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# The early topography of thalamocortical projections is shifted in *Ebf1* and *Dlx1/2* mutant mice

**Sonia Garel[1], Kyuson Yun[1], Rudolf Grosschedl[2] and John L. R. Rubenstein[1,*]**

[1]Nina Ireland Laboratory of Developmental Neurobiology, Department of Psychiatry, University of California San Francisco, San Francisco, CA 94143-0984, USA
[2]Gene Center and Institute of Biochemistry, University of Munich, Feodor Lynenstrasse 25, 81377 Munich, Germany
*Author for correspondence (e-mail: jlrr@cgl.ucsf.edu)

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## SUMMARY

**The prevailing model to explain the formation of topographic projections in the nervous system stipulates that this process is governed by information located within the projecting and targeted structures. In mammals, different thalamic nuclei establish highly ordered projections with specific neocortical domains and the mechanisms controlling the initial topography of these projections remain to be characterized. To address this issue, we examined *Ebf1*$^{-/-}$ embryos in which a subset of thalamic axons does not reach the neocortex. We show that the projections that do form between thalamic nuclei and neocortical domains have a shifted topography, in the absence of regionalization defects in the thalamus or neocortex. This shift is first detected inside the basal ganglia, a structure on the path of thalamic axons, and which develops abnormally in *Ebf1*$^{-/-}$ embryos. A similar shift in the topography of thalamocortical axons inside the basal ganglia and neocortex was observed in *Dlx1/2*$^{-/-}$ embryos, which also have an abnormal basal ganglia development. Furthermore, *Dlx1* and *Dlx2* are not expressed in the dorsal thalamus or in cortical projections neurons. Thus, our study shows that: (1) different thalamic nuclei do not establish projections independently of each other; (2) a shift in thalamocortical topography can occur in the absence of major regionalization defects in the dorsal thalamus and neocortex; and (3) the basal ganglia may contain decision points for thalamic axons' pathfinding and topographic organization. These observations suggest that the topography of thalamocortical projections is not strictly determined by cues located within the neocortex and may be regulated by the relative positioning of thalamic axons inside the basal ganglia.**

Key words: Topography, Thalamocortical axons, Internal capsule, Neocortex, Basal ganglia, *Dlx*, *Ebf1*, Mouse

## INTRODUCTION

In the mammalian brain, reciprocal connections between sensory nuclei of the dorsal thalamus and specific areas of the neocortex are essential for the relay and processing of visual, auditory, sensory and motor information (O'Leary et al., 1994; Levitt et al., 1997; Monuki and Walsh, 2001; Pallas, 2001; Ragsdale and Grove, 2001; Ruiz i Altaba et al., 2001; Sur and Leamey, 2001; O'Leary and Nakagawa, 2002). During development, thalamocortical and corticothalamic axons grow into the subcortical telencephalon, where they meet and continue on their paths to the cortex and thalamus, respectively (Miller et al., 1993; Metin and Godement, 1996; Molnar et al., 1998a; Auladell et al., 2000). In this process, thalamic axons of a given nucleus grow through the ventral thalamus, telencephalic stalk and basal ganglia to the specific presumptive neocortical areas that they will invade (Crandall and Caviness, 1984; Catalano et al., 1991; Kageyama and Robertson, 1993; Miller et al., 1993; Metin and Godement, 1996; Molnar et al., 1998a; Auladell et al., 2000). This first level of topographic organization (an inter-areal map), in which a given thalamic nucleus projects to a specific neocortical region, is important to relay visual, auditory, somatosensory and motor information to different neocortical areas. Within each neocortical area, thalamic axons establish connections that form an intra-areal topographic map, creating a second level of topographic organization (Schlaggar and O'Leary, 1994; Agmon et al., 1995). For example, the visual thalamus (dorsal lateral geniculate nucleus, dLGN) specifically projects to the occipital neocortex (visual cortical area), and within this region, a topographic map of visual space is generated through the distribution of axonal terminals (Kageyama and Robertson, 1993; O'Leary et al., 1994; Sur and Leamey, 2001).

The development of cortical-thalamic interconnections requires several pathfinding steps. For example, structures in the embryonic diencephalon and basal ganglia are implicated in providing signals that guide the growing axons, through attractants (Metin and Godement, 1996; Metin et al., 1997; Richards et al., 1997; Bagnard et al., 1998; Braisted et al., 1999; Braisted et al., 2000), repellents (Bagnard et al., 1998; Braisted et al., 1999; Bagri et al., 2002), or by producing pioneering axons (Mitrofanis and Guillery, 1993). In particular,

groups of cells in the basal ganglia have been proposed to be intermediate targets for cortical and thalamic axons, by producing attractants or forming transient projections to the thalamus (Mitrofanis and Guillery, 1993; Metin and Godement, 1996; Tuttle et al., 1999). In addition, it was shown that preplate and subplate cortical axons, which pioneer the corticofugal pathway, form a scaffold that guides thalamic axons into the neocortex (McConnell et al., 1989; Ghosh et al., 1990; Ghosh and Shatz, 1992; Ghosh and Shatz, 1993; Molnar et al., 1998b; McQuillen et al., 2002). These observations provided the basis of the 'handshake' hypothesis, which proposes that thalamic and cortical axons require each other to reach to their targets (Molnar and Blakemore, 1995; Molnar et al., 1998a). Support for this model comes from analysis of the *Tbr1* and *COUP-TFI* mutants, in which subplate defects impair the capacity of thalamocortical axons to reach the neocortex (Zhou et al., 1999; Hevner et al., 2001; Hevner et al., 2002). Similar results are also observed in *Emx1/Emx2* mutants (K. M. Bishop, S. G., Y. Nakagawa, J. L. R. R. and D. M. M. O'Leary, unpublished). Conversely, in *Gbx2* mutant mice, which have major thalamic defects, corticothalamic projections do not reach their target (Miyashita-Lin et al., 1999; Hevner et al., 2002). Taken together, these studies provide insights into how thalamic and cortical axons reach their respective target structure. However, very little is known about the mechanisms that control the targeting of thalamic axons to specific neocortical domains.

The chemoaffinity hypothesis stipulates that topographic projections are generated through the expression of molecules that mediate repulsion and/or attraction between the projecting axons and their target (Sperry, 1963; Goodhill and Richards, 1999). This mechanism was shown to play a key role in the establishment of retinotectal projections through Eph and ephrin protein interactions (Drescher et al., 1997; Goodhill and Richards, 1999; Feldheim et al., 2000) and is the prevailing model for the formation of topographic projections in the nervous system. It is implicated in the targeting of reticulogeniculate axons (Feldheim et al., 1998), of limbic thalamic axons to the limbic cortex (Barbe and Levitt, 1992; Mann et al., 1998) and hippocampal neurons to the septum (Gao et al., 1996), as well as in the formation of a topographic map within a neocortical area (Vanderhaeghen et al., 2000). Recent studies show that several genes, including genes encoding Eph/Ephrins, are expressed locally or in gradients in the neocortex before and shortly after the arrival of thalamic inputs (Bulfone et al., 1995; Gao et al., 1998; Nothias et al., 1998; Donoghue and Rakic, 1999; Mackarehtschian et al., 1999; Miyashita-Lin et al., 1999; Nakagawa et al., 1999; Rubenstein et al., 1999; Liu et al., 2000; Sestan et al., 2001), suggesting that the expression of localized cues within the neocortex may control the targeting of thalamic axons. Consistent with this model, the inactivation of *Emx2* and *COUP-TFI* transcription factor genes, that are expressed in high-caudal-low-rostral gradients in the cortical primordium, induces a change in neocortical molecular regionalization as well as a corresponding change in the pattern of thalamocortical connectivity (Bishop et al., 2000; Mallamaci et al., 2000b; Zhou et al., 2001; Muzio et al., 2002).

However, there is evidence that mechanisms operating outside of the neocortex may participate in regulating the development of thalamocortical topography. Explant culture experiments indicate that thalamic axons can innervate any region of the neocortex in vitro, suggesting the absence of an instructive code within the cortex (Molnar and Blakemore, 1991; Molnar and Blakemore, 1995). Based on a variety of studies, it has been proposed that molecular and/or temporal interactions between cortical and thalamic axons inside the internal capsule may regulate the regional specificity of thalamocortical connections (Molnar and Blakemore, 1991; Ghosh and Shatz, 1992; Ghosh and Shatz, 1993; Molnar and Blakemore, 1995; Molnar et al., 1998a).

So far, little is known about the mechanisms that control the initial targeting of thalamic axons to specific neocortical domains and the proposed models remain to be tested. To address this issue specifically, we searched for mouse mutants that exhibited projection defects in subpopulations of thalamic axons. We chose to analyze *Ebf1* mutant embryos because they have internal capsule pathfinding defects (Garel et al., 1999). *Ebf1* (also known as *Olf-1, O/E-1, Coe1*) encodes a HLH transcription factor (Hagman et al., 1993; Wang and Reed, 1993; Dubois and Vincent, 2001), and its inactivation affects basal ganglia development (Garel et al., 1999). We show that, in *Ebf1*$^{-/-}$ mutant embryos, axons from the dLGN are misrouted inside the basal ganglia and the projections that do form between thalamic nuclei and neocortical domains have a shifted topography. This shift occurs in the absence of an apparent change in thalamic or neocortical regionalization and is preceded by a shift in the positions of thalamic axons in the basal ganglia. These results indicate that thalamic projections from different nuclei are not formed independently of one another and raise the possibility that defects in the basal ganglia of *Ebf1*$^{-/-}$ embryos participate in shifting the early topography of thalamocortical axons. To test whether defects in structures located along the path of thalamic axons might shift thalamocortical topography, we analyzed *Dlx1/2* mutants that have defects in basal ganglia development (Anderson et al., 1997). *Dlx1* and *Dlx2* encode homeodomain transcription factors and are not expressed in cortical projection neurons or in the dorsal thalamus (Bulfone et al., 1993; Stühmer et al., 2002). In *Dlx1/2*$^{-/-}$ embryos, some thalamic axons fail to grow past the basal ganglia and, as in *Ebf1* mutants, thalamic axons that do reach the neocortex have a shifted topographic organization in the neocortex and basal ganglia. Taken together, our study suggests that the early topography of thalamocortical projections is not strictly and solely governed by information located within the neocortex and dorsal thalamus and that the positioning of thalamic axons within the basal ganglia may have an important role in organizing these projections.

## MATERIALS AND METHODS

### Mouse lines and genotyping

*Ebf1* heterozygous mice (Lin and Grosschedl, 1995) were maintained in a C57/Bl6 background and crossed to produce homozygous embryos. *Dlx1/2* heterozygous mice (Qiu et al., 1997) were maintained in mixed 129G and C57/Bl6 genetic background, and crossed to produce homozygous embryos. PCR genotyping of both lines was performed as described previously (Anderson et al., 1997; Qiu et al., 1997; Garel et al., 1999). Heterozygous embryos did not show any phenotype and were used as controls. For staging of embryos, midday of the day of vaginal plug formation was considered as embryonic day 0.5 (E0.5).

### In situ hybridization

Embryos were fixed overnight in 4% paraformaldehyde (PFA) at 4°C. In situ hybridization were performed on 80-100 μm thick vibratome sections as described previously (Garel et al., 1999) with the following probes: cadherin 6 (*Cdh6*) (a gift of M. Takeichi); *Cdh8* (a gift of M. Takeichi); *COUP-TFI* (a gift of M. Tsai); *Ebf1* (a gift of R. Grosschedl); *Emx2* (a gift of A. Simeone); *Epha4* (a gift of A. Nieto); *Epha7* (a gift of A. Wanaka); ephrin A2 (*Efna2* – Mouse Genome Informatics; a gift of J. Flanagan); ephrin A5 (*Efna5* – Mouse Genome Informatics; a gift of U. Drescher); *Fgfr3* (a gift of D. Ornitz); *Gbx2* (a gift of G. Martin); *Id2* (*Idb2* – Mouse Genome Informatics; a gift of M. Israel); *Lhx9* (a gift of S. Retaux); netrin 1 (*Ntn1* – Mouse Genome Informatics; a gift of M. Tessier-Lavigne); *Sema6a* (a gift of W. Snider). Sections were mounted in glycerol and analyzed under a dissection microscope.

### Axonal tracing

After overnight fixation in 4% PFA at 4°C, single crystals of the fluorescent carbocyanide dye DiI (1,1′-dioctadecyl 3,3,3′,3′-tetramethylindocarbocyanine perchlorate; Molecular Probes) or DiA (4-[4-(dihexadecyl amino)styryl]*N*-methyl-pyridinium iodide; Molecular Probes) were placed in single or multiple locations in the neocortex or dorsal thalamus (Godement et al., 1987; Metin and Godement, 1996). After 4-7 weeks at room temperature in 4% PFA to allow dye diffusion, the sample were embedded in 5% agarose and cut into 100 μm thick sections on a vibratome. Counterstaining was performed using Hoechst (Aldrich Chemicals) or SYTOX green (Molecular Probes) and digital images were taken using a Spot II camera on a fluorescent microscope or dissection microscope.

## RESULTS

### A specific subpopulation of thalamic axons is misrouted within the basal ganglia of *Ebf1*$^{-/-}$ mutant embryos

Previous analysis suggested that *Ebf1* inactivation may affect the navigation of specific populations of thalamic axons (Garel et al., 1999). To further characterize this phenotype, we performed axonal tracing experiments using DiI injections. Broad injections in the lateral part of the dorsal thalamus in E16 *Ebf1*$^{-/-}$ embryos showed that some thalamic axons are misrouted into the amygdalar region (Fig. 1A,B). These axons form a thick tract that ends in this region, except for a few axons that grow dorsally in the direction of the neocortex. On the contrary, at more rostral levels, thalamic axons appear normal within the internal capsule and grow into the neocortical intermediate zone (Fig. 1C,D). To identify the region of the thalamus that sends the misrouted axons, we placed a DiI crystal in the amygdalar region in E16.5 *Ebf1*$^{-/-}$ embryos. Retrogradely labeled cells were specifically found in a lateral thalamic area that has the characteristic shape and position of the presumptive dorsal lateral geniculate nucleus (dLGN) (Jones, 1985) (Fig. 1E,F). Thus, in the absence of *Ebf1*, dLGN axons are misrouted in the amygdalar region, whereas the rest of the thalamic axons normally grow into the internal capsule and the neocortex.

### The topography of connections between the dorsal thalamus and the neocortex is shifted in *Ebf1*$^{-/-}$ embryos

Axons from specific thalamic nuclei normally project towards particular neocortical domains (Crandall and Caviness, 1984; O'Leary et al., 1994; Sur and Leamey, 2001). We thus investigated in *Ebf1* mutants if dLGN axons reach their final target, the occipital neocortex and whether the general topography of thalamocortical projections is normal. We placed DiI crystals in three locations of the neocortex at E16.5 (Crandall and Caviness, 1984; Molnar et al., 1998a) (Fig. 2). In wild-type embryos, a DiI injection in the occipital neocortex labeled thalamic cell bodies and cortical axons in the dLGN (Fig. 2A,B,D,E). A DiI injection in the parietal neocortex, however, labeled cells and axon terminals in a more medial thalamic domain, where the

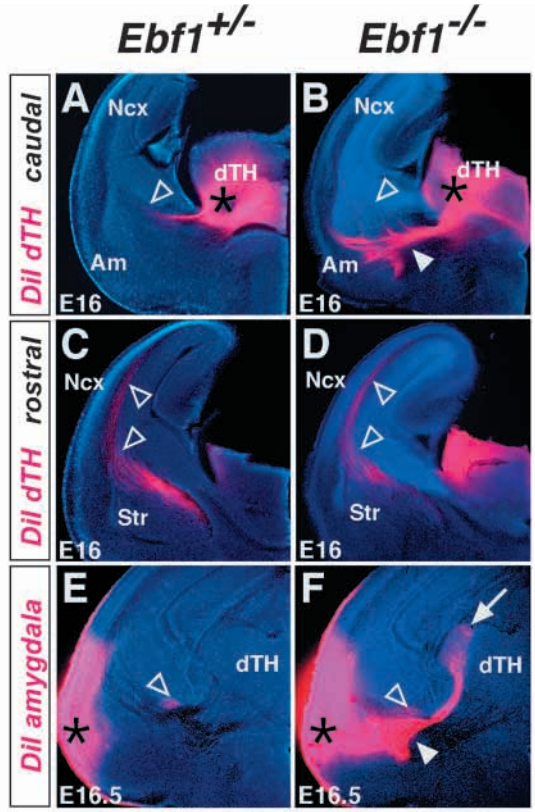


**Fig. 1.** dLGN axons are misrouted in the amygdalar region of *Ebf1*$^{-/-}$ embryos. DiI axonal tracing in controls (left) and *Ebf1*$^{-/-}$ mutant embryos (right). (A-D) Coronal hemisections of E16 brains in which a DiI crystal was placed in the lateral part of the dorsal thalamus (stars in A,B). In controls, DiI-labeled thalamic axons exit the dorsal thalamus and enter the internal capsule (open arrowhead in A). More rostrally, thalamic axons travel through the striatum and reach the neocortical intermediate zone (open arrowheads in C). In homozygous *Ebf1* mutants, at caudal levels, some thalamic axons grow ectopically into the amygdalar region (white arrowhead in B) and very few axons leave this aberrant tract to navigate through the striatum and towards the neocortex (open arrowhead in B). However, rostrally, *Ebf1* mutant thalamic axons are normally positioned in the striatum and neocortex (open arrowheads in D). (E,F) Coronal hemisections of E16.5 brains where a DiI crystal was placed in the amygdalar region (stars in E,F). The stria terminalis is stained in both controls and *Ebf1*$^{-/-}$ mutant embryos (open arrowhead in E,F). However, in *Ebf1*$^{-/-}$ embryos DiI labeling of the abnormal amygdalar tract (white arrowhead) retrogradely labels cells in a thalamic nucleus that has the shape and position of the dLGN (arrow). Am, amygdala; dTH, dorsal thalamus; Ncx, neocortex; Str, striatum.

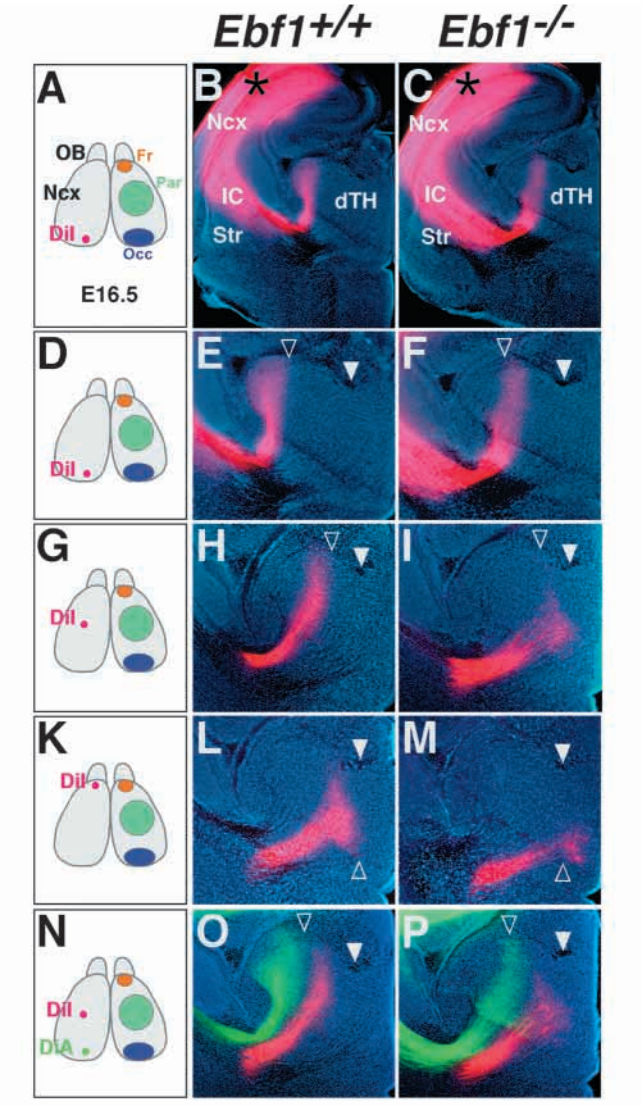


**Fig. 2.** *Ebf1* inactivation induces a shift in thalamocortical and corticothalamic connections. Coronal hemisections of E16.5 brains where crystals of DiI (A-M) or DiI and DiA (N-P) were placed in several regions of the neocortex of controls (left) and *Ebf1*$^{-/-}$ mutants (right). Schematic representations of a dorsal view of the brain (A,D,G,K,N) indicate the position of DiI and DiA crystals in the occipital (A,D), parietal (G), frontal (K), or parietal and occipital (N) neocortex. The insertion sites of DiI crystals are also visible in B and C (stars). The retroflexus tract, which can be used as a morphological landmark, is indicated by a white arrowhead (E,F,H,I,L,M,O,P). Open arrowheads indicate the medial boundary of the thalamic domain where cell bodies and axons are labeled in wild-type embryos (E,H,L,O). This wild-type boundary is indicated in *Ebf1*$^{-/-}$ embryos (open arrowheads) and shows that position of the labeled thalamic domains are shifted medially (F,I,M,P). dTH, dorsal thalamus; Fr, frontal neocortex; IC, internal capsule; Ncx, neocortex; OB, olfactory bulb; Occ, occipital neocortex; Par, Parietal neocortex; Str, striatum.

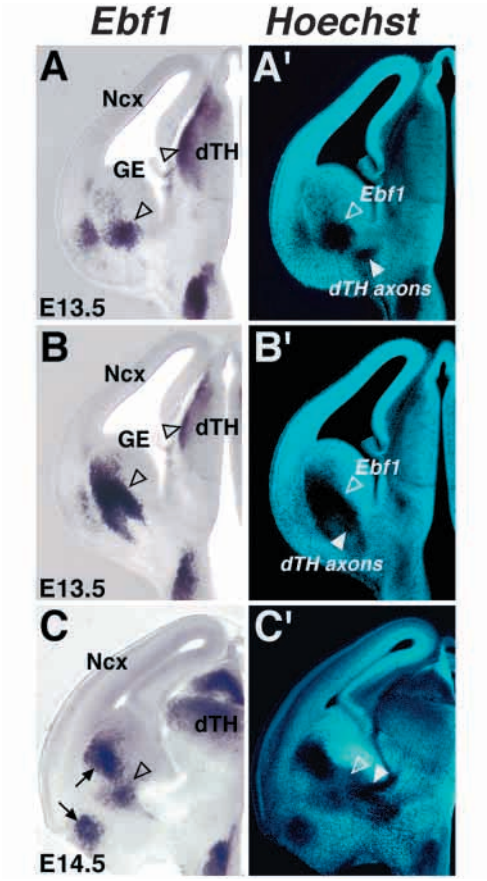


**Fig. 3.** *Ebf1* is expressed in the dorsal thalamus and in the basal ganglia, along the path of thalamic axons. Coronal hemisections of E13.5 (A,A′,B,B′) or E14.5 (C,C′) wild-type brains processed for *Ebf1* in situ hybridization and Hoechst fluorescent cell stain. On the left, brightfield pictures showing *Ebf1* expression are presented. On the right are fluorescent pictures of the same sections, where *Ebf1* expression (open arrowheads in A′,B′,C′) as well as low cell-density fiber tracts such as the thalamic axons (white arrowheads in A′,B′,C′) appear in black. At E13.5, *Ebf1* expression is observed in the mantle of the dorsal thalamus and of the ganglionic eminences (open arrowheads in A,B). Note that by E13.5, *Ebf1* expression is undetected in the layer I of the neocortex. At E14.5, *Ebf1* expression is observed in the amygdala and striatum (arrows in C) as well as in a group of cells located near the telencephalon-diencephalon boundary (open arrowhead in C). *Ebf1*-expressing cells are located on the path of thalamic axons at both ages (A′,B′,C′). dTH, dorsal thalamus; GE, ganglionic eminence; Ncx, neocortex.

presumptive ventrobasal complex (VB) is located (Jones, 1985) (Fig. 2G,H). Finally, a DiI injection in the frontal neocortex labeled cells and axons in an even more medial domain, which includes the presumptive ventromedial nucleus (VM) (Jones, 1985) (Fig. 2K,L). In *Ebf1*$^{-/-}$ mutant embryos, DiI injections in these three neocortical zones systematically labeled cell bodies and axons in a thalamic domain located more medially than in controls (Fig. 2A-M). Thus, thalamocortical and corticothalamic projections are shifted. This medial shift was confirmed by a double injection in the parietal and occipital neocortex with DiA and DiI, respectively (Fig. 2N-P). Taken together, in *Ebf1*$^{-/-}$ embryos, axons of the dLGN do not reach the occipital neocortex and the topography of thalamocortical projections is shifted with a given thalamic nucleus projecting towards a more caudal neocortical domain (Figs 2, 10).

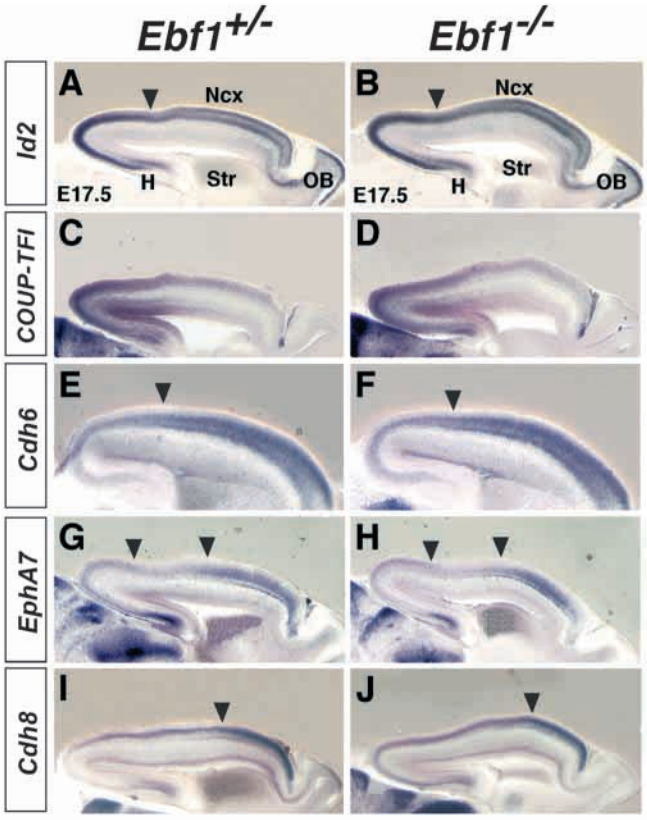


**Fig. 4.** Neocortical regionalization is not affected by *Ebf1* inactivation. Sagittal sections of E17.5 heterozygous (left) and homozygous (right) *Ebf1* mutants processed for in situ hybridization with the following probes: *Id2* (A,B), *COUP-TFI* (C,D), *Cdh6* (E,F), *Epha7* (G,H) and *Cdh8* (I,J). Arrowheads indicate rostrocaudal boundaries or changes in gene expression. These boundaries or gradients of expression are not changed in *Ebf1*$^{-/-}$ mutant embryos. H, hippocampus; Ncx, neocortex; OB, olfactory bulb; Str, striatum.

## No apparent defects in the dorsal thalamus and neocortex of *Ebf1*$^{-/-}$ embryos

How could such a shift in the topographic organization of projections occur? *Ebf1* is expressed in layer I of the neocortex between E10.5 and E12.5, in the mantle of the dorsal thalamus between E12.5 and E14.5, and in several nuclei in the embryonic basal ganglia (Wang and Reed, 1993; Garel et al., 1997; Garel et al., 1999) (Fig. 3). Thus, a straightforward explanation would be that *Ebf1* inactivation perturbs the positioning or specification of the various thalamic nuclei and/or affects regionalization within the neocortex.

Although the expression pattern of *Ebf1* does not suggest a role in neocortical regionalization, we nevertheless checked whether its inactivation affected patterning or lamination of the neocortex, based on gene expression patterns. At E12.5, *COUP-TFI*, *Emx2* and *Fgfr3* are expressed in gradients along the anteroposterior axis of the neocortex (Simeone et al., 1992; Liu et al., 2000; Ragsdale and Grove, 2001; Muzio et al., 2002) and participate in early cortical regionalization (Bishop et al., 2000; Mallamaci et al., 2000b; Zhou et al., 2001). Later in development, at E17.5, the expression pattern of *COUP-TFI*, *Id2*, *Cdh6*, *Cdh8*, *Epha5* and *Epha7* are in gradients or restricted to presumptive cortical areas and show specific laminar patterns (Suzuki et al., 1997; Donoghue and Rakic, 1999; Mackarehtschian et al., 1999; Miyashita-Lin et al., 1999; Nakagawa et al., 1999; Rubenstein et al., 1999; Liu et al., 2000). At both ages, we did not observe any changes in the cortical expression patterns of these genes in *Ebf1*$^{-/-}$ mutant embryos (Fig. 4; data not shown).

We next studied the morphology of the dorsal thalamus as well as the expression patterns of *Cdh6*, *Cdh8*, *COUP-TFI*, *Epha4*, *Epha7*, ephrin A2, ephrin A5, *Gbx2*, *Lhx9* and *Sema6a*. The expression of these genes is restricted to specific domains or nuclei in the dorsal thalamus, allowing us to establish the position and molecular properties of the developing thalamic nuclei (Suzuki et al., 1997; Zhou et al., 1997; Feldheim et al., 1998; Miyashita-Lin et al., 1999; Retaux et al., 1999; Liu et al., 2000; Nakagawa and O'Leary, 2001) (Fig. 5). In *Ebf1*$^{-/-}$ mutant embryos, we did not observe changes in the expression patterns of these genes between E14.5 and E16.5 (Fig. 5; data not shown).

Overall, our gene expression analysis shows that *Ebf1* inactivation does not severely perturb the molecular regionalization of the neocortex and dorsal thalamus. Thus, the shift in thalamocortical projections is unlikely to result from a general change in the positioning or molecular identity of the different thalamic nuclei.

## *Ebf1* inactivation affects early thalamic axon pathfinding in the basal ganglia

To better understand this phenotype, we examined how it develops, by performing DiI injections in the neocortex and dorsal thalamus in early embryos, before thalamic and cortical axons meet in the internal capsule. Using DiI injections in the neocortex at E13.5 and E14.5, we did not detect any defects in the growth and trajectory of cortical axons into the mantle zone of the ganglionic eminences, the basal ganglia primordium, in *Ebf1*$^{-/-}$ embryos (Miller et al., 1993; Metin and Godement, 1996; Molnar et al., 1998a; Auladell et al., 2000) (Fig. 6A-D). However, DiI injections in the dorsal thalamus at E13.5 showed that, in *Ebf1*$^{-/-}$ embryos, the thalamic axons growing into the ganglionic eminences do not make a sharp turn in direction of the neocortex (Fig. 6E,F). Instead, they are misrouted towards the pial surface (Fig. 6E,F). At E14.5 and E15.5, the misrouted thalamic axons begin to navigate towards the amygdala (Fig. 6G,H, Fig. 1A,B). Thus, whereas early cortical axons show normal navigation before encountering thalamic axons, thalamic axons are already misrouted when they enter the basal ganglia (Miller et al., 1993; Metin and Godement, 1996; Molnar et al., 1998a; Auladell et al., 2000).

We examined the different cell populations that have been proposed to guide thalamic axons in the ganglionic eminences. In wild-type embryos, thalamic DiI injections retrogradely label cells of the perireticular nucleus, which are located in the internal capsule and form transient projections with the thalamus (Mitrofanis and Guillery, 1993) (data not shown). In *Ebf1*$^{-/-}$, thalamic DiI injections label a group of basal ganglia cells that is located where the internal capsule axonal bundle would normally be in E13.5 embryos (arrow in Fig. 6F), indicating that at least some perireticular nucleus cells are normally positioned. Next, we examined the expression of axonal guidance molecules, such as *Sema6a* and netrin 1, which are implicated in regulating the growth of thalamic axons into and through the basal ganglia. *Sema6a* encodes a

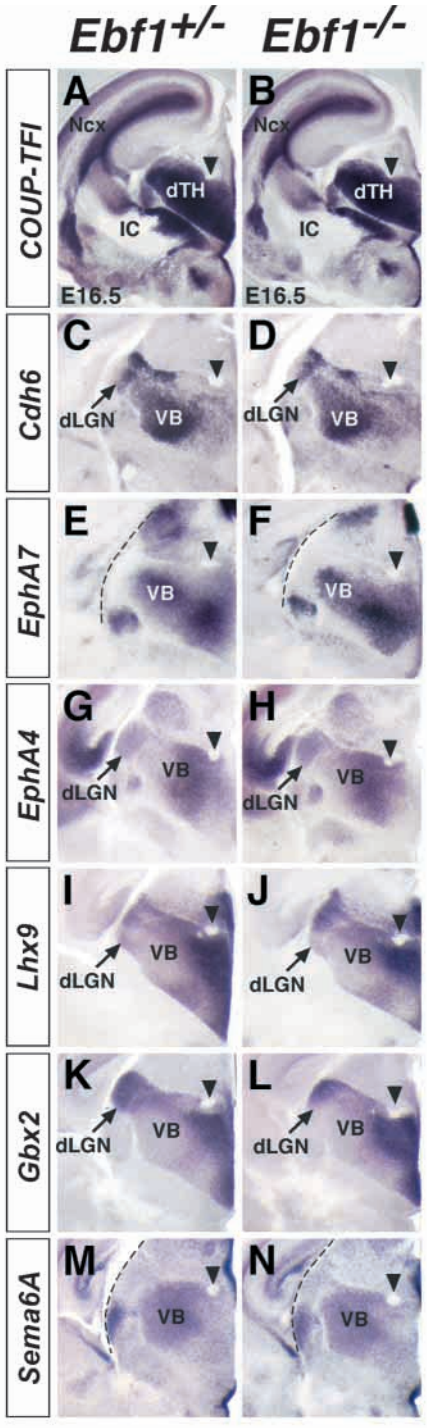


**Fig. 5.** *Ebf1* inactivation does not affect the molecular regionalization of the dorsal thalamus. Coronal hemisections of E16.5 heterozygous (left) and homozygous (right) *Ebf1* mutant brains processed for in situ hybridization with the following probes: *COUP-TFI* (A,B), *Cdh6* (C,D), *Epha7* (E,F), *Epha4* (G,H), *Lhx9* (I,J), *Gbx2* (K,L) and *Sema6a* (M,N). The expression domains of *COUP-TF1*, *Lhx9* and *Gbx2* cover large domains of the dorsal thalamus (A,I,K), *Epha7* and *Sema6a* are excluded from dLGN (E,M), *Cdh6* and *Sema6a* are low in medial nuclei (C,M), and *Epha4* shows a lateromedial gradient within VB (G). Expression of these genes is not apparently perturbed by *Ebf1* inactivation. Black arrowheads indicate the position of the retroflexus tract. A broken line indicates the pial surface of the thalamus in E,F,M,N. dLGN, dorsal lateral geniculate nucleus; dTH, dorsal thalamus; IC, internal capsule; Ncx, neocortex; VB, ventrobasal complex.

transmembrane semaphorin (Zhou et al., 1997) and its inactivation affects the pathfinding of a subset of thalamic axons in the caudal region of the basal ganglia (Leighton et al., 2001). *Sema6a* is expressed in the basal ganglia and in the dorsal thalamus (Zhou et al., 1997; Leighton et al., 2001) (Fig. 6I,K). In *Ebf1*$^{-/-}$ mutant embryos, *Sema6a* expression in the thalamus was unaffected at E13.5 and E16.5 (Fig. 6I-L). By contrast, *Sema6a* expression was greatly reduced in a group of cells located in the basal ganglia (Fig. 6I-L). These cells were located close to the entrance point of thalamic axons in the basal ganglia (Fig. 3A′-C′), where thalamic axons start to show pathfinding defects in *Ebf1* mutants (compare Fig. 6E-H with Fig. 6I-L). On the contrary, expression domains of the gene for netrin 1, which encodes a secreted molecule involved in the guidance of thalamic and cortical axons (Metin et al., 1997; Richards et al., 1997; Tuttle et al., 1999; Braisted et al., 2000), were not severely modified by *Ebf1* inactivation (Fig. 6M-P).

Overall, in *Ebf1* mutants, early thalamic axons fail to normally turn towards the cerebral cortex as they enter the basal ganglia, creating an abnormal tract in the amygdalar region. This defect may be due to a change in molecular properties in specific subsets of basal ganglia cells.

### Thalamic axons are caudally shifted in the internal capsule of *Ebf1*$^{-/-}$ embryos

Thalamic and cortical axons are topographically ordered along their trajectory in the internal capsule within the basal ganglia (Molnar et al., 1998a). We thus examined if the abnormal turn of thalamic axons inside the basal ganglia of *Ebf1*$^{-/-}$ embryos affected the topographic organization of axons inside the internal capsule.

In E16.5 wild-type embryos, double DiI and DiA injections in the frontal and parietal neocortex (Fig. 7A,B), or in the occipital and parietal neocortex (Fig. 7D,E), show that the axons originating from and projecting to a given neocortical domain form ordered bundles in the internal capsule (Fig. 7B,E). *Ebf1* mutants showed a normal organization of these axon bundles (Fig. 7A-F).

We next examined the position of axons inside the internal capsule by double DiI and DiA injections into the putative dLGN nucleus and VB complex, respectively. In wild-type embryos, the bundle of axons labeled by dLGN injections runs through the caudal part of the striatum and caudal regions of the neocortex (Fig. 7G,H,J). Conversely, axons labeled by VB injections grow at an intermediate anteroposterior position and invade the parietal neocortex (Fig. 7G,H,J). In *Ebf1*$^{-/-}$ embryos, dLGN axons form the abnormal tract that travels towards the amygdalar region (Fig. 7I). VB injections label a bundle of axons located inside the internal capsule; however, these are located more caudally, where dLGN-labeled axons would normally be in wild-type embryos (Fig. 7J,K).

Thus, altogether, these results indicate cortical axons originating from a given domain have a normal navigation and topography in the internal capsule in *Ebf1*$^{-/-}$ embryos. Conversely, thalamic axons show a shift in their position inside the internal capsule, and, consistent with their new trajectory

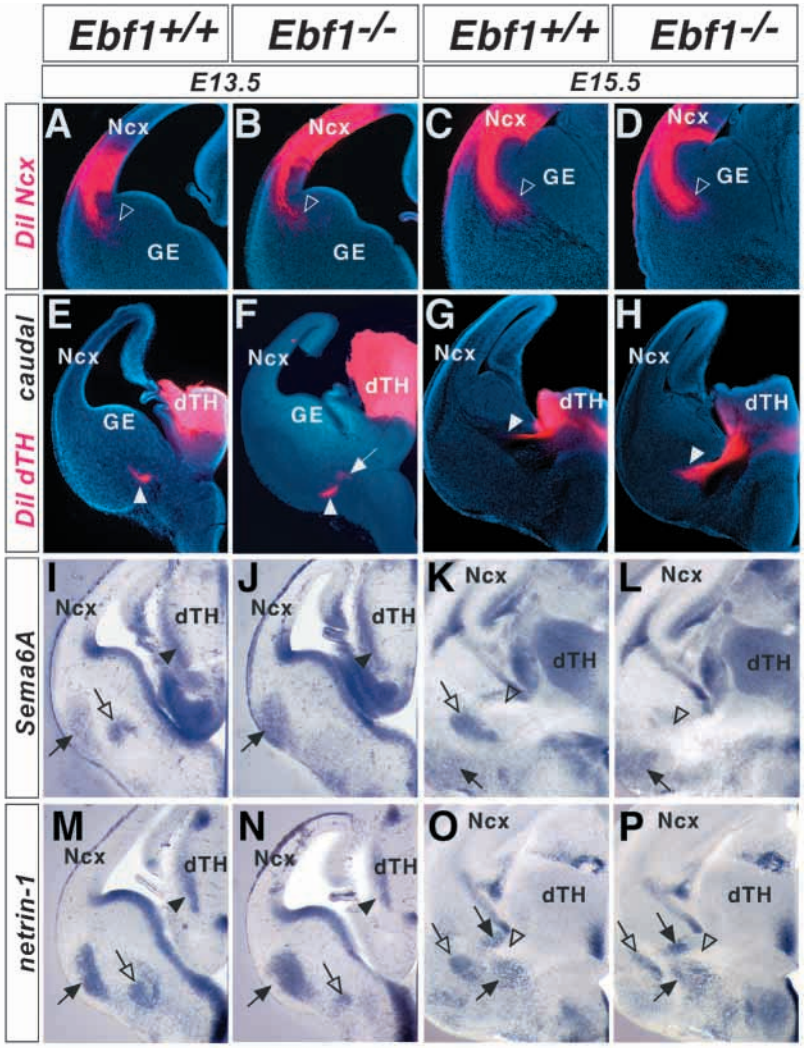


**Fig. 6.** *Ebf1* inactivation affects the pathfinding of early thalamic axons and *Sema6a* expression in the primordium of the basal ganglia. (A-H) Coronal hemisections of E13.5 (left) and E15.5 (right) wild-type and *Ebf1*$^{-/-}$ brains where a DiI crystal was introduced in the lateral part of the neocortex (A-D) or of the dorsal thalamus (E-H). In both wild-type and *Ebf1*$^{-/-}$ embryos, axons labeled by cortical DiI crystals make a sharp turn and enter the ganglionic eminences (open arrowhead in A-D). Crystals placed in the dorsal thalamus of wild-type embryos show that DiI-labeled thalamic axons (white arrowhead in E,G) make a sharp turn into the ganglionic eminences in the direction of the neocortex. On the contrary, in *Ebf1*$^{-/-}$ embryos thalamic axons run closer to the pial surface of the brain (white arrowhead in F,H). In E13.5 brains, a group of retrogradely labeled cells is detected (arrow in F) at the position where thalamic axons are located in wild-type embryos (compare arrow in F with arrowhead in E). These cells of the perireticular nucleus are intermingled with the thalamic axons in wild-type embryos. (I-P) Coronal hemisections of E13.5 (left) and E15.5 (right) wild-type and *Ebf1*$^{-/-}$ brains processed for *Sema6a* (I-L) and netrin 1 (M-P) in situ hybridization. In E13.5 controls (I), *Sema6a* is expressed in the mantle of the dorsal thalamus (black arrowhead), in a group of cells in the mantle of the ganglionic eminences (open arrow) and in the ventral pallium (black arrow). In *Ebf1*$^{-/-}$ mutant E13.5 embryos (J), although the thalamic expression is unaffected (black arrowhead), *Sema6a* expression in the mantle of the ganglionic eminences is greatly reduced. In E15.5 wild-type embryos (K), thalamic fibers (open arrowhead) grow dorsally to a group of *Sema6a*-expressing cells (open arrow). However in *Ebf1*$^{-/-}$ mutant embryos (L), *Sema6a* expression is strongly reduced and thalamic axons (open arrowhead) invade a zone that would be *Sema6a* positive in wild-type embryos. Netrin 1 expression, in E13.5 controls (M) is detected in the dorsal thalamus mantle (black arrowhead), in a group of cells in the mantle of the ganglionic eminences (open arrow) and in the ventral pallium (black arrow). At E15.5 (O), two groups of netrin 1-positive cells (black arrows) are located on both sides of the incoming thalamic axons (open arrowhead) and another group of labeled cells is located more laterally (open arrowhead). In *Ebf1*$^{-/-}$ mutant embryos (N,P) this expression pattern was not clearly changed, except for a slight reduction at E13.5, in the expression domain in the ganglionic eminences (compare M with N). dTH, dorsal thalamus; GE, ganglionic eminence; Ncx, neocortex.

invade a more caudal region of the neocortex. Thus, we observe a global shift in the position of thalamic axons just after their turn into the basal ganglia.

### *Dlx1/2* inactivation affects the topography of thalamocortical projections

Our analysis of the *Ebf1* phenotype indicates that a misguidance of specific thalamic axons and a general shift in the position of the others can occur in the absence of apparent defects in the neocortex and dorsal thalamus. As *Ebf1* is expressed in the dorsal thalamus, the possibility that its inactivation may affect thalamic neurons cannot be excluded. Nevertheless, our results raise the possibility that developmental defects in the basal ganglia of *Ebf1*$^{-/-}$ embryos may shift the topography of thalamocortical connections. To further examine if affecting structures on the path of thalamic axons can deviate the topography of thalamocortical projections, we examined *Dlx1/2* $^{-/-}$ mutant embryos (Qiu et al., 1997). *Dlx1* and *Dlx2* encode homeodomain transcription factors and are expressed in the basal ganglia and in the ventral thalamus, but not in neocortical projection neurons or in the dorsal thalamus (Bulfone et al., 1993). *Dlx1/2*$^{-/-}$ mutant embryos have a block in differentiation of the basal ganglia (Anderson et al., 1997). DiI injections in the neocortex and dorsal thalamus of E14 *Dlx1/2*$^{-/-}$ embryos shows that both cortical and thalamic axons fail to make a sharp turn into ganglionic eminences and are displaced towards the pial surface (Fig. 8A-F). This defect is probably due to the expansion of the subventricular zone (SVZ*) in the basal ganglia (Anderson et al., 1997) and results in the formation of a displaced and highly disorganized internal capsule, as shown by DiI injections in the dorsal thalamus of E16.5 embryos (Fig. 8G-J). Although some thalamic axons reach the neocortex, a large number of the misrouted axons remain in the basal ganglia (Fig. 8G-J). The identity of the thalamic nuclei generating the axons that fail to reach to cortex could not be unequivocally determined because of the disorganization of the internal capsule.

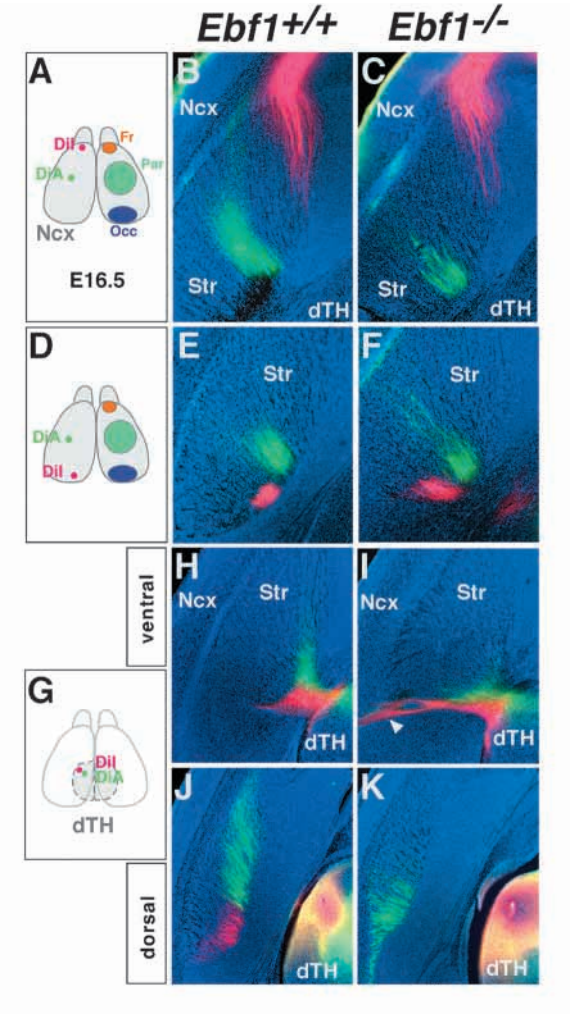


**Fig. 7.** A caudal shift of thalamic axons is detected inside the internal capsule of *Ebf1*$^{-/-}$ embryos. Horizontal hemisections of E16.5 wild-type (left) and *Ebf1*$^{-/-}$ mutant (right) brains in which one crystal of DiI and one of DiA were introduced in: (1) the frontal and parietal neocortex (A-C); (2) the occipital and parietal neocortex (D-F); and (3) in two putative thalamic nuclei – the dorsal lateral geniculate nucleus (dLGN) and ventrobasal complex (VB) (G-K). Schematic diagrams show the locations of DiI and DiA injection sites (A,D,G). (A-F) Horizontal sections of either frontal and parietal neocortex double injections (A-C) or occipital and parietal neocortex double injections (D-F). In wild-type embryos, bundles of labeled axons are restricted to regions of the internal capsule. The position of neocortical axons was not detectably altered in *Ebf1* mutants (compare B with C and E with F). (G-K) Horizontal sections at ventral levels (H,I) and more dorsal levels (J,K) of brains following a thalamic double injection. Even though the tracer crystals were relatively small, a large number of axons were stained in our experiments because of the small size of the thalamus. In wild-type animals, putative dLGN axons (red) and VB axons (green) turn into the striatum (H) and remain as two separate bundles in the caudal and intermediate regions of the internal capsule, respectively (J). In *Ebf1*$^{-/-}$ mutant embryos, dLGN axons are detected as an abnormal tract going towards the amygdala (white arrowhead in I). In dorsal sections, only VB axons are detected (green) and they are located in a more caudal region of the internal capsule than in controls (compare J with K). dTH, dorsal thalamus; Fr, frontal neocortex; Ncx, neocortex; Occ, occipital neocortex; Par, parietal neocortex; Str, striatum.

The topography of thalamocortical projections was examined by introducing DiI and DiA crystals in the occipital or parietal neocortex of E16.5 heterozygous and homozygous embryos (Fig. 9A-L). These experiments retrogradely labeled fewer cells and axons in homozygous embryos than in controls (Fig. 9A-L), confirming that numerous thalamic axons do not reach the cortex. However, DiI injections (Fig. 9A-I) or double DiI and DiA injections (Fig. 9J-L) in the neocortex of *Dlx1/2*$^{-/-}$ embryos systematically labeled cells located in a more medial domain (Fig. 9A-F) or in a wider domain that extends more medially (Fig. 9G-L) than in controls. Thus, the topography of thalamic projections into the neocortex is systematically shifted in *Dlx1/2*$^{-/-}$ mutants.

We next examined the positioning of thalamic axons within the internal capsule at E16.5 by placing DiI and DiA crystals inside the presumptive dLGN and VB (Fig. 9M-Q). In *Dlx1/2*$^{-/-}$ embryos, dLGN axons are present in ventral parts of the internal capsule (Fig. 9N,O) but most of these axons do not grow dorsally into the neocortex (Fig. 9P,Q). VB axons are shifted to a more caudal position within the internal capsule, particularly in its dorsal parts (Fig. 9P,Q). Thus, in *Dlx1/2*$^{-/-}$ mutants, a majority of dLGN axons fail to reach to neocortex and the topography of the remaining thalamocortical projections is shifted in the internal capsule and neocortex, as in the *Ebf1*$^{-/-}$ mutants.

## DISCUSSION

During embryogenesis, different nuclei of the dorsal thalamus and cortical domains establish precise interconnections that are key to the relay and processing of sensory and motor information. We provide new information on the mechanisms regulating the initial targeting of thalamocortical axons to specific cortical domains. Our analysis of *Ebf1* and *Dlx1/2* mutant embryos shows that individual thalamic projections are not formed independently of each other, and suggests that altering the development of structures located on the pathway of thalamic axons shifts the early topography of thalamocortical projections inside the internal capsule and in the neocortex. These results indicate that cues regionally restricted within the neocortex and dorsal thalamus do not precisely dictate the initial topography of thalamocortical projections. Furthermore, our study suggests that the positioning of thalamic axons inside the basal ganglia is important for regulating this topography.

### Thalamic projections are shifted in the absence of apparent thalamic and neocortical defects in *Ebf1* and *Dlx1/2* mutant embryos

We show that *Ebf1* inactivation drastically affects the pathfinding of a subset of thalamic axons and creates a shift in the topography of the remaining thalamocortical projections (Fig. 10). This shift is observed in the absence of apparent thalamic or neocortical defects between E13.5 and E17 (Figs 4, 5) and is first detected within the basal ganglia primordium (Fig. 10). Furthermore, during the period when thalamic axons are traveling through the basal ganglia primordium (E13.5-E15.5) (Miller et al., 1993; Metin and Godement, 1996; Molnar et al., 1998a; Auladell et al., 2000), this structure exhibits

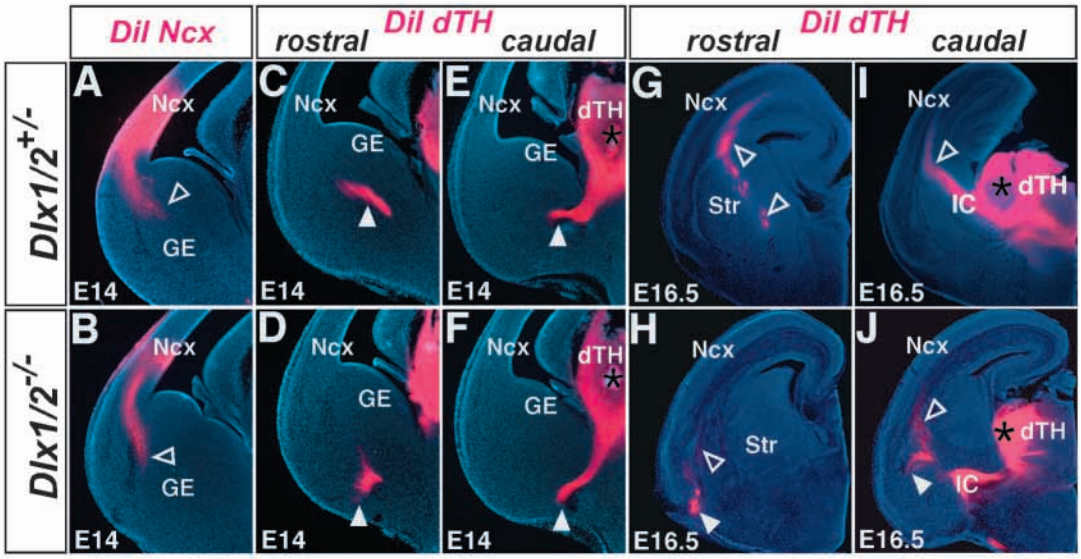


**Fig. 8.** Both cortical and thalamic axons pathfinding defects contribute to the formation of an abnormal internal capsule in *Dlx1/2*$^{-/-}$ embryos. Coronal hemisections of E14 (A-F) and E16.5 (G-J) *Dlx1/2* heterozygous (upper panel) and homozygous (lower panel) mutant brains where DiI crystals were introduced in the lateral part of the neocortex (A,B) or of the dorsal thalamus (C-J). Stars in E,F,I,J indicate DiI crystal positions. (A,B) DiI crystals in the lateral neocortex of control embryos label axons that make a turn and enter the ganglionic eminences (open arrowhead in A). In *Dlx1/2*$^{-/-}$ embryos, these axons, do not make a sharp turn and grow into the ganglionic eminences towards the pial surface (open arrowhead in B). (C-F) DiI crystals in the dorsal thalamus of control embryos label thalamic axons that make a sharp turn into the ganglionic eminences (white arrowhead in E). In more rostral sections (C), axons grow in direction of the neocortex (white arrowhead in C) reaching the region where cortical axons enter the ganglionic eminences (compare C with A). In *Dlx1/2*$^{-/-}$ embryos, thalamic axons fail to make a sharp turn (white arrowhead in F) and grow towards the pial surface of the ganglionic eminences (white arrowhead in D). Note that misrouted thalamic and cortical axons both travel in a more superficial domain of the ganglionic eminences (compare A with C and B with D). (G-J) A DiI crystal (star in I,J) in the lateral part of the dorsal thalamus labels the internal capsule, which is displaced closer to the pial surface in *Dlx1/2*$^{-/-}$ mutant embryos. Furthermore, in addition to axons that navigate to the neocortex (open arrowheads), axons diving into the amygdalar region (white arrowhead in J) were detected. These axons were detected in more rostral sections (H), running in the ventral pallium (white arrowhead), whereas axons of the superficially displaced internal capsule run dorsally into the necortex (compare open arrowheads in G,H). dTH, dorsal thalamus; GE, ganglionic eminences; IC, internal capsule; Ncx, neocortex; Str, striatum.

molecular defects (Garel et al., 1999), including an abnormal expression of *Sema6a* (Fig. 6I-L). Thus, although the loss of *Ebf1* expression in the dorsal thalamus may affect thalamic neurons, our molecular analysis shows that the shift in topography is not due to a general change in the molecular regionalization of the neocortex or dorsal thalamus. Furthermore, our results raise the possibility that this shift may be due to defects in the basal ganglia.

If defects in structures located on the path of thalamic axons can shift the topography of thalamocortical axons, we would expect to observe a similar phenotype in other mutant mice that have basal ganglia defects. We thus examined *Dlx1/2* mutants where differentiation of the basal ganglia and ventral thalamus is abnormal (Anderson et al., 1997; Marin et al., 2000; Yun et al., 2002). Furthermore, *Dlx1* and *Dlx2* are not expressed in the dorsal thalamus or in cortical projection neurons (Bulfone et al., 1993; Stuhmer et al., 2002). In *Dlx1/2* mutants, the formation of the internal capsule is perturbed, probably because of a block in basal ganglia differentiation (Anderson et al., 1997; Yun et al., 2002) and, as in *Ebf1* mutants, the topography of thalamocortical projections is shifted in the neocortex and internal capsule (Figs 9, 10). Thus, the phenotype of *Dlx1/2*$^{-/-}$ mice supports the possibility that affecting structures on the path of thalamic axons can shift the topography of thalamocortical projections. It could be argued that the reduction of cortical interneurons in *Dlx1/2* mutants (Anderson et al., 1997) might contribute to this phenotype. However, this is unlikely because *Nkx2.1* mutants, which also have a major deficit in neocortical interneurons, have normal thalamocortical projections (Marin et al., 2002).

Thus, our combined study of *Ebf1* and *Dlx1/2* mutants shows that the topography of thalamocortical axons can be systematically shifted in the absence of apparent abnormalities in the neocortex and dorsal thalamus, and suggests that this shift is due to defects in structures located on the path of the axons.

### The specificity of thalamic axons targeting is not dictated by cues located within the neocortex

Specific aspects of the phenotypes of *Ebf1* and *Dlx1/2* mutant embryos provide information on the mechanisms controlling the initial topography of thalamocortical projections. In particular, the systematic and coherent shift in the topography of thalamocortical axons indicates that projections of a given thalamic nucleus are not established independently of the projections of the other nuclei. In addition, this shift suggests that the mechanisms involved are likely to control the relative rather than absolute position of axons. This has been observed in the retinotectal system (Brown et al., 2000), suggesting that this feature may be a common characteristic for the formation of topographic projections.

Another aspect of the phenotypes of *Dlx1/2* and *Ebf1* mutants is that thalamic axons have a shifted position inside the basal ganglia before entering aberrant regions of the neocortex. If gradients of guidance cues within the neocortex were strictly governing the organization of thalamic inputs, we would have expected the thalamic axons to be redirected to

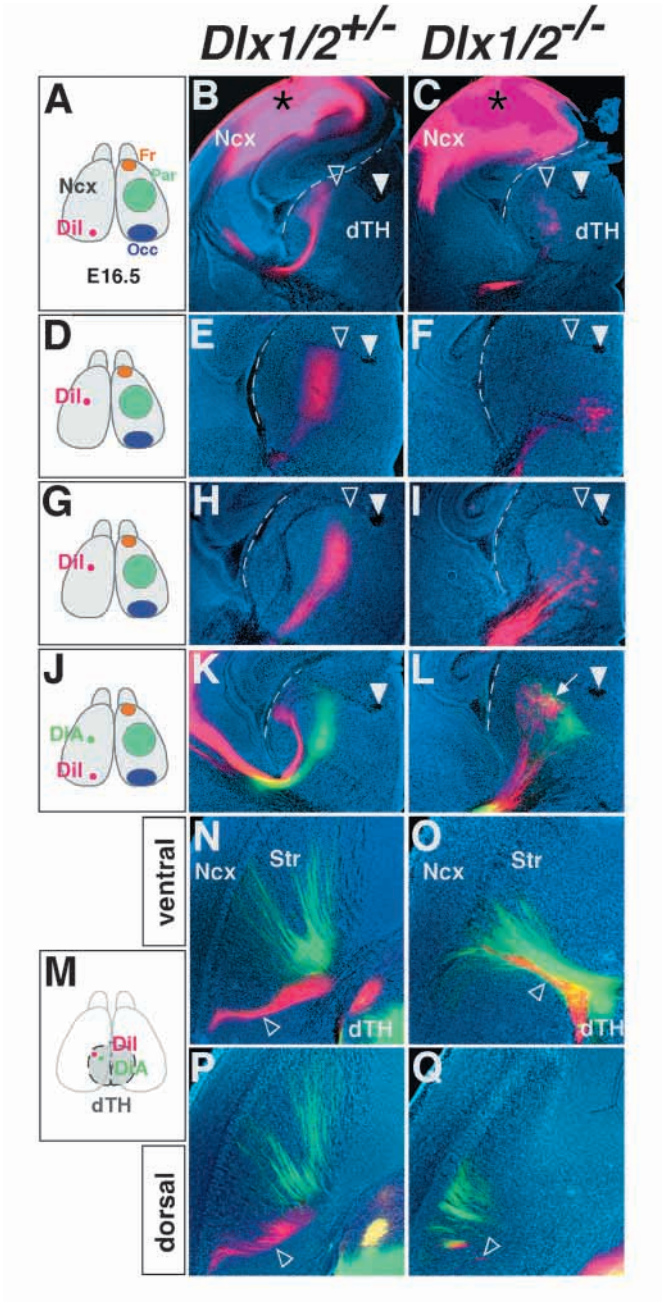


**Fig. 9.** The topography of thalamocortical projections is shifted in *Dlx1/2*$^{-/-}$ embryos. Coronal (A-L) or horizontal (M-Q) hemisections of E16.5 *Dlx1/2* heterozygous (left) and homozygous (right) mutant brains where DiI crystals (A-I) or DiI and DiA crystals (J-Q) were introduced in the occipital neocortex (A-C), the parietal neocortex (D-I), the occipital and parietal neocortex (J-L), or the putative dorsolateral geniculate nucleus (dLGN) and ventrobasal (VB) complex (M-Q). Schematic diagrams indicate the position of DiI and DiA crystals (A,D,G,J,M) and stars indicate their actual position in B,C. (A-L) Morphological landmarks, including the pial surface of the thalamus (broken line) and the retroflexus tract (white arrowhead), are used to position presumptive thalamic nuclei. In controls, injections in the occipital neocortex label cells in the putative dLGN (B,K) and injections in the parietal neocortex label cells in the VB complex (E,H,K). Open arrowheads indicate the medial boundary of the thalamic domain stained in wild-type embryos (B,E,H,K). This boundary is indicated in *Dlx1/2*$^{-/-}$ embryos and shows that thalamic domains labeled by occipital and parietal injections are shifted medially (C,F). Note that the number of cells labeled is reduced in homozygous mutant embryos (compare B with C and E with F). In some cases, the region containing labeled cells was broader in mutant embryos, partially including the domain labeled in controls as well as a more medial domain (H,I). Similarly, double occipital and parietal injections show that the labeled domains in mutant embryos are medially displaced (K,L). In this case there is some overlap in the regions labeled by each dye (arrow in L). (M-Q) Horizontal sections at ventral levels (N,O) and more dorsal levels (P,Q) of brains after a thalamic double injection in the presumptive dLGN and VB. Even though the tracer crystals were relatively small, a large number of axons were stained in our experiments because of the small size of the thalamus. In wild-type animals, putative dLGN axons (red) and VB axons (green) turn into the striatum and remain as two separate bundles in the caudal (open arrowheads in N and P) and intermediate regions of the internal capsule, respectively (N,P). In *Dlx1/2*$^{-/-}$ mutant embryos, dLGN axons are primarily detected ventrally (open arrowhead in O), and are mixed with VB axons. In more dorsal sections (Q), very few dLGN axons are visible (open arrowhead), indicating that they remain in ventral regions. On the contrary, a large number of VB axons are detected in a caudal region where normally dLGN axons travel (compare P with Q). dTH, dorsal thalamus; Fr, frontal neocortex; IC, internal capsule; Ncx, neocortex; Occ, occipital neocortex; Par, parietal neocortex; Str, striatum.

their appropriate destinations within the neocortex. Thus, positional information within the neocortex cannot correct the ectopic trajectory of the thalamic projections, at least at the early developmental stages we have studied. Therefore, either positional cues do not normally have a central role in regulating early thalamocortical projections, or perturbing axons on their way to the neocortex over-rides the activity of instructive gradients. These observations are in agreement with in vitro experiments in which thalamic axons can invade any region of the neocortex (Molnar and Blakemore, 1991). Furthermore, these data suggest that the initial organization of thalamocortical projections is not strictly regulated by a 'classical' chemoaffinity mechanism, where axons are guided by positional cues within the target structure (Drescher et al., 1997; Feldheim et al., 2000; Brown et al., 2000).

## The internal capsule: a decision point for the topography of thalamocortical projections?

Our results suggest that the positioning of thalamic axons inside the internal capsule is important for the topography of the initial projection, as changes in this position correlate with changes in the neocortical target domains. What mechanism(s) could underlie this process? There is evidence that thalamic axons use cortical subplate axons as a scaffold to enter the cortex (McConnell et al., 1989; Ghosh et al., 1990; Ghosh and Shatz, 1992; Ghosh and Shatz, 1993; Molnar et al., 1998b). The handshake model proposed that thalamic and cortical axons interact inside the internal capsule and guide each other to their final target, potentially through specific molecular interactions or through positional alignment within the internal capsule (Molnar and Blakemore, 1995; Molnar et al., 1998a). Neocortical patterning is likely to regulate the molecular properties and navigation trajectories of cortical axons that grow towards the thalamus. For example, both *Emx2* and *COUP-TFI* mutant mice, which show caudal-to-rostral

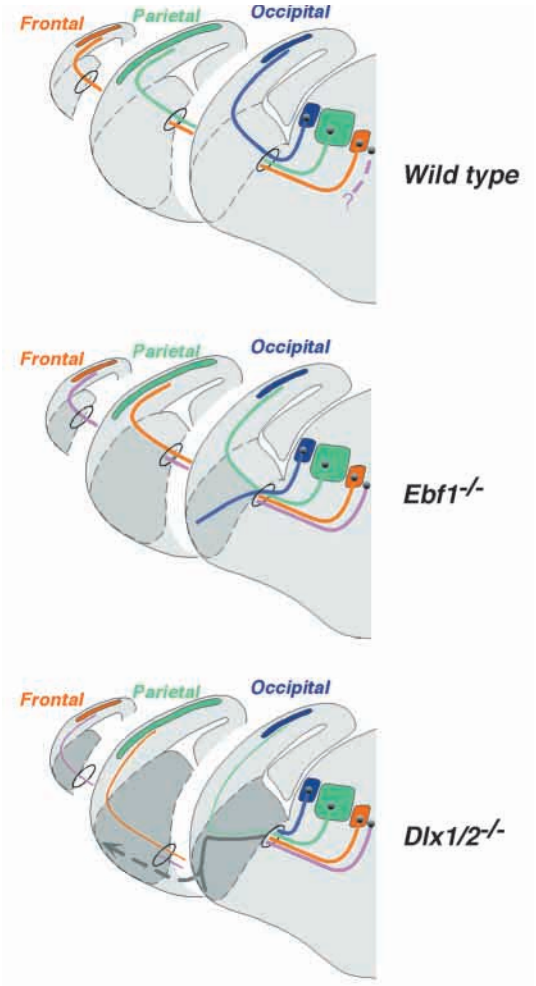


**Fig. 10.** Schematic representation of the phenotypes observed in *Ebf1*$^{-/-}$ and *Dlx1/2*$^{-/-}$ mutant embryos. Schematic coronal serial sections of the forebrain at the level of the occipital, parietal and frontal neocortex. In the wild-type brain, neocortical domains and thalamic nuclei that are normally interconnected have the same color and are connected by an axon tract of the same color. The axons grow through the internal capsule (open circle) and they pass through the basal ganglia (broken black lines). In *Ebf1*$^{-/-}$ embryos, the basal ganglia domain has molecular defects (indicated by the light gray), dLGN axons (dark blue) grow ectopically into the amygdalar region; the remainder of thalamic projection show a shift in their positions in the internal capsule and in the neocortex. In *Dlx1/2*$^{-/-}$ embryos, the basal ganglia develop abnormally (indicated by dark gray). The internal capsule is perturbed and numerous thalamic axons, the identity of which could not be clearly determined, grow into the amygdalar region and then travel rostrally (gray bundle). These probably contain dLGN axons, as these were not detected in the neocortex. Other axons grow towards the neocortex in the internal capsule; as in the *Ebf1* mutants, these axons show a shift in their position within the internal capsule and in the neocortical domain that they enter.

changes in the molecular properties and connectivity of the occipital neocortex (Bishop et al., 2000; Mallamaci et al., 2000b; Zhou et al., 2001), also have subplate defects (Zhou et al., 1999; Mallamaci et al., 2000a) that may contribute to the changes in thalamocortical connectivity.

In *Ebf1* mutants, we did not observe defects in the pattern or timing of subplate or cortical plate axonal outgrowth into the internal capsule (Fig. 6A-D). However, thalamic axons show pathfinding defects as they enter the basal ganglia, suggesting that the phenotype we observe may be due to a mismatch between normally positioned cortical axons and shifted thalamic axons. In *Dlx1/2* mutants, neocortical axons are displaced in the basal ganglia, and thus probably contribute to the disorganization of the internal capsule and to the reduction in the number of axons reaching their target structures (Fig. 8). However, the timing of their outgrowth, and their rostrocaudal distribution, does not seem affected. Thalamic axons, however, had a shifted position within the internal capsule (Fig. 9). Therefore, while defects in both thalamic and cortical axons could contribute to the shift in thalamocortical projections, our observations suggest that it may be primarily due to the displacement of the thalamic axons. Thus, if thalamic and cortical axons do directly interact ('handshake'), our data suggest that a displacement in thalamic axons, and in the alignment of thalamic and cortical axons, can induce a shift in their final target zone in the neocortex as well as a reciprocal shift in cortical projections. Overall, our study suggests that the position of thalamic axons in the basal ganglia, an intermediate structure located between the thalamus and neocortex, is an important decision point for the initial topography of thalamocortical projections.

### The role of basal ganglia in guidance and positioning of thalamic axons

Previous work has identified groups of cells in the basal ganglia that participate in the guidance of thalamic and cortical axons, by producing chemoattractants (Metin and Godement, 1996; Metin et al., 1997; Richards et al., 1997; Braisted et al., 1999; Braisted et al., 2000) and or by forming transient axonal scaffolds (Mitrofanis and Guillery, 1993). Defects in some of these cells, caused by the *Mash1* mutation, are correlated with the inability of thalamocortical axons to reach the neocortex (Tuttle et al., 1999). These results support the idea that the basal ganglia form an intermediate target for axons interconnecting the neocortex and thalamus (Metin and Godement, 1996). Our results provide evidence that the ordering of thalamic axons within the basal ganglia may play an important role in the final topographic organization of thalamocortical projections.

How is this order established or maintained? Although our study does not provide definitive answers, it allows us to discuss different hypotheses. One possibility is that an array of guidance cues regulates the spatial organization of thalamic axons inside the basal ganglia, by a chemoaffinity mechanism within this structure. However, so far, there is no obvious candidate molecule for such function. An alternative mechanism involves both guidance cues and the timing of thalamic axon outgrowth (Molnar and Blakemore, 1995; Molnar et al., 1998a). Indeed, there is a gradient of differentiation in the dorsal thalamus (Jones, 1985) and thalamic axons of different zones grow into the internal capsule at different times (Molnar et al., 1998a). Localized guidance cues or groups of cells may determine the position of pioneering thalamic axons. Candidate molecules include Sema6a: a subpopulation of thalamic axons is misrouted in the amygdalar region of *Sema6a* mutants (Leighton et al., 2001). These pioneer axons would provide a landmark for the next set

of incoming axons, which would navigate to an adjacent location. Thus,, as new axons arrive, they would stack in a temporally determined array. In both models, the shift in topography we observe in *Dlx1/2* and *Ebf1* mutant embryos could be a consequence of the axonal pathfinding defects of specific thalamic axons within the basal ganglia. This mechanism may also participate in the phenotype of *Emx2* and *COUP-TFI* mutant embryos, where a subset of thalamic axons do not reach the neocortex (Zhou et al., 1999; Mallamaci et al., 2000b; Lopez-Bendito et al., 2002).

## Conclusion

The favored model for the formation of topographic projections proposes that this process is regulated by cues located in the two interconnected structures (Sperry, 1963). This mechanism is implicated in the targeting of retinotectal (Drescher et al., 1997; Goodhill and Richards, 1999; Feldheim et al., 2000) and reticulogeniculate (Feldheim et al., 1998) axons, of limbic thalamic axons to the limbic cortex (Barbe and Levitt, 1992; Mann et al., 1998) and hippocampal neurons to the septum (Gao et al., 1996), as well as in the formation of a topographic map within a neocortical area (Vanderhaeghen et al., 2000). We have presented evidence that the initial topography of thalamocortical projections is not strictly determined by the information located inside the target structure and that the position of axons within intermediate structures may be important for the regulation of this topography. In particular, our results suggest a role for the basal ganglia in the organization of thalamic axons and the choice of their final target destination. More generally, these observations raise the possibility that intermediate structures, and/or the relative position of axons inside a fiber pathway, may regulate the formation of topographically organized long-range projections within the central nervous system.

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