Evidence From V1 Connections for Both Dorsal and Ventral Subdivisions of V3 in Three Species of New World Monkeys

DAVID C. LYON AND JON H. KAAS*
Department of Psychology, Vanderbilt University, Nashville, Tennessee 37203

ABSTRACT
We used patterns of connections of primary visual cortex (V1) to reevaluate differing proposals on the organization of extrastriate cortex in three species of New World monkeys. Several fluorescent tracers and the bidirectional tracer cholera toxin B subunit (CTB) were injected into dorsal V1 (representing the lower visual quadrant) and ventral V1 (representing the upper visual quadrant) of titi, squirrel, and owl monkeys. Labeled cells and terminals were plotted on brain sections cut parallel to the surface of flattened cortex and were related to architectonic boundaries. The results provided compelling evidence for both dorsal V3 with dorsal V1 connections and ventral V3 with ventral V1 connections. The connection pattern indicated that V3 represents the visual hemifield as a mirror image of V2. In addition, V3 could be recognized by a weak banding pattern in brain sections processed for cytochrome oxidase. V1 has connections with at least 12 subdivisions of visual cortex, with half of the connections involving V2 and 20% V3. Comparable results were obtained from all three species, suggesting that visual cortex is similarly organized. J. Comp. Neurol. 449:281–297, 2002.

Indexing terms: extrastriate cortex; neurons; primates; visual cortex

Although it is obvious after 30 years of research that the visual cortex of monkeys is subdivided into a considerable number of visual areas, the exact arrangement of areas remains uncertain. Only three areas, the first (V1) and second (V2) visual areas and the middle temporal (MT) visual area, seem to be universally accepted as valid and not subject to contention (see Kaas and Lyon, 2001). Other proposed areas vary according to research groups and species studied. In the present study, we injected tracers into primary visual cortex of three species of New World monkeys in order to use connection patterns to evaluate differing theories of extrastriate cortex organization. Our use of three species is part of an effort to address the possibility that different types of extrastriate organization exist in different primates. Our efforts were especially focused on the issue of whether these primates have the proposed third visual area, V3.

The concept of V3 as a subdivision of mammalian visual cortex stems from the early evidence in cats for a third visual area bordering V2 and mirroring V2 in retinotopic organization (Hubel and Wiesel, 1965). This concept seemed highly congruent with the proposal of Brodmann (1909) that V1 (area 17 or striate cortex) is surrounded by two ring-like bordering areas, areas 18 and 19. In cats, V2 and V3 corresponded nicely to Brodmann’s divisions (described from other carnivores), and it was natural to look for a comparable organization in primates. Both Cragg (1969) and Zeki (1969) soon provided evidence for V2 and V3 in macaque monkeys after lesions of dorsolateral striate cortex revealed projections to two locations in dorsal cortex (V2 and V3), as well as the region now known as MT or V5. Surprisingly, both V2 and V3 were well within the region Brodmann had included in area 18 of macaque monkeys.

The initial interpretation of this connection pattern in monkeys was challenged in two ways. First, microelectrode-mapping studies of visual cortex in New World owl monkeys indicated that much of the V3 region could be interpreted as part of a complete hemifield representation in a proposed dorsomedial visual area, DM (Allman and Kaas, 1975). This alternative interpretation was reinforced by...
studies of connections in New World squirrel monkeys that failed to reveal projections from V1 to the region of V3 (Spatz et al., 1970; Spatz and Tigges, 1972) and the lack of evidence for a V3 in other mammals, such as rats (see, e.g., Olavarria and Montero, 1984; Malach, 1989), squirrels (Kaas et al., 1989), and tree shrews (Sesma et al., 1984; Lyon et al., 1998). When early evidence for projections from dorsal V1 to cortex just rostral to dorsal V2 was obtained (Lin et al., 1982), this projection was judged to be in DM, an interpretation that was strengthened when both ventral and dorsal parts of V1 were found to have connections with the DM region just rostral to dorsal V2 (Krubitzer and Kaas, 1993).

Another problem for the hypothesis that primates have a V3 arose when investigators failed to find convincing evidence of connections of ventral V1 with the ventral V3 region of macaque monkeys (Weller and Kaas, 1983; Van Essen et al., 1986). Differences in the architecture and neuron response properties were noted as well (Burkhalter and Van Essen, 1986; Burkhalter et al., 1986; Newsome et al., 1986; Felleman and Van Essen, 1987). As a result, ventral V3 was redefined as another visual area, the ventral posterior area, VP (Newsome et al., 1986; Allman et al., 1980). This left macaque monkeys with two seemingly improbable visual areas, a dorsal V3 representing only the lower visual quadrant and a ventral VP representing only the upper visual quadrant. Other investigators continued to support the concept of a V3, although in modified forms.Gattass et al. (1988) used microelectrodes to explore the region in macaque monkeys and described a smaller V3 that was split into separate halves, where V4 came in contact with central V2. A smaller but unbroken V3 was later described from New World cebus monkeys (Sousa et al., 1991; Rosa et al., 2000).

Given this background, it seemed unclear whether a traditional or modified V3 existed and whether primate species varied in this regard. We started to reconsider this issue by studying V1 connections in New World marmoset monkeys (Lyon and Kaas, 2001). Marmosets have small brains with few fissures, so it was relatively easy to inject tracers into both dorsal and ventral parts of V1 and later flatten cortex for surface views of the distributions of transported label. The dense core of injected tracer included both supragranular and infragranular layers in all instances. One of the injections might have included the white matter and more of the underlying striate cortex (see discussion of Fig. 6B below). Uptake of the tracer by white matter appears unlikely. Pressure injections of 0.2–0.5 μl were made at about 30° from perpendicular of the medial wall using a stereotaxic syringe holder and 1.0 μl Hamilton syringes tipped with sealed glass micropipettes. Four other injections were placed on the lower bank were considered to be in the upper quadrant of VI, whereas injections along the upper bank were considered to be in the lower quadrant, based on retinotopic maps of squirrel monkeys (Cowey and Ellis, 1969) and owl monkeys (Allman and Kaas, 1971). Injections were placed by depth into the middle layer of cortex. The dense core of injected tracer included both supragranular and infragranular layers in all instances. One of the injections might have included the white matter and more of the underlying striate cortex (see discussion of Fig. 6B below). Uptake of the tracer by white matter appears unlikely. Pressure injections of 0.2–0.5 μl were made at about 30° from perpendicular of the medial wall using a stereotaxic syringe holder and 1.0 μl Hamilton syringes tipped with sealed glass micropipettes. Four other injections were placed on the dorsolateral surface of cortex, bringing the total to 17 injections. The four pressure injections were placed perpendicular to the cortical surface and were in either the lower (dorsal) or the central (lateral) visual representations of V1 (see Table 1, Fig. 1). Any leakage of tracer onto the surface from the injection site was removed with a swab during and after the injection to prevent any artificial spread.

Tracers used in this study included 0.2–0.5 μl of the retrogradely transported fluorochromes diamidino yellow (DY; 2%; Keizer et al., 1983), fast blue (FB; 3%; Kuypers et al., 1980), fluororuby (FR; 10%; Schmued et al., 1990), fluoroemerald (FE; 10%; Novikova et al., 1997), and the bidirectional tracer cholera toxin subunit B (CTB; 5%; Ericson and Blomqvist, 1988). Retrogradely labeled neurons and anterogradely labeled terminals from the same injection are known to overlap extensively, but the distributions of labeled cells can be somewhat broader (see Discussion). After injections, the dura was pulled over the exposed cortex and covered with a soft disposable contact lens. The opening of the skull was sealed with a thin cap of dental acrylic, the scalp was sutured, and the closed incision was treated with antibiotic ointment.
tic injections of penicillin (60,000 U/kg) and an analgesic, banamine (1 mg/kg), were given postoperatively. The monkeys were carefully monitored during recovery from anesthesia.

Histology and data analysis
After a postsurgical period of 9 days for the titi and owl monkeys and 12 days for the larger squirrel monkeys, each animal was anesthetized with ketamine hydrochloride (30 mg/kg) and xylazine (5 mg/kg) and then given a lethal dose of sodium pentobarbital (50 mg/kg). When areflexive, the monkeys were perfused transcardially with 0.9% phosphate-buffered saline (PBS; pH 7.4), followed by a solution of 2% paraformaldehyde fixative and subsequently 2% paraformaldehyde with 10% sucrose in PBS.

After removal of the brain, the cortical hemispheres were carefully separated from underlying structures, and the cortex was flattened as described elsewhere (Krubitzer and Kaas, 1990b, 1993). The flattened hemispheres were submerged for 12–36 hours in 30% sucrose in PBS while under a glass slide. Additional weight was added on top of the glass slide to help flatten the opened sulci. Next, the flattened hemispheres were frozen in 30% sucrose and cut parallel to the surface in 40 μm sections. A series of every fourth section was mounted on glass slides to observe fluorescent tracers. A second series was immunocytochemically processed to visualize the transported label of CTB (Bruce and Grolova, 1992; Angelucci et al., 1996; Sakai et al., 2000). A third series was processed for cytochrome oxidase (CO; Wong-Riley, 1979) and a fourth for myelin (Gallyas, 1979).

The locations and numbers of retrogradely labeled CTB and fluorescent cells and the locations of anterogradely labeled CTB terminals were digitized with an XY encoder attached to the stage of a fluorescent and brightfield microscope and plotted with the aid of Igor Pro (WaveMetrics, Inc.). The data were exported as EPS files from Igor Pro into Canvas 7 (Deneba) and printed. Architectonic borders derived from CO and myelin stains of adjacent sections were drawn on printed copies of each cortical section with plotted cells, scanned into Canvas 7, and redrawn digitally. Canvas 7 was used to align the adjacent cortical sections by using injection sites, blood vessel patterns, and section contours as landmarks. The percentage of labeled cells in each cortical area was derived by counting the number of labeled cells within each area and dividing by the total number of labeled cells in extrastriate cortex.

The images in Figures 2D and 3E were captured using a digital camera (Scion Corp.) and NIH Image software and adjusted for brightness and contrast within Canvas 7. Other images (in Figs. 2–5) were captured using a Spot 2 camera mounted on a Nikon E800 microscope and acquired through Adobe Photoshop 6.0 and adjusted for brightness and contrast. None of the images was altered in any other way.

RESULTS
The connections resulting from V1 injections in three species of New World monkeys demonstrate the existence of a retinotopically organized V3 strip of cortex along the outer border of V2. This V3 includes both dorsal and ventral regions (V3d and V3v; Fig. 1), representing lower and upper visual quadrants, respectively, as the mirror images of the retinotopy of dorsal and ventral V2. Our results also provide evidence for DM rostral to V3d and DL (V4) rostral to the lower portion of V3d, central V3, and V3v. Furthermore, the V1 connection patterns provide evidence for the existence of visual areas DL, MT, MTe, MST, and FST; two areas in posterior parietal cortex (VPP and LPP); and at least three subdivisions of IT cortex (ITc, ITm, and ITr). To illustrate the differences in the magnitudes of connections of extrastriate cortical areas with V1, we calculated the percentage of the total number of labeled cells within each area (Table 1).

Results are presented in four sections, beginning with CO architecture of the visual cortex to establish the relative locations of areas V1, V2, V3, and MT. In the remaining three sections, we show the tracer connection patterns of dorsal and ventral V1 injections in each of the three species examined. The first species described, the titi monkey, has not been previously studied for visual cortex organization. We show here that this species possesses a V3 (an area of contention in more commonly studied primates) as well as other more established primate visual areas. The second and third species presented, squirrel and owl monkeys, have been studied extensively for their visual cortex organization, and the evidence for the existence of many of the visual areas listed above has already been demonstrated. However, we present evidence for a new scheme of visual cortex organization for these monkeys that includes a previously omitted V3.

Architectonic identification of visual areas
We used tangential sections of flattened cortex that were processed for CO or myelin to identify borders of visual areas in the experimental monkeys. Such borders have been described before in similar preparations from owl and squirrel monkeys (Tootell et al., 1985; Krubitzer and Kaas, 1990a, 1993) but not titi monkeys.

In all three species, the borders of V1, V2, and MT were consistently identified in both CO-and myelin-stained sections. In CO-processed sections, V1 was obvious in sections through the superficial layers of cortex by the dot-like pattern of CO-dense blobs (Figs. 2–4). Sections through the middle layers of cortex were dense in CO and in myelin. V2 was characterized by a series of CO-dense bands that alternated with CO-light bands crossing the width of the area. At higher magnification, the CO bands appear as rows of CO-dense patches, as previously described (e.g., Wong-Riley and Carroll, 1984; Cusick and Kaas, 1988b). In myelin preparations, the bands in V2 were less evident but were sometimes apparent. At the widest V2 margins, nearer the dorsal and caudal poles, V2 extended 4–5 mm for titi and owl monkeys and as wide as 6–8 mm in squirrel monkeys. V2 was narrowest near the horizontal meridian and foveal representation, located adjacent to the V1 bulge on dorsolateral cortex (see Fig. 1). MT was most obvious in myelin-stained sections, where it appeared as a myelin-dense oval (Fig. 2C). In CO-stained sections, MT also appeared as a dense oval, but a substructure of CO-dense and CO-light patches was evident (Fig. 4). A belt of CO-dense patches surrounding much of MT identified the MT crescent (MTe; Kaas and Morel, 1993).

The two subdivisions of V3 (V3d and V3v) could be delimited in cortex along the rostral border of V2. Both V3d and V3v were characterized by CO-dense bands that were less obvious and less regular than those in V2 (Figs.
2–4). The V3 bands were also broader than those in V2. As with the V2 bands, those in V3 were rows composed of CO-dense patches (Figs. 2–4). To our knowledge, the bands in the V3 region have not been described before, except in our study of V3 in marmoset monkeys (Lyon and Kaas, 2001). However, remnants of these bands are evident in previous published photomicrographs of flattened visual cortex (see Fig. 2A of Tootell, et al., 1985; Fig. 2 of Cusick and Kaas, 1988b; Fig. 4A of Krubitzer and Kaas, 1990b). Because the V3 bands have been less obvious, and they differ in spacing and width from those in V2, the V3 region does not appear to be an extension of V2, and it was not included in V2 in previous studies in which V2 was defined by its banding pattern. Thus, our present architectonic definition of V2 is highly consistent with previous reports. The width of V3 was roughly two-thirds to one-half the width of V2 in the respective species. In owl monkeys, V3 appeared to become so narrow at the central representation (near the V1 bulge and the narrowest point of V2) that it may have separated into two halves.

Cortex in the DM region was darker in CO- and myelin-stained sections, but the banding pattern of V3d did not extend into DM (Figs. 2–4). Borders of DM were less obvious than those of V1, V2, V3, or MT. Reliable identification of DM was difficult because of the uneven flattening caused by the intraparietal sulcus (see Figs. 2D, 3E). To locate DM, myelin stains of multiple sections were required (not shown). Even so, the complete borders of DM were not consistently identified with confidence. However, information gained from the V1 connection patterns indicated the approximate extent of the area (see the following section). In previous descriptions, much of V3d appears to have been included in DM, because both regions are darker in CO and myelin preparations than the adjacent DL region between MT and V2.

Of the remaining visual areas described in this study, only DL (Allman and Kaas, 1974) was somewhat visible in the squirrel monkey CO pattern (Fig. 3A,B). Because of the lack of architectonic evidence, the regions of DL and areas DI, VPP, LPP, ITc, ITm, and ITr were based on V1 connection patterns (see below) in comparison with those described in other studies of New World monkeys (Weller and Kaas, 1983, 1985, 1987; Cusick and Kaas, 1988b, 1990b; Krubitzer and Kaas, 1990b, 1993; Beck and Kaas, 1998; Lyon and Kaas, 2001).

Cortical connections revealed by V1 injections

Titi monkeys. Three injections of tracers were placed in ventral V1 and a fourth in dorsal V1 of a single titi monkey (case 00–50; see Table 1). The major ipsilateral cortical connections of V1 were obvious from a CTB injection in ventral V1 even at low magnification (Fig. 5A). Around the edge of the densely labeled core of injected CTB, small clusters of labeled neurons and axon terminals

---

### Table 1. Percentages of Labeled Cells in Extrastriate Cortex in Identified Visual Areas for Each Injection in V1

| Sp.  | Case No. | V1 inj site | Tracer | Fig No. | No. of cells | V2 | V3 | MT | DL | IT | DM | DI | MTC | MST | FST | LPP | VPP |
|------|----------|-------------|-------|---------|-------------|----|----|----|----|----|----|----|-----|-----|-----|-----|-----|-----|-----|-----|
| Tit  | 00–50    | Ventral    | CTB   | 5       | 14,287     | 38 | 16 | 16 | 16 | 10 | 5  | 1  | 1  | 2  | <1 | <1 | |
| Tit avg % |         |             |       |         |             |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
| Sq   | 00–03    | Ventral    | CTB   | 7       | 13,176     | 58 | 16 | 16 | 16 | 5  | 7  | 1  | 2  | 1  | 1  | 1  | 1  | 1  | 1  |
| Sq avg % |         |             |       |         |             |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
| Owl  | 00–69    | Ventral    | CTB   | 8       | 6,066      | 36 | 28 | 14 | 14 | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 1  | 1  |
| Owl avg % |         |             |       |         |             |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |

---

**Fig. 1.** Summary of our current proposals of 16 visual cortex subdivisions shown on the caudal half of flattened cortex in three species of New World monkeys, titi monkey (A), squirrel monkey (B), and owl monkey (C). Better established areas are outlined; others are indicated. Outlined areas include primary visual cortex (V1), the second visual area (V2), a redefined third visual area (V3; V3d and V3v), the dorsomedial area (DM), the middle temporal area (MT), the medial temporal crescent (MTC), the medial superior temporal area (MST), and the dorsal and ventral divisions of the fundal area of the superior temporal sulcus (FSTd and FSTv). The borders for the rostral and caudal (DLr and DLc) divisions of the dorsolateral area (DL) are defined primarily by connection patterns and not architecture, so dashed lines are used. Representations of the upper (+) and lower (−) visual quadrants are indicated for some of the visual areas. Also, for some visual areas the zero horizontal (small open circles) and the zero vertical (small squares) meridians are marked. Solid circles represent estimated location of foveal vision in V1, V2, and MT. The locations of the dorsointermediate area (DI); caudal, medial, and divisions of inferior temporal cortex (ITc, ITm, and ITr); and posterior parietal (PP) visual regions ventral (VPP) and lateral parietal (LPP) are also indicated. Shading indicates cortex on the medial wall and ventral surface that was exposed by unfolding. Large dashed lines indicate the outline of the dorsolateral cortical surface. Small dashed lines indicate cortex of the opened intraparietal sulcus (IPS), lateral sulcus (LS), and superior temporal sulcus (STS). D: View of the medial and ventral surface of marmoset cortex prior to unfolding. The peripheral border of V1 is indicated, with the lower quadrant (−) of V1 on the upper bank of the calcarine sulcus and the upper quadrant (+) located on the calcarine's lower bank. CC, corpus callosum. Scale bars = 4 mm.
were visible, demonstrating a somewhat patchy distribution of local intrinsic connections within 1–2 mm of the injection core. A dense focus of labeled fibers was also apparent in ventral V2, indicating, as expected, that ventral V1 connects with ventral V2. As the injection core was centered in ventral V1 away from the representations of the horizontal and ventral meridians, the label in V2 was centered in the middle of the width of V2. Another dense patch of labeled cells and terminals was just rostral to the labeled portion of V2 in the expected location of ventral V3. The patch was clearly separate from the one in V2, providing strong evidence for the existence of a V3v in titi monkeys. Painter patches of label were visible at low magnification in MT and DM. The label in MT was ventral where the upper visual quadrant is represented, and rostralateral in DM, providing evidence that the upper visual quadrant is represented in this location.

Inspection of the same brain sections under higher magnification confirmed these locations of labeled neurons and terminals and revealed additional sites of label. The locations of labeled cells were plotted (Fig. 5B), and distributions of such neurons were apparent in ventral DLc and DLr and the regions of ITm, ITc, ITr, FST, MST, MTc, DI, VPP, and LPP. Scattered foci of labeled neurons in dorsal MT, MTc, and V3d were in inappropriate retinotopic locations. Comparable results were obtained from closely spaced injections of DY (green) and FR (red) in ventral V1 (Fig. 6A). Retrogradely labeled neurons were densely scattered around the injection cores in V1, demonstrating short intrinsic connections, whereas major patches of labeled neurons were located in nearby portions of ventral V2 and ventral V3. Because the DY injection was farther from the border of V1 than the FR injection, the DY-labeled patch was more rostral in V2 than the FR-labeled patch. This pattern was reversed in V3v. Thus, the connection pattern was consistent with the hypothesis that V2v forms a mirror-image representation of that in V1v, and V3v forms a mirror reversal of the V2v representation. The injection labeled a few neurons in ventral MT and ventral DM and a scattering of cells in other locations.

The fourth injection in this titi monkey was in dorsal V1, although the deeper part of the injection site also slightly involved an underlying portion of ventral V1. Most of the retrogradely transported label was in dorsal V2; dorsal V3; caudal portion of adjoining DM; and dorsal portions of DL, MT, and MTc. Labeled neurons were in locations expected from retinotopic patterns of connections. Other labeled cells were in ITm, ITc, VPP, DI, MST, and FST. Labeled neurons in V2v, V3v, and ventral DL most likely reflect the spread of the injection core into ventral V1.

**Squirrel monkeys.** In total eight injections of tracers were placed in V1 of two squirrel monkeys. Again the injections revealed short-range local connections within V1 and long-range connections with a number of extrastriate visual areas.

In squirrel monkey 00–03 (Figs. 6C, 7) four injections were placed in ventral V1, and one was placed near the representation of the horizontal meridian. The adjacent FE (green) and FR (red) injections in ventral V1 (Fig. 6C) produced a reversed pattern of labeled foci in V2v, and the pattern in V3v was reversed from that in V2v (although the FE label was continuous across the V2-V3 border, whereas only the FR label formed two distinct patches). A DY (blue) injection was made in dorsal V1, just dorsal to the calcarine sulcus and judged by location to be near the representation of the horizontal meridian (Fig 6C). This injection produced a dense patch of label dorsally along the border of V2 and V3, involving both areas. This location of label would be expected from an injection in the dorsal half of V1, just along the representation of the horizontal meridian. The three injections also labeled cells in MT, DL, DM, IT, FST, VPP, and LPP. The topographic pattern of label in DL and MT corresponded to the expected retinotopic sequence, but it was located more dorsal in MT than expected. Two other injections in ventral V1 of this case (Fig. 7C,D) also produced dense foci of labeled neurons in V2v and V3v as well as in MT, DL, DM, and other visual areas listed above.

In a second case (00–72; Figs. 6D, 7A,B), injections placed in the dorsolateral portion of V1 representing central vision also retrogradely labeled regions of extrastriate cortex. A dorsoventral sequence of three injected tracers labeled a dorsoventral sequence of partially overlapping arrays of labeled neurons along the rostral border of central V2 and rostrally adjacent cortex. The label in the V3 region suggests that dorsal and ventral halves of V3 may merge or nearly so. A comparable sequence of less densely labeled foci of neurons was seen along the central aspect of DL that may indicate the horizontal meridian shared by DLc and DLr. A similar sequence of labeled neurons was seen in caudolateral MT where central vision is represented. However, a scattering of labeled cells also occurred over the rest of MT. Label in caudolateral DM was consistent with the view that central vision is represented in that location. Other label was seen in ITc, ITm, ITr, MTc, MST, FST, VPP, and LPP.
Fig. 3. Scanned image of a section through superficial layers of flattened squirrel monkey caudal cortex processed for cytochrome oxidase (CO). An unlabeled image of the section (A) is duplicated and labeled (B). As expected, the rostral V1 border is most easily recognized because of a sharp decrease in CO density from V1 to V2. Areas V2 and V3 exhibit narrow dark and light CO bands. A lighter staining border separates the two areas. The V3 bands appear thicker than those in V2 and extend about 3 mm, roughly half the length of the V2 bands. The dashed white oval represents damage from injections for an unrelated experiment. The damaged cortex resulted in lighter CO staining for the middle region of the V3 strip. For a separate hemisphere, two more images of V1 blobs and the bands in dorsal V2 and V3 (C,D) show that the dark CO bands are formed by a series of blob-like patches. A black line indicates the V2/V3 border, whereas a white line marks the outer V3 border. Right is rostral; up is medial. E, top: Dorsolateral views of the right hemisphere of a squirrel monkey. V1 is actually visible as darker than the rest of cortex. E, bottom: View of the medial and ventral walls of the right cortical hemisphere of a squirrel monkey. For abbreviations see Figures 1 and 2. Scale bars = 5 mm.
Owl monkeys. Five different tracers were injected into V1 of one owl monkey (Fig. 8). As for titi and squirrel monkeys, the injections labeled fibers and neurons over short distances around the injection cores. These connections were nearly uniform in distribution along the margin of the core, but they became patchy just beyond the core, as previously described (Rockland and Lund, 1983; Cusick and Kaas, 1988a; Lyon and Kaas, 2001). This patchy array was most obvious after the CTB injection (Fig. 8B), which labeled terminals as well as neurons, and it was more obvious in owl monkeys than in titi or squirrel monkeys.

The locations of other labeled neurons provided evidence for both V3d and V3v. An injection of DY into ventral V1 resulted in dense foci of labeled cells in ventral V2 and immediately rostral in V3v (Fig. 8A). Other labeled neurons were in MT, DM, DL, IT, MST, FST, and portions of the posterior parietal cortex. A quite similar pattern of connections was seen after an injection of CTB into a slightly less ventral portion of ventral V1 (Fig. 8B).

When FB was placed at separate locations in paracentral V1 along the horizontal meridian and into dorsal V1, the distribution of labeled cells was broad, including both dorsal and ventral portions of V2, V3, MT, and DM (Fig. 8C). Other areas, including IT cortex and portions of posterior parietal cortex, were less densely labeled. Two injections placed in dorsal V1 labeled groups of neurons in V2d and V3v, as expected (Fig. 8D). Other foci of labeled cells were found in MT and DM, with scattered neurons elsewhere.

**Estimate of the connection magnitude of extrastriate visual areas with V1**

We estimated the relative strengths of connections between V1 and extrastriate visual areas by counting all labeled neurons in extrastriate cortex and assigning them to specific cortical fields (see Table 1). Because the boundaries of areas V2, V3, and MT were reliably determined, labeled neurons were assigned to these fields with confidence. More uncertainty exists for other fields, and other schemes of dividing cortex might locate neurons differently. In addition, we are not certain that all tracers are transported to all areas with equal effectiveness. Trans-
port may be less effective, for example, to more distant fields or over pathways with thinner axons.

The findings support several basic conclusions. First, roughly half of the extrastriate cells labeled by V1 injections were in V2. However, values for individual injection sites varied from a low of 25% in the titi monkey to a high of 80% in a squirrel monkey. Overall, proportionately more labeled cells were in V2 of squirrel monkeys (70%) than owl (41%) or titi (44%) monkeys. Second, V3 had the next highest proportion of labeled cells, about 20%. This was less in squirrel monkeys (with more in V2), at 13%, and more in owl (24%) and titi (25%) monkeys. This suggests that V2 has a greater role in processing V1 information and providing feedback in squirrel than in owl and titi monkeys. However, in all three primates, V3 had roughly half as many labeled cells as V2. Third, MT had the third highest proportional connections with V1, averaging just fewer than 10% and varying from a high of 16% in the owl monkey to a low of 5% in the squirrel monkeys. Fourth, DL and DM had about the same lower proportions of labeled cells (3–4%), with fewer DL cells in the owl monkey. Fifth, IT cortex had a scattering of labeled cells over three main subdivisions, comparable in total magnitude (4%) to DL and DM. Other areas (DI, MTc, MST, FST, VPP, and LPP), even when generously defined, had few labeled cells (0.3–1.7%).

Fig. 5. Connections of ventral V1 in titi monkey 00–50. Reconstructed borders of visual areas based on CO patterns and myeloarchitecture are superimposed onto either a captured image of CTB labeled terminals and cells in a superficial section of part of the flattened visual cortex (A) or the distribution of CTB labeled cells (B; black dots). The CTB injection (indicated by an arrow) was placed on the lower bank of the calcarine sulcus in the upper visual quadrant representation of V1 (see Figs. 1A, 2D). In flattening this hemisphere, a cut was made through the fundus of the calcarine sulcus, separating it into dorsal and ventral halves. Labeled terminals (A) are clearly present only in ventral V2 and V3v and sparsely in ventral DL, ventral MT, and DM, whereas labeled cells (B) are obvious in large numbers in ventral DL and ventral MTC; throughout areas DI, ITc, ITm, ITr, MST, VPP, LPP; and in the same locations as the labeled terminals. Other conventions as in Figure 1A. Scale bar = 2 mm.

Fig. 6. Distributions of labeled cells following injections of diamidino yellow (A; DY, green dots) and fluororuby (FR; red dots) in ventral V1 and fast blue (B; FB, blue dots) primarily in dorsal and partly in ventral V1 in the same titi monkey as shown in Figure 5 (case 00–50). The DY and FR injections were placed just ventral to the lip of the lower bank of the calcarine sulcus (see Fig. 2D) in the representation of the upper visual quadrant of V1 (see Fig. 1A), whereas FB was placed at the caudal end of the upper bank of the calcarine, primarily in the representation of the lower visual quadrant of V1, but the uptake zone also included some upper field representation. C: Distributions of labeled cells following injections of FE (green dots) and FR (red dots) in ventral V1 and DY (blue dots) in the lower quadrant of V1 in a squirrel monkey (case 00–03). The FE and FR injections were placed just ventral to the lip of the lower bank of the calcarine sulcus (see Fig. 3E) in the representation of the upper visual quadrant of V1 (see Fig. 1B), whereas FB was placed at the upper bank of the calcarine, in the representation of the lower visual quadrant of V1. D: Distributions of labeled cells following injections of CTB (pink dots) and DY (green dots) in dorsal V1 and FB (blue dots) in lateral V1 in a second squirrel monkey (case 00–72). All three injections were placed on the dorsolateral surface at 2 mm intervals; the CTB and FB are illustrated individually in Fig. 8A,B. In A–D, lines point to black ovals that represent the injection site and uptake zone of each fluorochrome. In A and C, there is evidence in these two species for a mirror reversal of the horizontal visual field from V1 to V2 (i.e., green-red flips to red-green), from V2 to V3v (i.e., red-green flips back to green-red), and again from V3v to DLc (i.e., green-red flips to red-green). Conventions as in Figure 5. Scale bars = 2 mm.
These percentages are reflected by the distributions of labeled cells following CTB and DY injections in squirrel monkey 00–72 (Figs. 6D, 7A). The number of CTB-labeled cells in V2, V3, and MT was rather large (9,706 cells in V2, 3,383 cells in V3, 1,849 cells in MT) compared with the number of DY-labeled cells (1,012 cells in V2, 297 cells in V3, 17 cells in MT). Even so, both tracers revealed that the largest percentage of labeled cells was in V2 (56% and 76%) and the second largest in V3 (20% and 22%; see Table 1).

**DISCUSSION**

In the present series of experiments, we used injections of tracers in primary visual cortex (V1) of titi, squirrel, and owl monkeys to reveal patterns of connections with extrastriate cortex. The results were similar for all three primates, and they provided compelling evidence for the existence of a third visual area, V3, that includes both a dorsal half (V3d) representing the lower visual quadrant and a ventral half (V3v) representing the upper visual quadrant. This V3 is about half the size of V2, and it has about half as many feedback connections with V1. V3 can be recognized in brain sections cut parallel to the surface of the cortex and processed for CO by a change from a distinct pattern of alternating dark and light bands in V2 to a less distinct pattern of broader bands in V3. Here we relate these findings to previous studies and concepts of visual cortex organization in monkeys. This report is part of an extended effort to determine similarities and differences in cortical organization in primate taxa.

**V3 in New World monkey**

A defining characteristic of V3 in monkeys is that it forms a mirror-image representation of V2 along the outer border of V2. The original evidence for V3 came from the projection pattern of dorsal V1 to extrastriate cortex (Cragg, 1969; Zeki, 1969). However, the existence of a V3 has been repeatedly questioned in subsequent reports and reviews (see below), especially for New World monkeys. Instead, the common conclusion was that the dorsal V3 region was occupied by several other visual areas, but largely DM, and the ventral V3 region constituted another visual area, VP (Allman and Kaas, 1975; Newsome and Allman, 1980; Lin et al., 1982; Krubitzer and Kaas, 1993; Rosa and Schmid, 1995; Beck and Kaas, 1998; Rosa and Tweedale, 2000). In other reports, a dorsal V3 was recognized but not a ventral V3 (Burkhalter and Van Essen, 1986; Burkhalter et al., 1986; Newsome et al., 1986; Van Essen et al., 1986; Felleman and Van Essen, 1987).

In the present study, we obtained clear evidence for connections that are highly consistent with the concept of a modified V3. In all three species of New World monkeys, ventral V1 connected with the region we now define as ventral V3, and dorsal V1 connected with the region of dorsal V3. The pattern of connections clearly indicated that this V3 represents the contralateral visual hemifield as a mirror reversal of the representation in V2. However, the connection pattern and the architectonic characteristics show that V3 is about half the width of V2, rather than being the same width as originally proposed. The length of V3 is less certain, but it does not appear to extend “dorsally” and “ventrally” as far as V2, and it narrows toward its center, where the two halves may or may not merge. Although the architectonic characteristics of V3 were less apparent in the central region, the connection pattern with central V1 provided some evidence for a continuation of a thinner central V3 between V3v and V3d in at least titi and squirrel monkeys, and this is shown in our illustrations. Consistently with the evidence that V3 is half the width of V2, V3 had about half as many connections with V1 as V2.

Although we used the bidirectional tracer CTB in all of the present cases, much of the evidence for connection patterns came from the use of fluorescent tracers that were transported from terminations in V1 to cell bodies in extrastriate areas. Although the CTB results provide strong evidence that the major extrastriate connections of V1, especially those with V2 and V3, are reciprocal, terminations were not obvious in some of the regions with sparse connections. We assume that V3 receives about half as many projections from V1 as from V2, as well as sending back half as many projections. Nonreciprocal connections have sometimes been reported for regions that are sparsely interconnected (for review see Felleman and Van Essen, 1991). However, this may simply reflect the greater ease of detecting a few labeled cells than of detecting a few labeled terminals. The major connection pathways in visual cortex have commonly been shown to be reciprocal and proportional (see Felleman and Van Essen, 1991; Rockland, 1997).

Why has the existence of V3 been uncertain in New World monkeys? The primary reason, in our judgment, was the lack of evidence for a connection pattern with V1 that is consistent with the concept of V3. More specifically, projections from V1 in early studies of New World monkeys were attributed to only V2 and MT (Spatz et al., 1970; Spatz and Tiggges, 1972; Tiggges et al., 1973). However, Martinez-Millan and Holländer (1975) did describe an additional projection to dorsomedial cortex just rostral to V2 in three of thirteen squirrel monkeys with injections in V1, and Lin et al. (1982) found similar projections in most but not all owl monkeys with V1 injections. These connections were attributed to the dorsomedial visual area (DM; Allman and Kaas, 1975) in the study of Lin et al. (1982) and in a subsequent study of V1 projections in owl monkeys and other New World monkeys (Krubitzer and Kaas, 1993). In a similar manner, studies of V1 projections in prosimian galagos failed to demonstrate connections other than to V2 and MT (Tiggges et al., 1973; Symonds and Kaas, 1978) or to V2, MT, and DM (Krubitzer and Kaas, 1993). In the prosimian slow lorises, projections from central V1 were localized in V2, MT, and caudal and rostral subdivisions of the dorsolateral visual area DL (Preuss et al., 1993). Even in macaque monkeys, there was only evidence of V1 projections to the region of dorsal V3 (see Weller and Kaas, 1983; Van Essen et al., 1991; Rockland, 1997).
Fig. 8. Distribution of labeled cells in visual cortex following V1 injections of five tracers in an owl monkey (case 00–68). Arrows point to white ovals that represent the injection site and uptake zone of each tracer. A,B: Two injections (DY and CTB) were on the lower bank of the calcarine sulcus in ventral V1 in the representation of the upper visual quadrant (see Fig. 1C). A third and rather large injection (FB) was placed on the dorsolateral surface near the V1 horizontal meridian. The large uptake zone included both upper and lower field representations of V1 and appeared to be close to the representation of central vision. D: Two more injections were placed on the upper bank of the calcarine, in the lower quadrant of V1. The FR injection (black) was placed nearest the V1/V2 border, whereas FE (grey) was placed farther caudally into V1. Conventions as in Figure 6. Scale bars = 2 mm.
1986), which could also be attributed to DM (Kaas and Lyon, 2001).

The lack of connectional evidence for V3v led to an interpretation of the V3v region as another visual area, VP, first in New World monkeys (Newsome and Allman, 1980) and then in macaque monkeys (Newsome et al., 1986). In contrast, Sousa et al. (1991) described V1 connections with cortex along the rostral borders of both dorsal and ventral V2 in New World cebus monkeys and attributed these connections to V3. Elsewhere, we described V1 connections in New World marmosets (Lyon and Kaas, 2001), Old World macaques (Lyon and Kaas, 2002b), and prosimian galagos (Lyon and Kaas, 2002a) and concluded that the evidence in these primates supports the concept of a smaller V3 with both dorsal and ventral halves, much like the V3 proposed in the present study.

There also is microelectrode mapping evidence for V3 as a retinotopically organized representation in macaques (Gattass et al., 1988) and some New World monkeys (Rosa et al., 2000), but this evidence is also largely consistent with the concept of a VP representing the upper visual quadrant and a V3d devoted to the lower visual quadrant.

The lack of compelling connectional evidence for a V3 in most reports is not surprising given that the connections with V2 are much denser and that the rostral border of V2 was usually uncertain, especially because cortex was not often cut parallel to the surface, where surface view patterns of connections and architectonic borders could be readily appreciated. Also, it was only in such preparations that the rostral border of V2 was clearly apparent from its banding pattern (see Livingstone and Hubel, 1983; Tootell et al., 1986; Krubitzer and Kaas, 1989; Tootell and Hamilton, 1989; Malach et al., 1994; Olavarria and Van Essen, 1997). Furthermore, although the banding pattern that identifies V2 became easy to recognize and delimit, architectonic features that identified a V3 were not obvious. However, Tootell et al. (1985), using CO stains of sections from flattened cortex, did describe a banding pattern in "V3PVA," of owl monkeys, which corresponds precisely to the present V3v. Here we suggest that a pattern of bands, broader but less obvious than those in V2, is useful in identifying V3 (also see Lyon and Kaas, 2001).

**Functional implications of the proposed V3**

The conclusion that a V3 with both ventral and dorsal halves exists in most, if not all, primates has several obvious implications for how visual information is processed in visual cortex. As originally proposed (Zeki, 1978), V3 is a third step in a cortical processing hierarchy. V3 does get direct input from V1 but is also a target of V2 (Van Essen et al., 1986; Felleman et al., 1997; Gattass et al., 1997; Lyon and Kaas, 2001), so it can be considered to be at a higher level than V2 (Rockland and Pandya, 1979; Maunsell and Van Essen, 1983; Felleman and Van Essen, 1991; Van Essen and DeYoe, 1995; Van Essen and Felleman, 1996; Hilgetag et al., 2000). Originally, V3 was seen as equal to V2 in size and significance as a third level of processing. Now it appears that V3 is about half the size of V2, with half of the V1 connections. V3 also distributes information to an array of at least 10 additional fields. Based on proportions of connections, V2 has the most interconnections with V1, and V3 has the next, but V3 is only one of the several areas that further process V1 information and only one of the several areas that process V2 information (see Stepniewska and Kaas, 1996). Thus, processing is much less serial than once proposed, and V3 is only one of the early distribution centers. The smaller size of V3 suggests a lesser role than V2, and individual neurons are likely to have larger receptive fields and process information over considerably more of the visual field, as others have shown (Felleman and Van Essen, 1987; Gattass et al., 1988; Gegenfurtner et al., 1997).

We suggest that some previous observations on V3 may be partially or largely contaminated by involvement of other visual areas, and such contaminations may have resulted in described asymmetries between V3d and VP (see Burkhalter et al., 1986). Additionally, results obtained from the caudal part of DM likely reflect the properties of V3 rather than DM. For instance, some injections thought to be in DM largely labeled neurons overlapping the CO blobs of V1, whereas other injections did not (see Beck and Kaas, 1998, 1999). Possibly, V3 has more connections with the blobs, modules of V1, and this would imply certain functional roles. Alternatively, or in addition, the evidence for a CO banding pattern in V3 suggests a modular organization, and the two or more sets of bands in V3 would have different connections and different functional roles.

As suggested above, analysis of the functional properties of V3 neurons has yielded varying results. Contrary to an earlier analysis that showed a predominance of color-selective cells in VP and direction-selective cells in V3d (Burkhalter and Van Essen, 1986; Felleman and Van Essen, 1987), more recent studies indicate that V3d has a large number of color- and motion-selective cells (Gegenfurtner et al., 1997). Furthermore, some cells attributed to V3 respond to more complex stimuli than input areas V1 and V2, such as pattern motion (Gegenfurtner et al., 1997; Smith et al., 1998). This function appears to be derived within V3, because cells in the input layer did not respond to the vector sum of plaid gratings (Gegenfurtner et al., 1997). Finally, the V3 region connects with both MT and DL (V4; Felleman and Van Essen, 1991; Felleman et al., 1997; Lyon and Kaas, 2001) and, thus, V3 contributes to both the dorsal and ventral processing streams (Ungerleider and Mishkin, 1982).

**Other cortical areas with V1 connections**

Although our focus was on patterns of V1 connections that related to the issue of the possible existence of V3, the V1 injections in all three primate species labeled neurons in regions of a number of proposed visual areas, including DLr, DLc, DM, ITc, ITm, ITr, DI, Mtc, MST, FST, VPP, and LPP. Whereas, very early studies provided evidence for V1 connections with only V2, MT, and sometimes V3, several subsequent studies, especially those of Doty (1983), Kennedy and Bullier (1985), and Perkel and colleagues (1986) in macaques and Sousa and colleagues (1991) in cebus monkeys, revealed a wider range of V1 connections, with distributions similar to those shown here. These additional patterns of connections likely reflect the use of more sensitive tracers.

**ACKNOWLEDGMENTS**

We thank Emily Grossman, Troy Hackett, and Mike Remple for helpful comments on the manuscript; Mary Feurtado for her assistance during sterile surgical proce-
dures and for postsurgical animal care; and Laura Trice and Mary Varghese for their help with histological procedures.

**LITERATURE CITED**

Allman JM, Kaas JH. 1971. A representation of the visual field in striate and adjoining cortex of the owl monkey (*Aotus tririgius*). Brain Res. 35:89–106.


NEW WORLD MONKEY V3 CONNECTIONS


