Intrinsic and extrinsic factors that shape neocortical specification

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Increasing evidence points to the importance of intrinsic molecular cues in specifying the regional identity of mammalian neocortex. Few such cues, however, have been found to be restricted to individual functionally defined cortical areas before the arrival of afferent information. In contrast, thalamocortical axons are specifically targeted to individual cortical areas, raising the possibility that they can instruct some aspects of cortical areal identity. Cortical structure and function can be altered by modifying the source or pattern of activity in thalamocortical afferents. In particular, studies of cross-modal plasticity have shown that in many respects, one sensory cortical area can substitute for another after a switch of input modality during development. Afferent inputs might therefore direct the formation of their own processing circuitry, a possibility that has important implications for brain development, plasticity and evolution.

In many ways, the cerebral cortex is the most impressive result of both brain development and brain evolution. Its marked expansion in primates such as ourselves is reflective of its important role in our behavioral complexity: Given the fascination that we humans have with our own minds, it is not surprising that the mammalian cerebral cortex has been a popular subject of study. One of the most obvious organizational features of the neocortex is its parcellation into a tremendous number of structurally and functionally distinct ‘areas’, such as primary sensory and motor areas, as well as higher order areas, such as language cortex. This localization of function has been noted since the time of ancient philosophers such as Galen, and was explored by Gall in the late 18th century. The mechanistic basis of the cortical pattern formation process, however, has been a matter of considerable and continuing debate.

The debate has centered on whether different cortical areas are programmed to be different early on, when neurons are being generated and are migrating into place (intrinsic control, Ref. 2), or whether later external events, such as the arrival of sensory inputs, instruct cortical identity (extrinsic control, Refs 3,4) (Fig. 1a,b). Available sources of intrinsic and extrinsic cortical patterning information include molecular factors intrinsic to the neocortical ventricular zone (where neurons are generated), to the neocortical subplate (through which the cortical cells migrate after their birth) or to the maturing neocortex itself; spontaneous, coordinated activity patterns intrinsic to the cortex; later arriving molecular factors extrinsic to the cortex, such as those deriving from afferent pathways; and extrinsically driven activity patterns (either sensory inputs or spontaneous activity extrinsic to cortex). The ultimate goal is to discover which of these factors operates when, where and to what extent, and, importantly, how they interact at each stage.

This article briefly outlines the debate and discusses recent data from gene knockout, cortical deletion and cross-modal plasticity experiments that shed new light on the issue. A synthetic view is emerging: that intrinsic and extrinsic patterning information act both separately and synergistically at different stages of neocortical development.

Time-course of specification of the mammalian neocortex

The sequence of events in cortical differentiation provides clues to its control, but there is no magic moment at which a cortical area comes into existence; rather there is a progressive differentiation of the forebrain, then the telencephalon, the dorsal pallium and finally the neocortex. At prenatal stages, several neocortical ‘regions’ can be distinguished by the differential expression of multiple gene families, before the arrival of extrinsic inputs5,6.

Peri- or postnatally, depending on the species, the neocortex becomes parcellated into smaller divisions, consisting of the individual sensory, motor and integrative ‘areas’ as defined by cytoarchitecture, connectivity and physiology. Whether the borders of the gene expression regions eventually form areal borders is unknown, and importantly, the regional expression patterns do not always exhibit sharp borders. Distinct areas can be detected close to the time that thalamocortical axons arrive at their target cortical layer 4, which occurs at approximately postnatal day (P) 3 in hamster and rat, P1 in mouse, P14 in ferret, embryonic day (E) 56 in cat, E91 in macaque, and E130 in human7–11. The targeting of the thalamocortical pathway is directed and area specific, even under conditions of cortical deafferentation12–14. These observations raise the possibility that regionalization is caused by the genes that drive the differential expression patterns, and that the thalamic inputs control the later areal subdivision stage, through either activity-dependent or activity-independent mechanisms. Recent experiments have shed new light on these ideas.

Intrinsic control of neocortical specification

Regionalization

The temporal correlation between neocortical regionalization and the appearance of restricted
patterns of gene expression is consistent with the hypothesis that one or more of the genes might be orchestrating the regionalization, independently of thalamocortical input. In fact, knockout mice that genetically lack a complete thalamocortical pathway exhibit normal, regionally specific prenatal expression patterns of several classes of downstream transcription factors and axon guidance molecules in the neocortex. This provides strong evidence that intrinsic cues are sufficient and that thalamic input is unnecessary to specify early regional identity, at least at the level of the gene expression patterns examined. One cannot infer from these results, however, that the particular genes examined direct the specification of cortical identity. In addition, because these knockout mice die at birth, before parcellation of individual neocortical areas, it has not been possible to determine if areal specification is affected; that is, whether the restricted gene expression patterns seen early on eventually produce borders between functional areas later in development. Suggestive evidence comes from the observation that the expression borders of the transcription factors Id2 and Thbr1 approximate the border between somatosensory and motor cortex, as defined by 5-HT and cytochrome oxidase staining patterns, but the causal factor(s) have not been determined.

The availability of gene knockout techniques has allowed manipulation of candidate cortical patterning genes, an approach that increases the likelihood of identifying causal links between gene expression patterns and the regional subdivision process. Knockout of the regulatory genes Emx2 or Pax6 results in the prenatal loss of caudal or rostral portions of the cortical epithelium, respectively, where these genes are normally expressed at high levels, and a disproportionate expansion of more rostral or caudal areas of cortex (Fig 1a,c). Further studies that incorporate independent assessments of regional and areal identity using anatomical and physiological as well as molecular methods are needed to determine whether these genes cause neocortical specification.

Arealization

After regional subdivisions are in place, individual areas are formed and begin to acquire their unique functional and anatomical characteristics. If regionally restricted molecules also control specification of individual functional cortical areas, then some areal borders would be expected to coincide with borders of molecularly defined cortical regions. Despite extensive searching, only a few genes whose expression is restricted to the confines of a single functionally defined cortical area before thalamocortical innervation have been found, suggesting that extrinsic factors might be required for this step. Adjacent cortical areas could acquire their borders through overlapping gradients of gene expression, however, rather than through unique genes within each individual area. It is difficult if not impossible to define areal borders in prenatal or early postnatal tissue, complicating the assertion that gene expression is restricted to functional areas prior to thalamocortical innervation.

Although some restricted gene expression patterns appear early, without benefit of extrinsic cues, and are sufficient to promote regional differences in the expression of downstream genes, thalamic input might be required to maintain them. Whether this means that regional identity is being maintained by thalamic input is not clear. Information established by restricted early gene expression patterns might also be malleable or even reversible at a later stage of development, perhaps in response to extrinsic information.

Cortical ablation produces compression of the fate map

A clever experiment by Huffman and colleagues suggests that the cortical fate map can be compressed, even if nonlinearly, or that areal fate can be specified or respecified by the thalamocortical inputs. Ablation of large parts of the presumptive neocortex before thalamocortical invasion presents
the thalamic nuclei with a much smaller target to parse. Nonetheless, the remaining cortical sheet develops visual, auditory and somatosensory cortical areas in a similar spatial relationship to that in normal animals, and the same thalamic nuclei as usual innervate these areas (Fig. 1d), although there is an increase in the spatial extent of multimodal cortex and a decrease in the extent of the visual area proportionate to the lesion size. The relative roles of intrinsic and extrinsic or molecular versus activity-dependent factors cannot be determined from these intriguing data, although the results are consistent with a crucial role for thalamocortical afferents in areal specification.

Extrinsic control of neocortical specification

If it is the case that early molecular events specify only regional identity and that extrinsic information is needed to establish areal identity, this information could be either molecular or activity based (or both). Recent studies suggest that at least some patterning characteristic of individual cortical areas, such as the development of orientation and ocular dominance modules in visual cortex, might be initially independent of sensory input, and sensation might be required only for maintenance or alteration of the original pattern. Activity-dependent patterning need not derive from a sensory organ, however. Coordinated spontaneous activity in the input pathway before sensory organ function drives the formation of crude circuits that are later refined by external sensory cues.

Thalamocortical axons are in an ideal position to provide molecular or activity-based patterning information to neocortex. Their access to the external world via the sensory organs, their scaffold-like projection specificity, and their early contact with neurons destined for layer 4 (Ref. 32) are important components of this hypothesis. Experimental approaches that have altered the activity pattern of thalamocortical inputs to cortex have provided support for the idea that thalamocortical axons are instructing areal identity.

Cross-modal ‘rewiring’ provides a valuable approach to the role of extrinsic influences on cortical specification

The cross-modal plasticity paradigm, which diverts retinal afferents into non-visual targets, was originally developed by Schneider and Frost in hamsters33,34, and then employed by Scalia and colleagues in frogs35 and by Sur and colleagues in ferrets36. In adult frogs, cutting and redirecting the regenerating optic nerve into the telencephalon results in direct, functional (but not topographic) retinal projections into the olfactory lobe37. In hamsters or ferrets, neonatal lesions of the retinorecipient layers of the superior colliculus combined with deafferentation of auditory or somatosensory thalamus results in functional, topographic retinal projections to the affected thalamic area,38-40, providing visual information indirectly to auditory or somatosensory cortex, respectively (Fig. 2). In the case of the redirected retinotthalamic projections, and unlike the frog paradigm, the modality and presumably the spatiotemporal pattern of activity provided to cortex is changed without changing the identity of the thalamic axons carrying the information, allowing an experimental decoupling of the source of thalamic activity from its pattern. Because the effect of the manipulation on areal specification is not tested until adulthood, the characteristics typically considered to define cortical areal identity, such as connectivity, topography and response properties, can be examined.

In ferrets, the retina-to-auditory thalamus projection comes from the W-type of retinal ganglion cell, in hamsters it is not known which ganglion cell types exhibit the plasticity, although they are likely to be the population that normally projects to the dorsal lateral geniculate nucleus44. As a result of redirecting the retinal axons to auditory thalamus (MGN) or somatosensory thalamus (VB), the modality and pattern of activity of afferents to primary sensory cortex is changed, but without changing the identity of the thalamic axons carrying the information.

Early ectopic retinal inputs ‘visualize’ the target

Redirection of visual information to non-visual primary sensory cortex results in a striking change in functional properties, in that the non-visual cortex comes to respond to visual stimulation in a surprisingly normal way. In the visual to somatosensory manipulation in hamsters, the superior–inferior axis of the retina is mapped in primary somatosensory cortex (S1). Visually responsive neurons in S1 have receptive field properties similar to those in visual cortex, such as movement sensitivity, and orientation and direction tuning. In ferrets with cross-modal rerouting to the auditory pathway, primary auditory cortex (A1) contains a two-dimensional map of visual space and neurons that, although sluggish (as expected from

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their W-input), are well tuned to various aspects of visual stimuli, such as the direction and velocity of movement and the orientation of visual edges. This is true despite the retention by A1 of its normal thalamocortical and corticocortical connections with other auditory structures. The presence of these two-dimensional responses in a cortical area that receives highly overlapped, one-dimensional thalamocortical input is unexpected, and suggests that the second dimension of the mapping information is reconstructed within A1 itself (Fig. 3). Recent data support this interpretation.

**Early visual input alters modular organization in auditory cortex**

Modular organization is an important characteristic of primary sensory cortex, and modules seen in visual cortex include ocular dominance columns and orientation ‘pinwheels’ (see Ref. 53 for a review). Auditory cortical modules organize sound frequency and binaural information. In both visual and auditory cortex, neurons that are matched in stimulus response characteristics are interconnected. It has been observed that the modular and topography-based connectivity patterns in cross-modal A1 are reorganized, perhaps allowing A1 to represent visuospatial information.

**Ocular dominance columns.** In MGN of cross-modal ferrets, left and right eye information is found in segregated, alternating regions or columns, as in the normal visual pathway. This might seem surprising, but given that eye-specific segregation can occur in other unexpected situations such as frogs in which a third eye has been implanted, it could be the natural outcome of any similar situation where afferents with non-coincident patterns of activity are competing for target space (see Ref. 65 for a review).

**Functional connectivity.** Callosal and horizontal connectivity patterns in normal ferret A1 are similar to those in cats, with banded callosal connections oriented along the tonotopic axis and horizontal connections clustered as a strip along the isofrequency axis (Fig. 4a). In cross-modal A1, callosal connections are arranged as small patches rather than bands and are excluded from the medial region. Horizontal connections are arranged as a semicircular collection of bouton clusters (Fig. 4b). Neurons in cross-modal A1 with similar visual stimulus orientation tuning are interconnected in a pinwheel-like organization of orientation tuning as in normal V1 (Ref. 69). Control ferrets that have been deafened as neonates have diffuse callosal and horizontal connectivity patterns, suggesting that the pattern seen in cross-modal does not result from deafferentation. These results represent the first evidence that changing only the modality and thus the spatiotemporal pattern of activity of sensory inputs, without changing the identity of the thalamocortical fibers carrying the information, can profoundly alter the pattern of cortical connectivity.

**Chemooarchitecture.** Orientation tuning in visual cortex arises in part through intrinsic GABAergic inhibition. In the cross-modal animals, changes are seen in the number and distribution of GABAergic neurons containing parvalbumin (PV) and calbindin (CB), and many CB neurons have an atypical morphology extending horizontally rather than vertically within a cortical column. Suppression of neighboring columns via these neurons could play a role in the ability of A1 to process visuospatial information such as oriented edges.

**Early visual input allows auditory cortex to mediate visual perception**

The aforementioned observations suggest that the early, anomalous visual inputs to the auditory
Fig. 4. Functional connectivity in the primary auditory cortex (A1) can be altered by visual input. (a) Callosal (interhemispheric) connectivity. Tangential pattern of retrograde and anterograde callosal label resulting from tracer (horseradish peroxidase) injections throughout the contralateral A1. Left: Normal animal. The regions receiving excitatory input from both ears (EE binaural bands) project callosally. Center: Deaf animal. Callosal label is widespread and diffuse. Right: Cross-modal animal. Connections are well-refined but distributed differently than in normal or deaf animals. (b) Horizontal (intracortical) connectivity. Schematic derived from plotting the distribution of labeled boutons following a small injection of the anterograde tracer BDA (blue circle) into A1. Horizontal connections in normal animals (left) are clustered along the isofrequency axis. In deaf animals, labeled boutons are widespread and unclustered (center). In cross-modal animals (right), boutons show clustering but have a different pattern, extending into medial A1.

Fig. 5. The morphology of many calbindin-immunoreactive neurons is altered in cross-modal primary auditory cortex (A1). Rather than arborizing within a column, as is typical (left), the dendrites of many calbindin neurons in cross-modal A1 extend laterally across columns (right), where they could affect overlapped inputs from medial geniculate nucleus (MGN). Scale bar, 50 μm.

Evolutionary significance

Taken together, these results suggest that if auditory cortex receives visual input during development, it acquires a modular organization resembling that seen in visual cortex in several important respects, and in addition, cortical circuits and their component neurons are substantially altered. This interpretation supports the contention that visual inputs can direct the construction of their own processing circuitry. It seems likely that this task would be more difficult in a non-sensory cortical area or particularly in a non-isocortical region. The results further suggest that alterations in sensory input pathways coupled with available cortical space could act as an important substrate for brain evolution. The exclusion of callosal projections from medial regions in cross-modal A1 suggests that it has been further subdivided, and that perhaps a ‘new’ cortical area has been created. The ability to induce formation of a new cortical area would provide a valuable model for the early stages of cortical parcellation driven by new inputs or by a modified pattern of activity during evolution.

Sensory deprivation can induce cross-modal plasticity: clinical significance

If it can be understood how sensory inputs instruct the development and plasticity of brain circuitry, then perhaps activity could be manipulated to encourage compensatory changes in brain-damaged infants. Such strategies have already proven valuable for within-modality compensation, such as therapies available for amblyopia and language impairment in children, and for motor recovery from stroke in older patients. Tantalizing evidence suggesting that directed cross-modal compensation might be possible comes from imaging studies on sensory substitution in deaf and blind individuals (see Ref. 89 for a review). For example, in early deaf individuals, non-auditory stimuli, including American Sign Language, can activate language cortex, and visual stimuli can activate putative auditory areas. In the early blind, improved auditory performance and auditory or somatosensory activation of occipital cortex have been reported.
cortical epithelium and provide regional cues that bias or perhaps even specify the parcellation of cerebral cortex into functionally specific regions. Later, extrinsic activity-based cues appear to play an instructive role in the organization, maintenance and plasticity of at least some cortical circuits, modules and areas. Experiments employing cross-modal plasticity show that it is not simply the source of the thalamocortical afferents, but the pattern of activity they convey that instructs cortical circuits, in turn influencing the collection of properties that we refer to as the functional ‘identity’ of cortical areas. In the future, it would be of great value to identify mutant mice with mistargeted or missing thalamocortical axon pathways that survive beyond the areal specification stage, and then determine the effect of the mistargeting on the areal specification process. This area of research holds great promise for understanding cortical development, evolution, and recovery from brain damage.

Concluding remarks
Intrinsic and extrinsic information sources are both important in brain development. Current evidence suggests that intrinsic instructions pattern the

Selected references

Fig. 6. Summary of the results from cross-modal plasticity experiments. (a) Normal, unimodal sensory pathways. (b) Redirection of visual afferents into the auditory thalamus allows the primary auditory cortex (A1) to respond ‘normally’ to visual stimuli, although sound-sensitive cells might still be present, either in an overlapping (red-purple stripes) or segregated pattern.


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