Review TRENDS in Neurosciences Vol.27 No.7 July 2004

Developmental mechanisms patterning thalamocortical projections: intrinsic, extrinsic and in between

Pierre Vanderhaeghen[1] and Franck Polleux[2]

[1]Institut de Recherche Interdisciplinaire en Biologie Humaine et Moléculaire (IRIBHM), University of Brussels (ULB), 808 Route de Lennik, B-1070 Brussels, Belgium
[2]Neuroscience Center, Department of Pharmacology, University of North Carolina, Chapel Hill, 105 Mason Farm Road, North Carolina 27599-7250, USA

Roger Sperry proposed 40 years ago that topographic neural connections are established through complementary expression of chemoaffinity labels in projecting neurons and their final targets. This led to the identification of ephrins as key molecular cues controlling the topography of retinotectal projections. Recent studies have revealed a surprising twist to this model, shedding light on the developmental mechanisms patterning the projections between the thalamus and the cortex: ephrins, unexpectedly expressed in an intermediate target, control the establishment of topography of axonal projections between these two structures. The same cues are re-used later to control the mapping of thalamocortical projections within a given cortical area, which strikingly illustrates how a limited set of genes can contribute to generate several levels of complexity of a neuronal network.

In the mammalian forebrain, the topographic projection of each thalamic nucleus to a unique set of cortical areas gives rise to the input specificity characterizing each sensory modality [1]. The level of organization of thalamocortical projections is far more complex than simple model systems such as retinotectal projections. Most thalamic nuclei project to specific cortical areas, providing the first level of inter-areal specificity of thalamocortical connections (Figure 1a). A second level of organization is achieved within each area, where projections from each individual thalamic nucleus display a precise intra-areal topographic organization (Figure 1b), allowing the generation of accurate spatial representations within each cortical area.

Although the importance of this multi-level organization of thalamic projections for normal cortical function has long been appreciated, the developmental mechanisms underlying its precise patterning remain largely unknown. In most species the inter-areal specificity of thalamocortical projections is achieved prenatally, whereas the intra-areal mapping of thalamocortical axons occurs postnatally [2]. Numerous models have been proposed to explain the patterning of thalamocortical projections, including correlated neural activity, temporal and/or spatial ordering of thalamic axon outgrowth, axon–axon interactions between corticothalamic and thalamocortical axons (the 'handshake' hypothesis), complementary axon guidance labels in the thalamus and cortex, as well as specific stop-signal cues in the developing subplate [2–8].

Topographic organization of thalamocortical projections

Numerous anatomical studies have provided a detailed description of the basic topographic rules of thalamocortical projections in mammals [9–11]. Thalamic projections are organized along the rostrocaudal and lateromedial axes of the mammalian cerebral cortex. First, along the rostrocaudal axis, axons originating from rostral thalamic nuclei project to rostromedial cortical areas, whereas caudal thalamic nuclei project caudolaterally in the cortex (Figure 1c). As an example, rostral thalamic nuclei of the anterior group and the ventrolateral nucleus (VL) project to medial cingulate cortex and frontal cortex (M1), respectively, whereas more caudal nuclei such as the lateral geniculate nucleus project to the caudal cortical pole, and target the primary visual area (V1). Second, along the lateromedial axis, axons originating from lateral thalamic nuclei tend to project caudally in the cortex, whereas axons originating from medial nuclei project more rostrally (Figure 1c).

These surprisingly simple topological rules apply to all primary thalamic nuclei and cortical areas, and to most of the thalamus and cortex, even though there are exceptions such as 'associative' thalamic nuclei that send more diffuse projections to the cortex [12]. These coordinate transformations lead to the basic pattern of thalamocortical projections linking each thalamic nucleus with a unique set of cortical areas in mammals including rodents, carnivores and non-human primates [11]. Remarkably, a recent study using a diffusion-tensor imaging technique has demonstrated that this topographic organization also applies to humans with a surprising level of similarity with other mammals [13] (Figure 1d).

Corresponding authors: Pierre Vanderhaeghen (pvdhaegh@ulb.ac.be), Franck Polleux (polleux@med.unc.edu).

Available online 4 June 2004

 0166-2236/$ - see front matter doi:10.1016/j.tins.2004.05.009

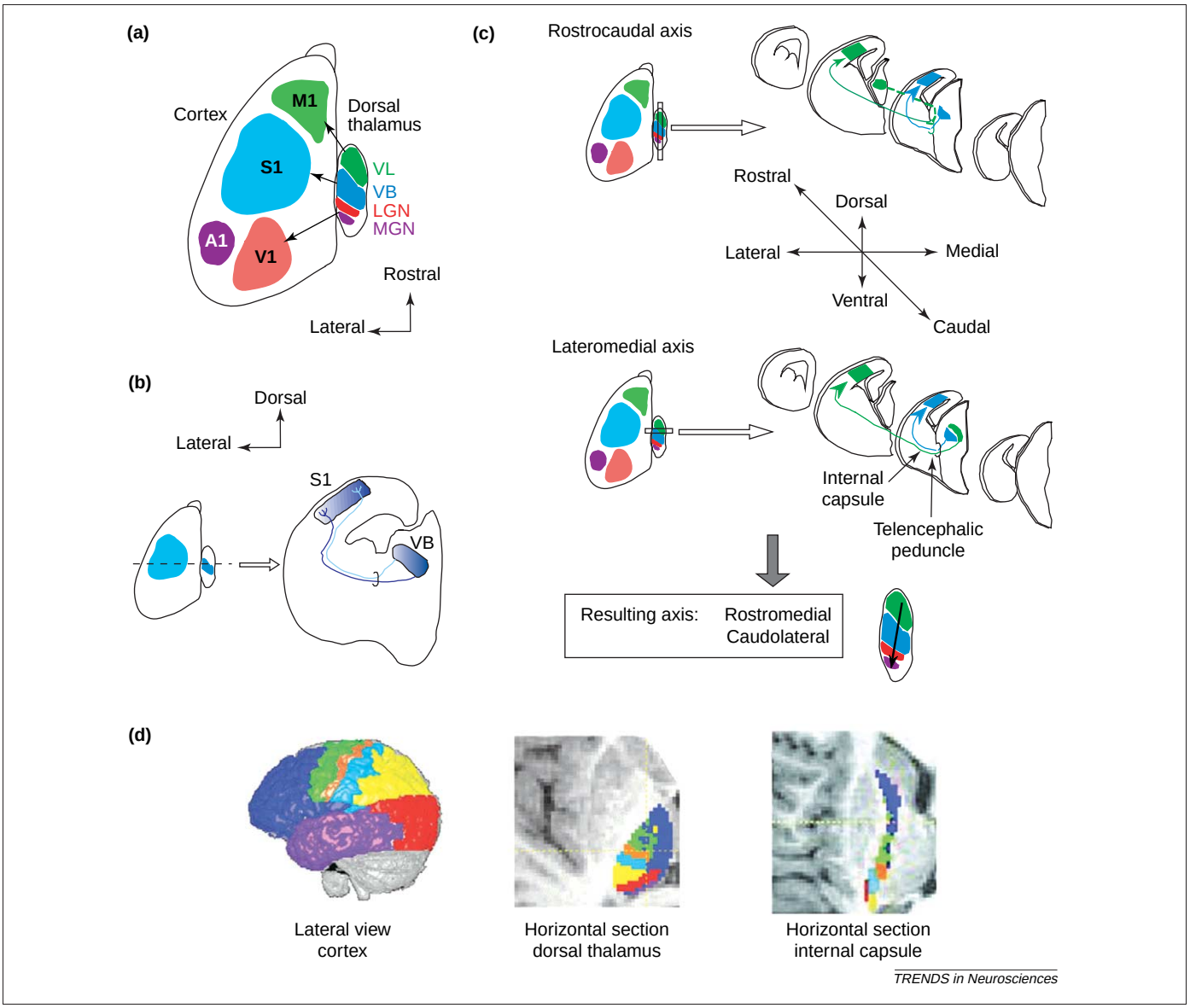

Figure 1. Thalamocortical connections display several levels of topographic specificity. **(a)** First, axons from individual thalamic nuclei project to a unique set of cortical areas (inter-areal specificity). **(b)** Second, axons project topographically within a given cortical area (intra-areal specificity), schematized here for the somatosensory system: axons from the lateral part of the primary somatosensory nucleus (the ventrobasal thalamic nucleus, VB; light blue) project to the medial part of the primary somatosensory area or barrel field (S1; dark blue), whereas axons from the medial part of the VB (dark blue) project to the lateral part of S1 (light blue). **(c)** The two main axes of projection defining the inter-areal topography of projections described in rodents, carnivores and non-human primates [9–11]. Numerous anatomical studies have provided a detailed description of the basic topographic rules of thalamocortical projections in mammals. Thalamic projections are organized along the rostrocaudal and lateromedial axes of the mammalian cerebral cortex. First, along the rostrocaudal axis, axons originating from rostral thalamic nuclei project to rostromedial cortical areas, whereas caudal thalamic nuclei project caudolaterally in the cortex (i). Second, along the lateromedial axis, axons originating from lateral thalamic nuclei tend to project caudally in the cortex, whereas axons originating from medial nuclei project more rostrally (ii). This results in a basic topological organization where a rostromedial-to-caudolateral axis in the thalamus is mapped to a rostrocaudal axis in the cortex. **(d)** Strikingly, the same topographic organization is preserved in humans. Modified, with permission, from Ref. [13]. Abbreviations: A1, primary auditory area; LGN, lateral geniculate nucleus; M1, primary motor area; MGN, medial geniculate thalamic nucleus; V1, primary visual area; VL, ventrolateral thalamic nucleus.

How is the inter-areal specificity of thalamocortical projections initiated during development?

The developmental mechanisms underlying the generation of cortical areas and their topographic organization remain poorly understood. In particular, it has remained unclear if the instructive cues patterning the cortical neuroepithelium reside primarily in extrinsic thalamic afferents or are intrinsic to the developing cortex.

Intrinsic mechanisms

Recently there has been a flurry of *in vivo* evidence indicating that the cortex contains intrinsic molecular determinants patterning the molecular identity and the position of cortical areas [5,8]. Importantly, graded expression of morphogens and transcription factors in the early embryonic cortex are required for the normal patterning of thalamocortical projections [14–16]. In an elegant series of experiments where *in utero* electroporation-mediated gene transfer was used to produce an ectopic posterior source of fibroblast growth factor 8 (FGF8), a morphogen involved in patterning the rostral cortical primordium, a second barrel-field was produced in the occipital pole [16]. Although these results await confirmation that the ectopic barrel field actually receives

the appropriate thalamic innervation by axons from the ventrobasal (VB) complex (the primary somatosensory thalamic nucleus), they strongly suggest that cortex-derived cues play a role in patterning the inter-areal specificity of thalamocortical projections (Figure 2a,b). In fact, *in vivo* grafting experiments have already pointed to the presence of cortical cues participating in the areal specificity of thalamocortical projections [17,18]. For example, when embryonic day (E)16 frontal cortex is grafted ectopically into the occipital cortex of neonatal rats, axons emerging from the VL and ventromedial (VM) thalamic nuclei not only project to their normal target (the frontal cortex) but also display a significant attraction towards the posterior frontal graft [17,18] (Figure 2d). This rewiring is found only for frontal-to-occipital grafts and not for occipital-to-occipital grafts (Figure 2c). Taken together, these results suggest that unidentified area-specific cues can attract thalamic axons towards their target cortical area *in vivo*.

Extra-cortical mechanisms

However, several studies recently provided evidence that the areal specificity of thalamocortical projections is likely to be initiated by extra-cortical cues. First, Garel *et al.* have shown that knockout mice for the transcription factors Ebf1 and Dlx1/2 display a defective topography of thalamocortical projections [19]. Interestingly, these transcription factors are expressed primarily in the ventral telencephalon, through which axons travel to form the internal capsule from the thalamus to the cortex and vice versa (Figure 3a). These results suggested that cues present in the ventral telencephalon could initiate thalamocortical topography, but their interpretation is limited by the fact that both *Ebf1* and *Dlx1/2* are expressed not only in the ventral telencephalon but also in the thalamus itself [20,21]. However, a second line of evidence also argues for the importance of extra-cortical cues in specifying the topography of thalamocortical projections: the analysis of mice presenting a hypomorphic allele of FGF8 revealed a prominent shift of the expression of early molecular markers defining cortical territories but, interestingly, does not produce a corresponding shift in the early targeting of thalamocortical projections at embryonic stages [22]. This result suggested for the first time a partial independence between the mechanisms controlling the cortical regionalization into specific areas and the mechanisms initiating the inter-areal specificity of thalamic axons targeting.

By contrast, both thalamocortical projections and cortical efferent projections are affected in knockout mice for transcription factors expressed in the developing cortex only (Tbr1), dorsal thalamus only (Gbx2), or both (Pax6, Emx2) [23–25]. Notably, the reciprocal pathfinding defects in these mutants are first found in the ventral telencephalon, through which axons travel to form the internal capsule (Figure 3a) from the thalamus to the cortex, and vice versa. Therefore, these results point to a potential role of the ventral telencephalon in the patterning of thalamocortical axons, although they would also be consistent with the 'handshake hypothesis', according to which thalamic axons might be patterned on their way to the cortex, following axon–axon interactions with cortical efferents axons in the internal capsule [2].

Taken together, these recent results suggested the existence of (i) intermediate, extra-cortical guidance cues, responsible for the initial specification of the topography of thalamocortical projections to different cortical domains, and (ii) intra-cortical cues, capable of attracting subsets of thalamic axons towards their appropriate cortical target. The idea of intermediate cues is not new, and many anatomical studies have speculated about the role of intermediate guidepost cells in the development of thalamocortical projections in mouse, rat and ferret [26–30]. However, the functional demonstration of the importance of ventral telencephalic cues initiating the topography of thalamocortical projections has emerged only very recently from a study of the role of the transcription factor neurogenin 2 (Ngn2) in the specification of neuronal connectivity [31]. Neurogenin 2 is a basic helix–loop–helix (bHLH) transcription factor expressed in the rostral part of the developing thalamus [31,32] and uniformly throughout the embryonic cortex [33]. Seibt *et al.* have explored the role of Ngn2 in the specification of the topography of projection of rostral thalamic neurons to

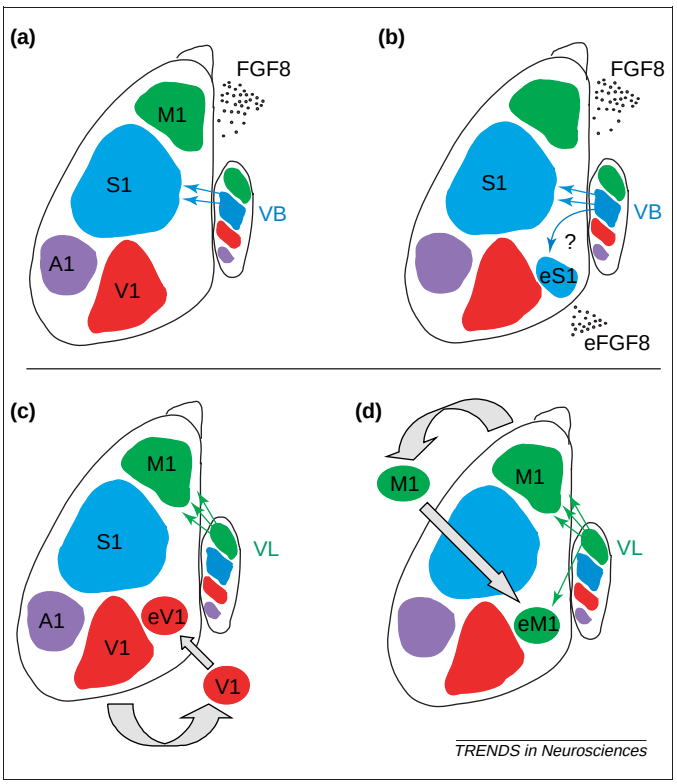

Figure 2. Thalamocortical patterning and intrinsic cortical cues. **(a,b)** Creating an ectopic source of fibroblast growth factor 8 (eFGF8), a morphogen normally expressed in the rostral organizer of the early cortical primordium (a), induces an ectopic caudal barrel field [eS1 in (b)] [16]. Although these results await confirmation using tracing experiments to demonstrate that axons from the ventrobasal thalamic nucleus (VB) actually project to the ectopic barrel-field [question mark in (b)], they strongly suggest that cortex-derived cues can play a crucial role in patterning the inter-areal specificity of thalamocortical projections. **(c,d)** Grafting experiments demonstrate that a frontal-to-occipital (d), but not occipital-to-occipital (c) transplant is innervated by thalamic axons from the ventrolateral thalamic nucleus (VL) [17,18], suggesting once more the existence of attractive area-specific cues in the control of the final targeting of thalamocortical projections. Abbreviations: A1, primary auditory area; eM1, ectopic primary motor area; eS1, ectopic primary somatosensory area; eV1, ectopic primary visual area; M1, primary motor area; S1, primary somatosensory area or barrel field; V1, primary visual area.

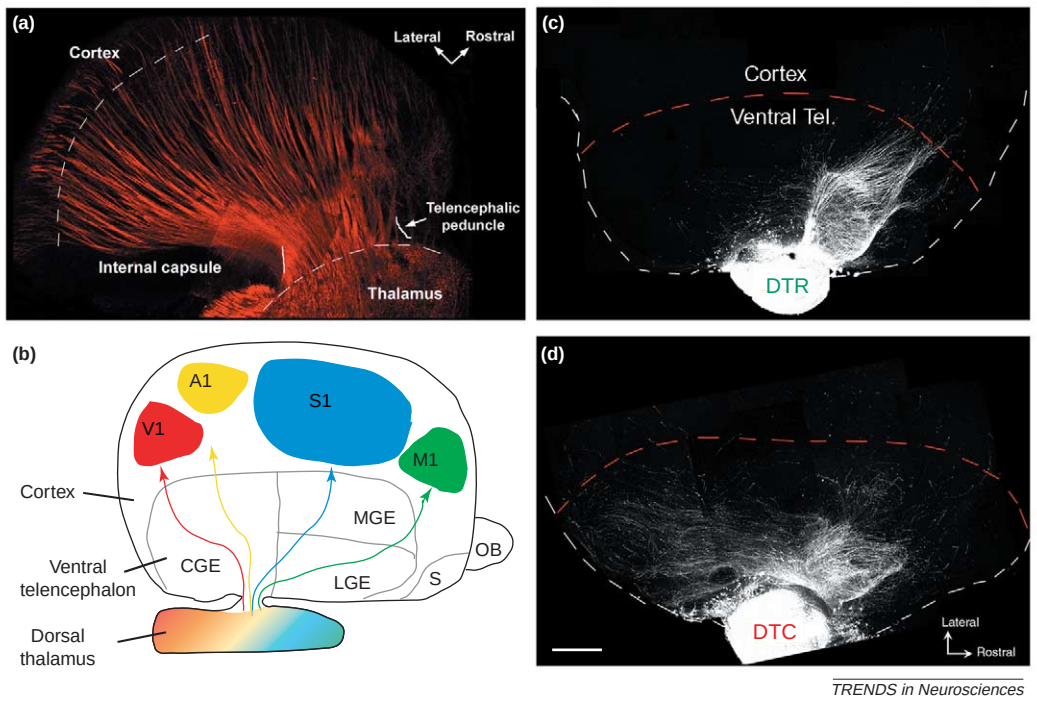

Figure 3. Thalamocortical patterning in the ventral telencephalon. **(a)** An oblique section of an embryonic day (E)17 mouse brain stained with a pan-axonal marker (neurofilament 165 kDa) reveals the outstanding array of millions of axons constituting the main input–output wiring to and from the cortex. Note that thalamocortical axons leave the thalamus through a narrow passage (telencephalic peduncle) and subsequently have to redistribute through the entire rostrocaudal extent of the ventral telencephalon to form the internal capsule, before invading the dorsal telencephalon (cortex). **(b)** Schematic view of a 'flattened' telencephalic vesicle illustrating the rostrocaudal sorting of axons originating from different rostrocaudal levels of the dorsal thalamus, allowing their targeting to distinct cortical domains [31]. **(c,d)** The 'telencephalic wholemount' assay recapitulates the topography of thalamocortical axons projection *in vitro*: in this assay, axons emerging from the rostral part [DTR in (c)] or the most caudal part [DTC in (d)] of E14.5 dorsal thalamus expressing green fluorescent protein (GFP) are co-cultured for three days *in vitro* with an isochronic telencephalic 'wholemount'. This assay reveals that different rostrocaudal levels of the thalamus respond differently to intermediate cues present in the ventral telencephalon. Scale bar for (c) and (d), 400 μm. Abbreviations: A1, primary auditory area; CGE, caudal part of the ganglionic eminence; LGE, lateral part of the ganglionic eminence; M1, primary motor area; MGE, medial part of the ganglionic eminence; OB, olfactory bulb; S, septum; S1, primary somatosensory area or barrel field; V1, primary visual area.

rostral cortical territories [31]. In this study, the authors developed a new *in vitro* assay where the projections of identified regions of the developing thalamus expressing green fluorescent protein (GFP) can be easily assessed while growing in wholemount telencephalic vesicles. Using this 'wholemount telencephalon' assay, the authors demonstrated that axons emerging from the anterior part of the dorsal thalamus at E14.5 preferentially target the anterior part of the ventral telencephalon, ultimately invading anterior cortical territories (Figure 3c). Conversely, axons emerging from progressively more caudal parts of the thalamus grow preferentially in more caudal parts of this intermediate target (Figure 3d). Importantly, the analysis of the thalamocortical projections in *Ngn2* knockout mice revealed a caudal shift in the topography of projection of rostral thalamic nuclei detected first in the ventral telencephalon and consequently in the cortex (Figure 4c). The study of Seibt *et al.* demonstrates for the first time: (i) that intermediate topographic cues distributed along the rostrocaudal axis of the ventral telencephalon are sufficient to initiate the specificity of thalamic axon projections to the appropriate cortical territories (Figure 3b), and (ii) that the dorsal thalamus is heterogeneous along its rostrocaudal axis with regard to the responsiveness of its axons to these ventral telencephalic cues (Figure 3c,d).

Identification of the extra-cortical topographic cues initiating thalamocortical projection topography

Based on these results, identification of the guidance cues involved in patterning of thalamocortical axons in the ventral telencephalon became a crucial issue. A recent study has succeeded in identifying some of these cues and has implicated ephrin–Eph signaling in the early sorting of thalamocortical axons in the ventral telencephalon [34]. The analysis of the expression pattern of Eph receptors in the early developing mouse forebrain provided a first hint that ephrins could be such sorting factors: three EphA receptors (EphA3, EphA4 and EphA7) were found to be expressed in high rostromedial to low caudolateral gradients in the early (E13 to E15) dorsal thalamus [34]. These gradients are very different from those previously described within individual nuclei of late embryonic (after E15) and early postnatal thalamus [35–37], as they encompass the boundaries between presumptive thalamic nuclei, thereby providing a potential substrate for specificity between individual nuclei (Figure 4a,b). Are there matching ligands in the telencephalon? Most strikingly, ephrin-A5, which is a ligand for these three receptors, is expressed in a complementary gradient (high caudal to low rostral) in the mantle zone of the ventral telencephalon, right at the zone of passage of thalamocortical axons in the internal capsule (Figure 3a). Importantly, the overall orientation of the ephrin–Eph gradients precisely matches the responsiveness of thalamic axons observed

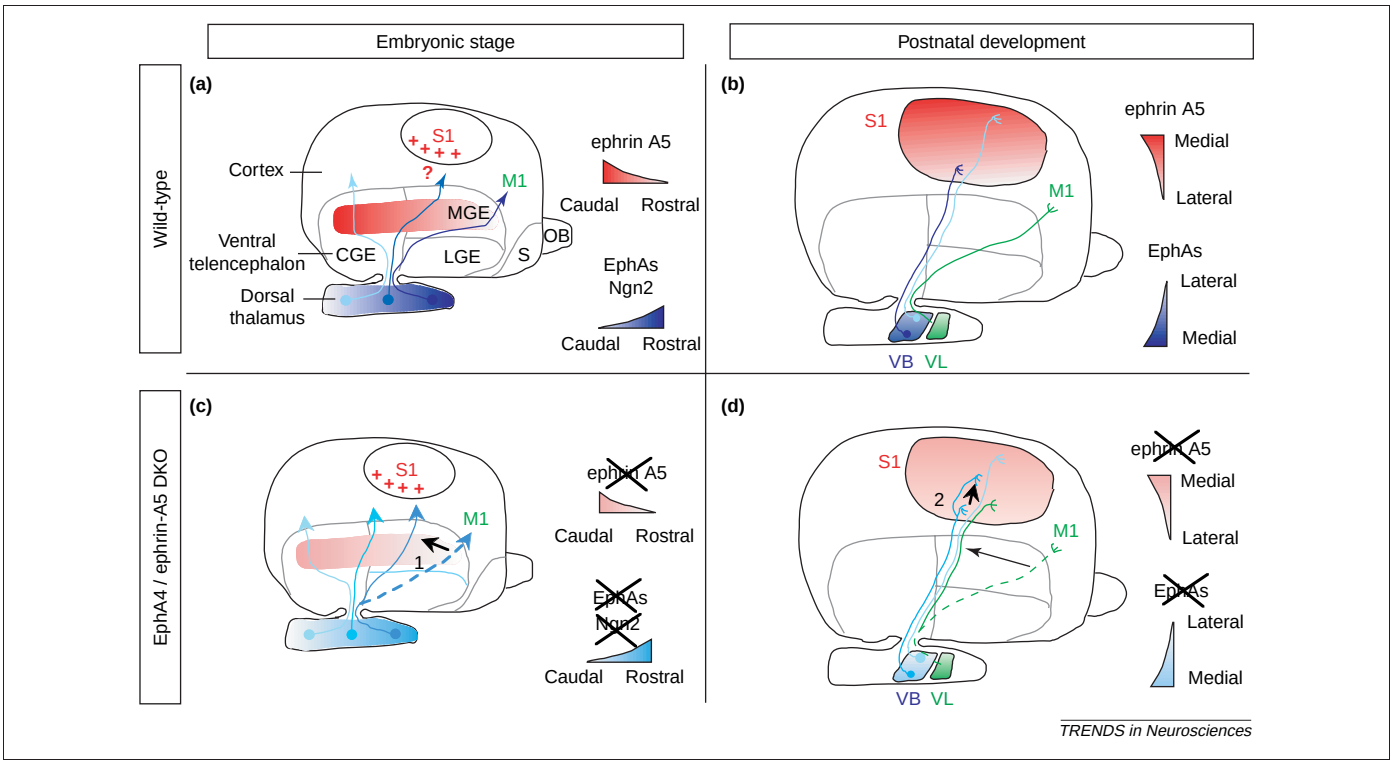

Figure 4. Model of sequential control of thalamocortical patterning. **(a)** Inter-areal thalamocortical topography is initiated at early embryonic stages in the ventral telencephalon. In wild-type mice, EphA receptors and the gene encoding neurogenin 2 (*Ngn2*) are expressed in the dorsal thalamus in a high rostromedial to low caudolateral gradient (blue). Ephrin-A5 is expressed in a complementary gradient, high caudal to low rostral, in the ventral telencephalon (red). Thalamocortical axons expressing high levels of EphA receptors from the rostromedial part of the thalamus (which gives rise to the ventrolateral thalamic nucleus, VL; axons in dark blue) travel through the rostral part of the ventral telencephalon, which expresses low levels of the ephrin-A5 ligands, and consequently invade the rostral part of the cortex (such as the primary motor area, M1). Axons from more caudolateral parts of the thalamus (which will become the ventrobasal thalamic nucleus, VB; axons in light blue) travel more caudally through the ventral telencephalon and eventually connect to more caudal cortical domains (such as the primary somatosensory area or barrel field, S1). Unidentified cues present in the dorsal telencephalon have been proposed to control the final areal targeting of thalamic axons in the dorsal telencephalon [7,16–18] (question mark; see also Figure 2). **(b)** Intra-areal topographic mapping of thalamocortical projections in the somatosensory system is also controlled by the same ephrin and Eph genes but with different spatial modalities. At postnatal ages, ephrin-A5 and EphA4 are expressed in complementary gradients in VB and S1, respectively. These gradients match one another, given the known VB-to-S1 topography, with the medial part of VB (expressing high levels of EphA receptors; axons in dark blue) projecting to the lateral part of S1 (expressing low levels of ephrin-A5; light red), and with the lateral part of VB (expressing low levels of EphA receptors; axons in light blue) projecting to the medial part of S1 (expressing high levels of ephrin-A5; dark red). **(c,d)** In the absence of appropriate ephrin–Eph signaling [in EphA4/ephrin-A5 double knockout (DKO) mice] or in the absence of *Ngn2* expression, rostromedial thalamocortical axons growing from the presumptive VL nucleus invade more caudal territories of the ventral telencephalon during embryonic stages [arrow 1 in (c)], resulting in an aberrant shift of their projections to more caudal cortical areas such as S1 [unnumbered arrow in (d)]. In addition, intra-areal mapping is also perturbed: axons from the medial VB send ectopic projections [arrow 2 in (d)] to medial domains of S1, although some topography is preserved, suggesting compensatory mechanisms involving additional ephrins, Eph receptors or as-yet unidentified genes. Abbreviations: CGE, caudal ganglionic eminence; LGE, lateral ganglionic eminence; MGE, medial ganglionic eminence; OB, olfactory bulb; S, septum.

in vitro and *in vivo* [31]: rostromedial thalamic axons that display high amounts of Eph receptors avoid the caudal domain of the ventral telencephalon enriched in ephrins (Figure 4a).

Dufour *et al.* took advantage of the thalamic–telencephalic co-culture assay to test for the requirement for ephrins in guiding thalamic axons in the ventral telencephalon [34]. As predicted from the expression patterns, addition of soluble inhibitors of ephrin As or EphA receptors to the culture medium resulted in a loss of topographic growth of rostral thalamic axons, and therefore in randomization of their pattern of invasion of the ventral telencephalon. Similar, albeit milder, effects were observed when using telencephalic wholemount preparations isolated from ephrin-A5 mutant embryos, suggesting that ephrin-A5 plays a role in patterning thalamocortical projections in the ventral telencephalon, along with other redundant cues remaining to be identified [34] (Figure 4c).

Together with the expression data, these results show that gradients of ephrins in the ventral telencephalon can act as topographically specific repellents for rostral thalamic axons, at least *in vitro*. Axon-tracing analyses of double knockout mice for the genes encoding ephrin-A5 and EphA4 enabled the relevance of these findings to be assessed *in vivo* [34]. Dufour and colleagues found aberrant projections from the rostral thalamus displaying a significant caudal shift in their projection, in both the ventral and the dorsal telencephalon. Similarly, retrograde axon-tracing analyses of postnatal compound mutant mice revealed strikingly aberrant projections from the VL nucleus to the caudally located somatosensory 'barrel' cortex, whereas in wild-type mice, VL strictly projects to rostrally located motor areas (Figure 4b). Injections in both the primary motor and the primary somatosensory cortex further confirmed that in the ephrin-A5/EphA4 double knockout mice, unlike in wild-type controls, the VL nucleus projects to both somatosensory barrel cortex and motor cortex, thus adopting an aberrantly divergent pattern of areal targeting (Figure 4d). Importantly, this tracing analysis was performed on postnatal day (P)15–P20 animals, providing direct

evidence that an early (embryonic) disruption of the sorting of thalamic axons in the ventral telencephalon can have a long-lasting influence on the mature pattern of thalamic innervation of the cortex. It will be interesting to determine whether this thalamocortical miswiring has any behavioral consequences, given that both grafting and re-wiring experiments have demonstrated a considerable plasticity of thalamocortical connectivity [3].

Although this work clearly demonstrates that ephrins in the ventral telencephalon play an instructive role in topographic mapping of thalamocortical projections, ephrins in the cortex have been proposed to control other aspects of inter-areal thalamocortical specificity, such as the targeting of specific thalamic nuclei to the limbic cortex [35,36,38,39]. Future work using region-specific conditional ephrin and/or Eph mutants should help determine the relative contribution of intermediate versus cortical ephrins in inter-areal mapping of thalamocortical projections.

Never change a winning team: ephrin and Eph genes and intra-areal mapping

Interestingly, the involvement of ephrin and Eph genes in thalamocortical development is not restricted to the specification of inter-areal targeting, as the very same ephrin and Eph genes are involved later, in development of the second level of organization of thalamocortical connections: the intra-areal topographic map organization, which allows the generation of accurate sensory representations within a specific cortical area (Figure 4b).

Even though a large body of evidence points to the importance of activity-dependent mechanisms in the generation and plasticity of cortical maps [40–43], recent work has suggested that activity-dependent mechanisms might not be essential for generating the crude topography of somatosensory and visual cortical maps, suggesting the involvement of guidance cues in this process [4,44,45]. Given their involvement in the development of topographic maps elsewhere in the CNS [46–51], ephrin and Eph genes were obvious candidates for controlling the topography of intra-areal mapping. Expression studies centered on the somatosensory system first revealed that ephrin-A5 and its receptor EphA4 are expressed in matching complementary gradients in the rodent primary somatosensory cortex (S1) and thalamus (the VB nucleus) at perinatal stages, which corresponds to the period of somatosensory map formation [37]. Using an *in vitro* axon guidance 'stripe assay', ephrin-A5 was shown to act as a topographically-specific repellent for thalamocortical axons from the medial VB, which display the highest amount of EphA4 receptor [37]. Analysis of ephrin-A5 mutants revealed an intriguing topographic distortion of the shape and size of the S1 map, consistent with a role in the mapping of somatosensory thalamic axons, but failed to show any disruption of the topographic, point-to-point precision of the projection, suggesting that genetic redundancy might be obscuring the full extent of the role of ephrin and Eph genes in this system [37,52]. Consistent with this hypothesis, analysis of ephrin-A5/EphA4 double knockout mice revealed a much more severe defect of thalamocortical projections between VB and S1 [34], where numerous axons from medial VB project ectopically to more medial domains in S1, thereby disrupting the normal point-to-point precision of the system (Figure 4d). Together, these results indicate that ephrin-A5 in the cortex acts as a graded repulsive cue for thalamocortical axons expressing graded levels of EphA receptors (including EphA4) to generate a precise topographic somatosensory map.

Remaining questions: interplay of guidance cues and activity?

Several questions remain concerning the cellular mechanisms of ephrin action in intra-areal mapping *in vivo*. Do ephrins modulate axonal branching and/or pruning [53], as they do in the retinotectal system [54], or do they rather control the guidance of thalamocortical axons when they invade the cortical plate? Analysis of the time-course of the defects found in ephrin and/or Eph mutants should help answer this question. The involvement of ephrins in development of cortical maps also raises the question of their relationship with activity-dependent mechanisms that are crucial for refinement of such maps [40–43]. Given the expression of ephrins in cortical barrels at postnatal stages [37], together with the recent implication of B ephrin in NMDA receptor signaling and synaptic plasticity in the adult hippocampus [55,56], it is tempting to speculate that the same genes are re-used during the postnatal activity-dependent refinement of cortical maps. Similarly, because inhibition of neuronal activity interferes with areal specificity of thalamocortical projections [57], it would be most interesting to test the effects of developmental patterns of activity on Eph and ephrin expression and signaling.

Sequential model for the patterning of thalamocortical projections

Historically, deciphering the mechanisms patterning thalamocortical projections in mammals has been a controversial issue. To reconcile some of the conflicting experimental evidence presented in this review, we would like to propose a simple model (Figure 4a,b) where the precise topography of projection of thalamic axons emerging from a given nucleus (e.g. VB) onto a unique cortical area (e.g. S1) is specified sequentially through the following steps:

(i) Extra-cortical cues, including ephrins, present in the ventral telencephalon initiate the topographic guidance of thalamic axons towards crudely defined cortical domains (frontal, parietal, temporal and occipital) (Figure 4a).

(ii) Cortical cues, including unidentified area-specific attractants (Figure 4a), play a role in the ultimate selection that thalamic axons make to target the appropriate cortical area (e.g. S1 versus S2 within the parietal domain).

(ii) Cortical ephrins, together with other presently unidentified cues and activity-dependent mechanisms, control the final intra-areal mapping of thalamic axons within their target cortical area (S1) (Figure 4b).

In the frame of this model, obvious future challenges in the field will be to identify (i) the transcription-dependent and transcription-independent mechanisms specifying

expression of the guidance cues present in the intermediate and terminal targets of thalamic axons, (ii) the mechanisms controlling the differential responsiveness of thalamocortical axons to these intermediate and terminal target-derived cues, (iii) the nature of the guidance cues, aside of ephrins, controlling thalamocortical patterning, and (iv) the timing and location of their cellular actions. This will enable us to determine the relative importance of each of the steps involved, and how they articulate with each other to generate the hierarchical complexity characterizing thalamocortical connectivity.

Acknowledgements

We apologize for not citing numerous studies because of space constraints and the focus of this review on recent literature. We thank members of our laboratories for helpful discussions. Work from our laboratories described in this review was funded by NIH-NINDS (F.P.), INSERM (F.P.), the Belgian FNRS (P.V.), FRSM (P.V.), Queen Elizabeth Medical Foundation (P.V.) and IUAP Federal Office for Scientific Affairs (P.V.), and an INSERM-CFB travel grant (F.P. and P.V.). P.V. is a Research Associate of the FNRS.

References

1 Jones, E.G. (1985) *The Thalamus*, Plenum Press
2 Lopez-Bendito, G. and Molnar, Z. (2003) Thalamocortical development: how are we going to get there? *Nat. Rev. Neurosci.* 4, 276–289
3 Sur, M. and Leamey, C.A. (2001) Development and plasticity of cortical areas and networks. *Nat. Rev. Neurosci.* 2, 251–262
4 Katz, L.C. and Crowley, J.C. (2002) Development of cortical circuits: lessons from ocular dominance columns. *Nat. Rev. Neurosci.* 3, 34–42
5 O'Leary, D.D. and Nakagawa, Y. (2002) Patterning centers, regulatory genes and extrinsic mechanisms controlling arealization of the neocortex. *Curr. Opin. Neurobiol.* 12, 14–25
6 Ghosh, A. *et al.* (1990) Requirement for subplate neurons in the formation of thalamocortical connections. *Nature* 347, 179–181
7 Ghosh, A. and Shatz, C.J. (1993) A role for subplate neurons in the patterning of connections from thalamus to neocortex. *Development* 117, 1031–1047
8 Grove, E.A. and Fukuchi-Shimogori, T. (2003) Generating the cerebral cortical area map. *Annu. Rev. Neurosci.* 26, 355–380
9 Caviness, V.S. Jr and Frost, D.O. (1980) Tangential organization of thalamic projections to the neocortex in the mouse. *J. Comp. Neurol.* 194, 335–367
10 Crandall, J.E. and Caviness, V.S. Jr (1984) Thalamocortical connections in newborn mice. *J. Comp. Neurol.* 228, 542–556
11 Hohl-Abrahao, J.C. and Creutzfeldt, O.D. (1991) Topographical mapping of the thalamocortical projections in rodents and comparison with that in primates. *Exp. Brain Res.* 87, 283–294
12 Jones, E.G. (2001) The thalamic matrix and thalamocortical synchrony. *Trends Neurosci.* 24, 595–601
13 Behrens, T.E. *et al.* (2003) Non-invasive mapping of connections between human thalamus and cortex using diffusion imaging. *Nat. Neurosci.* 6, 750–757
14 Bishop, K.M. *et al.* (2000) Regulation of area identity in the mammalian neocortex by Emx2 and Pax6. *Science* 288, 344–349
15 Mallamaci, A. *et al.* (2000) Area identity shifts in the early cerebral cortex of Emx2$^{-/-}$ mutant mice. *Nat. Neurosci.* 3, 679–686
16 Fukuchi-Shimogori, T. and Grove, E.A. (2001) Neocortex patterning by the secreted signaling molecule FGF8. *Science* 294, 1071–1074
17 Frappe, I. *et al.* (1999) Transplants of fetal frontal cortex grafted into the occipital cortex of newborn rats receive a substantial thalamic input from nuclei normally projecting to the frontal cortex. *Neuroscience* 89, 409–421
18 Frappe, I. *et al.* (2001) Attraction exerted *in vivo* by grafts of embryonic neocortex on developing thalamic axons. *Exp. Neurol.* 169, 264–275
19 Garel, S. *et al.* (2002) The early topography of thalamocortical projections is shifted in Ebf1 and Dlx1/2 mutant mice. *Development* 129, 5621–5634
20 Porteus, M.H. *et al.* (1994) DLX-2, MASH-1, and MAP-2 expression and bromodeoxyuridine incorporation define molecularly distinct cell populations in the embryonic mouse forebrain. *J. Neurosci.* 14, 6370–6383
21 Garel, S. *et al.* (1999) Ebf1 controls early cell differentiation in the embryonic striatum. *Development* 126, 5285–5294
22 Garel, S. *et al.* (2003) Molecular regionalization of the neocortex is disrupted in Fgf8 hypomorphic mutants. *Development* 130, 1903–1914
23 Pratt, T. *et al.* (2000) A role for pax6 in the normal development of dorsal thalamus and its cortical connections. *Development* 127, 5167–5178
24 Hevner, R.F. *et al.* (2002) Cortical and thalamic axon pathfinding defects in Tbr1, Gbx2, and Pax6 mutant mice: evidence that cortical and thalamic axons interact and guide each other. *J. Comp. Neurol.* 447, 8–17
25 Lopez-Bendito, G. *et al.* (2002) Role of Emx2 in the development of the reciprocal connectivity between cortex and thalamus. *J. Comp. Neurol.* 451, 153–169
26 Braisted, J.E. *et al.* (2000) Netrin-1 promotes thalamic axon growth and is required for proper development of the thalamocortical projection. *J. Neurosci.* 20, 5792–5801
27 Metin, C. and Godement, P. (1996) The ganglionic eminence may be an intermediate target for corticofugal and thalamocortical axons. *J. Neurosci.* 16, 3219–3235
28 Richards, L.J. *et al.* (1997) Directed growth of early cortical axons is influenced by a chemoattractant released from an intermediate target. *J. Neurosci.* 17, 2445–2458
29 Mitrofanis, J. and Guillery, R.W. (1993) New views of the thalamic reticular nucleus in the adult and the developing brain. *Trends Neurosci.* 16, 240–245
30 Molnar, Z. and Cordery, P. (1999) Connections between cells of the internal capsule, thalamus, and cerebral cortex in embryonic rat. *J. Comp. Neurol.* 413, 1–25
31 Seibt, J. *et al.* (2003) Neurogenin2 specifies the connectivity of thalamic neurons by controlling axon responsiveness to intermediate target cues. *Neuron* 39, 439–452
32 Nakagawa, Y. and O'Leary, D.D. (2001) Combinatorial expression patterns of LIM-homeodomain and other regulatory genes parcellate developing thalamus. *J. Neurosci.* 21, 2711–2725
33 Fode, C. *et al.* (2000) A role for neural determination genes in specifying the dorsoventral identity of telencephalic neurons. *Genes Dev.* 14, 67–80
34 Dufour, A. *et al.* (2003) Area specificity and topography of thalamocortical projections are controlled by ephrin/Eph genes. *Neuron* 39, 453–465
35 Gao, P.P. *et al.* (1998) Regulation of thalamic neurite outgrowth by the Eph ligand ephrin-A5: implications in the development of thalamocortical projections. *Proc. Natl. Acad. Sci. U. S. A.* 95, 5329–5334
36 Mackarehtschian, K. *et al.* (1999) Regional differences in the developing cerebral cortex revealed by ephrin-A5 expression. *Cereb. Cortex* 9, 601–610
37 Vanderhaeghen, P. *et al.* (2000) A mapping label required for normal scale of body representation in the cortex. *Nat. Neurosci.* 3, 358–365
38 Sestan, N. *et al.* (2001) Independent parcellation of the embryonic visual cortex and thalamus revealed by combinatorial Eph/ephrin gene expression. *Curr. Biol.* 11, 39–43
39 Uziel, D. *et al.* (2002) Miswiring of limbic thalamocortical projections in the absence of ephrin-A5. *J. Neurosci.* 22, 9352–9357
40 Katz, L.C. and Shatz, C.J. (1996) Synaptic activity and the construction of cortical circuits. *Science* 274, 1133–1138
41 Cline, H.T. (1998) Topographic maps: developing roles of synaptic plasticity. *Curr. Biol.* 8, R836–R839
42 Gaspar, P. *et al.* (2003) The developmental role of serotonin: news from mouse molecular genetics. *Nat. Rev. Neurosci.* 4, 1002–1012
43 Erzurumlu, R.S. and Kind, P.C. (2001) Neural activity: sculptor of 'barrels' in the neocortex. *Trends Neurosci.* 24, 589–595
44 Crair, M.C. (1999) Neuronal activity during development: permissive or instructive? *Curr. Opin. Neurobiol.* 9, 88–93
45 Crowley, J.C. and Katz, L.C. (2000) Early development of ocular dominance columns. *Science* 290, 1321–1324
46 Feldheim, D.A. *et al.* (1998) Topographic guidance labels in a sensory projection to the forebrain. *Neuron* 21, 1303–1313
47 O'Leary, D.D. and Wilkinson, D.G. (1999) Eph receptors and ephrins in neural development. *Curr. Opin. Neurobiol.* 9, 65–73
48 Brown, A. *et al.* (2000) Topographic mapping from the retina to the

midbrain is controlled by relative but not absolute levels of EphA receptor signaling. *Cell* 102, 77–88
49 Frisen, J. *et al.* (1998) Ephrin-A5 (AL-1/RAGS) is essential for proper retinal axon guidance and topographic mapping in the mammalian visual system. *Neuron* 20, 235–243
50 Wilkinson, D.G. (2001) Multiple roles of EPH receptors and ephrins in neural development. *Nat. Rev. Neurosci.* 2, 155–164
51 Flanagan, J.G. and Vanderhaeghen, P. (1998) The ephrins and Eph receptors in neural development. *Annu. Rev. Neurosci.* 21, 309–345
52 Prakash, N. *et al.* (2000) Malformation of the functional organization of somatosensory cortex in adult ephrin-A5 knock-out mice revealed by *in vivo* functional imaging. *J. Neurosci.* 20, 5841–5847
53 Mann, F. *et al.* (2002) Ephrins regulate the formation of terminal axonal arbors during the development of thalamocortical projections. *Development* 129, 3945–3955
54 Yates, P.A. *et al.* (2001) Topographic-specific axon branching controlled by ephrin-As is the critical event in retinotectal map development. *J. Neurosci.* 21, 8548–8563
55 Henderson, J.T. *et al.* (2001) The receptor tyrosine kinase EphB2 regulates NMDA-dependent synaptic function. *Neuron* 32, 1041–1056
56 Grunwald, I.C. *et al.* (2001) Kinase-independent requirement of EphB2 receptors in hippocampal synaptic plasticity. *Neuron* 32, 1027–1040
57 Catalano, S.M. and Shatz, C.J. (1998) Activity-dependent cortical target selection by thalamic axons. *Science* 281, 559–562