The development of cortical connections

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Abstract

The cortex receives its major sensory input from the thalamus via thalamocortical axons, and cortical neurons are interconnected in complex networks by corticocortical and callosal axons. Our understanding of the mechanisms generating the circuitry that confers functional properties on cortical neurons and networks, although poor, has been advanced significantly by recent research on the molecular mechanisms of thalamocortical axonal guidance and ordering. Here we review recent advances in knowledge of how thalamocortical axons are guided and how they maintain order during that process. Several studies have shown the importance in this process of guidance molecules including Eph receptors and ephrins, members of the Wnt signalling pathway and members of a novel planar cell polarity pathway. Signalling molecules and transcription factors expressed with graded concentrations across the cortex are important in establishing cortical maps of the topography of sensory surfaces. Neural activity, both spontaneous and evoked, plays a role in refining thalamocortical connections but recent work has indicated that neural activity is less important than was previously thought for the development of some early maps. A strategy used widely in the development of corticocortical and callosal connections is the early overproduction of projections followed by selection after contact with the target structure. Here we discuss recent work in primates indicating that elimination of juvenile projections is not a major mechanism in the development of pathways feeding information forward to higher levels of cortical processing, although its use is common to developing feedback pathways.

Introduction

The mechanisms that control the development of cortical connectivity probably include those that regulate axonal guidance and pathway refinement in simpler systems in both vertebrates and invertebrates. All mechanisms that have been proposed for axonal guidance are some combination of a few basic types of mechanism, classified according to the type of information they require. Two of these are probably involved in development of cortical connectivity. First, axons might acquire information from the environment through which they grow, including the target structure itself. Second, axons may receive insufficient guidance information during outgrowth but connections may be selected after contact with the target structure.

Most sensory input to the cerebral cortex comes via the thalamus. The early formation of the thalamocortical pathway is achieved by the growth cones of thalamic neurons navigating a complex three-dimensional pathway, initially ventrally through the prethalamus, then laterally into ventral telencephalon and finally dorsally towards cerebral cortex. Recent breakthroughs in understanding the development of this axonal pathway have involved the identification of cellular and molecular cues that help guide thalamic growth cones to their cortical targets. Work on the subsequent assembly of cortical maps, in particular feature maps encoding complex properties of the afferent system (such as ocular dominance in the visual system), has generated surprising new findings. Here we review advances in these two important areas.

The majority of inputs onto cortical neurons arise from other cortical neurons, either in the same hemisphere (corticocortical connections) or in the opposite hemisphere (callosal connections). The development of these pathways has been studied in a broad range of species over several decades (Innocenti & Price, 2005). Recent work in primates has identified the ways in which these pathways probably form in more complex brains while studies in rodents have started the hunt for the molecular mechanisms regulating their development. We review new insights gained from research on these topics.

The process by which axons innervate specific targets requires a way of guiding their trajectories, stopping growth at the target and, if necessary, eliminating entire axons or axonal processes that are somehow deemed to be in the wrong place (Price & Willshaw, 2000). In principle, the mechanisms for these tasks can use information from sources either intrinsic or extrinsic to the cell projecting the axon. Most hypotheses, however, consider that growing axons receive information from cues presented by their environment en route to, or at, their targets. Growth cones are programmed to respond to specific
Guidance of thalamocortical and corticothalamic axons through the early subdivisions of the forebrain

Regulating the navigational ability of thalamocortical growth cones

The early outgrowth of axons from the thalamus (Fig. 1) is probably directed by molecular cues between the thalamus and cortex (Molnár & Blakemore, 1995; Vanderhaegen et al., 2000; Fukuchi-Shimogori & Grove, 2001; Marin, 2003; Seibt et al., 2003; Garel & Rubenstein, 2004). Thalamic axons must be able to respond to such cues. The specification of thalamic cells is achieved during the early stages of forebrain patterning, before thalamocortical axons begin to grow. It involves the expression of two transcription factors, Pax6 and Gbx2, whose presence is required for thalamocortical development (Kawano et al., 1999; Miyashita-Lin et al., 1999; Pratt et al., 2000, 2002; Hevner et al., 2002; Jones et al., 2002). Gbx2 expression is restricted to the thalamus, which makes it probable that this gene is required within thalamic cells for them to respond to navigational cues (Miyashita-Lin et al., 1999; Hevner et al., 2002). Pax6 is expressed not only in the thalamus but also along the pathway taken by thalamocortical axons and in the cortex itself (Stoykova & Gruss, 1994; Pratt et al., 2000; Jones et al., 2002). Experiments by Pratt et al. (2000), in which mutant thalamus was confronted by wild-type telencephalon in vitro, indicated that thalamic cells lacking Pax6 have an inability to respond to navigational cues in the environment they grow through. A current hypothesis is that Pax6 and Gbx2 regulate the expression of downstream molecules essential for the thalamocortical axonal growth cone’s ability to navigate. It is not known what these downstream molecules are, although there is some information on which to base reasonable speculation. Work on the functions of Pax6 in the cortex and thalamus has shown that loss of Pax6 causes cell-autonomous defects of cell–cell adhesion (Pratt et al., 2002; Talamillo et al., 2003) and appropriate cell–cell adhesion is essential in axon guidance. Pax6 is required for normal cortical expression of members of the cadherin family of calcium-dependent cell adhesion glycoproteins (Stoykova et al., 1997; Bishop et al., 2000) and some semaphorins, including Sema3C and Sema5A (Jones et al., 2002). Cadherins present in the developing thalamocortical system include cadherin-6 and cadherin-8 (Rubenstein et al., 1999); their expression in the Pax6−/− thalamus has yet to be investigated.

Navigating thalamocortical growth cones interact with boundaries and reciprocal axonal tracts

As they advance, thalamocortical axons cross many anatomical and gene-expression boundaries between the thalamus and the cortex (Fig. 1). Thalamic fibres change their growth kinetics and fasciculation...
patterns as they traverse these distinct sectors of the embryonic forebrain. Studies in various mouse mutants have revealed at least two especially critical zones regulating thalamocortical axonal outgrowth in the embryonic forebrain (Hevner et al., 2002; Jones et al., 2002; López-Bendito et al., 2002; Garel & Rubenstein, 2004). One of these zones is at the diencephalic–telencephalic border and the other is at the pallial–subpallial boundary (PSPB, Fig. 1). Altered gene expression along the thalamocortical path can not only modify growth kinetics and fasciculation patterns but can also arrest growth at specific sites. If early developmental steps are perturbed, thalamic projections are often derailed at these sites and follow characteristic default pathways (López-Bendito & Molnár, 2003).

It has been suggested that the exit of thalamic axons from the diencephalon is dependent on the earlier development of normal projections from transient cells in the internal capsule (Métin & Godement, 1996). If this pathway is displaced (e.g. in Emx2+/-; López-Bendito et al., 2002) or missing (e.g. in Mash1−/−, Foxgl−/− or Pax6−/− mutants; Tuttle et al., 1999; Jones et al., 2002; Pratt et al., 2002), thalamic projections are derailed as they exit or are no longer able to exit at all.

The second region crucial for the early development of thalamocortical projections is the PSPB (Molnár & Butler, 2002). The early corticofugal projections reach and cross the PSPB before the thalamic cortical projections is the PSPB (Molnár & Butler, 2002). The early development of thalamocortical axons and fasciculation patterns but can also arrest growth at specific sites. If early developmental steps are perturbed, thalamic projections are often derailed at these sites and follow characteristic default pathways (López-Bendito & Molnár, 2003).

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The second region crucial for the early development of thalamocortical projections is the PSPB (Molnár & Butler, 2002). The early corticofugal projections reach and cross the PSPB before the thalamic projections (Fig. 1). They are thought to play an important role in the subsequent deployment of thalamic afferents through this region (Molnár & Blakemore, 1995). It has been postulated in the ‘handshake hypothesis’ that projections from the thalamus and from the early born cortical preplate cells intermingle in the basal telencephalon, such that thalamic axons can advance through the PSPB by contacting the scaffold of preplate axons (Molnár et al., 1998a). Although their contact is now well documented, it is important to address whether both sets of fibres depend on each other to advance normally toward their targets, or whether the growth of each set is controlled autonomously from within the cortex or thalamus. Direct evidence could be obtained from experiments where subplate projections are selectively eliminated before the arrival of thalamocortical axons in the internal capsule. Experimental findings to date in numerous mutant mice do, however, indicate that interactions between these axons are probably critical for guidance of thalamocortical fibres.

Mice lacking Tbr1, a transcription factor expressed in the cortex, show errors not only in corticothalamic but also in thalamocortical pathfinding within the region of the internal capsule (Hevner et al., 2001, 2002). Further support for the idea that it is necessary for thalamic axons to form an intimate relationship with the scaffold of preplate axons comes from studies on the reeler mouse and the Shaking Rat Kawasaki (Molnár et al., 1998a; Higashi et al., 2005). In normal embryos, at the time when the thalamic projections accumulate within the subplate, during the so-called waiting period, the overlying cortical plate is not permissive for thalamic fibre ingrowth (Hübener et al., 1995; Molnár & Blakemore, 1995, 1999). Nevertheless, in the reeler and Shaking Rat Kawasaki, thalamic axons do cross the cortical plate at this stage. The reason for this might stem from the observation that subplate cells in these mutants are displaced to form a superplate whose fibres descend through the cortical plate. These fibres might permit ascension thalamocortical axons to associate with them and so penetrate the cortical plate (Molnár et al., 1998b; Higashi et al., 2005). The importance of an interaction between corticothalamic and thalamocortical axons might explain why, in COUP-TFI mutant mice, premature subplate death is associated with the inability of most thalamocortical axons to grow out of the internal capsule, project into the intermediate zone and innervate the cortex (Zhou et al., 1999).

**Molecular cues important for navigating thalamocortical growth cones**

The population of thalamocortical axons rotates by 90° as it enters into the internal capsule and approaches the cortex, such that medial thalamic nuclei target anterior and lateral nuclei more posterior cortical regions. Anteroposterior movement in the thalamus corresponds to ventrodorsal shift in cortical targets. This simple correspondence is present from the time of thalamic fibre arrival, before the differentiation of individual thalamic nuclei (Molnár & Blakemore, 1995; Molnár et al., 1998a, b; Fig. 3A and B). These simple relationships must be considered when molecular gradients involved in thalamocortical guidance are considered.

Relatively little is known about the molecular cues that guide thalamocortical axons. There is evidence for the involvement of a variety of membrane-bound or diffusible molecules, including ligand-associated membrane protein (LAMP), cadherins, ephrins and Eph receptors, neurotrophins, netrin 1 and semaphorins (reviewed by López-Bendito & Molnár, 2003). Defects in embryos with null mutations in the corresponding genes are often subtle, indicating that these molecules either have minor roles in the development of thalamocortical axons or that they act in combination. Recent work has shown much more drastic anomalies in mice with inactivation of Celsr3, a gene encoding a protocadherin (Tissir et al., 2002, 2005), and Fzd3, a member of the Frizzled family encoding receptors for Wnt ligands (Wang et al., 2002).

The coordinated organization of cells within the plane of an epithelium, as first described in *Drosophila*, is referred to as planar cell polarity (PCP) and is controlled by mechanisms including a Frizzled signalling pathway. Both this and the classical Wnt signalling pathway act through Dishevelled and small G proteins, but then diverge downstream of Dishevelled (Pandur et al., 2002). In *Drosophila*, flamingo/starry night (fmi/stan), frizzled (fl), dishevelled (dsh), Van Gogh/strabismus (Vang/stbm) and prickle (pk) are implicated in the control of PCP, and form the group of so-called ‘core PCP genes’ (Chae et al., 1999; Lu et al., 1999; Usui et al., 1999; Adler & Lee, 2001; Lee et al., 2003; Senti et al., 2003; Fanto & McNeill, 2004; Amonlirdviman et al., 2005). The orthologs of core PCP genes in mice are Celsr1–3 (orthologs of fmi/stan), Fzd1–10, dishevelled 1, 2 and 3 (Dvl1–3) (Beier et al., 1992; Sussman et al., 1994; Lijam & Sussman, 1995; Greco et al., 1996; Tsang et al., 1996), Van Gogh-like 1 and 2 (VanGl1.2) (Kibar et al., 2001; Park & Moon, 2002; Katoh & Katoh, 2005) and prickle-like 1 and 2 (Bekman & Henrique, 2002; Katoh, 2003). In mice, inactivation of the PCP orthologs Celsr1 (Curtin et al., 2003) or VanGl2 (Kibar et al., 2001; Murdoch et al., 2001), or double mutations of Dvl1 and 2 (Hamblet et al., 2002), all perturb neural tube closure. Recent findings indicate that Celsr3 and Fzd3 are the first identified members of another PCP-like pathway which regulates connectivity between thalamus and cortex.

*Celsr3* and *Fzd3*-mutant mice develop normally but die within hours after birth of central ventilation failure (Wang et al., 2002; Tissir et al., 2005). In mutant animals the cortical wall is abnormally thin, mostly due to hypotrophy of the intermediate zone. The anterior commissure and internal capsule are absent, and no fibre bundle is found crossing the striatum (Fig. 2A–F). Neurofilament staining discloses the presence of a large aberrant tract that originates from the mutant thalamus but, instead of making a sharp turn to enter the internal capsule, descends ventrally and turns around the basal and lateral forebrain before entering the cortical marginal zone. Defects are also seen in other axonal pathways. In the spinal cord, early commissural fibres cross the midline normally at embryonic day (E)11.5 but, instead of turning rostrally as they do in normal embryos,
Celsr3- and Fzd3 (Lyuksyutova et al., 2003)-mutant axons stall after crossing and turn rostrally or caudally (Fig. 2G and H). Presumably, such anomalies of longitudinal association bundles in the hindbrain and spinal cord could contribute to defective ventilation and neonatal lethality.

In Celsr3- and Fzd3-mutant mice, the neocortex is entirely disconnected from subcortical structures, like a ‘cortex isolé’ in vivo.

Thalamic axons fail to reach their target areas in the cortex and corticofugal axons stall and degenerate in the intermediate zone and future white matter. Thalamic fibres depart at the normal time but, instead of turning towards the internal capsule, they continue ventrally along the hypothalamus and reach the basal forebrain. Axons from mutant cortical neurons originate normally, from the inferior pole of the cell, and course radially towards the white matter in which they run.

**Fig. 2.** Phenotype of normal and Celsr3-mutant mice. (A–F) At E15.5, carbocyanine dye (DiI) injections in temporal and basal cortical areas result in visualization of the anterior commissure in (A and F) normal but not in (B) mutant animals. Injections in the ganglionic eminence result in transport to the cortex and thalamus of (C and F) normal but not (D) mutant embryos. E and F are close views of areas boxed in A and C. Cx, cortex; Th, thalamus; AC, anterior commissure; GE, ganglionic eminence. (G and H) Injections in the spinal cord at E11.5 (*) label commissural axons in both genotypes, axons which (G) cross the midline (dotted lines) and turn rostrally (arrowheads) in normal embryos, but (H) fail to do so in mutant embryos.
tangentially. Preliminary evidence suggests that they fail to selectively
direct their growth cones towards the ganglionic eminence. In contrast
to normal cortical efferent fibres (Sheth et al., 1998), mutant axons
arrive at the PSPB boundary at E13.5, but they fail to progress further
and stall in the white matter where they seem to degenerate.

These findings indicate that Wnt/Frizzled signalling pathways are
critically important for the development of reciprocal connections
between the cortex and subcortical structures. Wnt/Frizzled path-
ways are well known for their diverse roles in normal tissue
morphogenesis and cancer but their roles in axon guidance are less
extensively studied. This area is probably a subject for increased
research in the future. The possibility that these molecules
simply act on forebrain patterning should be further examined.

The pallial–subpallial boundary and the telencephalic and dience-
phalic junctions are critical regions and slight variations in
patterning could elicit secondary changes in axon growth through
this region. The challenge is to dissociate these two roles in future
paradigms.

Development of thalamocortical topography and the roles
of intermediate targets

The thalamus and the cerebral cortex are interconnected in an ordered
fashion, such that adjacent cells in the presynaptic structure project,
as a general rule, to adjacent structures in the postsynaptic structure.

A start has been made towards discovering the molecular mechanisms
that regulate the development of this topography. One set of studies
has examined whether the molecular patterning of target cells in the
developing neocortex is important for the mapping of thalamocortical
projections onto it. This has been done by altering or abolishing the
normally graded expression of transcription factors and signalling
molecules across the cortical surface (Bishop et al., 2000, 2002,
2003; Fukuchi-Shimogori & Grove, 2001, 2003; Hamasaki et al.,
2004). The transcription factors Emx1 and Emx2 are expressed in
highly caudomedial to low rostral gradient in the neocortex, whereas
the transcription factor Pax6 is expressed in an opposite high
caudal to low rostral gradient. Loss of Emx expression results in a
caudomedial shift of cortical areas whereas loss of Pax6 results in
an opposite caudal to rostral shift in the cortex (Bishop et al., 2000).

Overexpression of Emx2 in cortical progenitors also produces a rostral shift of
cortical areas (Hamasaki et al., 2004). Evidence from Fukuchi-
Shimogori & Grove (2001, 2003) has indicated that FGF8 from a
source in rostral telencephalon regulates development of the cortical
areal map. Increasing this rostral FGF8 signal shifts areas caudally;
reducing the signal moves them rostrally anteriorly; introducing an
additional caudal source of FGF8 causes areal duplications.

Thalamocortical axons are ordered as they grow through the
ventral telencephalon towards the cortex, indicating that topography
is present in the tract before axons arrive at their final target
(reviewed in Garel & Rubenstein, 2004). It has been suggested that
interactions with corticofugal axons, with cells in the ventral
telencephalon and between growing thalamocortical axons them-

selves all play a role in establishing and maintaining this topography
(reviewed in López-Bendito & Molnár, 2003). Recent evidence has
indicated that members of the Eph/ephrin family of guidance
molecules are required for subcortical ordering of thalamocortical
growth (Dufour et al., 2003). Ephrin A5 has a gradient of expression
in the intermediate tissue through which thalamocortical axons grow
and EphA3, EphA4 and EphA7 have gradients of expression in the
thalamus. Analysis of mutant mice has shown that Eph/ephrin genes
control the topography of thalamocortical axons by sorting them in
the ventral telencephalon (Dufour et al., 2003). It is probable that
multiple molecular and cellular cues between the thalamus and the
cortex establish and refine topographic projections between these
structures.

Transition to the activity-dependent phase
of thalamocortical development

Is activity important for early targeting
of thalamocortical axons?

The second phase of thalamic development is believed to be
increasingly dependent on neural activity. Exactly when this phase
begins is not strictly defined. It is probable that a requirement for
neural activity develops gradually as neurons first develop sponta-
neous activity and later start to respond to stimuli in the external
environment. Peripheral sensory organs can generate patterns of
spontaneous activity at the time when sensory afferents first reach the
thalamus (Galli & Maffei, 1988; Meister et al., 1991). Functional
neural circuitry is already in place to relay these patterns to cortex
(Friauf et al., 1990; Friauf & Shatz, 1991; Herrmann et al., 1994;
Mooney et al., 1996; Hangata et al., 2002; Molnár et al., 2003). In
rodents this circuitry is present from before birth, as shown by optical
imaging using voltage-sensitive dyes using selective thalamic stimu-
lation in thalamocortical slice preparations from prenatal and perinatal
rodents (Higashi et al., 2002, 2005). Thus, early spontaneous neural
activity could influence early thalamocortical deployment in the

cortex. Direct evidence that neural activity is required for initial
targeting decisions made by thalamic axons as they traverse the
subplate, prior to their arbor formation in the cortex, came from work
by Catalano & Shatz (1998). When tetrodotoxin (TTX; a sodium
channel antagonist that blocks action potentials) was delivered into
the brain of cat fetuses at the time of arrival of thalamic projections to
the subplate, abnormal connections were established by geniculocortical
axons. Only a few thalamic fibres entered the visual cortex and an
aberrant topography was formed within the cortical plate. The exact
nature of the required neural activity is not known.

In mice, work on the role of activity in early target recognition has
come to a different conclusion. SNAP-25, together with syntaxin-1
and VAMP-2, form the core SNARE complex, which plays an
essential role in exocytotic release of neurotransmitter (Südhof, 1995).

Snap-25–/ mice develop to term, and fetal brain development appears
superficially normal, even though evoked neurotransmitter release is
entirely eliminated (Washbourne et al., 2002). SNAP-25-deficient
neurons extend axons that terminate in synapses where spontaneous,
action potential-independent release still occurs but action potentials
do not trigger neurotransmission. Thus, genetic ablation of SNAP-25
expression appears to selectively disable the vesicular processes
responsible for evoked synaptic transmission, leaving intact membrane
trafficking, for axon outgrowth, and excytosis, for spontaneous
neurotransmitter secretion. The development of thalamic cortical
projections in Snap-25–/ mice has been examined using carbocyanine
dyes (Molnár et al., 2002). These experiments demonstrated that
axons reach the cortex and start to invade it according to a normal
areal distribution in the cortex (Fig. 3). Further work is required to
examine whether there are species differences in the requirement
for neural activity in these processes or whether the different outcomes in
TTX-treated and Snap-25–/ embryos reflect different cellular effects
of these ablations.

Several in vivo and in vitro experiments have demonstrated the role
of neural activity in the termination of thalamocortical axons in
cortical layer 4 (Wilkermercy & Angelides, 1996; Catalano & Shatz,
The development of some of the complex circuitry required to give cortical neurons their receptive field properties, such as orientation and direction selectivity in the visual cortex, requires patterned activity rather than merely activity of sufficient level (Weliky & Katz, 1997). Work on the development of other features of cortical organization has suggested that neuronal activity is not required for the development of all receptive field properties and feature maps. Early studies of the formation of ocular dominance columns in the primary visual cortex suggested that geniculocortical terminals representing the two eyes are initially intermingled and segregate later into ocular dominance columns under the influence of retinal activity, either patterned or spontaneous (LeVay et al., 1978; Stryker & Harris, 1986; Katz & Shatz, 1996). However, recent work has challenged this conclusion.

The use of new tracing methods has shown that geniculostriate projections form ocular dominance columns at very early stages of development and, contrary to what was previously thought, column formation is very rapid in species such as ferret (Crowley & Katz, 2000; Crair et al., 1998; Fig. 4). Initially formed ocular dominance columns are resistant to imbalances in ascending activity, suggesting that the formation and later plasticity of ocular dominance columns are temporally and mechanistically distinct phenomena (Crair et al., 1998; Issa et al., 1999; Crowley & Katz, 2000; Crowley & Katz, 2002; Sengpiel & Kind, 2002). Moreover, Crowley & Katz (1999) showed that ocular dominance columns develop in the absence of any retinal input. In primates, Horton & Hocking (1996) showed that macaques delivered 1 week preterm in the dark have normal cortical ocular dominance patterns, suggesting that visual experience is not necessary for OD segregation. These findings suggest that mechanisms employing molecular cues might establish the ocular dominance columns.

Activity and the formation of feature maps in the visual system

Thalamocortical axons are deployed in an organized way in the cortex, not only to create topographic cortical representations of the order of cells in the thalamus (discussed above), but also to generate feature maps. These are more complex maps in which some attribute or feature of the activity in the presynaptic structure is represented in the cells of the target. Examples are the mapping of right and left eye inputs onto the visual cortex to form ocular dominance columns, or the ordered arrangement of orientation selective cells in the visual cortex. It has been known for decades that these maps can be altered by altering neural activity during brain development (Wiesel, 1982) and that activity, both spontaneous and patterned, has an important function in the normal development of cortical feature maps and receptive field properties (reviewed by Sengpiel & Kind, 2002).

Fig. 4. Two models for the development of ocular dominance columns. (A) In this model, the initially sparse innervation of the cortex by axons conveying information from the two eyes (right eye, shaded; left eye, solid) develops into extensively overlapping innervation. Through a process of retraction and regrowth, this resolves into segregated innervation. (B) In this model, the initially sparse innervation is elaborated by further growth such that segregation is maintained throughout (adapted from Crowley & Katz, 2002). Recent evidence indicates that cortical innervation develops as in model B in some species, including ferrets, but primate development may proceed closer to model A.

Fig. 3. Topography of thalamocortical projections in wild-type and Snap-25−/− rodents. (A) Injections of two different carbocyanine dyes side-by-side in the mediolateral direction in a wild-type E16 rat embryo produced the labelling shown in the horizontal section in (B). The two patches of thalamic label are orientated rostrocaudally. (C) Three injections of carbocyanine dyes in the rostrocaudal direction in a wild-type E16 rat embryo produced three patches of labelling orientated mediolaterally (D). The mapping of the thalamus and cortex are at ~90° to each other. (E) A similar topography in the connections between thalamus and cortex is seen in E16 Snap-25−/− mice, revealed by injections made as in C. Data from Molnár & Blakemore (1995); Molnár et al. (2002).

1998; Anderson & Price, 2002). Again, the exact role of neural activity is unclear. It may alter the expression of molecular factors, which subsequently mediate growth cone collapse and hence axon growth termination. Several cell surface and extracellular matrix molecules, including cadherins, semaphorins, Eph receptors and ephrin ligands, and chondroitin and heparan sulphate proteoglycans, are expressed in a lamina-specific fashion. These might account for some of the molecular differences between the cortical layers that influence the termination of thalamocortical projections (for review see Yamamoto et al., 2000; López-Bendito & Molnár, 2003).
The use of new labelling methods has led to similar conclusions regarding the formation of eye-specific layers in the lateral geniculate nucleus (LGN) (Rakic, 1976; Linden et al., 1981; Shatz, 1983). Study of this system has suggested that precise patterns of connectivity emerge from initially diffuse connections (Katz & Shatz, 1996; Stellwagen & Shatz, 2002). The segregation of retinal input is thought to depend on a dynamic and competitive interaction between left and right eye axons (Rakic, 1981; Rakic & Riley, 1983; Chalupa et al., 1984). Early experiments on binocular overlap in primates concluded that segregation of input from each eye occurs between E78 and E124. This work employed monocular injections of tritiated amino acids (Rakic, 1976) that can diffuse to label a broader region than that innervated from the injected eye. Using modern cholera toxin tracing methods, it has been shown that retinal axon segregation in the LGN is present at much earlier stages than previously thought (Huberman et al., 2002, 2005). Molecular signals might allow axons originating from temporal and nasal retina to sort out into right and left eye-specific layers.

The development of intracortical connections

Cortico-cortical axons interlink cortical regions on the two sides of the brain via the corpus callosum, and on the same side of the brain (ipsilaterally). A major strategy employed by many developing intracortical pathways in many species is the initial overproduction of projections followed by the selective elimination of some to yield the mature patterns of connections. For example, numerous studies in rodents, carnivores and primates have identified transient connections in the callosum of visual and somatosensory cortices, from auditory to visual cortex and between adjacent areas of the visual cortex (reviewed in Innocenti & Price, 2005; see also Fig. 5). The refinement of connections creates elaborate networks connecting specific sets of neurons together in a functionally appropriate way, for example linking regions with similar receptive field locations in the same or between the two cerebral hemispheres. The extent to which selective elimination of initially overabundant connections shapes the physiological function of the cerebral cortex has been addressed by recent work in primates. This work has tested whether the selective elimination of connections plays a profound role in shaping the hierarchical organization of the cortex, thereby determining the fundamental basis on which it functions.

Remodelling of connections and the development of the cortical hierarchy

Hierarchical organization of information processing in the brain has been an important issue in neurology since the work of Hughlings Jackson in the 1880s. The relevance of hierarchical processing for understanding both the physiology and the connectivity of the visual system was formalized in the work of Hubel & Wiesel (1962). More recently, a number of conceptually important studies have used mathematical treatment of connectivity data to address the hierarchical organization of the cortex using various combinations of graph theory and nonmetric multidimensional scaling (Young, 1992; Jouve et al., 1998; Clavagnier et al., 2004). In considering the hierarchical organization of the cortex, two types of projection can be defined: (i) those that feed information forward from the cortical inputs to higher levels; and (ii) those that feed information back the other way (Lund et al., 1975; Rockland & Pandya, 1979; Maunsell & van Essen, 1983; Kennedy & Bullier, 1985; Barone et al., 2000; Falchier et al., 2002). Feedback pathways originate largely from infragranular layers whereas feedforward pathways originate largely from supragranular layers. Knowledge of the laminar distributions of the cells of origin of a number of cortico-cortical pathways, i.e. the exact proportions of parent neurons that are supragranular vs. infragranular, allows them to be placed into a hierarchy (Barone et al., 2000; Felleman & van Essen, 1991).

Fig. 5. Development of cortico-cortical projections from area 17 to area 18 in the cat. (A) Injections of retrogradely transported tracers were made into area 18 of the visual cortex and brains were sectioned (broken line). (B) In neonates, injections (filled area) resulted in labelling of cells in continuous bands in superficial and deep cortical layers. Arrow marks the area 17/18 border. (C) By 3 weeks of age, cells projecting from area 17 to area 18 are distributed in patches and arise mainly from superficial layers. This developmental change is caused by the loss of early exuberant projections (Price & Blakemore, 1985a,b).
In cat, the immature cortex shows an absence of a hierarchical organization based on this concept (Batardiere et al., 1998). In primates, the laminar distributions of parent neurons of cortico-cortical pathways emerge from an early stage in which there is an excess of supragranular neurons (Kennedy et al., 1989). In developing cortico-cortical connections in monkey there is a concomitant increase in the numbers of infragranular projections and a decrease in the numbers of supragranular projections (Batardiere et al., 2002). The truly surprising result is that, despite the massive reorganization, there is a clear specification of hierarchy in the prenatal monkey cortex.

In developing primates, cortical areas which project to area V1 show 45–90% of supragranular layer neurons participating in cortical feedback pathways (Kennedy et al., 1989; Barone et al., 1995). In other work, it was shown that feedback pathways from the 14 cortical areas which project to area V4 exhibit 28–84% reduction in numbers of upper layer neurons. Thus, the developmental remodelling of cortico-cortical pathways is a distinctive feature of feedback pathways (Batardiere et al., 2002). In contrast, the development of the feedforward pathway from area V2 to area V4 has been shown to be complete early in prenatal life, to depend largely on directed growth and target recognition mechanisms and not to involve the large-scale elimination of inappropriate axons (Barone et al., 1998). Supragranular layer neurons constitute the major component of the feedforward pathway and their axons accurately target their final destination early in development. Despite the changes in the feedback projections, it was observed that the relative hierarchical organization of the visual system throughout development is similar to that in the adult. The early prenatal specification of feedforward connections could provide the neurophysiological substrate for the steady increase in visual capacities observed in infant monkeys during the first postnatal months (Blakemore et al., 1981). However, the adult laminar organization of feedback pathways is not present before the second postnatal month. These results show that feedback and feedforward projections develop by different processes but that hierarchical relations are established prenatally and independently of regressive phenomena. Given the evidence of the involvement of feedback projections in figure–ground discrimination (Zipser et al., 1996; Hupe et al., 1998), it is interesting to note that this psychophysical response emerges at the end of the first year of life and only becomes adult-like at ∼8–13 years of age in human (Sireteanu & Rieth, 1992). The searching question that remains is why would feedback pathways in the visually experienced infant include 28–84% additional supragranular layer projection neurons?

Mechanisms regulating the remodelling of connections

The mechanisms controlling the growth and, in many cases, remodelling of cortico-cortical and callosal pathways are unclear. Several factors have been implicated in the selection of projections from an early exuberant set. These include neuronal activity from the periphery and competition between axons for target-derived chemotrophic substances (reviewed in Innocenti & Price, 2005). Recent work has begun to study the expression of molecules that might identify targets for innervation by growing cortico-cortical axons or might cause the persistence of some axons and not others. At present, ideas about the way in which these molecules might operate are speculative.

Molecules that are expressed either in specific areas of developing cortex or with concentration gradients across the cortical surface have been described. These include signalling molecules (e.g. Eph/ephrins) and transcription factors (e.g. Emx2 and Pax6) and there is evidence that molecules such as these identify specific cortical areas to incoming thalamocortical afferents (Bishop et al., 2000, 2003; Sestan et al., 2001). It is conceivable that they do the same for developing cortico-cortical and callosal axons, allowing them to find their targets and/or selecting them as persistent projections. A recent study showed that the segregation of cortico-cortical connections into rostral and caudal sets in newborn mice corresponds with regional differences in the expression of the transcription factor Id2 and the orphan receptor RzRβ (Huffman et al., 2004). Further research is needed to discover whether the selection of cortico-cortical axons is affected in the cortex of mutants that lack or have altered expression of molecules crucial for cortical regionalization.

Conclusion

Cortical connections develop through the combined actions of activity-independent and activity-dependent mechanisms on the navigation of growth cones and the establishment and refinement of synaptic connections. It is widely accepted that activity-independent mechanisms predominate during the early phases of the generation of cortical circuitry. Interactions between growing axons and other cell types and guidance molecules are critical during early axonal pathfinding. Recent studies have indicated that spontaneous activity might also be required at these early stages of axon targeting. Later, stimulus-driven activity-dependent mechanisms operate to refine cortical connections. The pruning of initially exuberant cortico-cortical connections is a widespread phenomenon in cortical development, although recent work has indicated that it might not be a feature of developing feedforward projections in primates.

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Abbreviations

E, embryonic day; fz, frizzled; PCP, planar cell polarity; PSPB, pallial–subpallial boundary.; TTX, tetrodotoxin.

References


