Do cortical areas emerge from a protocortex?

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The adult mammalian neocortex consists of numerous 'areas' distinguished from one another largely on the basis of distinctions in cytoarchitecture and connections. The developing neocortex, though, lacks many of these area-specific distinctions, and is more uniform across its extent. This less differentiated structure, here termed the 'protocortex', undergoes considerable modification after neurogenesis which results in the emergence of well-defined neocortical areas. To what extent, then, are neocortical areas predetermined? This issue is considered in the context of recent findings on the generation of the neocortex and its subsequent parcellation into distinct areas.

The neocortex is unique to mammals. Although it differs greatly in complexity between mammalian species, in all mammals it can be divided on both morphological and functional grounds into a sizeable number of 'areas'[1,2]. There are phylogenetic differences in neocortical parcellation which reflect the addition of higher order 'associational' areas and an increase in the specialization of regions of neocortex to perform specific functions[3]. Much attention has been directed toward understanding the organization and operation of the neocortex. Recently, though, an increased amount of effort has been focused on determining how areas of the neocortex acquire their unique characteristics[4]. Although this question relates to an understanding of the mechanisms underlying the phylogenetic expansion of the neocortex in terms of the size and the number of definable areas, studies of neocortical development provide the best opportunity for answers. One can imagine two extreme positions of how distinct areas are developed: the neuroepithelium which gives rise to the neocortex may be regionally specified to generate area-unique lineages of neurons that reflect the area-specific features of the adult neocortex, or alternatively, the neocortical neuroepithelium may generate uniform lineages across its extent and rely on subsequent interactions to bring about the differentiation of areas. I will consider here an increasing body of evidence which suggests that many prominent features distinctive of the differentiation of areas of the neocortex are not determined at the time of neurogenesis, but rather are established through subsequent epigenetic interactions involving a variety of mechanisms.

Some distinctions and similarities between cortical areas in the adult

Areas of the adult neocortex are clearly dissimilar. Neocortical areas can be distinguished from one another by differences in connections, both outputs and inputs, as well as by distinctions in architecture, from different distributions of receptors for neurotransmitters to variations in cell sizes and densities. These area-specific characteristics contribute to the unique functional properties of the various neocortical areas. But, in spite of the many striking differences between areas, certain features are shared. The most obvious common feature is that by convention all neocortical areas have six primary layers. Although the appearance of individual layers changes at the borders between areas, the chief characteristics of each layer are retained. For example, the same basic scheme of laminar organization of sources of cortical outputs applies to all: neurons in layer 6 project to the thalamus and claustrum, neurons in layer 5 send their axons to all other subcortical targets, and layers 2 and 3 are the principal source of projections to other neocortical areas, ipsilaterally and contralaterally[5,6].

Even the basic cellular constituents seem to be consistent from one area to another. Although cortical thickness varies considerably, the number of neurons found in a 'radial traverse' through the six layers is surprisingly constant between diverse cortical areas within a species, as well as across species[7,8]. A notable exception is that the number of neurons found in a radial traverse in primary visual cortex (area 17) is higher than in other areas[7–9]. The proportion of cells classified by shape as pyramidal or non-pyramidal is also constant between two very different areas, the primary motor and visual areas[10]. Similarly, the predominant cortical inhibitory cell, the GABAergic neuron, is present in roughly equivalent proportions in all areas examined[8]. Cortical neurons that might use other neurotransmitters or modulators, for example those immunoreactive for choline acetyltransferase (the synthesizing enzyme for the neurotransmitter acetylcholine)[11], as well as interneurons of various peptide phenotypes[12–15], are also found in all neocortical areas. In short, all of the basic morphological and chemically defined types of cortical neurons identified to date are widely distributed within the adult neocortex.

Based on these and other structural and functional consistencies between areas of the adult neocortex, it has been proposed by both neuroanatomists and neurophysiologists, especially Lorente de No[16], Creutzfeld[17], Mountcastle[18], Powell[19] and Eccles[20], that different primary cortical areas share a common organizational scheme. This suggestion has been addressed experimentally in two independent sets of experiments in which somatosensory or auditory cortex was induced to process visual information by misrouting, during development, retinal axons to somatosensory thalamus[21] or to auditory thalamus[22] (Fig. 1). In these animals, the receptive field and response properties of cells in somatosensory or auditory cortex to visual stimuli resemble those normally seen in visual cortex. The most straightforward explanation for these findings is that the primary sensory areas of the neocortex normally process sensory information relayed through the thalamus in a fundamentally similar way, implying that the basic organization of cells and connections that underlie functional properties is also similar. This interpretation is supported by the finding that some cells in the somatosensory cortex to which visual input is directed can respond both to visual and somatosensory stimuli in modality-appropriate ways[21]. An alternative explanation is that the intrinsic organizations of neocortical

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sensory areas are not normally similar at mature stages, but that their development can be altered by visual input. However, even this suggests that primary sensory areas arise from regions of developing neocortex that are initially similar or to some extent pluripotent.

In summary, it appears that areas of the adult neocortex are constructed with the same basic set of cells organized in a fundamentally similar way, yet, by definition, each area has distinctive features.

Early events in cortical development

A discussion of differentiation of the areas of the neocortex should include the early stages of cortical development. Cortical neurons are generated in the neuroepithelium of the lateral ventricle. They migrate away from this site along the processes of radial glial cells and form the cortical layers in a deep-to-superficial sequence[4,24]. Previous studies have suggested that the young neurons are deposited in a radial fashion within the developing cortical plate. The first direct demonstration of this has come recently from studies in which a progenitor cell is infected with a recombinant retrovirus carrying a marker gene which allows for later identification of its progeny. Using this approach, it has been shown that clonally related cortical neurons are usually distributed in roughly radial arrays[25,26]. Occasionally, though, clonally related cells can be tangentially displaced within the cortex over distances that are substantial relative to the size of individual cortical areas[26]. These findings bear on the issue of area differentiation, since in its simplest form the concept of a specification of the neuroepithelium to give rise to specific cortical areas requires that neurons generated by a specific proliferative region remain segregated within the overlying cortical plate from neurons produced by neighboring proliferative regions[4]. A re-examination of this issue using two distinguishable viral markers that are now available[27] should allow for a firm determination of the frequency and magnitude of tangential displacements of clonally related neurons.

The findings from mouse chimera studies suggest that if the neocortical neuroepithelium does become regionally specified, specification must be a relatively late event. Neurons derived from blastula fusions of two strains of mice seem to be randomly dispersed within the mature neocortex[28], implying that proliferative cells mix within the neocortical neuroepithelium close to the time that the first neurons become postmitotic. At these and later stages, morphological distinctions that could suggest the subdivision of the neocortical neuroepithelium into regions are not apparent[29], while discontinuities indicative of the mosaic organization of certain other proliferative zones, for example the thalamic neuroepithelium, can be clearly discerned[30]. But any regional specification of the neocortical neuroepithelium should be revealed by a parallel expression of unique molecules in distinct patterns. Interestingly, antibodies to the peptides encoded by four proto-oncogenes (*sis-*, *src-*, *ras-* and *myc-*), and against the intermediate filament protein vimentin, co-stain patches of radial glial cells spanning the neocortical neuroepithelium of rats[31], whereas other antibodies to components of the neuroepithelium, D1.1[31,32] and Rat-401[33], stain it homogeneously. Presently, this is the best evidence for a structural or molecular regionalization within the neocortical neuroepithelium. However, since the patchy pattern of peptide staining emerges from a uniformly stained neuroepithelium only at very *late* stages of neurogenesis[31], it is not clear how such regionalization would play a role in an early specialization of the neuroepithelium.

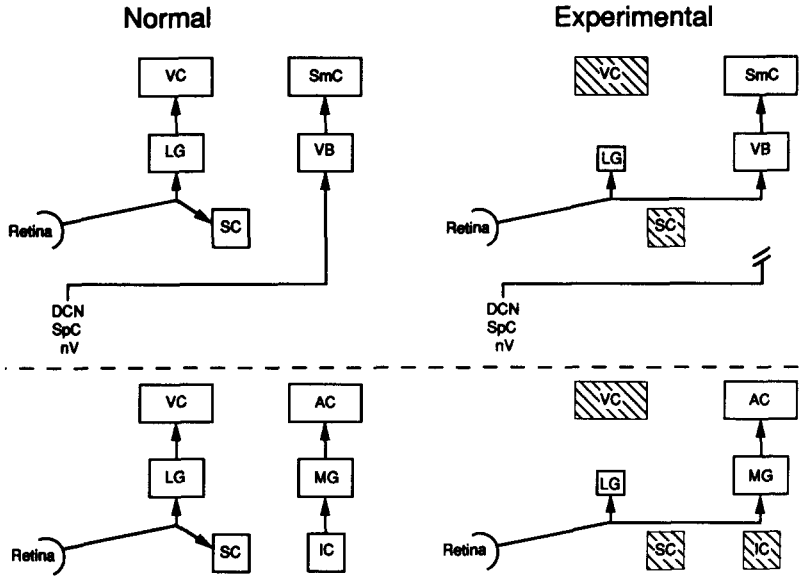

Fig. 1. *Aberrant routing of visual input into somatosensory and auditory cortex.* Top left: *in normal hamsters, the retina projects to the primary visual thalamic nucleus, the lateral geniculate nucleus (LG), and the superior colliculus (SC). The LG relays visual information to the visual cortex (VC). Somatosensory information is sent from the dorsal column nuclei (DCN), spinal cord (SpC) and the trigeminal nuclei (nV) to the primary somatosensory thalamic nuclei, termed the ventrobasal complex (VB), which in turn relays it to the somatosensory cortex (SmC).* Top right: *the retina can be induced to project to VB, by reducing its normal targets (by removing at birth the SC and the VC, which results in atrophy of the LG) and making terminal space available in the VB (by removing at birth its normal input). Under these conditions, SmC receives visual input from VB (see Ref. 21).* Bottom left: *in normal ferrets, the retina projects to the LG and the SC. The LG projects to several visual cortical areas. The primary auditory thalamic nucleus, the medial geniculate (MG), receives auditory information from the inferior colliculus (IC), and relays it to the auditory cortex (AC).* Bottom right: *the retina can be induced to project to MG by a similar strategy to that described above; retinal targets are reduced (by removing the SC and visual cortical areas 17 and 18, which results in atrophy of the LG) and terminal space is made available in MG (by removing IC). Under these conditions, AC receives visual input from MG (see Ref. 22).* (Figure modified from figures appearing in Refs 22 and 23.)

Generation of a 'protocortex'

The developing neocortex is distinct from the adult form in notable ways. First, it contains transient structures. The earliest recognized cortical structure is a cellular layer[34], termed the preplate, that does not persist into adulthood. The neurons that populate this layer are the first to be generated by the neocortical neuroepithelium, but die over the course of development[35]. Later generated neurons that form the cortical plate aggregate within the preplate and split it into two layers. The upper layer develops into layer 1, while the lower layer, termed the subplate, becomes part of the axon tracts underlying layer 6. Presently, it is not clear if the preplate is simply a phylogenetic remnant, or if it plays a critical role in cortical development before its demise[36].

TABLE I. Selected reports of developmentally widespread distributions of cortical projection neurons

Type of projection neuron	Mammalian order	Refs
Pyramidal tract/Corticospinal	Rodents	39, 40, 41
	Marsupials	42
	Lagomorphs	43
Corticotectal	Rodents	44
Callosal (commissural)	Rodents	45, 46
	Marsupials	47
	Lagomorphs	48
	Carnivores	49, 50, 51
	Primates	52, 53

Additionally, the neocortex is more uniform across its extent during development than at maturity, as it lacks many of the area-specific features characteristic of the adult. For instance, the primary somatosensory cortex of adult rodents contains a one-to-one representation of the mystacial vibrissae found on the muzzle, and sinus hairs present on the head and limbs, in the form of aggregations of layer 4 neurons and thalamic afferents referred to as barrels[37] (see Fig. 2). However, barrels are not apparent as the cortex is assembled, but emerge later from an initially uniform cortical plate[38]. Another example of uniformity in the developing neocortex can be taken from the development of area-specific outputs. In the adult neocortex, the unique outputs of specific areas are reflected in part by the limited distributions of types of cortical projection neurons, including those that send axons to subcortical targets such as the superior colliculus (corticotectal neurons), certain medullary nuclei and the spinal cord (pyramidal tract neurons), or through the corpus callosum to the contralateral cortex (callosal neurons). However, during development all of these classes of projection neurons are widely distributed across the neocortex (Table I). The restricted distributions of projection neurons in the adult, then, do not reflect regional differences in the ability of the neocortical neuroepithelium to generate general classes of cortical projection neurons.

Taken together, these comparisons of the organization of developing and adult neocortex lead to a reasonable conclusion that the entire extent of the neocortical neuroepithelium is competent to generate most, if not all, of the basic classes of cortical neurons, both permanent and transient. Further, early in its development, the neocortex not only contains large, transient populations of neurons, but also lacks the architectonic divisions characteristic of area diversity in the adult neocortex. The relative uniformity of the early neocortex compared with its adult form suggests that many of the area-specific features characteristic of the adult are not predetermined within the neuroepithelium. The neocortical neuroepithelium may generate a 'protocortex' from which well-defined areas gradually emerge in a manner dependent upon influences that operate after neurogenesis. If different regions of the protocortex are indeed similar and their differentiation is not rigidly predetermined, one would expect that they would be capable of considerable plasticity in their expression of area-specific features. In the following sections, this issue will be examined.

Development of area-specific outputs

The set of output projections of a given neocortical area in the adult is a subset of the projections that it originally elaborates. Although just a subset is retained by a given area, these early, widespread projections are made only to specific sets of targets appropriate for the general class of projection neuron from which they arise[54,55,57]; the subset retained in the adult varies between areas. The output of a neocortical area is remodeled chiefly through the selective elimination of particular axon collaterals or long distal segments of the primary axons without a concomitant death of the projection neurons. For example, in adult rats, pyramidal tract neurons (which extend a long axon through the pyramidal tract and innervate medullary nuclei and the spinal cord) are restricted to cortical layer 5, but of sensorimotor areas only. In neonates, though, while already limited to layer 5, these neurons are distributed throughout the entire neocortex[39,40]. Pyramidal tract neurons located in regions of developing neocortex completely devoid of them in adults, such as the primary visual and auditory areas, subsequently lose their pyramidal tract axons[39,56], but retain collateral branches to other subcortical targets appropriate for their cortical location[57]. The fate of this axon is not a fixed property of pyramidal tract neurons, but is dependent on the area location of the neuron in the developing cortex[58,59]. Thus, although the appropriate laminar position of cortical projection neurons is probably specified at or near the time they become postmitotic[60], their adult areal distribution is achieved through a process of selective axon elimination that occurs well after the cortex is assembled.

Selective axon elimination also brings about the developmental restriction of initially widely distributed populations of callosally projecting neurons[61–64]. The stabilization or elimination of callosal axons seems to be influenced by sensory input. This is suggested by the presence of an abnormally widespread distribution of callosal neurons in visual cortical areas of adult rodents, cats and primates in which visual input to the developing cortex was altered naturally by genetics (Siamese cats)[65] or experimentally by removing or changing, in a number of ways, retinal inputs to visual thalamus[66,67]. Such findings imply that thalamocortical input, or inputs relayed through thalamus, may regulate the process of selective axon elimination, and thus the output of a given region of neocortex.

Differentiation of area-specific architecture

The cytoarchitectural differentiation of a region of neocortex is not a fixed property, and is capable of considerable plasticity. To illustrate this point, we return to the barrel-field of rodents. Cortical barrels develop through an interaction with thalamic afferents that relay sensory information from the periphery. The existence of cortical barrels is the manifestation of a series of afferent-induced barrel-like parcellations beginning in the brainstem and passing through the thalamus to the cortex[38] (Fig. 2). A number of markers, including certain lectins, can reveal early stages of this process[68]. Manipulations of the sensory periphery, all of which modify or block sensory input through the trigeminal system, alter or even prevent

barrel formation[38,69]. Somatosensory cortex is able also to reorganize and form a normal pattern of barrels following small lesions made in the barrel-field during an early postnatal critical period[70]. Perhaps the best evidence that the barrel pattern is not predetermined comes from observations made on strains of mice inbred for abnormal sets of mystacial vibrissae. In these mice, supernumerary vibrissae are represented in the cortex through the induced formation of additional barrels, but only if the anomalous vibrissa follicle is innervated by a suprathreshold number of sensory axons[71]. These observations indicate that the differentiation of barrel morphology and the unique patterning of groups of barrels are not fixed properties of somatosensory cortex, but are induced by inputs relayed to the cortex from the sensory periphery, suggesting that at least this feature of cortical cytoarchitecture is not predetermined within the neuroepithelium.

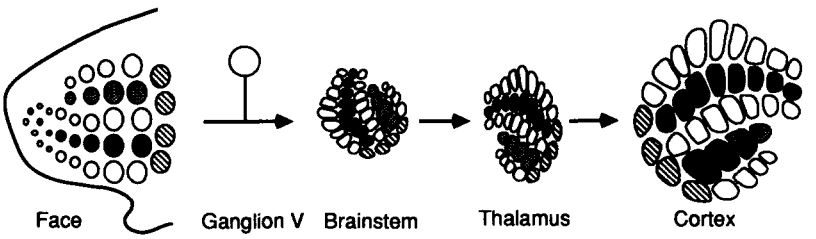

Fig. 2. *Patterning of cytoarchitectural units in somatosensory cortex. The pattern of 'barrels' in the posteromedial barrel subfield of somatosensory cortex of rodents is an isomorphic representation of the geometric arrangement of mystacial vibrissae found on the animal's face. Similar patterns are present in the brainstem and thalamic nuclei that relay inputs from the face to the barrel cortex. Alterations of the pattern of mystacial vibrissae, either genetically or by removal of vibrissae follicles during a critical period of development, result in a corresponding alteration of the cortical barrel pattern. Cutting the axons of trigeminal ganglion (Ganglion V) neurons (thus blocking the flow of sensory information from the periphery to the brainstem) early on prevents barrel formation (see Refs 38, 69–71).*

Are the borders between cortical areas fixed?

How is an extra barrel accommodated in somatosensory cortex? Does the area undergo some local or overall reorganization to allow for the space occupied by the barrel, or does the area expand its size at the expense of neighboring neocortical areas? Unfortunately, the size of an individual barrel is too small to make any firm statements. But recent findings in primates suggest that the border between primary visual cortex (area 17) and a secondary visual area (area 18) appears to be capable of a large shift with dramatic consequences on the subsequent differentiation of the affected piece of cortex[4,67]. Such a border shift seems to occur in macaque monkeys bilaterally enucleated at mid-fetal stages (see Fig. 3 legend, and Ref. 4 for arguments). This manipulation results in a 50% loss in the number of lateral geniculate neurons, the primary source of thalamic input to area 17. The total number of neurons in area 17, and its overall size, are correspondingly reduced, but the thickness and appearance of the layers are normal[72]. Features characteristic of area 17, including the unique laminar distributions of receptors for neurotransmitters and the presence of functional subunits specific to area 17, are retained within the reduced area identified as area 17 based on cytoarchitectural appearance[4]. But more importantly, a region of cortex normally contained within area 17 takes on the architectural appearance of area 18, and apparently lacks other characteristics which define area 17. Even the output of this region is altered and resembles that of area 18. A large number of callosally projecting neurons are present within area 18 up to its new border with area 17, with few or none found in the region cytoarchitecturally identified as area 17[67]; callosal neurons are rarely, if at all, encountered in area 17 of normal macaque monkeys[53]. These findings indicate that the biochemical, cytoarchitectural, and connectional differentiation of a part of neocortex can be developmentally controlled by epigenetic factors. Again, a critical, regulatory role for thalamic input in this phenomenon has been suggested[4].

Are cortical areas interchangeable?

Similar conclusions can be drawn from a set of studies that indicate that the regional location of a piece of developing neocortex has a decisive influence on the subsequent acquisition of many area-specific properties. This has been demonstrated by transplanting pieces of late fetal neocortex to heterotopic positions within the neocortex of newborn rodents. The layer 5 projections to subcortical targets permanently established by such transplants are dependent upon the transplant's position within the neocortex (Fig. 4). Visual cortical neurons transplanted to the sensorimotor region extend and permanently retain axons to the spinal cord, a subcortical target of sensorimotor cortex[58,59]. Conversely, sensorimotor cortical neurons transplanted to the visual region extend and then lose spinal axons, but retain a projection to a subcortical target of visual cortex, the superior colliculus[59]. The heterotopic transplants also establish callosal and thalamic connections, both input and output, appropriate for their *new* location[59,73,74]. In sum, the inputs and outputs of heterotopically transplanted neurons resemble those of the neurons normally present in that cortical location. Heterotopic transplants of neocortex can also take on the cytoarchitectural appearance of the host cortical region. For example, pieces of occipital (visual) cortex placed in the presumptive barrel-field of primary somatosensory cortex develop morphological features that resemble barrels when innervated by thalamic afferents[75]. From this observation it can be inferred that thalamic afferents are able to organize in a foreign piece of cortex, and that the transplanted cells can respond to the afferents in ways necessary to express the cytoarchitectural features appropriate to their new cortical locale. It can be concluded from this class of experiments that different regions of the protocortex are sufficiently alike that, if heterotopically placed in the developing neocortex, they will come to acquire many of the area-specific properties normally associated with their new location.

Mechanisms involved in the differentiation of the protocortex

All, or most, of the epigenetically influenced developmental processes that operate throughout the developing nervous system[76] are likely to play a role in the differentiation of the protocortex. Some of these processes elaborate upon existing components.

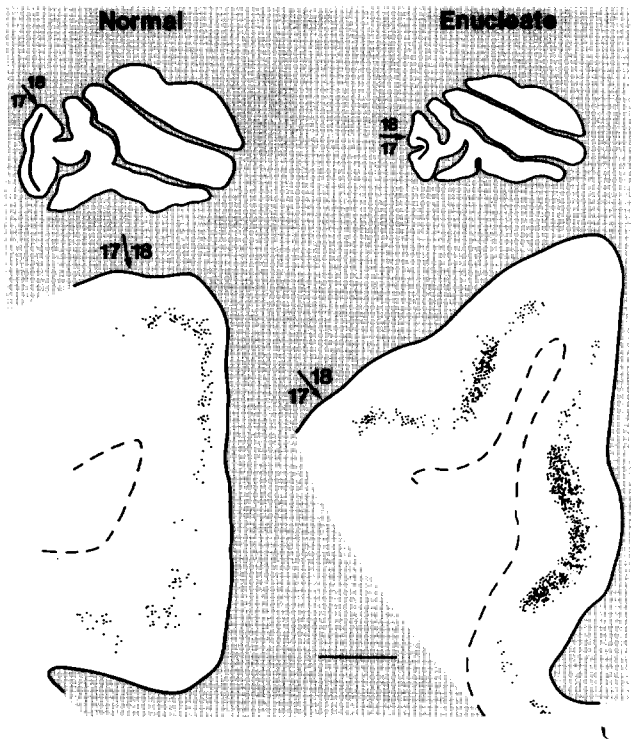

Fig. 3. *Borders between cortical areas are not fixed. A border shift between visual cortical areas 17 and 18 seems to occur in macaques bilaterally enucleated during the third month of gestation. The drawings are of sagittal sections of brains from normal (left) and bilaterally enucleated (right) newborn macaques. The top illustrations are low-power drawings to show the extent of area 17 (bold line) and the location of its border with area 18. The bottom drawings are higher power plots of callosally projecting neurons (which are normally present in area 18 but not area 17) taken from the same sections displayed above.* (Figure modified from Ref. 67.) *In similarly enucleated macaques analysed as adults, the total number of neurons is reduced by about 50% within the region defined by cytoarchitecture as area 17, but the thickness and appearance of the layers, the number of neurons in a traverse across the six layers, and cell density in this region are comparable to that in area 17 of normal adults[4,70]. The reduced number of neurons cannot be attributed to fewer cells generated since the enucleations are done after neurogenesis. Further, it is unlikely that the result is a consequence of increased cell death since a selective loss of entire columns of cortical neurons has never been observed (and is highly improbable), and neuronal death distributed across all of area 17 would result in a substantial reduction of the number of neurons per column and a thinning of the cortex rather than the observed reduction in surface area. Thus, a reasonable conclusion is that a part of cortex that normally would mature into area 17 has instead developed properties characteristic of the adjacent area 18.* (See Ref. 4 for detailed arguments in support of a border shift.)

For instance, the shapes and sizes of the dendritic arbors of specific classes of neurons, which contribute to differences in cytoarchitecture, can be greatly influenced by afferent input[38] and target-derived factors[77]. Other processes, such as selective axon elimination, synapse elimination and neuronal death, which can be thought of as regressive in nature, serve to remove, in a regionally specific manner, excess or functionally inappropriate components. We have already seen that selective axon elimination contributes to the development of area-specific outputs. Synapse elimination has been reported to occur in several diverse cortical areas[70], and is probably involved in the shaping of many cortical features. It is best documented as underlying the developmental remodeling of geniculocortical inputs to visual cortex[79], a process driven by a relative asynchrony in activity patterns among competing sets of inputs[80]. Neuronal death is a likely contributor to the sculpting of inter-area differences in the number of neurons found in specific layers. In rodents, about 30% of cortical neurons die[81]. Most of this loss occurs in the superficial layers, primarily in layers 2 and 3, and to a lesser extent in layer 4[9,81]. There is evidence that the number of cells in layer 4 is governed by the density of thalamic input, and that the number of cells present in the superficial layers is determined by a combination of differential cell loss and changes in neuronal differentiation[9,82], factors which are believed to contribute to the greater number of neurons found in a radial traverse in area 17 compared with other areas[9]. Although I have only briefly considered a few, clearly a wide range of developmental processes can act on the protocortex to establish area-specific features characteristic of the adult neocortex.

Regional differences in the protocortex

The processes just described contribute to the differentiation of the protocortex, but can they account for all of the differences seen between neocortical areas in the adult? Let us consider two features reported for area 17 of monkeys of the genus *Macaca*. First, in adult macaques area 17 has more than twice as many neurons in a radial traverse compared with other primary sensory areas, with the extra neurons contained in layers 4 and above[7,8]. The increased number of neurons in area 17 might reflect not only a reduced amount of cell loss, but also a regionally specific increase in neuronal production. Here it is worth recalling the patchy distribution of radial glial cells that stain in rats with antibodies to proto-oncogene peptides[31]. Within a patch, an increased proportion of neuroepithelial cells can be labeled with [^{3}H]thymidine (a marker of DNA synthesis), and the patches appear over the period that neurons which will populate the most superficial cortical layers are being generated. Although this observation can be interpreted in several ways, one intriguing possibility is that it reflects localized increases in the generation of superficial neurons[31]. Second, area 17 of macaques reportedly does not have a callosal projection even during development[53], and in this sense is unlike area 17 in all non-primate mammals examined to date, where, as mentioned earlier, neurons throughout area 17 transiently extend callosal axons. Macaques may have evolved a higher degree of specification of the output of area 17. If true, this feature may prove to be unique for area 17, since other cortical areas in macaques, namely the secondary visual area 18[53] and primary somatosensory cortex[52], do develop transiently widespread callosal connections. However, an argument against this possibility is the finding in macaques that parts of cortex that normally are contained within area 17 do have callosal connections following eye removal at mid-fetal stages[67].

Nevertheless, there are likely to be differences across the protocortex, whether present as subtle gradients or as sharp discontinuities. One likely possibility would be molecular distinctions between regions, and even within regions, of the protocortex, which promote the formation of appropriate connectional relationships, for example between thalamic nuclei and cortical areas, as well as underlie the topographic ordering of the input and output connections of the neocortex. These molecules would probably be present on the surfaces of select subsets of cells. To date, though, cells immunoreactive for antibodies that recognize distinct neuronal surface antigens are present in all neocortical areas, whether the number stained is substantial, as for Cat-301-positive neurons[83], or exceedingly small, as for Tor-23-positive neurons[84]. However, specific areas of limbic cortex (often termed allocortex and distinct from neocortex), do stain selectively for an antibody that recognizes a surface molecule (named limbic-associated membrane protein), both in the mature[85] and developing brain[86]. The same molecule is associated with subcortical components of the limbic system[85]. Similar markers will probably be found for neocortex.

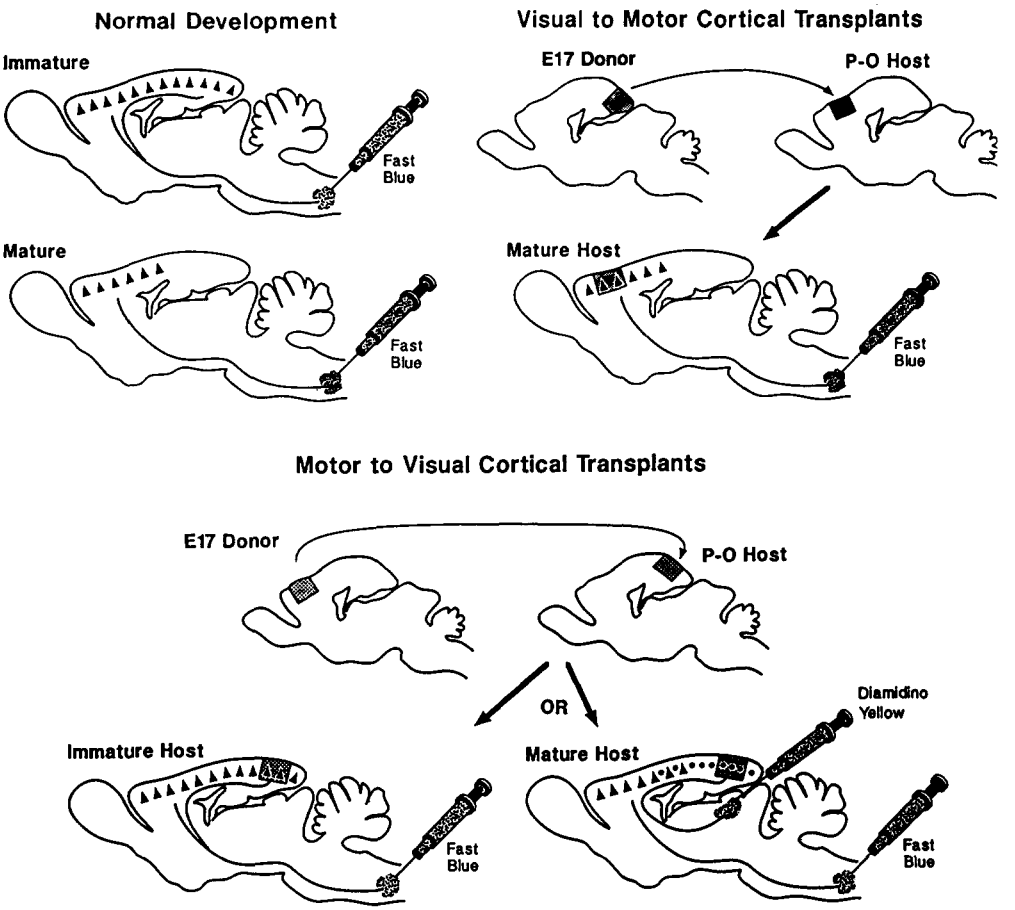

Fig. 4. *Development of area-specific outputs is not a fixed property of cortical areas.* Top left: *during normal development in rats, layer 5 neurons in all regions of the immature neocortex develop a pyramidal tract axon and can be labeled retrogradely with the dye Fast Blue injected into the pyramidal decussation. A similar injection made in mature rats labels layer 5 neurons confined to sensorimotor areas of cortex. The restriction from the widespread, immature distribution to the limited, mature one is achieved through a selective elimination of pyramidal tract axons without neuron loss (see Ref. 39). Heterotopic transplantation of fetal cortex shows that this elimination of pyramidal tract axons is dependent upon the cortical location of developing neurons (see Refs 58, 59).* Top right: *embryonic day 17 (E17) visual cortical neurons transplanted to the motor region of a newborn (P-0) host develop and permanently retain pyramidal tract axons as demonstrated by Fast Blue labeling from the pyramidal decussation after maturation.* Bottom: *motor cortical neurons transplanted to the visual region can be labeled with Fast Blue injected into the pyramidal decussation (PD) at immature stages, but not at maturity. However, in the same mature hosts, the transplanted neurons can be labeled with a second dye, Diamidino Yellow, injected into the superior colliculus, a permanent target of visual cortex but only a transient target of motor cortex. Thus, the transplants form permanent projections characteristic of their new cortical location.* (Figure modified from Ref. 59.)

Concluding remarks

The neocortical neuroepithelium generates a fairly uniform structure, here termed the protocortex, that does not have the architectonic divisions present in the adult neocortex. Many of the area-specific features characteristic of well-defined cortical areas emerge from the protocortex long after the conclusion of neurogenesis through a process that can be regulated by influences, for example afferent inputs, that vary across the developing neocortex. However, differences are likely to exist from one region of the protocortex to another – differences laid down at the time of neurogenesis that contribute to the development of area-specific properties. The extent to which area-specific properties are determined at the time of neurogenesis, that is, the degree to which the neocortical neuroepithelium is regionally specified to generate the definable characteristics of specific neocortical areas[4], is presently not resolved and may well vary from species to species. However, most studies relevant to this issue provide evidence for epigenetic regulation of area differentiation; the mere existence of cytoarchitectonically defined areas in the adult neocortex is presently the most compelling evidence for their predetermination within the neocortical neuroepithelium. The neocortex shows considerable plasticity in the development of area-specific features, but many of these findings are based on perturbation studies, and therefore such plasticity does not unequivocally demonstrate that area-specific features are not predetermined, but rather that they are not irreversibly predetermined. Nonetheless, different regions of the developing neocortex have the capability to acquire many of the area-specific characteristics normally associated with other cortical areas, indicating that there are significant similarities among these regions. These similarities may reflect a phylogenetic conservation of the ability of all parts of the neocortical neuroepithelium to generate the ensemble of basic structural components of the neocortex, thereby establishing a protocortex from which defined areas emerge.

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Selected references

1 Brodmann, K. (1909) *Lokalisationslehre der Groshirnrinde in ihren Pricipen dargestellt aus Grund des Zellenbaue*, Barth
2 Krieg, W. J. S. (1946) *J. Comp. Neurol.* 84, 221–275
3 Van Essen, D. C. (1985) in *Cerebral Cortex, Vol. 3: Visual Cortex* (Peters, A. and Jones, E. G., eds), pp. 259–330, Plenum
4 Rakic, P. (1988) *Science* 241, 170–176
5 Gilbert, C. D. (1983) *Annu. Rev. Neurosci.* 6, 217–248
6 Jones, E. G. (1984) in *Cerebral Cortex, Vol. 1: Cellular Components of the Cerebral Cortex* (Peters, A. and Jones, E. G. eds), pp. 521–553, Plenum
7 Rockel, A. J., Hiorns, R. W. and Powell, T. P. S. (1980) *Brain* 103, 221–244
8 Hendry, S. H. C., Schwark, H. D., Jones, E. G. and Yan, J. (1987) *J. Neurosci.* 4, 2497–2517
9 Finlay, B. L. and Slattery, M. (1983) *Science* 219, 1349–1351
10 Winfield, D. A., Gatter, K. C. and Powell, T. P. S. (1980) *Brain* 103, 245–258
11 Sofroniew, M. V., Campbell, P. E., Cuello, A. C. and Eckenstein, F. (1985) in *The Rat Nervous System, Vol. 1: Forebrain and Midbrain* (Paxinos, G., ed.), pp. 471–486, Academic Press
12 Fuxe, K., Hokfelt, T., Said, S. I. and Mutt, V. (1979) *Neurosci. Lett.* 5, 241–246
13 Hendry, S. H. C., Jones, E. G. and Emson, P. C. (1984) *J. Neurosci.* 4, 2497–2517
14 Peters, A., Miller, M. and Kimerer, L. M. (1983) *Neuroscience* 8, 431–448
15 Morrison, J. H., Benoit, P. J., Magistretti, P. J. and Bloom, F. E. (1983) *Brain Res.* 262, 344–351
16 Lorente de No, R. (1949) in *Physiology of the Nervous System* (Fulton, J. F., ed.), pp. 288–315, Oxford University Press
17 Creutzfeld, O. D. (1977) *Naturwissenschaft* 64, 507–517
18 Mountcastle, V. B. (1978) in *The Mindful Brain* (Mountcastle, V. B. and Edelman, G. M., eds), pp. 7–50, MIT
19 Powell, T. P. S. (1981) in *Brain Mechanisms and Perceptual Awareness* (Pompeiano, O. and Ajmone Marsan, C., eds), pp. 1–19, Raven
20 Eccles, J. C. (1984) in *Cerebral Cortex, Vol. 2: Functional Properties of Cortical Cells* (Jones, E. G. and Peters, A., eds), pp. 1–36, Plenum
21 Metin, C. and Frost, D. O. (1989) *Proc. Natl Acad. Sci. USA* 86, 357–361
22 Sur, M., Garraghty, P. E. and Roe, A. W. (1988) *Science* 242, 1437–1441
23 Frost, D. O. (1981) *J. Comp. Neurol.* 203, 227–256
24 Rakic, P. (1974) *Science* 183, 425–427
25 Luskin, M. B., Pearlman, A. L. and Sanes, J. R. (1988) *Neuron* 1, 635–647
26 Walsh, C. and Cepko, C. L. (1988) *Science* 241, 1342–1345
27 Galileo, D. S., Gray, G. E., Owens, G. C., Majors, J. and Sanes, J. R. *Soc. Neurosci. Abstr.* (in press)
28 Goldwitz, D. (1987) *Dev. Brain Res.* 35, 1–9
29 Smart, I. H. M. and Sturrock, R. R. (1979) in *The Neostriatum* (Divac, I. and Oberg, R. G. E., eds), pp. 127–146, Pergamon
30 Altman, J. and Bayer, S. A. (1988) *J. Comp. Neurol.* 275, 346–377
31 Johnson, J. G. and Van Der Kooy, D. (1989) *Proc. Natl Acad. Sci. USA* 86, 1066–1070
32 Levine, J. M., Beasley, L. and Stallcup, W. B. (1984) *J. Neurosci.* 4, 820–831
33 Frederikson, K. and McKay, R. D. G. (1988) *J. Neurosci.* 8, 1144–1151
34 Marin-Padilla, M. (1978) *Anat. Embryol.* 152, 109–126
35 Luskin, M. B. and Shatz, C. J. (1985) *J. Neurosci.* 5, 1062–1075
36 Shatz, C. J., Chun, J. J. M. and Luskin, M. B. in *Cerebral Cortex, Vol. 7* (Jones, E. G. and Peters, A., eds), Plenum (in press)
37 Woolsey, T. A. and Van Der Loos, H. (1970) *Brain Res.* 17, 205–242
38 Woolsey, T. A. in *Development of Sensory Systems in Mammals* (Coleman, J. R., ed.), Wiley (in press)
39 Stanfield, B. B., O'Leary, D. D. M., and Fricks, C. (1982) *Nature* 298, 371–373
40 Bates, C. A. and Killackey, H. P. (1984) *Dev. Brain Res.* 13, 265–273
41 O'Leary, D. D. M. and Stanfield, B. B. (1986) *Dev. Brain Res.* 27, 87–99
42 Cabana, T. and Martin, G. F. (1984) *Dev. Brain Res.* 15, 247–263
43 Distel, H. and Hollander, H. (1980) *J. Comp. Neurol.* 192, 505–518
44 Thong, I. G. and Dreher, B. (1986) *Dev. Brain Res.* 25, 227–238
45 Ivy, G. O. and Killackey, H. P. (1981) *J. Comp. Neurol.* 195, 367–389
46 Olavarria, J. and Van Sluyters, R. C. (1985) *J. Comp. Neurol.* 239, 1–26
47 Cabana, T. and Martin, G. F. (1985) *Anat. Embryol.* 17, 121–128
48 Chow, K. K., Baumbach, H. D. and Lawson, R. (1981) *Exp. Brain Res.* 42, 122–126
49 Innocenti, G. M., Fiore, L. and Caminiti, R. (1977) *Neurosci. Lett.* 4, 237–242
50 Innocenti, G. M. and Caminiti, R. (1980) *Exp. Brain Res.* 38, 824–827
51 Feng, J. Z. and Brugge, J. F. (1983) *J. Comp. Neurol.* 214, 416–426
52 Killackey, H. P. and Chalupa, L. M. (1986) *J. Comp. Neurol.* 244, 331–348
53 Dehay, C., Kennedy, H., Bullier, J. and Berland, M. (1988) *Nature* 331, 348–359
54 O'Leary, D. D. M. and Terashima, T. *Soc. Neurosci. Abstr.* (in press)
55 Koester, S. E. and O'Leary, D. D. M. *Soc. Neurosci. Abstr.* (in press)
56 O'Leary, D. D. M. and Terashima, T. (1988) *Neuron* 1, 901–910
57 O'Leary, D. D. M. and Stanfield, B. B. (1985) *Brain Res.* 336, 326–333
58 Stanfield, B. B. and O'Leary, D. D. M. (1985) *Nature* 313, 135–137
59 O'Leary, D. D. M. and Stanfield, B. B. (1989) *J. Neurosci.* 9, 2230–2246
60 McConnell, S. K. (1988) *J. Neurosci.* 8, 945–974
61 Innocenti, G. M. (1981) *Science* 212, 824–827
62 O'Leary, D. D. M., Stanfield, B. B. and Cowan, M. W. (1981) *Dev. Brain Res.* 1, 607–617
63 Ivy, G. O. and Killackey, H. P. (1982) *J. Neurosci.* 6, 735–743
64 Chalupa, L. M. and Killackey, H. P. (1989) *Proc. Natl Acad. Sci. USA* 86, 1076–1079
65 Shatz, C. J. (1977) *J. Comp. Neurol.* 173, 497–518
66 Innocenti, G. M. (1985) in *Cerebral Cortex, Vol. 5: Sensorymotor Areas and Aspects of Cortical Connectivity* (Jones, E. G. and Peters, A., eds), pp. 291–353, Plenum
67 Dehay, C., Horsburgh, G., Berland, M., Killackey, H. and Kennedy, H. (1989) *Nature* 337, 265–267
68 Cooper, N. G. F. and Steindler, D. A. (1986) *J. Comp. Neurol.* 249, 157–186
69 van der Loos, H. and Welker, E. (1985) in *Development, Organization, and Processing in Somatosensory Pathways*, pp. 53–67, Alan R. Liss
70 Seo, M. L. and Ito, M. (1987) *Exp. Brain Res.* 65, 251–260
71 Welker, E. and van der Loos, H. (1986) *J. Neurosci.* 6, 3355–3373
72 Rakic, P. and Williams, R. W. (1986) *Soc. Neurosci. Abstr.* 12, 1499
73 Chang, F. L. F., Steedman, J. G. and Lund, R. D. (1986) *J. Comp. Neurol.* 244, 401–411
74 O'Leary, D. D. M. (1988) *Soc. Neurosci. Abstr.* 14, 1113
75 Schlaggar, B. L. and O'Leary, D. D. M. (1988) *Soc. Neurosci. Abstr.* 14, 475
76 Purves, D. and Lichtman, J. W. (1985) *Principles of Neural Development* Sinauer
77 Voyvodic, J. T. (1989) *J. Neurosci* 9, 1997–2010
78 Rakic, P., Bourgeois, J. P., Eckenhoff, M. F., Zecevic, N. and Goldman-Rakic, P. S. (1986) *Science* 232, 232–235
79 LeVay, S., Wiesel, T. N. and Hubel, D. H. (1981) in *The Organization of the Cerebral Cortex* pp. 29–45, MIT
80 Stryker, M. P. and Harris, W. A. (1986) *J. Neurosci.* 6, 2 17–2133
81 Huemann, D., Lueba, G. and Rabinowicz, G. (1978) *J. Hirnforsch.* 19, 385–393
82 Windrem, M. S., Jan de Beur, S. M. and Finlay, B. L. (1986) *Soc. Neurosci. Abstr.* 12, 867
83 Hendry, S. H. C., Jones, E. G., Hockfield, S. and McKay, R. D. G. (1988) *J. Neurosci.* 8, 518–542
84 Stephenson, D. T. and Kushner, P. D. (1988) *J. Neurosci.* 8, 3035–3056
85 Levitt, P. (1984) *Science* 223, 299–301
86 Horton, H. L. and Levitt, P. (1988) *J. Neurosci.* 8, 4653–4661