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Cortical integration of parallel pathways in the visual system of primates

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Injections of wheatgerm agglutinin conjugated with horseradish peroxidase (WGA-HRP) were placed in the middle temporal visual area (MT) of squirrel monkeys to reveal the distributions of interconnections with functionally distinct modules in areas 17 and 18. In agreement with previous reports, brain sections cut parallel to the surface of manually flattened cortex and reacted for cytochrome oxidase (CO) revealed CO dense blobs in area 17 and alternating CO dense thick and thin bands separated by CO light interbands in area 18. Alternate sections stained for myelin indicated that the CO light interblobs and interbands are more densely myelinated than the CO dense blobs and bands. Our major finding is that projections from MT to areas 17 and 18 are both to modules projecting to MT and modules projecting to other targets. In area 17, the cells in the middle layers projecting to injection sites in MT typically were distributed in several short merging and diverging rows, suggesting the convergence of projections from several matched orientation columns in area 17 to the restricted injection site in MT. Backward projections from MT to more superficial layers in area 17 were distributed more evenly across cortex and over a wider area of cortex. These terminations were dense throughout the interblob cortex which includes all orientation columns and neurons projecting to area 18, but were light over the blobs. As previously reported, neurons in area 18 projecting to MT were located in one set of the CO dense bands. However, these bands appeared to be thin rather than thick. More superficial label reflecting projections from MT to area 18 was distributed over both thin bands projecting to MT and the thick bands projecting elsewhere. The results suggest that an important function of backward connections is to integrate information across diverging processing streams.

The visual system of primates includes subcortical stations and a number of cortical areas that are interconnected and process information in a semihierarchical fashion[6,13,17,18]. In addition, some information is processed in parallel, and two major streams of processing that originate in the retina, the magnocellular and parvocellular pathways, further differentiate in primary visual cortex into 3 distinct subsystems. Within these subsystems there are both forward connections which activate neurons in the next stations, and backward connections of less certain functions. Results from the present study both confirm the anatomical distinctiveness of the parallel stream that is relayed from subsets of neurons in area 17 (V-I) and area 18 (V-II) to the middle temporal visual area (MT), and provide anatomical evidence that an important function of the backward connections of MT is to integrate processing across major streams.

The methods followed those described in detail elsewhere[5]. Under aseptic conditions, single injections of 0.05–0.1 μl of 1% WGA-HRP were placed in MT of 6 squirrel monkeys (*Saimiri sciureus*) anesthetized with ketamine hydrochloride supplemented with acepromazine. Two to three days later, the squirrel monkeys received a lethal dose of sodium pentobarbital and were perfused with saline, followed by 1% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). Cortex was removed, manually flattened, stored overnight between glass plates in phosphate buffer with 30% sucrose, frozen, and cut parallel to the surface into 40 μm sections. Alternate sections were reacted with tetramethyl benzidine for HRP[19]. Remaining sections were reacted for cyto-

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chrome oxidase (CO) activity[10,27], or stained for myelinated fibers[8].

Both the sections reacted for CO and the sections stained for myelin revealed MT and modular subdivisions of areas 17 and 18. MT is known to be densely myelinated[2,18,20,26] and to have high levels of CO reactivity[5,23]. Thus, MT was easily identified, and in each case the injection site was completely confined to MT. The dense injection cores were approximately one-third or less the size of MT, and the pattern of transport indicated that the effective uptake zones were considerably smaller. Sections through the superficial layers of cortex also revealed the regularly spaced ovals of dense CO activity in area 17 previously described as patches or blobs[10,15,23,27]. The Gallyas silver stain for myelin[8] produced a negative image of these CO activity patterns (Fig. 1B, 1C). Thus, the regions of the CO blobs in the superficial layers of area 17 stained less densely for the myelin than the interblob regions. In middle layers of area 18, CO dense bands separated by light interbands were apparent (Figs. 1A, 3C). Close inspection allowed the thin bands (arrows, Fig. 1A) to be distinguished from the alternating thick bands, although a

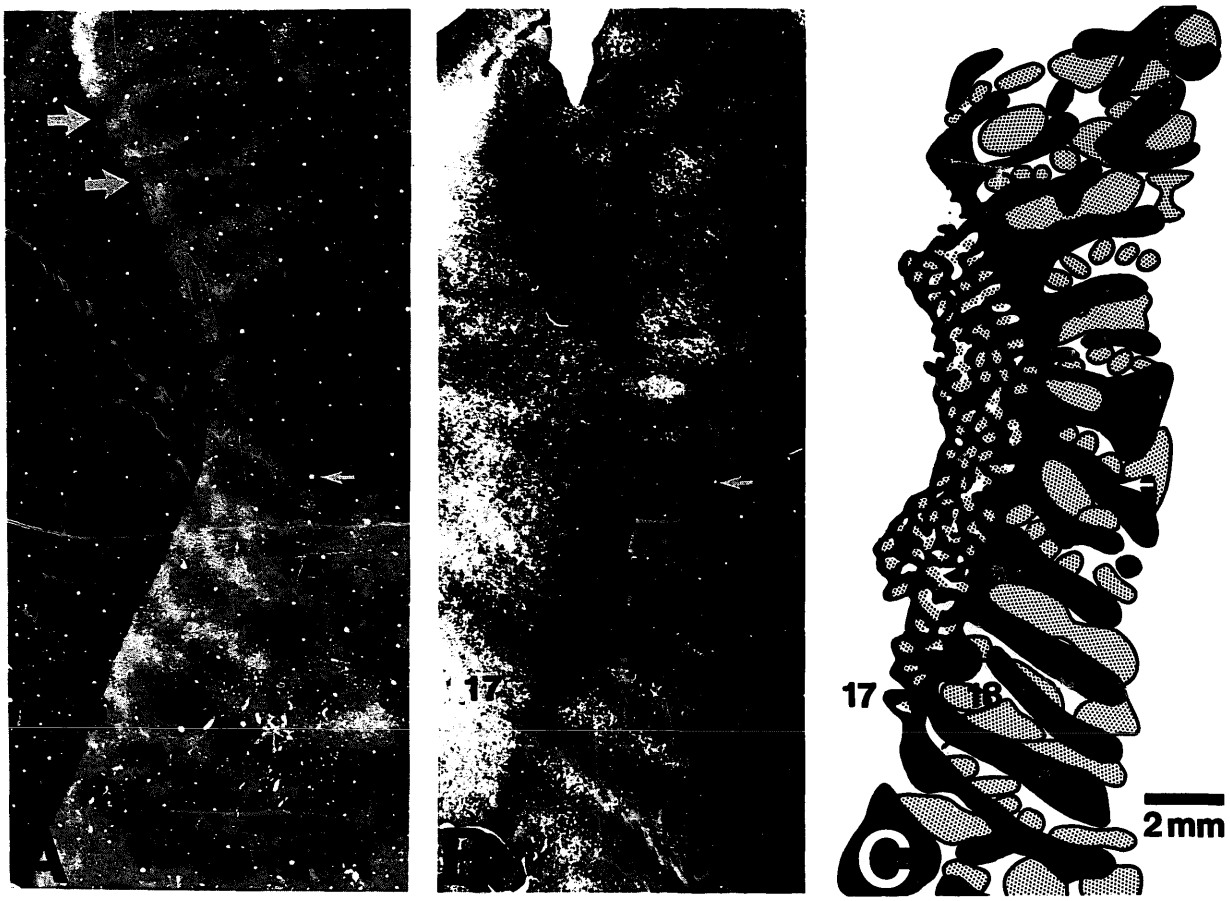

Fig. 1. Patterns of cytochrome oxidase (CO) and myelin dense regions in areas 17 and 18 of squirrel monkeys in brain sections cut parallel to the surface of manually flattened cortex. A: a section reacted for CO. Note the sharp boundary between areas 17 and 18, and the CO dense bands in area 18. Upper arrows mark the caudal borders of two thin bands that are separated by a thick band. Other thick and thin bands are also apparent. Although thin distinction is sometimes difficult to make, the overall pattern suggests that thick and thin bands of dense CO activity alternate. In area 17, the section is too deep to fully reveal the CO dense blobs, but blobs are faintly apparent in some portions of the section. B: an adjacent brain section stained for myelin. Note the alternation of myelin dense and myelin light bands in area 18, and the dense myelination surrounding myelin-light ovals in area 17. C: a schematic superimposition of the CO (shaded) and myelin (black) patterns showing that they are basically negative images of each other. The comparison indicates that the CO blobs and bands are lightly stained for myelin, while the interblob regions and the interbands are densely myelinated. An arrow in area 18 in A, B and C marks one of the blood vessels that were used to align the adjacent brain sections.

difference in appearance was not always obvious. The interbands in area 18 were densely myelinated to form a negative image of the CO bands (Fig. 1C). Previously, Tootell et al.[24] noted that myelin dense bands could be seen in area 18 in unstained, freshly cut sections, and concluded from sections stained with Luxol fast blue that the CO bands in area 18 and blobs in area 17 are more densely myelinated. This difference in results suggests that Luxol fast blue and the Gallyas silver stain can darken different components of areas 17 and 18.

Each injection produced label in restricted portions of areas 17 and 18 that varied in location in correspondence with the retinotopic organizations of area 17, area 18, and MT in primates (for review see ref. 25). Thus, injections in more rostral portions of MT representing more peripheral vision labelled more caudal portions of area 17 and medial and lateral parts of area 18 also representing more peripheral vision. Injections centered in MT produced zones of label in area 17 that were well displaced from the 17/18 border, while injections nearer the outer border of MT resulted in label in area 17 that was closer to the 17/18 border.

Injections in MT produced zones of label in area 17 that were quite different in distribution in the middle and superficial layers (Fig. 2). In the middle layers of cortex (layer 3C of Hassler[9]; layer 4B of Brodmann[4]), neurons and fine processes were labelled in a pattern that was discontinuous and often included 3–5 merging and diverging rows of cells within a 2–3 mm zone of cortex (Fig. 2B). While the significance of this pattern is uncertain, one possibility is that small injections in MT can be restricted to a region with inputs from several nearby orientation columns of similar selectivity in area 17. Thus, the bands of label in area 17 could reflect the spacing and shapes of orientation columns in area 17. In macaque monkeys, orientation columns of the same selectivity, merge and diverge, but have an average separation distance of about 600 μm[3,12]. MT has an orderly arrangement of columns of cells selective for direction of movement[1], and a given column would be expected to have converging inputs from several matched orientation columns in area 17 (see ref. 13).

More superficially in area 17, injections in MT produced a more even pattern of anterograde label that included fine processes but not cell bodies. This label

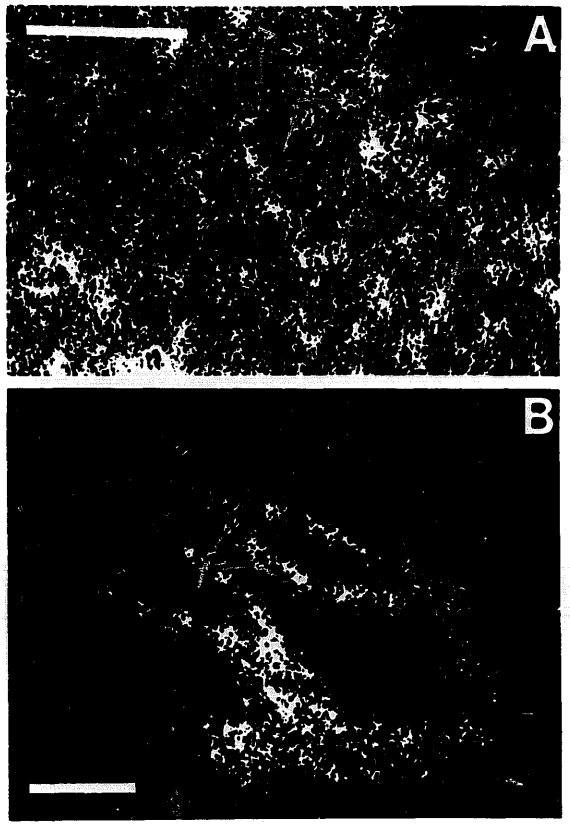

Fig. 2. Superficial (A) and deep (B) label in area 17 from a case with a restricted injection of WGA-HRP in MT. Careful superimpositions with alternate sections reacted for cytochrome oxidase (CO) revealed that the widespread superficial label (A) was dense over the interblob cortex, but light over the CO blobs. The deeper label (B), which included neurons projecting to MT in layer 3C, was restricted to several merging and diverging rows or bands that are reminiscent of the orientation columns. Marker bar = 1 mm.

was dense in the interblob regions and sparse over the blobs throughout the labelled zone (Fig. 2A). Thus, the backward projections[18] from restricted locations in MT to area 17 were distributed across orientation columns of all selectivities, but there was a tendency to avoid the blobs which contain neurons that are not orientation selective[15].

The pattern of label in area 18 resulting from an MT injection is shown in Fig. 3. In both superficial sections from the first 200 μm of cortex (Fig. 3A) and deeper sections from the middle layers of cortex (Fig. 3B), the label was almost completely confined to clumps aligned in rows that were superimposed on the CO dense bands. As previously reported[6,22], the

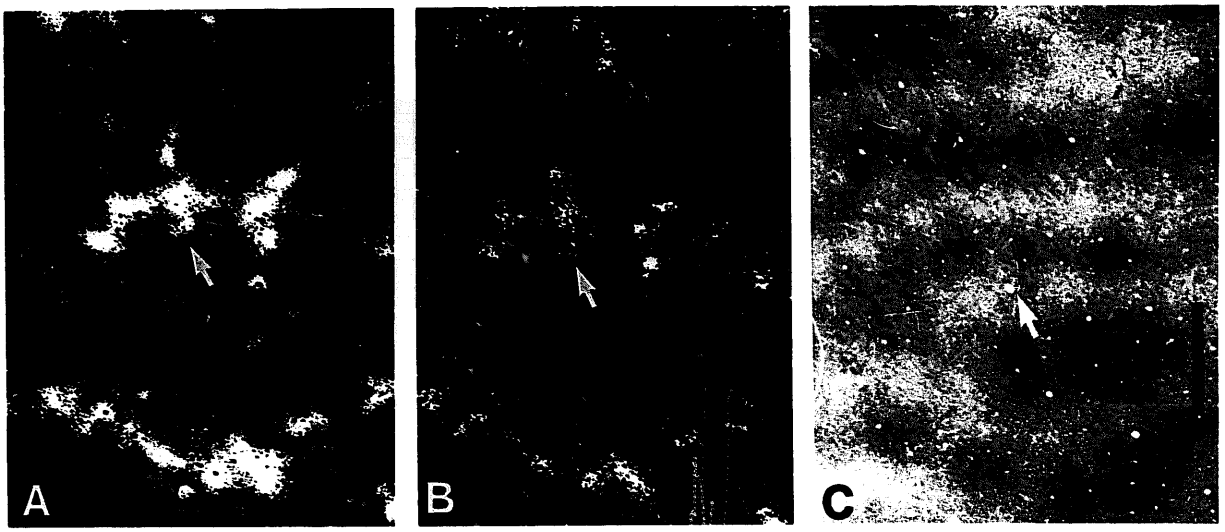

Fig. 3. The relationship of superficial (A) and deep (B) label to CO dense bands (C) in area 18 after an injection of WGA-HRP in MT. The superficial label, reflecting terminations from MT, was in both the thin and thick sets of CO dense bands, while the deeper label, reflecting output neurons to MT, was in only the thin CO bands. Arrows in A, B and C mark one of the blood vessels used to align the three sections. Marker bar = 1 mm.

deeper label, including retrogradely labelled cells, was restricted to every other CO dense band within the region of transport. Similar results were obtained in the 5 other squirrel monkeys. That these labelled neurons were within the CO thin bands, was an unexpected finding since the thick bands project to MT in macaque monkeys[6,22], and the thin bands project to V4 (DL)[6,22]. We have recently extended these studies (unpublished observations) and find that the bands that project to MT in owl monkeys are thin, while the bands that project to MT in marmosets are about the same width as those projecting to DL. Interestingly, Livingstone and Hubel[15] note that the physiological properties of neurons associated with thick and thin bands are the reverse in some macaque monkeys of those in other macaque monkeys. Thus, in some macaque monkeys, thin rather than thick bands have neurons with characteristics that appear compatible with a projection to MT. Although a consistent finding across all monkeys studied is that every other CO dense band projects to MT, width may not be a consistent distinguishing feature of the bands projecting to MT across and perhaps even within species.

In more superficial sections, the label in area 18 was found in fine processes rather than cell bodies. The densest superficial label was located in the bands that project to MT, but sparser superficial label was in the bands that presumably project to DL (Fig. 3A). If the superficial label is the result of backward connections from MT, as evidenced by the lack of labelled neurons and the location of label in supragranular layers known to receive input from MT[18,20,25], then feedback from MT relates to neurons in two distinct processing streams in area 18, the thin and the thick bands.

A remaining question is whether the outputs from both areas 17 and 18 to MT reflect the information that is relayed through the magnocellular portion of the lateral geniculate nucleus as seems to be the case for macaque monkeys[7,13,17]. In squirrel monkeys, Livingstone and Hubel[16] report that neurons in layer 4B (III C of Hassler[9]) of area 17, a layer devoted to the magnocellular pathway, project to the thick CO stripes of area 18, while present results indicate that thin stripes project to MT.

While MT appears to receive inputs largely from one channel in area 17 and one channel in area 18, the backward projections of MT appear to relate to all three of the major processing streams emanating from these fields. In area 17, the widespread feedback to interblob regions could modulate outputs from both the layer 3C neurons projecting to MT and more superficial neurons projecting to interbands in

area 18 that relay to DL[6]. Backward projections from MT to the two sets of CO bands in area 18 could modulate outputs to both MT and to DL. Thus, the backward connections of MT have the potential of mediating interactions between processing streams that may be critical in creating a unified perception of the world.

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1 Albright, T.D., Desimone, R. and Gross, C.G., Columnar organization of directionally selective cells in visual area MT of the macaque, *J. Neurophysiol.*, 51 (1984) 16–31.
2 Allman, J.M. and Kaas, J.H., A representation of the visual field in the caudal third of the middle temporal gyrus of the owl monkey (*Aotus trivirgatus*), *Brain Research*, 31 (1971) 85–105.
3 Blasdel, G.G. and Salama, G., Voltage-sensitive dyes reveal a modular organization in monkey striate cortex, *Nature (Lond.)*, 32 (1986) 579–585.
4 Brodmann, K., *Vergleichende Lokalisationslehre der Grosshirnrinde*, Barth, Leipzig, 1909.
5 Cusick, C.G. and Kaas, J.H., Cortical connections of area 18 and dorsolateral visual cortex in squirrel monkeys, *Visual Neurosci.*, 1 (1988) 211–237.
6 DeYoe, E.A. and Van Essen, D.C., Segregation of efferent connections and receptive field properties in visual area V2 of the macaque, *Nature (Lond.)*, 317 (1985) 58–61.
7 DeYoe, E.A. and Van Essen, D.C., Concurrent processing streams in monkey visual cortex, *Trends Neurosci.*, 11 (1988) 219–226.
8 Gallyas, F., Silver staining of myelin by means of physical development, *Neurol. Res.*, 1 (1979) 203–209.
9 Hassler, R., Comparative anatomy of the central visual systems in day and night-active primates. In R. Hassler and H. Stephan (Eds.), *Evolution of the Forebrain*, Thieme, Stuttgart, 1966, pp. 419–434.
10 Horton, J.C., Cytochrome oxidase patches: a new cytoarchitectonic feature of monkey visual cortex, *Philos. Trans. R. Soc. Lond. (Ser. Biol.)*, 304 (1984) 199–253.
11 Hubel, D.H. and Livingstone, M.S., Segregation of form, color, and stereopsis in primate area 18, *J. Neurosci.*, 7 (1987) 3378–3415.
12 Hubel, D.H., Wiesel, T.N. and Stryker, M.P., Anatomical demonstration of orientation columns in macaque monkey, *J. Comp. Neurol.*, 177 (1978) 361–380.
13 Kaas, J.H., The structural basis for information processing in the primate visual system. In J.P. Pettigrew, W.R. Levick and K.J. Sanderson (Eds.), *Visual Neuroscience*, Cambridge University Press, 1986, pp. 315–340.
14 Krubitzer, L.A. and Kaas, J.H., Connections of modular subdivisions of cortical visual areas 17 and 18 with the middle temporal area, MT, in squirrel monkeys, *Soc. Neurosci. Abstr.*, 13 (1987) 3.
15 Livingstone, M.S. and Hubel, D.H., Anatomy and physiology of a color system in the primate visual cortex, *J. Neurosci.*, 4 (1984) 309–356.
16 Livingstone, M.S. and Hubel, D.H., Connections between layer 4B of area 17 and the thick cytochrome oxidase stripes of area 18 in the squirrel monkeys, *J. Neurosci.*, 7 (1987) 3371–3377.
17 Livingstone, M.S. and Hubel, D.H., Segregation of form, color, movement and depth: anatomy, physiology and perception, *Science*, 240 (1988) 740–749.
18 Maunsell, J.H.R. and Van Essen, D.C., The connections of the middle temporal visual area (MT) and their relationship to a cortical hierarchy in the macaque monkey, *J. Neurosci.*, 12 (1983) 2563–2586.
19 Mesulam, M.-M., Tetramethylbenzidine for horseradish peroxidase neurohistochemistry: a non-carcinogenic blue reaction-product with superior sensitivity for visualizing neural afferents and efferents, *J. Histochem. Cytochem.*, 26 (1978) 106–117.
20 Spatz, W.B. and Tigges, J., Experimental-anatomical studies in the 'middle temporal visual area (MT)' in primates. I. Efferent cortico-cortical connections in the marmoset *Callithrix jacchus.*, *J. Comp. Neurol.*, 146 (1972) 451–464.
21 Schiller, P.H. and Malpeli, J.G., The effect of striate cortex cooling on area 18 cells in the monkey, *Brain Research*, 126 (1977) 366–369.
22 Shipp, S. and Zeki, S., Segregation of pathways leading from area V2 to areas V4 and V5 of macaque monkey visual cortex, *Nature (Lond.)*, 315 (1985) 322–325.
23 Tootell, R.B.H., Hamilton, S.L. and Silverman, M.S., Topography of cytochrome oxidase activity in owl monkey cortex, *J. Neurosci.*, 5 (1985) 2786–2800.
24 Tootell, R.B.H., Silverman, M.S., DeValois, R.L. and Jacobs, G.H., Functional organization of the second cortical visual area in primates, *Science*, 220 (1983) 737–739.
25 Van Essen, P.C., Functional organization of primate visual cortex. In A. Peters and E. Jones (Eds.), *Cerebral Cortex, Vol. 3, Visual Cortex*, Plenum, New York, 1985, pp. 259–329.
26 Weller, R.E., Wall, J.T. and Kaas, J.H., Cortical connections of the middle temporal visual area (MT) and the superior temporal cortex in owl monkeys, *J. Comp. Neurol.*, 228 (1984) 81–104.
27 Wong-Riley, M.T.T. and Carroll, E.W., Quantitative light and electron microscopic analysis of cytochrome oxidase-rich zones in V-II prestriate cortex of the squirrel monkey, *J. Comp. Neurol.*, 222 (1984) 18–37.