How do thalamic axons find their way to the cortex?

Zoltán Molnár and Colin Blakemore

A cascade of simple mechanisms influences thalamic innervation of the neocortex. The cortex exerts a remote growth-promoting influence on thalamic axons when they start to grow out, becomes growth-permissive when the axons begin to invade, and later expresses a 'stop signal', causing termination in layer 4. However, any part of the thalamus will innervate any region of developing cortex in culture, and the precise topographic distribution of thalamic fibres in vivo is unlikely to depend exclusively on regional chemoaffinity. The 'handshake hypothesis' proposes that axons from the thalamus and from early-born cortical preplate cells meet and intermingle in the basal telencephalon, whereafter thalamic axons grow over the scaffold of preplate axons, and become 'captured' for a waiting period in the subplate layer below the corresponding part of the cortex. The bizarre pattern of development of thalamic innervation in the mutant reeler mouse provides strong evidence that thalamic axons are guided by preplate axons.


Any developmental neuroscientist with a tendency to masochism is encouraged to contemplate the development of thalamocortical projections. In general, each thalamic relay nucleus projects precisely and topographically to a particular cortical territory. However, the routes taken by individual fibres in the adult brain can be tortuous, with sharp bends and even rotations of the array around one axis but not the orthogonal. Moreover, single thalamic nuclei can project to more than one cortical area; each cortical field can have several thalamic inputs; and the topographic order of projections from a single nucleus can change abruptly at the borders of neighbouring cortical areas (such as the reversal of the retinotopic map between primary and secondary visual areas).

How could this bewildering complexity and precision be generated by the simple mechanisms that are familiar to developmental neuroscience, such as tropism, selective adhesion and competitive interaction? This question assumes special significance because of the growing evidence that the input from each major thalamic nucleus can act as a local 'extrinsic signal', influencing the fate of the cortex it innervates by setting the boundaries of the cortical field, delivering its afferent input, and influencing its regional differentiation.

In considering how neural systems form, it is important not to infer complexity during development from the appearance in the mature animal. Many of the perplexing features of thalamocortical organization (especially the convoluted paths taken by individual axons, the rotations of axon arrays and irregular topology between thalamus and cortex) might be produced after the initial establishment of connections. The ultimate imbroglio of thalamocortical organization might be generated by a cascade of individually simple mechanisms.

In an attempt to simplify the problem, a number of studies have been performed on rodents, since they have small cerebral hemispheres in which adjacent neocortical fields generally receive their projections from neighbouring thalamic nuclei. Table 1 shows the chronicle of thalamocortical development for rat and mouse. As in all eutherian mammals, the major events take place in utero, and are initiated before the thalamus is innervated by the sensory pathways.

How the cortex is built

The cells of the cerebral cortex originate in the proliferative neuroepithelium that lines the forebrain vesicle. From this mitotic factory, immature neurones migrate along the processes of radial glia towards the pial surface to generate the layers of the cortex. The first post-mitotic neurones form the primordial plexiform zone or preplate, which is later split by the invasion of true cortical neurones that are generated subsequently. The latter are distributed in an inside-out sequence to create layers 6–2 of the cortex, which are sandwiched between the superficial and deep components of the original preplate [the marginal zone (cortical layer 1) and the subplate, respectively (see below)].

Neurones of the preplate mature precociously, sending out axons, and expressing a variety of transmitter and receptor systems, long before the cells of the cortical plate mature. However, a substantial proportion of these neurones die after the arrival of thalamic axons, and the completion of cortical neuronogenesis. These characteristics provide strong circumstantial evidence that preplate cells play a role in the development of the cortex. Of particular interest is the mutant mouse reeler. In this autosomal-recessive mutant, whose aberrant gene product has been cloned very recently, the cortex forms in a roughly outside-in sequence, leaving the entire preplate stranded as a 'superplate' above the inverted layers of the cortical plate; however, the basic topographic interrelations between thalamus and cortex develop essentially normally.
TABLE I. Chronology of thalamocortical development in rat and mouse

<table>
<thead>
<tr>
<th>Age</th>
<th>Rat</th>
<th>Mouse</th>
<th>Major events (for occipital cortex and LGN)</th>
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<tbody>
<tr>
<td>E12</td>
<td>E11</td>
<td></td>
<td>Start of cortical neurogenesis</td>
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<tr>
<td>Early E14</td>
<td>Early E13</td>
<td>E13</td>
<td>First preplate cells complete their migration</td>
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<tr>
<td>E14</td>
<td></td>
<td></td>
<td>Commencement of axon outgrowth from preplate and thalamus</td>
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<tr>
<td>E15</td>
<td>E14</td>
<td></td>
<td>Arrival of first cells of the cortical plate</td>
</tr>
<tr>
<td>Early E15</td>
<td>Early E14</td>
<td>E15</td>
<td>Preplate and thalamic axons meet in basal telencephalon</td>
</tr>
<tr>
<td>E16</td>
<td>E15</td>
<td></td>
<td>Thalamic axons arrive under cortex</td>
</tr>
<tr>
<td>E18–19</td>
<td>E17–18</td>
<td></td>
<td>Major invasion of the cortical plate begins</td>
</tr>
<tr>
<td>E21/P0</td>
<td>E21/P0</td>
<td></td>
<td>Birth</td>
</tr>
<tr>
<td>P1</td>
<td>P0</td>
<td></td>
<td>Arrival of thalamic axons in layer 4</td>
</tr>
</tbody>
</table>

Approximate ages in rat and mouse (including the rodent mutant) are given for the main events in the development of the occipital cortex, and projections from the lateral geniculate nucleus (LGN), the visual relay in the dorsal thalamus. The 'plug date' is taken as embryonic day zero (E0) for the dating of fetuses, and the first postnatal day as P0. Cortical-cell generation finishes before birth but migration into the upper layers continues until the end of the first postnatal week (L. T. B.). In the tangential direction, across the developing cerebral cortex, there is another distinct neurogenic gradient, the anteroventral areas of the hemisphere preceding the caudaldorsal in maturity by more than a day (L. T. B.).

The timing and pattern of thalamic-axon outgrowth

In an early study, using degeneration techniques, Lund and Mustari described the outgrowth of fibres from the fetal rat thalamus, and their accumulation under the appropriate region of the cortex for a waiting period of a few days before invasion of the cortex. Although the insensitivity of their methods led them to overestimate the age by a couple of days at each stage, this general sequence has been confirmed in recent studies using the acutely sensitive carbocyanine dyes to trace axons in fixed fetal brains (L. T. B., see Table I). Catalano and colleagues also reported that fibres from the ventral thalamus arrive under the somatosensory cortex early, and enter the cortical plate before birth, but they demonstrated axons that penetrate the lower layers of the cortex immediately after their arrival, and questioned the existence of a waiting period in rodents. However, the consensus seems to be that many thalamic fibres accumulate under the true cortical plate but for a shorter time than the prolonged waiting periods that are observed in carnivores and primates.

The sequence and topography of initial outgrowth from the dorsal thalamus to the occipital cortex is illustrated in Figs 1 and 2. Figure 1 shows two bundles of axons that have been labelled by placement of small crystals of different dyes in neighbouring regions of the dorsal thalamus of an embryonic day 14.5 (E14.5) rat fetus. Even at this early stage, when the major nuclei of the thalamus are just beginning to be distinguishable, and the cortex consists of preplate alone, thalamic axons have grown down through the diencephalon in reasonable topographic order, and out through the primitive internal capsule under the anlage of the corpus striatum, and are coursing up into the intermediate zone of the telencephalon. Figure 2 illustrates the subsequent events for both normal and reeler mice. In the normal animal, thalamic axons approach the cortex but then gather in the subplate layer. Very few thalamic axons penetrate the cortical plate until a couple of days before birth, when they suddenly turn and invade radially en masse, branching and terminating mainly in layer 4. The pattern seems very different in the reeler mutant, in which the neurons that are equivalent to the normal subplate remain above the gathering cortical plate. Thalamic fibres stream obliquely upwards in fascicles through the cortical plate, and accumulate for a few days in the superplate layer above. They then plunge down and terminate, presumably on cells that are equivalent to the normal layer 4 (Refs 26 and 27). Could a single developmental programme explain both patterns of innervation?

A cascade of signals from the cortex is revealed in vitro

Organotypic co-culture provides a simplified system in which to study the expression of signals that might play a part in the initiation, guidance and termination of thalamic innervation of the developing
In our own studies, thalamic explants from rat embryos (from E14.5 to E21, but mostly E16) were co-cultured in serum-free medium with slices of neocortex from other animals aged between E14.5 and postnatal day 11 (P11). These ages were chosen to span the natural period of thalamic-fibre outgrowth, arrival, accumulation and ingrowth. For most experiments, thalamic blocks were taken from the region of the putative lateral geniculate nucleus (LGN), and cortical slices were taken from the occipital region, which is assumed to be the precursor of the primary visual cortex. The thalamic explants were pre-incubated with 1,1'-dioctadecyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate (diI) to label outgrowing axons. Three patterns of axon growth occurred, depending on the age at which the cortical slice was taken.

**The cortex exerts a remote growth-promoting influence from the earliest stage**

When isolated thalamic explants are cultured in serum-enriched medium, there is considerable (and variable) outgrowth of neurites. This is not obviously enhanced by the presence of a nearby slice of cortex, which led Bolz and colleagues to conclude that the cortex does not produce diffusible substances that affect extension of thalamic axons. However, serum contains a variety of factors that can stimulate growth of neurites, making it difficult to interpret any additional effect from an added cortical slice. In serum-free medium, there is very little extension of neurites from a thalamic block cultured alone, but if a cortical slice is placed within about 2 mm when cultured on a collagen-coated membrane (or 4 mm on laminin), axons stream out of the thalamic explant on all sides, and a mass of fibres fills the gap between the explants. This target-dependent outgrowth of axons is seen even for E15 rat cortical slices, suggesting that the cortex produces a diffusible substance that has a growth-promoting influence on thalamic axons, from the beginning of formation of the cortical plate until after the age at which innervation is complete.

There is no evidence for an explicit effect on the direction of growth of individual axons that would be indicative of a true tropic influence. However, if the culture chambers were left undisturbed, neurites usually extended further from the explant, and more densely on the side facing the cortical slice, presumably because of the establishment of a concentration gradient. Thus, in vivo, the diffusible substance might not only initiate outgrowth of axons from the thalamus but also influence its direction, perhaps enhancing growth towards the primitive internal capsule.

**The cortex becomes growth permissive at the end of the natural waiting period**

When an E15–16 rat thalamic explant is cultured with a cortical slice taken before about E20, thalamic axons grow out across the floor of the culture chamber at about 1 mm per day. When the axons reach the slice, most of them encircle it, and some run over its surface, but few invade it (Fig. 3), suggesting that thalamic fibres do not find early embryonic cortex an attractive environment for growth.

At about E19–20 in the rat, the tissue of the cortical slice becomes growth permissive, and it is penetrated readily by thalamic axons. With a cortical slice taken at E20, P3 fibres grow profusely out of an E16 thalamic block, through the ventricular surface of the slice and onward, in a radial direction, across the intermediate zone and subplate, and into the cortical plate itself. Growing at approximately 1 mm per day, they pass through the superficial layer of newly arrived immature neurones (the dense cortical plate),

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**Referring to the image**

**Fig. 2. Thalamic axons arrive, wait and invade in normal and reeler mice.** These fluorescence micrographs of the lateral part of the occipital cortex (lateral is up; ventral to the left) show the sequence of events as thalamic axons approach, pause and enter the cortical plate in the normal mouse (left) and in the mutant reeler mouse (right), in which the cells of the preplate remain as the 'superplate' above the arriving cells of the cortical plate, rather than being split into marginal zone (mz) and subplate (sp). Thalamic axons have been labelled with a crystal of 1,1'-dioctadecyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate (diI) placed in the dorsal thalamus. (A and D) At embryonic day 14.5 (E14.5), they are approaching the subplate in the normal mouse but are growing in oblique fascicles through the cortical plate in the reeler mouse. (C and D) At E18 (three days before birth), they are still concentrated densely in the subplate layer in the normal mouse but have paused in the superplate of the reeler mouse. Before birth in both phenotypes, they start to leave the waiting compartment and enter the cortical plate, turning up in the normal mouse and down in the reeler mouse. By postnatal day three (P3), most thalamic axons have terminated in layer 4 or its equivalent in the reeler mouse. Scale bars, 200 μm for A, B, E and F; 100 μm for C and D.
and reach the marginal zone after about three to four days in culture, without terminating or even hesitating en route (Fig. 3). Some axons then turn and run tangentially through the cortex but many penetrate the surface, and run along it, directly below the pia itself, forming a fasciculated band of fibres that is similar to the circling axons that are observed at earlier stages.

**Layer 4 expresses a 'stop signal' after about P2−3**

In rat cortical explants taken after about P2, thalamic axons grow less quickly. After only four days in culture, most of them have branched locally, lost their growth cones, and terminated approximately 300 μm below the pial surface, at a depth corresponding to layer 4 (Refs 17,29,31,33 and 39) (see Fig. 3). A few axons extend up to the marginal zone, as observed in vivo.

It has been suggested that around two to three days after birth in the rat, layer 4 starts to express a 'stop signal' that causes thalamic fibres to branch and terminate in vivo31. The failure of most thalamic axons to grow beyond layer 4 in cortex that is older than P2 is not due merely to the later-forming upper layers of the cortex lacking growth-permissive properties, since axons grow readily down through the upper layers, and terminate in the presumptive layer 4 if the thalamic block is placed against the pial surface of the cortical slice31,32.

**Thalamic innervation in vitro is not obviously regionally selective**

In principle, chemospecific variation between regions in the cortex might determine the innervation of cortical areas by particular thalamic nuclei. However, it is difficult to imagine how regional differences in a diffusible growth-promoting substance could establish such ordered patterns of thalamic outgrowth as are observed in vivo (see Fig. 1). Furthermore, the growth-permissive property is not expressed until about E20, three or four days after thalamic axons have distributed themselves in a fairly precise pattern under the cortical plate (Fig. 2).

Despite these reasons for scepticism, the possibility of regional chemoaffinity was tested by culturing thalamic blocks in a choice paradigm in which a central thalamic explant was flanked by two slices, one from the appropriate target area, and the other from an inappropriate region33. These procedures were capable of revealing target preferences: when confronted with a slice of E18–P6 cerebellum, small numbers of thalamic axons encircled the slice but very few penetrated it. However, when flanked with two different explants of neocortex, one from the correct target, and the other from a region that the chosen region of thalamus would never approach in vivo, there were no obvious differences in the patterns of innervation34 (Fig. 4). This experiment does not exclude entirely the
possibility of a subtle gradient of signals across the cortex, nor the expression of regionally specific factors at the tips of early corticofugal axons (see below), but it does suggest that thalamic axons do not identify their particular region of the cortical plate on the basis of chemosensitivity. Although the cascade of cortical properties that are revealed in vitro might play a part in determining the timing of outgrowth, waiting, innervation and termination of thalamic axons in vivo, it seems that other explanations must be sought for the topographic pattern of their guidance.

Do early corticofugal projections from the preplate guide thalamocortical fibres?

Shatz and colleagues reported that neurones of the subplate (those first-generated preplate cells that remain below the thickening cortical plate) send pioneering axons to the diencephalon in cat and ferret, and suggested that these fibres might play a part in the establishment of ascending and other descending projections.

If descending fibres are entirely responsible for guiding thalamic axons to their correct cortical target regions, it should be possible to show that axons from the preplate of each cortical region pioneer a route to the corresponding thalamic nucleus, that they form their guidance projections before axons leave the thalamus, and that thalamic axons grow over the preplate scaffold to their targets.

The subplate scaffold is organized topographically.

The outgrowth and topography of the early axon projection from preplate cells can be revealed by placing small crystals of carbocyanine dye into the cortex of perfused fetal rats and mice of known gestational age and, after sufficient incubation to enable diffusion of the dye along axons, and then examining sections by fluorescence and confocal microscopy.

Immediately after the first post-mitotic neurones have migrated to the outer edge of the cerebral wall, axons can be observed leaving these preplate cells. From each region of cortex, the axons grow through the intermediate zone towards the basal telencephalon (Fig. 5). After 24 h (for fibres from occipital cortex), they curve medially beneath and through the developing corpus striatum towards the primitive internal capsule. Interestingly, the appearance of the corticofugal projection is initially indistinguishable in normal rodents and in reeler mice, because the cells of the true cortical plate (whose bizarre aberrant distribution characterizes reeler mice), are not yet present.

Labelling with parasagittal or coronal rows of dye placements of different colour shows that each region sends a quite tightly organized bundle of axons towards the internal capsule, with little or no intermixing of axons from neighbouring cortical areas even where the array narrows as it approaches the internal capsule. At this early stage, each labelled bundle has uncomplicated geometry, simply curving in an arc without twists or sharp bends and taking the shortest route through the intermediate zones. Thus, axons from the preplate do indeed seem to establish a reasonably well-ordered topographic scaffold.

Outgrowths of preplate and thalamic axons are synchronized, not sequential

Unfortunately, the second expectation for the simple theory of preplate-axon guidance of thalamic fibres is not fulfilled. Outgrowth of axons from each thalamic nucleus is approximately synchronized with that from the preplate of the corresponding cortical area, rather than being delayed until the completion of the preplate scaffold (Table 1). Implantations of dye crystals in the cortex only two days after the initial outgrowth of preplate axons (E16 for rat occipital cortex) leads to labelling of cell bodies in the matched thalamic nucleus, presumably because of retrograde diffusion along thalamic axons that have already reached the cortex on that age.

 Fibres from different thalamic nuclei project towards the internal capsule as an ordered array (Fig. 1). Even fibres that originate in an individual nucleus maintain reasonable topography, some apparently making corrective detours, as they stream towards and through the internal capsule, and into the telencephalon, without any possibility of guidance from preplate axons. Recent evidence suggests that, during this initial part of their growth, thalamic fibres might be associated with other transient axon systems. However, the preplate array could play a role during the distal part of the trajectory of thalamic axons.

Fig. 4. Thalamic axons prefer cortex to cerebellum but show no obvious regional preference within the cortex. Results of the 'choice' paradigm in which an explant of embryonic day 16 rat lateral geniculate nucleus (E16 LGN) was cultured next to two different slices (A). Camera lucida drawings show 1,1'-dioctadecyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate-labelled thalamic axons after five days in vitro. (A) Given the choice between postnatal day 6 occipital cortex (P6 OCC), which is the appropriate target, and a slice of P6 cerebellum (CER), which the thalamic does not innervate in vivo, axon invasion was restricted largely to the cortical slice, in which most fibres terminated in what appears to be layer 4, 300-400 μm below the pial surface. Of the few axons that sprouted toward the cerebellar slice, most ran around the border of the slice or in fascicles across its surface. (B) Confronted with P6 occipital cortex and frontal cortex (FRO), axons from the LGN explant showed no obvious preference, despite the fact that they do not innervate frontal cortex in vivo. In 398 co-cultures, no consistent differences were observed in the pattern or density of innervation, whatever region of thalamus and neocortex were combined. Scale bar, 1 mm.
Thalamic axons grow over the preplate axon scaffold: the 'handshake hypothesis'

We have suggested that thalamic axons meet subplate axons, and intermingle with them en route to the subplate layer\textsuperscript{17,31}. Indeed, this 'handshake' between the two arrays might be needed not only for the final phase of topographic guidance of thalamic axons but also for the initiation of the waiting period. The fact that thalamic axons in co-culture show no tendency to accumulate within the subplate layer suggests that the two axon systems need to establish some sort of intimate association in the environment where they normally meet (the basal telencephalon or intermediate zone) if, subsequently, thalamic axons are to stop and form temporary synapses within the subplate layer\textsuperscript{48}.

This handshake hypothesis can be tested simply by labelling the two axon systems with different dyes, and observing whether they intermingle. Using this test, Miller and colleagues\textsuperscript{45} concluded that the two fibre systems grow in adjacent compartments. However, interpretation is not straightforward. If the pathways are labelled too early, they will not have met; if too late, the corticofugal projection will include the axons of true cortical cells of layers 6 and even 5, which certainly follow different paths\textsuperscript{37,48}. More importantly, it is essential to label precisely matched cortical and thalamic loci. Since the spatial relationships of thalamic nuclei change somewhat during development, it is not easy to know that regions have been labelled that will correspond in the adult. After implanting different carboxycyanine dyes into the dorsal thalamus and the occipital cortex in E15–15.5 fetal rats, we too have observed labelled bundles running separate and parallel to each other. However, this can probably be attributed to misalignment of the two dye placements because, in many such experiments, intermingling has been observed\textsuperscript{16,17,44}. Although Bicknese and colleagues\textsuperscript{46} suggested that thalamic axons tend to lie superficial to preplate axons as the former approach the cortex, Bicknese and Pearlman\textsuperscript{46} have also demonstrated interdigitation of descending and ascending axons in the internal capsule.

Figure 6A shows an example of thalamic and subplate axons, running in opposite directions through the intermediate zone but contributing to the same fascicles. The theory of co-fasciculation is also supported by the fact that crystal implantation at any point in the cortex after the arrival of thalamic axons, labelling both descending and ascending projections, stains discrete, tight bundles of fibres rather than separate groups of afferent and efferent axons\textsuperscript{17} (Fig. 6B).

Evidence from the reeler mouse

The proposal that thalamic fibres complete their journey to the cortex by growing over preplate axons, and that this intimate association is the essential prelude to their accumulation within the waiting compartment, is supported strongly by the peculiar pattern of innervation in the reeler mouse (Fig. 2B, D and F). The earliest stages of formation of the preplate, the outgrowth of a topographically ordered array of descending axons from the preplate cells (Fig. 5), the outgrowth of thalamic axons through the primitive internal capsule and their tangential distribution in association with the scaffold of corticofugal fibres towards the appropriate cortical regions all occur in the reeler mouse, just as in the normal mouse\textsuperscript{39}. The two genotypes begin to diverge in appearance only after cells of the cortical plate itself start to arrive (about E15 for the occipital cortex). In the reeler mouse, cortical-plate cells gather below the 'superplate' cells, whose pioneer axons then come to lie in oblique fascicles that are surrounded by the thickening cortical plate.

The rat cortex does not become growth permissive until some days after thalamic axons arrive under the cortical plate in vivo\textsuperscript{37,38} (see Fig. 3). However, when thalamic axons in the reeler mouse reach the cortex,
they do not wait below but run up diagonally in fascicles through the cortical plate. They then gather over the superplate for some days before growing downwards and arborizing towards the bottom of the plate among cells that presumably would have been destined normally for layer 4 (Refs 26 and 27) (Fig. 2). It seems very likely that thalamic fibres grow through the hostile environment of the cortical plate by following the oblique fascicles of preplate axons laid down earlier. The superficially very unusual pattern of growth of thalamic axons in the reeler mouse, and their aberrant appearance in the adult⁹, could be explained by the same algorithm of interaction and guidance that we propose for the normal animal.

Conclusions and questions

The timing of outgrowth, accumulation, invasion and termination of thalamic axons, and the establishment of the waiting period, are at least partly regulated by a cascade of simple signals that is expressed by the cortex. The ordered outgrowths from both preplate and thalamus might depend on interaction with extracellular guidance cues or other fibre systems, but the sequence in which fibres leave the cortex and the thalamus might also play a part in establishing fibre order. Even in rodents, where the whole process of cortical neurogenesis is complete in a week or less, the anteroventral areas of the hemisphere are more than a day ahead of the caudodorsal areas in maturity⁸, and a similar temporal gradient probably exists in the developing thalamus. These gradients might impose 'программ,' patterns on the outgrowth of axons, each wave of axons being laid down on the surface of the pre-existing axon array, and thus determine the sequence in which the two fibre arrays arrive and interact in the basal telencephalon (Fig. 7). Of course, time is uni-dimensional, and cannot determine the entire two-dimensional pattern of thalamocortical innervation. The other axis of the map might depend on some sort of gradient of molecular signals on the preplate and thalamic-axon arrays, or merely on the spatial separation of parts of the growing scaffolds.

It must be emphasized that the basic topography that is established by the early guidance of thalamic axons is likely to be refined, both during and after the waiting period. This could be achieved through the selective retraction of side-branches within the subplate layer⁵, and by local refinement within the cortex through axonal rearrangement, competitive interaction, dendritic restructuring and synaptic modification, much of which is likely to depend on afferent activity⁴.

It would be wrong to disguise the many complexities of thalamocortical innervation that remain to be explained, especially in animals with larger and more complex cerebral hemispheres. However, we begin to see how the basic pattern of thalamocortical innervation might be generated by the successive combination of a number of individually simple mechanisms.
Acknowledgements
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Fig. 1. 'How chronotopy' and the 'handshake' could determine thalamocortical topography. These diagrams summarize observations on the relationship between fibres from the preplate and the thalamus and show the way in which temporal gradients of maturation could contribute to the establishment of early topography. They represent coronal sections through the left hemisphere of the rat at the level of the internal capsule (IC) of the normal rodent shortly after the appearance of the preplate (A), after the start of formation of the cortical plate in the lateral part of the hemisphere (B), and near the start of the waiting period (C). The preplate is shown as pink, the cortical plate is shown as green. Effrent projections from the cortical plate are not shown. Preplate cells (red) send out their axons towards the internal capsule in order, reflecting the ventral-to-dorsal gradient of maturation. If axons tend to grow on the surface of pre-existing axons, without crossing them, this chronological sequence of outgrowth would establish one row of a topographic array. If there is a matched temporal gradient of outgrowth from the thalamus (light blue), this could determine the order in which thalamic fibres (dark blue) and axons from the preplate of the corresponding area of the cortex meet and associate with each other in the handshake region of the basal telencephalon. This sequential establishment of contact with fibres of the preplate scaffold might aid the construction of one axis of the topographic distribution of thalamic axons to the cortex.

Z. Molnár and C. Blakemore — Development of thalamocortical innervation
Exuberant development of connections, and its possible permissive role in cortical evolution

Giorgio M. Innocenti

The callosal visual connections of the cat provide a model for studying the phenotypes of cortical axons and their differentiation. The terminal arbor of a callosal axon develops in several successive stages. At each stage, the arbor approximates the adult phenotype more closely. This is achieved through two mechanisms: (1) exuberant, but increasingly constrained, growth and (2) partial deletion of previously generated parts of the arbor. This differentiation is controlled by interactions of the axon with its cellular environment, and by visual experience. It might have played a permissive role in the evolution of the cerebral cortex by enabling adjustments of cortical connectivity to changes in the number, size, internal organization and cellular composition of cortical areas.


CORTICAL AREAS – and within each area, layers, cortical columns and types of neurones – have different and characteristic connectivity. In the course of evolution, the number and sizes of areas and layers\(^1\)–\(^2\), and the types and numbers of columns and neurones, have changed. The concomitant evolution of the connectivity patterns required flexible developmental processes. Two of these processes are retained in extant mammals\(^3\)–\(^4\). The first is specification of cortical areas by afferents, particularly the thalamic afferents. The second is exuberant axonal growth in development, that is, the fact that axons that originate or terminate, or both, in cerebral cortex initially form excessive branches and synapses; these are distributed somewhat diffusely, and refined by selective pruning (for review, see Ref. 7). The key feature of these two processes is that fundamental aspects of the cortical phenotype are not narrowly pre-programmed but instead result from interactions between cortical neurones and their cellular environment.

Here we focus on adult cortical organization and the development of visual callosal axons. Evidence suggests that several aspects of the development of this system of connections can be assumed to apply to other connections in the cerebral cortex and elsewhere\(^5\)–\(^8\). The notion of ‘axonal phenotype’ refers to an open-ended set of features, some of which are considered below with respect to their functional significance and differentiation. These features have only recently become accessible to investigation, with the introduction of new anterograde tracers that enable the visualization of individual axons, including the long axons (for examples, see Refs 8–12).

Two facets of axonal phenotype

Two facets of axonal phenotype are important because they implement computational strategies that are typical of the CNS. First is the spatial distribution of the terminal arbor, which determines the topographical relationship that the axon establishes between its parent soma and its synaptic targets. This aspect of the axonal phenotype implements the spatial transformations (mapping) between interrelated neuronal assemblies, and has been the main focus of past and recent theories of the development of neural connections (for recent reviews, see Refs 13–16). The mapping algorithm is usually complex. An axon rarely establishes ‘point-to-point’ connections. Instead, its terminal arbor diverges more or less widely within a volume where it distributes selectively to certain neurones, and to certain subcellular compartments of these neurones. The second facet of the morphological phenotype of an axon includes the diameter of the axon trunk and the length, diameter and spatial relationships of its branches\(^9\). These geometrical properties of the axon determine its conduction properties; therefore, they provide the structural basis of the temporal transformations between interrelated neuronal assemblies.

These two facets of axonal phenotype became clear in recent studies of biocytin-labelled callosal axons, interconnecting visual areas 17 and 18 of the cat. Computer techniques were used for the three-dimensional reconstruction of axons, and for the simulation of their activity\(^7,17,18\).

It was found that most callosal axons terminate in radially oriented clusters of pre-terminal branches

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