Reappraisal of DL/V4 Boundaries Based on Connectivity Patterns of Dorsolateral Visual Cortex in Macaques

We placed injections of 3–5 distinguishable tracers in different dorsolateral locations in the visual cortex of four macaque monkeys to help define the extent of the dorsolateral visual complex (DL) commonly known as area V4. Injections well within DL/V4 region labeled neurons in V2, V3, MT, IT, and sometimes V1. In contrast, injections in caudal area 7a dorsal to current descriptions of DL/V4 produced a different pattern of labeled neurons largely involving posterior parietal and adjoining occipital cortex, as well as cortex of the medial wall. Injections placed in the dorsal prelunate cortex (DP), near the expected location of the dorsal border of DL/V4, labeled neurons in a third pattern, including regions of the posterior parietal and occipital cortex, inferior temporal (IT) cortex, and sometimes parts of dorsal area V2, DL/V4 complex and MT. Injections placed near or ventral to previous estimates of the ventral border of the rostral divisions of DL (DLr) and near the expected rostroventral border of V4 with TEO labeled cells in a pattern distinctively different from either central DL/V4 injections or those dorsal to DL/V4. Injections placed rostroventral to DL/V4 labeled neurons over a large extent of the IT cortex, while failing to label neurons in V1, V2 and MT. Injections that partially involved the rostroventral border of DL/V4 produced a similar pattern of labeled neurons, but also labeled a few cells in ventral V1 and V2, as well as many in DL/V4. Dorsal and rostroventral injections also labeled different regions of the prefrontal cortex, but only DL/V4 injections that included area DP labeled neurons in the prefrontal cortex. The results revealed contrasting and transitional connection patterns for four regions of the dorsolateral visual cortex, and they provided evidence for the locations of dorsal and rostroventral borders of the DL/V4 complex.

Keywords: extrastriate cortex, inferior temporal cortex, neuronal tracers, posterior parietal cortex, primates

Introduction

The principle goal of the present study was to inject different, distinguishable tracers into the dorsal, middle and rostroventral portions of the dorsolateral visual cortex of the same macaque monkeys so that cortical connection patterns of these regions could be directly compared. In macaque monkeys, the cortex between the representation of foveal vision in V1 and the middle temporal visual area, MT or V5, was defined as the fourth visual area, V4, on the basis of connections with the second visual area, V2 (Zeki, 1971). In New World owl monkeys, a similar region was termed the dorsolateral visual area, DL, after microelectrode recordings revealed a systematic representation of the contralateral visual hemifield, with the lower visual quadrant represented dorsal to the upper visual quadrant (Allman and Kaas, 1974b). Although V4 and DL were first described in different primates, the early depictions of the extent of the two areas were so similar (Allman and Kaas, 1974b; Van Essen and Zeki, 1978; Van Essen and Maunsell, 1983; Van Essen, 1985) that it seemed reasonable to conclude that DL and V4 were equivalent terms for the same area. However, there have been several proposals for dividing the V4 region into two or more areas (for a review, see Stepniewska and Kaas, 1996), and the depicted boundaries of the DL/V4 complex have varied across proposals (see Discussion). In particular, starting with the microelectrode mapping study of Gattass et al. (1988), most reports have illustrated a V4 that extends much further ventrally than in early depictions and has a larger representation of the upper visual quadrant than the lower visual quadrant (e.g. Desimone and Ungerleider, 1989; Boussaoud et al., 1990, 1991; Felleman and Van Essen, 1991; Fize et al., 2003; see Discussion). The redefined V4 has been called an ‘improbable’ visual area, with the suggestion that the overrepresentation of the upper quadrant is the result of including parts of other areas in V4 (Kaas, 1993). As microelectrode mapping studies, as well as functional magnetic resonance imaging (fMRI) studies of retinotopic patterns, do not easily identify the borders between areas where these borders are retinotopically congruent, the proposed territories of the DL/V4 complex could include parts of other visual areas. However, adjoining visual areas should differ in how they connect with other regions of cortex in ways that reflect their different roles in processing of visual information. In the present experiments different tracers were placed in DL/V4 and near the proposed dorsal and rostroventral boundaries in an effort to reveal changes in connection patterns that could identify areal borders.

Materials and Methods

Connection patterns were studied by placing 3–5 different tracers in the dorsolateral visual cortex of four adult macaque monkeys (Macaca mulatta). Tracers were placed in different dorsal to ventral locations in cortex exposed on the brain surface, just anterior to the lunate sulcus and the inferior occipital sulcus that delineate V1. Our intention was to place injections both within and outside the region we had previously defined as the dorsolateral visual complex (DL) (Stepniewska and Kaas, 1996), possibly the equivalent of V4. The goal was to compare connection patterns revealed by injections centered in DL with those reflecting injections near or outside the presumptive dorsal and ventral borders of DL. The experimental procedures were approved by the Vanderbilt University Animal Use Committee and adhered to National Institutes of Health guidelines.

Tracer Injections

Surgical procedures were performed under aseptic conditions on monkeys anesthetized with 2% isoflurane. The dorsolateral visual cortex was exposed and injections of different fluorescent dyes — 3% Fast Blue (FB), 2% Diamidino Yellow (DY), 10% Fluororuby (FR) and 10% Fluorolissmridine (FL) in distilled water — as well as 2% wheatgerm agglutinin conjugated to horseradish peroxidase (WGA-HRP; Sigma Chemicals) and 1% cholera toxin subunit B (CTB; Sigma Chemicals) in...
distilled water or 10% solution of biotinylated dextran amine (BDA; Molecular Probes) in phosphate buffer were made with a micropipette coupled to a Hamilton syringe. Injections of 0.5–1 µl of fluorescent dyes, BDA or CTB and 0.05 µl of WGA-HRP were placed in several locations in the dorsolateral visual cortex just rostral to the lunate and inferior occipital sulci and caudal to the superior temporal sulcus (Fig. 1). The most dorsal injection was between the tip of superior temporal sulcus (STS) and intraparietal sulcus, and the most ventral injection was just rostral and ventral to the lower bank of the inferior occipital sulcus. Other injections were distributed along the prelunate gyrus. Injections were placed 1–1.5 mm deep into the cortex to avoid damage and uptake of tracers by underlying fibers. All injection locations and extents were later verified in the prepared histological material.

Histology
After 7–10 days of survival (or 5 days in case 99-36 when WGA-HRP was injected), monkeys were administered a lethal dose of sodium pentobarbital and perfused intracardially with warm phosphate buffered saline followed by fixative (2% paraformaldehyde and then 2% paraformaldehyde with 10% sucrose), and their brains were removed. The posterior part of the cortex, from the lateral sulcus backward was carefully separated from the rest of the brain, and sulci were unfolded (Fig. 2). The cortex was flattened by gentle pressure between two glass slides and placed in 30% sucrose in buffered saline overnight for cryoprotection (see Krubitzer and Kaas, 1990). The next day, the cortex was cut parallel to the cortical surface into 40- or 50-µm-thick frozen sections. Sections were separated into five series. A set of unprocessed sections was mounted for examination of fluorochromes, and other series of sections were processed to reveal BDA (in case 98-66), BDA/CTB (in case 98-51) or WGA-HRP/CTB (in case 99-36). Processing procedures for BDA, BDA/CTB and WGA-HRP and CTB follow those described elsewhere (Sakai et al., 2000; Stepniewska et al., 2003). In each case, an additional series of sections was processed for myelin (Gallyas, 1979) or cytochrome oxidase (CO) (Wong-Riley, 1979) to reveal the architecture of cortical regions.

Data Analysis
Neurons labeled with fluorescent tracers were plotted at high resolution under ultraviolet illumination with a Leitz microscope coupled to an X-Y plotter (Igor Pro 3.14, Wave Metrics, Inc.). Neurons labeled with WGA-HRP and CTB were plotted under dark (WGA-HRP) or bright field (CTB) illumination with a Wild microscope with a drawing tube attached. For each case the injection sites and label were drawn from individual sections, which were superimposed with adjacent sections. Data Analysis: Deconvolution Neurons labeled with fluorescent tracers were plotted at high resolution.

Identification of Cortical Areas and Boundaries
Interpretations of the connection patterns in the present cases were greatly aided by the identification of the boundaries of several visual areas by architectonic criteria as reference areas, and the estimation of the locations and extents of other areas based on widely accepted depictions. In our processed brain sections of flattened visual cortex, we were able to reliably identify three visual areas that are components of all current proposals of visual cortex organization (for a review, see Kaas and Lyon, 2001). V1 was easily identified and delineated in both CO and myelin preparations due to the pattern of dark CO blobs and CO and myelin dense middle cortical layers. V2 was obvious over much of its extent as a result of its pattern of alternating CO dark and CO light bands, and MT was clearly visible as a CO and myelin dark oval (for photomicrographs, see Stepniewska and Kaas, 1996). The boundaries of other visual areas were largely or completely estimated from previous descriptions. The position of V3 was based on the recent description of Lyon and Kaas (2002). In part, V3 was also identified in favorable sections by thicker but less obvious CO bands than in V2 (Lyon and Kaas, 2002). The location of the dorsomedial visual area, DM, corresponds to that estimated from V1 connection patterns and myeloarchitecture (Lyon and Kaas, 2002). In the present material, DM also appeared darker than adjoining areas in CO and myelin preparations, but borders were somewhat uncertain. The dorsolateral visual complex, DL, consists of rostral and caudal divisions with similar but somewhat different connections (see Discussion). The borders of these two areas were based on our earlier description (Stepniewska and Kaas, 1996). DLc is somewhat more densely myelinated than Dlr, and this difference was apparent in some of our brain sections. Cortex ventral to DL was considered to be inferotemporal cortex, and rostral (ITr) and caudal (ITc) divisions (Weller and Kaas, 1987) were denoted without an attempt to mark a boundary between the two. Much of ITc could be

Figure 1. The locations of injection sites on lateral views of the left hemisphere of a macaque brain for four studied cases. Injections were placed in central DL in cases 99-36, 98-66 and 00-51, dorsally in the VPP or DP areas in all cases and ventrally in the inferotemporal cortex in cases 99-36, 98-66 and 98-102. Other injections were made at the PP–DL border region in cases 98-66, 00-51 and 98-102. Dashed line marks approximate border of area DL. CS, central sulcus; IPS, intraparietal sulcus; IOS, inferior occipital sulcus; LS, lateral sulcus; LuS, lunate sulcus; STS, superior temporal sulcus.
included in TEO of previous reports (e.g. Boussaoud et al., 1991), and the dorsal part of ITc could be included in V4 (see Discussion). The location of the middle superior temporal (MST) area was approximated based on the description by Desimone and Ungerleider (1986). As a moderately myelinated area, MST could sometimes be visualized in our sections. The fundal superior temporal area (FST) is estimated from the report of Desimone and Ungerleider (1986). Dorsal (FSTd) and ventral (FSTv) portions are distinguished as they have different connections (Kaas and Morel, 1993). This region is also moderately myelinated. A narrow stripe of cortex along the outer boundary of MT, the MT crescent (MTc) of Kaas and Morel (1993) was faintly apparent as a band of myelinated patches. Cortex between the dorsal end of the superior temporal sulcus and the intraparietal sulcus is designated ventral posterior parietal cortex (VPP). This region of cortex has been variously included in area 7a and the dorsal prelunate area (DP), or given other terms (for a review, see Lewis and Van Essen, 2000). Several subdivisions of the cortex of intraparietal sulcus have been proposed, but we only approximate locations of the lateral (LIP), ventral (VIP) and posterior (PIP) intraparietal areas (see Lewis and Van Essen, 2000).

The locations of areas in the prefrontal cortex were made in reference to the previous architectonic study of Preuss and Goldman-Rakic (1991). We use the numbers of Walker (1940) for fields largely to aid discussion, and have not attempted to define boundaries.

Results

Patterns of ipsilateral cortical connections were determined for a total of 15 injections in different locations in four macaque monkeys. The distributions of labeled neurons were summarized on surface-view illustrations of flattened cortex after plots of cells on individual brain sections were analyzed and compiled. Differences in connection patterns revealed by injections in and near the DL region provided clear evidence for the locations of the dorsal and ventral borders of the DL/V4 complex in macaques. Although neurons could be located relative to the surface in the sections from flattened cortex, such preparations are not ideal for determining the laminar locations of labeled neurons. Thus, the present focus was on areal patterns of label.

Connection Patterns in Visual Cortex

Here we briefly describe the connection patterns produced by the 15 injection locations in the four monkeys illustrated in Figures 3–6. All injections were limited to gray matter, and the white matter revealed by myelin staining in the deep sections was not contaminated by the tracers. In monkey 99-36 (Fig. 3), several closely spaced injections of fluorogold (FE) were placed in dorsolateral prelunate cortex in a location that corresponds to the dorsal half of DLc of our terminology (e.g. Stepniewska and Kaas, 1996), and V4 of basically all V4 descriptions (see Discussion). The injection was just dorsal to the presumptive representation of the zero horizontal meridian,

Figure 2. Cortex flattening procedure. (A–D) Dorsal, ventral, lateral and medial views of the left hemisphere with marked sulci. (E–G) The procedure used to flatten the visual cortex of the caudal part of the hemisphere. In (F) and (G), thick black lines mark cuts made along the calcarine sulcus on the medial wall and the intraparietal sulcus on the dorsal aspect of the hemisphere. In (G), thin black lines mark contours of opened sulci. CaS, calcarine sulcus; OTS, occipitotemporal sulcus; POS, parietooccipital sulcus; ExCaS, external calcarine sulcus; PMTS, posteromedial temporal sulcus. Other abbreviations are as in Figure 1.
judged to course from the rostral peak of V1 to the ventral margin of MT, and was thus somewhat within the representation of the lower visual quadrant in dorsal DL or V4 as defined by Gattass et al. (1988). The location of the injection was approximately the same as injections in caudal V4 of macaque monkeys by Shipp and Zeki (1985) and DeYoe and Van Essen (1985). These two groups of investigators found that their injections labeled neurons in a sequence of bands in dorsal V2 that largely correspond with the thin CO stripes and interstripes of V2 (see also Zeki and Shipp, 1989). Likewise, our dorsal DL...
injections labeled a sequence of bands in dorsal V2 representing the visual hemifield from $-2^\circ$ to $4^\circ$ (Gattass et al., 1981). Such V2 connections are expected to be an identifying feature of classically defined V4 (Zeki, 1971). A few labeled neurons appeared to be in retinotopically mismatched locations in ventral V2. Our injections in dorsal DLc also labeled neurons over much of the dorsal DLc and dorsal DLr, and even the ventral DLr. The more widespread pattern in DLr provides further support for considering the two regions as separate areas (see Stepniewska and Kaas, 1996). Other labeled neurons were in dorsal V3, and more sparsely in retinotopically mismatched ventral V3. Labeled neurons were largely within ventrocaudal MT, corresponding to paracentral vision of the lower visual quadrant, with fewer neurons in ventrorostral MT,
corresponding to paracentral vision of the upper visual quadrant (Gattass and Gross, 1981). Labeled neurons were also in the MTc region, and in IT\(r\) of the temporal lobe. A few labeled cells were in IT\(c\), FST and the cortex rostral to FST within the superior temporal sulcus. The connections with the IT cortex are consistent with the evidence that such connections are another defining feature of V4 (e.g. Shipp and Zeki, 1995; Felleman et al., 1997b). The separate regions of labeled neurons in IT\(r\) and IT\(c\) suggest that these are functionally distinct regions, and they also identify regions of temporal cortex that are unlikely to be parts of V4.

The other injections in case 99-36 were judged by location to be dorsal and ventral to DL, and they failed to label neurons in a pattern comparable to central DL injections. The locations of the injections of WGA-HRP and FR in the VPP and DP regions, respectively, would be considered to be dorsal to V4 by all
current investigators, and most of the expected targets of V4 were not labeled. Thus, no labeled neurons were detected in V2, V3 or MT. However, the FR injection did label neurons in the ITc region, not far from those labeled by the DLc injection and just outside V3v. The labeled neurons in ITc further define the ventral limit of DL/V4. The FR injection also labeled neurons over much of posterior parietal cortex, including the cortex of the intraparietal sulcus, and DM more caudally and deep in the lunate sulcus (Beck and Kaas, 1999). The more rostral WGA-HRP injections also failed to label neurons in V2, V3 and MT while labeling neurons over much of the posterior parietal cortex and a large number of neurons in the cortex of the medial wall, a region known to have connections with the injected region of posterior parietal cortex (Vogt and Pandya, 1999).
A few small groups of labeled neurons were on the rostral bank of the superior temporal sulcus.

The injection of CTB in case 99-36 was judged from the connection pattern to be just ventral to DLr (Fig. 3). This injection failed to label the targets that define V4. Thus, no labeled neurons were detected in V2 or MT. However, labeled neurons were found in ventral V3, suggesting that the injection was in a part of IT that represents the upper visual quadrant. This could also account for the labeled cells that were judged to be in the ventral extreme of DLr, providing evidence for DLr projections to the temporal cortex. A small focus of label in dorsal DLr would appear to represent retinotopically mismatched connections. Other labeled neurons were widely scattered over ITr and ITc, the visual cortex of the medial wall, and FST and the adjoining cortex in the caudal bank of the superior temporal sulcus.

Injections of tracers in five different locations in case 98-66 further identified the connection pattern of DLc (caudal V4) and the boundaries of DL/V4 (Fig. 4). An injection of FE into a dorsal portion of DLc was similar in location to the DLc injection of the previous case, but smaller in extent. Thus fewer cells were labeled. However, V2, V3 and MT were again labeled, although only a few labeled neurons were in V3. The locations of these labeled neurons in V2, V3 and MT all corresponded to parts representing the central few degrees of the lower visual quadrant. As central V4 has been described as having inputs from central V1 (Zeki, 1978; Nakamura et al., 1993), it was not surprising to find a few labeled neurons in V1 corresponding to central vision of the lower quadrant near the representation of the vertical meridian. Other labeled neurons were in dorsal DLc, in dorsal and ventral, but mainly ventral, DLr (a retinotopic mismatch) and in ITr cortex.

The other four injection sites in case 98-66 (Fig. 4) helped define the dorsal and ventral boundaries of DL/V4. Injections of BDA and FB in the VPP region were clearly well dorsal to DL, and they labeled neurons in the VPP region, the intraparietal sulcus (possibly VIP, LIP and especially PIP), the visual cortex of the medial wall and the locations of MST in the superior temporal sulcus. An injection of DY was judged to be largely dorsal to DL, but involving the dorsal border of DLc. Thus, there was some label in dorsal V2 in a portion representing ~30° into the lower visual quadrant (Gattass et al., 1981). As the label was along the V1/V2 border, the injection site likely involved the representation of the vertical meridian. Microelectrode recordings have identified neurons with receptive fields on the vertical meridian (Youakim et al., 2001) in this region of the cortex where V4 is postulated to adjoin a dorsal prelunate visual area termed DP (Maguire and Baizer, 1984; Van Essen, 1985; Andersen et al., 1990). A few clumps of labeled cells were in V1 and others were in caudomedial MT, where the paracentral and peripheral lower visual quadrant is represented. Other labeled neurons were in rostral DM, MST, dorsal DLr, several locations in ITr, scattered locations in the occipito-temporal sulcus and visual cortex of the medial wall, as well as in the region of the frontal eye field (Fig. 7). The connections with cortex of the medial wall identify the injection location as largely outside of DL (Andersen et al., 1990).

In ventral prelunate cortex of case 98-66, an injection of FR was judged to be largely ventral to DLr, but possibly including some of the ventral extreme of DLr. The possibility that the injection was almost completely ventral to DL was supported by the lack of labeled neurons in MT and V3 and the occurrence of only a few labeled neurons in V2 and V1. However, the FR injection labeled large numbers of neurons in DLr and DLc, and this distribution is consistent with the view that DL/V4 constitutes the main relay to the IT cortex. The FR injection also labeled large numbers of neurons in several locations across the IT cortex.

In case 00-51 injections were placed in central DL, near the dorsal border of DL, and dorsal to DL in the dorsal prelunate (DP) region (Fig. 5). The injection of CTB was judged to include the representation of central vision along zero horizontal meridian in DLc. This injection labeled neurons in portions of dorsal and ventral V1, V2, V3 and MT representing central vision, as well as central and ventral parts of DLr. Other label was in several locations in the IT cortex. All of these connections conform to those expected from central DLc (V4). While the FR injections in case 00-51 would be placed by current estimates in the DP region well dorsal to V4, some neurons were labeled in dorsal V2. As we did not find labeled cells in V2 in other cases with injections in the DP region, it seems unlikely that this cortex is part of V4. Instead, we conclude that DP injections may sometimes label a few scattered neurons in dorsal V2. The FR injection also labeled some neurons along the dorsal border of DLc, a few in dorsal MT and a few groups of cells in the IT cortex and in near the occipito-temporal sulcus. Other labeled neurons were densely distributed in the parietal and intraparietal cortex near the injections, and in the regions of DM and M. More laterally, an injection of BDA near the presumed dorsal border of DLc labeled a similar distribution of neurons, with more neurons labeled in DLc and DLr, possibly as a result of the injection slightly involving DLC.

In a fourth case, 98-102, injections were placed dorsal to DL in DP, at the expected dorsal border of DL and at the expected ventral border of DL (Fig. 6). None of the injections labeled neurons in a pattern that is characteristic of DL/V4, consistent with our judgement that the locations of the injections were largely or completely outside DL. However, the CTB injection did label some neurons near the extreme dorsal end of V2. Cortex in this location could be area prostriata, which borders part V1 representing peripheral vision (see Rosa, 1997). The injections of DY near the dorsal border of DLc failed to label neurons in V2 or V3, and only a single clump of neurons was found in MT. Other labeled neurons were in the DP and VPP regions, the dorsal extents of DLc and DLr, and ITr, with a few cells in DM and the superior temporal sulcus. The FE injection near the ventral border of DLr failed to label neurons in V2, V3 and MT. Thus, the injection did not appear to involve DL. A few labeled neurons next to the injection site may have been in DL. Other labeled neurons distant from the injection site were in separate IT locations. As the injection site was small, it may not have revealed the complete connection pattern.

Connection Patterns in the Frontal Cortex

Some of the injections in the present cases labeled neurons in prefrontal cortex (Fig. 7) in the region of the frontal eye field in the arcuate sulcus (areas 8Am and 45), and in the cortex 8Ar and cortex of the principal sulcus (46d and 46v). Thus, injections in DP that appeared to extend into dorsal DLc (cases 98-66 and 98-102) labeled patches of neurons in 8Am and 8Ar, but injections in central DLc (00-51) did not. However, some of the injections dorsal and ventral to DL labeled neurons in the frontal lobe. Injections in ITc just ventral to DLr labeled neurons in areas 45.
and 8Ar (98-66; 99-36, Fig. 7), while injections in VPP and DP labeled neurons in 8Am and area 46 (98-102; 00-51; 99-36; Fig. 7). Thus, injections of tracers into posterior parietal cortex and inferior temporal cortex labeled separate locations in the prefrontal cortex.

Discussion
In the present report, we describe and illustrate the connection patterns in the ipsilateral cortex resulting from each of 15 different injection sites in the dorsolateral visual cortex. The injection sites, just rostral to the lunate and inferior occipital sulci, were intended to help identify and delimit V4, an area or complex of areas originally defined by projections from V2 and V3 (Zeki, 1971). As the results are illustrated on flat surface views of cortex, they allow direct comparisons of connection patterns revealed by injections of different tracers in several areas of cortex in the same cases. They also provide an extensive and detailed comparison of the connections of V4 with areas dorsal and ventral to V4.

The V4 region of the visual cortex is also called the dorsolateral visual area, DL, after an early microelectrode
mapping study demonstrated systematic representation of the contralateral visual hemifield in the cortex between V2 and MT of New World owl monkeys (Allman and Kaas, 1974b). Here we refer to the region as the DL/V4 complex. In both New World and Old World monkeys there is considerable evidence that the DL/V4 region contains at least three visual areas that parallel each other in visuotopic organization: the MT crescent (MTC) or V4 transitional (V4t), in cortex that borders MT, the adjacent rostral division of DL (DRL) or V4A, and a caudal division of DL (DLC) or V4 proper (for a review, see Stepniewska and Kaas, 1996). All three areas represent the contralateral visual hemifield from lower quadrant to upper quadrant in a dorsoventral sequence. Because neurons in these areas have relatively large receptive fields and there is considerable receptive fields scatter, microelectrode mapping studies have provided weaker evidence for the three fields than studies of differences in connection patterns. Many investigators include the territories of V4A and V4 within the single representation, V4. As an added complication, Shipp and Zeki (1995) redefined V4A from part of V4 to much of TEO (see Zeki, 1996).

There are also uncertainties over the extents of the three areas. V4t has been described as a lightly myelinated area bordering only the portion of MT representing the lower visual quadrant (Desimone and Ungerleider, 1986), thus representing only the lower quadrant, while MTC has been described as a more extensive complete representation (Kaas and Morel, 1993). The V4 region, when considered as a single area, has been portrayed as having various extents (Fig. 8), especially in its ventral extension into the temporal lobe. The principle goal of the present study was to compare connection patterns of regions of the cortex well within DL/V4 with those possibly outside of DL/V4 to see if similarities and differences in connection patterns could help to define the borders of the complex. The defining differences between DRL (V4A) and DLC (V4 proper) connections are not considered here, but the two related fields share many connections, including V2 and V3 (see Stepniewska and Kaas, 1996).

Zeki (1971) originally defined V4 and V4A as separate targets of V2 and V3 projections (Fig. 8B). V4A appeared to be about the same width (or slightly more) as V4, and a narrow strip of cortex separated the V4 complex from area V5 (MT). Although the dorsal and ventral extents of V4 and V4A were not determined, the widths of the three fields corresponded quite well with our current proposal (Fig. 8A; also see Krubitzer and

Figure 8. Different proposals of the organization of extrastriate visual cortex in the macaque monkey. Each diagram is drawn as a flat reconstruction of the left hemisphere. The summary diagrams of other reports have been redrawn to conform to the prototype in (A) for ease of comparison, but the original diagrams in different formats may more accurately reflect the intentions of the authors. HM, horizontal meridian. Other conventions are as in Figure 4.
Kaas, 1993; Stepniewska and Kaas, 1996). In owl monkeys, Sereno and Allman (1990) and Allman et al. (1994) provided microelectrode mapping evidence for parallel retinotopic representations in each of these areas, which they described as posterior (DLp), intermediate (Dli) and anterior (DLa) divisions of DL. Early surface view descriptions of the extent of V4 depicted an area largely dorsal to a line connecting the representations of foveal vision in V2 and MT (e.g. Van Essen and Maunsell, 1983), suggesting that V4 may be largely devoted to representing the lower visual quadrant. Soon after, the possibility of a large ventral extension of V4 was considered (Fig. 8C; Van Essen, 1985) on the basis of evidence of the region having V2 connections (Ungerleider et al., 1983; Gattass et al., 1997). In a well-known microelectrode mapping study, Gattass et al. (1988) described an expanded V4 that included the territory of V4A and parts of temporal lobe within a single, but crude retinotopic organization (Fig. 8D). A similar V4 was later described for New World cebus monkey (Pinon et al., 1998). The Gattass et al. (1988) report apparently was influential in following depictions (Fig. 8E; Felleman and Van Essen, 1991). Much or most of the expanded V4v region appears to be within the territories of ventral V2 projections (Fig. 8F; Gattass et al., 1997).

However, the present results on connection patterns suggest that the DL/V4 complex is not as extensive either dorsally or rostroventrally (Fig. 8A), as proposed for V4 in some of the current summary diagrams. Differences in connection patterns distinguish the TEO region (Boussaoud et al., 1991), also known as the redefined V4A region (Zeki, 1996), from DL/V4.

The Connections of DLa/V4

Injections that were well centered in DL (DLc) labeled neurons in V2, V3, MT, IT, other parts of the DL complex and sometimes V1 (Fig. 9). The major connections were those commonly described for DL/V4. In particular, the injections labeled band-like arrays of neurons in V2 that are known from previous studies to project to DL/V4 (De Yoe and Van Essen, 1985; Shipp and Zeki, 1985; Weller and Kaas, 1985; Cusick and Kaas, 1988; Zeki and Shipp, 1989; Nakamura et al., 1993; Felleman et al., 1997b; Xiao et al., 1999). V2 projects to both divisions of the complex (DLr/V4A and DLa/V4), as originally described by Zeki (Zeki, 1971), but in different, parallel retinotopic patterns. The connections with DLr are less dense and the representation is more compressed (Stepniewska and Kaas, 1996). V2 has connections with other regions of extrastriate cortex (e.g. Gattass et al., 1997), including the IT (TEO) region (Zeki, 1971) that appears to have been partly included in the expanded version of V4 (Zeki, 1996; for a review, see Stepniewska and Kaas, 1996). V3 connections with DLc/V4 are less firmly established due to difficulties in defining V3 (Kaas and Lyon, 2001), but such connections have been described (for a review, see Felleman et al., 1997a) since the original report of Zeki (1971).

In the present cases, MT was consistently labeled after all injections centered in DL, and some sparse label was observed in FST. Connections between DL/V4 and MT have been well established in previous studies (Rockland and Pandya, 1979; Maunsell and Van Essen, 1983; Weller and Kaas, 1985; Ungerleider and Desimone, 1986; Morel and Bullier, 1990; Tanaka et al., 1990) although sometimes these connections have been described as weak (Shipp and Zeki, 1995). FST, but not MST, has been reported to have connections with DL/V4 (Weller et al., 1984; Boussaoud et al., 1990; see also Felleman and Van Essen, 1983).

Connections of the DL/V4 region with the IT or TEO region of the temporal lobe are well established (e.g. Zeki, 1977; Seltzer and Pandya, 1978; Rockland and Pandya, 1979; Desimone et al., 1980; Weller and Kaas, 1985; Weller and Steele, 1992; Distler et al., 1993; Felleman et al., 1997b). Connections of V1 with DL/V4 have been described, but they appear to be variable in magnitude and largely from foveal V1 (Allman and Kaas, 1974a; Zeki, 1978; Van Essen et al., 1986; Nakamura et al., 1993; for a review, see Casagrande and Kaas, 1994). Connections of other parts of V1 with DL/V4 have been also described, and V1 has some connections with the IT cortex (e.g. Lyon and Kaas, 2002). DL/V4 has not been considered as having significant connections with the frontal eye field (Schall et al., 1995), but a small patch of label was found in the anterior bank of the inferior arcuate sulcus (area 45) after large injections in V4 representing the lower quadrant of the visual field in a study by Ungerleider et al. (1989). Label after an injection involving the DL/V4 border with DP in two of our cases also raises the possibility of connections between DL/V4 and the frontal eye field (Fig. 7).

Connections of Cortex Near or Beyond the Dorsal Border of DL/V4

We placed different tracers at a total of nine different sites just rostral to the lunate sulcus that were judged to be near the dorsal border of most depictions of V4 or clearly dorsal to that border (Fig. 1). Cortex dorsal to V4 on the exposed brain surface is generally divided into a dorsal prelunate region that
adjoints V4 (area DP of Maguire and Baizer, 1984 and Asanuma et al., 1985) and the nearby caudal extension of area 7a (e.g. Andersen et al., 1990; Felleman and Van Essen, 1991), which we have labeled ventral posterior parietal cortex, VPP (Figs 3–6). Neither of these regions constitutes a well-defined area of cortex, but the connections of both regions have been studied. Our injections in VPP (7a) were in the more caudal portion that Pandya and Seltzer (1982) defined as area Opt. The injections labeled the cortical region around the injection sites, cortex in the intraparietal sulcus (LIP, PIP), the DP region, MST, the more distant cortex on the medial wall of the cerebral hemisphere, the region of the frontal eye field, and the cortex of the principal sulcus of the frontal lobe (Fig. 9). Previously, Andersen et al. (1990) described connections of the caudal portion of area 7a as largely with DP and LIP. Other connections were with MST and the parietal occipital area (PO), which Colby et al. (1988) considered to be the same as the previously defined medial area M (Allman and Kaas, 1976). Our more caudal VPP injections (Figs 5 and 6) labeled both of these regions. As in the present study, connections with area 8a and 46 of the frontal lobe were previously noted (Andersen et al., 1990; Schall et al., 1995). As the connections of the 7a region have been described in a number of other reports, including studies with lesions or injections in 7a and studies labeling 7a after injections in other areas, a number of proposed visual areas have been related to area 7a (for a review, see Andersen et al., 1990). Our VPP injections labeled the regions most consistently described as having connections with caudal 7a. None of the current schematics of cortical organization (Fig. 8) include the 7a region of cortex in V4 or the DL complex, and the distinctly different connection patterns of 7a and DL/V4 support this conclusion.

Other injections in the prelunate cortex were either centered in DP or along the expected border of DP with DL (Stepniewska and Kaas, 1996). Injections centered in DP (FR, Fig. 3) labeled neurons throughout DP and in adjoining VPP (7a). DM, PIP, LIP, IT and cortex of the medial wall (Fig. 9). Andersen et al. (1990) reported a similar connection pattern of DP, including 7a, V3A (DM), LIP (but not PIP), as in the present study, but also V4, MST and PO(m). Connections with the medial wall and IT cortex were not reported (but see Zhong and Rockland, 2003). However, a slightly more lateral injection in DP (FR, Fig. 5) did label neurons in the M (PO), MST and dorsal DLc (V4) regions, thus including a few more of the targets that Andersen et al. (1990) reported for DP. Again, neurons were labeled in IT and the cortex of the medial wall, and a few neurons were labeled in dorsal V2, suggesting that these areas have connections with parts of DP, although such connections have been previously unreported. Other described connections of the DP region with VP (V3v) (Van Essen, 1985) were not seen in our two cases with central DP injections.

Injections near or on the DP/DL (V4) border labeled neurons in parts of dorsocaudal MT and dorsal V2 that represents peripheral vision of the lower visual quadrant, as did a dorsal DL injection, while labeling locations throughout DP, and DP targets such as the intraparietal cortex, DM and MST. We took this mixed pattern of connections as evidence that injection sites included bordering parts of DP and DL/V4. Thus, the injections of DY in case 98-66 (Fig. 4), BDA in case 00-51 (Fig. 5) and DY in case 98-102 (Fig. 6) all appear to define the locations of the DP-DL (V4) border. This border corresponds well to our previous estimate of its location based on V2 connections (Stepniewska and Kaas, 1996). The dorsal border of DL/V4 based on present results closely approximates or is somewhat ventral to other current estimates of this border (Fig. 8). The present evidence suggests that DL/V4 does not extend all the way to the dorsal end of the superior temporal sulcus, as in some proposals, but only to a location just ventral to the dorsal end. The present location corresponds to a proposed dorsal boundary of V4 where microelectrode recordings revealed a representation of the zero vertical meridian (Youakim et al., 2001; also see Maguire and Baizer, 1984). However, in a previous study where injections were placed in dorsal V4 and possibly in DP, a difference in connection patterns was not noted (Tanaka et al., 1990).

**Connections of Cortex Near the DL/V4-IT Border**

Our three injections near the DLr and ITc (TEO) border (Figs 3, 4 and 6) all appeared to be mainly in ITc, while slightly involving DLr in two of the cases (Figs 4 and 6). All injections labeled neurons in scattered locations across IT cortex and a few neurons across the border into the ventral tip of DLr (Fig. 9). One case (Fig. 3) also labeled neurons in V3v, FSTd, and cortex caudally adjacent to FSTd, while another case (Fig. 4) also labeled neurons in DLc, FSTv and cortex adjacent to FST. A few neurons were labeled along the ventral V2–V1 border. Because none of the injection labeled neurons in rostral MT, representing the upper visual quadrant as does ventral DL/V4, and only one injection labeled any cells in V2v, the injection sites were judged to be completely or nearly completely ventral to DL/V4. As the three injection sites were located rostral to the lip of the inferior occipital sulcus, the injection sites likely define the dorsal border of IT with DLr (original V4A) rather than DLc (V4 proper). Thus, DLc (V4 proper), but not DLr, may extend further into the inferior occipital sulcus. A much greater ventral extent of DLc did not seem likely in our previous estimates based on assumptions about the total V2 projection pattern (Stepniewska and Kaas, 1996); but projections from ventral V2 to cortex as ventral as the occipito-temporal sulcus have been described as belonging to V4 (Gattass et al., 1997). As these most ventral connections appear to conform to a single retinotopic pattern, they are unlikely to be a part of the weak V2 connections with IT (Zeki, 1971; Stepniewska and Kaas, 1996). Previously, large injections in the ITc region have extensively labeled the V4 (DL region and other parts of the temporal lobe, with weak labeling of parts of V2 and V3 (Morel and Bullier, 1990; Baizer et al., 1991; Weller and Steele, 1992; Distler et al., 1993; Webster et al., 1994). Moreover, injections of ITc in previous and present studies labeled prefrontal areas 8 and 45 within the frontal eye-field (Ungerleider et al., 1989; Webster et al., 1994; Schall et al., 1995).

**Conclusions**

The present study contributes to our understanding of visual cortex organization in two main ways. First, injections of tracers in the presumptive DL/V4, VPP (7a), DP and ITc (TEO) regions of cortex of macaque monkeys allowed detailed descriptions of the cortical connections of these regions of cortex. Because injections of distinguishable tracers were placed in as many as five locations in a single monkey, the connection patterns of several cortical regions could be directly compared in the same monkey. In addition, cortex was artificially flattened and cut parallel to the surface in the present cases so that surface-view patterns of labeled neurons could be accurately plotted and...
reconstructed across a limited number of brain sections that were locally aligned with precision. These connection patterns were related to the borders of those visual areas that can be reliably identified histologically (MT, V1, V2). Thus, the distortions in surface-view illustrations that result from laborious reconstructions based on a long series of brain sections cut in traditional planes were avoided, and detailed and accurate descriptions of the connection patterns of four regions of cortex were obtained.

Second, differences in connection patterns provided strong evidence for the dorsal and rostroventral limits of the DL/V4 complex. The results indicate that neither DLr nor DLc extend dorsally much past the level of the dorsal tip of MT, and even more clearly they do not extend to the dorsal tip of the superior temporal sulcus. This conclusion is consistent with a number of previous observations. Differences in connection patterns revealed by ventral injection sites provided evidence for a ventral limit of DLr with ITc (TEO). The results support the conclusion that the dorsal and ventral halves of DLr, divided by a line of foveal vision extending from foveal V1 to foveal MT and representing the upper visual quadrant ventrally and the lower visual quadrant dorsally, are approximately equal in size. Thus, there is no overrepresentation of the upper visual quadrant in DLr. While the results did not define the ventral limit of DLc, they did establish a width of DLc of ~8 mm from the border of V3v to the rostral lip of the inferior occipital sulcus. Our previous study of V2 connections with DLc (Stepniewska and Kaas, 1996) revealed a symmetrical pattern of representations in the dorsal and ventral portions, and suggested a ventral extent of DLc only slightly (5 mm) past the ventral extent of DLr. However, a more extensive study of the projection patterns of V2 (Gattass et al., 1997) provided evidence for a much more ventral extension, and a disproportionately large upper field representation. While no other interpretation of the Gattass et al. (1997) data is obvious, the disproportionate representation of the upper field in DLc-V4 seems improbable from a functional point of view, and further studies may yet identify part of the V4v region as belonging to another area.

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Address correspondence to Jon Kaas, Department of Psychology, Vanderbilt University, 301 Wilson Hall, Nashville, TN 37203, USA. Email: jon.kaas@vanderbilt.edu.

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