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Mechanisms controlling the guidance of thalamocortical axons through the embryonic forebrain

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Abstract

Thalamocortical axons must cross a complex cellular terrain through the developing forebrain, and this terrain has to be understood for us to learn how thalamocortical axons reach their destinations. Selective fasciculation, guidepost cells and various diencephalic and telencephalic gradients have been implicated in thalamocortical guidance. As our understanding of the relevant forebrain patterns has increased, so has our knowledge of the guidance mechanisms. Our aim here is to review recent observations of cellular and molecular mechanisms related to: the growth of thalamofugal projections to the ventral telencephalon, thalamic axon avoidance of the hypothalamus and extension into the telencephalon to form the internal capsule, the crossing of the pallial–subpallial boundary, and the growth towards the cerebral cortex. We shall review current theories for the explanation of the maintenance and alteration of topographic order in the thalamocortical projections to the cortex. It is now increasingly clear that several mechanisms are involved at different stages of thalamocortical development, and each contributes substantially to the eventual outcome. Revealing the molecular and cellular mechanisms can help to link specific genes to details of actual developmental mechanisms.

General introduction

In 2011, the 'handshake hypothesis' celebrated its 21st birthday. This hypothesis was formulated by Blakemore & Molnár (1990) as a way to explain how ascending thalamic axons navigate to their appropriate cortical targets with help from reciprocal descending cortical axons (Molnár & Blakemore, 1995). It was based on *in vivo* observations demonstrating an intimate anatomical relationship between developing thalamic and early cortical axons (Molnár *et al.*, 1998a,b) and on *in vitro* findings that cortical explants from different regions accept innervations from any region of the thalamus (Molnár & Blakemore, 1991). It has been suggested that a mechanism such as guidance from descending axons, which is present *in vivo* but disrupted in explant culture, might be necessary to achieve specific patterns of thalamocortical connectivity (Molnár & Blakemore, 1991, 1999). The original formulation of the hypothesis stated that 'the descending and ascending axons each pioneer the pathway through their own segment of the brain and, after a ''handshake'' near the internal capsule, each may guide the growth of the other over the distal part of its trajectory …' (Molnár & Blakemore, 1991).

However, this hypothesis was not supported by several observations. Some of the major objections are related to the separate route of the thalamic and corticofugal projections observed in adults. Indeed, the layer 6 and layer 5 projections take separate routes between any one thalamic nucleus and its cortical areas, and and each component involves complex crossing that appears to occur at different sites for the thalamocortical and the corticothalamic components (Adams *et al.*, 1997). We have very limited information about these points in the adult, and need more tracing studies at the single-cell level to resolve them (Lozsádi *et al.*, 1996; Grant *et al.*, 2012). Furthermore, there is great deal of difference between layer 5 and layer 6 cell axons that target the core and the matrix thalamic neurons in the adult (Jones, 2001, 2007; Sherman & Guillery, 2005; Sherman, 2007). The handshake hypothesis only accounted for the earliest corticofugal projections and the thalamic projections, and their encounter in the internal capsule at the time of crossing the pallial–subpallial boundaries (PSPBs). However, even these early interactions were questioned

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in several tracing studies (Miller *et al.*, 1993; Bicknese *et al.*, 1994), and in dissociated cultures thalamic and cortical growth cones often extended along axons of their own kind, and, after contacts between cortical and thalamic fibres, in most cases growth cones collapsed and retracted (Bagnard *et al.*, 2001). However, the co-fasciculation of the early thalamic and early corticofugal projections has been demonstrated in mouse and rat (Molnár *et al.*, 1998a,b), and the close association of these fibres was apparent in the fascicles crossing the embryonic cortical plate in the reeler mutant (Molnár *et al.*, 1998b). The relationship between thalamic and early corticofugal projections has not been observed in organotypic cultures in their natural environment.

Over the last 21 years, the handshake hypothesis has remained influential because of its attractive simplicity, while being challenging to test experimentally. As the thalamocortical projections traverse the entire telencephalon, a better understanding of patterning within the diencephalon and telencaphalon was required to reveal possible guidance mechanisms. We now know that thalamic and early corticofugal projections do not pioneer their own growth towards the internal capsule; they are aided by other cells with projections (in the prethalamus and internal capsule) or with migratory paths defined by corridor cells. Nevertheless, the interactions between early corticofugal projections and thalamic fibres at the PSPB are still postulated to explain various phenotypes in mouse knockouts (Hevner *et al.*, 2002; López-Bendito & Molnár, 2003). Many of these questions were not readily testable experimentally 21 years ago. However, with the generation of mouse lines that express reporter genes in selected cell groups (Jacobs *et al.*, 2007; Piñon *et al.*, 2009), and the increased understanding of selective gene expression patterns of subplate and other cell populations (Ayoub & Kostovic, 2009; Hoerder-Suabedissen *et al.*, 2009; McKellar & Shatz, 2009; Osheroff & Hatten, 2009; Oeschger *et al.*, 2012), we now have the chance to revisit this issue. Moreover, our understanding of the molecular and cellular aspects of telencephalic development is also increasing rapidly, and with this background we can refine our questions on axon guidance. We have come a long way since the handshake hypothesis was first suggested but, despite new knowledge of many additional mechanisms, the idea still retains considerable importance for a particular stage and segment of thalamocortical development (Fig. 1A and B).

Molecular patterning of the early thalamus

The thalamic region of the diencephalon comprises three functionally distinct zones, the prethalamus, the thalamus proper, and the pretectum, which extends in a rostrocaudal fashion (Larsen *et al.*, 2001; Puelles & Rubenstein, 2003). Traditionally, the thalamus was described as having two major components, a ventral thalamus and a dorsal thalamus, with the latter being the component that processes and relays most sensory information from the periphery to the cerebral cortex. This nomenclature is confusing, as the ventral thalamus, in fact, lies rostral to the dorsal thalamus along the curved axis of the neural tube. In recent years, in developmental studies it has become increasingly common to use the terms prethalamus and thalamus to describe the ventral thalamus and dorsal thalamus respectively (Puelles & Rubenstein, 2003). A major advantage of this prethalamus/thalamus nomenclature is that the regional descriptors 'ventral' and 'dorsal' can then be applied in their descriptive meaning without confusion. Therefore, we adopt this nomenclature here.

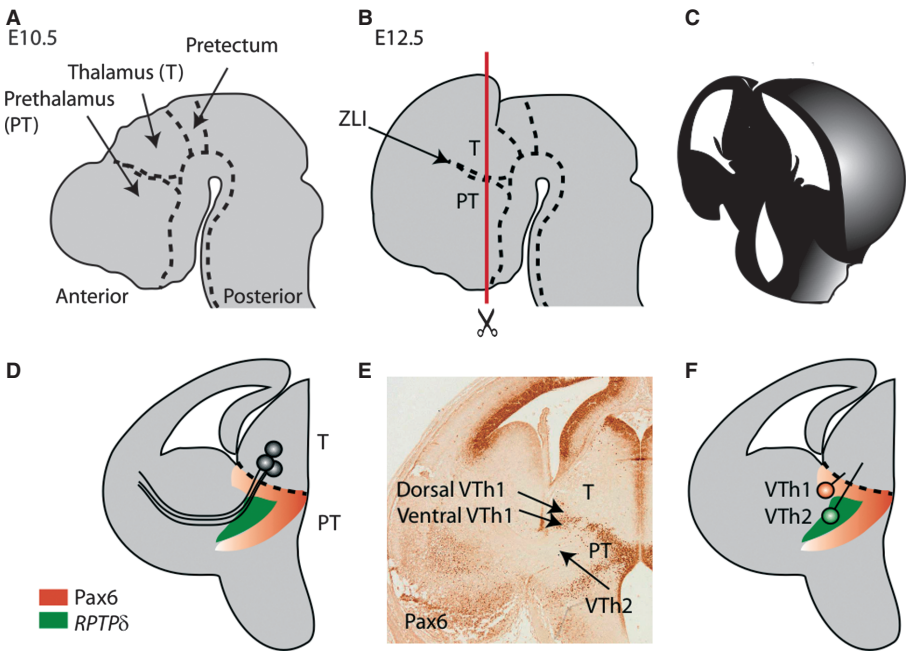

FIG. 1. The anatomy of the developing forebrain and the location of prethalamic cell groups providing guidance for TCAs in embryonic mouse brain. (A) A sagittal view of the brain around E10.5 showing the pretectal, thalamic and prethalamic anlagen. (B) By E12.5, the telencephalic vesicles expand over the diencephalon; note that the prethalamus (PT) lies anterior to the thalamus (T). These two structures are separated by the ZLI. (C) The appearance of the forebrain when cut as shown by the red line in B at E14 (D) TCAs grow from the thalamus, through the prethalamus, and into the telencephalon. The prethalamus contains cells that express the markers Pax6 and RPTPδ (Tuttle *et al.*, 1999). (E) An example of a section stained with an antibody for the Pax6 TF (E14). The positions of prethalamic groups of cells proposed by Tuttle *et al.* (1999) to project to the thalamus and provide guidance to TCAs are shown. These groups were originally called VTh1 and VTh2, with VTh1 split into a dorsal and a ventral domain. The dorsal domain of VTh1 expresses a low level of Pax6, whereas the ventral domain of VTh1 expresses a high level of Pax6. (F) VTh2 does not express Pax6 but does express RPTPδ.

The mammalian thalamus is composed of dozens of morphologically and functionally distinct nuclei (Jones, 2007). Some of these nuclei project topographically to specific areas of the cortex, relaying sensory input from the periphery and playing a critical role in sensory functions (Jones, 2001; Clascá *et al.*, 2009; Sherman & Guillery, 2011). In contrast, the prethalamus, comprising the zona incerta, reticular nucleus, and ventral lateral geniculate nucleus, does not project to the cortex (Jones, 2007). The zona limitans intrathalamica (ZLI) separates the prethalamus and the thalamus.

The thalamus develops from neural progenitor cells located within the p2 domain of the alar plate of the caudal diencephalon between embryonic day (E)10.5 and E16.5 (Angevine, 1970; Puelles & Rubenstein, 1993, 2003). Although recent studies have identified molecules that may influence the patterning of the diencephalon, it has remained largely unknown how the distinct, postmitotic thalamic nuclei emerge from discrete developmental units (Scholpp & Lumsden, 2010). As occurs in the neocortex and other brain regions, molecules secreted by signalling centres between tissue compartments organize the patterning and growth of specific tissues. The ZLI expresses members of the Sonic hedgehog (Shh) signal molecule family, together with other secreted factors such as Wnts and fibroblast growth factors (FGFs), and has been demonstrated to act as a local organizer for thalamic development. Although Wnt signalling is important for setting up the initial anteroposterior regionalization (Salinas & Nusse, 1992; Murray *et al.*, 2007; Quinlan *et al.*, 2009), it remains unknown whether this is directly required for thalamic specification. FGF signalling has also been implicated in organizing diencephalic development. FGF15 and FGF19 have been shown to function downstream of Shh in the thalamus, and are therefore implicated in some aspects of thalamic development (Miyake *et al.*, 2005; Gimeno & Martinez, 2007). On the basis of elegant *in utero* manipulations in the thalamus, recent reports have added FGF8 activity to this scenario, and have shown that FGF8 activity controls the patterning of thalamic nuclei (Kataoka & Shimogori, 2008).

Nevertheless, several studies have shown that Shh is the principal requirement for cell fate specification during thalamic development. Indeed, there are at least three Shh-dependent steps in patterning of the thalamic anlage. These include the induction of specific sets of transcription factors (TFs), through which Shh determines cell specification during thalamic development (Scholpp & Lumsden, 2010). Moreover, elimination of Shh activity in both chick and zebrafish results in the loss of genetic fate determinants and cell identity in both the prethalamus and the thalamus (Kiecker & Lumsden, 2004; Scholpp *et al.*, 2006). A recent study has determined that ectopic activation of the Shh signalling pathway induces the expression of thalamic markers such as Gbx2, oligodendrocyte transcription factor-2, neurogenin-2 (Neurog2) and oligodendrocyte transcription factor-3 in the mouse pretectum, demonstrating that Shh plays a crucial role in patterning thalamic progenitor domains (Vue *et al.*, 2009).

Transcriptional control of thalamocortical axon (TCA) guidance

Several TFs are expressed in distinct but often overlapping patterns in the thalamus, suggesting that they cooperate to control the specification and differentiation of thalamic nuclei and cell types. One of the first attempts to look at the cell-autonomous role of TFs in thalamocortical pathfinding was the work by Pratt *et al.* (2000, 2002). They showed that the development of the thalamus is compromised in *Pax6*$^{-/-}$ embryos, and that the thalamus exhibits abnormalities of differentiation and of the projection of axons (also see Jones *et al.*, 2002). Gbx2 is expressed broadly and early in the thalamus (Bulfone *et al.*, 1993), and later it is required for the differentiation of a subset of nuclei and the development of TCA projections (Miyashita-Lin *et al.*, 1999; Jones *et al.*, 2002). A recent study has demonstrated that Gbx2 plays a cell-non-autonomous role in controlling the segregation of postmitotic thalamic neurons from the neighbouring brain structures that do not express Gbx2 (Chen *et al.*, 2009). Another key piece of work on the transcriptional control of TCA pathfinding came from the study by Seibt and colleagues, demonstrating that the basic helix–loop–helix TF Neurog2 cell-autonomously specifies the projection of thalamic neurons to frontal cortical areas (Seibt *et al.*, 2003). Neurog2-knockout mice are characterized by a targeting shift in the TCA projections that occurs initially in the ventral telencephalon (VTel) (Seibt *et al.*, 2003), suggesting that Neurog2 regulates the guidance receptors in these axons, which read ventral telencephalic cues. However, to date, no downstream targets of Neurog2 have been identified.

It remains unclear how distinct pools of thalamocortical projecting neurons are topographically specified, and which TFs regulate the growth of their axons (López-Bendito & Molnár, 2003; Shimogori & Grove, 2005; Price *et al.*, 2006). TFs expressed in postmitotic neurons are responsible for specifying neuronal identity and for activating specific axon guidance programmes in other neuronal pathways. Genetic studies in mice have demonstrated the role of the LIM homeodomain (LIM-HD) proteins in determining the identity of motor neurons (Jurata *et al.*, 2000; Kania *et al.*, 2000; Lee & Pfaff, 2001; Kania & Jessell, 2003). Moreover, a specific combination of TFs from the LIM-HD family regulates the topographic targeting of distinct pools of axons to specific muscles in the limb mesenchyma (Sharma *et al.*, 1998; Kania *et al.*, 2000). In recent years, several candidate genes have been identified as potential downstream effectors of these TFs. For example, Lim1 expression in lateral motor column neurons in the spinal cord regulates the expression of the tyrosine kinase EphA4, a protein that is essential for the final targeting of axons to the limb (Kania & Jessell, 2003). Similarly, the transcription factor Zic2 regulates midline crossing by retinal axons in conjunction with another member of the Eph family, EphB1 (Lee *et al.*, 2008; García-Frigola & Herrera, 2010). These molecular pathways may also be important for thalamocortical pathfinding.

In the thalamus, the Lhx2 TF is a member of the LIM-HD family of proteins, and is strongly expressed during development (Retaux *et al.*, 1999; Nakagawa & O'Leary, 2001). Severe thalamocortical pathfinding defects have been described in *Lhx2* null mice (Lakhina *et al.*, 2007), implicating this TF in the guidance of these axons. However, the death of these mice at early embryonic stages precludes *in vivo* studies of the role of *Lhx2* in later aspects of TCA connectivity. As *Lhx2* is also expressed in other forebrain areas, such as the neocortex, it is essential to restrict the loss of Lhx2 to thalamic neurons in order to precisely determine the role of this TF in thalamocortical development. Disruption of Lhx2 regulatory activity only in thalamic neurons leads to axonal pathfinding defects in TCAs, with fewer axons ultimately reaching their cortical targets (Marcos-Mondéjar *et al.*, 2012).

Mice deficient in *Robo1*, *Robo2* or both show prominent defects in TCA guidance during development, including abnormal axonal invasion of the hypothalamus (Andrews *et al.*, 2006; López-Bendito *et al.*, 2007). Overexpression of *Lhx2* in rostral and intermediate thalamic neurons by *in utero* electroporation results in the abnormal invasion of the hypothalamus by electroporated axons (Marcos-Mondéjar *et al.*, 2012). Moreover, this study demonstrated that Lhx2 is a direct repressor of Robo1 and Robo2 receptors, as their thalamic

expression is altered in the absence of this TF. The list of TF pathways involved in the early differentiation of the thalamus and the early guidance of TCAs is impressive, but it is most probably far from complete.

Guidance from the thalamus to the subpallium

The role of prethalamic and ventral telencephalic projections to the thalamus in the early guidance of TCAs

The molecular mechanisms that guide the first axons from the thalamus and into the prethalamus, which they must cross to access the border between the diencephalon and the telencephalon, are poorly understood. Coordinated control of the polarity of newly differentiating thalamic neurons might ensure that the first axonal extensions grow towards the boundary of the thalamus and prethalamus, but it is also likely that projections from the prethalamus to the thalamus (PTh–Th; Appendix and Table 1) and projections from the VTel to the thalamus (VTel–Th) provide guidance (Fig. 2). A study by Métin & Godement (1996) in hamsters showed that as axons grow from the thalamus they intermingle with reciprocal projections from the prethalamus (PTh–Th) and ventral telencephalon (VTel–Th) to the thalamus. Equivalent prethalamic neurons were subsequently discovered in rat embryos (Molnár *et al.*, 1998a; Molnár & Cordery, 1999), in a region described by Mitrofanis (1992) as the perireticular nucleus (Fig. 2). Braisted *et al.* (1999) also examined this region in embryonic mice, and suggested that VTel–Th neurons project axons into the thalamus at around the time at which the first TCAs reach the VTel (E13–E14). These authors suggested that the VTel–Th neurons probably belong to the globus pallidus (GP) rather than being perireticular cells. This suggestion stemmed from the fact that perireticular cells were retrogradely labelled from the thalamus in postnatal but not embryonic rats (Mitrofanis & Baker, 1993) and ferrets (Mitrofanis, 1994a,b). Despite these and other differences in the details of the various studies, the spatial and temporal features of the axonal projection of the VTel–Th neurons are consistent with the idea that this axonal projection, and possibly the cell bodies themselves, may act as a scaffold to guide TCAs through the developing prethalamus and towards the diencephalic–telencephalic border or other axons in the opposite direction, or perhaps both. Consistent with this hypothesis, in *Ascl1*$^{-/-}$ and *Pax6*$^{-/-}$ embryos this population of ventral telencephalic VTel–Th cells appears to be missing, and TCAs fail to extend into the VTel (Tuttle *et al.*, 1999; Pratt *et al.*, 2002) (Fig. 3). There are decreases in the number and displacement of these cells in *Lhx2*$^{-/-}$ and *Emx2*$^{-/-}$ mutants, respectively, and these are associated with guidance defects of TCAs (Tuttle *et al.*, 1999; Bishop *et al.*, 2000, 2003; López-Bendito *et al.*, 2002; Lakhina *et al.*, 2007).

A study by Tuttle *et al.* (1999) further subdivided the PTh–Th projections into two groups. Tuttle *et al.* (1999) named these VTh1 and VTh2, with VTh1 split into a dorsal and a ventral domain. The location of these groups is shown in Fig. 1E and F. Their nomenclature is, of course, now confusing, because VTh stands for ventral thalamus, and as 'prethalamus' is preferred to 'ventral thalamus', they might be better renamed PTh–Th1 and PTh–Th2. The dorsal domain of VTh1/PTh–Th1 expresses a low level of the TF Pax6, whereas the ventral domain of VTh1/PTh–Th1 expresses a high level of Pax6; VTh2/PTh–Th2 does not express Pax6 (Fig. 1E). There is much less functional information on the possible roles of the PTh–TH groups in TCA guidance than for the VTel–Th cells.

In the case of both the PTh–Th and VTel–Th groups of axons, an association between the loss of these cells and TCA pathfinding defects in mutants cannot be taken to imply causation. At present, we know very little about these projections: their embryological origins, molecular identities, fates and potential roles in TCA guidance remain to be determined. We do not have the tools to interfere selectively with their function, as molecular markers that distinguish them have not been identified.

Repulsive activity from the hypothalamus

As thalamic axons traverse the prethalamus at E11–E13 in the mouse or E12–E14 in the rat, they grow in the direction of the hypothalamus before they turn laterally towards the internal capsule. Tuttle *et al.* (1999) showed cells in the hypothalamus with projections to the thalamus, but, clearly, these projections do not succeed in drawing the

TABLE 1. Suggested nomenclature for guidepost neurons that cross the early diencephalic and telencephalic subdivisions. For further description see appendix.

Cell group	Synonymous name	References
Neurons with projections from the prethalamus to the thalamus (PTh–Th)	TRN Ventral thalamus	Mitrofanis & Guillery (1993) Métin & Godement (1996) Molnár *et al.* (1998a,b); Molnár & Cordery (1999) Tuttle *et al.* (1999) Mitrofanis (1992) as the perireticular nucleus. Braisted *et al.* (1999)
Neurons with projections from the VTel to the thalamus (VTel–Th)	Perireticular cell Perireticular nucleus Internal capsule guidepost cells	Mitrofanis & Guillery (1993) Métin & Godement (1996) Molnár *et al.* (1998a,b); Molnár & Cordery (1999) Tuttle *et al.* (1999) Mitrofanis (1992) as the perireticular nucleus. Braisted *et al.* (1999)
Neurons with projections from the VTel to the cortex (VTel–Cx)	Nucleus basalis Métin & Godement (1996) and Adams & Baker (1995) described numerous cells from the pallidum with projections to the cortex; they associated these labelled cells with the perireticular nucleus (Admas and Baker, 1995)	Adams & Baker (1995) Coleman & Mitrofanis (1999) Métin & Godement (1996)
Corridor cells	The adult equivalent of these cells is not clear. We do not know the proportions of the surviving cells. The distinction between corridor cells and other populations of guidepost neurons is not fully resolved.	López-Bendito *et al.* (2006); Bielle *et al.* (2011a)

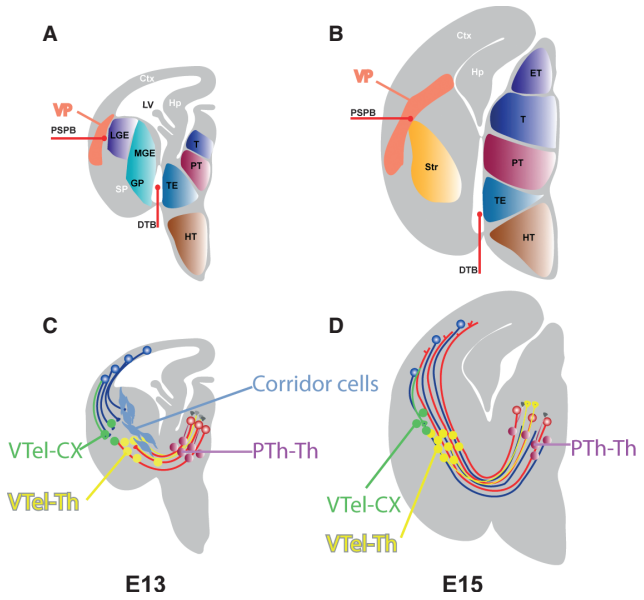

FIG. 2. The position of various cell populations guide thalamic axons. (A and B) The various subdivisions in the diencephalon (ET, epithalamus; HT, hypothalamus; PT, prethalamus, T, thalamus; TE, thalamic eminence) and telencephalon (Ctx, cerebral cortex; SP, subpallium; Str, striatum; VP, ventral pallium) and their boundary (DTB, diencephalic–telencephalic boundary). (C and D) The early connectivity in the telencephalon and diencephalon. Prethalamic (PTh–Th) and ventral telencephalic (internal capsule, VThel–Th) cells with thalamic projections (purple and yellow, respectively) are instrumental in early thalamic axon guidance. The panel in C illustrates the migration of the corridor cells and their interactions with the thalamocortical projections. Corridor cells (light blue) originate from the LGE at E12, and migrate tangentially towards the diencephalon, where they form a permissive 'corridor' for the thalamic projections (red) to navigate them through the internal capsule. Modified from López-Bendito & Molnár (2003) and Hanashima *et al.* (2006). Hp, hippocampus; LV, lateral ventricle.

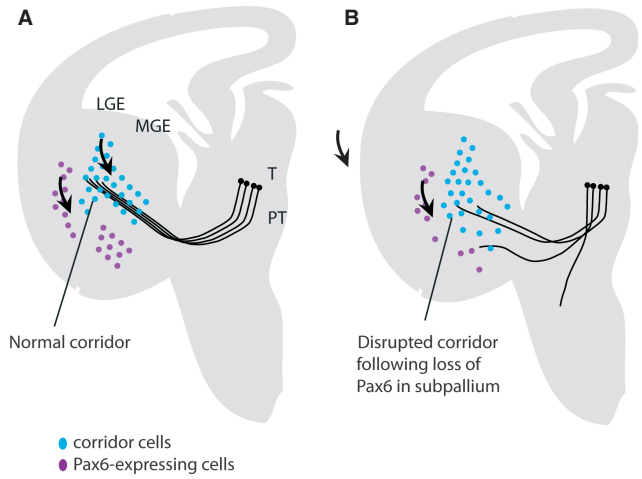

FIG. 3. Evidence that Pax6 plays a role in corridor formation. (A) Normally, Pax6-expressing cells (purple) are located ventral to the corridor/developing internal capsule in the MGE; those located laterally form the lateral cortical stream migrating from the PSPB (arrow). Islet1-expressing cells (green) migrate from the progenitor layer of the LGE (arrow) to form the corridor through which TCAs grow (arrow). (B) In conditional mutant embryos with selective reduction of Pax6 in specifically the VTel but not in the thalamus or cortex, there are fewer Pax6-expressing cells ventral to the corridor than normal (other populations of Pax6-expressing cells outside the region of Pax6 deletion are not shown, as they are not affected). Cells from the LGE migrate to form a corridor that is abnormally broad, with a lower peak density of Islet1-expressing cells; many Islet1-expressing cells stray into the area depleted of Pax6 expression. Many thalamic axons fail to enter this abnormal corridor, or exit it along its length. Data are taken from Simpson *et al.* (2009). PT, prethalamus, T, thalamus.

thalamic axons to the hypothalamus. On the contrary, thalamic axons turn very sharply away from the hypothalamus into the internal capsule in the direction of the diencephalic–telencephalic border. Several studies have shown that: (i) the hypothalamus expresses high levels of Slits, which are generally chemorepellent for growing axons; (ii) hypothalamic explants repel thalamic axons in explant cultures; and (iii) in both *Slit2*$^{-/-}$ and *Slit1*$^{-/-}$;*Slit2*$^{-/-}$ mutants, a large number of thalamic projections fail to enter the telencephalon, and instead descend into the hypothalamus (Braisted *et al.*, 1999, 2009; Bagri *et al.*, 2002; López-Bendito *et al.*, 2007; Bielle *et al.*, 2011b). These findings provide quite compelling evidence that thalamic axons, which express Robo receptors through which Slits signal, deviate away from the hypothalamus and across the diencephalic–telencephalic boundary, owing to Slit-mediated repulsion.

The role of tangentially migrating 'corridor' cells in delineating the internal path of TCAs

More recently, a distinct population of guidepost cells has been identified that controls the precise pathfinding of TCAs along an internal trajectory within the subpallium (López-Bendito *et al.*, 2006). These cells are GABAergic neurons that migrate tangentially from the lateral ganglionic eminence (LGE) into the medial ganglionic eminence (MGE) and form a cellular 'corridor' between the proliferative zones of the MGE and the GP (Fig. 2; Appendix; Table 1) (López-Bendito *et al.*, 2006). Accordingly, they are located in the MGE, but express molecular markers of LGE-derived neurons, such as *Islet1*, *Ebf1*, and *Meis2*, and do not express MGE molecular markers such as *Nkx2.1*. These neurons, named 'corridor cells', migrate from E11.5 to E14 in the superficial mantle of the subpallium in a ventral direction, superficially to the large stream of MGE-derived interneurons that migrate towards the cerebral cortex. *In vitro* analysis in embryonic brain slices has shown that corridor cells constitute a permissive territory for the internal growth of TCAs through MGE-derived cell groups, which are otherwise non-permissive for TCAs. Although the factors controlling the non-permissive activities of MGE-derived territories remain to be determined, corridor cells were shown to express a membrane-bound isoform of neuregulin-1. TCAs express the neuregulin-1 receptor ErbB4, and gain-of-function experiments in embryonic slices as well as in telencephalic conditional neuregulin-1 mutants and constitutive ErbB4 mutants indicate that this signalling pathway regulates the pathfinding of TCAs throughout the corridor. These findings show that the migration of corridor neurons generates a neuregulin-1-permissive domain that is essential for the internal pathfinding of TCAs within the subpallium. Thus, corridor cells are immature neurons that act via contact or a short-range activity to position an axonal tract, and constitute genuine guidepost cells. Interestingly, these corridor cells are conserved in diverse species, and show distinct positioning that could underly evolutionary changes in the positioning of TCAs in the subpallium (Bielle *et al.*, 2011a).

What is the relationship between corridor cells and perireticular/internal capsule cells? These two proposed guidepost cell populations are not located in exactly the same regions, as some perireticular cells are in the prethalamus or in its vicinity, whereas corridor cells are in the MGE (Table 1; Fig. 2). Accordingly, perireticular cells have been proposed to regulate the entrance of TCAs into the subpallium (Métin & Godement, 1996; Molnár *et al.*,

1998a,b; Molnár & Cordery, 1999; Tuttle *et al.*, 1999), whereas corridor cells orient the internal pathfinding of TCAs inside the MGE (López-Bendito *et al.*, 2006). However, some back-labelled neurons from the thalamus are also found in the corridor and LGE, raising the possibility that perireticular and corridor cells may be related to some extent (Fig. 2). Further analyses are needed for the determination of the molecular identity of back-labelled cells in the internal capsule, and thereby reveal whether some corridor cells may settle in that region and act by axon-mediated contact.

Guidance of TCAs across the VTel

The subpallium is a main intermediate target for TCAs

In contrast to the hypothalamus, the subpallium attracts TCAs and corticofugal axons, and constitutes a main intermediate target for these projections (Métin & Godement, 1996; Braisted *et al.*, 1999; Garel & Rubenstein, 2004). Analyses of mutant mice in which the regionalization and development of the subpallium has been affected (Marín *et al.*, 2002) have started an assessment of the relative importance of the LGE and MGE in corticofugal and TCA pathfinding. In particular, mutations affecting the development of the LGE, such as in *Ebf1* or $Gsh1^{-/-}$;$Gsh2^{-/-}$ double mutants, severely impair TCA navigation, in contrast to mutations that perturb MGE development, such as in *Nkx2.1* mutants (Garel *et al.*, 1999; Sussel *et al.*, 1999; Marin *et al.*, 2002; Yun *et al.*, 2003). As previously mentioned, *in vitro* experiments in embryonic brain slices have revealed that the GP and the MGE proliferative zones exert repulsive activities that are likely to channel TCAs along an internal route (López-Bendito *et al.*, 2006).

In parallel with the identification of the structures regulating TCA pathfinding through the subpallium, several studies have been conducted to determine the molecular nature of the guidance mechanisms involved. In particular, analyses of mice carrying mutations for guidance cues or their receptors have implicated netrin-1, Slit1 and Slit2, and their receptors Robo1 and Robo2, as well as semaphorin 6A in the general pathfinding of TCAs in the subpallium (Braisted *et al.*, 2000, 2009; Leighton *et al.*, 2001; Bagri *et al.*, 2002; Bonnin *et al.*, 2007; López-Bendito *et al.*, 2007; Powell *et al.*, 2008; Little *et al.*, 2009). In addition, members of the protocadherin family were shown to play essential roles in TCA guidance and internal capsule formation (Tissir *et al.*, 2005; Uemura *et al.*, 2007; Zhou *et al.*, 2008, 2009). In particular, Celsr3 is a seven-pass cadherin orthologue of *Drosophila* flamingo, which acts both in the planar cell polarity pathway and in relation to neurite outgrowth, and is widely expressed in the mantle of the telencephalon and forebrain. Its specific experimental inactivation in the subpallium and prethalamus severely impairs the formation of the thalamocortical connections: TCAs stall in the ventral subpallium just after crossing the telencephalic–diencephalic boundary, whereas corticofugal axons stall after crossing the PSPB in the proximal part of the LGE (Zhou *et al.*, 2008, 2009). These studies revealed an absolute requirement for Celsr3 expression by an intermediate target that acts at short range, and also demonstrated an *in vivo* function of these intermediate targets. Constitutive mutants for the *Frizzled3* gene, which, in *Drosophila*, participates in the planar cell polarity pathway with flamingo, have a very similar phenotype in the pathfinding of the internal capsule, suggesting that the two genes also cooperate in mice during this major axonal wiring event (Wang *et al.*, 2002, 2006).

Collectively, these experiments show that the subpallium is a major intermediate target for TCAs, and that the LGE is particularly involved in their guidance. At the molecular level, a series of secreted and transmembrane molecules expressed in the subpallium contribute to TCA pathfinding, and their inactivation in mice has provided definitive evidence for the *in vivo* requirement for this intermediate target.

Molecular determinants in the subpallium specify intermediate sorting of TCAs

As TCAs travel internally through the subpallium, they diverge rostrocaudally along a fan-like structure, allowing distinct thalamocortical/thalamofugal axons, which are already segregated inside the tract, to navigate towards different cortical areas. Analyses of mutants in which the development of the subpallium or thalamus has been affected have revealed that this initial topography is largely independent of cortical regionalization; instead, it is chiefly controlled by information contained within the subpallium (Garel *et al.*, 2002, 2003; Dufour *et al.*, 2003; Seibt *et al.*, 2003; Shimogori *et al.*, 2004). At the molecular level, initial topographic sorting of pre-segregated TCAs inside the internal capsule is mediated by countergradients of distinct ligand–receptor systems expressed by TCAs and cells in the VTel (Fig. 4). Key determinants of initial TCA divergence include ephrinAs/EphAs, netrin-1/DCC/Unc5a–c, class III semaphorins (Sema3s)/neuropilins (Npns), and the L1 family of cell adhesion molecules (L1-CAMs), which specify sorting of distinct TCA contingents (Vanderhaeghen & Polleux, 2004).

Appropriate targeting of motor thalamic axons from the ventrolateral (VL) nucleus of the dorsal thalamus to the primary motor cortex (M1) is enabled by repellent TCA guidance in the subpallium mediated by countergradients of ephrinA5/EphAs. TCAs in the rostral dorsal thalamus express high levels of EphA4 and EphA7 receptors, and are repelled from a high-caudolateral to low-rostromedial gradient of ephrinA5 expressed in the subpallium (Dufour *et al.*, 2003; Egea *et al.*, 2005; Torii & Levitt, 2005) (Fig. 4). In mice deficient in EphA4, EphA7, or both ephrinA5 and EphA4, contingents of VL axons become shifted caudally in the subpallium and misproject to the primary somatosensory cortex (S1) (Dufour, Seibt *et al.*, 2003; Dufour *et al.*, 2006). EphrinA5 knockout mice also show a caudal misprojection of a portion of afferents from the laterodorsal thalamic nucleus to S1 (Uziel *et al.*, 2002). *In vitro* studies indicate that ephrinA5 can act as a repellent (Gao *et al.*, 1998) or attractant cue for different populations of thalamic and cortical axons (Castellani *et al.*, 1998; Mann *et al.*, 2002). Within the cortex, deletion of ephrinA5 decreases the arborization of thalamic axons (Uziel *et al.*, 2008), and may promote compensatory dendritic branching of thalamocortical recipient cells, as shown for spiny stellate cells in layer 4 of S1 (Guellmar *et al.*, 2009). Netrin-1 provides a counterforce to ephrinA5-induced TCA repulsion, playing a dual role in attracting rostral TCAs and repelling caudal TCAs (Braisted *et al.*, 2000; Bonnin *et al.*, 2007; Powell *et al.*, 2008) (Fig. 4). The opposing responses are mediated by different expression levels of the netrin-1 receptors DCC (deleted in colorectal carcinoma) and Unc5a,c on TCAs, and modulated by serotonin (Bonnin *et al.*, 2007).

L1-CAMs [L1, close homologue of L1 (CHL1), and neuron-glial related cell adhesion molecule (NrCAM)] are immunoglobulin-class axon guidance molecules that regulate pathfinding of TCAs by mediating repellent responses to gradients of ephrinAs and Sema3A–G (Maness & Schachner, 2007). Sema3s are secreted ligands that promote axon repulsion or attraction by binding Npn-1/2 receptors. These receptors recruit plexinA (PlexA) subunits 1–4 and stimulate Rac1-GTPase, an activity that is intrinsic to PlexAs (Tran *et al.*, 2007; Pasterkamp & Giger, 2009). In turn, Rac1 is capable of inducing

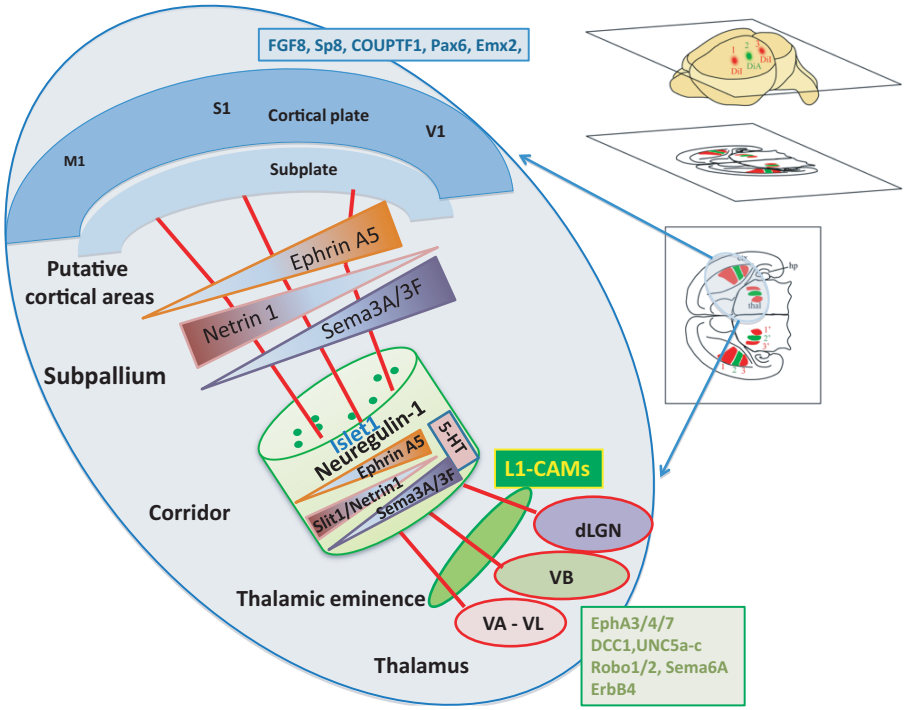

FIG. 4. Scheme of TCA trajectories from thalamic nuclei through the subpallium/VTel to distinct neocortical areas. Upper right panels: schematic diagram illustrating multiple carbocyanine dye placements in the cerebral cortex positioned along an anterioposterior axis, showing the arrangements of backfilled dorsal thalamic neurons in a mediolateral fashion. The schematic panels indicate the appropriate sections with labelling. The right hemisphere is enlarged to illustrate some of the molecular mechanisms that are involved in guidance of the thalamic axons across the thalamic eminence, corridor and subpallium to reach the appropriate regions in the cortex. TCAs from different nuclei in the thalamus (VA/VL, ventroanterior/ventrolateral nuclei; VB, ventrobasal complex; dLGN, dorsal lateral geniculate nucleus) emerge at the thalamic eminence en route to the neocortex, and are sorted within a corridor of Islet1-positive cells in the subpallium/VTel along the rostrocaudal axis (E13.5–E15.5 in the mouse). Within the corridor, TCAs expressing different combinations of axon guidance cue receptors (listed in the box within the dorsal thalamus) are guided by gradients of repellent and attractant cues (ephrinA5, netrin-1, Sema3A, Sema3F, and Slit1), influenced by neuregulin-1 and serotonin (5-HT, 5-hydroxytryptamine). In the ventral pallium, with the exception of Slit1, similar gradients are present. The thalamic axons target cortical areas that will specialise to M1, S1, and V1, at the time of their arrival and accumulation below the putative cortical areas their entry to the cortex is regulated by the subplate. Some of the various gradients in the subplate and cortical plate are listed (FGF8, Sp8, COUPTF1, Pax6, and Emx2). Within the neocortex, additional molecular cues and activity-dependent mechanisms promote the final synaptic targeting of TCAs. This simple initial topography can be considerably rearranged at the time of entry to the cortical plate.

repellant responses in growth cones by promoting rearrangements of actin filaments. Deletion of CHL1 or Npn-1 in mice causes a caudal shift of axon contingents from the ventrobasal (VB) complex within the subpallium, resulting in mistargeting to the primary visual cortex (V1) (Gu *et al.*, 2003; Wright *et al.*, 2007). CHL1 normally binds Npn-1 to enable repellent guidance from the caudal-high gradient of Sema3A in the VTel, so that TCAs correctly target S1 (Wright *et al.*, 2007). Sema3A-induced growth cone collapse depends on binding of ezrin–radixin–myosin cytoskeletal adaptors to the CHL1 cytoplasmic domain (Mintz *et al.*, 2008; Schlatter *et al.*, 2008). In an analogous mechanism, NrCAM and Npn-2 direct TCA contingents from more rostral thalamic nuclei [ventroanterior (VA)/ventrolateral (VL)] to M1 (Demyanenko *et al.*, 2011a). NrCAM associates with Npn-2, but not Npn-1, to mediate growth cone collapse induced by Sema3F, which is expressed in a caudal-high gradient in the subpallium. Semaphorin 6A also functions in the thalamocortical projection, enabling dorsal lateral geniculate nucleus axons to turn within the VTel to enter the neocortex (Leighton *et al.*, 2001). Functional consequences of mistargeting to incorrect cortical areas have been revealed in NrCAM null mice, which display impaired visual acuity and impaired binocular interactions, owing to impaired V1 cortical responses (Demyanenko *et al.*, 2011a).

L1, like CHL1 (Wright *et al.*, 2007), binds the Npn-1 required for growth cone collapse induced by Sema3A (Castellani *et al.*, 2000). Unlike deletion of CHL1, deletion of L1 in mice does not alter area-specific topographic targeting of TCAs. However, when both L1 and CHL1 are deleted in mice, a more severe phenotype is observed (Demyanenko *et al.*, 2011b), in which TCAs from both rostral (VA/VL) and VB nuclei mistarget to V1. The double mutant phenotype suggests a cooperative role for L1 and CHL1 in mediating repellent responses to Sema3A or to ephrinA5 (Demyanenko *et al.*, 2011b). L1 and CHL1 coimmunoprecipitate with the principal ephrinA5 receptors in the dorsal thalamus (EphA3, EphA4, and EphA7), and mediate ephrinA5-induced growth cone collapse (Demyanenko *et al.*, 2011b). Why does genetic deletion of CHL1 (Wright *et al.*, 2007), NrCAM (Demyanenko *et al.*, 2011a), or L1/CHL1 (Demyanenko *et al.*, 2011b), or of their interacting partners Npn-1/2 and Sema3A/3F, result in caudal misprojection of TCAs? Caudal misprojection of TCAs also occurs in mouse mutants deficient in EphA4/ephrinA5 (Dufour *et al.*, 2003), netrin-1 (Powell *et al.*, 2008), and semaphorin 6A (Little *et al.*, 2009). One hypothesis is that caudal mistargeting of TCAs in the absence of caudal repellent cues may result from gradients of unidentified rostral repellents or caudal attractants in the VTel.

The complex patterns of expression of semaphorins, ephrins, netrins, their receptors, and L1-CAMs may serve to precisely direct TCA subpopulations to cortical targets (Wright *et al.*, 2007; Demyanenko *et al.*, 2011a,b). These ligand–receptor complexes may be localized in distinct subdomains of the growth cone membrane. Within these growth cone subdomains, downstream signalling from activated receptors may impinge asymmetrically on actin filaments (Zhang *et al.*, 2003; Marquardt *et al.*, 2005; Burnette *et al.*, 2008), resulting in

localized retraction of filopodia and lamellipodia (Schaefer *et al.*, 2008), thus specifying directional navigation. An important goal for the future will be to identify the intracellular signalling pathways activated by each guidance receptor system at the crucial choice points along the thalamocortical pathway. Furthermore, it is likely that many more axon guidance cues and receptors will cooperate to guide TCAs at various decision points en route to the cortex.

Although these guidance cues were initially proposed to act mainly in the LGE-derived striatum, *in vitro* experiments in slices have revealed that corridor neurons probably act proximally to the striatum in orienting TCAs along the rostrocaudal axis, where many of the guidance cues are also expressed (Bielle *et al.*, 2011a). *In vivo* and *in vitro* analyses of Slit1 and Robo1/2 mutant mice have confirmed that localized cues in the corridor act to orient pathfinding of intermediate and rostral axons, indicating that, in this system, guidepost corridor neurons participate not only in the internal navigation of TCAs, but also in the formation of their fan-shaped topographic arrangement within the intermediate target (Bielle *et al.*, 2011a,b).

Further studies investigating potential crosstalk among the different guidance signalling pathways, as well as the molecular mechanisms involved, may provide decisive information for understanding how TCAs are initially topographically ordered. Furthermore, how this intermediate subpallial topography interacts with positional information located in the neocortex to control the final spatial arrangement of TCAs remains to be explored. In the adult, the topographic order is not based on a single principle (outlined in Fig. 4); many thalamocortical interconnections involve mirror reversals between the thalamus and cortex (Adams *et al.*, 1997), suggesting substantial rearrangements at a later stage, closer to the termination sites. There are examples of such rearrangements in the adult (see Nelson & LeVay, 1985; reviewed by Grant *et al.*, 2012).

How TCAs enter the cerebral cortex

The PSPB, which TCAs must cross to reach the cortex, is first established as a gene expression boundary. By the age at which thalamic axons approach the pallium (E13 for mouse; see Fig. 2C), the PSPB has developed a striking radial glial fascicle that runs across the trajectory of TCAs and has a high density of cells, including those of the lateral cortical stream, which migrate across the path of TCAs (Chapouton *et al.*, 2001; Carney *et al.*, 2006, 2009). It has been suggested that these features make this region relatively hostile to the passage of thalamic axons and that descending corticofugal axons from the cortex interact with ascending thalamic axons and assist them across this region (Molnár *et al.*, 1998a,b; Molnár & Butler, 2002). The pioneer corticofugal axons arrive at the PSPB before the thalamic axons, and tracing studies suggest that at least the earliest cohort of these fibres can cross the PSPB without thalamic axons. It is possible that a breakdown of this interaction explains TCA defects in some strains of mutant mice (Hevner *et al.*, 2002; Jones *et al.*, 2002; López-Bendito *et al.*, 2002; López-Bendito & Molnár, 2003; Dwyer *et al.*, 2011).

Recently, this hypothesis has been tested with conditional mutagenesis to assess the effects of blocking corticofugal axonal development without disrupting the thalamus, subpallium or PSPB in the *Emx1Cre;APCloxP/loxP* mutants (Chen *et al.*, 2012). It was found that, whereas thalamic axons still traversed the subpallium in topographic order, they did not cross the PSPB (Fig. 5B). Normal cortex and mutant cortex stimulate the growth of axons from the thalamus by equal amounts in culture experiments (Fig. 5C). This suggests that the inability of thalamic axons to cross the PSPB in *Emx1Cre;APCloxP/loxP* mutants is unlikely to be explained by long-range chemorepulsion by mutant cortex. By providing evidence against alternative explanations and by showing that replacement of mutant cortex with control cortex restored corticofugal efferents and allowed thalamic axons from conditional mutants to cross the PSPB (Fig. 4D), this work provided the most compelling evidence to date that cortical efferents are required to guide TCAs across the PSPB. The molecular mechanisms involved require further investigation. These studies are aided by our better understanding of embryonic subplate and thalamus gene expression patterns (Osheroff & Hatten, 2009; Oeschger *et al.*, 2012).

Guidance of TCAs within the cortex

Early topography during accumulation below the cortical plate

Thalamocortical projections arrive at the cerebral cortex prior to the birth of the majority of cortical neurons and before their migration is complete (Rakic, 1976; Shatz & Luskin, 1986). At this stage, the peak of cerebral cortical neurogenesis and neural migration, the cortical germinal and intermediate zones undergo highly dynamic changes. Meanwhile, the cortical plate is increasing in thickness, and new cells are added to it in an inside-first and outside-last fashion. The subplate zone, which is generated earliest, can be considered to be a relatively stable platform in the developing cortex during this period (Marin-Padilla, 1971; Lund & Mustari, 1977; de Carlos & O'Leary, 1992). The subplate zone contains postmigratory, mature neurons that are the first to express neuronal markers and develop functional synapses (Molliver & Van der Loos, 1970; Kostovic & Rakic, 1990; Friauf & Shatz, 1991; Higashi *et al.*, 2001). The ingrowing thalamocortical projections start to accumulate in this zone for considerable periods, depending on the species (Rakic, 1976; Shatz & Luskin, 1986; Catalano *et al.*, 1991; Molnár *et al.*, 1998a,b). Thalamic afferents overshoot their targets and develop transient side-branches on more proximal segments of their path, through delayed branching (Naegele *et al.*, 1988). These side-branches within the intermediate zone and subplate extend over considerable distances, and have been considered to be the anatomical substrate for the rearrangements of cortical maps during both experimentally induced and normal development (Molnár *et al.*, 2000; Shimogori & Grove, 2005). However, the mechanisms that deliver the thalamic projections and initiate their accumulation below the cortical plate are considered to be largely autonomous (Price *et al.*, 2006). Studies in mice with the SNARE complex knocked out suggest that the early ingrowth of the thalamic axons does not depend on early neuronal communication transmitted through regulated or spontaneous vesicular release mechanisms (Molnár *et al.*, 2002; López-Bendito & Molnár, 2003; Blakey *et al.*, 2012, in this issue), but that, after this initial entry, an activity-dependent mechanism may start to dominate (Catalano & Shatz, 1998; Molnár *et al.*, 2003; Uesaka *et al.*, 2006, 2007; Yamada *et al.*, 2010).

Areal differences in the topographic organization after thalamic fibre entrance to the cortex

Thalamic organization changes during the process of normal development, and it can be altered through: (i) manipulations of the early guidance mechanisms in the subpallium; (ii) manipulations of the early cortical regionalization; or (iii) changing the flow of sensory input from the sense organs.

Although the subpallium controls the early guidance of TCAs, cortical regionalization, which is controlled by the morphogen FGF8 and gradients of TFs (Pax6, COUP-TFI, Emx2, and Sp8), is sufficient to reorient the thalamocortical map within the neocortex (Garel *et al.*,

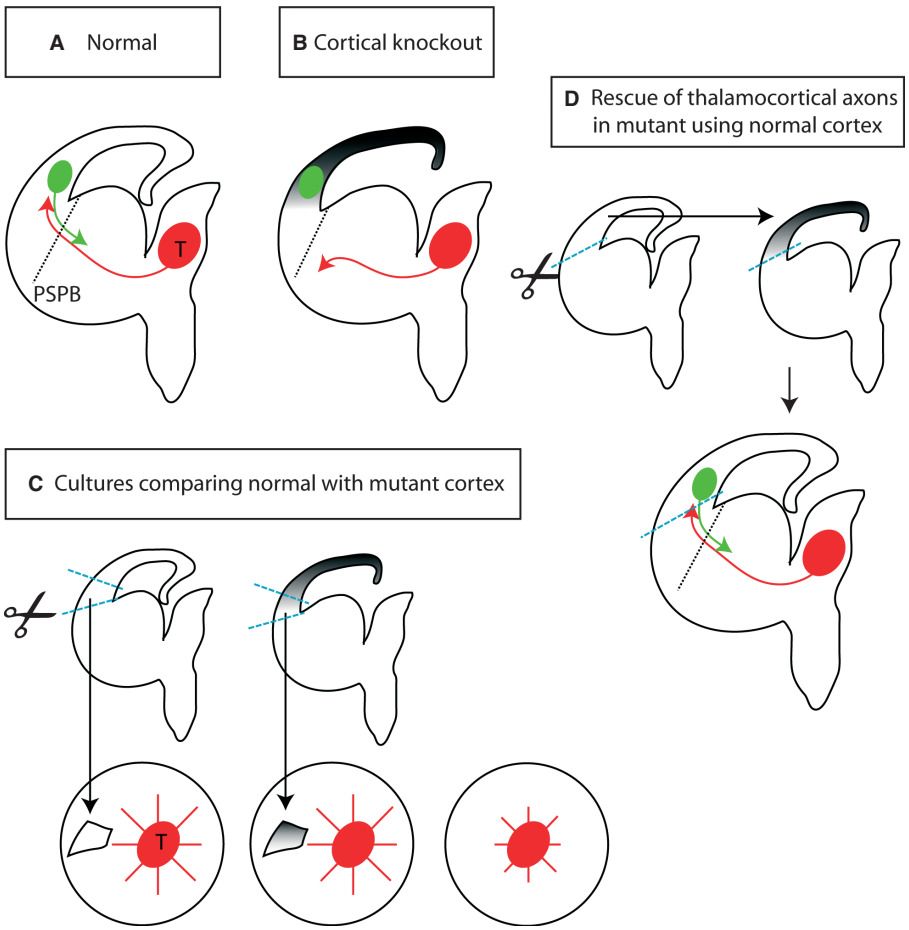

FIG. 5. A summary of recent experiments testing the importance of corticofugal axons for thalamic axon crossing of the PSPB carried out by Chen *et al.* (2012). (A) Normally, axons from the thalamus (T; red) cross the PSPB in close association with descending axons from the cortex (green). (B) In conditional *Emx1Cre;APCloxP/loxP* mutants, the development of cortical neurons and hence of corticofugal axons is blocked, but, although the thalamus and VTel are unaffected, thalamic axons do not cross the PSPB. (C) Culture experiments showed that both normal cortex and mutant cortex stimulate the growth of axons from the thalamus by equal amounts. This suggests that the inability of thalamic axons to cross the PSPB in *Emx1Cre;APCloxP/loxP* mutants is unlikely to be explained by long-range chemorepulsion by mutant cortex. (D) When normal cortex was substituted for mutant cortex in slice cultures from the brains of *Emx1Cre;APCloxP/loxP* embryos, corticofugal axons were restored, and thalamic axons were able to cross the PSPB. These results provide evidence for the importance of corticofugal axons in allowing thalamic axons to cross the PSPB.

2003; Rash & Grove, 2006; O'Leary & Sahara, 2008). The deployment and initial entry of thalamocortical projections to the subplate zone is considerably modified as the TCAs enter the cortical plate in cortical areas, such as V1 of rodents (Naegele *et al.*, 1988; Krug *et al.*, 1998; Ravary *et al.*, 2003). There are areal differences in the density, topographic precision and maturity of thalamocortical projections. Whereas TCAs undergo significant rearrangements in the cortex after entry into V1 of rodents, in the rodent S1 the topography is essentially established immediately after entry (Agmon *et al.*, 1993, 1995). The period during which the thalamocortical projections can be rearranged after sensory manipulations shows considerable variation. Altering early cortical gene expression patterns of FGF8 imposes shifts or even two opposing cortical gradients, with corresponding shifts and duplications of thalamocortical projections (Shimogori & Grove, 2005), whereas changes in Pax6 gradients fails to elicit substantial changes in thalamocortical topography (Piñon *et al.*, 2008). FGF8 gradient alterations can lead to duplication of the thalamic input from the same VB nucleus into multiple areas. The thalamocortical projections develop additional branches within the white matter, a region that corresponds to the location of subplate neurons (Shimogori & Grove, 2005). The earliest thalamocortical interactions and the eventual thalamocortical entry into the cortical plate are orchestrated by the subplate (Allendoerfer & Shatz, 1994; Kanold & Luhmann, 2010). The recognition of the ultimate target neurons within layer 4 of the cerebral cortex and the maturation of these connections relies on multiple cellular and molecular mechanisms (see Blakey *et al.*, 2012 and Yamamoto & López-Bendito, 2012; both in this issue of EJN). The transient circuits between subplate neurons, thalamic afferents and layer 4 neurons are now widely recognized as constituting a key mechanism for early circuit formation (Kanold & Luhmann, 2010). The subplate neurons integrate into the cortical circuits in an age-specific and area-specific dynamic fashion (Piñon *et al.*, 2009; Hoerder-Suabedissen & Molnár, 2012; Tolner *et al.*, 2012; Viswanathan *et al.*, 2012).

Concluding remarks

The development of thalamocortical connections relies on multiple mechanisms. Early connectivity or migrating cell populations shape the trajectory of this axonal tract and assist the crossing of several boundaries. The diencephalic–telencephalic boundary and the PSPB are considered to be the most vulnerable sectors of the pathway, with various guidance defects and several default pathways being reported in mutants with TF factor or axon guidance molecule defects. The

initial topography is also guided by several factors, some establishing gradients in the VTel. The initial deployment of thalamic projections and their accumulation below the cortex is orchestrated by gradients in the subplate. There are several examples of substantial rearrangements in this region before the final ingrowth into the cortical plate, through either manipulations of the early cortical regionalization or changes in the flow of sensory input from sense organs.

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Abbreviations

CHL1, close homologue of L1; E, embryonic day; FGF, fibroblast growth factor; GP, globus pallidus; L1-CAM, L1 cell adhesion molecule; LGE, lateral ganglionic eminence; LIM-HD, LIM homeodomain; M1, primary motor cortex; MGE, medial ganglionic eminence; Neurog2, neurogenin-2; Npn, neuropilin; NrCAM, neuron-glial related cell adhesion molecule; PlexA, plexinA; PSPB, pallial–subpallial boundary; PTh–Th, prethalamus to thalamus; S1, primary somatosensory cortex; Sema3, class III semaphorin; Shh, Sonic hedgehog; TCA, thalamocortical axon; TF, transcription factor; V1, primary visual cortex; VA, ventroanterior; VB, ventrobasal; VL, ventrolateral; VTel, ventral telencephalon; VTel–Th, ventral telencephalon to thalamus; ZLI, zona limitans intrathalamica.

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Appendix. Complexities of the current nomenclature: neurons that cross the early diencephalic and telencephalic subdivisions

The thalamocortical and corticofugal projections start to develop while the various sectors of the telencephalon and diencephalon are being generated. Numerous cell groups migrate tangentially (perpendicular to the orientation of the radial glia) from the pallidum, LGE, MGE, and CGE (de Carlos *et al.*, 1996; Marín & Rubenstein, 2002; Parnavelas, 2002) ; there are streams of cells leaving the LGE and tangentially migrating along the ventral pallium to enter the diencephalon (López-Bendito *et al.*, 2006).

In addition to these tangentially migrating neuronal populations, there are several precocious groups of neurons in the diencephalon and telencephalon whose axons and possibly cell bodies provide guidance for thalamic axons. These cell groups have mostly been identified by their connectivity (Mitrofanis & Guillery, 1993; Métin & Godement, 1996; Molnár *et al.*, 1998a,b; López-Bendito *et al.*, 2006). Unfortunately, they have not been named consistently in the literature. We propose a simple nomenclature based on abbreviations for the sites of the cell bodies and their target tissues: for example, a transient axonal projection from the VTel to the thalamus is called VTel–Th, one from the VTel to the cortex is called VTel–Cx, and one from the prethalamus to the thalamus is called PTh–Th (Table 1; Fig. 2). In this way, we are not confined by insufficient knowledge of their origin, gene expression, or other little known factors.