ity. Also, GTP-binding proteins might increase accuracy of protein sorting. Coupling GTP hydrolysis with vesicle fusion might serve as a proofreading mechanism (7), similar to that proposed for elongation factor Tu in protein translation (*23*).

REFERENCES AND NOTES

1. G. Palade, *Science* **189**, 347 (1975).
2. S. R. Pfeffer and J. E. Rothman, *Annu. Rev. Biochem.* **56**, 829 (1987).
3. N. Segev, J. Mulholland, D. Botstein, *Cell* **52**, 915 (1988).
4. P. Novick, C. Field, R. Schekman, *ibid.* **21**, 205 (1980); B. Goud, A. Salminen, N. C. Walworth, P. Novick, *ibid.* **53**, 753 (1988); A. Salminen and P. J. Novick, *ibid.* **49**, 527 (1987).
5. D. Gallwitz, C. Donath, C. Sander, *Nature* **306**, 707 (1983); H. D. Schmitt, P. Wagner, E. Pfaff, D. Gallwitz, *Cell* **47**, 401 (1986).
6. H. Haubruck, C. Disela, P. Wagner, D. Gallwitz, *EMBO J.* **6**, 4049 (1987); N. Touchot, P. Chardin, A. Tavitian, *Proc. Natl. Acad. Sci. U.S.A.* **84**, 8210 (1987).
7. D. Baker, L. Wuestehube, R. Schekman, D. Botstein, N. Segev, *Proc. Natl. Acad. Sci. U.S.A.* **87**, 355 (1990).
8. R. A. Bacon, A. Salminen, H. Ruohola, P. Novick, S. Ferro-Novick, *J. Cell Biol.* **109**, 1015 (1989).
9. R. D. Burgoyne, *Trends Biochem. Sci.* **14**, 394 (1989).
10. P. Chavrier, R. G. Parton, H. P. Hauri, K. Simons, M. Zerial, *Cell* **62**, 317 (1990); B. Goud, A. Zahraoui, A. Tavitian, J. Saraste, *Nature* **345**, 553 (1990); F. Darchen *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **87**, 5692 (1990); G. Fischer v. Mollard *et al.*, *ibid.*, p. 1988.
11. R. D. Burgoyne and A. Morgan, *FEBS Lett.* **245**, 122 (1989); J. P. Doucet, S. Fournier, M. Parulekar, J. M. Trifaro, *ibid.* **247**, 127 (1989); G. M. Widey, M. J. Im, C. J. Homcy, R. M. Graham, *J. Cell Biol.* **109**, 293 (1989); J. Rubins, T. M. Shannon, B. F. Dickey, *ibid.*, p. 298; P. J. Padfield and J. D. Jamieson, *ibid.*, p. 299.
12. P. Melancon *et al.*, *Cell* **51**, 1053 (1987).
13. D. Baker, L. Hicke, M. Rexach, M. Schleyer, R. Schekman, *ibid.* **54**, 335 (1988).
14. H. Ruohola, A. K. Kabcenell, S. Ferro-Novick, *J. Cell Biol.* **107**, 1465 (1988); C. J. M. Beckers and W. E. Balch, *ibid.* **108**, 1245 (1989); L. S. Mayorga, R. Diaz, P. D. Stahl, *Science* **244**, 1475 (1989); Y. Goda and S. R. Pfeffer, *Cell* **55**, 309 (1988).
15. S. A. Tooze, U. Weiss, W. B. Huttner, *Nature* **347**, 207 (1990).
16. M. E. Groesch, H. Ruohola, R. Bacon, G. Rossi, S. Ferro-Novick, *J. Cell Biol.* **111**, 45 (1990).
17. N. Segev, unpublished data.
18. R. Wright, M. Basson, L. D'Ari, J. Rine, *J. Cell Biol.* **107**, 101 (1988).
19. H. D. Schmitt, M. Puzicha, D. Gallwitz, *Cell* **53**, 635 (1988).
20. G. Jedd and N. Segev, unpublished data.
21. A. Franzusoff and R. Schekman, *EMBO J.* **8**, 2695 (1989).
22. H. R. Bourne, *Cell* **53**, 669 (1988).
23. J. J. Hopfield, *Proc. Natl. Acad. Sci. U.S.A.* **71**, 4135 (1974); R. C. Thompson, *Trends Biochem. Sci.* **13**, 91 (1988).
24. The microsome-based transport reaction contained microsomes, phs, and cytosol. Their preparation and the transport reaction were described (7). A typical transport reaction contained microsomes (2 μg), phs (20 μg), and cytosol (100 μg). The intermediate vesicle fraction was the supernatant recovered after centrifugation at 14,000*g* (10 min at 4°C). The *Saccharomyces cerevisiae* strains used were: GPY60 (*Matα, ura3-52, trp1, leu2, his4, pep4::URA3*); NSY12 (*Matα, ura3-52, trp1, leu2, his4, pep4::URA3, ypt1-1*). Anti-Ypt1 Fab fragments were prepared from anti-trpE-Ypt1 as described (7). The inhibition by the antibody was shown to be specific for Ypt1 (*3*, 7). The analysis of core- and outer chain-glycosylated pro-α factor and the materials used for the in vitro system were described (*7*, *13*).
25. Protease protection experiment (Fig. 2, C and D): Intermediate was isolated after incubation of a transport reaction in the presence of anti-Ypt1 (as described in Fig. 1). The samples were then treated with trypsin (50 μg/ml) for 1 hour at 4°C in the presence or absence of detergent (Triton X-100, 0.1%). The protease reaction was terminated by the addition of trypsin inhibitor (100 μg/ml). SDS gel electrophoresis (11%) and Laemmli buffer system (*26*) were used for all protein electrophoresis. Proteins were electroblotted to nitrocellulose and radiographic detection was performed with affinity-purified antibodies and ^{125}I-labeled protein A (*27*).
26. U. K. Laemmli, *Nature* **227**, 680 (1970).
27. W. N. Burnette, *Anal. Biochem.* **112**, 195 (1981).

28. I thank A. Adams, N. Hay and C. Palfrey for helpful discussions and R. Wright for the antiserum to HMG1, L. Westehube and M. Rexach for advice, G. Jedd for assistance, and R. Miller, F. Tamanoi, A. Adams, and N. Hay for reading the manuscript. Supported by grants from the Cancer Research Foundation, the Louis Block Fund and the Sprague Foundation.

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Potential of Visual Cortex to Develop an Array of Functional Units Unique to Somatosensory Cortex

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The identification of specialized areas in the mammalian neocortex, such as the primary visual or somatosensory cortex, is based on distinctions in architectural and functional features. The extent to which certain features that distinguish neocortical areas in rats are prespecified or emerge as a result of epigenetic interactions was investigated. Late embryonic visual cortex transplanted to neonatal somatosensory cortex was later assayed for "barrels," anatomically identified functional units unique to somatosensory cortex, and for boundaries of glycoconjugated molecules associated with barrels. Barrels and boundaries form in transplanted visual cortex and are organized in an array that resembles the pattern in the normal barrelfield. These findings show that different regions of the developing neocortex have similar potentials to differentiate features that distinguish neocortical areas and contribute to their unique functional organizations.

THE FUNCTIONAL ORGANIZATION OF the mammalian neocortex is based on its parcellation into numerous anatomically and functionally distinct areas (*1*). In spite of interest in the processes that control the differentiation of neocortical areas during development (*2*, *3*) and throughout evolution (*4*), fundamental issues remain unresolved. Adult neocortical areas can be distinguished by differences in their connections and by their architectural features. These differences underlie the unique functional properties of each area and are related to the anatomically identified functional units. These specializations include the "blobs" of the primary visual area of primates (*5*) and the "barrels" of the primary somatosensory area of rodents (*6*). However, the developing neocortex lacks many of the features that distinguish adult neocortical areas. Early in neocortical development, but after the generation and migration of cortical neurons, cytoarchitecture is uniform between areas that will later have an abrupt border in the adult (7); major classes of projection neurons are not restricted to their limited distributions across neocortical areas as in adults (*8*); and functional units are only beginning to emerge (*9*, *10*). To define the mechanisms responsible for the differentiation of neocortical areas, it is necessary to determine the extent to which each area's characteristics are prespecified or are caused by extrinsic influences that operate as the cortex is assembled.

To address this issue, we used the primary somatosensory area of rodent parietal cortex, which contains discrete functional units, termed "barrels." Barrels are isolated aggregates of layer 4 neurons, innervated by clusters of ventrobasal thalamic afferents, and arranged in a pattern isomorphic to that of the sensory hairs on the rodent's body surface (*10*). Therefore, barrels are not only indicative of the functional organization of the rodent's somatosensory cortex but are also an example of the architectural differentiation that distinguishes neocortical areas. Barrels develop gradually from a uniform cortical plate midway through the first week after birth (*10*, *11*). Experimental studies have demonstrated that the formation of barrels depends on input from an intact sensory periphery during a critical period of development (*10*, *12*). Although these studies demonstrated that barrel formation can be modified, they did not address whether

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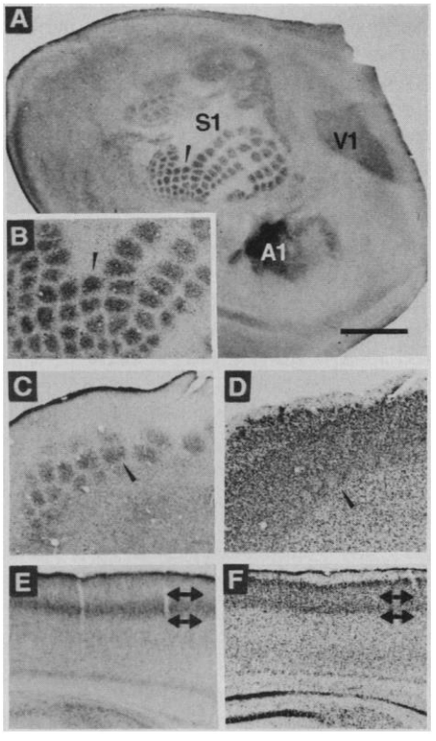

Fig. 1. Normal features of rat somatosensory and visual cortex assayed for AChE and stained by the Nissl technique. (**A**) An AChE-reacted, tangential section through layer 4 of P12 rat cortex shows the distribution of thalamocortical afferents from the principal sensory thalamic nuclei to the primary visual (V1), somatosensory (S1), and auditory (A1) cortex. In young rats, the AChE reaction product labels the termination patterns of the thalamocortical afferents that originate from the principal sensory thalamic nuclei, including the ventrobasal, lateral geniculate, and medial geniculate nuclei (*16*). In visual (occipital) cortex, geniculocortical afferents distribute in a homogeneous pattern, whereas in somatosensory (parietal) cortex, the pattern is disjunctive, reflecting the clustering of ventrobasal thalamic afferents in barrels. (**B**) Higher magnification of barrels in somatosensory cortex. Arrowhead points to the same barrel as in (A). (**C** through **F**) Differences in laminar cytoarchitecture between cortical areas are best seen in a parasagittal plane. Mature somatosensory and visual cortical areas each have a prominent, granular layer 4 composed of a dense population of stellate neurons that receive a substantial thalamic input. These features are typical of sensory cortical areas. However, the distributions of neurons and afferents in layer 4 differ between these areas. (C) AChE-positive puffs (arrowhead indicates an example) reflect the segregation of ventrobasal afferents in the somatosensory cortex coincident with (D) aggregates of Nissl-stained layer 4 cells (arrowhead). Because of their lateral location, these sections cut obliquely across layer 4 and reveal more than a single row of barrels. (E) In the visual cortex, the AChE reaction product marks the uniformly dense geniculocortical termination pattern coincident with (F) a continuous, prominent band of layer 4 cells. Paired double-headed arrows in (E) and (F) define the extent of layer 4. Scale bar represents 1.50 mm in (A); 0.60 mm in (B); 0.80 mm in (C through F).

parietal cortex is uniquely prespecified to develop barrels or the characteristic patterning of groups of barrels.

We used transplantation studies to test the potential of other neocortical areas to form barrels (*13*). Pieces of late fetal occipital (visual) cortex were transplanted to the presumptive barrelfield of parietal (somatosensory) cortex in newborn rats (*14*) before the development of cytoarchitecturally defined barrels (*11*) and before ventrobasal thalamic afferents segregate in a barrel-related manner (*15*). We later assayed the host and transplanted cortex for barrels by analyzing brain sections for the distribution of the acetylcholinesterase (AChE) reaction product, an early marker for primary sensory thalamocortical afferents (*16*), and by using Nissl staining to delineate the cortical cytoarchitecture (*17*). These methods revealed the differences between somatosensory and visual cortex both in cytoarchitecture and in the distribution of thalamocortical afferents (Fig. 1).

We find that barrel-like morphologies develop in occipital-to-parietal transplants (Fig. 2). These morphologies resemble the normal barrels of rat somatosensory cortex (Fig. 1, C and D); discrete puffs of AChE reaction product that mark the disjunctive pattern of ventrobasal thalamic afferents (*16, 18*) fill the cell-sparse centers of cellular aggregations. Such cellular aggregations are not seen in normal occipital cortex nor are they seen in occipital-to-parietal transplants when ventrobasal thalamic afferents fail to invade the transplant (indicated by a paucity of AChE reaction product). Homotopic transplants of occipital and parietal cortex have distributions of cells and thalamic afferents appropriate for their locations (*19*). The development of barrels in occipital-to-parietal transplants indicates that ventrobasal thalamic afferents are able to cluster appropriately in layer 4 of a foreign piece of cortex and that the transplanted layer 4 cells redistribute into aggregates in response to the thalamic afferents as the cells of normal barrels do. Therefore, the ability to form barrels is not unique to parietal cortex, the region that forms barrels in situ, but is also possessed by the embryonic occipital cortex.

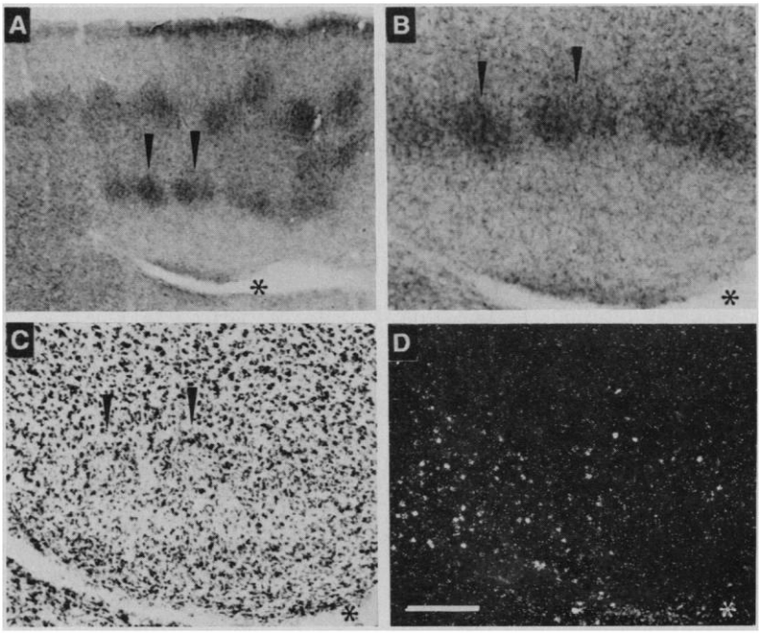

Fig. 2. Barrel-like morphologies develop in occipital (visual) cortex transplanted to the presumptive barrelfield of parietal (somatosensory) cortex. (**A**) An AChE-reacted parasagittal section through P12 rat parietal cortex with an occipital cortical transplant. As in Fig. 1C, the section cuts obliquely across layer 4, revealing two "rows" of AChE-positive puffs: barrels in the "upper row" are in the host parietal cortex, those in the "lower row" (two are marked with arrowheads) are in the occipital-to-parietal transplant and are shown at a higher magnification in (**B**). (**C**) A Nissl-stained section reveals cellular aggregates that correspond to the AChE-positive puffs in the adjacent section pictured in (B). Two AChE-positive puffs in (A and B) and their corresponding cellular aggregates in (C) are marked with arrowheads. These cellular aggregates, which have cell-dense sides and cell-sparse centers, resemble barrels in the anterolateral barrel-subfield of normal rat somatosensory cortex shown in Fig. 1D. (**D**) A dark-field autoradiogram reveals cells labeled with [^{3}H]thymidine in a section adjacent to that in (C). These thymidine-labeled cells mark the transplanted tissue. Only a small proportion of the transplanted cells appears labeled because only those generated at the time the [^{3}H]thymidine was administered incorporate the [^{3}H]thymidine into their DNA and only those nuclei in the upper 2 to 3 μm of the 40-μm section would appear labeled because of the short pathlength of the β-emission particle. The asterisks in (A through D) are placed in corresponding locations for orientation. Scale bar represents 0.40 mm in (A); 0.20 mm in (B) through (D).

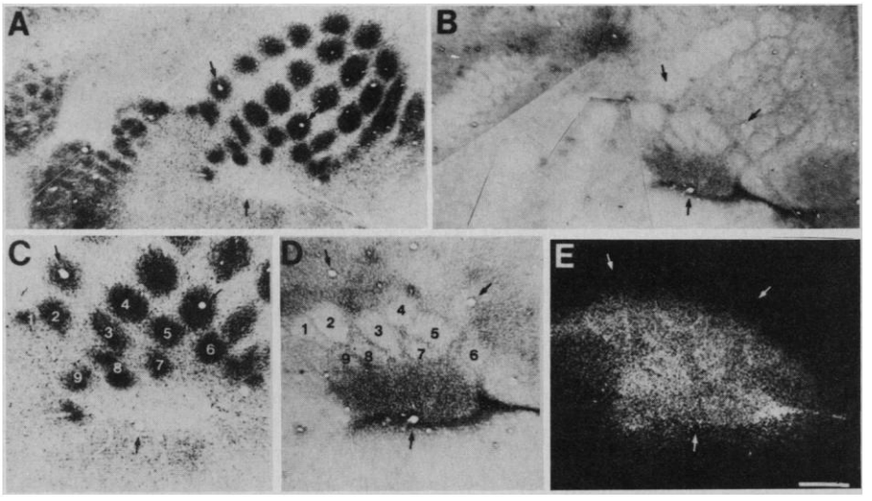

Fig. 3. Appropriate patterns of barrels and glycoconjugate boundaries develop in occipital (visual) cortex transplanted to the barrelfield of parietal (somatosensory) cortex. (**A**) In an AChE-reacted tangential section through the host somatosensory and transplanted visual cortex of a P8 rat, a normal-appearing barrel pattern is evident. The transplant is located in a region comparable to that shown in Fig. 1B. (**B**) Photomontage of PNA-stained sections adjacent to that shown in (A). The overall PNA-binding pattern resembles that in normal barrel cortex and complements the AChE-positive pattern in (A). (**C**) Some of the AChE-positive barrels formed in the transplanted occipital cortex are numbered in this higher magnification of the section in (A). (**D**) In this higher magnification of the section shown in (B), glycoconjugate boundaries are identified by the same numbers that mark the corresponding AChE-positive barrels in (C). (**E**) Fluorescence micrograph of the same section shown in (A) taken before processing for AChE. Cells labeled with bisbenzimide mark the transplant. Dense aggregates of labeled cells are distributed in a pattern that parallels the labeling revealed by AChE histochemistry and PNA binding. The arrows in (A through E) indicate the same blood vessels located just outside of the transplant. Scale bar represents 0.50 mm (A and B); 0.30 mm in (C) through (E).

This demonstration that barrel-like morphologies can develop in a foreign piece of neocortex does not address whether patterning information intrinsic to parietal cortex is necessary to establish the normal arrangement of barrels. Therefore, we examined additional heterotopic occipital-to-parietal transplants in brains sectioned tangential to the cortical surface in order to optimize visualization of the barrel array. In this set of experiments, barrels were identified by AChE histochemistry; alternate sections were treated with the lectin peanut agglutinin (PNA) that in normal parietal cortex reveals a pattern of glycoconjugate boundaries that complements the barrel array (*20*). This pattern of glycoconjugated molecules is apparent as barrels emerge (*20*) and is not observed in other areas of cortex. An occipital-to-parietal transplant case sectioned tangentially to the cortical surface demonstrates the same overall barrel pattern (Fig. 3) as that seen in normal cortex (Fig. 1A) (*21*). In host parietal and transplanted occipital cortex, discrete puffs of AChE reaction product (Fig. 3, A and C) are outlined by rings of PNA binding (Fig. 3, B and D). The patterns of AChE-positive puffs (*16*) and PNA-labeled glycoconjugates are complementary, resemble those in normal parietal cortex, and maintain continuity across borders between the host and transplanted cortex. This observation demonstrates that the puffs of AChE-positive ventrobasal thalamocortical afferents and associated glycoconjugate boundaries that develop in the transplant do not form randomly; rather their patterning is appropriate for that region of the barrelfield.

What is the source of information that guides the differentiation of an appropriate barrel array in the transplanted visual cortex? It is unlikely that patterning information is conferred on the transplant by cells that migrate into the transplant from host parietal cortex. Astrocytes, but not neurons, do migrate between transplanted and host cortex. However, astrocytes do not cross the border between the transplant and host cortex until 7 days after transplantation and migrate thereafter at a rate of ~200 μm/day (*22*). Because barrel arrays that span the transplanted occipital cortex are apparent by 8 days after transplantation and probably even earlier, cells migrating from the remaining host parietal cortex into the transplant cannot account for our findings. A straightforward interpretation is that the patterning of the barrel array and its associated glycoconjugate boundaries are not unique properties of parietal cortex, indicating that development of the barrel array does not require patterning information intrinsic to cortex. Instead, both the barrel-related cellular and glycoconjugate patterns are imposed by ventrobasal thalamic afferents on a region of cortex, whether it is normal parietal cortex developing in situ or occipital cortex transplanted to the parietal region (*23*).

Our findings lead to several conclusions. First, ventrobasal thalamic afferents can organize into clusters in a foreign piece of cortex that normally lacks a disjunctive pattern of thalamocortical terminations. Therefore, this capacity for organization must be a property of the afferent system. Second, layer 4 cells in the transplant, which are uniformly distributed in the donor embryonic visual cortex and would remain so in the adult, redistribute in response to the clustered ventrobasal thalamic afferents, mimicking the behavior of layer 4 cells in somatosensory cortex. These afferents may operate directly, or through an intermediary (*24*), to shape the dendritic morphologies of layer 4 neurons in both host somatosensory (*25*) and transplanted visual cortex and thereby influence cytoarchitecture. Therefore, the formation of individual barrels does not require information inherent to parietal cortex but is governed by ventrobasal thalamic afferents. Third, the normal patterning of the barrel array does not require cues prespecified uniquely in parietal cortex. However, cues intrinsic to cortex may be necessary to develop the appropriate matching between thalamic nuclei and specific regions of cortex (*26*) and to establish the orientation of topographic maps in thalamocortical projections. But the emergence of a somatotopic patterning of ventrobasal thalamic afferents after the afferents invade layer 4 must reflect their active responses to influences originating at the sensory periphery; these influences must therefore provide sufficient information to initiate the differentiation of both the barrel array and the complementary pattern of glycoconjugate boundaries, independent of cortical cues.

Experiments have demonstrated that late embryonic neocortex transplanted heterotopically can develop the efferent connections (*27*) characteristic of the host locale. The present study shows that such transplants can develop patterned afferent connections and architectural features appropriate to the host locale. Therefore, the differentiation of the features that distinguish cortical areas in adults must not be prespecified at the time the cortex is assembled. Thus, with respect to these features, the regions of the developing neocortex that give rise to diverse areas in the adult initially must have similar potentials. Studies also suggest that the development of "blobs," functional units unique to the primary visual cortex of primates (*5*), is controlled by influences extrinsic to cortex. Blobs develop in

the primary visual cortex of monkeys even in the absence of retinae (*2, 28*), indicating that, unlike barrels, the differentiation of blobs may not require an intact sensory periphery. However, blob development seems to be dramatically influenced by thalamocortical afferents. After mid-gestational enucleation, blobs are found in an attenuated region of cortex; this region has the cytoarchitecture of primary visual cortex and presumably receives afferents from the primary visual thalamic nucleus, the lateral geniculate, which is also reduced in size (*2, 28*). Regions of cortex adjacent to the attenuated primary visual cortex, which under normal circumstances would probably differentiate into primary visual cortex, fail to develop the architecture characteristic of that area (*2, 28*). These observations imply that blob development occurs only in regions of cortex innervated by thalamocortical afferents from the lateral geniculate nucleus.

Our results suggest that regions of the developing neocortex are interchangeable and indicate that factors extrinsic to cortex control the differentiation of areas. We conclude that the neocortical neuroepithelium is specified to generate fundamental cortical features such as laminated structure (*13*), a variety of cortical cell types (*29*), and a common local circuitry (*30*) but that this epithelium lacks a rigid specification of architectural features and functional organizations that distinguish cortical areas. We infer that the neocortical neuroepithelium generates comparable populations of neurons across the entire neocortex that, when exposed to the same external cues, can respond in comparable ways (*31*). Such a framework diminishes the need for prespecification of features that distinguish cortical areas and provides a parsimonious model for the developmental differentiation of cortical areas, the phenomenon of cortical plasticity after early trauma, and the emergence of new cortical areas during evolution.

REFERENCES AND NOTES

1. K. Brodmann, *Lokalisationslehre der Groshirnrinde in Ihren Principen Dargestellt aug Grund des Zellen Baue* (Barth, Leipzig, FRG, 1909); W. J. S. Krieg, *J. Comp. Neurol.* **84**, 221 (1946); D. C. Van Essen, in *Cerebral Cortex*, vol. 3, *Visual Cortex*, A. Peters and E. G. Jones, Eds. (Plenum, New York, 1985), pp. 289–330.
2. P. Rakic, *Science* **241**, 170 (1988).
3. D. D. M. O'Leary, *Trends Neurosci.* **12**, 400 (1989); H. Killackey, *J. Cognitive Neurosci.* **2**, 1 (1990).
4. J. H. Kaas, in *Higher Brain Function: Recent Exploration of the Brain's Emergent Properties*, S. Wise, Ed. (Wiley, New York, 1986), pp. 347–378.
5. M. S. Livingstone and D. H. Hubel, *J. Neurosci.* **4**, 309 (1984); J. C. Hutton and T. E. Hedley-White, *Philos. Trans. R. Soc. London* **304**, 255 (1984).
6. T. A. Woolsey and H. Van Der Loos, *Brain Res.* **17**, 205 (1970).
7. I. Kostovic and P. Rakic, *J. Neurosci.* **4**, 25 (1984).
8. G. M. Innocenti, *Science* **212**, 824 (1981); D. D. M. O'Leary, B. B. Stanfield, W. M. Cowan, *Dev. Brain Res.* **1**, 607 (1981); B. B. Stanfield, D. D. M. O'Leary, C. Fricks, *Nature* **298**, 371 (1982); G. O. Ivy and H. P. Killackey, *J. Neurosci.* **2**, 735 (1982); I. G. Thong and B. Dreher, *Dev. Brain Res.* **25**, 227 (1986).
9. D. Purves and A.-S. Lamantia, *Proc. Natl. Acad. Sci. U.S.A.* **87**, 5764 (1990).
10. T. A. Woolsey, in *Development of Sensory Systems in Mammals*, J. R. Coleman, Ed. (Wiley, New York, 1990), pp. 461–516.
11. S. P. Wise and E. G. Jones, *J. Comp. Neurol.* **178**, 187 (1978).
12. Literature exists implicating ventrobasal thalamic afferents in the development of a normal barrelfield [H. Van der Loos and T. A. Woolsey, *Science* **179**, 395 (1973); T. A. Woolsey and J. R. Wann, *J. Comp. Neurol.* **170**, 53 (1976); H. P. Killackey and G. R. Belford, *ibid.* **183**, 285 (1979); D. Jeanmonod, F. L. Rice, H. Van der Loos, *Neuroscience* **6**, 1503 (1981); D. R. Dawson and H. P. Killackey, *J. Comp. Neurol.* **256**, 246 (1987)]. Vibrissae follicle cauterization or infraorbital nerve cut, during a critical period, lead to an abnormal-appearing cortical barrelfield, as revealed by a number of anatomical methods (*10*) including the binding of PNA [N. G. F. Cooper and D. A. Steindler, *Brain Res.* **489**, 167 (1989)] and the immunohistochemical identification of J1/tenascin (*32*), also called cytotactins and, of cytotactin-binding protein [K. L. Crossin, S. Hoffman, S.-S. Tan, G. M. Edelman, *Dev. Biol.* **136**, 381 (1989)]. Strains of mice with supernumerary vibrissae often have a corresponding cortical barrel, but only if a threshold number of nerve fibers innervates the extra vibrissa [E. Welker and H. Van Der Loos, *J. Neurosci.* **6**, 3355 (1986)].
13. Pieces of embryonic rat neocortex transplanted to the brains of neonatal rats continue to generate neurons (*33*), develop lamination in the characteristic inside-out fashion (*33, 34*), and establish connections with the host brain [F.-L. F. Chang, J. G. Steedman, R. D. Lund, *Dev. Brain Res.* **13**, 164 (1984); M. K. Floeter and E. G. Jones, *ibid.* **22**, 19 (1985); A. J. Castro, N. Tonder, N. A. Sunde, J. Zimmer, *Exp. Brain Res.* **66**, 533 (1987)]. Heterotopic transplantation of developing cortex has been useful for studying factors that influence the development of regional differences in cortical efferents (*27*).
14. Rat pups and fetuses of timed, pregnant Sprague-Dawley dams (Harlan Sprague-Dawley) were used for all experiments. Embryonic day E0 refers to day of insemination. P0 refers to the first 24 hours after birth. Pups from eight litters served as host animals; fetuses from four rats were used to harvest donor tissue. On E17 or E18, pups were removed from an anesthetized dam (3.5% chloral hydrate, 10 mg per 100 g of body mass), and their brains were dissected and placed into cold Ham's F-10 medium (Gibco). A 1 mm by 2 mm piece of cortex, spanning from pia to ventricle, from either the occipital or parietal region was dissected. Transplants were fitted, with their pial surface up, into rectangular cavities made in the occipital or parietal cortex of host pups (P0) anesthetized by hypothermia. When making the transplantation cavity, we took care to remove a 1 mm by 2 mm portion of the parietal cortical gray matter and to spare the underlying white matter. After closure of the cranium and scalp and recovery from hypothermia, pups were returned to the dam. For later verification of the transplant in the host brain, donor tissue was labeled with either ^{3}H-labeled thymidine or bisbenzimide before transplant; pregnant females were injected intraperitoneally with [^{3}H]thymidine (10 μCi per 1 g of body mass; specific activity of 6.7 Ci/mmol; ICN ImmunoBiologicals) on E15 during ongoing cortical neuronogenesis. Alternatively, after dissection, donor tissue was placed for 5 min in 0.02% bisbenzimide (Hoechst 33258, Sigma), dissolved in cold Ham's F-10 medium, and then washed for 5 min in several rinses of cold Ham's F-10 medium before transplantation. [^{3}H]thymidine is only incorporated in cells in the mitotic S phase during the short time that the thymidine is available for uptake. Bisbenzimide rapidly labels the nuclei of most, if not all, cells.
15. Donor tissue was taken from occipital cortex after the generation of layer 4 neurons [R. D. Lund and M. J. Mustari, *J. Comp. Neurol.* **173**, 289 (1977)] and before geniculocortical fibers invade the occipital cortical plate [B. S. Reinoso and D. D. M. O'Leary, *Soc. Neurosci. Abstr.* **14**, 1113 (1988); *ibid.* **16**, 493 (1990)]. P0 host pups were used because in utero transplantation was not practical. Thalamic fibers begin to invade the parietal cortical plate on E19 (*35*), but this does not prevent them from later reextending into the transplanted tissue. Thalamic fibers segregate into a barrel-related, somatotopic pattern 2 days after birth (*35*). Glycoconjugate boundaries are evident by P3 (*36*). Cytoarchitecturally defined barrels are not apparent in rats until after P4 (*12*).
16. The dense AChE reaction product in layer 4 of primary sensory areas in rat cortex marks the termination of thalamocortical afferents from the principal sensory thalamic nuclei. AChE is transiently expressed by the neurons of the ventrobasal, lateral geniculate, and medial geniculate thalamic nuclei and, when histochemically treated, the reaction product heavily labels the axons, including their terminal arborizations in the primary somatosensory, visual, and auditory cortical areas, respectively [R. T. Robertson, *Neurosci. Lett.* **75**, 259 (1987); ———, M. A. Hanes, J. Yu, *Dev. Brain Res.* **41**, 1 (1988); D. A. Kristt, *J. Comp. Neurol.* **186**, 1 (1979); *Neuroscience* **29**, 27 (1989); R. T. Robertson, F. Mostamand, G. H. Kageyama, K. A. Gallardo, J. Yu, *Dev. Brain Res.* **58**, 81 (1991)].
17. P8 to P12 host pups (six litters) were perfused transcardially with 0.9% buffered saline, followed by 10% buffered formalin, then fixed in 15% sucrose in 10% buffered formalin. Brains that appeared to have surviving transplants (~90%) were frozen and sagittally sectioned at 50 μm on a microtome and collected in four series. We processed two series of sections for AChE to delineate the thalamocortical termination pattern of the principal sensory thalamic nuclei (*16*). We processed a third series for autoradiography [W. M. Cowan, D. I. Gottlieb, A. E. Hendrickson, J. L. Price, T. A. Woolsey, *Brain Res.* **37**, 21 (1972)] to verify the survival of the transplant by the presence of thymidine-labeled neurons. We Nissl-stained a fourth series with 0.25% cresyl violet to show cellular distributions.
18. Retrograde tracing from heterotopic transplants of late-fetal occipital (visual) cortex into the cortex of newborn host rats show that such transplants receives afferent input from the thalamic nuclei that normally innervate the host cortical location, but not from the lateral geniculate nucleus, the source of thalamic input to the visual cortex in situ [(*34*); D. D. M. O'Leary, *Soc. Neurosci. Abstr.* **14**, 1113 (1988)].
19. Homotopic transplantations of both parietal and occipital cortex were performed as control experiments [B. L. Schlaggar and D. D. M. O'Leary, *Soc. Neurosci. Abstr.* **14**, 475 (1988); *ibid.* **15**, 1050 (1989)]. Parietal-to-parietal transplants develop barrel-like morphologies similar to those that form in occipital-to-parietal transplants. {Barrels also develop in parietal cortex removed and then placed directly back into the same site in neonatal murine brain [F. L. Andres and H. Van Der Loos, *Dev. Brain Res.* **20**, 115 (1985)]}. Occipital-to-occipital transplants develop architecture and uniform thalamocortical afferent terminations appropriate to visual cortex. Thus, in each type of transplant performed, morphological features develop in a manner appropriate to the site, not to the origin of the cortical tissue. The absence of barrel-like morphologies in these control transplants reinforces our conclusion that transplantation per se does not produce barrel-like morphologies.
20. The glycoconjugates revealed by PNA-binding are densely distributed in barrel sides and septae but sparsely distributed in the termination sites of ventrobasal thalamic afferents in barrels. These boundaries precede the appearance of cytoarchitecturally defined barrels (*15*). These findings have led to the suggestion that the glycoconjugate boundaries constitute a framework that is a prespecified feature intrinsic to parietal cortex and that may influence the patterning of ventrobasal thalamic afferents and the distribution of cortical cells and their processes [N. G. F. Cooper and D. A. Steindler, *J. Comp. Neurol.*

249, 157 (1986); *Brain Res.* **380**, 341 (1986); D. A. Steindler, N. G. F. Cooper, A. Faissner, M. Schachner, *Dev. Biol.* **131**, 243 (1989); (*36*)].
21. Transplants were done as described (*14*). At P8, host pups were anesthetized (3.5% chloral hydrate, 10 mg/100 g of body mass) and perfused transcardially with 0.9% buffered saline, followed by 4% paraformaldehyde and 2% glutaraldehyde in 0.2 M cacodylic acid buffered to pH 7.3. Brains were removed and cortices were flattened for tangential sectioning at 40 or 50 μm on a Vibratome. Every other section was assayed for AChE (*16*). We processed the remaining sections for the binding of PNA to reveal the distribution of glycoconjugated molecules in host and transplanted tissue (*20*). Before these procedures, we examined selected sections and photographed them under ultraviolet illumination to identify the bisbenzimide-marked transplant.
22. H. F. Zhoue, L. H.-C. Lee, R. D. Lund, *J. Comp. Neurol.* **292**, 320 (1990).
23. Our findings indicate that ventrobasal afferents control the formation of the glycoconjugate boundaries from an initially homogeneous distribution of glycoconjugates. In the developing barrelfield, the glycoconjugate boundaries revealed by lectin-binding emerge from a homogeneous binding pattern (*20*) and are coincident with AChE-positive, thalamocortical terminations (Fig. 3). We find that, elsewhere in the cortex, regions of dense, AChE-positive, thalamocortical terminations correspond to regions of diminished or absent lectin-binding. In rat, this feature is most notable in primary visual and auditory areas of the cortex. As in the barrelfield, these patterns emerge from an initially homogeneous pattern of lectin-binding (B. L. Schlaggar and D. D. M. O'Leary, unpublished observations).
24. Evidence from the development of the ferret retinogeniculate system [J. B. Hutchins and V. A. Casagrande, *J. Comp. Neurol.* **298**, 113 (1990)] and the insect olfactory glomeruli [L. A. Oland, G. Orr, L. P. Tolbert, *J. Neurosci.* **10**, 2096 (1990)] suggests an intermediary role for glia in receiving morphogenic information from afferents and creating boundaries that delimit future distributions of cells and their processes. An intermediary role of this type has been proposed for the glial-associated glycoconjugate expression in the parietal barrelfield [D. A. Steindler, A. Faissner, M. Schachner, *Comments Dev. Neurobiol.* **1**, 29 (1989); (*32*)].
25. R. M. Harris and T. A. Woolsey, *J. Comp. Neurol.* **196**, 357 (1981); A. Peinado and L. C. Katz, *Soc. Neurosci. Abstr.* **15**, 960 (1989); *ibid.* **16**, 1127 (1990).
26. A. Ghosh, A. Antonini, S. K. McConnell, C. J. Shatz, *Nature* **347**, 179 (1990).
27. B. B. Stanfield and D. D. M. O'Leary, *ibid.* **313**, 135 (1985); D. D. M. O'Leary and B. B. Stanfield, *J. Neurosci,* **9**, 2230 (1989).
28. R. O. Kuljis and P. Rakic, *Proc. Natl. Acad. Sci. U.S.A.* **87**, 5503 (1990).
29. S. K. McConnell, *J. Neurosci.* **8**, 945 (1988); *Trends Neurosci.* **12**, 342 (1989).
30. M. Sur, P. E. Garraghty, A. W. Roe, *Science* **242**, 1437 (1988); C. Metin and D. O. Frost, *Proc. Natl. Acad. Sci. U.S.A.* **86**, 357 (1989).
31. This position should not be interpreted to mean that there is evidence that all cortical neurons are equipotential. Rather, neurons of the same class distributed across the cortex have similar developmental potentials, limited to a set of potentials unique to that class.
32. D. A. Steindler *et al.*, *Exp. Neurol.* **109**, 35 (1990).
33. C. B. Jaeger and R. D. Lund, *Exp. Brain Res.* **40**, 265 (1981).
34. F.-L. F. Chang, J. G. Steedman, R. D. Lund, *J. Comp. Neurol.* **244**, 401 (1986).
35. R. S. Erzurumlu and S. Jhaveri, *Dev. Brain Res.* **56**, 229 (1990).
36. C. McCandlish, R. S. Waters, N. G. F. Cooper, *Exp. Brain Res.* **77**, 425 (1989).
37. Experiments were approved by the Animal Care Committee of our institution. Supported by NIH grants P01 NS17763 and R01 EY07025. We thank C. Go for technical assistance, J. Christensen for help with the PNA-binding method, and J. W. Lichtman and T. A. Woolsey for comments on the manuscript.

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A Distinct Potassium Channel Polypeptide Encoded by the *Drosophila eag* Locus

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Many of the signaling properties of neurons and other electrically excitable cells are determined by a diverse family of potassium channels. A number of genes that encode potassium channel polypeptides have been cloned from various organisms on the basis of their sequence similarity to the *Drosophila Shaker* (*Sh*) locus. As an alternative strategy, a molecular analysis of other *Drosophila* genes that were defined by mutations that perturb potassium channel function was undertaken. Sequence analysis of complementary DNA from the *ether à go-go* (*eag*) locus revealed that it encodes a structural component of potassium channels that is related to but is distinct from all identified potassium channel polypeptides.

THE *DROSOPHILA SH* LOCUS WAS THE first K^+ channel structural gene to be isolated (*1*). Since then, a number of additional K^+ channel genes have been cloned from *Drosophila* and other organisms (*2*). The primary strategy researchers used to clone most of these genes was cross-hybridization with DNA probes derived from *Sh*. However, other K^+ channel genes less closely related to *Sh* may not have been identified by this approach. As an alternative route to isolate genes affecting K^+ channel structure and function, we made no assumptions about sequence conservation and focused on mutations that disrupt K^+ channel activity in *Drosophila* (*3*).

One of these genes is the X-linked *eag* locus, which was originally identified in *Drosophila* on the basis of mutations that cause a leg-shaking phenotype (*4*). Electrophysiological studies revealed that *eag* mutations cause spontaneous repetitive firing in motor axons and elevated transmitter release at the larval neuromuscular junction (*3, 5*). In *eag Sh* double mutants, transmitter release at the larval neuromuscular junction persists for at least an order of magnitude longer than in either single mutant, resulting in large plateau-shaped synaptic potentials. These prolonged synaptic potentials are correlated with long trains of action potentials in motor axons. Whereas *Sh* mutations eliminate I_A, a fast, transient, voltage-gated K^+ current (*6*), voltage-clamp experiments indicated that I_K, a slow non-inactivating, voltage-gated K^+ current, is reduced in *eag* mutants (*7*). Furthermore, some *eag* mutations diminish I_A, as well as I_{CF} and I_{CS}, which are fast and slow Ca^{2+}-activated K^+ currents, respectively (*7, 8*). However, it was unclear whether *eag* encoded a structural component of K^+ channels or affected the activity of these channels by another mechanism.

To elucidate the molecular basis of the *eag* phenotype, we cloned this locus by chromosome jumping and walking and identified cDNAs that correspond to the *eag* transcript (*9, 10*). These cDNAs span over 35 kb of genomic DNA and encompass the molecular lesions associated with four mutant *eag* alleles.

We determined the complete nucleotide sequence of cDNA CH20 (*10*), which is 4061 nucleotides in length. Although this cDNA is incomplete relative to the 10-kb *eag* transcript (*9*), it contains a complete open reading frame, beginning at nucleotide 464 and terminating at nucleotide 3985, thus encoding a deduced polypeptide of 1174 amino acids (Fig. 1). Translation was assumed to begin at nucleotide 464 because this is the first ATG codon of the open reading frame, and the sequence immediately preceding this codon matches the consensus translational start site for *Drosophila* (*11*). Computer analyses of the amino acid and nucleotide sequences of *eag* failed to detect any similarities with sequences of other genes in three databases (*12*). However, hydropathy analysis (Fig. 2) of the *eag* polypeptide (Eag) indicated the presence of seven potential membrane-spanning domains, reminiscent of the structure of known K^+ channel polypeptides (*1, 2*). Furthermore, comparison with a K^+ channel consensus sequence derived from the *Sh* superfamily revealed that each hydrophobic domain of Eag shared similarity with the corresponding domain of the consensus sequence (Fig. 3). The sequence similarities in two of these regions, S4 and H5, are noteworthy. A feature of most voltage-gated channels is an S4 domain, which is thought to represent the voltage sensor of these channels (*13*). This domain consists of a string of positively charged residues at every third position that are each separated by two hydrophobic residues. The fourth hydro-

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