Dividing Up the Neocortex

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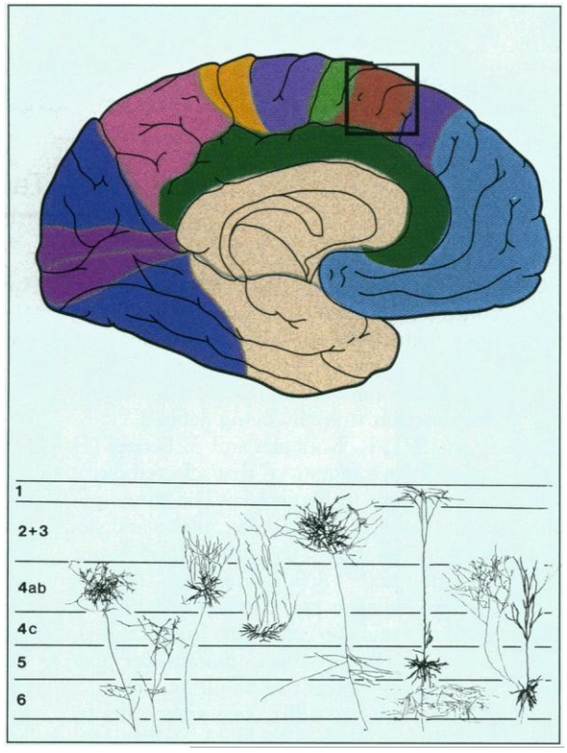
The mammalian neocortex is an extraordinary computational machine that is systematically divided into over 40 functionally distinct areas. Elucidation of the process by which these areas and their characteristic and unique set of axonal connections from the thalamus are established poses a major problem for neurobiology.

The presence, in the adult brain, of such precision in the parcellation of neocortex into separate areas has fueled much speculation about how these subdivisions emerge during development. The question is especially intriguing because, initially, the neocortex appears uniform and it is near impossible to discern areal boundaries. One idea is that cells belonging to each cortical area are intrinsically different, specified at the outset within the germinal zone at the ventricular surface (*1*). In other words, the germinal epithelium may contain a "protomap" of the neocortex, with cells already committed to a given areal identity very early in development, even before they migrate (*2*).

This idea is very attractive because of the enticing analogy between the parcellation of neocortex and the parcellation of other regions of the neuraxis during vertebrate and invertebrate development, where sets of regulatory genes (homeobox genes) control the formation of spatially distinct compartments. For example, in the hindbrain, a segmented structure, both the expression pattern of homeobox genes and the movements of cells strictly observe segmental boundaries (*3*, *4*). Not only are some of these particular genes expressed in very restricted regional patterns during development, but the fate of cells expressing some of these genes can be maintained in a novel environment after transplantation (*4*) (that is, their fate is determined).

Could similar mechanisms be responsible for the subdivision of neocortex into areas? There are a number of experiments that suggest this is not likely. When forming visual cortex is transplanted to somatosensory cortex in neonatal rats, it develops into cortex that resembles somatosensory rather than visual cortex (*5*), suggesting that local and dynamic extrinsic influences specify the identify of the cortex. Cell lineage tracing studies with retroviruses have also shown that each area of the forming cerebral cortex can contain mixtures of cells that have originated from quite distant regions of the germinal zone (*6*). Moreover, studies of cell movements with time-lapse imaging demonstrate that a significant amount of tangential mixing occurs within the neocortex (*7*). These observations suggest that, early in development, neocortical areas are not likely to be divided into compartments.



Organization of the cerebral cortex. (**Top**) Areal subdivisions of the cortex. Box indicates region shown in detail below. (**Bottom**) Cellular components of cortical layers. [Adapted from C. D. Gilbert, *Annu. Rev. Neurosci.* **6**, 217 (1983)]

Equally fascinating are the results of many studies that have intentionally searched for region-specific molecular markers that might be associated with the identity of discrete neocortical areas. However, at present there are no clear examples in which such markers are restricted to a particular area of the neocortex. Rather, these markers appear to be associated with broader subdivisions of the telencephalon (*8*). Among the first examples was the discovery of a limbic system–associated membrane protein (LAMP), expressed almost exclusively within limbic system structures of the cerebral cortex. Pieces of limbic cortex retain their LAMP immunoreactivity even when transplanted to a different region of the telencephalon (*9*), implying that cells in the limbic cortex have an early commitment to a limbic fate and demonstrating that this particular region of the cerebral cortex can be uniquely identified on the basis of a particular protein. There is now also a growing catalog of genes, mostly members of the homeobox gene family and other regulatory genes, whose expression is restricted to the telencephalon and therefore would seem to represent prime candidates for the regional specification of the telencephalon (*10*, *11*). However, although many of these genes are indeed restricted to a subdivision of the telencephalon, for example the basal ganglia (*11*), none are restricted in their expression to an area of the neocortex. An alternative, that a gene family might have a nested distribution of expression across neocortex similar to that found for the Hox genes in mouse spinal cord (*3*), has not been reported either, although an example of nested expression of two related homeobox genes, *EMX-1* and *EMX-2*, across large portions of the telencephalon has been demonstrated (*12*). All of these observations imply that a good number of genes involved in the regionalization of the telencephalon into its major subdivisions (such as the basal ganglia or limbic system) have probably now been identified, and it will be very exciting to learn more about their exact functions in the future.

Thus, despite progress, major questions still remain concerning the mechanism of dividing the neocortex into areas and also of endowing neurons of the six cortical layers with their laminar identities.

Within each area of neocortex, the neurons reside in a highly organized radial array of six cellular layers, from the pial surface to the white matter, that differ strikingly in their composition, functional properties, and sets of connections. For instance, the neurons of layer 4 receive the major input from the thalamus, while layer 6 neurons send the major output back to the thalamus. These six layers are found everywhere within neocortex, but the pattern of thalamic connections, along with slight local variations in cellular composition and connectivity, define rather precise boundaries between each cortical area.

There may be important differences between mechanisms that specify the radial

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(layers) and the tangential (areas) domains of neocortex. Whereas transplantation experiments indicate a lack of stable positional markers for neocortical areas (*13*), similar transplants reveal that the identity of premigratory neurons destined for a particular cortical layer is retained after transplantation (*14*), unless the cells are transplanted very early—before the time of cell division in the ventricular germinal zone, when their laminar fates can in fact be altered. Although no experiments have revealed clear molecular differences between neocortical areas, the expression of POU genes is layer-specific (*15*). Even the sequence of steps in the formation of specific sets of axonal connections differs in the radial and tangential dimensions of cortex. The formation of radial connections between the layers appears to be highly specific at the outset, with axons apparently avoiding growth into inappropriate cortical layers (*16*). In vitro, too, cocultures of pieces of cortex and thalamus form axonal connections that obey the laminar-specific rules present in vivo, with the thalamus connecting to layer 4 and layer 6 of cortex connecting back to the thalamus (*17*).

The rules governing the formation of horizontal connections within neocortex may be very different. The establishment of axonal connections between thalamus and cortex requires the presence of ongoing interactions between the ingrowing axons and subplate neurons; in the absence of subplate neurons, appropriate thalamic axons cannot select and invade their cortical target areas even though the cells of the cortical plate are still present directly above (*18*). Later in development, the formation of ocular dominance columns in visual cortex or of the long distance patchy horizontal connections within cortical layers 3 and 5 is accomplished by extensive axonal remodeling and pruning, which is thought to require neural activity (*19*). Indeed, the results of these experiments serve as a reminder that very precise and discrete boundaries can form from initially diffuse sets of axonal inputs, and they emphasize the point that dynamic competitive interactions, rather than initially highly restricted spatial cues, can also produce sharp boundaries as a developmental outcome.

This comparison between the tangential and radial development of neocortical connections suggests that perhaps it is in the radial domain of cortex—in the formation of cortical layers—that the underlying developmental mechanisms will turn out to be similar to those that operate to form segmental boundaries and compartments in the nervous systems of other species, or in the hindbrain of vertebrates—as if each layer functions as a separate region, segment, or domain. On the other hand, just how the neocortex is subdivided into discrete cytoarchitectonic areas, and how the appropriate subsets of axons from the thalamus form the correct sets of connections with cortex, is still a major unsolved puzzle in developmental neurobiology.

REFERENCES AND NOTES

1. P. Rakic, *Science* **241**, 170 (1988).
2. D. D. M. O'Leary, *Trends Neurosci.* **12**, 400 (1989).
3. C. Stern and R. J. Keynes, *ibid.* **11**, 191 (1988); A. Graham, N. Papalopulu, R. Krumlauf, *Cell* **57**, 367 (1989); D. G. Wilkinson and R. Krumlauf, *Trends Neurosci.* **13**, 335 (1990); S. Fraser, R. Keynes, A. Lumsden, *Nature* **344**, 431 (1990).
4. R. Keynes and A. Lumsden, *Neuron* **12**, 1 (1990); S. Guthrie *et al.*, *Nature* **356**, 157 (1992).
5. B. Schlaggar and D. D. M. O'Leary, *Science* **252**, 1556 (1991).
6. C. Walsh and C. L. Cepko, *ibid.* **255**, 434 (1992).
7. N. A. O'Rourke, M. E. Daily, S. J. Smith, S. K. McConnell, *ibid.* **258**, 299 (1992); G. Fishell, C. A. Mason, M. E. Hatten, *Soc. Neurosci. Abstr.* **18**, 926 (1992).
8. The neocortex is only one part of the telencephalon—the basal ganglia, the amygdala, and the phylogenetically older cortex of the hippocampus and limbic lobe are others.
9. M. F. Barbe and P. Levitt, *J. Neurosci.* **11**, 519 (1991).
10. M. Price *et al.*, *Neuron* **8**, 241 (1992); W. Tao and E. Lai, *ibid.*, p. 957.
11. M. Porteus, A. Bulfone, R. D. Ciaranello, J. L. R. Rubenstein, *ibid.* **7**, 221 (1991).
12. A. Simeone *et al.*, *EMBO J.* **11**, 2541 (1992).
13. D. D. M. O'Leary and B. B. Stanfield, *J. Neurosci.* **9**, 2230 (1989).
14. S. K. McConnell and C. E. Kaznowski, *Science* **254**, 282 (1991).
15. X. He *et al.*, *Nature* **340**, 35 (1989); G. D. Frantz, I. M. Burstein, A. P. Bohner, S. K. McConnell, *Soc. Neurosci. Abstr.* **18**, 957 (1992).
16. L. C. Katz, *Eur. J. Neurosci.* **3**, 1 (1991).
17. J. Bolz, N. Novak, M. Gotz, T. Bonhoeffer, *Nature* **346**, 359 (1990); Z. Molnar and C. Blakemore, *ibid.* **351**, 475 (1991).
18. A. Ghosh, A. Antonini, S. K. McConnell, C. J. Shatz, *ibid*, **347**, 179 (1990).
19. E. M. Callaway and L. C. Katz, *J. Neurosci.* **10**, 1134 (1990); C. J. Shatz, *Neuron* **5**, 745 (1990).

Circadian Clock Genes Are Ticking

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The nature and function of *per*, a gene that controls circadian rhythms in *Drosophila*, has remained a wonder and an enigma ever since its discovery. Now, 20 years later, the *per* gene remains a wonder but its biochemical function is finally being defined.

In 1971, R. Konopka and S. Benzer (*1*) isolated three mutations that affected circadian rhythms in *Drosophila*. The three mutations were, unexpectedly, allelic and defined a new gene, the *period* (*per*) locus. Normal per^+ flies expressed 24-hour rhythms; whereas per^S mutants had 19-hour rhythms, and per^L mutants had 29-hour rhythms. The arrythmic mutant per^0 behaved phenotypically as a null mutant.

From 1984 to 1987, two groups led by M. Young at Rockefeller and J. Hall and M. Rosbash at Brandeis independently cloned *per* in a series of groundbreaking experiments that were the first to show that germline transformation with DNA could rescue a complex behavioral program (*2*). At the time, the primary sequence of Per and the three mutant alleles did not reveal any clues to its function. Early work focused on an unusual threonine-glycine (TG) repeat region that gave Per the ignominious label as a proteoglycan. Collectively we wondered what an extracellular matrix-like protein could be doing in the circadian clock? Disgust was the most frequent response to the notion of Per as a proteoglycan. Most were relieved to learn later that the TG repeat was not necessary for circadian rhythms (*3*). A second hypothesis that proposed Per as a modulator of gap junction coupling has also apparently not passed the test of time (*4*).

New clues to the nature of the *per* gene product have appeared with the recent discovery of three genes that share sequence homology with Per (see figure). The *Drosophila single-minded* protein (Sim) (*5*), the human aryl hydrocarbon receptor nuclear transporter (ARNT) (*6*), and the aryl hydrocarbon receptor (AHR) (*7*) all share with Per a domain called PAS (for Per, ARNT, Sim). The PAS domain encompasses approximately 270 amino acids of similar sequence that contain two 51–amino acid direct repeats (A and B in the figure) (*5*). In Sim, ARNT, and AHR, the PAS domain lies adjacent to a basic region (BR) helix-loop-helix (HLH) domain, which functions as a DNA-binding and dimerization region in transcriptional regulator proteins (*8*). ARNT and AHR are thought to dimerize with each other and together regulate transcription after ligand-binding (*6*, *7*). It is interesting that the ligand-binding domain of AHR and the per^L mutation both map to the PAS domain (*2*, *7*). Burbach and colleagues (*7*) speculate that the BR/HLH-PAS domain may function as a dimerization domain analogous to BR/HLH–leucine zipper motif proteins such as Myc and Max, and MyoD and E2A (*9*). Per, however, does not possess a BR/HLH domain or any other

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