THALAMOCORTICAL DEVELOPMENT: HOW ARE WE GOING TO GET THERE?

Guillermina López-Bendito and Zoltán Molnár

The arealization of the mammalian cortex is believed to be controlled by a combination of intrinsic factors that are expressed in the cortex, and external signals, some of which are mediated through thalamic input. Recent studies on transgenic mice have identified families of molecules that are involved in thalamic axon growth, pathfinding and cortical target selection, and we are beginning to understand how thalamic projections impose cytoarchitectonic differentiation on the developing cortex. By unravelling these mechanisms further, we should be able to increase our understanding of the principles of cortical organization.

EPIGENETIC
Describes a change in phenotype that is brought about by changes in the regulation of gene expression or changes in the function of gene products, rather than by a change in genotype.

VENTRICULAR ZONE
The proliferative inner layer of the developing brain and spinal cord.

SUBVENTRICULAR ZONE
A layer of cells in the developing brain that is generated by the migration of neuroblasts from the adjoining ventricular zone.

Department of Human Anatomy and Genetics, University of Oxford, South Parks Road, Oxford OX1 3QX, UK. Correspondence to Z.M. e-mail: zoltan.molnar@anat.ox.ac.uk
doi:10.1038/nrn1075

The mechanisms that control neocortical regionalization — or arealization — have been the subject of much debate. Intrinsic mechanisms, such as differential gene expression that is autonomous to the neocortex, and extrinsic influences, such as input from thalamocortical afferents, have both gained support from recent studies[1]. Although some components of local specialization are determined by differential patterns of commitment at an early stage of development (perhaps even during mitosis), there is considerable evidence that the developing cortex is partly multipotential, and that some localized extrinsic signal is needed as an EPIGENETIC cue to control the ultimate differentiation of each area[2,3]. The obvious candidate for this extrinsic signal, at least for primary sensory areas, is the input from the appropriate thalamic nucleus.

Sense organs (except for the olfactory organs) and the subcortical motor centres provide input to one or more thalamic nuclei, and these nuclei have well-defined reciprocal connections with the cortical regions through which they process sensory information (FIG. 1a,b). The reciprocal connections have area and lamina specificity, they are remarkably similar for all cortical areas, and are highly conserved between species. Most of the thalamic input terminates in layer IV of the neocortex, although there are some terminations in layers I, II/III and VI. Layer VI neurons of each area send corticofugal projections back to the corresponding thalamic nucleus, and layer V sends projections to additional nuclei[4] (FIG. 1c).

This article provides an overview of the exciting recent progress that has been made in the field of thalamocortical development. The transgenic mouse has proved to be a very powerful tool to increase our understanding of the principal developmental mechanisms, and it has helped us to dissect the causal relationships between thalamocortical fibre targeting and areal specialization in the cortex. Through the analysis of these mouse mutants, we are beginning to identify the importance of various forms of communication between the cortex and thalamus, and the roles that these interactions have during embryonic and early postnatal development.

Early organization of the cortex and thalamus

Formation of the cortical layers. The largest region of the cerebral cortex, the six-layered neocortex, is the part of the mammalian brain that has shown the most extensive expansion and specialization during evolution[5,6]. Most neocortical neurons, including the projection neurons, are generated within the VENTRICULAR and SUBVENTRICULAR ZONES (VZ/SVZ) of the lateral ventricle. The first postmitotic neurons accumulate below the pial surface, forming a new layer called the preplate.

© 2003 Nature Publishing Group

Neurons that are subsequently generated in the VZ/SVZ migrate along radial glial processes to form the cortical plate, which splits the preplate into a superficial marginal zone (MZ) and a deep subplate. The cortical plate gradually differentiates in a deep to superficial, or 'inside-out', pattern, forming layers VI to II of the adult neocortex[7]. The cortex also becomes patterned along anteroposterior and mediolateral axes[7].

A largely transient population of neurons that is located in the subplate has been proposed to have a crucial role in cortical development[8]. These neurons send local and long-distance projections, pioneering the INTERNAL CAPSULE (IC) and the main input and output projection paths between the cortex and the rest of the central nervous system (CNS)[8–10].

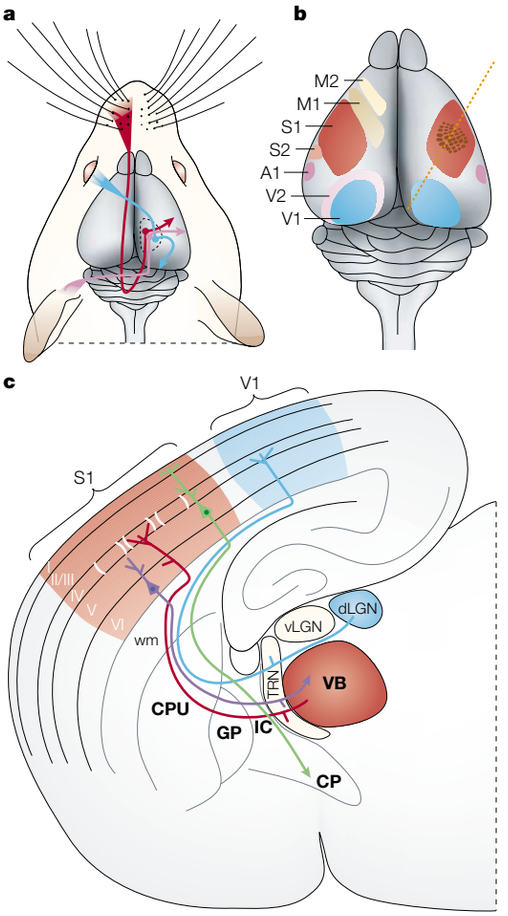


Figure 1 | **Sensory modalities reach the cerebral cortex through different thalamic nuclei. a** | Visual input from the retina (blue line) is relayed through the dorsal lateral geniculate nucleus (dLGN) to reach the visual cortex (V1, blue area in **b**). Somatosensory information from the whiskers (red line) reaches the ventrobasal complex (VB) through the brainstem, and is relayed to the barrel field of the primary somatosensory cortex (S1, red area in **b**). Acoustic information (purple line) arrives at the medial geniculate nucleus through numerous relays, and is then relayed to the primary auditory cortex (A1, purple area in **b**). **b** | The sensory and motor cortical areas of the mouse brain. Orange dashed line indicates plane of section in part **c**. **c** | Forebrain section, showing S1 and VB and the pathways that link them. The red line indicates the thalamocortical fibre running from VB to layer IV of S1 (red shading). The blue line indicates thalamocortical projections to the anterior segment of V1 (blue shading). Neurons in layer VI of the same area project to VB. Layer V extends projections to the cerebral peduncle (CP). White brackets in layer IV represent septa of barrels. CPU, caudate putamen; GP, globus pallidus; IC, internal capsule; M1, primary motor area; M2, secondary motor area; S2, secondary somatosensory cortex; TRN, thalamic reticular nucleus; vLGN, ventral lateral geniculate nucleus; WM, white matter.

Formation and differentiation of thalamic nuclei. The thalamus and cortex develop synchronously. Most of the thalamic neurons in the rat are born between embryonic day (E) 13 and E19 (REF. 11), which coincides with the period of neuron generation in the cortex. The lateral geniculate nucleus (LGN) is generated over two days between E12 and E14 (REF. 12), and the thalamus and hypothalamus can be distinguished after E12. On E13, a wedge-shaped enlargement appears in the diencephalic wall, with further furrows defining the developing dorsal and ventral thalamus. At around E16/E17, nuclear differentiation begins in the thalamus, starting with the epithalamus and ventral thalamus, and later spreading to the dorsal thalamus[4]. The thalamic reticular nucleus is considerably larger during development, and a substantial proportion of its cells disappear by adulthood[13]. Only dorsal thalamic neurons send projections to the cortex; the epithalamus and ventral thalamus do not[4]. In addition to the cerebral cortical projections, distinct dorsal thalamic regions send axons to the STRIATUM, the OLFACTORY TUBERCLE, parts of the AMYGDALA, the piriform cortex and the hippocampal formation[4]. It has been suggested that the different subsets of thalamic projections find their targets by recognizing specific signals in these regions, and on reaching the appropriate target, they establish topographically ordered representations[8,14].

Thalamocortical and corticothalamic projections

In rodents, the forebrain undergoes extensive changes during the second and third week of gestation. It differentiates into distinct domains — prosomeres — each with specific morphological features and gene expression patterns[15,16]. Around the same time, the neocortex and dorsal thalamus start to link with each other through reciprocal connections — in mice this occurs between E13 and E18. Thalamocortical and corticothalamic projections have to cross several emerging boundary zones to reach their ultimate target cells (FIG. 2). These include the diencephalic–telencephalic and the pallial–subpallial boundaries (DTB and PSPB, respectively), which are demarcated by distinct molecular properties[17] (FIG. 2). For example, the PSPB has been characterized by the absence of expression of both *Emx1* and *Dlx1* (REF. 17), and the presence of *Pax6* (REF. 18). This zone is believed to have an important role during development and evolution, acting as a transient barrier zone for fibres and a corridor for migrating neurons[19].

It has been shown that fibre trajectories and fasciculation patterns change as thalamocortical and corticofugal axons cross these sharp gene expression boundaries[20]. For example, both thalamocortical and corticofugal

INTERNAL CAPSULE
A large bundle of axons that reciprocally connects the cortex with the subcortical structures of the brain.

STRIATUM
Part of the subpallium and one of the components of the striatopallidal complex. It comprises deep (caudate nucleus, putamen and nucleus accumbens) and superficial (olfactory tubercle) parts.

OLFACTORY TUBERCLE
A structure in the base of the telencephalon that acts as a relay centre for olfactory information. It was initially classed as a component of the olfactory (or piriform) cortex, but its cellular architecture more closely resembles that of the striatum, with which it shares a common developmental origin in the lateral ganglionic eminence. It is particularly prominent in species that rely heavily on their sense of smell, such as rodents.

AMYGDALA
A small almond-shaped structure, comprising 13 nuclei, buried in the anterior medial section of each temporal lobe.

© 2003 Nature Publishing Group

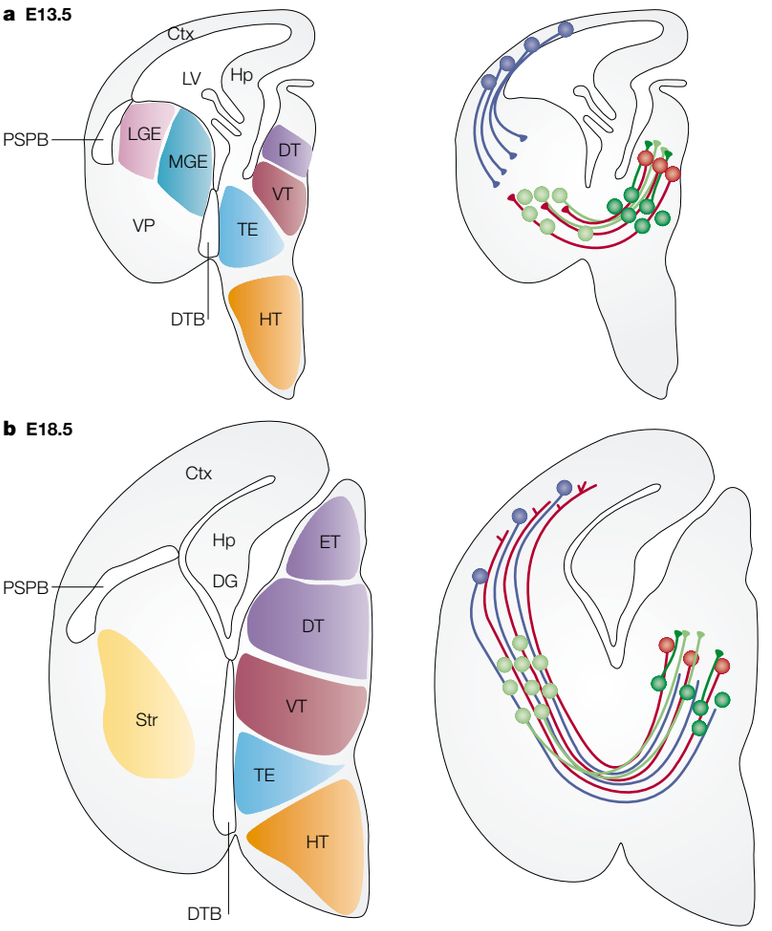


Figure 2 | **Thalamocortical axon growth through the developing mouse forebrain.** During embryonic development, thalamic axons travel long distances to reach their target cortical areas. **a** | At embryonic day (E) 13.5, thalamic axons (red lines) leave the dorsal thalamus (DT) descend among ventral thalamic cells (dark green) and cross the boundary between the diencephalon and the telencephalon (DTB). Then, they extend through the medial and lateral ganglionic eminences (MGE and LGE, respectively). Cells located at the internal capsule and thalamic reticular nucleus (light and dark green circles, respectively), and their projections (green lines), seem to have a close relation with thalamic projections *en route* to the cortex (Ctx). At the same time, corticofugal axons (blue lines) leave the cortex and transiently pause at the pallial–subpallial boundary (PSPB). **b** | At E18.5, both sets of projections are approaching their targets. Thalamic axons are located mainly at the intermediate zone and subplate of the cortex, and some of their side branches have started to invade the deep cortical plate. Corticofugal axons arrive at the dorsal thalamus in a topographically organized manner. DG, dentate gyrus; ET, epithalamus; Hp, hippocampus; HT, hypothalamus; LV, lateral ventricle; Str, striatum; TE, thalamic eminence; VP, ventral pallium; VT, ventral thalamus.

projections show puzzling behaviour at the DTB and PSPB. The developing thalamocortical axons first proceed ventrally from the dorsal thalamus and then turn dorsolaterally at the DTB, where they enter the IC by E13 in the mouse (FIG. 2a). They advance rapidly to a largely transient population of cells in this region[21], where they pause before traversing the PSPB at E15. Projections that originate from the preplate in the neocortex also pause at the PSPB at around E14 (REF. 22). Although projections from different cortical regions arrive at this zone asynchronously according to the cortical developmental gradient[7], the front of the early corticofugal projection lines up along the PSPB[22]. After their interaction at this boundary, thalamocortical and corticofugal fibres resume their advance, intimately associated with each other, and proceed towards their targets[20] (FIG. 2b). In contrast to these well-defined boundaries, the exact nature of forebrain prosomeres remains controversial, so it is not yet clear how closely thalamocortical and corticofugal axons respect their boundaries.

Growth of thalamocortical axons

Guidance molecules in the thalamocortical pathway. The early steps of thalamocortical fibre deployment depend on various factors. The indication that a patterned cortex might direct the ingrowth of thalamic afferents came from observations that certain macromolecules that are generated in this region seem to participate directly in setting up the correct thalamocortical connectivity[23–26]. Distinct gene expression patterns show regions of the developing brain that express a diverse set of diffusible or membrane bound molecules, which guide the establishment of early connectivity. Some of these molecules have several functions at different developmental stages (FIG. 3).

The release of attractive and repulsive factors, and axon guidance molecules, from the cortex is believed to have an important role in channelling the growing projections though the forebrain, and also in the focusing of specific sets of axons to particular cortical areas[3]. Limbic-associated membrane proteins (LAMP) (FIG. 3), the ORPHAN NUCLEAR RECEPTOR Coup-tfi, Cadherin 6 (Cdh6), Cdh8 and Cdh11, and the ephrins and Eph receptors are expressed in distinct regions of the cerebral cortex, and could attract or repel specific sets of thalamocortical projections. A recent study, in which neurotrophin 3 (NT3) was specifically deleted in the neocortex, provided evidence for a target-derived function of neurotrophins in the establishment of synaptic interactions between thalamic axons and cortical neurons[27]. Additionally, the p75 neurotrophin receptor is expressed in a low-rostral to high-caudal gradient within the subplate during the period of thalamocortical targeting. In p75-knockout mice, the thalamic input to the visual cortex is reduced, implying a role for neurotrophins and their receptors in the area-specific targeting of thalamic axons[28].

One molecule that has been implicated in thalamocortical pathfinding is netrin 1 (REF. 29) (FIG. 3a). It is expressed in the ventral telencephalon when thalamocortical axons are navigating through this region[30], and *in vitro* it has been shown to act as a chemoattractant for cortical axons[30]. The existence of a ventral telencephalic chemoattractant activity for dorsal thalamic axons has recently been demonstrated using *in vitro* axon guidance assays[31,32]. It has also been suggested that the hypothalamic area releases a chemorepellent signal for thalamic axons[31], but this signal has not yet been identified. Molecules such as Slits have been suggested to play a prominent part in the development of thalamocortical connections, by preventing them from invading the hypothalamus and from crossing the midline[33].

Recent studies have shown that netrin 1 is expressed by cells in the IC region, where it acts as an attractant for thalamocortical axons that travel through this ventral

ORPHAN NUCLEAR RECEPTOR
A receptor for which a ligand has not been identified.

© 2003 Nature Publishing Group

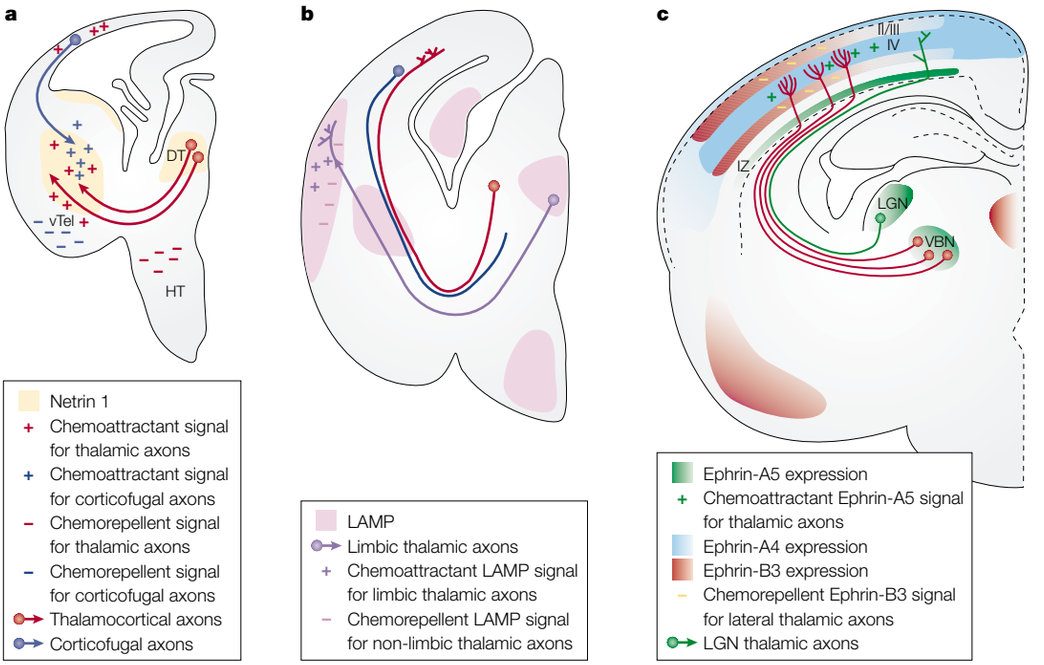


Figure 3 | **Signals, receptors and guidance molecules postulated in the development of area-specific thalamocortical connectivity.** The deployment of thalamic fibres through different telencephalic and diencephalic regions requires a meticulous organization of attractive and repulsive signals and other guidance molecules. Certain molecules have been proposed to act as chemoattractant or chemorepellent signals to modulate the paths of thalamocortical (red lines) and corticofugal (blue lines) projections. **a** | Netrin 1 is a strong candidate for an early attractive signal in the internal capsule. It is expressed early in the ventral telencephalon (vTel) and dorsal thalamus (DT), when thalamocortical axons are navigating through these regions. It has also been shown *in vitro* to act as a chemoattractant signal for early cortical axons (blue +). The existence of a ventral telencephalic and a cortical chemoattractant activity (red +) for dorsal thalamic axons has been demonstrated. Moreover, a chemorepellent signal for thalamic axons (red –) by the hypothalamic area (HT) has been also indicated. **b** | The limbic system-associated membrane protein (LAMP) might also be involved in the regional specification of a subset of thalamocortical projections. LAMP is selectively expressed in the perirhinal and frontal limbic cortex and medial limbic thalamic nuclei (purple shadows) at early developmental stages. In the limbic cortex, LAMP attracts limbic thalamic axons (purple +) and also serves as a repulsive cue to prevent non-limbic thalamic axons from innervating inappropriate cortical regions (pink –). **c** | Ephrin-A5, Ephrin-A4 and Ephrin-B3 are thought to play a later part in generating the correct thalamic invasion and arborization pattern in the appropriate cortical layer. Ephrin-A5 is expressed in the somatosensory cortex, with stronger expression in layers IV and VI (blue area). Ephrin-A4 is enriched (green area) in the lateral geniculate nucleus (LGN), in the ventrobasal complex of the thalamus (VB) and in the intermediate zone (IZ) of the cortex. Ephrin-B3 (red area) is highly expressed in the embryonic ventral telencephalon at late embryonic ages (embryonic day 17). In the neonate, it is expressed in the amygdala, piriform area and entorhinal cortex.

telencephalic domain *en route* to the cortex[30] (FIG. 3a). In *netrin 1*-deficient mice, however, thalamocortical axons initially project along their normal path and can reach the dorsal cortex, implying that netrin 1 is not required by the thalamocortical axons to cross the DTB to reach the IC[32]. However, the pathway of thalamic axons through the IC region is disorganized in the *netrin 1* mutant mice and fewer thalamic axons reach the cortex[32]. Taken together, these observations indicate that netrin 1 is a chemoattractant molecule that is required for the development of normal thalamocortical projections, but it might act in combination with other guidance molecules to control thalamocortical pathfinding.

Recent studies have also provided evidence that ephrins and their receptors have a key role in guiding thalamocortical axons to their appropriate target cells in the developing cortex (FIG. 3c). In mice that lack the *ephrin-A5* gene, the relative scale of the different regions within the body representation was altered in the primary somatosensory area[34]. Ephrin-A5 has also been implicated in the patterning of thalamocortical connections between areas of the neocortex and the limbic cortex[26,35]. In addition, blocking endogenous ephrin-A ligands abolishes the preferential branching of thalamic projections on substrates from their target layer, and ephrin-A5, which is expressed in layer IV of the somatosensory cortex, induces collateral formation of thalamic axons[36]. This indicates that ephrin-As are instrumental not only for areal targeting, but also for the formation of terminal arborizations of thalamic axons in their target cortical layer. Recent *in vivo* evidence has implicated ephrin-A5 in the patterning of thalamocortical connections between areas of the neocortex and limbic cortex[37].

Further support for a role for ephrins and their receptors in the formation of region-specific thalamocortical connections has come from a recent *in vitro* study, using membranes containing ephrin-B3, which is highly expressed in the limbic cortex[38]. Membranes prepared

© 2003 Nature Publishing Group

from this region specifically inhibited axonal growth in thalamic neurons that expressed EphA4, which are normally targeted to the dorsal cortex (FIG. 3b,c).

Another important set of guidance signals in the forebrain is the semaphorins — a large family of transmembrane and secreted proteins[39–41]. They have been shown to selectively inhibit the formation of terminal arborizations, to prevent branching and maintain fasciculation[42], and to influence pathway formation[43]. Previous work also provided evidence that semaphorins can act as both attractive and repulsive guidance cues during the initial patterning of corticofugal projections. A repellent axonal guidance molecule, semaphorin 3A (Sema3a), is expressed in the IC when projections between the thalamus and cortex are being established[44]. Time-lapse imaging studies have shown that growth cones can distinguish between specific cues expressed on axonal surfaces; the addition of Sema3a to the substrate increases the rate of HOMOTYPIC fasciculation and enhances HETEROTYPIC growth cone retractions of thalamic axons[44].

Recent tracing studies have shown interesting defects in thalamocortical development in *Sema6a* mutant mice[45]. Some thalamocortical axons fail to turn dorsally after crossing the DTB through the IC, and instead project aberrantly to the amygdaloid region[45]. *Sema6a* expression has been described in thalamocortical neurons and in the IC[46] at the time when thalamic axons grow through different regions and boundaries[45]. This might indicate that the guidance defects that are observed in *Sema6a*-knockout mice could be partially cell-autonomous, but further investigations in this mutant mouse are required to definitively answer this question.

Forebrain patterning and thalamocortical development. Recent studies have started to define candidate molecules that control the expression patterns of regulatory genes and transcription factors in the forebrain. These in turn control the expression of membrane-bound or soluble guidance molecules, which modulate the pathfinding of thalamocortical axons. Signalling molecules, such as fibroblast growth factor 8 (Fgf8), Sonic hedgehog (Shh), bone morphogenetic proteins (BMPs) 2, 4, 6 and 7, and Wnts 2b, 3a, 5a and 7a, control the regional expression of specific transcription factors in well-defined signalling centres[47–49]. Graded or restricted expression of different genes that code for transcription factors, nuclear receptors, cell adhesion molecules, axon guidance receptors and ligands has been described in defined areas of the embryonic forebrain[15,50–52]. For example, numerous transcription factors, including *Pax6, Mash1, Tbr1, Foxg1, Ngn2, Ebf1, Emx2, Dlx2* and *Otx2*, are expressed in distinct patterns in the ventral PALLIUM, whereas *Gbx2* and *Emx1* transcripts are absent from this region. These expression patterns probably modulate molecular patterning at the PSPB, which in turn influences axonal pathfinding.

Recently, abnormalities of thalamocortical development have been described in several mutants lacking transcription factors that are expressed along their route of navigation (FIG. 4). The most severe phenotypes were observed in *Gbx2*, *Mash1*, *Pax6*$^{Sey/Sey}$ and *Pax6/LacZ*-knockout mice, where thalamic axons failed to innervate the cortex[53–56] (FIG. 4). Moreover, in *Tbr1*, *Emx2*, *Pax6*$^{Sey/Sey}$, *Pax6/LacZ* and *Gbx2* mutants, pathfinding errors also affect the reciprocal corticofugal projections. In *Tbr1* and *Gbx2* mutants, the errors occur first in the subpallium during the formation of cortical projections within the IC (FIG. 4). These findings indicate that cortical and thalamic projections become dependent on each other for axon guidance in the IC, having grown into the subpallium independently by other mechanisms. The guidance cues in the internal capsule have not yet been identified.

In *Gbx2* and *Mash1*-knockout mice, the embryonic regionalization of the neocortex remains normal in the absence of thalamic innervation, as shown by markers that reveal regional boundaries, including *Cad6, Cad8, Cad11, Emx1, Lhx2* and *Id2* (REFS 50,51). In *Emx2*-knockout mice, a shift in thalamic targeting is matched with an altered cortical gene expression pattern, but in *Pax6* mutants, in which thalamic projections do not reach the cortex, an opposite shift of cortical arealization has been observed[57–59]. These results indicate that the shift in cortical arealization might be independent of the early alteration of thalamic targeting, and that it is driven by the altered *Emx2* and *Pax6* expression in the cortex.

Recent analysis of *Dlx1/2* and *Ebf1*-knockout mice has implied that the early topography of thalamocortical axons is not governed by information within the neocortex and dorsal thalamus. In *Ebf1* mutant mice, in which the early cortical and thalamic gene expression pattern seems to be normal, thalamocortical axons shift towards more caudal neocortical domains, and projections from the dorsal part of the lateral geniculate nucleus (dLGN, or the visual thalamic nucleus) do not reach the OCCIPITAL CORTEX[46]. Similar results are found in the *Dlx1/2* double knockout mice, in which the majority of dLGN axons fail to reach the neocortex, and a shifted topography of thalamic projections is found in the IC and neocortex[46]. These experiments indicate that the relative positions of thalamic axons that traverse the basal ganglia and ventral telencephalon might have an important role in organizing the cortical targeting of these projections[46]. The fact that there is no shift in embryonic cortical gene expression pattern in *Ebf1* and *Dlx1/2* mutants implies that thalamocortical axon targeting is not strictly governed by cortical signals, and supports the idea that early thalamic axons are not responsible for setting up these early cortical gene expression gradients.

The previously mentioned studies (FIG. 4) showed that the development of thalamocortical interconnections requires the correct expression of a specific set of transcription factors along their pathway, especially at the DTB and PSPB. However, caution is needed when interpreting the results in these mutants, as defects in thalamocortical pathfinding might not be due to defects in the path along which the axons grow, but rather to defects in the thalamic cells themselves[60]. For example, the axons might lack the ability to respond to perfectly normal cues along the pathway, and this could explain why pathfinding defects are sometimes seen at target

HOMOTYPIC
A term that refers to interactions between cells or molecules of the same type.

HETEROTYPIC
A term that refers to interactions between cells or molecules of different types.

PALLIUM
The roof of the telencephalon. It contains both cortical structures (for example, hippocampus and neocortex) and deep-lying nuclear structures (for example, claustrum and parts of the amygdala). Pallium is not synonymous with cortex.

OCCIPITAL CORTEX
The most caudal of the four main subdivisions of the cerebral cortex.

© 2003 Nature Publishing Group

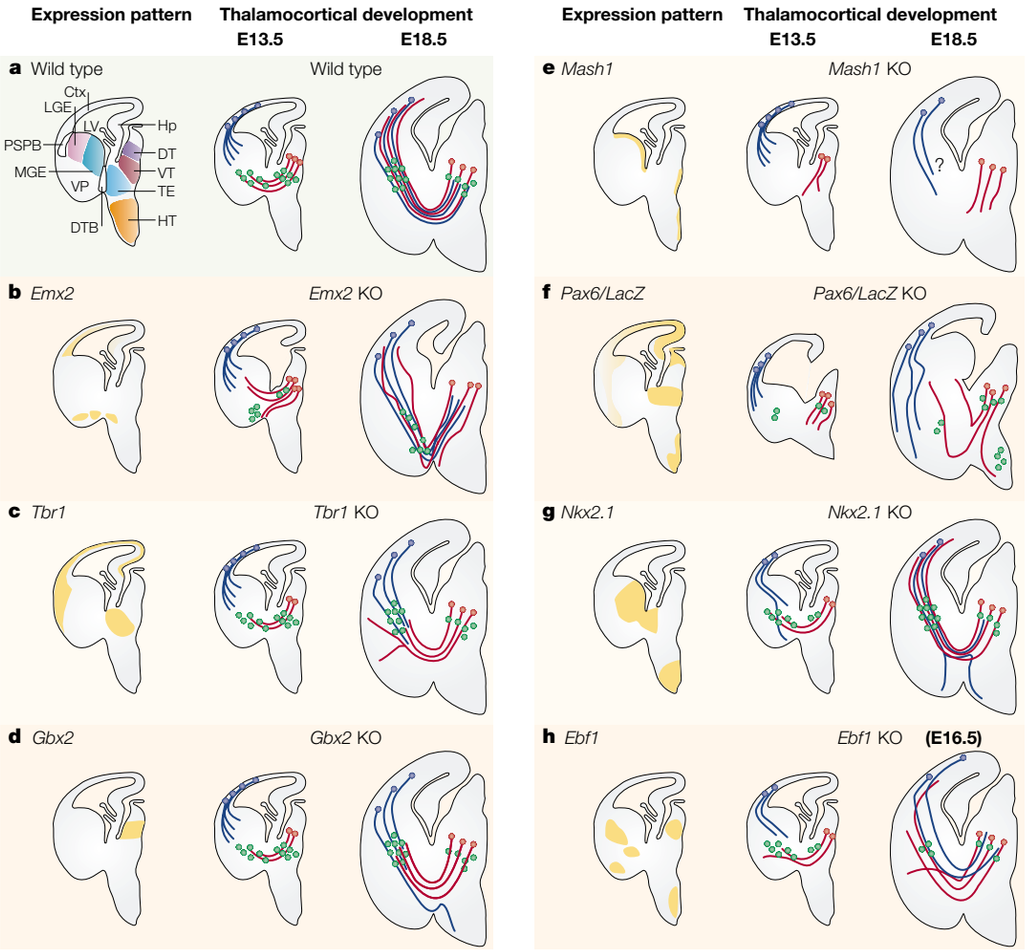


Figure 4 | **Abnormalities in thalamic and corticofugal development in transcription factor gene mutants.** Several transcription factors are expressed in the embryonic forebrain (**b**–**h**) along the routes of thalamocortical (red lines) and corticofugal (blue lines) fibres (**a**). **b** | *Emx2* is expressed in the dorsal cortex in a low-rostral to high-caudal and a low-lateral to high-medial gradient, and also in the ventral telencephalon. Loss of this homeobox gene in knockout (KO) mice causes both corticofugal and thalamocortical axons to follow an aberrant route through the ventral telencephalon (VT). This altered development occurs in conjunction with a displacement of internal capsule cells (green circles) and their early dorsal thalamic projections. **c** | Loss of *Tbr1*, which is expressed in the dorsal pallium and the thalamic eminence (TE), produces errors in the targeting of both thalamic and corticofugal axons. Some thalamic fibres deviate from their normal route to the cortex (Ctx) and invade the amygdaloid region. Loss of either *Gbx2* (**d**), *Mash1* (**e**) or *Pax6* (**f**) leads to a complete loss of thalamic innervation. Defects in corticofugal pathfinding also occur in *Pax6* and *Gbx2* KO mice, but normal corticofugal development was described in *Mash1* KO mice. **g** | Thalamocortical axons seem to develop normally in *Nkx2.1* mutant mice, but corticofugal projections from layer V neurons develop abnormally and extend to the ventral telencephalon and hypothalamus (HT). Projections from layer VI neurons to the dorsal thalamus (DT) develop normally. **h** | Dorsal lateral geniculate nucleus (dLGN) axons are misrouted in the amygdala of *Ebf1*$^{-/-}$ embryos. *Ebf1* inactivation induces a shift in thalamocortical and corticothalamic connections. DTB, diencephalic–telencephalic boundary; LGE, lateral ganglionic eminence; LV, Lateral ventricle; MGE, medial ganglionic eminence; PSPB, pallial–subpallial boundary; VP, ventral pallium.

PERIRHINAL CORTEX
One of the subdivisions of the medial temporal lobe. It is involved in learning and memory, and is believed to be particularly important for object memory.

GANGLIONIC EMINENCE
The proliferative zone of the ventral telencephalon, which gives rise to the basal ganglia, and also generates some cortical neurons and glia. It consists of lateral, caudal and medial subdivisions.

sites where the mutated genes are not even expressed[54]. Therefore, it is difficult to conclude whether the primary cause of axonal growth defects resides within or outside a particular forebrain structure.

Interactions with other cells and fibres. The earliest generated cells of the mammalian forebrain are in the preplate, PERIRHINAL CORTEX, thalamic reticular nucleus and the ventral part of the GANGLIONIC EMINENCE[7–9]. These are also among the first cells to develop connections in the mammalian forebrain[10,61–64]. As these cells and their connections are situated along the future path of thalamocortical connectivity, it has been suggested that they guide thalamic axons along their trajectory to their cortical target. Cells with a similar function have been described in various species, including hamster[21], rat[64] and human[65].

It has also been suggested that the early outgrowth of thalamocortical axons from the diencephalon might be governed by pioneering projections from cell groups that are located in the ventral telencephalon and ventral thalamus[20,21]. Several studies have implicated the projections

© 2003 Nature Publishing Group

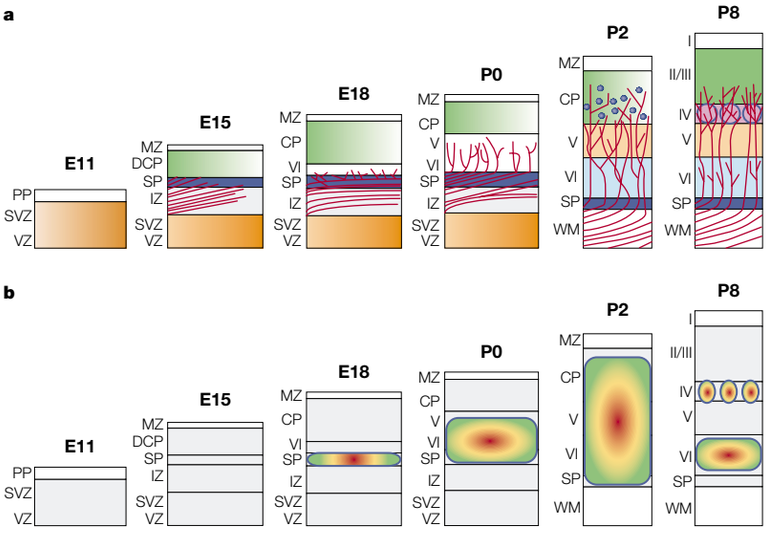


Figure 5 | **First contact with the cortex. a** | Maturation of cortical lamination and ingrowth of thalamic fibres summarized on schematic coronal sections of mouse cortex. At embryonic day (E) 11, the first postmitotic cells migrate to the outer edge of the cerebral wall to form the preplate (PP), which is subsequently split into the marginal zone (MZ, the future layer I) and the subplate (SP) by the arrival of neurons in the true cortical plate (CP). When thalamic axons (red lines) arrive at the cortex at E15, only a densely packed cortical plate is present. The axons start to accumulate in SP, although some axons and side branches penetrate the deep part of the cortical plate (DCP). During the early postnatal period (P0), most thalamic fibres invade the CP and layers V and VI. Thalamic axons assume their characteristic periphery-related pattern and impose a barrel arrangement on cortical layer IV neurons. **b** | Optical recording with voltage-sensitive dyes revealed that thalamocortical projections can conduct action potentials at E15, and also that direct thalamic stimulation begins to elicit sustained depolarization in the internal capsule and SP at E18–E19. This depolarization spreads to almost the entire thickness of the cortex shortly after birth. By the end of the first postnatal week (P8), the characteristic periphery-related clustering of activation appears in layer IV. IZ, intermediate zone; SVZ, subventricular zone; VZ, ventricular zone; WM, white matter. Adapted, with permission, from REF. 70 © (2000) Graham Publishing.

from IC cells in the guidance of thalamic axons through different boundaries and telencephalic subdivisions[14]. The strongest evidence for a role for IC cells and their projections in guiding thalamocortical axons has come from mutant mice. In *Mash1*-knockout mice, the IC cells with thalamic projections are missing, and thalamocortical axons fail to enter this region[53] (FIG. 4e). In *Emx2* and *Pax6* homozygous mice, the early IC projections take an aberrant ventral route at the DTB, and some of the thalamocortical axons follow them as they traverse that region[56,59].

Interactions between thalamic and cortical axons. The 'handshake hypothesis' postulates that projections from the thalamus and the early-born cortical preplate cells meet and intermingle in the basal telencephalon, such that thalamic axons grow over a scaffold of preplate axons[14]. Although their contact is now well documented[20,54], it is still not known whether both sets of fibres depend on each other to advance, extend and develop normally towards their targets, or whether their paths and destinations are autonomously controlled at the cortex and thalamus. Recent results in *Ebf1* and *Dlx1/2* mutant mice, in which a shift in thalamocortical and corticofugal connectivity has been described[46], strongly argue for a guiding role for the IC. It has been suggested that in these mutants, thalamic projections might change their relative position with their corticofugal counterparts, and that this, in turn, causes a shift in the cortical targeting of thalamic axons[46].

One important stipulation of the handshake hypothesis is that the development of thalamocortical and corticofugal connections and forebrain regionalization has to be synchronized. Errors in corticothalamic and thalamocortical pathfinding within the region of the IC were described in mice with mutations in transcription factor genes that are expressed in the cortex (*Tbr1*), thalamus (*Gbx2*) or in both (*Pax6*)[18,54,56]. In these mutants, both thalamic and corticofugal connections are aberrant and do not arrive at their final targets. This supports the idea that it is necessary for thalamic axons to form an intimate relationship with the scaffold of preplate axons.

Further support for this model has come from analysis of the *Coup-tfi* mutant, in which subplate defects impair the capacity of thalamocortical axons to reach the neocortex[66]. In *Coup-tfi* mutant mice, few thalamocortical axons are able to grow out of the IC, project into the intermediate zone and innervate the cortex[66]. This abnormal thalamocortical development might be explained by altered expression of guidance molecules and the premature death of subplate cells after E16.5 (REF. 66). Although these recent results are indicative, it has still not been unequivocally demonstrated that selective fasciculation of the two fibre sets is essential for their mutual guidance. In *Coup-tfi* mutants, the subplate scaffold is initially present, but this is not sufficient to guide thalamocortical axons to their target[44,66]. More direct evidence for the handshake hypothesis could be obtained from experiments where subplate projections are selectively eliminated before E14 in mice — before the arrival of thalamocortical axons in the IC.

Recently, an interesting idea has come from studies in *Nkx2.1* mutant mice. In these mice, thalamocortical axons seem to develop normally, but corticofugal projections from layer V extend aberrant connections at the ventral telencephalon and hypothalamus[67]. This implies that corticofugal projections from layer V might require different guidance mechanisms for their subcortical targeting than layer VI or subplate projections. So, it is possible that only the guidance of layer VI and subplate projections relies on an intact thalamocortical projection. The two sets of corticofugal projections could have a different affinity for the thalamic reticular nucleus, which might be involved in the sorting of these projections[13]. It is not clear whether corticofugal projections from the subplate and/or layer VI accumulate here temporarily before advancing to the core of the dorsal thalamus[22].

First communication with the cortex

In mammals, thalamic fibres arrive at the appropriate cortical regions before their ultimate target neurons are born[12,68,69], and they have to wait for two or three days (E16–E19 in rodents) before they can continue their growth and establish their final innervation pattern

© 2003 Nature Publishing Group

within the cortical plate (FIG. 5a). The early deployment of thalamocortical connectivity is established in an autonomous fashion before the afferents from the sensory periphery reach the dorsal thalamus. However, the sensory periphery could start to modify this juvenile topography after the initial targeting, during the process of thalamic fibre ingrowth and arborization[70]. It has been proposed that while the thalamic axons accumulate in the subplate, they engage in activity-dependent interactions with these cells, and this might lead to their realignment before they enter the cortex[71,72].

Thalamic axons are known to develop numerous transient side-branches as they accumulate in the subplate[73–75]. This side-branch formation might be regulated by electric fields that are generated by activity along the axons. Interestingly, when tetrodotoxin (a sodium channel antagonist that blocks action potentials) was delivered into the brain of cat fetuses at the same time as the arrival of thalamic projections at the subplate, abnormal connections were established by the LGN axons[72]. Only a few thalamic fibres entered the visual cortex, and an aberrant topography was formed within the cortical plate[72]. Although these findings indicate that even the initial phases of thalamocortical targeting might depend on early activation patterns, the exact nature of the required neural activity is not known.

It has also been shown that early thalamic projections can elicit sustained depolarization patterns in the subplate at the time of side-branch formation[76,77] (FIG. 5b). This early interaction is different from mature postnatal activation, being relatively small but much longer lasting.

Shortly before birth, most of the thalamic axons start to detach from the subplate and grow into the cortical plate, forming branches and synapses in the appropriate layer (FIG. 5a). It has been suggested that a 'stop' and/or a 'branch' signal in the cortex plays a part in the specific targeting of thalamic axons to their appropriate layer[14]. *In vitro* studies indicate that LGN axons recognize their target cell layers in the cortex. In organotypic co-cultures, composed of LGN and visual cortex explants, thalamocortical connections form with essentially the same laminar specificity as they do *in vivo*[78–81]. The existence of the stop signal has been shown more directly using time-lapse imaging in LGN axon co-cultures[82]. Most thalamic axons form branches in layer IV, regardless of their direction of entry into the cortical explants, indicating that the stop behaviour might be produced by a relative difference in molecular signals between layer IV and the adjacent layers, rather than being an intrinsic property of layer IV itself.

There are several possible molecular mechanisms that could underlie the axonal stop in layer IV. Contributing factors might include a decrease in growth-promoting activity in layer IV, or the detection of relative changes in the local concentrations of some molecules by LGN axons. Evidence for these hypotheses comes from experiments using chemically fixed cortical slices in combination with enzymatic perturbation[83]. More thalamic axons grow in deep layers (V and VI) than in superficial layers (II–IV). The inhibition of growth is markedly reduced by a pretreatment with phosphatidylinositol-specific phospholipase C (PI-PLC). Therefore, an inhibitory factor or factors might contribute to the axonal termination by decreasing the growth rate of thalamic axons that reach layer IV (REF. 84).

Several cell surface and extracellular matrix molecules, including cadherins[25,85], semaphorins[40,41,86], Eph receptors and ephrin ligands[26,35,52], and CHONDROITIN and HEPARAN SULFATE PROTEOGLYCANS[87,88], are expressed in a lamina-specific fashion, and these might account for some of the molecular differences between the cortical layers that influence the termination of thalamocortical projections. The molecules that are required for thalamic branch formation are also beginning to be identified, and Fgf2 (REF. 89) and Slit2 (REFS 90,91) are prime candidates.

Thalamocortical axons and cortical patterning

Intrinsic molecular determinants of the proliferative zone are proposed to play a part in the early broad regionalization of the developing neocortex[92,93]. Using co-culture studies, several groups have demonstrated that the initial expression of region-specific markers is probably independent of thalamic innervation[94–97]. Several genes, including *Tbr1* (REF. 98), *Pax6* (REFS 18,99), *Emx2* (REFS 100,101), *Latexin*[102], *Cad6* and *Cad8* (REF. 50), *Coup-tfi*, *Chl1* (REF. 103) and *Id2* (REF. 98), are expressed in a region- and lamina-specific manner before thalamic afferents invade the cerebral cortex. For example, *Pax6* and *Emx2* are expressed in opposing gradients in the dorsal telencephalic neuroepithelium — the proliferative zone that gives rise to cortical neurons — and *Cad6* and *Cad8* are markers of the somatosensory and motor areas respectively. Therefore, some aspects of early cortical regionalization do not seem to require extrinsic influences. Support for this hypothesis came from studies in *Mash1* and *Gbx2*-knockout mice[51,54]. *Mash1* and *Gbx2* are not expressed in the neocortex, and mice that are deficient for either gene fail to develop a correct thalamocortical projection to the cortex[53,54]. Despite the lack of cortical innervation by thalamic axons, prenatal region-specific cortical gene expression develops normally[51].

However, numerous studies have shown that the differentiation of many of the anatomical features that distinguish different cortical areas depends to a large extent on the input of thalamocortical axons[6,92]. Several studies have shown that thalamocortical projections can influence the size and even the identity of specific cortical areas[6,104,105]. For example, reduction of thalamic input to the cortex following BINOCULAR ENUCLEATION alters cortical areal fate by creating a 'hybrid' visual cortex in place of area 17 (REFS 106–108). Similarly, early ablation of thalamic nuclei leads to an alteration in the size and cell number in the corresponding area of the neocortex[109]. However, a recent study examined the thalamic connectivity formed by grafts of embryonic (E16) parietal or occipital cortex placed homo- or heterotopically into the neocortex of newborn rats[110]. This showed that although E16 parietal or occipital cortical grafts attract thalamic projections, their cells do not have the capacity to differentiate and maintain the organization of the BARREL cortex[110].

CHONDROITIN SULPHATE PROTEOGLYCANS
Important components of the extracellular matrix and connective tissue. These proteins contain hydrophilic, negatively charged polymers of glucuronic acid and sulphated *N*-acetyl glucosamine residues.

HEPARAN SULPHATE
A glycosaminoglycan that consists of repeated units of hexuronic acid and glucosamine residues. It usually attaches to proteins through a xylose residue to form proteoglycans.

BINOCULAR ENUCLEATION
Surgical removal of both of the eyeballs.

BARREL
A cylindrical column of neurons found in the rodent neocortex. Each barrel receives sensory input from a single whisker follicle, and the topographical organization of the barrels corresponds precisely to the arrangement of whisker follicles on the face.

© 2003 Nature Publishing Group

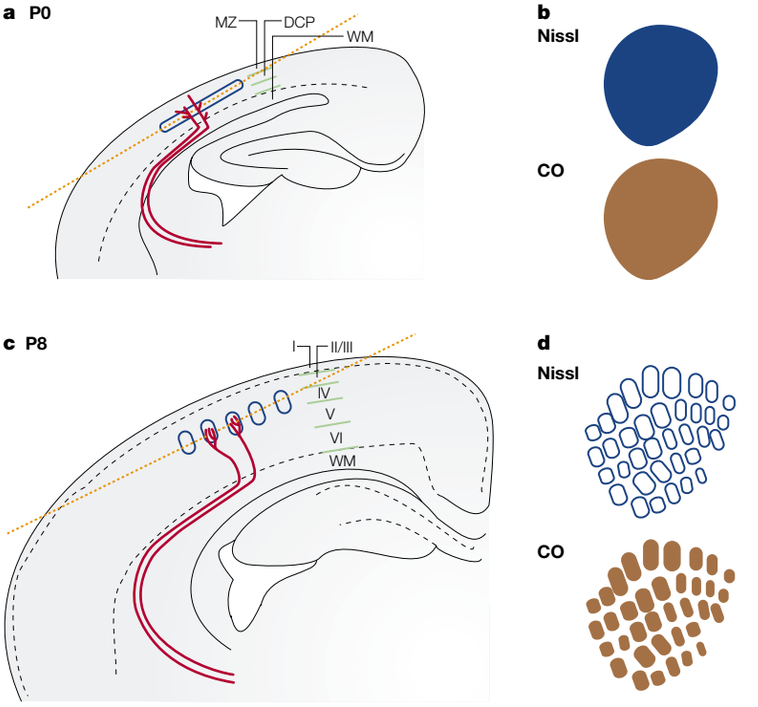


Figure 6 | **Formation of the periphery-related pattern in the somatosensory cortex.** **a** | By birth (P0), thalamic projections have entered the cortex and started to form branches, but these do not yet segregate in a periphery-related fashion. **b** | A tangential section, cut at the level of layer IV (orange dashed line in **a**). The distribution of these branches in layer IV (shown through cytochrome oxidase, CO, staining) and the cells in this layer (Nissl staining) seems to be homogeneous. **c** | At the beginning of the second postnatal week (P8), the organization of cortical layers is complete. Thalamic axon arbors cluster according to a periphery-related pattern in S1 and layer IV cells organize around the thalamic projections in a barrel pattern (blue barrels). **d** | A tangential section (orange dashed line in **c**) in layer IV shows the distribution of the thalamic arbors, revealed by CO, and the cytoarchitecture of the target layer IV cells, revealed by Nissl staining. The periphery-related pattern formed by thalamic axons reflects the organization of the whiskers at the periphery. DCP, deep cortical plate; MZ, marginal zone; WM, white matter.

These observations indicate that cortical regionalization is initially created by the graded expression of various genes, and that thalamic input controls the later stages of areal subdivision through activity-dependent or independent mechanisms.

The role of thalamic projections in determining the identity of cortical areas and establishing their architecture might not be limited to later stages, however. Recent *in vitro* studies indicate that thalamic afferents release a diffusible factor that promotes proliferation of neurons and glia in the proliferative zones of the cortex[111]. Moreover, neuronal migration in the cortex is facilitated by thalamic fibres in organotypic thalamus–cortex co-cultures[112]. If a similar mechanism operates *in vivo*, this early influence of thalamocortical axons on corticogenesis could contribute to area-specific differences in cytoarchitecture that become evident later in postnatal development.

The remodelling of cortical circuitry during thalamic fibre invasion is a complex process, in which expression of surface molecules and growth factors, plus patterns of afferent and local activity, seem to play an important part[113]. The primary somatosensory cortex of the rodent is an excellent model system in which to investigate the influence of the periphery on cortical development. A huge area of this region is devoted to the representation of the whiskers of the snout. Stimulation of a single whisker results in the activation of a well-defined cortical region, corresponding to that particular whisker. Blocking or changing the flow of sensory input during the first postnatal week alters the pattern of cortical representation according to the peripheral pattern. Thalamic axon arbors are aligned according to the periphery-related pattern, and they are targeted into the middle of the layer IV cells, where they become arranged into a circular pattern of barrels[114].

Understanding the formation of the barrels gave us considerable insight into the interactions between thalamocortical projections and the cortex. During the first two days after birth in mice, thalamic axons grow through the lower layers of the primary somatosensory cortex to form arbors in a periphery-related pattern[115,116] (FIG. 6). Subsequently, layer IV neurons become displaced to form the barrel walls, and they reorient their dendrites into a cell-sparse region in the centre of the barrel, where they receive synaptic contacts from segregated groups of thalamic axon terminals.

Several lines of evidence indicate that the morphological reorganization and differentiation of cortical neurons to form barrels depends, at least in part, on signals that are conveyed by invading thalamic axons (see later discussion). However, molecules released from telencephalic centres seem to set up the early general patterning of different cortical areas. A recent study has shown that Fgf8 is involved in the regulation of the anteroposterior neocortical pattern[117]. Expanding or reducing the endogenous Fgf8 source shifts cortical areas posteriorly or anteriorly, respectively, and if a second Fgf8 source is introduced into the posterior cortical primordium, a duplicate somatosensory barrel field develops with mirrored reversal to the original area. Therefore, there is direct evidence for a secreted signalling molecule that is involved in the regulation of the neocortical area map, but whether this feature has a direct or indirect relationship with changes in thalamocortical projections remains unknown.

Thalamocortical segregation in the barrel cortex

Gene deletion studies and *in vitro* assays are beginning to disclose the molecular signals that direct topographic thalamocortical connections and the patterning of maps in sensory cortical areas[118,119]. In mice with null mutations in either the *monoamine oxidase A* (*Maoa*) gene or the *adenylyl cyclase 1* (*Adcy1*) gene, ingrowing thalamic axons fail to segregate to form the primordial periphery-related pattern, and cortical cells do not rearrange as cytoarchitectonic entities[120–122] (FIG. 7). A disruption of thalamocortical patterning is caused by elevated levels of 5-hydroxytryptamine (5-HT, serotonin) but not other amines, in the *Maoa*-knockout mouse. Genetic removal of 5-HT_{1B} receptors, or pharmacological blockade of 5-HT synthesis in this mutant mouse restores normal patterning of thalamic axon terminals and near-normal segregation of cortical barrels[121,123].

© 2003 Nature Publishing Group

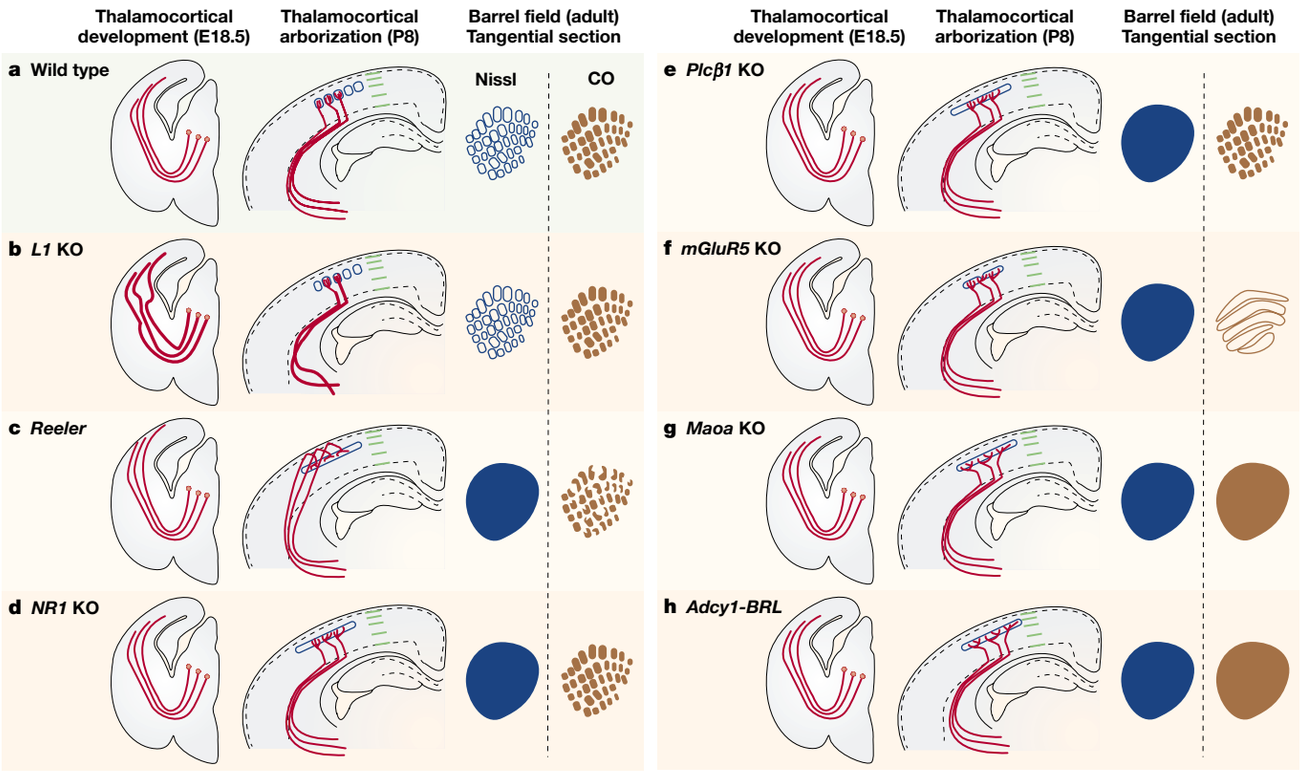


Figure 7 | **Relationship between thalamocortical connectivity and cortical map formation.** Thalamic axons (red lines) traverse the telencephalon and enter the lower layers of the cortex by birth in mice. During the first few days of postnatal life, they organize themselves into clusters to form a periphery-related pattern that will induce the cytoarchitectonic barrels. **a** | In normal mice, most layer IV neurons become displaced to form the barrel walls (as seen by Nissl staining), and they reorient their dendrites into the cell-sparse region in the centre of the barrel, where they receive synaptic contacts from the corresponding group of thalamic axons (as seen by cytochrome oxidase, CO, staining). **b** | In cell adhesion molecular *L1* mutant mice, abnormally thick thalamic bundles were observed along the internal capsule and striatum. However, thalamic axons arrive at layer IV, where they form a normal periphery-related pattern of barrels. **c** | In the *reeler* mouse embryo, the thalamic fibres penetrate the cortical plate and run up diagonally to the abnormal unsplit preplate (the superplate) at the top of the cortex. From this aberrant position, thalamocortical axons descend and arborize in the middle of the cortical plate and assume an almost normal periphery-related pattern. The barrels are not visible in Nissl stained sections. **d** | Deletion of the *N*-methyl-D-aspartate (NMDA) receptor 1 gene, *NR1*, in excitatory cortical neurons prevents the formation of cytoarchitectonic barrels. However, despite a marked reduction in NMDA-mediated synaptic activation, the periphery-related thalamic terminal patterning remains intact. **e** | In mice lacking the *phospholipase C-β1* (*Plcβ1*) gene, the embryonic thalamocortical axon development seems normal, but postnatal defects in barrel formation have been described. **f** | In *metabotropic glutamate receptor 5* (*mGluR5*)-knockout (KO) mice, segregation of thalamocortical axons into periphery related patterns does not occur normally — rows are apparent in layer IV and no individual barrels are formed. **g**, **h** | In mice with null mutations in either the *monoamine oxidase A* (*Maoa*) or the *adenylyl cyclase 1* (*Adcy1*) gene, ingrowing thalamic axons fail to segregate to form the periphery-related pattern, and cortical layer IV cells do not form cytoarchitectonic entities as was originally demonstrated in barrelless (BRL) mice. E, embryonic day; P, postnatal day.

Group I metabotropic glutamate receptors (mGluR1 and mGluR5) have been implicated in cortical and hippocampal plasticity[124]. Postsynaptic molecules, such as mGluR5, and the receptor-activated G-protein-coupled phosphodiesterase Plcβ1, also seem to be involved in cytoarchitectonic differentiation imposed by thalamocortical axons within the cortex. Mice with a deletion of the *mGluR5* gene display normal segregation of large whisker thalamocortical axons, but these only form in rows, not individual patches within rows, and these mice also lack barrels[125]. The fact that deletion of *mGluR5* leads to the loss of barrels indicates that mGluR5 signalling is also crucial for transmitting the periphery-related pattern from thalamic axons to their postsynaptic target in the cortex (FIG. 7f). It is known that thalamocortical arbors in layer IV are segregated into a periphery-related pattern shortly after they colonize the cortical plate[116,120], but whether mGluR5 has a role in this initial phase of segregation of thalamic axons, or whether its role is limited to the maintenance of segregation and cytoarchitectonic pattern formation in layer IV, still remains unclear.

Interestingly, in mice that lack the *Plcβ1* gene, defects in barrel formation have been described in the absence of defects in the patterning of thalamocortical axons[125] (FIG. 7e). The expression of Plcβ1 in the developing cortex is mainly postsynaptic (T. Spires, personal communication), and phosphoinositide hydrolysis following activation of the group I mGluRs is dependent on Plcβ1 expression during the first postnatal week, indicating that mGluR5 activation of Plcβ1 is crucial for barrel formation. The mechanism responsible for the segregation of thalamic axons according to the peripheral pattern is not known. Activity mediated through *N*-methyl-D-aspartate receptors (NMDARs) does not seem to be involved, because deletion of the *NMDAR1* (*NR1*) gene

© 2003 Nature Publishing Group

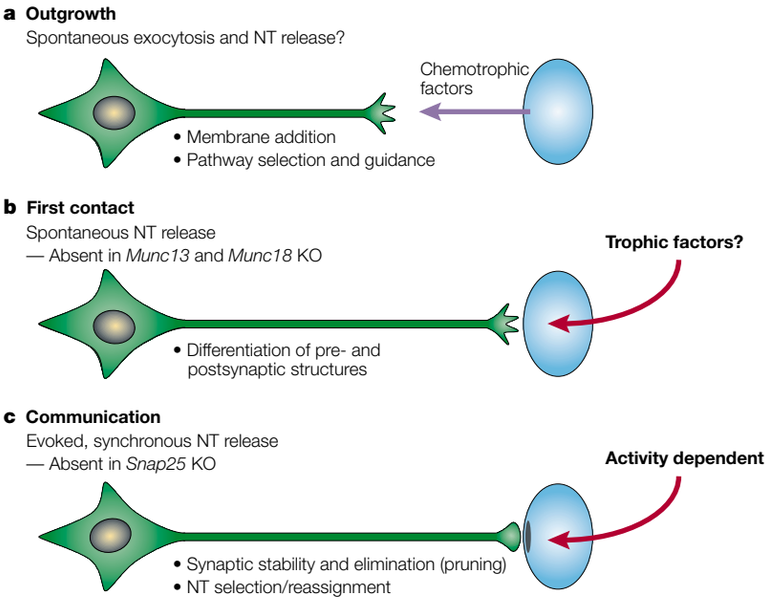


Figure 8 | **When does neural activity play a part in thalamocortical development?** Thalamic fibres undergo topographic refinement as they approach the cortex and start to accumulate in the subplate layer, and also as they enter the cortex and reach layer IV. The neural activity in the cortical network, or in the thalamic fibres themselves, is believed to play a part in this process. Different types of intercellular communication might be involved in each step of axonal guidance and the establishment of synapses. During axonal outgrowth, spontaneous exocytosis and neurotransmitter (NT) release might have a role in the selection of different pathways and guidance towards the target. As the axons approach their target, trophic factors released by the postsynaptic cells could be also involved. As the selection of the target is made, the final communication and synapse formation might depend on evoked, synchronous NT release. Activity-dependent mechanisms are believed to be crucial in this process. KO, knockout.

in excitatory cortical neurons did not completely prevent the formation of the periphery-related pattern of thalamocortical axons, but cytoarchitectonic barrels failed to form[126,127] (FIG. 7d).

Is the early topography crucial? It seems that a precise trajectory of thalamic axons might not be crucial for the final pattern of cortical targeting, and that thalamic projections are capable of re-aligning themselves after altered delivery. In the *reeler* mouse, in which the preplate fails to split and remains at the pial surface[128], the thalamocortical projections show a spectacular rearrangement. They penetrate the cortical plate and run diagonally up to the abnormal unsplit preplate, where they wait before turning down again into the plate itself[129,130] (FIG. 7c). During early postnatal development, lectin binding delineates slightly abnormally shaped barrels, but they appear according to the normal pattern in the somatosensory cortex[131]. The thalamic barreloid complex, however, reveals a disposition of glycoconjugates that is completely normal. So, the *reeler* mouse provides an example in which thalamic projections arrive at the cortex through an aberrant route, but they form a normal periphery-related pattern in the primary somatosensory cortex.

It will be interesting to study further the question of whether abnormalities in thalamocortical pathfinding could modify the relative position of the periphery-related pattern. Abnormalities in axonal guidance have been described in mice with mutations in *Sema6a* or the gene that codes for the cell adhesion molecule L1 (REFS 45,132), and these could provide good models to follow development into adulthood.

Activity and thalamocortical arborization

There are three known forms of neurotransmitter release: regulated synaptic vesicle release, spontaneous vesicle release and PARACRINE non-vesicular release (FIG. 8a). It has been shown that peripheral neurons are already generating spontaneous activity patterns by the time that sensory afferents begin to reach the thalamus[133]. These activity patterns could elicit excitatory postsynaptic potentials on thalamic projection neurons, and these patterns could influence the formation of terminals within the subplate and layer IV (REFS 134,135). A controversial question in this field is whether the activity arriving from thalamic axons, or that of the target network, has a crucial role in the correct deployment, subsequent entrance from the subplate, and precise arborization of thalamic axons in the proper layer and area[72,136,137].

Extensive studies of the components of the machinery that triggers neurotransmitter release have indicated that the SNARE (*N*-ethylmaleimide-sensitive fusion protein (NSF)-attachment protein receptor) proteins and Munc18 are required for membrane fusion during exocytosis, and attractive models to investigate their function have been developed[138]. Snap25, a member of the SNARE complex, is present in the presynaptic membrane terminal. The *Snap25*-deficient mouse, in which action potential-regulated synaptic vesicle release is disrupted[139], provides a model system to evaluate the contribution of regulated and spontaneous neurotransmitter release to embryonic cortical development. Surprisingly, a recent study in this mutant indicated that axonal growth and early topographic arrangement of the thalamic fibre pathway do not rely on activity-dependent mechanisms that require evoked neurotransmitter release[140]. However, spontaneous vesicle release and paracrine non-vesicular release are still present in the *Snap25*-knockout mice. Unfortunately, these mice die at birth, probably due to respiratory failure, so the role of synaptic activity in the later phases of thalamocortical development, when invasion of and branching within the cortical layers are occurring, remains unknown.

The Munc13 and Munc18 proteins, which are mammalian homologous of the *Caenorhabditis elegans* unc-13 and unc-18 respectively[141], are involved in the docking of secretory vesicles to the plasma membrane. Mutations in these proteins completely block membrane fusion, and in *Munc13* and *Munc18* mutant mice there is a complete absence of spontaneous vesicle release, leaving paracrine non-vesicular release only. A recent study has identified *Munc13-3* as a candidate gene for critical period neuroplasticity in the visual cortex[142].

Further *in vivo* and *in vitro* studies using these mutants could be important in establishing which mechanisms are required for different periods of cortical and thalamocortical development. With these powerful

PARACRINE
Signalling process that involves the secretion of molecules from a cell, which act on other cells in the immediate neighbourhood that express appropriate receptors, rather than acting on the same cell (autocrine signalling) or on remote cells (endocrine signalling).

© 2003 Nature Publishing Group

tools, it should be possible to define the various forms of neuronal interaction that are important for the development of a precise thalamocortical connectivity and cortical network.

Concluding remarks

Cortical development involves shaping the fate of the cortical epithelium into discrete cortical areas with specific input, output and networks. There are continuous interactions between the thalamus and the cortex in moulding these features. During the last few years, considerable progress has been made towards understanding the early development of thalamocortical projections and their interactions within the developing cortical circuitry. These advances have mainly been made in mutants with selective deletions of genes that are expressed in distinct forebrain regions or at the thalamocortical synapse. Although some aspects of embryonic cortical gene expression patterns are not altered if the thalamocortical input is absent or displaced, the intact pre and/or postsynaptic machinery of thalamocortical transmission seems to be essential to impose cortical cytoarchitectonic patterns postnatally. Our challenges now are to interpret the observations obtained in these mutants, and to establish the causal relationships between the signals, receptors and trophic factors that are expressed and the thalamic and cortical patterns that are established.

1. Rubenstein, J. L., Rakic, P. Special issue: genetic control of cortical development. *Cereb. Cortex* **9**, 521–901 (1999).
2. O'Leary, D. D., Schlaggar, B. L. & Tuttle, R. Specification of neocortical areas and thalamocortical connections. *Annu. Rev. Neurosci.* **17**, 419–439 (1994).
3. O'Leary, D. D. & Nakagawa, Y. Patterning centers, regulatory genes and extrinsic mechanisms controlling arealization of the neocortex. *Curr. Opin. Neurobiol.* **12**, 14–25 (2002).
   A comprehensive review on the early gene expression patterns and cortical regionalization.
4. Jones, E. G. *The Thalamus* (Plenum, New York, 1985).
5. Northcutt, R. G. & Kaas, J. H. The emergence and evolution of mammalian neocortex. *Trends Neurosci.* **9**, 373–379 (1995).
6. Krubitzer, L. & Huffman, K. J. Arealization of the neocortex in mammals: genetic and epigenetic contributions to the phenotype. *Brain Behav. Evol.* **55**, 322–335 (2000).
7. Bayer, S. A. & Altman, J. *Neocortical Development* (Raven, New York, 1991).
8. Allendoerfer, K. L. & Shatz, C. J. The subplate, a transient neocortical structure: its role in the development of connections between thalamus and cortex. *Annu. Rev. Neurosci.* **17**, 185–218 (1994).
9. Molnár, Z. Development and evolution of thalamocortical interactions. *Eur. J. Morphol.* **38**, 313–320 (2000).
10. McConnell, S. K., Ghosh, A. & Shatz, C. J. Subplate neurons pioneer the first axon pathway from the cerebral cortex. *Science* **245**, 978–982 (1989).
    The first detailed description of the pioneering projections from subplate to the internal capsule.
11. Altman, J. & Bayer, S. A. Development of the diencephalon in the rat. V. Thymidine-radiographic observations on internuclear and intranuclear gradients in the thalamus. *J. Comp. Neurol.* **188**, 473–499 (1979).
12. Lund, R. D. & Mustari, M. J. Development of the geniculocortical pathway in rats. *J. Comp. Neurol.* **173**, 289–306 (1977).
13. Mitrofanis, J. & Guillery, R. W. New views of the thalamic reticular nucleus in the adult and the developing brain. *Trends Neurosci.* **16**, 240–245 (1993).
14. Molnár, Z. & Blakemore, C. How do thalamic axons find their way to the cortex? *Trends Neurosci.* **18**, 389–397 (1995).
15. Puelles, L. & Rubenstein, J. L. Expression patterns of homeobox and other putative regulatory genes in the embryonic mouse forebrain suggest a neuromeric organization. *Trends Neurosci.* **16**, 472–479 (1993).
16. Nakagawa, Y. & O'Leary, D. D. Combinatorial expression patterns of LIM-homeodomain and other regulatory genes parcellate developing thalamus. *J. Neurosci.* **21**, 2711–2725 (2001).
17. Puelles, L. *et al.* Pallial and subpallial derivatives in the embryonic chick and mouse telencephalon, traced by the expression of the genes *Dlx-2*, *Emx-1*, *Nkx-2. 1*, *Pax-6*, and *Tbr-1*. *J. Comp. Neurol.* **424**, 409–438 (2000).
18. Stoykova, A. & Gruss, P. Roles of Pax-genes in developing and adult brain as suggested by expression patterns. *J. Neurosci.* **14**, 1395–1412 (1994).
19. Molnár, Z. & Butler, A. B. The corticostriatal junction: a crucial region for forebrain development and evolution. *Bioessays* **24**, 530–541 (2002).
20. Molnár, Z., Adams, R. & Blakemore, C. Mechanisms underlying the early establishment of thalamocortical connections in the rat. *J. Neurosci.* **18**, 5723–5745 (1998).
21. Métin, C. & Godement, P. The ganglionic eminence may be an intermediate target for corticofugal and thalamocortical axons. *J. Neurosci.* **15**, 3219–3235 (1996).
    The first description of the guidepost cells situated in the internal capsule with early dorsal thalamic projections.
22. Molnár, Z. & Cordery, P. Connections between cells of the internal capsule, thalamus, and cerebral cortex in embryonic rat. *J. Comp. Neurol.* **413**, 1–25 (1999).
23. Donoghue, M. J. & Rakic, P. Molecular evidence for the early specification of presumptive functional domains in the embryonic primate cerebral cortex. *J. Neurosci.* **19**, 5967–5979 (1999).
24. Barbe, M. F. & Levitt, P. Attraction of specific thalamic input by cerebral grafts depends on the molecular identity of the implant. *Proc. Natl Acad. Sci. USA* **89**, 3706–3710 (1992).
25. Suzuki, S. C., Inoue, T., Kimura, Y., Tanaka, T. & Takeichi, M. Neuronal circuits are subdivided by differential expression of type-II classic cadherins in postnatal mouse brains. *Mol. Cell. Neurosci.* **9**, 433–447 (1997).
26. Gao, P. P. *et al.* Regulation of thalamic neurite outgrowth by the Eph ligand ephrin-A5: implications in the development of thalamocortical projections. *Proc. Natl Acad. Sci. USA* **95**, 5329–5334 (1998).
27. Ma, L. *et al.* Neurotrophin-3 is required for appropriate establishment of thalamocortical connections. *Neuron* **36**. 623–634 (2002).
28. McQuillen, P. S., DeFreitas, M. F., Zada, G. & Shatz, C. J. A novel role for p75NTR in subplate growth cone complexity and visual thalamocortical innervation. *J. Neurosci.* **22**, 3580–3593 (2002).
29. Serafini, T. *et al.* Netrin-1 is required for commissural axon guidance in the developing vertebrate nervous system. *Cell* **87**, 1001–1014 (1996).
30. Métin, C., Deleglise, D., Serafini, T., Kennedy, T. E. & Tessier-Lavigne, M. A role for netrin-1 in the guidance of cortical efferents. *Development* **124**, 5063–5074 (1997).
    The first study indicating the role of netrin 1 in the development of early cortical connectivity.
31. Braisted, J. E., Tuttle, R. & O'Leary, D. D. Thalamocortical axons are influenced by chemorepellent and chemoattractant activities localized to decision points along their path. *Dev. Biol.* **208**, 430–440 (1999).
32. Braisted, J. E. *et al.* Netrin-1 promotes thalamic axon growth and is required for proper development of the thalamocortical projection. *J. Neurosci.* **20**, 5792–5801 (2000).
33. Bagri, A. *et al.* Slit proteins prevent midline crossing and determine the dorsoventral position of major axonal pathways in the mammalian forebrain. *Neuron* **33**, 233–248 (2002).
34. Vanderhaeghen, P. *et al.* A mapping label required for normal scale of body representation in the cortex. *Nature Neurosci.* **3**, 358–365 (2000).
35. Mackarehtschian, K., Lau, C. K., Caras, I. & McConnell, S. K. Regional differences in the developing cerebral cortex revealed by *Ephrin-A5* expression. *Cereb. Cortex* **9**, 601–610 (1999).
36. Mann, F., Peuckert, C., Dehner, F., Zhou, R. & Bolz, J. Ephrins regulate the formation of terminal axonal arbors during the development of thalamocortical projections. *Development* **129**, 3945–3955 (2002).
37. Uziel, D. *et al.* Miswiring of limbic thalamocortical projections in the absence of ephrin-A5. *J. Neurosci.* **22**, 9352–9357 (2002).
38. Takemoto, M. *et al.* Ephrin-B3-EphA4 interactions regulate the growth of specific thalamocortical axon populations *in vitro*. *Eur. J. Neurosci.* **16**, 1168–1172 (2002).
39. Matthes, D. J., Sink, H., Kolodkin, A. L. & Goodman, C. S. Semaphorin II can function as a selective inhibitor of specific synaptic arborizations. *Cell* **81**, 631–639 (1995).
40. Raper, J. A. Semaphorins and their receptors in vertebrates and invertebrates. *Curr. Opin. Neurobiol.* **10**, 88–94 (2000).
41. Skaliora, I., Singer, W., Betz, H. & Puschel, A. W. Differential patterns of semaphorin expression in the developing rat brain. *Eur. J. Neurosci.* **10**, 1215–1229 (1998).
42. Messersmith, E. K. *et al.* Semaphorin III can function as a selective chemorepellent to pattern sensory projections in the spinal cord. *Neuron* **14**, 949–959 (1995).
43. Bagnard, D., Lohrum, M., Uziel, D., Puschel, A. W. & Bolz, J. Semaphorins act as attractive and repulsive guidance signals during the development of cortical projections. *Development* **125**, 5043–5053 (1998).
44. Bagnard, D., Chounlamountri, N., Puschel, A. W. & Bolz, J. Axonal surface molecules act in combination with semaphorin 3a during the establishment of corticothalamic projections. *Cereb. Cortex* **11**, 278–285 (2001).
45. Leighton, P. A. *et al.* Defining brain wiring patterns and mechanisms through gene trapping in mice. *Nature* **410**, 174–179 (2001).
46. Garel, S., Yun, K., Grosschedl, R. & Rubenstein, J. L. The early topography of thalamocortical projections is shifted in *Ebf1* and *Dlx1/2* mutant mice. *Development* **129**, 5621–5634 (2002).
    This study supports the idea that early thalamic targeting in the cortex depends on signals within the ventral telencephalon and that it might be independent from early cortical gene expression.
47. Crossley, P. H. & Martin, G. R. The mouse *Fgf8* gene encodes a family of polypeptides and is expressed in regions that direct outgrowth and patterning in the developing embryo. *Development* **121**, 439–451 (1995).
48. Grove, E. A., Tole, S., Limon, J., Yip, L. & Ragsdale, C. W. The hem of the embryonic cerebral cortex is defined by the expression of multiple *Wnt* genes and is compromised in *Gli3*-deficient mice. *Development* **125**, 2315–2325 (1998).
49. Rubenstein, J. L. & Beachy, P. A. Patterning of the embryonic forebrain. *Curr. Opin. Neurobiol.* **8**, 18–26 (1998).
50. Nakagawa, Y., Johnson, J. E. & O'Leary, D. D. Graded and areal expression patterns of regulatory genes and cadherins in embryonic neocortex independent of thalamocortical input. *J. Neurosci.* **19**, 10877–10885 (1999).
51. Miyashita-Lin, E. M., Hevner, R., Wassarman, K. M., Martinez, S. & Rubenstein, J. L. Early neocortical regionalization in the absence of thalamic innervation. *Science* **285**, 906–909 (1999).
    References 50 and 51 provided the first evidence indicating that the early cortical gene expression pattern is independent of embryonic thalamic input.
52. Donoghue, M. J. & Rakic, P. Molecular gradients and compartments in the embryonic primate cerebral cortex. *Cereb. Cortex* **9**, 586–600 (1999).
53. Tuttle, R., Nakagawa, Y., Johnson, J. E. & O'Leary, D. D. Defects in thalamocortical axon pathfinding correlate with altered cell domains in *Mash-1*-deficient mice. *Development* **126**, 1903–1916 (1999).
    This study provides strong support for the early guiding role of the internal capsule cells and their projections in the early development of thalamic projections.

© 2003 Nature Publishing Group

54. Hevner, R. F., Miyashita-Lin, E. & Rubenstein, J. L. 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 (2002).
The experiments of this study indicate that an interaction between early corticofugal and thalamocortical projections is necessary for the correct development of both sets of fibres.
55. Stoykova, A., Fritsch, R., Walther, C. & Gruss, P. Forebrain patterning defects in *Small eye* mutant mice. *Development* **122**, 3453–3465 (1996).
56. Jones, L., López-Bendito, G., Gruss, P., Stoykova, A. & Molnar, Z. *Pax6* is required for the normal development of the forebrain axonal connections. *Development* **129**, 5041–5052 (2002).
57. Bishop, K. M., Goudreau, G. & O'Leary, D. D. Regulation of area identity in the mammalian neocortex by *Emx2* and *Pax6*. *Science* **288**, 344–349 (2000).
58. Mallamaci, A., Muzio, L., Chan, C. H., Parnavelas, J. & Boncinelli, E. Area identity shifts in the early cerebral cortex of *Emx2*$^{-/-}$ mutant mice. *Nature Neurosci.* **3**, 679–686 (2000).
59. López-Bendito, G., Chan, C. H., Mallamaci, A., Parnavelas, J. & Molnár, Z. Role of *Emx2* in the development of the reciprocal connectivity between cortex and thalamus. *J. Comp. Neurol.* **451**, 153–169 (2002).
60. Pratt, T. *et al.* A role for *Pax6* in the normal development of dorsal thalamus and its cortical connections. *Development* **127**, 5167–5178 (2000).
Using an elegant co-culture essay, this study provides evidence that thalamic cells are directly influenced by the lack of *Pax6*, which might contribute to the pathfinding defects of early thalamocortical axons.
61. Molnár, Z. *Development of Thalamocortical Connections* (Springer, Heidelberg, 1998).
62. McConnell, S. K., Ghosh, A. & Shatz, C. J. Subplate neurons pioneer the first axon pathway from the cerebral cortex. *Science* **245**, 978–982 (1989).
63. De Carlos, J. A. & O'Leary, D. D. Growth and targeting of subplate axons and establishment of major cortical pathways. *J. Neurosci.* **12**, 1194–1211 (1992).
64. Mitrofanis, J. & Baker, G. E. Development of the thalamic reticular and perireticular nuclei in rats and their relationship to the course of growing corticofugal and corticopetal axons. *J. Comp. Neurol.* **338**, 575–587 (1993).
65. Letinic, K. & Kostovic, I. Transient neuronal population of the internal capsule in the developing human cerebrum. *Neuroreport* **7**, 2159–2162 (1996).
66. Zhou, C. *et al.* The nuclear orphan receptor COUP-TFI is required for differentiation of subplate neurons and guidance of thalamocortical axons. *Neuron* **24**, 847–859 (1999).
This study describes the premature death of subplate neurons in *Coup-tfi*-deficient mice, which leads to a reduction in thalamic axons growth through the internal capsule.
67. Marín, O., Baker, J., Puelles, L. & Rubenstein, J. L. Patterning of the basal telencephalon and hypothalamus is essential for guidance of cortical projections. *Development* **129**, 761–773 (2002).
68. Rakic, P. Prenatal genesis of connections subserving ocular dominance in the rhesus monkey. *Nature* **261**, 467–471 (1976).
69. Shatz, C. J. & Luskin, M. B. The relationship between the geniculocortical afferents and their cortical target cells during development of the cat's primary visual cortex. *J. Neurosci.* **6**, 3655–3668 (1986).
70. Molnar, Z., Higashi, S., Adams, R. & Toyama, K. in *Plasticity of Adult Barrel Cortex* (ed. Kossut, M.) 47–79 (Graham Publishing, Johnson City, Tennessee, 2000).
71. Krug, K., Smith, A. L. & Thompson, I. D. The development of topography in the hamster geniculo-cortical projection. *J. Neurosci.* **18**, 5766–5776 (1998).
72. Catalano, S. M. & Shatz, C. J. Activity-dependent cortical target selection by thalamic axons. *Science* **281**, 559–562 (1998).
This study shows that blocking action potentials during early stages of development in the occipital cortex disrupts the entry and areal targeting of thalamic projections.
73. Naegele, J. R., Jhaveri, S. & Schneider, G. E. Sharpening of topographical projections and maturation of geniculocortical axon arbors in the hamster. *J. Comp. Neurol.* **277**, 593–607 (1988).
74. Ghosh, A. & Shatz, C. J. Involvement of subplate neurons in the formation of ocular dominance columns. *Science* **255**, 1441–1443 (1992).
75. Catalano, S. M., Robertson, R. T. & Killackey, H. P. Individual axon morphology and thalamocortical topography in developing rat somatosensory cortex. *J. Comp. Neurol.* **367**, 36–53 (1996).
76. Friauf, E. & Shatz, C. J. Changing patterns of synaptic input to subplate and cortical plate during development of visual cortex. *J. Neurophysiol.* **66**, 2059–2071 (1991).
77. Higashi, S., Molnár, Z., Kurotani, T. & Toyama, K. Prenatal development of neural excitation in rat thalamocortical projections studied by optical recording. *Neuroscience* **115**, 1231–1246 (2002).
Demonstrates the spatial and temporal distribution of cortical activation after direct thalamic stimulation in thalamocortical slices using optical recording of voltage-sensitive dyes.
78. Yamamoto, N., Kurotani, T. & Toyama, K. Neural connections between the lateral geniculate nucleus and visual cortex *in vitro*. *Science* **245**, 192–194 (1989).
79. Yamamoto, N., Yamada, K., Kurotani, T. & Toyama, K. Laminar specificity of extrinsic cortical connections studied in coculture preparations. *Neuron* **9**, 217–228 (1992).
80. Molnár, Z. & Blakemore, C. Lack of regional specificity for connections formed between thalamus and cortex in coculture. *Nature* **351**, 475–477 (1991).
81. Bolz, J., Novak, N. & Staiger, V. Formation of specific afferent connections in organotypic slice cultures from rat visual cortex cocultured with lateral geniculate nucleus. *J. Neurosci.* **12**, 3054–3070 (1992).
82. Yamamoto, N., Higashi, S. & Toyama, K. Stop and branch behaviors of geniculocortical axons: a time-lapse study in organotypic cocultures. *J. Neurosci.* **17**, 3653–3663 (1997).
83. Yamamoto, N. *et al.* Characterization of factors regulating lamina-specific growth of thalamocortical axons. *J. Neurobiol.* **42**, 56–68 (2000).
84. Yamamoto, N. Cellular and molecular basis for the formation of lamina-specific thalamocortical projections. *Neurosci. Res.* **42**, 167–173 (2002).
85. Redies, C. & Takeichi, M. Expression of N-cadherin mRNA during development of the mouse brain. *Dev. Dyn.* **197**, 26–39 (1993).
86. Polleux, F., Giger, R. J., Ginty, D. D., Kolodkin, A. L. & Ghosh, A. Patterning of cortical efferent projections by semaphorin-neuropilin interactions. *Science* **282**, 1904–1906 (1998).
87. Oohira, A., Katoh-Semba, R., Watanabe, E. & Matsui, F. Brain development and multiple molecular species of proteoglycan. *Neurosci. Res.* **20**, 195–207 (1994).
88. Watanabe, E. *et al.* A membrane-bound heparan sulfate proteoglycan that is transiently expressed on growing axons in the rat brain. *J. Neurosci. Res.* **44**, 84–96 (1996).
89. Szebenyi, G. *et al.* Fibroblast growth factor-2 promotes axon branching of cortical neurons by influencing morphology and behavior of the primary growth cone. *J. Neurosci.* **21**, 3932–3941 (2001).
90. Wang, K. H. *et al.* Biochemical purification of a mammalian slit protein as a positive regulator of sensory axon elongation and branching. *Cell* **96**, 771–784 (1999).
91. Ozdinler, P. H. & Erzurumlu, R. S. Slit2, a branching-arborization factor for sensory axons in the Mammalian CNS. *J. Neurosci.* **22**, 4540–4549 (2002).
92. Rakic, P. Specification of cerebral cortical areas. *Science* **241**, 170–176 (1988).
93. Rubenstein, J. L. *et al.* Genetic control of cortical regionalization and connectivity. *Cereb. Cortex* **9**, 524–532 (1999).
94. Cohen-Tannoudji, M., Babinet, C. & Wassef, M. Early determination of a mouse somatosensory cortex marker. *Nature* **368**, 460–463 (1994).
95. Levitt, P., Eagleson, K. L., Chan, A. V., Ferri, R. T. & Lillien, L. Signaling pathways that regulate specification of neurons in developing cerebral cortex. *Dev. Neurosci.* **19**, 6–8 (1997).
96. Nothias, F., Fishell, G. & Ruiz i Altaba, A. Cooperation of intrinsic and extrinsic signals in the elaboration of regional identity in the posterior cerebral cortex. *Curr. Biol.* **8**, 459–462 (1998).
97. Gitton, Y., Cohen-Tannoudji, M. & Wassef, M. Specification of somatosensory area identity in cortical explants. *J. Neurosci.* **19**, 4889–4898 (1999).
98. Bulfone, A. *et al.* T-brain-1: a homolog of Brachyury whose expression defines molecularly distinct domains within the cerebral cortex. *Neuron* **15**, 63–78 (1995).
99. Walther, C. & Gruss, P. *Pax-6*, a murine paired box gene, is expressed in the developing CNS. *Development* **113**, 1435–1449 (1991).
100. Gulisano, M., Broccoli, V., Pardini, C. & Boncinelli, E. *Emx1* and *Emx2* show different patterns of expression during proliferation and differentiation of the developing cerebral cortex in the mouse. *Eur. J. Neurosci.* **8**, 1037–1050 (1996).
101. Mallamaci, A. *et al.* EMX2 protein in the developing mouse brain and olfactory area. *Mech. Dev.* **77**, 165–172 (1998).
102. Arimatsu, Y. *et al.* Early regional specification for a molecular neuronal phenotype in the rat neocortex. *Proc. Natl Acad. Sci. USA* **89**, 8879–8883 (1992).
103. Liu, Q., Dwyer, N. D. & O'Leary, D. D. Differential expression of *COUP-TFI*, *CHL1*, and two novel genes in developing neocortex identified by differential display PCR. *J. Neurosci.* **20**, 7682–7690 (2000).
104. Pallas, S. L. Intrinsic and extrinsic factors that shape neocortical specification. *Trends Neurosci.* **24**, 417–423 (2001).
105. Kaas, J. H., Florence, S. L. & Jain, N. Subcortical contributions to massive cortical reorganizations. *Neuron* **22**:657–660 (1999).
106. Rakic, P., Suner, I. & Williams, R. W. A novel cytoarchitectonic area induced experimentally within the primate visual cortex. *Proc. Natl Acad. Sci. USA* **88**, 2083–2087 (1991).
107. Dehay, C., Giroud, P., Berland, M., Killackey, H. & Kennedy, H. Contribution of thalamic input to the specification of cytoarchitectonic cortical fields in the primate: effects of bilateral enucleation in the fetal monkey on the boundaries, dimensions, and gyrification of striate and extrastriate cortex. *J. Comp. Neurol.* **367**, 70–89 (1996).
An interesting study that indicates a direct link between thalamic input and cortical neural proliferation.
108. Kahn, D. M. & Krubitzer, L. Massive cross-modal cortical plasticity and the emergence of a new cortical area in developmentally blind mammals. *Proc. Natl Acad. Sci. USA* **99**, 11429–11434 (2002).
109. Windrem, M. S. & Finlay, B. L. Thalamic ablations and neocortical development: alterations of cortical cytoarchitecture and cell number. *Cereb. Cortex* **1**, 230–240 (1991).
110. Gaillard, A. & Roger, M. Early commitment of embryonic neocortical cells to develop area-specific thalamic connections. *Cereb. Cortex* **10**, 443–453 (2000).
111. Dehay, C., Savatier, P., Cortay, V. & Kennedy, H. Cell-cycle kinetics of neocortical precursors are influenced by embryonic thalamic axons. *J. Neurosci.* **21**, 201–214 (2001).
112. Edgar, J. M. & Price, D. J. Radial migration in the cerebral cortex is enhanced by signals from thalamus. *Eur. J. Neurosci.* **13**, 1745–1754 (2001).
This study demonstrates that thalamic input stimulates cortical neural migration.
113. Katz, L. C. & Shatz, C. J. Synaptic activity and the construction of cortical circuits. *Science* **274**, 1133–1138 (1996).
114. Woolsey, T. A. & Van der Loos, H. The structural organization of layer IV in the somatosensory region (SI) of mouse cerebral cortex. The description of a cortical field composed of discrete cytoarchitectonic units. *Brain Res.* **17**, 205–242 (1970).
115. O'Leary, D. D., Ruff, N. L. & Dyck, R. H. Development, critical period plasticity, and adult reorganizations of mammalian somatosensory systems. *Curr. Opin. Neurobiol.* **4**, 535–544 (1994).
116. Agmon, A., Yang, L. T., Jones, E. G. & O'Dowd, D. K. Topological precision in the thalamic projection to neonatal mouse barrel cortex. *J. Neurosci.* **15**, 549–561 (1995).
117. Fukuchi-Shimogori, T. & Grove, E. A. Neocortex patterning by the secreted signaling molecule FGF8. *Science* **294**, 1071–1074 (2001).
118. Erzurumlu, R. S. & Kind, P. C. Neural activity: sculptor of 'barrels' in the neocortex. *Trends Neurosci.* **24**, 589–595 (2001).
A nice review on the pre- and post-synaptic elements involved in thalamocortical patterning and cytoarchitectonic differentiation in the barrel cortex in the rodent primary somatosensory cortex.
119. Molnár, Z. & Hannan, A. J. Development of thalamocortical projections in normal and mutant mice. *Results Probl. Cell Differ.* **30**, 293–332 (2000).
120. Rebsam, A., Seif, I. & Gaspar, P. Refinement of thalamocortical arbors and emergence of barrel domains in the primary somatosensory cortex: a study of normal and monoamine oxidase a knock-out mice. *J. Neurosci.* **22**, 8541–8552 (2002).
121. Cases, O. *et al.* Lack of barrels in the somatosensory cortex of *monoamine oxidase A*-deficient mice: role of a serotonin excess during the critical period. *Neuron* **16**, 297–307 (1996).
122. Vitalis, T. *et al.* Effects of monoamine oxidase A inhibition on barrel formation in the mouse somatosensory cortex: determination of a sensitive developmental period. *J. Comp. Neurol.* **393**, 169–184 (1998).
123. Salichon, N. *et al.* Excessive activation of serotonin (5-HT) 1B receptors disrupts the formation of sensory maps in monoamine oxidase a and 5-ht transporter knock-out mice. *J. Neurosci.* **21**, 884–896 (2001).
124. Bortolotto, Z. A., Fitzjohn, S. M. & Collingridge, G. L. Roles of metabotropic glutamate receptors in LTP and LTD in the hippocampus. *Curr. Opin. Neurobiol.* **9**, 299–304 (1999).

© 2003 Nature Publishing Group

125. Hannan, A. J. *et al.* PLC-β1, activated via mGluRs, mediates activity-dependent differentiation in cerebral cortex. *Nature Neurosci.* **4**, 282–288 (2001).
Together with reference 126, this is the first description of a separate alteration in the periphery-related patterning of thalamic projections from the cytoarchitectonic differentiation of barrels in the absence of Plcβ1 and mGluRs.
126. Iwasato, T. *et al.* Cortex-restricted disruption of NMDAR1 impairs neuronal patterns in the barrel cortex. *Nature* **406**, 726–731 (2000).
127. Datwani, A., Iwasato, T., Itohara, S. & Erzurumlu, R. S. NMDA receptor-dependent pattern transfer from afferents to postsynaptic cells and dendritic differentiation in the Barrel cortex. *Mol. Cell. Neurosci.* **21**, 477–492 (2002).
128. Caviness, V. S. Jr, Crandall, J. E. & Edwards, M. A. in *Cerebral Cortex* Vol. 7 (eds Jones, E. G. & Peters, A.) 59–89 (Plenum, New York, 1998).
129. Caviness, V. S. Jr. Development of neocortical afferent systems: studies in the reeler mouse. *Neurosci. Res. Program Bull.* **20**, 560–569 (1982).
130. Molnár, Z. & Blakemore, C. in *Development of the Cerebral Cortex* Symposium 193 (eds Bock, G. & Cardew, G.) 127–149 (Wiley, Chichester, UK, 1995).
131. O'Brien, T. F., Steindler, D. A. & Cooper, N. G. Abnormal glial and glycoconjugate dispositions in the somatosensory cortical barrel field of the early postnatal reeler mutant mouse. *Brain Res.* **429**, 309–317 (1987).
132. Cohen, N. R. *et al.* Errors in corticospinal axon guidance in mice lacking the neural cell adhesion molecule L1. *Curr. Biol.* **8**, 26–33 (1998).
133. Lund, R. D. & Bunt, A. H. Prenatal development of central optic pathways in albino rats. *J. Comp. Neurol.* **165**, 247–264 (1976).
134. Mooney, R., Penn, A. A., Gallego, R. & Shatz, C. J. Thalamic relay of spontaneous retinal activity prior to vision. *Neuron* **17**, 863–874 (1996).
This study supports the idea that early patterns of peripheral activity in sensory organs can elicit activation of thalamic projection neurons, which could propagate to the cortex at early stages.
135. Shatz, C. J. & Kirkwood, P. A. Prenatal development of functional connections in the cat's retinogeniculate pathway. *J. Neurosci.* **4**, 1378–1397 (1984).
136. Ghosh, A. & Shatz, C. J. A role for subplate neurons in the patterning of connections from thalamus to neocortex. *Development* **117**, 1031–1047 (1993).
137. Herrmann, K. & Shatz, C. J. Blockade of action potential activity alters initial arborization of thalamic axons within cortical layer 4. *Proc. Natl Acad. Sci. USA* **92**, 11244–11248 (1995).
138. Rizo, J. & Sudhof, T. C. Snares and Munc18 in synaptic vesicle fusion. *Nature Rev. Neurosci.* **3**, 641–653 (2002).
139. Washbourne, P. *et al.* Genetic ablation of the t-SNARE SNAP-25 distinguishes mechanisms of neuroexocytosis. *Nature Neurosci.* **5**, 19–26 (2002).
140. Molnár, Z. *et al.* Normal development of embryonic thalamocortical connectivity in the absence of evoked synaptic activity. *J. Neurosci.* **22**, 10313–10323 (2002).
141. Brenner, S. The genetics of *Caenorhabditis elegans*. *Genetics* **77**, 71–94 (1974).
142. Yang, C. B., Zheng, Y. T., Li, G. Y. & Mower, G. D. Identification of *munc13-3* as a candidate gene for critical-period neuroplasticity in visual cortex. *J. Neurosci.* **22**, 8614–8618 (2002).

Acknowledgements
We are grateful to O. Marín, R. Hevner and C. Métin for thoughtful comments on this manuscript. We also would like to thank all members of our laboratory for their help, comments and support during the realization of this article. This work was supported by the European Community, The Wellcome Trust, The Royal Society, the Swiss National Science Foundation and the Oxford McDonnell Centre for Cognitive Neuroscience (North American Network Grant). We also thank M. Wilson for inspiring figure 8.

Online links

DATABASES
The following terms in this article are linked online to:
LocusLink:
Adcy1 | Cdh11 | Cdh6 | Cdh8 | Coup-tfi | *Ebf1* | *Emx1* | Ephrins | Eph receptors | Fgf8 | *Gbx2* | L1 | *Maoa* | *Mash1* | mGluR1 | mGluR5 | *Munc13-3* | Munc18 | netrin 1 | *Nkx2.1* | *Pax6* | Plcβ1 | semaphorins | Snap25 | *Tbr1* |
Access to this interactive links box is free online.

© 2003 Nature Publishing Group