24, medial vtel = 2168/3721, dt = 2613/4390; JC58, medial vtel = 2135/3325, dt = 2427/3465; Pax6−/−, Tg− → Pax6+/−/medial vtel; JC56, medial vtel = 1412/3484, dt = 692/3179; JC61, medial vtel = 1513/3591, dt = 511/3633. These primary counts were divided by the tissue-specific correction factors (1.16 for Pax6+/− for the medial ventral telencephalon, 1.07 for the dorsal thalamus) to give the corrected hybridization index, which gives a true estimate of the percentage of Tg+ cells in the tissue for comparison with the global Tg+ contribution (see Materials and Methods). Large variation between tissue-specific and global Tg+ contribution, indicating a requirement for Pax6, is seen only in the dorsal thalamus of Pax6−/− → Pax6+/+ chimeras (marked with *), where Pax6−/− cells are also abnormally distributed (Fig. 3). vtel, Ventral telencephalon.

In E15.5 control embryos, DiI crystals placed in the optic cup labeled a tract leaving the retina (Fig. 6a), coursing over the ventral surface of the brain, and continuing dorsally and caudally to the lateral dorsal thalamus (Fig. 6b,c). These axons were not seen entering telencephalic tissue (Fig. 6d–e). In E15.5 Foxg1−/− embryos, DiI crystals placed in the optic cup label a tract in which the majority of axons followed the same trajectory as that seen in control embryos to reach the dorsal thalamus (Fig. 6f–j). Unlike in the wild type, a subpopulation of axons deviated into telencephalic structures (Fig. 6g–j).
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+/− and Pax6−/− 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−/− thalamus is a likely mechanism for its failure to receive transient innervation from the ventral telencephalon. That Pax6−/− and Pax6−/−/− cells did not intermingle in the thalamus of E12.5 Pax6−/− → Pax6−/−/− 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,

Figure 5. Dil labeling in Foxg1−/− embryos at E12.5 (a–d), E14.5 (e–g), and E15.5 (h–j). a–h, Dil injections into dorsal thalamus. a, A caudal E12.5 section showing the injection site and absence of Dil labeling from telencephalic structures (the Foxg1−/− telencephalon lacks recognizable ventral structures so is marked cc*). b, A higher magnification of the top boxed area in a showing that the very few axons which approach the telencephalon are disorganized. c, A higher magnification of the bottom area boxed in a showing that labeled axons grow ventrally and medially rather than turning laterally into the telencephalon. d, A more rostral E12.5 section showing that no retrogradely labeled cells or axons can be detected in the rostral telencephalon. e–g, Caudal to rostral series of sections at E14.5 show the injection site and the trajectory of thalamic efferents. These do not penetrate the telencephalon; arrows mark lateral limit of the tract in each section. h, By E15.5 the tract has not penetrated the telencephalon but has continued ventrally within the thalamus toward the hypothalamus (marked with arrow). i, j, Dil injection into telencephalic structures at E15.5. i, Although no substantial tract leaves the telencephalon in Foxg1−/− embryos, higher magnification of the area boxed in j shows that a few disorganized axons are able to cross the telencephalic/diencephalic boundary. All sections were cut in the coronal plane. cc* and e* denote Foxg1−/− correlates of Foxg1+/+ cerebral cortex and eye; dt, dorsal thalamus; ht, hypothalamus. Scale bars: a, d–h, 500 μm; b, c, 50 μm.

Figure 6. Dil labeling of axons from the optic cup in Foxg1+/+ (f–j) and control (a–e) embryos at E15.5. a–c, In control embryos, a rostral to caudal series shows the tract leaving the optic cup (a), growing over the lateral surface of the hypothalamus (b), and reaching the lateral aspect of the dorsal thalamus (c). Boxed areas in b and c are shown at higher magnification in d and e. d, Axons do not penetrate into telencephalic structures. e, Axons form a smooth tract running in a dorsoventral direction in the lateral dorsal thalamus. f–j, In Foxg1−/− embryos, a rostral to caudal series shows the tract leaving the optic cup (f), growing over the lateral surface of the hypothalamus (g) (unfilled yellow arrow in g shows axons penetrating the telencephalon), and reaching the lateral aspect of the dorsal thalamus (h). Boxed areas in g and h are shown at higher magnification in i and j. i, Some axons leave the main tract and penetrate into telencephalic structures (unfilled yellow arrow, j), whereas the majority continue caudally (filled yellow arrow). Scale bars: a–e, f–j, 500 μm; d, e, i, j, 50 μm.
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; Meech 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/H11002 and Pax6/H11001 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/H11002 embryos**

The thalamocortical phenotype of Mash1/H11002 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/H11002 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/H11002 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–thalamic boundary in Foxg1/H11002 embryos because our injections into the Foxg1/H11002 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/H11002 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/H11002 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/H11002 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/H11002 embryos, that the Foxg1/H11002 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/H11002 mutant, some retinal axons (which are normally excluded from the ventral telencephalon) are able to penetrate in the Foxg1/H11002 mutant. It is tempting to speculate that the loss of Foxg1 from the developing telencephalon results in the simultaneous loss of attractive cues for thalamic 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/H11002 optic tract reflect an autonomous requirement for Foxg1 in a subset of retinal axons. These issues deserve examination in a future study.

**REFERENCES**


Mastick GS (2001) Pioneer axon guidance errors in Pax6 mutant mouse