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The sublaminar organization of corticogeniculate neurons in layer 6 of macaque striate cortex

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Abstract

We examined the laminar distribution of corticogeniculate neurons in the macaque striate cortex labeled by axonal transport following injections of retrograde tracers into the lateral geniculate nucleus (LGN). Large injections of retrograde tracers involving all layers of the LGN resulted in a distinctive bilaminar distribution of labeled cells in cortical layer 6. One tier of labeled neurons was located along the layer 5-6 border and a second was located near the bottom of the layer, leaving the middle of layer 6 largely free of labeled neurons. Following injections of tracers that were restricted to the magnocellular layers of the LGN, almost all of the labeled neurons were located in the lower tier. In contrast, following injections of retrograde tracers confined to the parvocellular layers of the LGN, labeled cells were found in both tiers, with the greatest number in the upper tier. Thus, layer 6 of macaque striate cortex consists of three distinct sublayers only two of which are the source of descending projections to the LGN: an upper tier that projects exclusively to the parvocellular layers and a lower tier that projects to both magnocellular and parvocellular layers.

Keywords: Visual cortex, Area 17, Parallel pathways, Corticothalamic, Lateral geniculate nucleus

Introduction

Judged by their connections, neurons in layer 6 of striate cortex are in a strategic position to influence the flow of information from the lateral geniculate nucleus (LGN) to the striate cortex. Not only is layer 6 the source of descending projections that terminate in the LGN (Gilbert & Kelly, 1975; Lund et al., 1975; Hendrickson et al., 1978), it is also the source of intrinsic projections that terminate in cortical layer 4 (Lund & Boothe, 1975; Fitzpatrick et al., 1985), the principal target of LGN axons. An important and unresolved issue about the organization of layer 6 in primates is its relationship to the parallel pathways that relay through different layers of the LGN: the parvocellular pathway, which conveys information necessary for color perception and high acuity form vision; and the magnocellular pathway, which conveys information well-suited for the perception of motion (DeYoe & Van Essen, 1988; Livingstone & Hubel, 1988; Merigan & Maunsell, 1993).

Previous studies in macaques have suggested that layer 6 is divisible into two "stream-specific" halves: an upper half whose connections are associated mostly with the parvocellular layers of the LGN and a lower half whose connections are associated with the magnocellular layers (Lund, 1988). One line of evidence in support of this view comes from experiments in which injections of retrograde tracers were made into these two LGN subdivisions (Lund et al., 1975). Following injections of HRP into the parvocellular layers, labeled neurons were located preferentially in the upper half of layer 6; in contrast, following injections of retrograde tracers into the magnocellular layers, labeled neurons were located preferentially in the lower half of layer 6. Golgi studies of intrinsic projections also support this view; neurons in the upper part of layer 6 appear to project primarily to 4Cβ and 4A, the principal targets of parvocellular LGN axons, while those in the lower half of layer 6 project primarily to 4Cα, the target of magnocellular LGN axons (Lund & Boothe, 1975). Finally, a third line of evidence comes from examining LGN terminations within layer 6. Although these projections are sparse, those from the parvocellular layers terminate primarily in the upper half of layer 6, whereas those from the magnocellular layers terminate primarily in the lower half of layer 6 (Hendrickson et al., 1978; Blasdel & Lund, 1983).

Given the evidence for a bipartite subdivision of layer 6, we were surprised to find that large injections of retrograde tracers placed into the macaque LGN revealed three distinct tiers within layer 6: two tiers along the edges of layer 6 that contained the bulk of the corticogeniculate cells and a middle region that was largely devoid of labeled neurons (Fitzpatrick & Einstein, 1989). This observation suggested that the sublaminar organization of layer 6 was more complex than originally thought, and it led us to re-examine the relationship between a neuron's posi-

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tion in layer 6 and site of projection within the LGN. Our results suggest that the upper third of layer 6 projects exclusively to the parvocellular layers, while the lower third projects to both the magnocellular and parvocellular layers. We consider the significance of this sublaminar organization for understanding the contribution of layer 6 to the functions of the magnocellular and parvocellular streams.

Methods

Animal preparation

Eight macaque monkeys were used in this study. Prior to surgery, animals were anesthetized with a mixture of ketamine and xylazine (10 mg/kg; 0.3 mg/kg; i.m.), shaved, scrubbed, intubated, and placed in a stereotaxic apparatus. Body temperature was maintained at 37°C by using a thermostatically controlled heating blanket. Neosporin ointment was placed in the eyes to avoid damage to the corneas. A mixture of nitrous oxide and oxygen (2:1) and halothane (1–2%) was administered throughout the surgical procedure to maintain a deep level of anesthesia. Using aseptic technique, a midline scalp incision was made and the wound margins were infused with a long-lasting local anesthetic (bupivacaine). The scalp and muscles were pulled back to expose the bone over the parietal lobes. Small craniotomies were made in the appropriate locations and the dura was reflected. Following the injection of tracer substances (described below), the dura was replaced, the openings in the skull were filled with Gelfoam, and the scalp was sutured. The animals were then given injections of antibiotics and allowed to recover from surgery.

Injections of WGA-HRP, Fast Blue, DiI, and Fluoro-Ruby

Injections of WGA-HRP were made into the LGN in six cases. The WGA-HRP injections were made by iontophoresis (2–5 μA, pulsed for 30–60 min) through glass micropipettes 5–20 μm in diameter. The WGA-HRP (Sigma Chemical Co., St. Louis, MO; 5%) was dissolved in 0.9% saline. In some cases, the solution also contained 0.1% poly-L-ornithine (Sigma Chemical Co., St. Louis, MO), which acts to limit the size of the injection site (Hadley & Trachtenberg, 1978).

Pressure injections of Fast Blue (Illing GmbH and Co., Germany; 7% in deionized H_2O) or DiI (Molecular Probes, Eugene, OR; 10% in dimethyl sulfoxide) were made in two cases each. Injections were made through micropipettes 30–50 μm in diameter that were attached to the end of a 1.0-μl Hamilton syringe. Injection volumes for both tracers ranged from 0.2–0.3 μl. Injections of Fluoro-Ruby (tetramethylrhodamine dextran amine; Molecular Probes, Eugene, OR; 10% in saline) were made in two cases by iontophoresis (3 μA, pulsed for 30 min) through glass micropipettes 15–20 μm in diameter.

For all injections, the LGN was initially located using stereotaxic coordinates. Multiunit responses of LGN neurons to stimulation of the ipsilateral and contralateral eye were then used to position the pipettes in specific layers. These recordings were made through the injection pipette or through another pipette containing 2 M NaCl.

Following a 2–4 day survival for the WGA-HRP experiments and a 4–14 day survival for the fluorescent tracer experiments, animals were sacrificed with an overdose of sodium pentobarbital (80 mg/kg; i.p.). Following cessation of breathing, animals were perfused with saline followed by either 4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4 (PB) or 2.5% paraformaldehyde and 0.2% glutaraldehyde in PB.

Histology

For the WGA-HRP experiments, the striate cortex and thalamus were cryoprotected by immersion in a 25% solution of sucrose in PB and then sectioned at 50 μm on a freezing microtome. Sections were processed for HRP using tetramethylbenzidine as the chromogen (Mesulam, 1977), then mounted onto gel-subbed slides, lightly counterstained with thionin, quickly dehydrated with alcohols, cleared with xylene, and coverslipped.

For the Fast Blue and DiI experiments, the striate cortex and thalamus were initially sectioned at 300 μm on a vibratome. These cases were part of experiments where the retrogradely labeled cells were intracellularly filled with the dye Lucifer Yellow (Fitzpatrick & Einstein, 1989). After the intracellular injection procedure, some of these thick sections were resectioned on a freezing microtome at 60 μm. Labeled cells in the thick or thin sections were examined by placing the sections on slides and coverslipping them in a drop of PB.

For the Fluoro-Ruby experiments, cryoprotected blocks of striate cortex and thalamus were sectioned at 50 μm on a freezing microtome and collected in PB. These sections were then mounted from PB onto gel-coated slides and allowed to dry.

Data analysis

WGA-HRP labeled neurons were examined with both bright- and dark-field illumination. Fast Blue, DiI, and Fluoro-Ruby labeled neurons were examined with epifluorescent illumination using rhodamine and UV filter cubes. Drawings of the locations of labeled neurons from representative sections were made using a camera lucida and a 20× or 63× objective.

The sublaminar distribution of labeled cells following injections of WGA-HRP into different LGN layers was quantified using a videometrics M-100 graphics system attached to an IBM PC. For selected samples, we measured the distance (perpendicular to the surface) from the center of each labeled cell to the layer 5/6 border. To adjust for variation in the thickness of layer 6 in different sections and in different animals, this value was standardized by dividing it by the total thickness of layer 6 in the same section and multiplying by 100. This gives a figure that represents the location of the cell expressed as a percentage of the total distance from the top of the layer (0% represents the layer 5/6 border, 100% represents the layer 6 white matter border).

The distribution of corticogeniculate neurons across the depth of layer 6 was analyzed in one animal with a large injection of WGA-HRP into the LGN by plotting the location of all of the labeled cells in a 2-mm-wide sample through the region of cortex that appeared to contain the highest density of labeled cells. The total distribution of neurons in layer 6 (both labeled and unlabeled) was examined in this same animal by using a 63× oil-immersion objective to draw all of the neurons in a 730-μm-wide sample through the same region of cortex. This sample was also used to estimate the percentage of layer 6 neurons that project to the LGN.

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Results

Distribution of corticogeniculate neurons in layer 6

Injections of WGA-HRP into the lateral geniculate nucleus resulted in the retrograde labeling of cell bodies in layer 6 and anterograde labeling of axon terminals in the overlying cortical layers. Because the laminar pattern of projections from the LGN to the striate cortex has been well described in the macaque (Hubel & Wiesel, 1972; Hendrickson et al., 1978; Blasdel & Lund, 1983), the pattern of anterograde labeling in the cortex could be used to confirm which layers of the LGN had been involved in the injection site. This turned out to be especially useful for evaluating large injection sites which involved all layers of the LGN. These injections inevitably include topographically matched parts of the magnocellular and parvocellular layers, but they often extend into nonmatching parts as well. The pattern of anterograde label in the striate cortex makes it possible to distinguish these regions: some regions will show a "complete" pattern of anterograde label that includes the targets of all LGN relay cells, while others show only part of this pattern, that associated with either the magnocellular or parvocellular terminal distribution.

Fig. 1 shows an example of the distribution of labeled cells and terminals in striate cortex following a large injection that involved all layers of the LGN. This region of the cortex was chosen for illustration because the pattern of labeled terminals indicates that all of the LGN layers that innervate this region have been included in the injection site; i.e. a thick dark band of labeled terminals fills the depth of layer 4C; a thin discontinuous ribbon of labeled terminals is present in layer 4A; and under dark-field illumination, discrete patches of labeled terminals are present in layer 3. Within layer 6, labeled cells were found in two distinct tiers: an upper tier that lies adjacent to the layer 5–6 border and a lower tier that lies in the deepest part of layer 6. These two tiers are separated by a zone that is largely free of labeled cells.

Line drawings of the injection site and a plot of the distribution of labeled cells in layer 6 resulting from this injection are shown in Fig. 2A. Fig. 2B shows in quantitative form the relative numbers of labeled neurons located at different distances from the top of layer 6. To generate this histogram, we chose a 2-mm sample of layer 6 and measured the distance of each labeled cell from the top of layer 6. These values were then divided by the total thickness of layer 6, multiplied by 100 and binned in 5% increments. The distribution of corticogeniculate neurons is strongly biased towards the edges of layer 6 forming two well-defined peaks, one in the upper third and the other in the lower third of the layer. Together the upper and lower thirds of layer 6 contain over 90% of the labeled corticogeniculate neurons. Similar patterns of label were found in two other cases with large injections of WGA-HRP involving both the magnocellular and parvocellular layers of the LGN.

The distribution of corticogeniculate neurons differs markedly from the total distribution of neurons in layer 6. Fig. 2C shows the distribution of all neurons (labeled and unlabeled) that were present in a 730-μm-wide sample through the depth of layer 6. This distribution is broad and relatively flat over the middle third of the layer and declines near the edges. As a result, corticogeniculate cells appear to be most numerous in those parts of layer 6 that have relatively low cell densities. This point is especially clear for the lower tier: in counterstained sections the lower tier of corticogeniculate neurons often stood out as a cell-sparse zone lying below the bulk of layer 6.

Since corticogeniculate neurons are absent from the middle of layer 6 and they are most abundant in the regions with lower cell density, it follows that they make up a relatively small percentage of the total number of layer 6 neurons. In our sample of 464 layer 6 neurons, 62 were labeled by transport of WGA-HRP from the LGN, suggesting that corticogeniculate neurons make up roughly 13% of the layer 6 neurons.

Similar distributions of labeled neurons were found following large injections of the fluorescent tracers Fast Blue and DiI into the LGN. Labeled cells were found at the top and bottom of layer 6 while the middle of layer 6 was conspicuously free of labeled cells. Because these cases were used for intracellular filling experiments, and during this procedure the label faded, no attempt was made to quantify the distributions. However, these observations indicate that the absence of labeled neurons in the middle of layer 6 cannot be attributed to some failure of these neurons to incorporate or transport WGA-HRP.

Comparison of the distribution of layer 6 cells projecting to the magnocellular and parvocellular layers of the LGN

Injections of WGA-HRP that involved the magnocellular layers while sparing the parvocellular layers labeled cells that were almost entirely restricted to the lower tier of layer 6. This pattern was noted in the large-injection cases in regions of the cortex where only the target of the magnocellular layers (4Ca) was labeled, and in two cases where the injection site was smaller and largely restricted to the magnocellular layers. Fig. 3A shows the injection site and the distribution of layer 6 cells labeled from one of these cases. The histogram presented in Fig. 3B shows that the peak of this distribution corresponds in depth to the lower tier of cells that was labeled following large injections involving all layers of the LGN.

Injections of WGA-HRP that involved the parvocellular layers while sparing the magnocellular layers labeled cells in both the upper and lower tiers of layer 6. This pattern was observed in the large-injection cases in regions of the cortex where only the targets of the parvocellular layers (4Cb and 4A) contained labeled terminals and in three additional cases with injections restricted to the parvocellular layers. Fig. 4A shows the injection site and the distribution of labeled cells from a case with a small injection of WGA-HRP centered in parvocellular layer 4. The histogram presented in Fig. 4B shows that the majority of the labeled cells were located in the upper tier; roughly 25% were located in the lower tier. The other two restricted parvocellular injections were made with the fluorescent tracer Fluoro-Ruby. One of these was centered in LGN layer 4, the other in LGN layer 6. Both injections produced similar patterns of labeled cells in cortical layer 6.

Discussion

These results demonstrate that layer 6 of macaque striate cortex is divided into three distinct tiers: two tiers near the edges of layer 6 that contain the bulk of the corticogeniculate population and a central tier that is largely devoid of LGN projecting neurons. This organization of LGN projecting neurons seems to be unique to Old World primates; it is not present in the New World species (squirrel or owl monkey) nor is it found in prosimians (Fitzpatrick et al., 1983; Diamond et al., 1985; Conley

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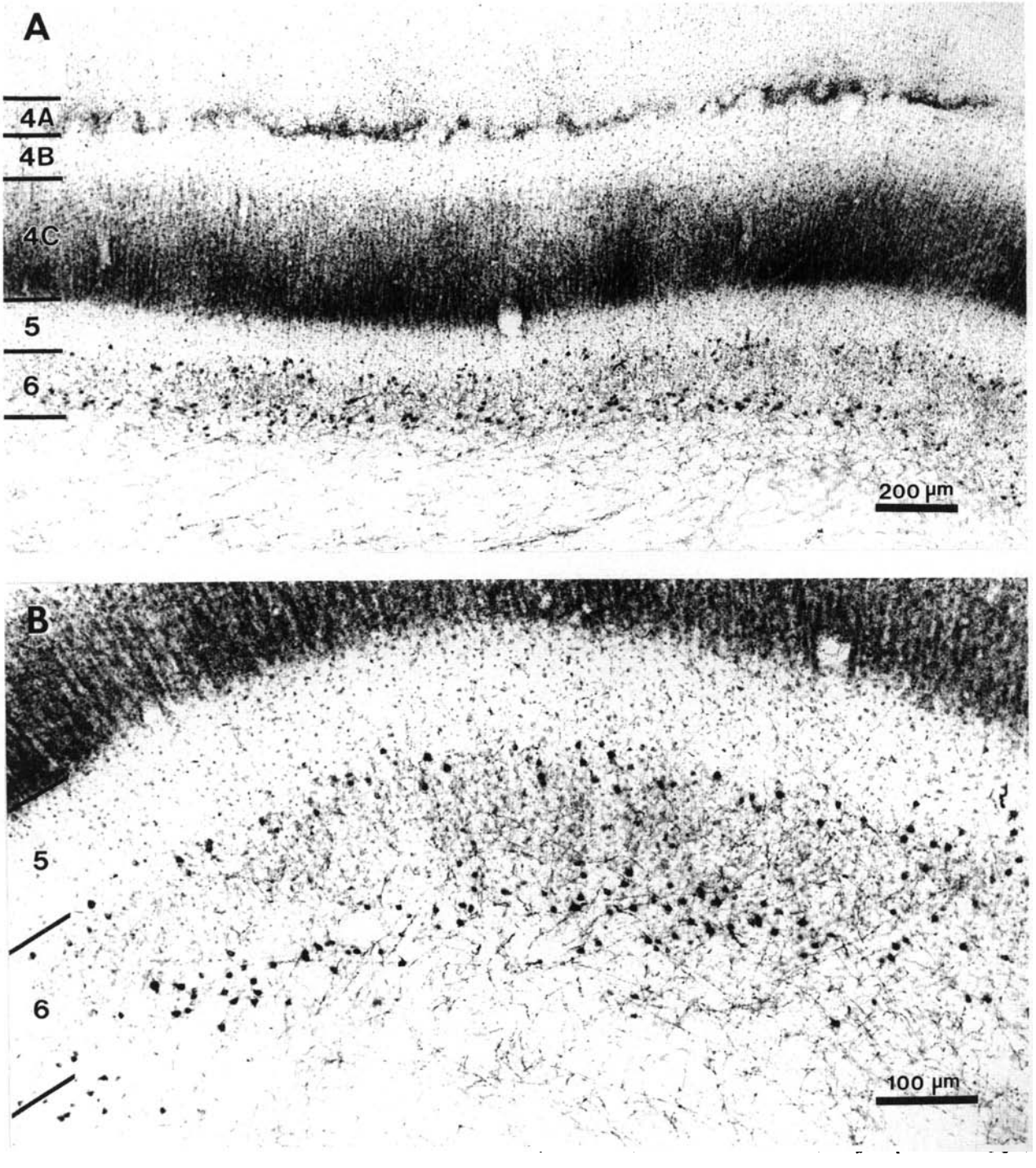

Fig. 1. Photomicrograph of labeled corticogeniculate neurons and axon terminals in striate cortex following a large injection of WGA-HRP that involved all layers of the LGN. A: Retrograde-labeled neurons are located in the upper and lower thirds of layer 6. Anterograde-labeled axon terminals are present throughout layers 4C and 4A. Under darkfield illumination, patches of labeled terminals could also be visualized in layers 2 and 3. B: High-power view of the distribution of labeled corticogeniculate neurons across the depth of layer 6. Labeled neurons are restricted to the upper and lower thirds of layer 6 and appear absent from the middle.

& Raczkowski, 1990). In the following paragraphs, we consider the functional implications of this tripartite subdivision and the relation between the two tiers of corticogeniculate neurons and the magnocellular and parvocellular layers of the LGN.

A tripartite subdivision of layer 6

The paucity of corticogeniculate neurons in the middle of layer 6 indicates that these neurons send their axons to some other

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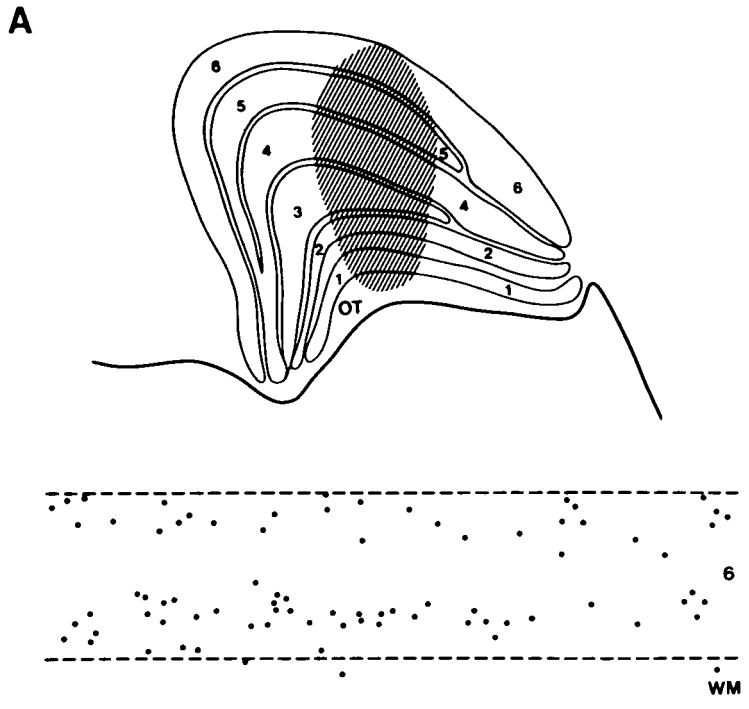

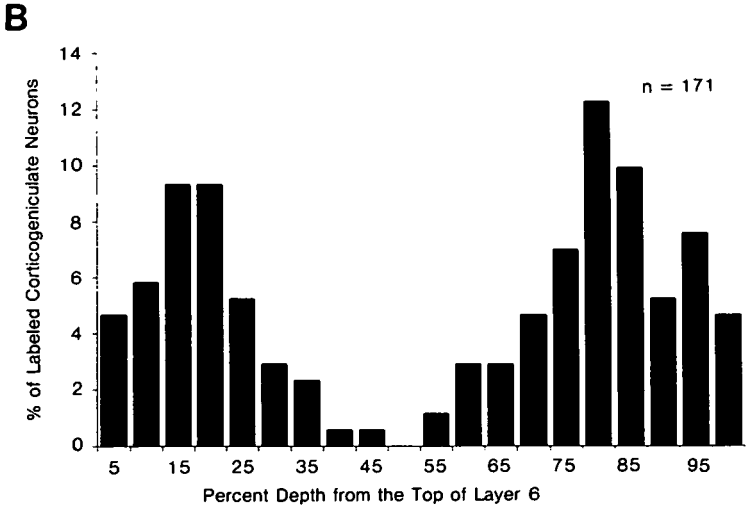

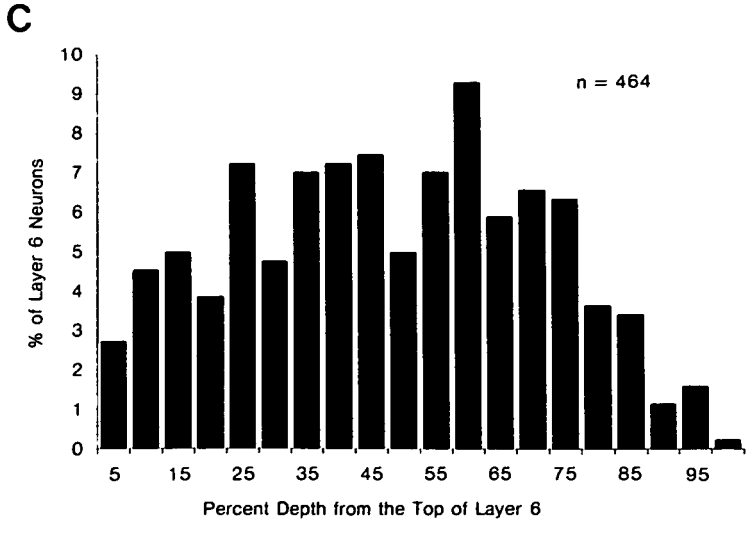

Fig. 2. Line drawing of a WGA-HRP injection that involved all layers of the LGN and the distribution of labeled corticogeniculate neurons across the depth of layer 6 of striate cortex. A: The injection site included every layer of the LGN and labeled neurons were found in both the upper and lower thirds of layer 6 with the middle of layer 6 free of labeled neurons. B: Histogram showing the distribution of labeled neurons as a function of depth. The horizontal axis represents the distance from the layer 5/6 border expressed as a percentage of the total width of layer 6. The vertical axis represents the number of labeled neurons expressed as a percentage of the total sample (171 neurons). The distribution of corticogeniculate neurons has two peaks at the outer edges of layer 6. C: Histogram showing the total distribution of neurons in layer 6 (labeled and unlabeled) as a function of depth. Conventions are the same as those in B. On the whole, neurons in layer 6 have a rather broad even distribution through the middle, decreasing in number at the edges. There is no hint of the bimodal distribution of corticogeniculate neurons in the overall distribution of layer 6 neurons.

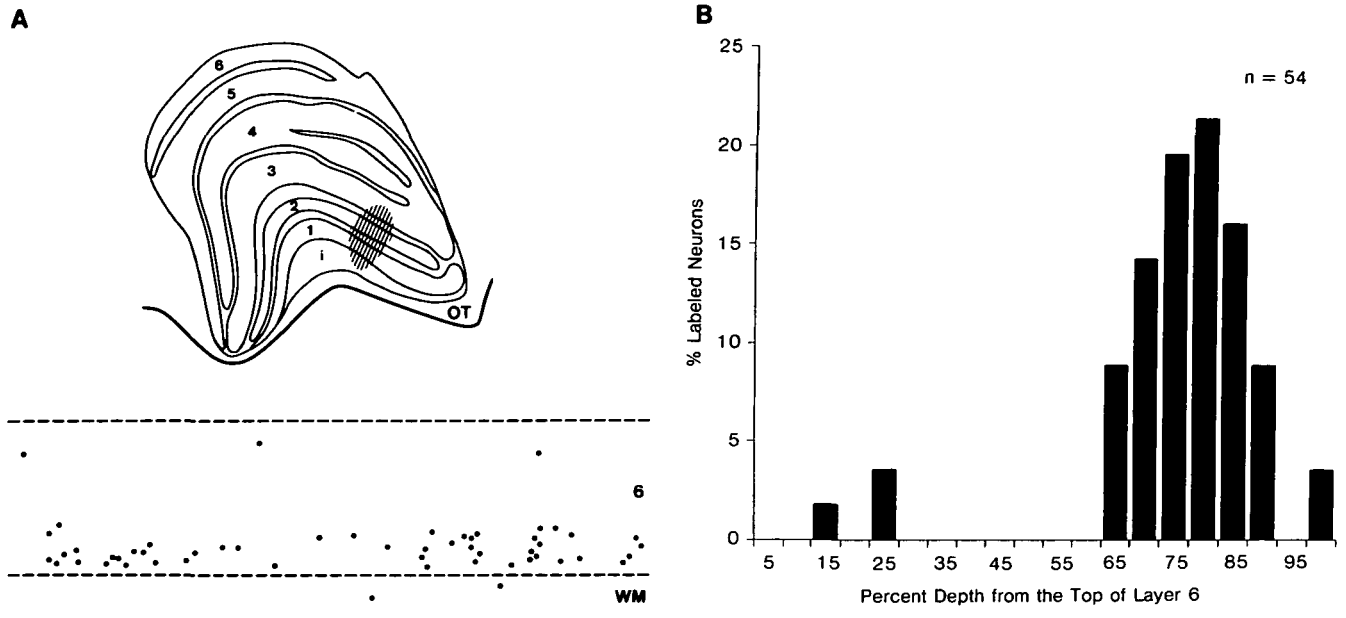

Fig. 3. Line drawing of a WGA-HRP injection that was centered in the magnocellular layers of the LGN and the distribution of labeled corticogeniculate neurons across the depth of layer 6 of striate cortex. A: The injection site included LGN magnocellular layers 1 and 2 and encroached slightly upon parvocellular layer 3. Almost all of the labeled neurons were located in the bottom of layer 6. B: Histogram of the distribution of corticogeniculate neurons across the depth of layer 6. The peak of this distribution corresponds to the lower tier of labeled cells seen after large injections of WGA-HRP involving all LGN layers.

target; the question is where? One obvious possibility is the claustrum. The projections to the claustrum and the LGN have been shown to originate from separate populations of layer 6 neurons in other species and, in the cat at least, claustral projections arise from cells that tend to lie in the middle of the layer (Carey et al., 1980; LeVay & Sherk, 1981; Katz, 1987). Perhaps the separation of claustrum- and LGN-projecting neurons in the macaque is more complete than that in the cat where the

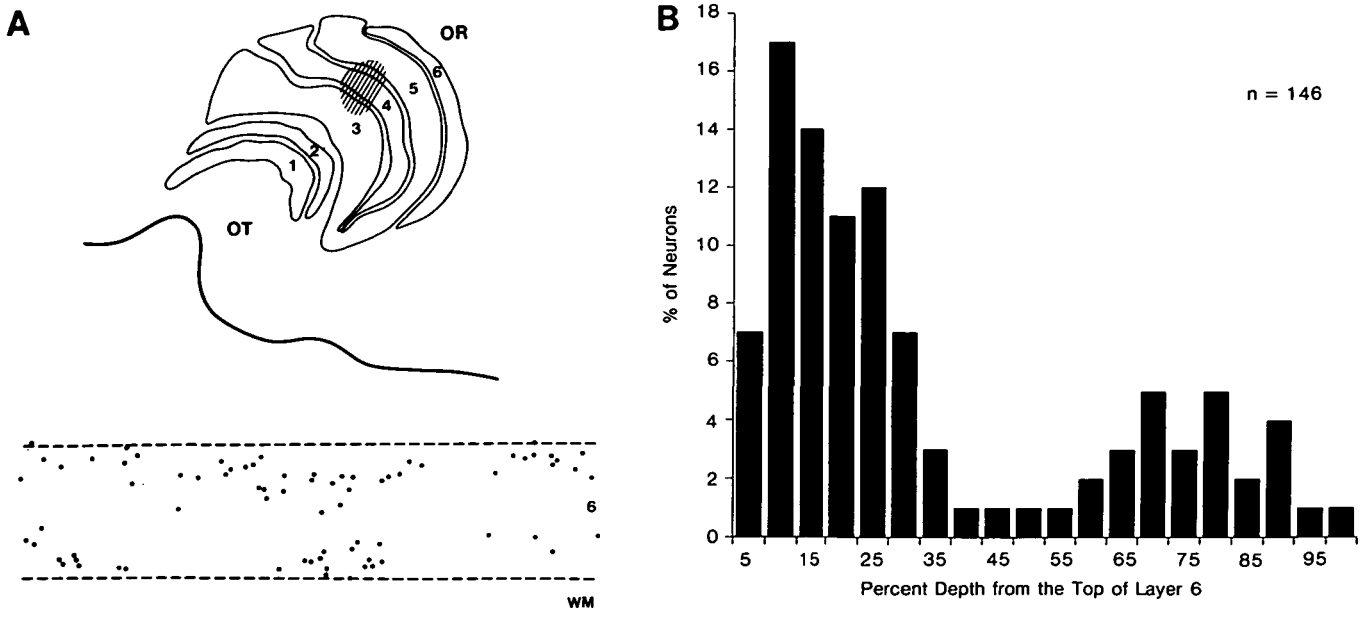

Fig. 4. Line drawing of a WGA-HRP injection that was centered in the parvocellular layers of the LGN and the distribution of labeled corticogeniculate neurons across the depth of layer 6 of striate cortex. A: The injection site was centered in layer 4 and spread to layers 3 and 5. While most of the labeled neurons are located in the upper third of layer 6, a substantial number are also present in the lower third. B: Histogram showing the distribution of corticogeniculate neurons across the depth of layer 6. Labeled neurons are located primarily (71%) in the upper third of layer 6. However, the bottom third of layer 6 also contains a significant number of labeled neurons (24%).

LGN-projecting population extends evenly throughout the depth of layer 6.

However, even if claustrum-projecting cells are found to occupy the middle of layer 6 in macaques, it seems unlikely that they would account for all of the pyramidal cells in this region. In the cat, claustrum-projecting cells make up only 3.5–5% of the neurons in layer 6 (LeVay & Sherk, 1981; Katz, 1987), far less than the percentage of cells that occupy the middle third of layer 6 (see Fig. 2). Other known targets for neurons in layer 6 of macaque are cortical areas V2 and MT (Maunsell & Van Essen, 1983; Fries et al., 1985; Kennedy & Bullier, 1985), but the neurons projecting to these targets are sparse and are found primarily in the upper part of layer 6. Recent work in the prosimian Galago has raised the possibility that some layer 6 neurons may be a source of projections to the pulvinar nucleus of the thalamus (Conley & Raczkowski, 1990), but whether this pathway exists in the macaque remains unknown. It is not even clear that neurons in the middle of layer 6 are projection neurons: a large percentage of the pyramidal neurons in the middle of layer 6 may be local circuit neurons. While pyramidal cells, as a class, are generally regarded as projection neurons, pyramidal cells with strictly local axon arbors have been described in the visual cortex of the monkey as well as the cat (Lund & Boothe, 1975; Martin & Whitteridge, 1984; Katz, 1987; Lund, 1988).

Whether they are local circuit neurons or not, there is no doubt that neurons in the middle of layer 6, like those at the edges, give rise to intrinsic axon arbors that terminate in the cortical layer 4C (Lund & Boothe, 1975; Fitzpatrick et al., 1985). As such, one of the most important functional implications of the tripartite subdivision of layer 6 is that a large portion of the excitatory input to layer 4C from layer 6 derives from neurons that do not project to the LGN. This is a significant departure from what is found in other species, where most of the layer 4 projections arise as collaterals from the axons of corticogeniculate neurons (Katz, 1987; Usrey & Fitzpatrick, 1993*a*,*b*). Perhaps in the evolution of higher primates, the role of layer 6 in modulating the transfer of activity from the LGN to layer 4 has diversified, requiring a pathway from layer 6 to layer 4 that is independent of the corticogeniculate system. If so, then one might expect to find some differences in the laminar and/or synaptic organization of the layer 4 projections that originate from cells in the middle and at the edges of layer 6.

Another issue related to the tripartite subdivision of layer 6 is the small percentage of layer 6 neurons that actually contribute to the corticogeniculate pathway. Although one cannot be certain that even a large tracer injection labels all of the corticogeniculate neurons in a given region of striate cortex, we estimate that corticogeniculate neurons comprise roughly 13% of the neurons in layer 6 of the macaque, far less than the estimate of 50% for corticogeniculate neurons in the cat (Gilbert & Kelly, 1975; Katz, 1987) and 35% in the prosimian Galago (Conley & Raczkowski, 1990). At first glance, this finding might be interpreted as a sign of a relatively diminished role for the corticogeniculate pathway in higher primates. However, such comparisons can be misleading because they fail to take into account other factors that differ across species such as the total number of neurons in layer 6, the number of LGN neurons available for contact, the amount of divergence of individual corticogeniculate axon arbors within the LGN, and the total number of synapses on a single LGN neuron. It seems fair to conclude that feedback to the LGN is only one of the contributions that layer 6 neurons make to visual processing. The other contributions, whatever they might be, appear to occupy a larger proportion of layer 6 neurons in the macaque than in other species.

Sublaminar organization of projections to the magnocellular and parvocellular layers of the LGN

With regard to the projections to the magnocellular and parvocellular layers of the LGN, our results confirm the earlier work suggesting that the upper part of layer 6 contains neurons that project primarily to the parvocellular layers of the LGN (Lund et al., 1975). Indeed, our results indicate that the upper third of layer 6 contains neurons that project almost exclusively to the parvocellular layers.

The projections of neurons in the lower tier are more difficult to assess. Clearly, almost all of the projections to the magnocellular layers of the LGN arise from neurons in this lower tier; and yet there were a significant number of neurons in this tier after injections that were restricted to the parvocellular layers. It seems unlikely that the labeling in the lower tier after parvocellular layer injections can be dismissed as an artifact produced by labeling damaged fibers bound for the magnocellular layers. It has been our experience that WGA-HRP, unlike HRP alone, is not readily taken up by fibers of passage and we found no signs of fiber of passage labeling in the distribution of LGN terminal fields in the striate cortex. Injections of WGA-HRP involving the parvocellular layers labeled terminals in layer 4Cβ and 4A, leaving 4Cα, the target of the magnocellular layers, terminal free.

Since we did not perform double-label experiments, we cannot say whether any of the neurons in the lower tier of layer 6 send collaterals to both the magnocellular and parvocellular layers. However, we think it is unlikely that *all* of the neurons in the lower tier give rise to collaterals that terminate in both LGN subdivisions. Following injections of tracers that involve all layers of the LGN, almost equal numbers of neurons were labeled in the upper and lower tiers. In contrast, following injections that were restricted to the parvocellular layers, the number of labeled cells in the lower tier dropped to roughly one-third the number in the upper tier. Thus, many of the corticogeniculate neurons in the lower tier are not labeled after injections of the parvocellular layers, suggesting that they project exclusively to the magnocellular layers.

The intercalated layers: another potential target for corticogeniculate fibers

This discussion has focused on comparisons of the magnocellular and parvocellular layers as if these were the only potential targets for corticogeniculate axon terminals. There is, however, another potential target that was included in the injection site when either LGN subdivision was injected: the small cells that occupy the interlaminar zones within the parvocellular layers and those that lie on either side of the magnocellular layers (Guillery & Colonnier, 1970; Kaas et al., 1978). While they are often overshadowed by the larger and darker staining neurons in the principal layers, results in New World primates and in prosimians indicate that these intercalated neurons deserve the same status as those in the principal layers in as much as they relay information from a distinct class of retinal ganglion cells to the striate cortex (Itoh et al., 1982; Norton & Casagrande,

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1982). Unlike their counterparts in the main layers that send their axons to terminate in cortical layer 4, those in the intercalated layers terminate in layer 3, and constitute the principal source of thalamic inputs to the cytochrome-oxidase rich blobs (Fitzpatrick et al., 1983; Weber et al., 1983; Diamond et al., 1985; Lachica & Casagrande, 1992).

There seems little doubt that the small cells that make up the intercalated layers are targets of cortical axon terminals. These regions are heavily labeled following injections of anterograde tracers into striate cortex in prosimians and New World monkeys (Lin & Kaas, 1977; Symonds & Kaas, 1978) and profiles with the ultrastructural characteristics of cortical terminals are the most abundant type of profile in the macaque interlaminar zones (Wilson & Hendrickson, 1981). In recent studies of the corticogeniculate pathway in the tree shrew, where we have been able to reconstruct the terminal fields of individual axons, we found that the projections to the interlaminar zones and to the main layers arise from separate populations of layer 6 cells (Usrey & Fitzpatrick, 1992, 1993*b*). Furthermore, neurons giving rise to projections to the interlaminar zones appear to be concentrated in the deepest parts of layer 6. Thus, it is tempting to suggest an alternative explanation for the cells that are labeled in the lower tier of layer 6 following injections into the parvocellular layers: these neurons may represent a separate class of corticogeniculate neuron that selectively contacts the small cells surrounding the magnocellular and parvocellular layers. If so, then each of the ascending pathways that relays through the LGN could be the recipient of a separate descending pathway.

Sources of inputs to corticogeniculate neurons

Ultimately, the significance of these two tiers of corticogeniculate neurons rests not only on differences in their LGN targets, but on their sources of inputs. Evidence that parvocellular and magnocellular axons terminate in the upper and lower parts of layer 6, respectively, suggests a selectivity in the pattern of inputs that is matched, to some degree, with that of the outputs (Hendrickson et al., 1978; Blasdel & Lund, 1983). However, one should not conclude from this that neurons in the upper and lower tiers are driven exclusively by one or the other ascending pathway. LGN projections to layer 6 are relatively sparse and the sublaminar distribution of terminals from other sources, especially layer 4C, is largely unknown (Lund, 1988). Furthermore, corticogeniculate neurons are not limited to these two tiers for their inputs. The basilar dendritic processes of neurons in the upper tier extend well into the middle third of layer 6, and the apical dendrites of neurons in the lower tier rise through the upper tier on their way to more superficial layers (Fitzpatrick & Einstein, 1989). Also, both populations of neurons have apical dendrites that sample from more superficial layers, especially layer 5. Thus, in order for each tier to be under the influence of a single ascending pathway would require a high degree of specificity in dendritic sampling, both inside and outside of layer 6. It may well be that the functional properties of neurons in each tier reflect the integration of information from both the magnocellular and parvocellular streams.

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References

Blasdel, G. & Lund, J.S. (1983). Termination of afferent axons in macaque striate cortex. *Journal of Neuroscience* **3**, 1389–1413.

Carey, R.G., Bear, M.F. & Diamond, I.T. (1980). The laminar organization of the reciprocal projections between the claustrum and striate cortex in the tree shrew, *Tupaia glis*. *Brain Research* **184**, 193–198.

Conley, M. & Raczkowski, D. (1990). Sublaminar organization within layer VI of the striate cortex in *Galago*. *Journal of Comparative Neurology* **302**, 425–436.

DeYoe, E.A. & Van Essen, D.C. (1988). Concurrent processing streams in monkey visual cortex. *Trends in Neuroscience* **11**, 219–226.

Diamond, I.T., Conley, M., Itoh, K. & Fitzpatrick, D. (1985). Laminar organization of geniculocortical projections in *Galago senegalensis* and *Aotus trivirgatus*. *Journal of Comparative Neurology* **242**, 584–610.

Fitzpatrick, D. & Einstein, G. (1989). Laminar distribution and morphology of area 17 neurons projecting to the lateral geniculate nucleus in the macaque. *Society for Neuroscience Abstracts* **15**, 1398.

Fitzpatrick, D., Itoh, K. & Diamond, I.T. (1983). The laminar organization of the lateral geniculate body and the striate cortex in the squirrel monkey (*Saimiri sciureus*). *Journal of Neuroscience* **3**, 673–702.

Fitzpatrick, D., Lund, J.S. & Blasdel, G.G. (1985). Intrinsic connections of macaque striate cortex. Afferent and efferent connections of lamina 4C. *Journal of Neuroscience* **5**, 3329–3349.

Fries, W., Keizer, K. & Kuypers, H.G.J.M. (1985). Large layer VI cells in macaque striate cortex project to both superior colliculus and prestriate visual area V5. *Experimental Brain Research* **58**, 613–616.

Gilbert, C.D. & Kelly, J.P. (1975). The projections of cells in different layers of the cat's visual cortex. *Journal of Comparative Neurology* **163**, 81–106.

Guillery, R.W. & Colonnier, M. (1970). Synaptic patterns in the dorsal lateral geniculate nucleus of the monkey. *Zeitschrift für Zellforschung* **103**, 90–108.

Hadley, R.T. & Trachtenberg, M.C. (1978). Poly-l-ornithine enhances the uptake of horseradish peroxidase. *Brain Research* **158**, 1–14.

Hendrickson, A.E., Wilson, J.R. & Ogren, M.P. (1978). The neuroanatomical organization of pathways between the dorsal lateral geniculate nucleus and visual cortex in Old World and New World primates. *Journal of Comparative Neurology* **182**, 123–136.

Hubel, D.H. & Wiesel, T.N. (1972). Laminar and columnar distribution of geniculo-cortical fibers in the macaque monkey. *Journal of Comparative Neurology* **146**, 421–450.

Itoh, K., Conley, M. & Diamond, I.T. (1982). Retinal ganglion cell projections to different layers of the lateral geniculate body in *Galago crassicaudatus*. *Journal of Comparative Neurology* **205**, 282–290.

Kaas, J.H., Huerta, M.F., Weber, J.T. & Harting, J.K. (1978). Patterns of retinal terminations and laminar organization of the lateral geniculate nucleus of primates. *Journal of Comparative Neurology* **182**, 517–554.

Katz, L.C. (1987). Local circuitry of identified projection neurons in cat visual cortex brain slices. *Journal of Neuroscience* **7**, 1223–1249.

Kennedy, H. & Bullier, J. (1985). A double-labeling investigation of the afferent connectivity to cortical areas V1 and V2 of the macaque monkey. *Journal of Neuroscience* **5**, 2815–2830.

Lachica, E.A. & Casagrande, V.A. (1992). Direct W-like geniculate projections to the cytochrome oxidase (CO) blobs in primate visual cortex: axon morphology. *Journal of Comparative Neurology* **319**, 141–158.

LeVay, S. & Sherk, H. (1981). The visual claustrum of the cat. I. Structure and connections. *Journal of Neuroscience* **1**, 956–980.

Lin, C.S. & Kaas, J.H. (1977). Projections from cortical visual areas 17, 18, and MT onto the dorsal lateral geniculate nucleus in owl monkeys. *Journal of Comparative Neurology* **173**, 457–474.

Livingstone, M. & Hubel, D. (1988). Segregation of form, color, movement, and depth: Anatomy, physiology, and perception. *Science* **240**, 740–749.

Lund, J.S. (1988). Anatomical organization of macaque monkey striate visual cortex. *Annual Review of Neuroscience* **11**, 253–288.

Lund, J.S. & Boothe, R. (1975). Interlaminar connections and pyramidal neuron organization in the visual cortex, area 17, of the macaque monkey. *Journal of Comparative Neurology* **159**, 305–334.

Lund, J.S., Lund, R.D., Hendrickson, A.E., Bunt, A.H. & Fuchs, A.F. (1975). The origin of efferent pathways from the primary visual

cortex, area 17, of the macaque monkey. *Journal of Comparative Neurology* **164**, 287–304.

MARTIN, K.A.C. & WHITTERIDGE, D. (1984). Form, function and intracortical projections of spiny neurones in the striate visual cortex of the cat. *Journal of Physiology* **353**, 463–504.

MAUNSELL, J.H.R. & VAN ESSEN, D.C. (1983). The connections of the middle temporal visual area (MT) and their relationship to a cortical hierarchy in the macaque monkey. *Journal of Neuroscience* **3**, 2563–2586.

MERIGAN, W.H. & MAUNSELL, J.H.R. (1993). How parallel are the primate visual pathways? *Annual Review of Neuroscience* **16**, 369–402.

MESULAM, M.-M. (1977). Differential sensitivity between blue and brown reaction products of HRP neurohistochemistry. *Neuroscience Letters* **5**, 7–14.

NORTON, T.T. & CASAGRANDE, V.A. (1982). Laminar organization of receptive-field properties in the lateral geniculate nucleus of bush-baby (*Galago crassicaudatus*). *Journal of Neurophysiology* **47**, 715–741.

SYMONDS, L.L. & KAAS, J.H. (1978). Connections of striate cortex in the prosimian *Galago senegalensis*. *Journal of Comparative Neurology* **181**, 477–512.

USREY, W.M. & FITZPATRICK, D. (1992). Intrinsic and extrinsic projections of neurons in layer VI of striate cortex: Anatomical evidence for selective influence on parallel geniculocortical pathways. *Society for Neuroscience Abstracts* **18**, 297.

USREY, W.M. & FITZPATRICK, D. (1993*a*). The sublaminar organization of layer VI projections to layer IV in tree shrew striate cortex. *Investigative Ophthalmology and Visual Science* (Suppl.) **34**, 1175.

USREY, W.M. & FITZPATRICK, D. (1993*b*). Parallel streams in the corticogeniculate pathway: Intrinsic and extrinsic projections of neurons in layer VI of striate cortex. In *Thalamic Networks for Relay and Modulation*, ed. MINCIACCHI, D., MOLINARI, M., MACCHI, G. & JONES, E.G. New York: Pergamon Press (in press).

WEBER, J.T., HUERTA, M.F., KAAS, J.H. & HARTING, J.K. (1983). The projections of the lateral geniculate nucleus of the squirrel monkey: Studies of the interlaminar zones and the S layers. *Journal of Comparative Neurology* **213**, 135–145.

WILSON, J.R. & HENDRICKSON, A.E. (1981). Neuronal and synaptic structure of the dorsal lateral geniculate nucleus in normal and monocularly deprived *Macaca* monkeys. *Journal of Comparative Neurology* **197**, 517–539.

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