EXUBERANCE IN THE DEVELOPMENT OF CORTICAL NETWORKS

Giorgio M. Innocenti and David J. Price‡*

Abstract | The cerebral cortex is the largest and most intricately connected part of the mammalian brain. Its size and complexity has increased during the course of evolution, allowing improvements in old functions and causing the emergence of new ones, such as language. This has expanded the behavioural and cognitive repertoire of different species and has determined their competitive success. To allow the relatively rapid emergence of large evolutionary changes in a structure of such importance and complexity, the mechanisms by which cortical circuitry develops must be flexible and yet robust against changes that could disrupt the normal functions of the networks.

**Department of Neuroscience, Karolinska Institutet, Retzius väg 8, S-17177 Stockholm.*
‡Developmental Biology Laboratory, Biomedical Sciences, The University of Edinburgh, Hugh Robson Building, George Square, Edinburgh EH8 9XD, UK.
Correspondence to G.M.C.
e-mail: giorgio.innocenti@neuro.ki.se
doi:10.1038/nrn1790
Published online
1 November 2005

During the course of development, structures in the brain and elsewhere undergo transformations according to sequentially implemented rules (algorithms). Examples of algorithms in brain development include the mapping rules that dictate the routes of migrating neurons and growing axons, axo-axonal competition, chemotropism and Hebbian-like synaptic potentiation. These algorithms can be embodied in computer simulations of developmental processes, an area that is likely to expand rapidly in the coming years given the current interest in the creation of bio-inspired, self-organizing, man-made information-processing devices.

The exuberant development of connections — that is, the overproduction of axons, axonal branches and synapses, followed by selection — is one of the algorithms that underlie the development of biological neural networks. This algorithm has been broadly applied to the production of artificial neural networks. However, the artificial neural network field has often implemented selection processes in networks that — unlike biological networks — lack any significant degree of initially pre-specified, selective connectivity.

In this review, we aim to establish the relative roles of pre-specified connectivity and exuberance-selection in the formation of neural circuits, drawing on data from biological experiments. We also evaluate the mechanisms that regulate the selection of persistent connections from an exuberant population in the construction of neural circuits, and discuss the advantages of exuberance-selection and the likelihood that it might have favoured flexible development and evolution of the brain.

What is developmental exuberance?

The term 'developmental exuberance' was first introduced to describe the formation of transient callosal projections between the visual areas of the cat brain during development[1] (FIG. 1). Since then, two types of exuberance have been described in the development of cortical circuits in different systems and species (see Supplementary information S1 (table)). Macroscopic exuberance refers to the formation of transient projections between macroscopic brain parts. It includes transient afferent and efferent projections between a cortical site and one or more other brain regions, such as cortical areas, subcortical nuclei, the spinal cord or the cerebellum. Microscopic exuberance refers to the formation of transient structures that are involved in communication between neurons within a restricted cortical territory. This includes the formation of transient axonal or dendritic branches, synapses

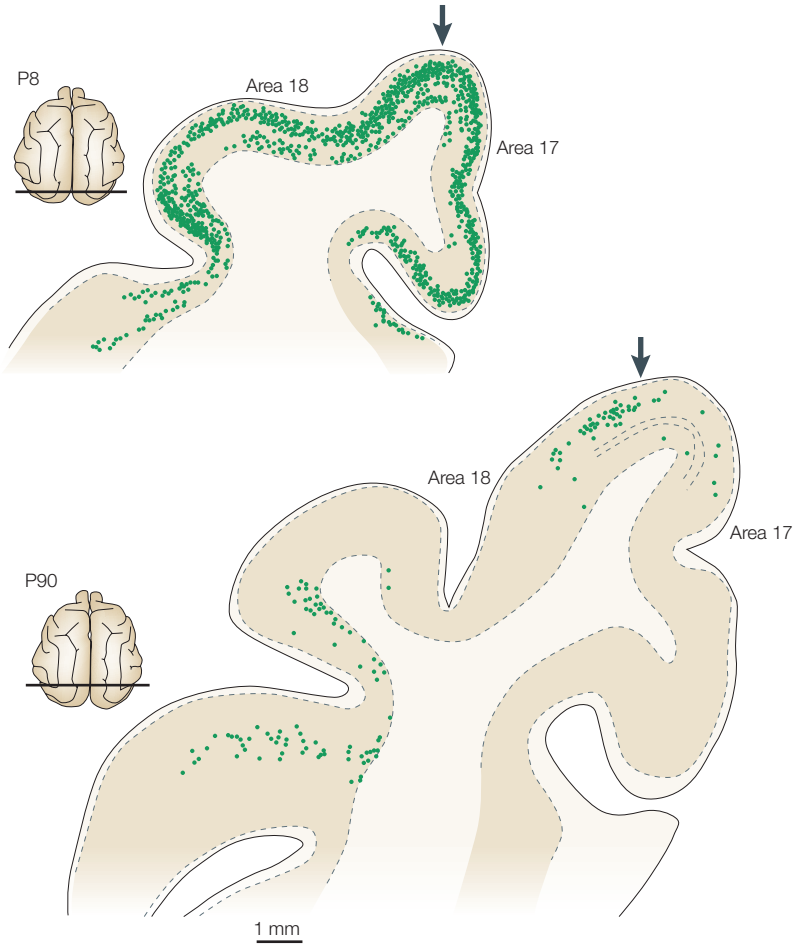


Figure 1 | **Exuberant projections into the corpus callosum from the visual areas of the cat.** Drawings of coronal sections showing the distribution of neurons (green dots) labelled by injections of a retrograde tracer into the visual areas of the opposite hemisphere at two developmental ages. Arrows point to the border between areas 17 (to the right of the arrow) and 18 (to the left). During the first postnatal week the projection originates from the whole of area 17 and from the other visual areas, whereas by day 90 the origin of the projection has become focused to two regions, near the border of areas 17 and 18, and around the bottom of the suprasylvian sulcus. Modified, with permission, from REF. 68 © (1979) Macmillan Magazines Ltd.

TARGET
(of growing axons). The site, or structure, towards which an axon grows — ultimately one or more neurons.

OCULAR DOMINANCE
The neuronal property of responding preferentially to stimuli presented to one eye or the other.

TRACER
A tracer denotes a substance that is actively transported or diffuses along axons. Anterograde tracers move from neuronal cell bodies towards axon terminals, whereas retrograde tracers move from axon terminals (or damaged axons) towards neuronal cell bodies. Many tracers move in both directions.

and/or dendritic spines in layers and/or columns in which they will not be found in the adult, and also their overproduction at appropriate topographical locations.

Both types of exuberance imply that neural structures involved in interneuronal communication are formed in a labile juvenile state and undergo a subsequent transition into a stable adult state. The distinction between the two types of exuberance is not always clear-cut. In particular, evidence of exuberant synaptogenesis is based on counts performed in electron-microscopic preparations that do not allow the origin of the supernumerary synapses to be determined. Interestingly, for some projections both types of exuberance appear at different times in development. That is, the projections become increasingly focused, as if the TARGET is reached by progressive topographical approximation (see below).

Developmental exuberance is widespread

It is now well established that large numbers of transient projections are produced at all levels during the development of cortical networks, from the initial guidance of thalamocortical axons to their targets, to the refinement of connections between cortical areas and from the cortex to subcortical regions. Early findings have been reviewed previously[2–4]; here we deal essentially with data and concepts developed over the past 10–15 years (see Supplementary information S1 (table) for a summary of both sets of data).

Thalamocortical projections. As a general rule, adjacent points in each thalamic nucleus in the adult brain project to adjacent points in their corresponding cortical sensory area(s), and cortical areas receive inputs from adjacent thalamic nuclei. Superimposed on this point-to-point topographic mapping are more complex arrangements for the ordering of different functional properties of cortical neurons, such as their OCULAR DOMINANCE or their selectivity for the orientation or colour of objects. Development of the thalamocortical pathway requires the delivery of thalamic axons along a complex three-dimensional route to the cortex, the arrival of thalamic axons from each nucleus at the correct regions of the cortex and the creation of feature maps. Transient projections are prominent during these processes (FIG. 2).

Several studies have shown that some thalamocortical axons send transient collaterals to regions of the cortex that they will not persistently innervate[5,6]. These include exuberant thalamic projections to subdivisions of somatosensory areas of the monkey and to prefrontal and somatosensory cortices of the rat. These transient projections might allow incoming axons to sample the nature of the cortical regions through which they pass.

With regard to the refinement of feature maps in the cortex, in 1976 Rakic[7] described the overlap of ocular dominance columns in layer 4 of area 17 of the prenatal monkey. Similar findings have been reported in the kitten[8], but more recent work has indicated that results could have been contaminated by spill-over of TRACER across the eye-specific laminae of the lateral geniculate nucleus[9–11] (LGN; BOX 1). The tracing of individual geniculocortical axons[12] provided evidence for modest overlap of axons belonging to different eye domains in the early stages in the formation of ocular dominance columns.

Cortico-cortical projections. Cortico-cortical axons mostly course through white matter to link regions of cortex on the two sides of the brain (contralaterally), through the corpus callosum, and/or on the same side of the brain (ipsilaterally). The first report of exuberance of cortical connections[1] showed that parts of areas 17 and 18 that are devoid of callosal connections in the adult cat form transient projections to the contralateral hemisphere at birth (FIG. 1). These projections are rapidly eliminated, the majority by postnatal day 21, although some might persist beyond day 30. Subsequent work led to the discovery of transient callosal connections in the

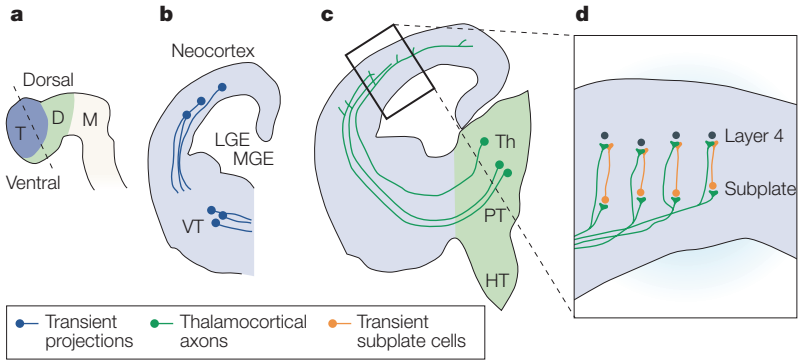


Figure 2 | **Transient structures present during thalamocortical development.** **a** | Embryonic anterior neural tube. A section through the dashed line is shown in panel **b**. **b** | The left side of the embryonic brain showing projections from the developing cortex, including from the subplate and the ventral telencephalon (VT). These projections are thought to help guide thalamic axons to their cortical targets; subplate cells and ventral telencephalic cells projecting to the thalamus later disappear. **c** | Thalamocortical axons growing under the cortical plate produce many transient branches to cortical regions that they will not persistently innervate. **d** | Thalamocortical afferents innervate the cortical plate, terminating on cortical layer 4 cells and transient subplate cells that innervate layer 4 cells and so regulate the early development of cortical circuitry. D, diencephalon; HT, hypothalamus; LGE, lateral ganglionic eminence; M, mesencephalon; MGE, medial ganglionic eminence; PT, prethalamus; T, telencephalon; Th, thalamus.

somatosensory cortex of the rat, cat and monkey (for reviews, see REFS 2,3), transient long-range projections from the auditory to the visual cortex on both sides of the kitten's brain[13] and exuberant projections between adjacent areas of the kitten's visual cortex[14] (for reviews, see REFS 4,5) (FIG. 3). More recently, the concept has been extended to several other species and systems, including transient projections from the temporal cortex to the limbic system of the monkey[15] and transient intra-areal projections in the cat and monkey[16–18]. Although the phenomenon seems to be widespread, the magnitude of exuberance-selection might vary in different species, systems and types of connection (for an example, see REF. 19). Caution is needed when drawing comparisons between species, given the difficulties in the quantification of the transient projections (BOX 1), and differences in the duration and timing of cortical development and the speed of axonal development.

Cortico-subcortical projections. Developmental exuberance was first identified in cortico-subcortical projections from the occipital cortex to subcortical structures, including the cerebellum and the spinal cord, in the rabbit and rat[20,21]. Subsequently, exuberant cortico-subcortical projections were found to the spinal cord, superior colliculus, pons, nucleus ruber, trigeminal nuclei and other medullary structures in several species (for reviews, see REFS 2–4,22–25).

The functions of transient projections

Several of the transient projections that are associated with the development of thalamocortical connections have been ascribed functions in the construction of cortical circuitry. In rodents, injected retrograde tracers have been used to identify a transient population of thalamic afferents that originate in the ventral TELENCEPHALON, in the region of the internal capsule, before the thalamocortical tract forms[26–28]. These projections are thought to guide thalamocortical axons as they make a sharp lateral turn across the boundary between the DIENCEPHALON and telencephalon (FIG. 2). In mouse mutants that lack the transcription factors MASH1, PAX6 or FOXG1, the transient afferents do not form and the thalamocortical tract fails to enter the telencephalon[29,30].

A second transient axonal population derives from the cortical subplate (FIG. 2). This is a group of highly differentiated neurons that are generated early[31], many of which are fated to die. This population is larger and more highly developed in phylogenetically more advanced species, although it can be identified even in rodents[32]. These neurons form a rich network of connections with both cortical and subcortical structures. Several studies have indicated that one of the functions of this population is to generate PIONEER PROJECTIONS towards the thalamus that might guide thalamocortical axons from the internal capsule to the cortex (for reviews, see REFS 31,33). Subplate neurons receive synaptic input from thalamocortical axons and layer 4 neurons, which are the main final targets of thalamocortical axons and have reciprocal functional connections with the subplate[34–36]. Ablation of this complex but transient cortical circuitry has shown that it is important for the establishment of ocular dominance columns, orientation maps and orientation tuning[37,38].

The origin of many exuberant synapses that form during the development of the cerebral cortex (for an example, see REF. 39) is unknown. Their functions, and what can be achieved by their elimination, are, therefore, unclear. Visual callosal connections show no important changes in topography before or after the period during which individual axons shed synaptic boutons, indicating that the overproduction and elimination of synapses might be limited to modulating the strength of the connections between the axons and their target neurons[40,41]. Different axons of a given projection, and the different branches of an axon, might undergo different degrees of synaptic overproduction and elimination. Therefore, the elimination of synaptic boutons could have important consequences for information processing by differentiating the strength of the connections between neuronal populations. Abnormally located (ectopic) RECEPTIVE FIELDS are found in area 17 of kittens but not in adult cats, and might result from exuberant long-range tangential projections[42]. Electrophysiological studies have indicated that exuberant inhibitory connections in the ferret visual cortex undergo remodelling, which might relate to the emergence of selective response properties[43]. Transient functional connections are also found in the developing corticospinal tract[44].

It seems unlikely that most transient projections form mature synaptic contacts. Indeed, most exuberant cortico-cortical projections do not seem to form synaptic boutons and do not grow to any great extent into the grey matter. The latter is also true for some transient projections to the spinal cord (for data and references, see below).

TELENCEPHALON
One of the major components of the forebrain; thalamocortical axons grow through its ventral part to reach its dorsal part, where the cerebral cortex forms.

DIENCEPHALON
The component of the forebrain in which the thalamus develops.

PIONEER PROJECTIONS
(Or axons). Axons that precede the growth of others to a given target, and are thought to guide later-growing projections.

RECEPTIVE FIELD
A region in the periphery that, when stimulated in an appropriate way, produces a response in a particular sensory neuron.

Box 1 | **Methodological issues**

The comparison of juvenile and adult connections is complicated by variations with age in the uptake, transport and diffusion of axonally transported substances that are used to trace them. Some tracers (for example, lipophilic molecules such as carbocyanine dyes) label young, unmyelinated axons well but older, myelinated axons much less effectively. Other tracers tend to be less effectively taken up and/or transported by young axons, preventing the detection of connections that can be readily visualized when they are more mature. In some studies, the same projections were studied at different ages using several tracers[1,13,51], but, unfortunately, most studies did not do this, raising the possibility that juvenile connections might have been missed owing to insufficiently sensitive tracing methods. Another problem is that the young brain contains tracer-permeable gap junctions and leaky membranes, so trans-neuronal diffusion of tracers might suggest the presence of exuberant projections where none exists. This source of artefact might underlie controversies about the maturation of geniculocortical projections. For several decades it was believed that ocular dominance columns emerge, by a process of axonal sorting, from initially exuberant overlapping innervation from the two eyes. This was based mainly on the injection of tritiated proline into the eye, from where it diffuses trans-neuronally into the lateral geniculate nucleus (LGN)[8]. However, recent work using injections of labelled dextrans into the LGN showed that ocular dominance columns form through the selective elaboration of initially accurately targeted projections, rather than through elimination of initially incorrectly targeted projections[9]. A powerful methodological innovation was the use of tracers that can remain in neurons for a long time[46], such as fast blue and fluorescent beads. These allow investigators to take snapshots of the state of the same connection at different developmental stages. Such tracers can circumvent the difficulty posed if certain connections appear to shift their location during development, either because the neurons involved are still migrating, or because the structures in which they are embedded change size or shape. These tracers also allow neuronal death to be distinguished from axonal elimination as the cause of the deletion of transient connections.

The processes of selection and elimination can be modulated by several factors. Perhaps the most important function of the overproduction and selection of connections in development is to provide a high degree of flexibility in the formation of cortical circuits. The same potential for developmental plasticity might have favoured the evolution of the cerebral cortex — in particular the emergence of new cortical areas — by facilitating their insertion into functional networks[45]. These possibilities are discussed further below.

Mode of elimination and quantification

Two mechanisms can cause elimination of transient projections: neuronal death and the selective deletion of axons, axonal branches or synapses. Cell death is important for the removal of many transient projections during development of the thalamocortical pathway, the best known example being the postnatal death of subplate cells in many species[31,32]. Transient projections from the ventral telencephalon to the thalamus disappear prenatally and their fate(s) are not clear. So far, there is no specific molecular marker for this population of cells and the use of long-lasting retrograde tracers to label and follow them[46] is technically difficult in the embryo. Increased cell death has been reported in the thalamus after cortical innervation and might be a crucial step in the matching of cell numbers in the presynaptic and postsynaptic structures[47].

Axonal elimination seems to be the main — or only — mechanism for the elimination of transient axonal pathways that originate in the cortex. The data that support this idea were obtained by first labelling transient projections with long-lasting fluorescent retrogradely transported tracers and then, after the transient projections had been eliminated, injecting different tracers into the same or different structures. This allowed researchers to confirm whether the cell bodies labelled by the early injection had shed the transient axons and/or to find out where they were projecting to at this time[2,14,19,46]. This approach does not exclude the possibility that some of the projections might be eliminated by neuronal death.

The analysis of single axons that were anterogradely labelled with biocytin provided clues to the cellular mechanism of axonal elimination. Callosal axons originating from the peripheral parts of area 17, which is devoid of callosal connections in the adult, initially branch extensively in the white matter, among the neurons of the subplate. During the period of projection elimination, nerve endings that could be interpreted as collapsed GROWTH CONES, or retracting or degenerating axons were found at some axonal terminals[48]. Transient populations of macrophages that have been found in the white matter might be involved in clearing the debris of the eliminated axons (for a review, see REF. 4).

Methodological difficulties (BOX 1) have often prevented precise estimates of the magnitude of axonal elimination. However, it is clear that some projections are fully eliminated. This is the case, for example, for the corticofugal projections to the spinal cord or cerebellum. The most satisfactory quantification comes from electron microscopic studies that have shown losses of 70% of the callosal axons in both cats and monkeys[49,50]. This figure might still underestimate the real extent of axonal loss, because axonal elimination and the addition of new axons to the corpus callosum might overlap in time, although a few of the axonal profiles counted in the corpus callosum might actually be short intracallosal branches[51].

Exuberance versus pre-specification

The exuberant development of cortical connections is restricted by mechanisms that guide growing axons along specific pathways and determine their targets. Early observations on the development of callosal connections in the cat indicated the existence of these early specificities and provided a model whose principles extended successfully to the development of other connections. First, the connections, including the exuberant connections, are organized in topographical order from the earliest stages. Injections of tracers in the anteroposterior direction over all the areas of one hemisphere label territories that are progressively spaced in the same direction in the other hemisphere[52]. Similar evidence has been found for topographic mapping in newly forming projections in other regions, such as between areas of the visual cortex, although exuberance reduced its precision[53,54] (FIG. 3).

GROWTH CONE
A highly dynamic structure at the growing end of an axon (or dendrite) that steers axonal (or dendritic) growth by decoding cues in the environment.

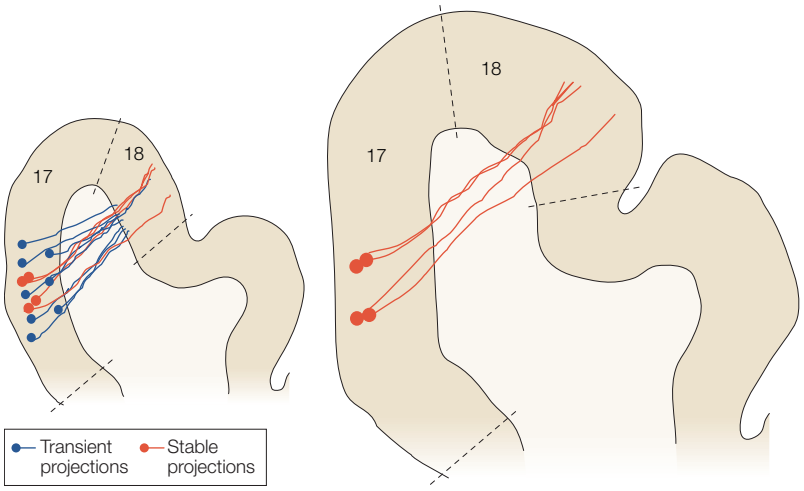


Figure 3 | **Development of ipsilateral cortico-cortical connections from visual area 17 to area 18 in the cat.** Shortly after birth, axons project from cells in the superficial and deep layers of area 17 to the superficial and deep layers of area 18 (left panel). The diagram shows a small number of these axons projecting to a small region of area 18. Only some of these axons will persist (red); these arise from a narrower domain of area 17 than that producing the overall population projecting to area 18. The projections that persist as the animal matures derive from clusters of cells in the superficial layers of area 17 and terminate in clusters in the superficial layers of area 18 (right panel).

Second, laminar specificity in the origin of the cortical projections is present from the earliest stages of development. Callosal axons originate mainly from cortical layers 3 and 6, although the contribution of the two layers to a given projection often changes with age, usually by the elimination of exuberant projections from layer 3 (REFS 52,55). This is also the case in the development of callosal connections in monkeys[56,57]. Even from the earliest stages, ipsilateral cortico-cortical connections in the cat also originate from cells above and below layer 4, although the contribution from deep layers reduces with age[14]. In these pathways, the precision of the initial topographic mapping between the superficial layers is much greater than that connecting the deep layers[53,54].

Third, studies using retrogradely transported tracers have shown that cortico-cortically projecting neurons in layer 3 consist of several subclasses, each projecting selectively, albeit in some cases transiently, to different cortical districts in the ipsilateral or contralateral hemisphere[13,41,58] (FIG. 4). Similar observations apply to the organization of callosal connections from area 18 in the monkey, although in that species there is a clear sublamination between callosal neurons to contralateral area 18 and association neurons to area 17 (REF. 57). In the rat, callosal and other corticofugal projection neurons are also separate populations from the start[59].

Origin-to-target specificity also occurs when axons grow near and into their terminal sites, as is best demonstrated by the study of individual axons (FIG. 5). Transient callosal and intrahemispheric axons reach the subplate, where they branch profusely, albeit transiently. The axons that will persist then invade the grey matter, where they develop terminal arbors and synapses, whereas the transient axons remain in the white matter and are subsequently eliminated[48]. The origin of projections to each point in the white matter is more widespread than the projection to each point in the grey matter in callosal connections and association connections in the visual areas of kittens[16,41,48]. This finding was predicted by the different labelling obtained with retrograde tracers injected in the grey versus white matter (for a review, see REF. 2) and was extended to association projections from area V2 in the monkey[19]. However, it is possible that a few of the transient axons do invade the grey matter to some extent[60]. Similar concepts apply to the development of the corticospinal projections. Most of the transient corticospinal projections appear not to enter the grey matter, although some might (for reviews, see REFS 4,61; for an alternative view, see REF. 44).

Once in the cortex, axons show further patterns of specific growth. Axonal branching and the formation of synapses become progressively more focused to the sites of adult termination, although transient branches and synapses are formed close to or in the territories of adult termination[40,41] (FIG. 5). The micro-exuberance of the intrinsic connections in the visual and prefrontal cortices of primates[18,62] resembles that shown by cat visual callosal axons in the cortical grey matter. In the callosal connections of the cat, overproduction of synaptic boutons and their partial elimination occur in the absence of massive changes in the topography of connections[40,41].

Factors that affect axonal selection

The selection of persistent projections from a juvenile exuberant set of axons is a hierarchical, sequential process (BOX 2). This process begins with the selective growth of axons from within defined white matter compartments towards specific targets. Subsequent growth into the targets progressively restricts the focus of axonal growth and leads to the establishment of terminal arbors and synapses at specific regional and cellular sites. All of these selections depend crucially on factors outside the projecting cells themselves, which trigger intracellular pathways and cell-autonomous, genetic programmes. Indeed, the overall change in projecting axons from a juvenile, labile state to an adult, stable state might result from extrinsic epigenetic control of cell-intrinsic programmes that cause the maturation of cytoskeletal axonal proteins, in particular the heavy subunit of neurofilaments (for a review, see REF. 2) and the Tau proteins[63].

The regulation of specificity in the initial growth and targeting of thalamocortical and cortico-cortical axons is poorly understood, but probably involves a combination of mechanisms that have been shown to guide the general development of axonal pathways. These cues include cells, many of which are transient, that provide guidance (as discussed above). A recently described transient neuronal population in the developing corpus callosum might be involved in axonal guidance, but this role remains to be defined[64]. The guidance of callosal axons into the grey matter seems to involve interactions with radial glia[65].

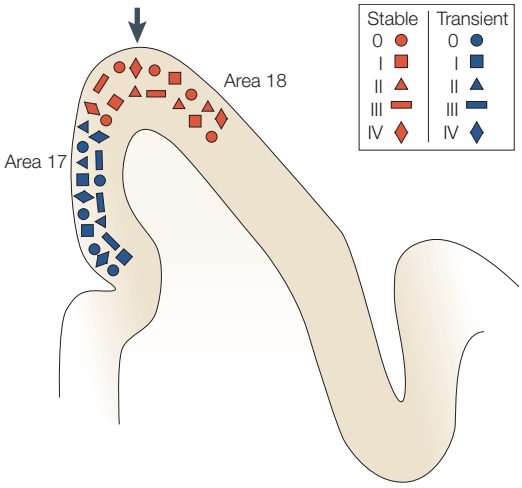


Figure 4 | **Types of callosally projecting neuron in areas 17 and 18 of the cat visual cortex.** From the earliest ages studied, the callosally projecting neurons in areas 17 and 18 of the cat consist of at least five different types (0–IV), which are defined by the site of termination of their axons in the contralateral hemisphere. Type 0 projects to the area 17/18 border, type I to the lateral border of area 19, type II to the border between two suprasylvian areas, type III to two areal borders between suprasylvian areas and type IV to the lateral border of area 19 (as type I) in addition to the border between two suprasylvian areas (as type II). The same types of neuron are found in parts of the visual areas that are destined to form permanent callosal projections (near the border of areas 17 and 18, arrow) and in parts of area 17 that form only transient projections. Therefore an initial, precise connectional typology is expressed in cortical neurons before the fate of the connection (maintenance or elimination) is decided. The initial connectional typology is determined by cell-intrinsic, probably genetic, information uniformly distributed throughout the cortex, whereas the final fate of the connection depends on positional information, for example, on the position of the neuron in the cortex. Modified, with permission, from REF. 41 © (1999) Wiley InterScience.

The following factors are involved in the selection of projections from the juvenile, exuberant set: information from the periphery that depends on sensory input; competition among axonal systems or between neurons for chemotrophic substances in the target structure; hormones, in particular thyroid hormones; and the expression of molecules that identify targets as appropriate for persistent innervation.

Input from the periphery. Shatz provided the first evidence[66] that the development of callosal connections might be controlled by thalamocortical input, conveying information from the periphery, and in particular the retina. Before the developmental exuberance of callosal connections was known, she described abnormal callosal connections in the Siamese cat as a consequence of the abnormal crossing of retinal axons in this species (see also REF. 67). Subsequent work showed a loss of callosal connections in kittens that were binocularly deprived of vision by eyelid suture, eye enucleation, dark rearing or sectioning of the optic chiasm[68–71] (for a review, see REF. 2).

More recent work showed the importance of thalamocortical axons and normal sensory activity for the normal selection of axons in ipsilateral cortico-cortical pathways[72,73] and confirmed that bilateral enucleation in the cat decreases the number of callosally projecting neurons[74]. This work failed to show persistence of an enlarged callosally projecting band of cells straddling the border between areas 17 and 18, which conflicts with the results of eye enucleation studies in rats (for a review, see REF. 2). Dark rearing and enucleation have less prominent effects on callosal projections in the extrastriate areas (area 19 and lateral suprasylvian) of the cat[75].

Callosal axons projecting to areas 17 and 18 that survive visual deprivation have severely stunted terminal arbors, as evaluated by their total length, number of branches and number of boutons[76] (FIG. 6). A period of normal vision lasting for as little as 10 days, beginning after the onset of natural eye opening, is sufficient to stabilize the normal complement of visual callosal connections[77]. A comparable short period of normal vision also leads to fully developed callosal terminal arbors in the adult[76]. Surprisingly, at the end of the short period of normal vision the arbors are far from having reached their adult state. Therefore, after a short and early period of vision, development can continue, apparently normally, in the absence of vision. The fact that normal vision is required early to trigger maturation, rather than to maintain developed arbors or to instruct their development, is supported by the finding that deprivation of ~1 month, before any normal vision, also results in stunted development of the axonal arbors and local connections in primary visual areas[76].

The fact that callosal connections in several visual areas are stabilized by information that is contained in cortical representations of retinal topography is implicit in the finding that, across species, the connections are restricted close to the representation of the visual midline[78]. Considerable refinement of this concept came from work relating callosal connectivity to ocular dominance. This generated the prediction that callosal connections are stabilized by correlated activity within each hemisphere that is driven by inputs from both temporal retinae[79]. However, the relationships between retinotopy and callosal connections can be modified during development — they were shown to be altered when retinotopic maps were disrupted by early cortical lesions[80]. Other factors, such as axonal competition, might, therefore, override information from the periphery.

Axo-axonal competition. The idea that axons might compete with each other during development is well grounded in the results of early lesion experiments in many systems and species. For example, there is evidence that the reduction in numbers of cells projecting from the thalamus to the cortex in the developing thalamocortical system results from competition for brain-derived neurotrophic factors (BDNFs) in the cortex[81]. This concept might also apply to the development of cortico-cortical connections (for a review, see REF. 2) and continues to be supported by the results of selective lesion studies. For example, in the monkey, lesions of

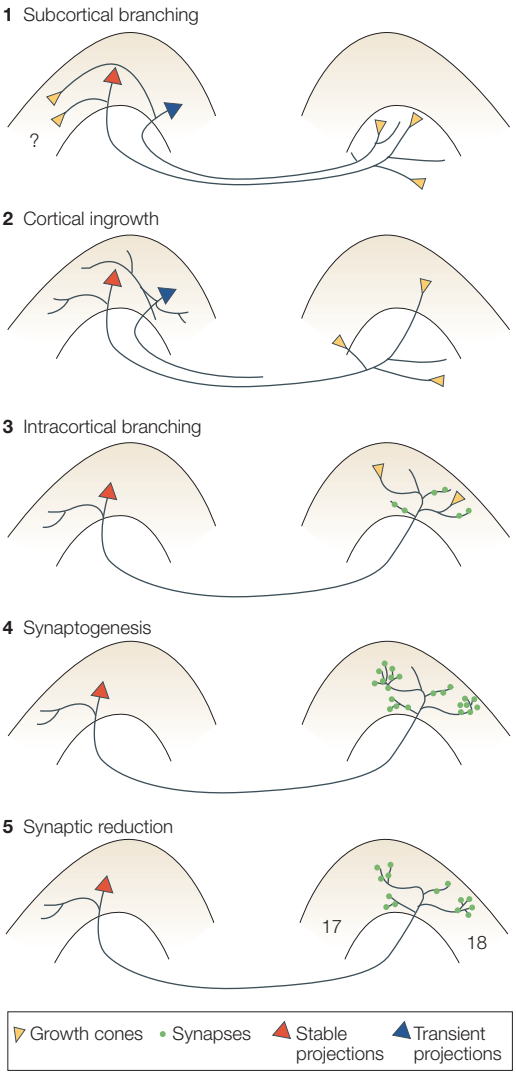


Figure 5 | **The growth of callosal axons into their site of termination is a multi-stage process.** Initially, both axons that are to be eliminated and those that will become permanent branch profusely in the white matter (1). Later, only or mainly axons destined to become permanent grow into the grey matter (2), where they develop terminal branches and synapses (3,4). Note that at all stages some transient structures, such as main axons, axonal branches or synapses, are formed. Note also that, from the onset, synapses are formed in specific layers and columns. The stage of synaptic reduction does not much alter, if at all, the original distribution of synapses (5). Modified, with permission, from REF. 45 © (1995) Elsevier Science.

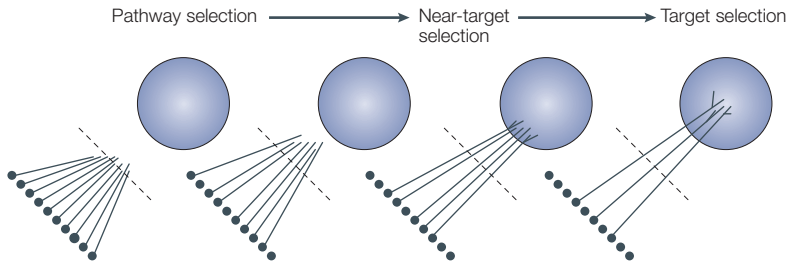
the inferior temporal area resulted in the maintenance of exuberant projections to the lateral basal nucleus of the amygdala (and the expansion of projections to the dorsal part of the lateral nucleus)[82]. This reorganization might be the substrate for the preserved visual recognition function in monkeys with early lesions of the inferior temporal area[83]. Bilateral (normally transient) cortico-rubral connections were also maintained after early unilateral cortical lesions[24]. Perinatal lesions of the posterior parietal and visual cortices in the ferret resulted in the formation of aberrant projections from the primary somatosensory areas on the side of the lesion to the intact posterior parietal cortex of the other hemisphere[80]. Whether such projections result from the maintenance of juvenile projections or *de novo* formation was not determined. Interestingly, the results indicate that callosal axons from parietal and visual areas, which were eliminated by the lesion, can compete during development with somatosensory callosal axons for termination in an area such as the parietal cortex, where somatosensory and visual inputs merge. Another point of interest is that the thalamocortical projections to the remaining visual and parietal areas were not altered, which seems to rule out the possibility that reorganization of the thalamocortical projections caused reorganization of the callosal connections.

Thyroid hormones, alcohol syndrome. Hypothyroid rats fail to eliminate exuberant visual and auditory callosal connections[84,85]. In an experimental model of severe congenital hypothyroidism[85], callosally projecting neurons remained distributed continuously across the cortex, failing to achieve the normal clustered distribution. Congenitally hypothyroid rats also fail to eliminate the normally transient corticospinal projections from the occipital cortex[86].

The selection of juvenile axonal projections is also altered in fetal alcohol syndrome. In monkeys whose mothers had received ethanol during pregnancy, the anterior half of the corpus callosum was thicker and contained an increased number of axons[87]. The numbers of callosal neurons in the somatosensory cortex, corticospinal neurons and descending corticospinal axons were also increased in rat models of fetal alcohol syndrome (quoted in REF. 87). These results differ from those reported in humans, in whom fetal alcohol syndrome has been more frequently associated with dysgenesis, or even agenesis, of the corpus callosum, and a reduction in brain size (for data and discussion, see REF. 87).

Markers of targets for persistent innervation. The molecular markers that allow target cells to select incoming axons have not been identified. Various molecules are expressed either in specific areas of the developing cortex or with concentration gradients across the cortical surface. These molecules, which include signalling molecules such as ephrins and Eph receptors, and transcription factors such as EMX2 and PAX6, have been postulated to confer cortical area-specific identity to incoming thalamocortical afferents[88,89]. These molecules might also be important in the selection of persistent projections from an earlier exuberant population. One recent study showed that the segregation of most intrahemispheric cortico-cortical connections into rostral and caudal sets in newborn mice corresponds to regional differences in the expression of genes such as *Id2* and *Rzr*β (REF. 90), which encode inhibitory helix–loop–helix and orphan nuclear receptors, respectively. This correspondence was not altered in mutant mice that lacked thalamocortical inputs, which suggests that molecular differences that are intrinsic to the cortex are important. However, it

Box 2 | **Exuberance and axonal selection by targets**



Exuberance and axonal selection by targets are two intimately interrelated processes that are involved in the construction of cortical circuits. The diagram shows how an axonal population is progressively restricted down to its final composition by successive selections, first along the pathway, then near the target, and finally within the target. One difference between selection along the pathway and selection near or at the target is that at the later two selection stages the axons are beginning to form terminal arbors. Therefore, the selection affects not only the parent axon but also its branches and/or synapses. Macroscopic exuberance is associated with the selection of main axons, microscopic exuberance with the selection of axonal branches and/or synapses near or at the target. During the formation of neural circuits, axonal selection by the target seems to apply to neurons with axons that travel over a long distance — such as pyramidal cortical neurons, the axons of which project across the callosum — to other cortical areas or subcortical and thalamocortical neurons. The terminal arbors of these projections show stereotypical geometry[109]. By contrast, local interneurons, many of which are inhibitory, seem to actively seek their targets using more detailed local guidance cues[110].

was affected in newborn mice with reduced fibroblast growth factor 8 (FGF8) signalling, in which the molecular properties of rostral and caudal neocortical cells are changed. Further research will be needed to discover whether and how the selection of axons from an exuberant set is affected in the cortex of mutants either lacking or with changes in the expression domains of molecules that are crucial for cortical regionalization.

Developmental exuberance in the human brain

It seems possible to extend information learnt from animal studies to human development. In humans, one of the most studied systems of cortical connections is the corpus callosum. The human corpus callosum undergoes a period of cross-sectional reduction during the end of gestation and the first and second postnatal months[91]. It has been suggested that axonal elimination contributes to this[91], because in the cat and monkey the reduction in the cross-sectional area of the corpus callosum corresponds to, and is probably caused by, the massive period of axonal elimination that precedes axonal myelination. One of the advantages of the corpus callosum for morphological studies is that it can be easily visualized and measured in the human brain, both in post-mortem tissue and through *in vivo* brain imaging. Changes in the gross morphology of the corpus callosum are not directly related to changes in the number of axons, as the size of the adult structure is determined by both the number of axons and their size, including the thickness of the myelin sheaths. Nevertheless, variations in the size and/or shape of the corpus callosum are probably an index of variations in interhemispheric connectivity. Individual variations in the morphology of the corpus callosum have been reported in both healthy individuals and in pathological conditions (see below).

A much-debated issue is that of gender differences in the size and/or shape of the corpus callosum, which could be interpreted as evidence of hormonally controlled development of interhemispheric connectivity. The issue was raised in a seminal paper by DeLacoste-Utamsing and Holloway[92], and has acquired support from the animal literature[93,94]. However, despite two decades of research, the conclusions remain controversial (for a review, see REF. 95; see also REFS 96,97). Another important line of investigation, started by the work of Witelson, has led researchers to suggest that differences in callosal size are related to handedness[98–100].

Although the effects of gender and brain laterality on the size and shape of the corpus callosum remain controversial and hard to interpret anatomically, most studies of pathology-related morphological changes in the corpus callosum take into account sex and handedness as two potentially associated variables. It should be stressed that the changes in cortical connectivity that are indicated by callosal morphology are probably not specific to this structure, but are rather one aspect of more generalized changes in cortico-cortical connectivity, which, as discussed above, obeys similar developmental principles.

Perspectives

Almost 30 years after the discovery of the formation of transient projections in cortical development, several key concepts are becoming well established. One is the generality of the phenomenon across systems and species, albeit with a few possible exceptions. The second is the fact that the stabilization of cortico-cortical axons can be modified by several factors, in particular by functional deprivation and by early lesions.

The two concepts mentioned above indicate the validity of extending results from animal work to work on the development of the human brain, which aims to ensure optimal development of cortical connectivity despite adverse or pathological conditions. Indeed, alterations in the morphology of cortical connections, in particular callosal connections, have been reported in several conditions caused by abnormal development, including attention deficit hyperactivity disorder (ADHD)[101,102], dyslexia[103] and schizophrenia (for a review, see REF. 104). Our understanding of the protection of developing cortical connectivity requires several issues to be addressed, three of which, mentioned below, seem to be particularly crucial.

Mechanics of axonal maintenance and elimination. Real-time imaging of axonal development[105] has confirmed previous evidence[48] suggesting that the elimination of exuberant axons involves two separate mechanisms — that is, retraction of branches over short distances and degeneration of long axonal branches. This imaging method offers a promising step towards

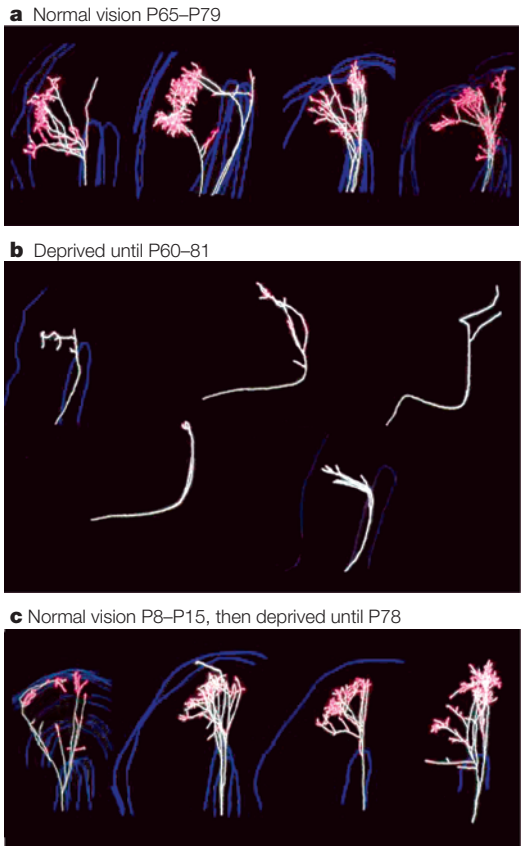


Figure 6 | **Consequences of visual deprivation by bilateral eyelid suture on visual callosal axons originating near the area 17/18 border in the cat.** Pink dots denote synaptic boutons. Continued deprivation for up to 81 days leads to severely stunted terminal arbors compared with normal development (**a**,**b**). Surprisingly, one week of vision is sufficient to trigger apparently normal development of the arbors (**c**). P, postnatal day. Modified, with permission, from REF. 76 © (1999) Blackwell Publishing.

unravelling the molecular mechanisms involved in the elimination of exuberant connections, which remain largely unknown.

The extracellular factors that trigger axonal retraction include molecules known to be chemorepulsive to growth cones in other systems, such as the semaphorins, Slits and ephrins. In support of this idea, Bagri *et al.*[106] found that mice that are mutant for semaphorin receptors show defective pruning of hippocampal mossy fibres. How such molecules might be deployed in the developing cortex to choreograph the events that sculpt intricate patterns of cortical connections from initially exuberant connections is unclear. In other systems, axonal remodelling depends on factors that include correlated neural activity and gradients of chemoattractants and chemorepellents. For example, the development of retinotopic maps in the tectum or superior colliculus, which involves significant axonal elimination, depends on the balance between nitric oxide and BDNF[107] as well as on waves of spontaneous activity initiated in the retina and on gradients of Ephs and ephrins in both the retina and its target[108,109]. Although the importance of neural activity for the selection of cortical axons is recognized and molecular gradients are being identified in the cortex[88,110], the subtle differences in levels or combinations of molecules and neural activity that could generate the complexity of callosal and cortico-cortical connections have not yet been detected. Equally little is known about the intracellular signalling pathways that result in the loss of cortical axons, their branches or synapses. RhoA, a small green fluorescent protein (GFP)-binding protein, is one of the few intracellular signalling molecules whose activation is implicated in the retraction of neuronal processes in other systems[111]. Substrates downstream of RhoA include some that interact with cytoskeletal proteins that are likely to bring about the withdrawal of unselected axons.

Cell-intrinsic versus -extrinsic control of axonal geometry. Although axonal geometry is an essential element in cortical computation[45] and it depends on axonal growth and pruning, the roles of cell-intrinsic versus -extrinsic signals during its maturation remain unclear. Axons show class-specific geometries, although sometimes the differences between different axonal classes are surprisingly small[112,113]. The fast, vision-independent stabilization and maturation of arbors of callosal and intra-areal axons that follow short periods of normal vision[76,77] suggest that the fast release of trophic molecules, in particular BDNF, as reported in the development of retinotectal and cortical connections[107,114], might trigger a cell-intrinsic programme of axonal development. It seems likely that activity still controls the fine structure of the axonal arbors by interacting with the cell-intrinsic developmental programmes, but this remains to be determined by further studies of axonal morphologies and/or their functional consequences. In any case, myelination, and therefore the axonal conduction properties that are crucial to the timing of cortical computation, might be heavily dependent on cell-extrinsic factors, including activity[115].

Functional consequences of axonal geometry and connectivity. The relationships between axonal geometry and connectivity and their functional consequences are beginning to be explored using *in vitro* animal models[116]. However, extending the concept to the human brain remains far more challenging. Several difficulties are of a methodological nature, and might be solved in the future. Diffusion tensor imaging offers formidable possibilities for detecting changes in cortical pathways and their development (for an example, see REFS 117–119). Transported manganese[120] might allow us to further refine our knowledge of cortical connectivity. Finally, electrophysiological and imaging techniques that reveal functional connectivity are being implemented, in some cases in combination (for examples, see REFS 121–123).

Investigating the relationship between brain structure and function, particularly in the human brain, remains one of the most urgent and exciting tasks in the field of neuroscience. No rules seem to be available to allow us to predict the functional consequences of a change in cortical structure or to infer changes in cortical structure from functional data. On the contrary, in some cases the nature of the relationship between cortical structure and function, as, for example, in a recently reported case of preserved visual function despite a severe congenital abnormality (microgyria) of the primary visual areas[124,125], is puzzling. The flexibility in circuit construction that developmental exuberance affords provides a possible explanation for such paradoxes. The understanding and exploitation of this flexibility might provide a way of tackling problems of defective brain structure and function by following in the footsteps of evolution's achievement.

1. Innocenti, G. M., Fiore, L. & Caminiti, R. Exuberant projection into the corpus callosum from the visual cortex of newborn cats. *Neurosci. Lett.* **4**, 237–242 (1977).
2. Innocenti, G. M. The development of projections from cerebral cortex. *Prog. Sens. Physiol.* **12**, 65–114 (1991).
3. O'Leary, D. D. M. Development of connectional diversity and specificity in the mammalian brain by the pruning of collateral projections. *Curr. Opin. Neurobiol.* **2**, 70–77 (1992).
An important review that complements this one, describing how the pruning of collateral projections first became recognized as a fundamental and widespread mechanism for the development of specific axonal connections.
4. Stanfield, B. B. The development of the corticospinal projection. *Prog. Neurobiol.* **38**, 169–202 (1992).
Another important review describing early studies of exuberance in the rodent's developing corticospinal system, which became established as a powerful model in this field.
5. 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).
6. 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).
7. Rakic, P. Prenatal genesis of connections subserving ocular dominance in the rhesus monkey. *Nature* **261**, 467–471 (1976).
8. LeVay, S., Stryker, M. P. & Shatz, C. Ocular dominance columns and their development in layer IV of the cat's visual cortex: a quantitative study. *J. Comp. Neurol.* **179**, 223–244 (1978).
9. Crowley, J. C. & Katz, L. C. Early development of ocular dominance columns. *Science* **290**, 1321–1324 (2000).
10. Crair, M. C., Horton, J. C., Antonini, A. & Stryker, M. P. Emergence of ocular dominance columns in cat visual cortex by 2 weeks of age. *J. Comp. Neurol.* **430**, 235–249 (2001).
11. Crowley, J. C. & Katz, L. C. Ocular dominance development revisited. *Curr. Opin. Neurobiol.* **12**, 104–109 (2002).
12. Antonini, A. & Stryker, M. P. Development of individual geniculocortical arbors in cat striate cortex and effects of binocular impulse blockade. *J. Neurosci.* **13**, 3549–3573 (1993).
13. Innocenti, G. & Clarke, S. Bilateral transitory projection to visual areas from the auditory cortex in kittens. *Dev. Brain Res.* **14**, 143–148 (1984).
14. Price, D. J. & Blakemore, C. Regressive events in the postnatal development of association projections in the visual cortex. *Nature* **316**, 721–724 (1985).
15. Webster, M. J., Ungerleider, L. G. & Bachevalier, J. Connections of inferior temporal areas TE and TEO with medial temporal-lobe structures in infant and adult monkeys. *J. Neurosci.* **11**, 1095–1116 (1991).
This remains one of the most striking examples of exuberant cortical connections in the monkey.
16. Assal, F. & Innocenti, G. M. Transient intra-areal axons in developing cat visual cortex. *Cereb. Cortex* **3**, 290–303 (1993).
17. Galuske, R. A. W. & Singer, W. The origin and topography of long-range intrinsic projections in cat visual cortex: a developmental study. *Cereb. Cortex* **6**, 417–430 (1996).
18. Callaway, E. M. Prenatal development of layer-specific local circuits in primary visual cortex of the macaque monkey. *J. Neurosci.* **18**, 1505–1527 (1998).
19. Barone, P., Dehay, C., Berland, M. & Kennedy, H. Role of directed growth and target selection in the formation of cortical pathways: prenatal development of the projection of area V2 to area V4 in the monkey. *J. Comp. Neurol.* **374**, 1–20 (1996).
20. Distel, H. & Holländer, H. Autoradiographic tracing of developing subcortical projections of the occipital region in fetal rabbits. *J. Comp. Neurol.* **192**, 505–518 (1980).
21. Stanfield, B. B., O'Leary, D. D. M. & Fricks, C. Selective collateral elimination in early postnatal development restricts cortical distribution of rat pyramidal tract neurones. *Nature* **298**, 371–373 (1982).
22. Curfs, M. H. J. M., Gribnau, A. A. M. & Dederen, P. J. W. C. Selective elimination of transient corticospinal projections in the rat cervical spinal cord gray matter. *Dev. Brain Res.* **78**, 182–190 (1994).
23. Galea, M. P. & Darian-Smith, I. Postnatal maturation of the direct corticospinal projection in the macaque monkey. *Cereb. Cortex* **5**, 518–540 (1995).
24. Murakami, F., Kobayashi, Y., Uratani, T. & Tamada, A. Individual corticorubral neurons project bilaterally during postnatal development and following early contralateral cortical lesions. *Exp. Brain Res.* **96**, 181–193 (1993).
25. Del Caño, G. G., Gerrikagoitia, I., Goñi, O. & Martínez-Millán, L. Sprouting of the visual corticocollicular terminal field after removal of contralateral retinal inputs in neonatal rabbits. *Exp. Brain Res.* **117**, 399–410 (1997).
26. Metin, C. & Godement, P. The ganglionic eminence may be an intermediate target for corticofugal and thalamocortical axons. *J. Neurosci.* **16**, 3219–3235 (1996).
27. Molnar, Z., Adams, R. & Blakemore, C. Mechanisms underlying the early establishment of thalamocortical connections in the rat. *J. Neurosci.* **18**, 5723–5745 (1998).
28. 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).
29. 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).
30. Pratt, T. *et al.* Disruption of early events in thalamocortical tract formation in mice lacking the transcription factors Pax6 or Foxg1. *J. Neurosci.* **22**, 8523–8531 (2002).
31. 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).
32. Price, D. J., Aslam, S., Tasker, L. & Gillies, K. Fates of the earliest generated cells in the developing murine neocortex. *J. Comp. Neurol.* **377**, 414–422 (1997).
33. Lopez-Bendito, G. & Molnar, Z. Thalamocortical development: how are we going to get there? *Nature Rev. Neurosci.* **4**, 276–289 (2003).
34. Friauf, E., McConnell, S. K. & Shatz, C. J. Functional synaptic circuits in the subplate during fetal and early postnatal development of cat visual cortex. *J. Neurosci.* **10**, 2601–2613 (1990).
35. Herrmann, K., Antonini, A. & Shatz, C. J. Ultrastructural evidence for synaptic interactions between thalamocortical axons and subplate neurons. *Eur. J. Neurosci.* **6**, 1729–1742 (1994).
36. Hanganu, I. L., Kilb, W. & Luhmann, H. J. Functional synaptic projections onto subplate neurons in neonatal rat somatosensory cortex. *J. Neurosci.* **22**, 7165–7176 (2002).
37. Ghosh, A. & Shatz, C. J. Involvement of subplate neurons in the formation of ocular dominance columns. *Science* **255**, 1441–1443 (1992).
38. Kanold, P. O., Kara, P., Reid, R. C. & Shatz, C. J. Role of subplate neurons in functional maturation of visual cortical columns. *Science* **301**, 521–525 (2003).
39. Rakic, P., Bourgeois, J.-P., Eckenhoff, M. F., Zecevic, N. & Goldman-Rakic, P. S. Concurrent overproduction of synapses in diverse regions of the primate cerebral cortex. *Science* **232**, 232–235 (1986).
A seminal paper on exuberant and synchronous synaptogenesis in different cortical areas of the monkey.
40. Aggoun-Zouaoui, D., Kiper, D. C. & Innocenti, G. M. Growth of callosal terminal arbors in primary visual areas of the cat. *Eur. J. Neurosci.* **8**, 1132–1148 (1996).
41. Bressoud, R. & Innocenti, G. M. Topology, early differentiation and exuberant growth of a set of cortical axons. *J. Comp. Neurol.* **406**, 87–108 (1999).
Documents exuberant development of individual axonal arbors and ways in which exuberant development is constrained by what seems to be specific growth of different axonal types.
42. Luhmann, H. J., Greuel, J. M. & Singer, W. Horizontal interactions in cat striate cortex: III. Ectopic receptive fields and transient exuberance of tangential interactions. *Eur. J. Neurosci.* **2**, 369–377 (1990).
43. Chen, B., Boukamel, K., Kao, J. P. Y. & Roerig, B. Spatial distribution of inhibitory synaptic connections during development of ferret primary visual cortex. *Exp. Brain Res.* **160**, 496–509 (2005).
44. Curfs, M. H. J. M., Gribnau, A. A. M., Dederen, P. J. W. C. & Bergervoet-Vernooij, H. W. M. Transient functional connections between the developing corticospinal tract and cervical spinal interneurons as demonstrated by c-*fos* immunohistochemistry. *Dev. Brain Res.* **87**, 214–219 (1995).
45. Innocenti, G. M. Exuberant development of connections, and its possible permissive role in cortical evolution. *Trends Neurosci.* **18**, 397–402 (1995).
46. Innocenti, G. M. Growth and reshaping of axons in the establishment of visual callosal connections. *Science* **212**, 824–827 (1981).
47. Lotto, R. B., Asavaritikrai, P., Vali, L. & Price, D. J. Target-derived neurotrophic factors regulate the death of developing forebrain neurons after a change in their trophic requirements. *J. Neurosci*. **21**, 3904–3910 (2001).
48. Aggoun-Zouaoui, D. & Innocenti, G. M. Juvenile visual callosal axons in kittens display origin- and fate-related morphology and distribution of arbors. *Eur. J. Neurosci.* **6**, 1846–1863 (1994).
49. Berbel, P. & Innocenti, G. M. The development of the corpus callosum in cats: a light- and electron-microscopic study. *J. Comp. Neurol.* **276**, 132–156 (1988).
50. LaMantia, A.-S. & Rakic, P. Axon overproduction and elimination in the corpus callosum of the developing rhesus monkey. *J. Neurosci.* **10**, 2156–2175 (1990).
51. Kadhim, H. J., Bhide, P. G. & Frost, D. O. Transient axonal branching in the developing corpus callosum. *Cereb. Cortex* **3**, 551–566 (1993).
52. Innocenti, G. M. & Clarke, S. The organization of immature callosal connections. *J. Comp. Neurol.* **230**, 287–309 (1984).
53. Price, D. J., Ferrer, J. M., Blakemore, C. & Kato, N. Postnatal development and plasticity of corticocortical projections from area 17 to area 18 in the cat's visual cortex. *J. Neurosci.* **14**, 2747–2762 (1994).
54. Caric, D. & Price, D. J. The organization of visual corticocortical connections in early postnatal kittens. *Neuroscience* **73**, 817–829 (1996).
55. Innocenti, G. M., Berbel, P. & Clarke, S. Development of projections from auditory to visual areas in the cat. *J. Comp. Neurol.* **272**, 242–259 (1988).
56. Kennedy, H., Bullier, J. & Dehay, C. Transient projection from the superior temporal sulcus to area 17 in the newborn macaque monkey. *Proc. Natl Acad. Sci. USA* **86**, 8093–8097 (1989).
57. Meissirel, C., Dehay, C., Berland, M. & Kennedy, H. Segregation of callosal and association pathways during development in the visual cortex of the primate. *J. Neurosci.* **11**, 3297–3316 (1991).
58. Innocenti, G. M. & Clarke, S. Multiple sets of visual cortical neurons projecting transitorily through the corpus callosum. *Neurosci. Lett.* **41**, 27–32 (1983).

59. Koester, S. E. & O'Leary, D. D. M. Connectional distinction between callosal and subcortical projecting cortical neurons is determined prior to axon extension. *Dev. Biol.* **160**, 1–14 (1993).
60. Ding, S.-L. & Elberger, A. J. Confirmation of the existence of transitory corpus callosum axons in area 17 of neonatal cat: an anterograde tracing study using biotinylated dextran amine. *Neurosci. Lett.* **177**, 66–70 (1994).
61. Kuang, R. Z. & Kalil, K. Development of specificity in corticospinal connections by axon collaterals branching selectively into appropriate spinal targets. *J. Comp. Neurol.* **344**, 270–282 (1994).
62. Woo, T. U., Pucak, M. L., Kye, C. M., Matus, C. V. & Lewis, D. A. Peripubertal refinement of the intrinsic and associational circuitry in monkey prefrontal cortex. *Neuroscience* **80**, 1149–1158 (1997).
63. Riederer, B. M. & Innocenti, G. M. Differential distribution of Tau proteins in developing cat cerebral cortex and corpus callosum. *Eur. J. Neurosci.* **3**, 1134–1145 (1991).
64. Riederer, B. M., Berbel, P. & Innocenti, G. M. Neurons in the corpus callosum of the cat during postnatal development. *Eur. J. Neurosci.* **19**, 2039–2046 (2004).
65. Norris, C. R. & Kalil, K. Guidance of callosal axons by radial glia in the developing cerebral cortex. *J. Neurosci.* **11**, 3481–3492 (1991).
66. Shatz, C. J. Anatomy of interhemispheric connections in the visual system of Boston Siamese and ordinary cats. *J. Comp. Neurol.* **173**, 497–518 (1977).
67. Tremblay, F., Ptito, M., Miceli, D. & Guillemot, J.-P. Distribution of visual callosal projection neurons in the siamese cat: an HRP study. *J. Hirnforsch.* **28**, 491–503 (1987).
68. Innocenti, G. M. & Frost, D. O. Effects of visual experience on the maturation of the efferent system to the corpus callosum. *Nature* **280**, 231–234 (1979).
69. Innocenti, G. M. & Frost, D. O. The postnatal development of visual callosal connections in the absence of visual experience or of the eyes. *Exp. Brain Res.* **39**, 365–375 (1980).
70. Frost, D. O. & Moy, Y. P. Effects of dark rearing on the development of visual callosal connections. *Exp. Brain Res.* **78**, 203–213 (1989).
71. Boire, D., Morris, R., Ptito, M., Lepore, F. & Frost, D. O. Effects of neonatal splitting of the optic chiasm on the development of feline visual callosal connections. *Exp. Brain Res.* **104**, 275–286 (1995).
72. Price, D. J., Ferrer, J. M. R., Blakemore, C. & Kato, N. Postnatal development and plasticity of corticocortical projections from area 17 to area 18 in the cat's visual cortex. *J. Neurosci.* **14**, 2747–2762 (1994).
73. Caric, D. & Price, D. J. Evidence that the lateral geniculate nucleus regulates the normal development of visual corticocortical projections in the cat. *Exp. Neurol.* **156**, 353–362 (1999).
Obtained direct evidence from lesion experiments that showed the role of thalamocortical connections in the selection of cortico-cortical afferents from the initial exuberant stock.
74. Olavarria, J. F. & Van Sluyters, R. C. Overall pattern of callosal connections in visual cortex of normal and enucleated cats. *J. Comp. Neurol.* **363**, 161–176 (1995).
75. Olavarria, J. F. The effect of visual deprivation on the number of callosal cells in the cat is less pronounced in extrastriate cortex than in the 17/18 border region. *Neurosci. Lett.* **195**, 147–150 (1995).
76. Zufferey, P. D., Jin, F., Nakamura, H., Tettoni, L. & Innocenti, G. The role of pattern vision in the development of cortico-cortical connections. *Eur. J. Neurosci.* **11**, 2669–2688 (1999).
77. Innocenti, G. M., Frost, D. O. & Illes, J. Maturation of visual callosal connections in visually deprived kittens: a challenging critical period. *J. Neurosci.* **5**, 255–267 (1985).
78. Manger, P. R. *et al.* The representation of the visual field in three extrastriate areas of the ferret (*Mustela putorius*) and the relationship of retinotopy and field boundaries to callosal connectivity. *Cereb. Cortex* **12**, 423–437 (2002).
79. Olavarria, J. F. Callosal connections correlate preferentially with ipsilateral cortical domains in cat area 17 and 18, and with contralateral domains in the 17/18 transition zone.
J. Comp. Neurol. **433**, 441–457 (2001).
This work makes an important contribution to the concept that the topographic projections from the retina guide the establishment of callosal connections.
80. Restrepo, C. E., Manger, P. R., Spenger, C. & Innocenti, G. M. Immature cortex lesions alter retinotopic maps and interhemispheric connections. *Ann. Neurol.* **54**, 51–65 (2003).
A recent paper that confirms and extends the concept that competition among cortico-cortical axons is involved in shaping cortico-cortical networks.
81. Lotto, R. B., Asavaritikrai, P., Vali, L. & Price, D. J. Target-derived neurotrophic factors regulate the death of developing forebrain neurons after a change in their trophic requirements. *J. Neurosci.* **21**, 3904–3910 (2001).
82. Webster, M. J., Ungerleider, L. G. & Bachevalier, J. Lesions of inferior temporal area TE in infant monkeys alter cortico-amygdalar projections. *Neuroreport* **2**, 769–772 (1991).
83. Webster, M. J. & Bachevalier, J. in *Emotion*, *Memory and Behavior* (ed. Nakajima, T.) 3–15 (CRC, USA, 1995).
84. Gravel, C., Sasseville, R. & Hawkes, R. Maturation of the corpus callosum of the rat: II. Influence of thyroid hormones on the number and maturation of axons. *J. Comp. Neurol.* **291**, 147–161 (1990).
85. Berbel, P. *et al.* Organization of auditory callosal connections in hypothyroid adult rats. *Eur. J. Neurosci.* **5**, 1465–1478 (1993).
86. Li, C.-P., Olavarria, J. F. & Greger, B. E. Occipital cortico-pyramidal projection in hypothyroid rats. *Dev. Brain Res.* **89**, 227–234 (1995).
87. Miller, M. W., Astley, S. J. & Clarren, S. K. Number of axons in the corpus callosum of the mature *Macaca nemestrina*: increases caused by prenatal exposure to ethanol. *J. Comp. Neurol.* **412**, 123–131 (1999).
88. 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).
89. Bishop, K. M., Garel, S., Nakagawa, Y., Rubenstein, J. L. & O'Leary, D. D. *Emx1* and *Emx2* cooperate to regulate cortical size, lamination, neuronal differentiation, development of cortical efferents, and thalamocortical pathfinding. *J. Comp. Neurol.* **457**, 345–360 (2003).
90. Huffman, K. J., Garel, S. & Rubenstein, J. L. R. Fgf8 regulates the development of intra-neocortical projections. *J. Neurosci.* **24**, 8917–8923 (2004).
91. Clarke, S., Kraftsik, R., Van der Loos, H. & Innocenti, G. M. Forms and measures of adult and developing human corpus callosum: is there sexual dimorphism? *J. Comp. Neurol.* **280**, 213–230 (1989).
92. DeLacoste-Utamsing, C. & Holloway, R. L. Sexual dimorphism in the human corpus callosum. *Science* **216**, 1431–1432 (1982).
93. Kim, J. H. Y. & Juraska, J. M. Sex differences in the development of axon number in the splenium of the rat corpus callosum from postnatal day 15 through 60. *Dev. Brain Res.* **102**, 77–85 (1997).
94. Nunez, J. L., Nelson, J., Pych, J. C., Kim, J. H. Y. & Juraska, J. M. Myelination in the splenium of the corpus callosum in adult male and female rats. *Dev. Brain Res.* **120**, 87–90 (2000).
95. Bishop, K. M. & Wahlsten, D. Sex differences in the human corpus callosum: myth or reality? *Neurosci. Biobehav. Rev.* **21**, 581–601 (1997).
96. Dubb, A., Gur, R., Avants, B. & Gee, J. Characterization of sexual dimorphism in the human corpus callosum. *Neuroimage* **20**, 512–519 (2003).
97. Hwang, S. J. *et al.* Gender differences in the corpus callosum of neonates. *Neuroreport* **15**, 1029–1032 (2004).
98. Witelson, S. F. The brain connection: the corpus callosum is larger in left-handers. *Science* **229**, 665–668 (1985).
99. Witelson, S. F. & Goldsmith, C. H. The relationship of hand preference to anatomy of the corpus callosum in men. *Brain Res.* **545**, 175–182 (1991).
100. Habib, M. *et al.* Effects of handedness and sex on the morphology of the corpus callosum: a study with brain magnetic resonance imaging. *Brain Cogn.* **16**, 41–61 (1991).
101. Giedd, J. N. *et al.* Quantitative morphology of the corpus callosum in attention deficit hyperactivity disorder. *Am. J. Psychiatry* **151**, 665–669 (1994).
102. Magara, F., Ricceri, L., Wolfer, D. P. & Lipp, H.-P. The acallosal mouse strain I/LnJ: a putative model of ADHD? *Neurosci. Biobehav. Rev.* **24**, 45–50 (2000).
103. von Plessen, K. *et al.* Less developed corpus callosum in dyslexic subjects—a structural MRI study. *Neuropsychologia* **40**, 1035–1044 (2002).
104. Innocenti, G. M., Ansermet, F. & Parnas, J. Schizophrenia, neurodevelopment and corpus callosum. *Mol. Psychiatry* **8**, 261–274 (2003).
105. Portera-Calliau, C., Weimer, R. W., De Paola, V., Caroni, P. & Svoboda, K. Diverse modes of axon elaboration in the developing neocortex. *PLoS Biol.* **3**, e272 (2005).
106. Bagri, A. Cheng, H. J., Yaron, A., Pleasure, S. J. & Tessier-Lavigne, M. Stereotyped pruning of long hippocampal axon branches triggered by retraction inducers of the semaphoring family. *Cell* **113**, 285–299 (2003).
107. Ernst, A. F., Gallo, G., Letourneau, P. C. & McLoon, S. Stabilization of growing retinal axons by the combined signalling of nitric oxide and brain-derived neurotrophic factor. *J. Neurosci.* **20**, 1458–1469 (2000).
108. McLaughlin, T., Hindges, R., Yates, P. A. & O'Leary, D. D. M. Bifunctional action of ephrin-B1 as a repellent and attractant to control bidirectional branch extension in dorso-ventral retinotopic mapping. *Development* **130**, 2407–2418 (2003).
109. McLaughlin, T., Torborg, C. L., Feller, M. B. & O'Leary, D. D. M. Retinotopic map refinement requires spontaneous retinal waves during a brief critical period of development. *Neuron* **40**, 1147–1160 (2003).
110. Muzio, L. & Mallamaci, A. *Emx1*, *Emx2* and *Pax6* in specification, regionalization and arealization of the cerebral cortex. *Cereb. Cortex.* **13**, 641–647 (2003).
111. Nakayama, A. Y., Harms, M. B. & Luo, L. Small GTPases Rac and Rho in the maintenance of dendritic spines and branches in hippocampal pyramidal neurons. *J. Neurosci.* **20**, 5329–5338 (2000).
112. Tettoni, L., Gheorghita-Baechler, F., Bressoud, R., Welker, E. & Innocenti, G. M. Constant and variable aspects of axonal phenotype in cerebral cortex. *Cereb. Cortex* **8**, 543–552 (1998).
Shows that, surprisingly, axons from different species, systems and those that form different types of connection share several common features and relatively few variations of a common 'Bauplan'.
113. Thomson, A. M. & Morris, O. T. Selectivity in the inter-laminar connections made by neocortical neurons. *J. Neurocytol.* **31**, 239–246 (2002).
114. Lein, S. & Shatz, C. J. Rapid regulation of brain-derived neurotrophic factor mRNA within eye-specific circuits during ocular dominance column formation. *J. Neurosci.* **20**, 1470–1483 (2000).
115. Bengtsson, S. I. *et al.* Extensive piano practicing has regionally specific effects on white matter development. *Nature Neurosci.* **8**, 1148–1150 (2005).
116. Shepherd, G. M. G., Stepanyants, A., Bureau, I., Chklovskii, D. & Svoboda, K. Geometric and functional organization of cortical circuits. *Nature Neurosci.* **8**, 782–790 (2005).
117. Klingberg, T. *et al.* Microstructure of temporo-parietal white matter as a basis for reading ability: evidence from diffusion tensor magnetic resonance imaging. *Neuron* **25**, 493–500 (2000).
118. Zhang, J. *et al.* Mapping postnatal mouse brain development with diffusion tensor microimaging. *Neuroimage* **26**, 1042–1051 (2005).
119. Snook, L., Paulson, L. A., Roy, D., Phillips, L. & Beaulieu, C. Diffusion tensor imaging of neurodevelopment in children and young adults. *Neuroimage* **26**, 1164–1173 (2005).
120. Saleem, K. S. *et al.* Magnetic resonance imaging of neuronal connections in the macaque monkey. *Neuron* **34**, 685–700 (2002).
121. Knyazeva, M. G. & Innocenti, G. M. EEG coherence studies in the normal brain and after early-onset cortical pathologies. *Brain Res. Rev.* **36**, 119–128 (2001).
122. Carmeli, C., Knyazeva, M. G., Innocenti, G. & De Feo, O. Assessment of EEG synchronization based on state-space analysis. *Neuroimage* **25**, 339–354 (2005).
123. Knyazeva, M. G., Fornari, E., Meuli, R., Innocenti, G. M. & Maeder, P. Imaging a synchronous neuronal assembly in the visual brain. *Neuroimage* 20 Sep 2005 [epub ahead of print].
124. Innocenti, G. M., Maeder, P., Knyazeva, M., Fornari, E. & Deonna, T. Functional activation of microgyric visual cortex in man. *Ann. Neurol.* **50**, 672–676 (2001).
125. Zesiger, P., Kiper, D., Deonna, T. & Innocenti, G. M. Preserved visual function in a case of occipito-parietal microgyria. *Ann. Neurol.* **52**, 492–498 (2002).

Competing interests statement
The authors declare no competing financial interests.

Online links

DATABASES
The following terms in this article are linked online to:
Entrez Gene: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene
BDNF | EMX2 | FGF8 | FOXG1 | *Id2* | PAX6 | *Pzr*β

FURTHER INFORMATION
Genetic control of brain development: http://129.215.8.155/cnsdb/
Price's laboratory: http://www.bms.ed.ac.uk/research/idg/gendev/price/

SUPPLEMENTARY INFORMATION
See online article: S1 (table)
Access to this interactive links box is free online.