Intermediate targets in formation of topographic projections: inputs from the thalamocortical system

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Topography of axonal projections has been generally thought to arise from positional information located within the projecting and targeted structures, independent of events along the path or within the axonal bundle. Recent evidence suggests that in the projection from the dorsal thalamus to the neocortex, initial rostrocaudal targeting of axons is regulated at the level of an intermediate target, the subcortical telencephalon. In this system, thalamic axons are spatially positioned within the subcortical telencephalon, partly via interactions between EphAs and ephrin-As, and this positioning apparently determines the rostrocaudal level of the neocortex that the axons will initially target.

Functioning of the nervous system relies on the precision of its wiring pattern. Development of connections often requires that axons navigate over long distances and make multiple changes in direction before reaching their target. For instance, formation of the projection from the retina to the optic tectum (superior colliculus) proceeds by sequential pathfinding steps involving multiple intermediate structures: retinal axons grow into the optic nerve, cross or do not cross the midline at the optic chiasm, grow caudally along the diencephalon and finally enter the tectum [1]. At each step, guidance cues orient and guide the growth cone towards its next guidepost, until axons reach their final target. Retinotectal axons have topographic innervation of their target that reflects the location of the cell bodies in the retina. In particular, nasal retinal neurons project to the posterior part of the tectum, whereas retinal neurons located further away from the nose project to more rostral parts of the tectum. Eph tyrosine kinase receptors and their ligands, the ephrins, have been shown to control the formation of this topographic map via complementary expression gradients within the retina and tectum, respectively [2].

The success in identifying the molecular cues controlling retinotectal topography has made this system the paradigm for conceptualizing how topographic maps are generated in other regions of the nervous system. Thus, whereas long-distance axon pathfinding relies on guidance from intermediate targets, the acquisition of topography has been thought to rely on positional information located in the projecting and targeted structures. Surprisingly, results from three papers provide evidence that an intermediate target, the subcortical telencephalon (ST) or basal ganglia primordium, has a key role in controlling the initial topography of thalamic projections to the neocortex [3–6]. This article presents an overview of the development and topographic organization of the thalamocortical system, and discusses evidence for an emerging role of the ST in routing of thalamic axons to specific cortical areas.

Subcortical telencephalon: an intermediate target guiding thalamocortical axons towards the cerebral cortex

Processing and integration of sensory and motor information is essential to perception, appropriate responses and cognition. This task is performed in part by the cerebral cortex, which receives sensory and motor information from the dorsal thalamus via the thalamocortical projection. During development, thalamocortical axons (TCAs) follow a complex path (Figure 1a): they make a sharp turn at the diencephalic–telencephalic boundary to enter the ST, and make another turn at the cortical–subcortical telencephalic boundary to enter the cerebral cortex [7–10]. Throughout their journey, TCAs navigate in either the mantle or intermediate zone of the territories they traverse, avoiding the proliferative and subpial layers.

Signals in the ST regulate the trajectory of TCAs through the production of secreted axon guidance cues as well as by contact-mediated interactions [9,10] (Figure 1a). The mantle zone exerts an attractive activity on growing TCAs, which is likely to contribute, together with a repulsive activity produced by the hypothalamus, to turning of TCAs into the ST [11–13]. This attractive activity is mimicked by the secreted factor netrin-1, which is produced by the mantle [13]. Other factors, such as netrin-G1 ligand (NGL-1) and hepatocyte growth factor (HGF/SF), have been recently shown to promote thalamic axons outgrowth into the lateral ganglionic eminence (LGE) and medial ganglionic eminence (MGE) parts of the ST, respectively [14,15]. Furthermore, navigation of TCAs...
in the ST is regulated by the secreted factors slit1 and slit2 [16]. In Slit2 and Slit1, Slit2 double mutant mice, some TCAs aberrantly grow towards and across the telencephalic ventral midline and other axons abnormally enter the superficial ST instead of turning into the cortex [16]. Both regions express netrin-1 and are likely to attract TCAs in the absence of Slit2 or of both Slit1 and Slit2. Finally, Sema6A inactivation [17] and in vitro experiments [18] have implicated members of the semaphorin family in TCA pathfinding. Thus, a balance of attractive and repulsive guidance cues has been involved in the guidance of TCAs into, within and out of the ST. Furthermore, transient axonal projections generated by ST neurons have been postulated to guide growing TCAs by fasciculation [11,19] (Figure 1a). Consistent with this hypothesis, an absence or displacement of these transient projections in Mash1, Pax6 or Emx2 mutant mice correlates with abnormalities in the trajectory of TCAs [20–22]. Although cues and cell populations that potentially guide TCAs have been identified, the respective in vivo roles of these factors and of the different ST nuclei remain to be fully defined. For instance, the medial part of the ST (the MGE) has been proposed to contain several specific groups of cells that guide TCAs entering the ST [11,20]. Nevertheless, inactivation of Nkx2.1, which leads to transformation of the MGE into its lateral counterpart (the LGE), does not seem to have a major effect on TCA navigation [23].

In addition to its direct role in the guidance of TCAs, the ST might exert a role on thalamocortical pathfinding indirectly via the control of corticofugal projections. Similar to its effect on TCAs, the ST guides corticofugal projections, which include the corticothalamic projection and cortical fibers descending to the brainstem and spinal cord [16,24–26]. There is evidence that subplate axons, which are pioneers of the corticofugal projection, interact closely with TCAs in the ST and guide them into the cortex [27–31] (Figure 1a). Consistent with this evidence, mutant mice with subplate abnormalities, such as Tbr1, COUP-TFI and p75 mutants, show defects in the reciprocal thalamocortical projection [30–33]. Tbr1 and p75 mutants exhibit TCA pathfinding defects after crossing the ST, at the cortical–subcortical boundary, suggesting that cortical axons are important for the entrance of TCAs into the cortex [30,31,33]. Just as the pathfinding of TCA projections appears to rely on cortical projections, pathfinding of corticothalamic projections appears to rely on the integrity of TCA projections. Mice with defects in TCAs, such as Gbx2 mutants, exhibit abnormalities in corticofugal projections near the ST–diencephalon boundary [31]. Thus, in addition to its role in providing pathfinding signals, the ST might also provide a permissive environment for interactions between cortical and thalamic axons [9,34] that enables these fibers to exit the ST. Therefore, the ST has a key role in the guidance of both thalamic and cortical projections. However, none of these guiding activities has been shown to regulate the topography of thalamocortical projections.

**Topography in the thalamocortical system and its relationship with cortical regionalization**

The topography of TCAs is closely related to the modular functional organization of the cerebral cortex. The neocortex is composed of a mosaic of specialized subdivisions

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**Figure 1.** Pathfinding and organization of thalamocortical axons (TCAs). (a) A coronal section of embryonic-day (E)14.5 mouse brain, the plane of which is depicted in the top left corner. TCAs (red) grow out of the dorsal thalamus (DT) through the ventral thalamus (VT), turn into the subcortical telencephalon (ST) and finally enter the neocortex (Ncx). Attractive (+) and repulsive (−) axon guidance cues, as well as transient axonal projections from the ST and the cortical subplate (dark gray), guide TCAs along their path. (b) Inter-areal topography of TCAs is represented on a horizontal E18.5 mouse brain section, the plane of which is pictured in the top left corner. TCA bundles are schematized to summarize their topographic organization. Dorsal thalamic nuclei project to distinct neocortical primary areas: the dorsal lateral geniculate nucleus (dLGN) projects to the visual cortex (blue), the ventrobasal complex (VB) to the somatosensory cortex (green), and the ventrolateral nucleus (VL) to the motor cortex (orange). TCAs targeting different rostrocaudal levels of the cortex have distinct trajectories within the ST. Other abbreviations: Cort–subcort, cortical–subcortical boundary; Di–tel, diencephalic–telencephalic boundary; Hyp, hypothalamus.
Regionalization might not be the only regulator of initial cortical navigation entirely be due to a change in cortical regionalization [9,34,55]. The handshake hypothesis: thalamic axons target a given area through a specific interaction with cortical axons originating from this area [9,34,55]. This finding, and the observation that thalamic and cortical axons grow in close contact, provided the basis for the ‘handshake hypothesis’: thalamic axons target a given area through a specific interaction with cortical axons originating from this area [9,34,55]. The handshake hypothesis is still under debate but there is accumulating evidence that events occurring outside the neocortex participate in the initial targeting of TCAs. For instance, in Emx2 and COUP-TFI mutants, TCAs pathfinding defects are observed far away from the cortex, suggesting that aberrant TCA navigation in these mice might not entirely be due to a change in cortical regionalization [22,32,46–48]. Consistent with the idea that cortical regionalization might not be the only regulator of initial TCA topography, a change in the molecular identity of the rostral neocortex of Fgf8 hypomorphic mutants does not seem to affect initial targeting of TCAs [56].

Subcortical telencephalon: a regulator of thalamocortical inter-areal topography

Demonstration that Ebf1 and Dlx1, Dlx2 double mutant mice have inter-areal topography defects provided new insights into the role of the ST in TCA pathfinding [3]. Both mutants have abnormalities in ST development and both exhibit a shift in the inter-areal topography of TCAs at birth (Figure 1). Ebf1 encodes an atypical helix-loop-helix (HLH) transcription factor that is expressed in the LGE and caudal ganglionic eminence (CGE) of the ST, in the cortical marginal zone and in the dorsal thalamus [57]. Dlx1 and Dlx2 encode homeodomain transcription factors that control differentiation of GABAergic neurons in the forebrain, and they are not expressed in the glutamatergic neurons of the dorsal thalamus or cortex [58]. Ebf1 inactivation disrupts maturation of LGE neurons and the pathfinding of TCAs in the ST [57]. In Ebf1−/− embryos, axons from the dorsal lateral geniculate nucleus abnormally grow into the amygdalar region and stay in the ST; the other TCAs do reach the cerebral cortex but show a global caudal shift in topography [3] (Figure 2). This shift occurs in the apparent absence of defects in cortical or thalamic regionalization or in the initial outgrowth of cortical axons. Furthermore, this global shift is already observed in the trajectory of TCAs within the ST of Ebf1−/− embryos. These results strongly suggest that the positioning of axons in the ST controls the rostrocaudal targeting of TCAs, independently of cortical regionalization. Support for this model came from analysis of mutant mice carrying a single deletion of the locus containing both Dlx1 and Dlx2 genes [3], which have differentiation defects in the ST and ventral thalamus. In Dlx1/2−/− embryos, some TCAs abnormally stay in the ST, whereas others are able to grow into the cerebral cortex. TCAs that do reach the cerebral cortex have a caudal shift in rostrocaudal topography similar to that observed in Ebf1−/− embryos [3]. In contrast to the Ebf1 mutation, inactivation of Dlx1 and Dlx2 cannot affect cortical or thalamic projections cell-autonomously. These results therefore indicate that affecting the relative positioning of TCAs within the ST modifies the cortical area they will initially target.

Together, these studies show that the path followed by TCAs within the ST controls their initial targeting to specific rostral, intermediate or caudal cortical areas at birth. However, they did not address the mechanisms underlying this process. The ST could have an instructive role, by the display of guidance cues and/or a permissive cue, for instance by maintaining the pre-existing order of TCAs within the axonal bundle. Explant cultures experiments have recently brought support for an instructive role [5] (Figure 3). In an open-book assay of telencephalic vesicles co-cultured with dorsal thalamus explants, TCAs invade the ST and grow toward the cerebral cortex. When explants are dissected from different rostrocaudal levels of the dorsal thalamus, axons tend to grow into different directions within the ST: rostral TCAs grow rostrally, caudal TCAs grow caudally and intermediate TCAs grow...
at an intermediate level. This assay supports the hypothesis that positional information within the dorsal thalamus and ST controls the positioning of TCAs. Nevertheless, this rostrocaudal positional information does not match the topography of TCAs in vivo, where medial nuclei tend to project more rostrally and lateral nuclei caudally [39,40]. Further in vitro experiments, as well as dorsal thalamus fate mapping, will be needed to resolve this apparent discrepancy.

Molecular determinants controlling the targeting of rostral thalamocortical projections

What are the factors mediating positional information and controlling TCA sorting in the ventral telencephalon? Recent experiments have revealed the roles of neurogenin2 (ngn2), EphAs and ephrin-As in targeting a subset of TCAs to the frontal cortex, which includes motor areas [45] (Figure 4). Ngn2 is a basic HLH transcription factor involved in neuronal determination and differentiation,
whose sites of expression include the cerebral cortex, the cortical–subcortical telencephalic boundary and the dorsal thalamus [5]. Its expression in differentiating cells of the dorsal thalamus is restricted to rostral thalamic nuclei [5,59], which project to the frontal and medial cortex. Inactivation of ngn2 did not appear to have a major effect on thalamic regional specification but did modify the pathfinding of rostral TCAs in two ways: (1) instead of targeting the frontal cortex, TCAs navigated through the ST towards a more caudal cortical region, and (2) the TCAs stopped at the cortical–subcortical telencephalic boundary and did not enter the cortex [5]. Co-cultures of ngn2−/− rostral thalamus explants with wild-type telencephalic vesicles in the open-book assay showed that ngn2 specifies cell-autonomously these two aspects of rostral TCA pathfinding. This analysis demonstrates that ngn2 controls the response properties of a subset of thalamic neurons to positional information within the ST.

Further insights into the molecular control of TCA targeting to the frontal cortex have come from analysis of EphA4, ephrin-A5 double mutants [4]. Indeed, in mice lacking this receptor–ligand pair, some axons from the ventrolateral thalamic nucleus that normally project to the frontal cortex project to the more caudal somatosensory cortex. A similar caudal shift is observed in EphA7−/− mice. This shift in TCA trajectory is detected in the ST, suggesting that EphAs and ephrin-As control the pathfinding of rostral TCAs within this region. Consistent with this model, ephrin-A5 shows a gradient of expression within the ST, and EphA4, EphA3 and EphA7 exhibit a rostrocaudal gradient of expression in differentiating cells of the dorsal thalamus. In vitro analysis using the open-book assay demonstrated that reducing ephrin activity, either by adding an EphA3-Fc or by using an ephrin-A5−/− telencephalic vesicle, induced a caudal shift in the trajectory of wild-type rostral thalamic neurons within the ST. Thus, gradients of Eph receptors in the dorsal thalamus and of ephrins in the ST appear to be regulating the positioning and targeting of rostral thalamic axons to the frontal cortex.

These experiments open the way to the dissection of the molecular pathways controlling TCA positioning in the ST and their subsequent areal targeting. Strikingly, Eph receptors and ephrins are once again involved in the control of topography, but this time at the level of an intermediate target. The role of these factors in the targeting of more caudal or intermediate TCAs, as well as their role in the phenotype of ngn2−/− mice, remains to be addressed.

**Conclusions and perspectives**

TCAs originating from specific thalamic nuclei are segregated within the fiber tract, follow a distinct pathway within the ST, target a specific cortical area (inter-areal targeting) and form a representation of a sensory or motor field (intra-area targeting). As reviewed here, there is new evidence that the ST operates as a routing center that controls the initial rostrocaudal targeting of thalamocortical projections (Figure 5). Indeed, in vivo and in vitro studies show that positional information controls the trajectory of TCAs within the ST, thereby imposing the initial rostrocaudal targeting of TCAs in the cortex [3,5]. Furthermore, experiments show that ngn2 and EphAs in the dorsal thalamus and ephrin-As in the ST encode this positional information for TCA targeting to the frontal cortex [4,5]. Thus, although several lines of evidence indicate that regional cues in the neocortex shape, refine and remodel thalamic projections [49,51–54,60], new studies support a role for the ST, an intermediate structure, in initial TCA targeting. These findings raise two important questions about (1) the respective roles of different ST subdivisions (LGE, MGE and CGE) and cell populations in TCA navigation; and (2) the putative role of

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**Figure 4.** Neurogenin2 (ngn2), Ephs and ephrins control the targeting of rostral thalamocortical axons (TCAs). The phenotypes of wild-type (a), Ngn2−/− (b) and EphA4−/−, ephrin-A5−/− (c) mice (the large horizontal section in each panel), and the in vitro analysis of rostral TCA pathfinding in each case (right). Ngn2 expression in the rostral dorsal thalamus and cortex, EphA4 expression in the dorsal thalamus and ephrin-A5 expression in the subcortical telencephalon (ST) are represented on the left (blue). (Note that ephrin-A5 expression in the cortex and dorsal thalamus and EphA4 expression in the cortex and ST are not shown here.) In Ngn2−/− mice, rostral thalamic axons, instead of growing rostrally, grow into an intermediate position within the basal ganglia primordium and stop at the cortical–subcortical boundary. Co-cultures of rostral thalamic axons from Ngn2−/− mice with wild-type telencephalic vesicles show that these two defects are cell-autonomous. Rostral TCAs also grow more caudally in EphA4−/−, ephrin-A5−/− mice and subsequently invade more caudal regions of the neocortex. Ephrin-A5−/− telencephalic vesicles co-cultured with wild-type rostral thalamic explants show that this caudal shift is reproduced by a decrease of ephrin signaling in the telencephalon.
other intermediate structures, such as the ventral thalamus in TCA pathfinding and orientation. The fact that the path followed by TCAs in the ST determines the region they will target has implications for the mechanisms controlling neocortical regionalization. Recent key experiments have shown that cortical regionalization is controlled in part through intrinsic molecular mechanisms before the arrival of thalamic afferents [37,38,41,42]. However, the initial targeting of TCAs into their final topographic location in the ST (purple gradient) is regulated in part through intrinsic molecular mechanisms, including the ST, and thus is not solely dictated by cortical signals. Further studies are needed to establish how this initial targeting might influence the final topographic projections of thalamic axons, and whether this targeting has an instructive role on cortical development or on the reciprocal corticothalamic projection.

Although, early aspects of thalamocortical topographic organization are regulated within an intermediate target, Eph receptors and ephrins appear once again as mediators of this process. Whereas these molecules seem so far to be systematically associated with formation of topographic maps in vertebrates, it will be of great interest to determine whether the role of intermediate targets in control of topography is unique to the thalamocortical system or is a more general mechanism controlling axonal tract formation in the nervous system.

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