Molecular differences among neurons reveal an organization of human visual cortex

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ABSTRACT   Monoclonal antibody Cat-301 recognizes a cell-surface proteoglycan on subsets of neurons in several areas of the cat and macaque monkey central nervous system. In striate and extrastriate visual cortex of the macaque, the distribution of Cat-301-positive neurons demonstrates features of cellular organization that correlate with previously described functional subdivisions. Here we show that Cat-301 recognizes an antigen in human cortex that is closely related, if not identical, to the antigen in laboratory animals. Further, we use Cat-301 to demonstrate an organization of molecularly defined neurons in primary and secondary visual cortex (cortical areas V1 and V2) of the human. The organization demonstrated with Cat-301 in human area V1 correlates with the organization of ocular dominance columns demonstrated by cytochrome oxidase histochemistry. The organization demonstrated with Cat-301 in human area V2 correlates with the thick stripes of the cytochrome oxidase pattern. The present observations provide evidence for a visual pathway in human cortex homologous to the magnocellular pathway in macaque, a pathway involved in processing the low-contrast, achromatic, and moving components of visual stimuli.

Studies of the organization and function of the human cerebral cortex have relied extensively on extrapolations from experiments done on laboratory animals. The experimental manipulations used to establish many of the connectional and physiological properties of cortical neurons require invasive techniques that cannot be used to study the human brain. While recent advances in imaging technology (1) have begun to reveal the functional organization of the human central nervous system (CNS) directly, the resolution of these techniques is still poor. One approach to the identification of functional subdivisions of the human CNS at the single-cell level is to generate molecular markers for functional classes of neurons in experimental animals. The conservation of molecular species through evolution might then allow the analysis of functional and molecular architecture to be extended to the human.

The presence of functional subdivisions within the primate visual system is now well accepted. From retina through several orders of cortical processing, the primate visual system can be thought of as composed of two parallel channels of information processing, the magnocellular channel (the "motion," "achromatic," or "low-contrast" channel) and the parvocellular channel (the "form/color" channel), which are distinct from each other both anatomically and functionally (for review, see refs. 2–5). Neurons in the magnocellular channel have much greater sensitivity to low contrasts of light and to the direction of a moving stimulus and lesser sensitivity to color than do neurons in the parvocellular channel. In the macaque monkey (*Macaca fascicularis*), monoclonal antibody Cat-301 (6, 7) preferentially

labels magnocellular neurons in four hierarchically related cortical areas, thus demonstrating that neurons within a functionally and anatomically distinct pathway share molecular characteristics (8, 9, 27).

Here we report a first step in extending the analysis of the functional organization of the monkey visual cortex to that of the human. We demonstrate that Cat-301 immunoreactivity is present in human CNS and that the subcellular distribution and biochemical properties of the antigen recognized by Cat-301 in the human are similar to those observed in other species. Moreover, we show that the organization of Cat-301-positive neurons in human visual cortex is strikingly similar to that of the functional pathway previously described in macaque through the use of Cat-301.

METHODS

Human postmortem brain from three neurologically normal individuals was obtained without fixation. To view the organization of the occipital cortex in coronal sections, coronal slabs (0.5–1.0 cm thick) of occipital cortex were fixed by immersion in a fixative of 4% paraformaldehyde in 0.1 M sodium phosphate buffer at pH 7.4. Coronal sections (50 μm thick) were immunohistochemically stained for Cat-301 by using horseradish peroxidase-conjugated anti-mouse antibody (Cappel Laboratories) to visualize the mouse monoclonal antibody Cat-301. Details of the staining methods have been described (6).

To view the organization of neurons in the tangential plane (that is, within a given cortical layer), the occipital third of the cerebral cortex was flattened as previously described (10). Briefly, dissecting away the overlying pia and blood vessels and the underlying white matter allows the cortex to flatten. The flattened cortex was then fixed (in the same fixative described above) under weights between foam sponges overnight. Fifty-micrometer-thick sections of the flattened cortex were made tangential to the pial surface on a freezing sliding microtome and were stained for Cat-301 immunoreactivity.

For biochemical analyses, DEAE-enriched fractions from urea extracts of unfixed cat or human cortex were electrophoresed in 3–8% polyacrylamide gels, transferred to nitrocellulose, probed with Cat-301, and visualized with an alkaline phosphatase-conjugated anti-mouse antibody (Fisher). In some cases, the antigen was immunoprecipitated with Cat-301-loaded goat anti-mouse beads before electrophoresis. Detailed methods for the biochemical analyses were described by Zaremba *et al.* (11).

RESULTS

One of the most characteristic features of Cat-301 staining is the association of immunoreactivity with the neuronal surface (6). At high magnification at the light microscopic level,

Abbreviation: CNS, central nervous system.
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antibody staining is distributed inhomogeneously over the surface of the cell, leaving small unstained circular fenestrae. Electron microscopic analysis has shown that these fenestrae represent the site of synaptic boutons where the synaptic cleft is not stained by Cat-301 (6, 7). In the human CNS, Cat-301-positive neurons display the same surface-associated staining observed in other species (Fig. 1*A*). Furthermore, human neurons, like cat and monkey neurons, display the characteristic fenestrated staining pattern (Fig. 1*B*). The similarity in subcellular localization of Cat-301 immunoreactivity suggests that the antigen recognized by Cat-301 in the human may be similar to the antigen observed in other species.

Our biochemical studies provide further evidence that the human antigen is closely related to the antigen we have studied in hamster, guinea pig, and cat. Previously we demonstrated that the Cat-301 antigen in experimental animals is a chondroitin sulfate proteoglycan and that its biochemical properties suggest that it is a component of CNS extracellular matrix (11). Biochemical studies of human cerebral cortex show that the human antigen has chromatographic and electrophoretic properties, as well as enzymatic sensitivities, that are identical to those of the Cat-301 antigen from the other species. The human antigen binds to DEAE ion-exchange columns in 8 M urea at pH 6 and is eluted at a sodium chloride concentration of about 0.25 M. On Western blots of SDS/polyacrylamide gels the human antigen (Fig. 2, lane 2) is seen as a broad band with a staining intensity centered at 680 kDa, virtually indistinguishable from the cat antigen (lane 1). Electrophoresis as a broadly migrating species is frequently indicative of the presence of carbohydrates. The human antigen is sensitive to digestion by chondroitinase, which shifts its apparent molecular mass to ≈580 kDa (lane 3). These biochemical data, together with our histological data, indicate that the human antigen, like the hamster, guinea pig, and cat antigen, is an extracellular chondroitin sulfate proteoglycan and suggest that both the polypeptide and the carbohydrate structure of the antigen are likely to be similar in all species. The histological and
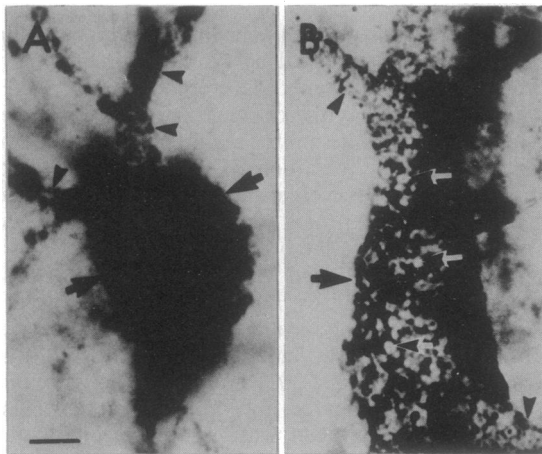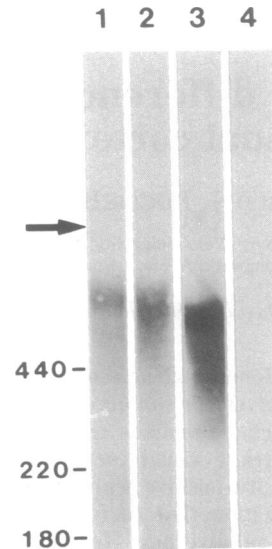

FIG. 2. The human Cat-301 antigen has electrophoretic properties similar to those of the antigen from experimental animals. The intact antigen from both cat (lane 1) and human (lane 2) migrates to an estimated molecular mass of 680 kDa. Following digestion with chondroitinase, the human antigen migrates to an estimated molecular mass of 580 kDa (lane 3). Chondroitinase alone shows no immunoreactivity (lane 4). The antigen in lanes 2 and 3 was immunoprecipitated with Cat-301-loaded goat anti-mouse beads before electrophoresis. Arrow indicates the boundary between the stacking gel and the resolving gel. Molecular mass markers were laminin α subunit (440 kDa), laminin β subunit (220 kDa), and clathrin heavy chain (180 kDa).

biochemical similarities suggest that the Cat-301 antigen may subserve related biological functions in all these species.

We next asked whether related populations of neurons might be recognized in monkey and human. In the macaque monkey, each area of cortex that contains Cat-301-positive neurons has a characteristic distribution of antibody-positive cells (7–9, 27). Primary visual cortex (area V1) contains two intensely stained bands of Cat-301-positive neurons, in layers 4 and 6 (Fig. 3*A*, left side). Recent analyses have shown that the band of staining in layer 4 includes both layers 4B and 4Ca (27). Layers 2, 3, and 5 also contain antibody-positive neurons, but at a far lower density than layers 4 and 6. Secondary visual cortex (area V2) contains two bands of antibody-positive neurons, a dense band in layer 3 and a lighter band in layer 5 (Fig. 3*A*, right side).

In coronal sections of human occipital cortex, areas V1 and V2 contain antibody-positive neurons with laminar distribution virtually identical to that seen in macaque (Fig. 3*B*). As in the macaque, human area V1 contains two bands of intensely stained neurons, in layers 4 and 6 (Fig. 3*B*, left side). Precise laminar assignments are difficult in human cortex, but based on cell density, assignments can be tentatively made. By this criterion, the band of staining in layer 4 occupies roughly the deeper three-fourths of layer 4B and extends into the superficial part of the underlying layer 4Ca. Cat-301-positive neurons are also found in the superficial layers, 2 and 3, but at a far lower density than in the deeper layers. In area V2, layer 3 contains a dense band of Cat-301-positive neurons, while the more superficial and deep layers contain fewer antibody-positive neurons (Fig. 3*B*, right side).

Differences can also be observed in Cat-301 staining between monkey and human. In area V1 of the macaque (Fig. 3*A*), immunoreactivity in layer 6 is more intense than in layer 4, but in the human (Fig. 3*B*), the band in layer 6 is less intense than the band in layer 4. Because the stained cell types and their laminar distribution seem consistent between species, the laminar differences in staining intensity may reflect a

FIG. 1. Human cortical neurons stained with Cat-301 demonstrate surface-associated immunoreactivity. (*A*) When the section passes through an antibody-positive neuron, a dark ring of staining (arrows) surrounds the unstained cytoplasm (c). Staining also extends out along proximal dendrites (small arrowheads). (*B*) When the section grazes the surface of an antibody-positive neuron, the distribution of immunoreactivity over the cell surface can be appreciated. Immunoreactivity is inhomogeneously distributed over the surface. Small, unstained circular profiles dot the surface of the neuron (small white-and-black arrows). The continuity of staining from cell body (black arrow) to dendrites can be seen at the small arrowheads. (Bar = 10 μm in *A* and *B*.)
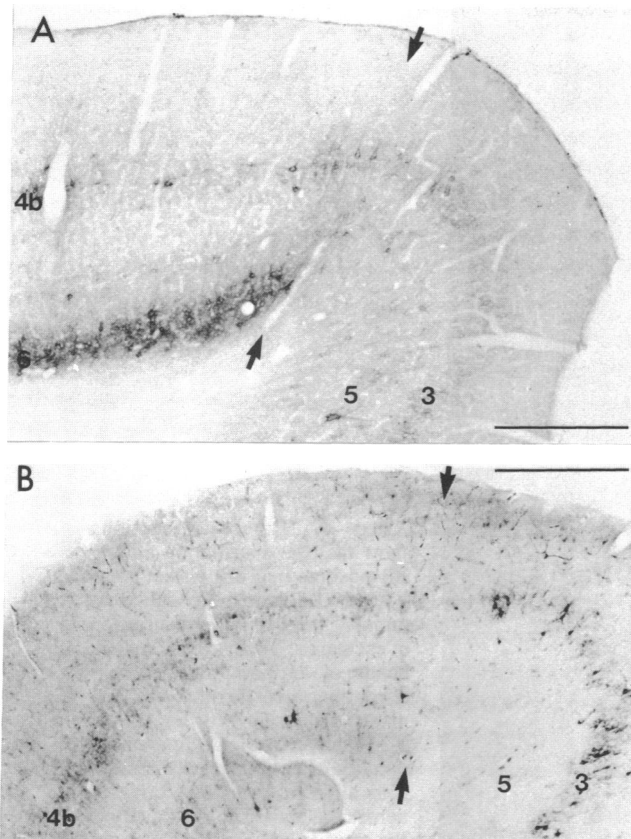
FIG. 3. The laminar distribution of Cat-301-positive neurons in areas V1 (to the left side in each panel) and V2 (to the right side in each panel) is the same in macaque (*A*) and human (*B*), although there are between-species differences in staining intensity. (*A*) In the macaque, area V1 contains two bands of labeled neurons, in layers 4 and 6. The V1/V2 border (arrows) is marked by an abrupt change in the distribution of antibody-positive neurons. (Bar = 0.5 mm.) (*B*) In human visual cortex, area V1 contains two bands of labeled cells, in layers 4 and 6, and the border with area V2 (arrows) is marked by a change in laminar distribution of antibody-positive neurons. As in the macaque, human area V2 contains a dense band of Cat-301-positive cells in layer 3 and a lighter band in layer 5. (Bar = 1 mm.)

difference in the relative density of a particular class of neuron.

In addition to the characteristic laminar distribution of antibody-positive neurons in macaque areas V1 and V2, the distribution of Cat-301-positive neurons *within* the layers of each area reflects previously described features of organization. Within the plane of any one cortical layer (that is, parallel to the cortical surface), both V1 and V2 have characteristic topographic patterns. In area V1 the ocular dominance columns (12) reflect the segregated right-eye and left-eye inputs from the lateral geniculate nucleus in the tangential plane of layer 4C. In tangential sections of macaque area V1, Cat-301-positive neurons form patches that align into rows within layers 4 and 6, and these patches are in vertical register with the center of the ocular dominance columns of layer 4C (7, 13).

Tangential sections of human area V1 stained with Cat-301 show topographic features similar to those seen in the macaque. Human area V1 also contains patches of antibody-positive neurons that align in rows within layers 4 and 6 (Fig. 4*B*). The periodicity of these rows is ≈1 mm, matching the width of ocular dominance columns described previously in human (14, 15), and approximately double the width of ocular dominance columns of monkey area V1.

Cytochrome oxidase histochemistry demonstrates the major organizational features in the tangential plane of macaque area V2. Darkly reactive stripes run perpendicular to the V1/V2 border and have a characteristic pattern of alternating dark thick and thin stripes with interposed light regions (10). This banding pattern reflects the organization of functionally distinct pathways within V2 (10, 16, 17). Previous work showed that in tangential sections of macaque area V2 stained with Cat-301, darkly immunoreactive neurons also form stripes that run perpendicular to the V1/V2 border. Superimposition of the Cat-301 pattern on the cytochrome oxidase pattern reveals that the Cat-301 stripes are in register with the thick cytochrome oxidase stripes (8, 27).

The organization of human area V2 has been less well described. Cytochrome oxidase histochemistry shows alternating light and dark stripes, but the resolution of this technique in human V2 is poor (18). Cat-301 identifies a subset of V2 neurons with an organization similar to that described in macaque. In tangential sections of human area V2, Cat-301 densely stains groups of neurons that form thick stripes across the area. These stripes of Cat-301-positive neurons are oriented perpendicular to the V1/V2 border (Fig. 4*A*). Each stripe is approximately 1–3 mm thick and 1.5–3 cm long. The periodicity of the Cat-301-positive stripe is 4–8 mm, approximately double that described for the thick cytochrome oxidase (and Cat-301-positive) stripes in macaque. These results raise the possibility that the thick, Cat-301-positive stripes in human area V2 are homologous to the thick stripes in macaque.

DISCUSSION

The studies we describe here demonstrate several similarities between the properties of Cat-301-positive neurons in macaque and human. Two lines of evidence suggest that the human Cat-301 antigen is related, if not identical, to the antigen in monkey and cat: (*i*) the relatively unusual subcellular distribution of Cat-301 binding sites is the same in all species and (*ii*) the biochemical properties of the Cat-301 antigen in all species suggest that it is a chondroitin sulfate proteoglycan. Furthermore, the laminar and intralaminar organization of Cat-301-positive neurons in human visual cortex matches that previously demonstrated in macaque. These data suggest that the functional properties of Cat-301-positive neurons in human visual cortex may be related to those in macaque.

Many recent studies suggest that in primates, visual stimuli are processed in parallel channels, each channel carrying information related to different components of a visual stimulus. In somewhat simplified terms, the magnocellular channel preferentially carries information about dim, low-contrast, achromatic, and moving stimuli, while the parvocellular channel preferentially carries information about color and fine spatial detail. This separation of visual processing into two channels is maintained from the retina through a series of relays in the dorsal lateral geniculate nucleus and through several sequentially related areas in cerebral cortex (2, 3, 5).

Previous work has shown that in the macaque the distribution of Cat-301-positive neurons in areas V1 and V2 correlates with the distribution of neurons in the magnocellular pathway (7, 8, 27). In area V1, layers 4B, 4Ca, and 6 contain a high proportion of neurons with physiological and connectional properties characteristic of cells in the magnocellular pathway (16, 19–21). Similar analyses have shown that in area V2 the thick, dark cytochrome oxidase stripes represent part of the magnocellular pathway (10). The similarity in distribution of Cat-301-positive neurons in macaque and human provides evidence for a magnocellular pathway in human areas V1 and V2. Cat-301 recognizes subsets of
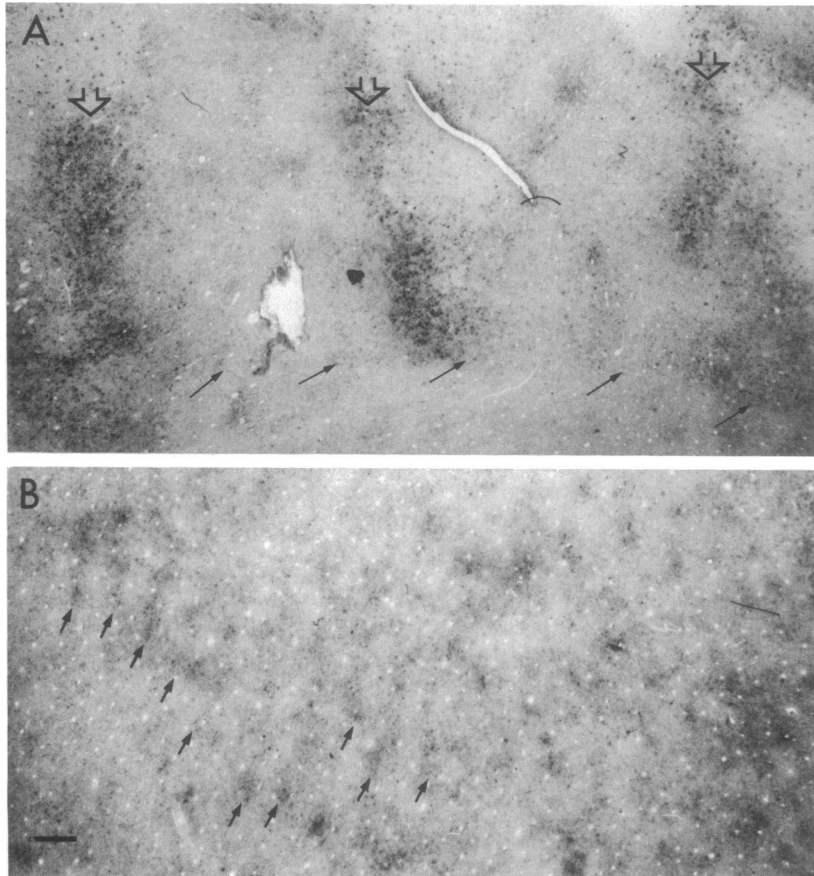
FIG. 4. Tangential sections show that the distribution of Cat-301-positive neurons within areas V1 and V2 demonstrate major features of the cellular organization of each area. (*A*) In area V2, thick bands of antibody-positive cells (large open arrows) run perpendicular to the border between V1 and V2 (small arrows). (*B*) In area V1, patches of antibody-positive neurons align to form rows (arrows) across the area. The periodicity of the rows is ≈1 mm. (Bar = 1 mm in *A* and *B*.)

neurons in areas of the CNS not related to processing visual stimuli (7, 9). While a detailed analysis of other areas of the CNS has not yet been carried out, the distribution of Cat-301-positive neurons in non-visual areas of human CNS also appears similar to that observed in macaque.

Previous studies have shown that the anatomical organization and some aspects of connectivity of human cortex are similar to those of macaque (14, 18). Since Cat-301-positive neurons demonstrate the same areal, laminar, and intralaminar distribution in human as in macaque, it is probable that they represent functionally homologous cell types. Furthermore, it is likely that their distribution in human visual cortex reveals components of the magnocellular channel. These data provide molecular evidence in support of the existence of parallel processing streams in the human visual system, as has been suggested by clinical observations and psychophysical experiments (22, 23).

Another intriguing feature of the Cat-301 antigen is the regulation of its expression by neuronal activity during the early postnatal period. It has been shown in two systems (24, 25) that deprivation of normal activity patterns early in development dramatically reduces Cat-301 immunoreactivity, whereas deprivation in adults does not. This regulation of Cat-301 by activity correlates with previously described "critical periods" in development. The influence of early activity on neuronal maturation has profound implications for the timing and choice of therapy for abnormalities present in human infants at birth (such as strabismus). Reagents like Cat-301 may help to define the parameters of critical periods in human development.

Our results suggest that probes for conserved molecular features between experimental animals and humans can permit the appreciation of a functional architecture of human cortex. Monoclonal antibody Cat-301 is currently one of the few molecular probes that demonstrate such dramatic features of neuronal organization as an ocular dominance-like perio-

dicity of area V1 and the thick stripes of area V2. Since Cat-301 also selectively identifies neurons in other cortical visual areas in the macaque (26, 27), it may also be possible to define additional cortical areas in human extrastriate visual cortex with Cat-301. The Cat-301-positive neurons represent only a small subset of cortical neurons and the identification of the antigen as an extracellular proteoglycan suggests that molecules of similar chemical nature might be expressed by Cat-301-negative neurons. The isolation of related molecules may lead to the demonstration of other functionally related subsystems within the human cerebral cortex.

1. Fox, P., Miezen, F. M., Allman, J. M., VanEssen, D. C. & Raichle, M. E. (1987) *J. Neurosci.* **7,** 913–922.
2. Maunsell, J. H. R. & Newsome, W. T. (1987) *Annu. Rev. Neurosci.* **10,** 363–401.
3. Mishkin, M., Ungerleider, L. G. & Macko, K. A. (1983) *Trends Neurosci.* **5,** 414–417.
4. VanEssen, D. & Maunsell, J. (1983) *Trends Neurosci.* **6,** 370–375.
5. Livingstone, M. & Hubel, D. H. (1988) *Science* **240,** 740–749.
6. Hockfield, S. & McKay, R. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 5758–5761.
7. Hockfield, S., McKay, R., Hendry, S. & Jones, E. G. (1983) *Cold Spring Harbor Symp. Quant. Biol.* **48,** 877–889.
8. DeYoe, E., Garren, H., Hockfield, S. & VanEssen, D. (1986) *Neurosci. Abstr.* **12,** 130.
9. Hendry, S., Jones, E. G., Hockfield, S. & McKay, R. (1988) *J. Neurosci.* **8,** 518–542.
10. Tootell, R., Silverman, M. S., DeValois, R. L. & Jacobs, G. H. (1983) *Science* **220,** 737–739.
11. Zaremba, S., Guimaraes, A., Kalb, R. G. & Hockfield, S. (1989) *Neuron* **2,** 1207–1219.

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12. Hubel, D. H. & Weisel, T. N. (1978) *Proc. R. Soc. London Ser. B* **198**, 1–59.
13. Hendry, S., Hockfield, S., Jones, E. G. & McKay, R. (1984) *Nature (London)* **307**, 267–269.
14. Horton, J. C. & Hedley-Whyte, E. T. (1984) *Philos. Trans. R. Soc. London Ser. B* **304**, 255–272.
15. Hitchcock, P. F. & Hickey, T. L. (1980) *Brain Res.* **182**, 176–179.
16. Livingstone, M. & Hubel, D. H. (1987) *J. Neurosci.* **7**, 3371–3377.
17. Hubel, D. H. & Livingstone, M. (1987) *J. Neurosci.* **7**, 3378–3415.
18. Burkhalter, A. & Bernardo, K. L. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 1071–1075.
19. Dow, B. (1974) *J. Neurophysiol.* **37**, 927–946.
20. Tootell, R., Hamilton, S. L. & Switkes, E. (1988) *J. Neurosci.* **8**, 1594–1609.
21. Lund, J. (1988) *Annu. Rev. Neurosci.* **11**, 253–288.
22. Damasio, A. (1985) in *Principles of Behavioral Neurology*, ed. Mesulam, M. (Davis, Philadelphia), pp. 259–288.
23. Livingstone, M. & Hubel, D. H. (1987) *J. Neurosci.* **7**, 3416–3468.
24. Kalb, R. & Hockfield, S. (1988) *J. Neurosci.* **8**, 2350–2360.
25. Sur, M., Frost, D. & Hockfield, S. (1988) *J. Neurosci.* **8**, 874–882.
26. McGuire, P., Hockfield, S. & Goldman-Rakic, P. (1989) *J. Comp. Neurol.* **288**, 280–296.
27. DeYoe, E., Hockfield, S., Garren, H. & VanEssen, D. (1990) *Visual Neurosci.*, in press.