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The Morphology of the Koniocellular Axon Pathway in the Macaque Monkey

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The key objective of this study was to determine the distribution and morphology of koniocellular (K) lateral geniculate nucleus (LGN) axons in primary visual cortex (V1) of the macaque monkey. In particular, we were interested in understanding whether subpopulations of K axons exist in this species and, if so, if these subpopulations arise from different K layers of the LGN. Restricted injections of the tracers, biotinilated dextran amine, or *Phaseolus vulgaris* leucoagglutinin were targeted to specific LGN K layers under electrophysiological guidance and immunocytochemistry was used to visualize labeled axons in cortex that were subsequently reconstructed through serial sections. A total of 36 complete axons and 166 axon segments were reconstructed. Our results identified at least 2 main subpopulations of K axons in macaque V1 based on branching patterns and bouton distribution. Axons that arise primarily from LGN layers K1 and K2 are morphologically simple and tend to branch in cortical layers 1 and 3A. These axons give rise to fewer boutons than seen in axons arising from the dorsal K LGN layers K3–K6. Axons that arise from LGN layers K3–K6 terminate as complex, focused arbors in the cytochrome oxidase (CO) blobs in layer 3Bα, with only occasional simple projections to the more superficial layers of cortex. Combined with previous observations, our data suggest that there are at least 3 subclasses of K LGN axons in macaque monkey that are similar to K axons identified earlier in both nocturnal simian owl monkeys (Ding and Casagrande 1997) and in prosimian, bush babies (Lachica and Casagrande 1992) suggesting that the LGN K channels that terminate in the CO blobs and in layer 1 are not unique to macaque monkeys but are a common primate feature.

Keywords: geniculocortical pathway, lateral geniculate nucleus (LGN), parallel pathway, primate, striate cortex

Introduction

In studying the visual system of primates, much attention has been focused on the idea of parallel processing of visual information. Starting at the level of the retina, different aspects of the visual scene are channeled separately through different classes of retinal ganglion cells to lateral geniculate nucleus (LGN) layers and then to the first synapse in primary visual cortex or striate cortex (referred to henceforth as V1) (Casagrande and Xu 2004). Substantial evidence in macaque monkeys supports the idea that the magnocellular (M) and parvocellular (P) LGN cells receive axonal input from separate classes of retinal ganglion cells, referred to as parasol and midget ganglion cells, respectively (Watanabe and Rodieck 1989).

Parasol and midget ganglion cells are not the only ganglion cells that send axons to the LGN in macaque monkeys. Large injections of retrograde tracers into the LGN have revealed that at least 8 other ganglion cell classes are labeled and therefore must provide visual information via LGN to cortex (Rodieck and Watanabe 1993; Dacey et al. 2003). At the level of the LGN other cell classes besides M and P cells have been distinguished neurochemically and morphologically in macaque monkeys and in other primates by immunostaining for the alpha subunit of calmodulin-dependent protein kinase 2 (αCAMKII) or the calcium-binding protein, calbindin (Hendry and Yoshioka 1994; Johnson and Casagrande 1995; Hendry and Casagrande 1996; Goodchild and Martin 1998; Xu et al. 2001). These cells are referred to collectively as koniocellular (K) cells. In bush babies, owl monkeys, and squirrel monkeys tracer injections into these neurochemically defined LGN K layers have revealed populations of axons that terminate principally in the cortical layers above layer 4 (4C of Brodmann 1909), the primary target layer of P and M LGN axons (Fitzpatrick et al. 1983; Diamond et al. 1985; Lachica and Casagrande 1992; Ding and Casagrande 1997). Especially prominent in the latter studies was the projection from K LGN axons to the cytochrome oxidase (CO) blobs in layer 3B. Recent data in both macaque monkey and marmoset have suggested that a subset of K LGN axons carry chromatic signals from S cones but whether these particular axons terminate in the CO blobs remains unclear (Martin et al. 1997; White et al. 1998; Chatterjee and Callaway 2003). Because S cones do not exist in bush babies and owl monkeys, K axons to the CO blobs in these primates carry some other signals (Jacobs et al. 1996; Ding and Casagrande 1997). Presumably there are several different classes of K LGN cells, a conclusion supported by physiological studies in several primate species (Norton and Casagrande 1982; Irvin et al. 1986; White et al. 1998, 2001; Xu et al. 2001; see also Casagrande 1994; Hendry and Reid 2000 for review).

Taken together, the above findings raise significant questions about K LGN cells in macaque monkey. How many morphological types of K LGN cells are there? Do different LGN K layers have morphologically distinct axons in V1? Can we distinguish K LGN cell axons in V1 in trichromatic Old World macaque monkeys from K axons described in monochromatic primates? We aimed to address these questions in the present morphological study. Some of the results described here were reported earlier in abstract form (Casagrande et al. 1997).

Materials and Methods

Subjects

Tissue for this experiment was collected from both hemispheres of 2 adult macaque monkeys (*Macaca fascicularis*). All procedures involving these monkeys were done according to the *National Institutes of Health Guide for the Care and Use of Laboratory Animals* as well as

the guidelines and procedures of the Vanderbilt University Animal Care Committee using an approved protocol.

Surgical Procedures

All procedures were performed under aseptic conditions. Atropine sulfate (0.1 mg/kg) was given to the monkeys prior to surgery to inhibit salivation along with Dexamethasone (0.5 mg/kg) and Gentamicin (5.0 mg/kg) as an anti-inflammatory and antibiotic, respectively. The animals were then intubated and anesthetized using 3–4% isofluorane in oxygen, and maintained with the same gas mixture at 1–2%. A water circulating heating pad was used to keep the monkeys warm throughout surgery. In addition, heart and respiration rates were continuously monitored and reflexes were tested periodically. If there were any indications that the monkeys were not well anesthetized the percent of isofluorane was increased.

Once deeply anesthetized, the monkeys were secured in a stereotaxic apparatus. Retinal landmarks were plotted using a reversible ophthalmoscope on a tangent screen 57 cm distant and used to determine more precisely the location of tracer injections. After exposing the skull, a bilateral craniotomy was performed and an electrode, glued to a pipette filled with tracer (10% biotinilated dextran amine [BDA] in saline or 4% *Phaseolus vulgaris* leucoagglutinin [PHA-L] in 0.05 M phosphate buffer, both at pH 7.4), was lowered into the LGN based on stereotaxic coordinates. Layers of the LGN were identified by eye dominance shifts determined from visually evoked recorded activity to a flashing light. In each case, the center of the injection in one hemisphere was targeted to LGN layer K1 and in the other hemisphere to LGN K3. In one animal, PHA-L was used for both injections and in the other BDA was used for both injections.

After the K LGN layer was located tracers were injected iontophoretically through the pipette. PHA-L was injected at 1–2 μA for 10–15 min and BDA was injected at 2 μA for 10–15 min. After the injections were complete in both LGN hemispheres, the dural flaps were replaced and the skin was sutured. Postoperatively, the animals were given 0.02 mg/kg of an analgesic (Banamine) as needed and 300 000 units/kg of long-acting penicillin (Flocillin). The monkeys were carefully and continuously monitored until fully awake and were then given soft palatable foods and water.

Histology and Immunocytochemistry

After a 21-day survival period, the animals were deeply anesthetized with an overdose of Nembutal. They were then perfused transcardially first with a rinse of saline and then with a fixative containing 2% paraformaldehyde, 0.2% glutaraldehyde, and 0.2% saturated picric acid (v/v) in 0.1 M phosphate buffer (pH 7.4), followed with the same fixative containing 10% sucrose. A final rinse then followed with 0.05 M phosphate buffer (pH 7.4). The brains were allowed to equilibrate in a 30% sucrose buffer overnight at 4 °C, and then were frozen. Each visual cortex was cut parasagitally at 52 μm and the piece of thalamus containing the LGN was cut coronally at the same thickness.

Sections reacted to reveal the PHA-L label were incubated at 4 °C for 72 h in an antibody against PHA-L at a 1:2000 dilution. Sections were then rinsed 3 times in Tris-buffered saline (TBS) and then placed in a linking antibody (biotinylated rabbit anti-goat) at a 1:200 dilution in TBS with 0.5% Triton X 100 and 2% normal rabbit serum for 2.5 h at room temperature. Next the sections were placed in avidin–biotin Standard Elite, (ABC, Vector Laboratories, Burlingame, CA) in TBS plus 0.5% Triton X for 2.5 h, followed by 3, 5-min TBS rinses. Finally, the sections were incubated in the diaminobenzidine (DAB) chromagen (Sigma D-5637). The DAB reaction was heavy metal enhanced in a mixture of TBS, 0.02% DAB, 0.6% nickel ammonium sulfate, 0.05 M imidazole, and 0.0004% H_2O_2 according to the Tago et al. (1987) method.

For the BDA reaction, sections were rinsed initially in TBS and left overnight. Sections then were placed in ABC for 48 h at 4 °C. Finally, the sections were rinsed 3 times with TBS and reacted with DAB and enhanced using the same Tago method (see above).

Some labeled sections in both cases were subsequently counterstained with 1% Cresyl Violet to reveal layers or were reacted for CO in order to see termination patterns of K axons in relation to the CO blobs and the layers of V1 using methods described previously (Boyd and Matsubara 1996; Ding and Casagrande 1997).

Reconstruction of Axons and Analysis

Axons were selected for reconstruction after examination of all V1 sections in which any terminal label could be identified. Selected axons met the following criteria: 1) the axon appeared to be completely filled with obvious boutons, 2) the axon could be traced both to its finest terminal branches and to the white matter through serial sections using blood vessels and other landmarks, and 3) the main terminals of the axonal branches were above layer 4 in sections that showed little label in layer 4 proper, suggesting that the injection location involved mainly a K layer.

Axons were reconstructed using a light microscope and *camera lucida* drawing tube. Low-magnification tracings (10× and 50×) were made first in order to determine the axon's location in cortex. Then, high-power 1200× (oil-immersion objective) drawings were made through serial sections making sure to include landmarks such as blood vessels and cortical layer boundaries by comparing high and low power drawings. Tracings were aligned on a light box using these landmarks and collapsed onto a single 2D drawing showing the complete axon. Axons were analyzed for bouton number and cortical layer or layers of termination and their morphologies were compared with other K axon morphologies published by our laboratory in owl monkeys and bush babies (Lachica and Casagrande 1992; Ding and Casagrande 1997). The extent of the injection sites in each LGN was also reconstructed through serial sections.

Figures 5 and 9 are photomontages that were created from multiple images taken at different focal planes (as many as 90 in some cases), opened in Photoshop in layers and the portions of each Photoshop layer that were not in focus erased to create an in-focus montage.

Reconstruction of Axonal Segments and Analysis

Given the difficulty inherent in confining injections to single K LGN layers without involvement of adjacent P or M layers while still obtaining completely filled axons, we also analyzed segments of filled axons in layers above layer 4 in one hemisphere where the injection in the LGN was large and involved parts of each LGN layer. For this analysis we took advantage of 5 points: 1) LGN axons innervate V1 of macaque monkey with tight topographic precision, 2) the LGN injection extended along the electrode tract at an angle through the LGN involving different layers at different visuotopic representations in each layer in the nucleus, 3) P LGN layers send axonal projections to cortical layer 4β, whereas M LGN layers send projections to cortical layer 4α, 4) individual P and M LGN layers innervate layer 4 as ocular dominance patches, and 5) K LGN axons terminate above the label in layer 4 (Hendry and Yoshioka 1994).

Using this information we developed a set of hypotheses described below (see also Fig. 1) about the K layer of origin of the axon segments that we reconstructed in the cortical layers 3Bα and layer 1. We analyzed all areas of V1 with clear label in layer 4 and well-labeled axon segments in the same cortical column as label in cortical layer 4. As the same area of visual space is represented by cortical axons in approximately 1- to 2-mm-wide columns of cortex, we never analyzed axonal segments in columns that were closer to each other than 1 mm measured tangential to cortex and always restricted our analysis to columns of less than 1.0 mm. If ocular dominance patches were labeled in layer 4 we tried to restrict our analysis of axon segments in layers 3Bα or cortical layer 1 to the vertical column involving this patch of label in layer 4. In this manner we avoided sampling axon fragments that were not in columns shared with label in layer 4.

As shown in Figure 1 we made 6 predictions about the K LGN layer of origin of reconstructed axons involved in the original injection site as follows:

1. Ocular dominance patches of label in layer 4α must originate only from one M layer. If the label is limited to 4α but continuous then both M layers must be involved. In either case axon segments labeled in the same column could only arise from LGN K1, K2 and possibly K3.
2. If the layer 4 label forms an ocular dominance pattern that spans both sublayers, 4α and 4β, then only the adjacent ipsilaterally innervated LGN M and P layers and the intervening K3 layer could give rise to the cortical label.

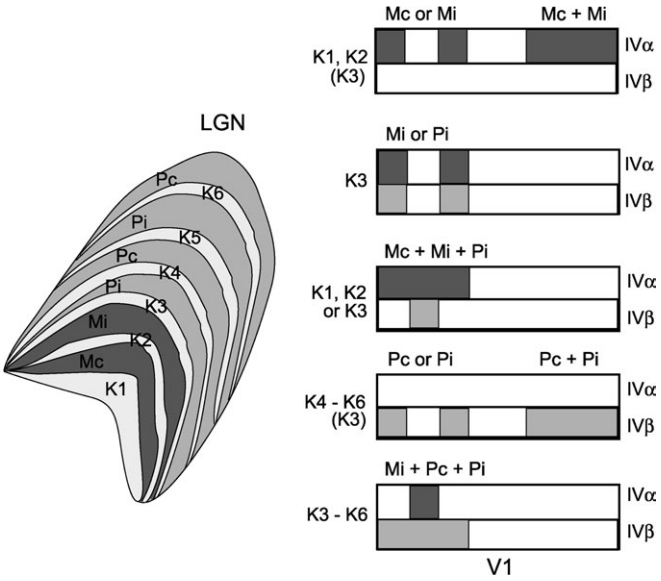


Figure 1. Possible patterns of axonal label in V1 cortical layer 4 and predicted geniculate layers involved in the original injection site. These predictions are as follows top to bottom. 1) Ocular dominance patches of label only in layer 4α must originate from either LGN layer Mc or Mi but not both. Therefore, label could only involve additional layers K1 or K2 with a slight possibility of involvement of K3. If the label in 4α shows no sign of patchy ocular dominance separation (solid line) then both LGN layers Mc and Mi must be involved in the injection. 2) If the label forms an ocular dominance pattern that spans both layers 4α and 4β then only LGN layers Mi and Pi and the intervening K3 layer could be involved. 3) If label is patchy in layer 4β and continuous above in 4α then LGN layers Mc and Mi are involved in the injection as well as LGN layer Pi and the intervening K layers K1–3. 4) If there is patchy ocular dominance label in 4β then only a single P layer (Pc or Pi) is involved and the adjacent K layers. If the label is continuous in 4β then both a Pc and a Pi layer must be involved with adjacent K layers. Therefore, layers K4–K6 could be involved and possibly K3. 5) If the label is continuous in layer 4β but patchy in layer 4α, then LGN layers Mi, Pi, and Pc are involved and LGN layer K3 is definitely involved in the injection along with LGN layer K4 and possibly also K5 and K6. See text for further explanation. i, ipsilateral; c, contralateral.

3. If label is patchy in cortical layer 4β and continuous in cortical layer 4α, then both LGN M layers, the adjacent ipsilaterally innervated P layer, and the intervening K layers K1–K3 could give rise to the cortical label.
4. If there is patchy ocular dominance label in cortical layer 4β, then only a single P layer and adjacent K layers could give rise to the cortical label.
5. If the label is continuous in 4β and absent in 4α, then a contralaterally and an ipsilaterally innervated LGN P layer and adjacent K layers give rise to the cortical label.
6. If the label is continuous in layer 4β but patchy in layer 4α, then only the ipsilaterally innervated M LGN layer and the adjacent 2 P layers and K layers between could give rise to the cortical label.

Using these predictions we reconstructed axon segments in the primary targets of the LGN K layers, namely cortical layer 1 and cortical layer 3Bα and performed several chi-square analyses to try to distinguish between K axon classes that might be associated with specific K LGN layers. All axon segments were subclassified for analysis based on the pattern of label observed in cortical layer 4. In the first analysis we wished to determine if the cortical laminar distribution of axon segments in either layer 1 or 3Bα, that were predicted to arise from layer K3 alone (associated with patchy label in layer 4), could be distinguished from axon segments predicted to arise from LGN layers K1/K2 (associated with label restricted to 4α). In order to do this we first divided the axon segments found in both layer 1 and layer 3Bα into 3 groups: those likely arising from geniculate layers K1–K2, those likely arising from layer K3, and those that did not fall into either of these categories, which we labeled "unknown." The unknown category would be any axon segment found in either layer 1 or 3Bα that was associated with a pattern of label in layer 4 that could not distinguish whether the axon arose from K3 or K1/K2 (see Fig. 1). A chi-square analysis was performed to determine whether the distributions of these 3 axon segment groups differed significantly when segments in layers 1 and layer 3Bα were analyzed. A follow-up chi-square analysis was conducted in order to determine whether there were significant differences in the distribution in either layer 1 or layer 3Bα.

A second analysis looked at the issue in a different way. This time axon segments in layers 1 and 3Bα axons were classified according to whether label in layer 4 was restricted to layer 4α (K1, K2), was restricted to layer 4β (K4–6) or was unknown. Again chi-square analyses were performed to determine if there was a significant difference between the axon segment distributions within the 3 groups and follow-up analyses were conducted to look at the distributions in either layer 1 or layer 3Bα.

Both of these statistical sets of tests were designed to examine if layer 1 cortical K axons were more likely to come from geniculate layers K1–K2, and if K axons in 3Bα were more likely to originate in LGN layers K3 or K4–K6. An alpha level of 0.05 or less was considered significant.

Results

Thirty-six K axons were completely reconstructed in V1 through serial cortical sections to the white matter. In addition, 166 presumed K labeled axon segments were reconstructed in cortical layers 1 and 3Bα. The nomenclature used here for identifying visual cortical layers is a modification of one originally proposed by Hässler (1967) and used originally by Fitzpatrick et al. (1983). The reason we use this nomenclature is that it is more appropriate for comparison across primates given that layer 4B of Brodmann gets no thalamic input and layer 4A of Brodmann appears to be a specialization of V1 layer 3 found only in some primate species (see also Casagrande and Kaas 1994 for more detailed justification). A comparison between Brodmann's (1909) more commonly used laminar designations is indicated in parenthesis to the left of the Nissl-counterstained V1 section shown in Figure 2(*A*). Figure 2(*B*) shows the appearance of cortical layers in a V1 section stained for CO. Note that the periodic patches of dark CO label (the CO blobs) are located in layer 3Bα. These CO blobs are the sites of termination of the majority of the K axons we reconstructed which are described in detail below.

Injection Sites

Figure 3(*A*) shows a Nissl-stained section through the LGN of one case with the LGN layers indicated. As can be seen in this example K LGN layers are very thin in comparison with the P and M LGN layers. K layers are numbered from K1 to K6 starting at the optic tract adjacent to the contralaterally innervated M layer.

All 4 of our injections labeled more than one LGN layer. In 2 cases, the injections resulted in label in the portion of V1 representing central vision (10° < eccentricity). In one case, the label in V1 was located in the portion of cortex representing approximately 17–20° eccentricity. In the fourth case, several discrete patches of label were seen in widely separated regions of cortex representing both central and peripheral vision. Figure 3(*B*) shows a counterstained single section showing the extent of the label in 2 dorsal P layers. In this individual section, the label appears to involve only a single K layer, K6. In one LGN shown in Figure 3(*C*), the serially reconstructed extent of the label in the LGN involved mainly K layers 1 and 2 and the adjacent M layers. In 2 other hemispheres, the label involved the dorsal 4 K layers and adjacent P layers as shown by

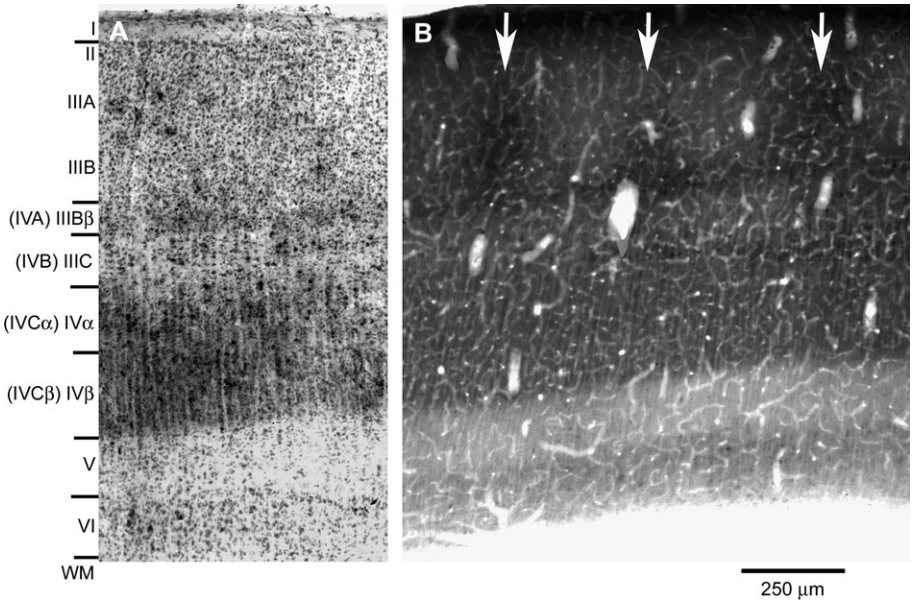


Figure 2. Laminar designations of V1 used in this paper of macaque monkey V1 with Brodmann's (1909) nomenclature (where different) indicated in parentheses. (*A*) Nissl-stained V1 section (*B*), CO-stained section with arrows showing CO blobs located in layer IIIBα.

the serial reconstruction in one hemisphere shown in Figure 3(*D*). In the fourth hemisphere, the injection was larger and involved portions of all LGN layers, perhaps because the label was dragged back along the tract from an intended K1 injection. Note that the serial injection site reconstructions show the maximum extent of the label collapsed onto one section. In each individual LGN section, as shown in Figure 3(*B*), the label often was confined to a single LGN layer which, given the tight retinotopic connections between LGN layers and cortex, allowed us to distinguish axons in cortex that originated in single LGN layers.

Morphological Varieties of K Axons

Figures 4 and 6 show examples of the variety of branching patterns seen following the 3 more restricted LGN injections targeted at either LGN K3 (Fig. 4) or LGN K1 (Fig. 6). Photomicrographic montages of 2 axons are shown in Figure 5. Reconstructions of these axons appear in Figures 4 and 6. All of the axons, regardless of cortical layer of termination, shared the following features in common. To begin with, they were all very fine in caliber in comparison with P and M axons. The latter terminate primarily in cortical layer 4. These axon size differences were qualitatively obvious, especially in the white matter viewed at high power. We did not attempt to quantify axon size differences, however, given that the axon diameters for K cell axons are sometimes close to the resolution limits of the light microscope.

A second feature that unified K axons was that they almost never exhibited side branches in layers 4 (4C of Brodmann 1909) or layer 6. The 2 exceptions show tiny twigs in layer 4 for axon 2 in Figure 4 and axon 5 in Figure 6.

Axons originating from injections targeted at LGN K3 were different, as a group, from those originating from K1 (compare axons shown in Figure 4 with those in Fig. 6). The former exhibited more robust branching patterns that generally ended in a single CO blob or blob column in cortical layer 3 (see also Fig. 5*A*). In some cases, as shown for axon 5 in Figure 4, the axon branched in 2 CO blobs but this was an exception. Axons arising from the LGN K3 injections also could be seen branching occasionally above the CO blobs including cortical layers 3A and 1. Because the CO blobs lie directly above cortical layer 3Bβ (4A of Brodmann) axon branches from axons terminating in the blobs proper also had some branches in layer 3Bβ. Axons with heavy terminations in 3Bβ were a very diverse population and distinct from the axons described here and, therefore, are described separately (see Yazar et al. 2004).

Axons arising from injections targeted at LGN layer K1 tended to confine their axonal arbors principally to cortical layer 1 where they sometimes extended for a considerable distance tangential to the cortical surface across more than one column (defined by the CO blobs below) as seen for axon #3 in Figure 6. Axons arising from injections targeted at K1 also tended to end in simpler arbors with fewer branches and boutons than those ending in the CO blobs and arising from the more dorsal LGN K layers (see Fig. 5*B*).

Figures 7 and 8 summarize the relative branching patterns of all 36 completely reconstructed axons from the 2 cases. In case #1 (Fig. 7), the 9 axons shown at the left were all reconstructed from an injection targeted at LGN K1 but which involved both K1 and K2, whereas the 9 axons shown on the right (from the other hemisphere) arose from an injection targeted at LGN K3 but involving, to varying degrees, the 4 dorsal K layers (K4–6). In Figure 8, all 18 K axons are from an injection that involved the 4 dorsal K layers. The axonal termination patterns shown in Figures 7 and 8 reinforce the conclusion that K LGN axons can exhibit a variety of termination patterns that target all cortical layers that lie dorsal to cortical layer 4.

Whether these K axons would fall out more clearly into subtypes related to each K layer remains unclear. Nevertheless, bouton counts by layer from these axons (see Tables 1 and 2) support the idea of at least 2 main subclasses of K axons, those arising from K layers adjacent to the M layers and those arising

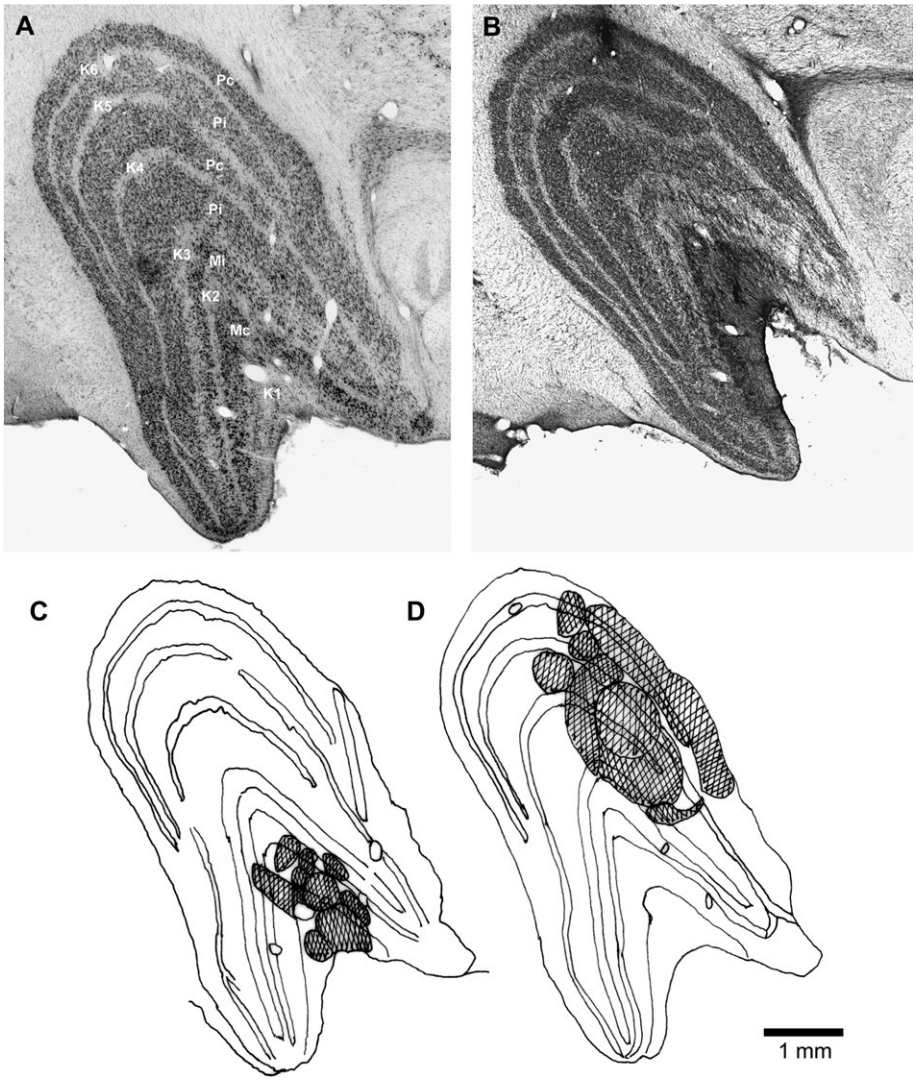


Figure 3. (*A*) Nissl-stained section showing LGN lamination. Laminar designations are as follows. i, ipsilateral, c, contralateral, P, parvocellular, K, koniocellular, M, Magnocellular. Numerals refer to different K layers. (*B*) A section showing part of the label which is shown as a composite in the reconstruction shown in (D). (*C*) Serial reconstruction of the label involving mainly LGN layers K1 and K2 and both LGN M layers from one hemisphere, collapsed onto a single drawing. (*D*) Serial reconstruction of the label involving mainly layers K3–K6 and the P layers from one hemisphere, collapsed onto a single drawing.

from their counterparts adjacent to the P layers. Table 1 shows that in the K1 and K2 axons the vast majority of boutons are in cortical layers 1 and 3A with only 15% of boutons from this population in the other cortical layers. Table 2, shows that, by contrast, axons arising from LGN layers K3–6 have boutons almost exclusively in the CO blobs of cortical layer 3Bα, with only 7% of boutons seen in other cortical layers.

Analysis of K Axon Segments

As described in the Methods we preformed several chi-square analyses to test the hypothesis that following a large LGN injection the number of labeled axon segments located in the same cortical column in cortical layers 1 and 3Bα arise from different LGN K layers. Examples of the patterns of label in layer 4 used to guide the selection of axon segments in cortical layers 1 and 3Bα are shown schematically in Figure 1 and in a series of photomicrographs in Figure 9. In the chi-square analysis performed between axon segment populations in layer 1 and layer 3Bα to determine whether they could be subclassified as K1–K2, K3, or were unknown, we first determined whether the number of unknowns differed between the 2 axon populations. Twenty out of 81 axons in the layer 1 axon population fell into the "unknown" category, whereas 20 out of 85 layer 3Bα axons were classified as unknown, yielding $\chi^2 = 0.03$, $P = 1$. The number of axons in the unknown axon segment populations, therefore, did not differ significantly, and thus these axon segments were disregarded in the remaining analyses. The next question addressed was whether the distribution of K1–K2 versus K3 axon populations differed significantly in terminations in cortical layers 1 or 3Bα. The likelihood of being a potential K1–K2 axon segment versus a K3 axon segment differed significantly between the populations found in layer 1 and layer 3Bα, χ^2 ($N = 126$) = 9.06, $P < 0.01$. In other words, there were less potential K3 axons and more potential K1–K2 axons in the layer 1 population than in the layer 3Bα population. Follow-up analyses indicated that the distribution of axon segments in the layer 1 group by itself was not significantly different than chance (χ^2 ($N = 61$) = 1.33, $P = 0.25$). The

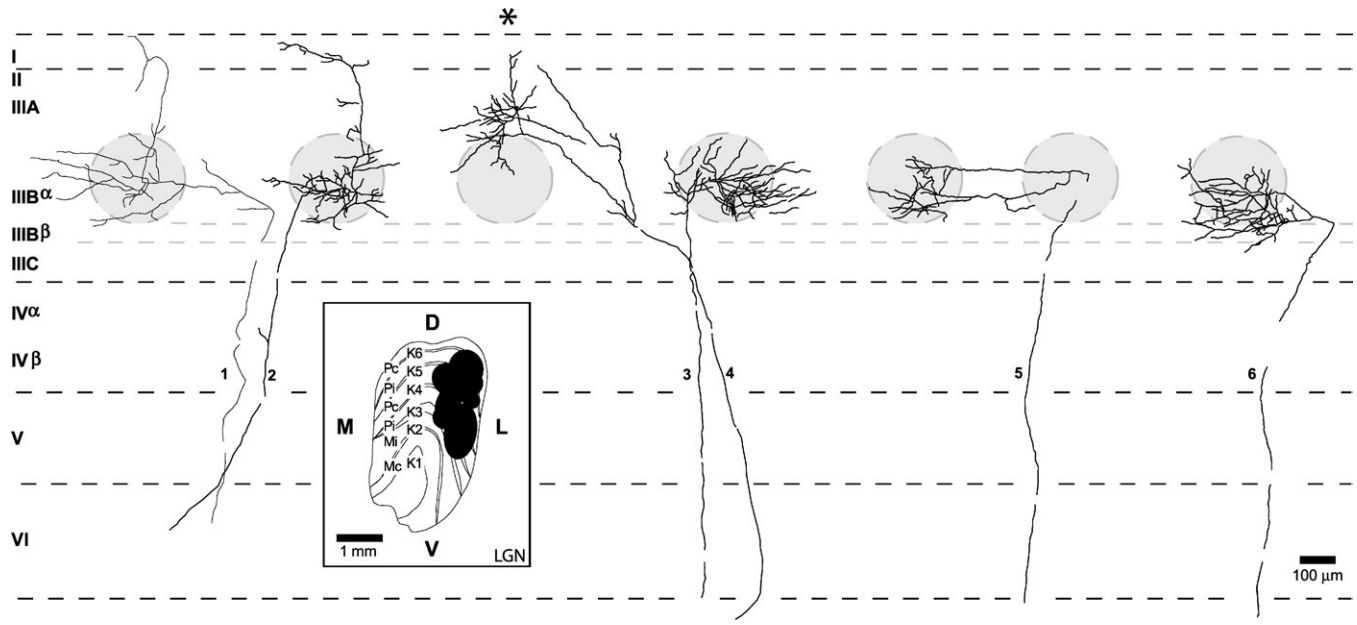


Figure 4. Examples of different types of reconstructed axons from an injection involving LGN layers K3–K6. Roman numerals represent cortical laminar designations, and hatched circles represent CO blobs located in cortical layer IIIBα. In the insert, the extent of the injection site collapsed onto a single LGN section is indicated in black. Note that label in many sections involved only one or 2 LGN layers. D, dorsal; L, lateral; M, medial; V, ventral. Numerals refer to each axon. In the insert i, ipsilateral; c, contralateral; *indicates axon pictured in Figure 5(*A*).

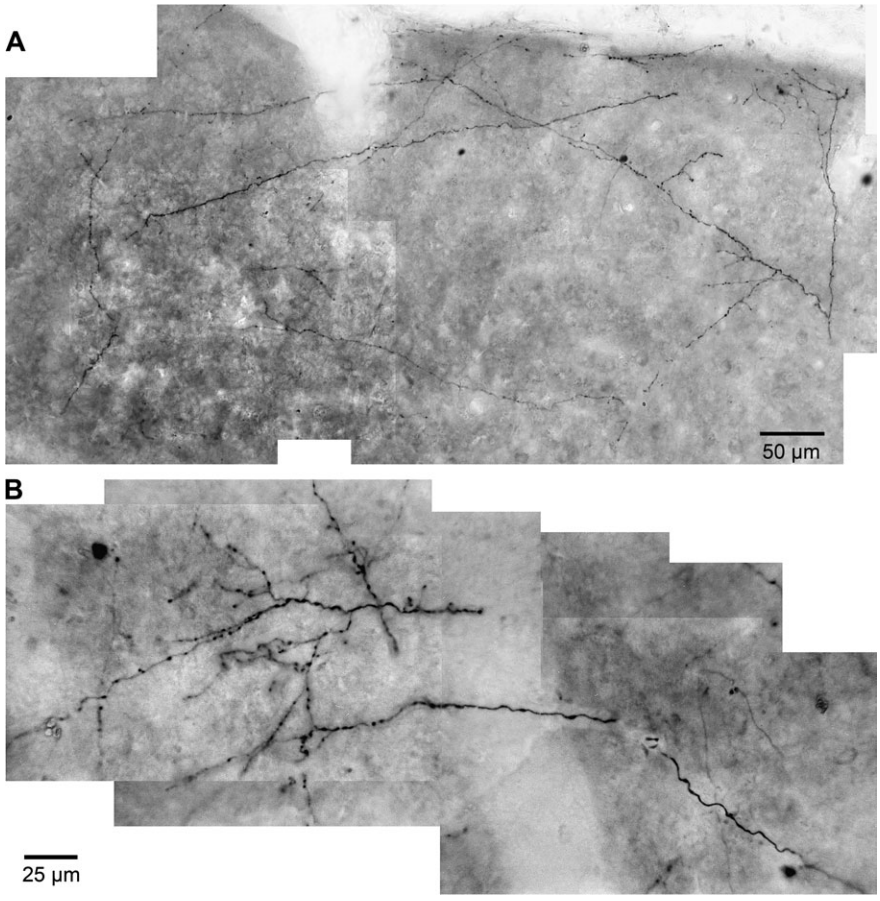


Figure 5. Photomicrographic montages of portions of single reconstructed axons in layer 3Bα (*A*) and layer 1 (*B*). (*A*) A portion of the axon designated * in Figure 4. (*B*) A portion of the axon designated * in Figure 6. Note that each axon was photographed at a different magnification. Each photomontage consisted of a large number of individual photographs taken while focusing through the section to trace each axon.

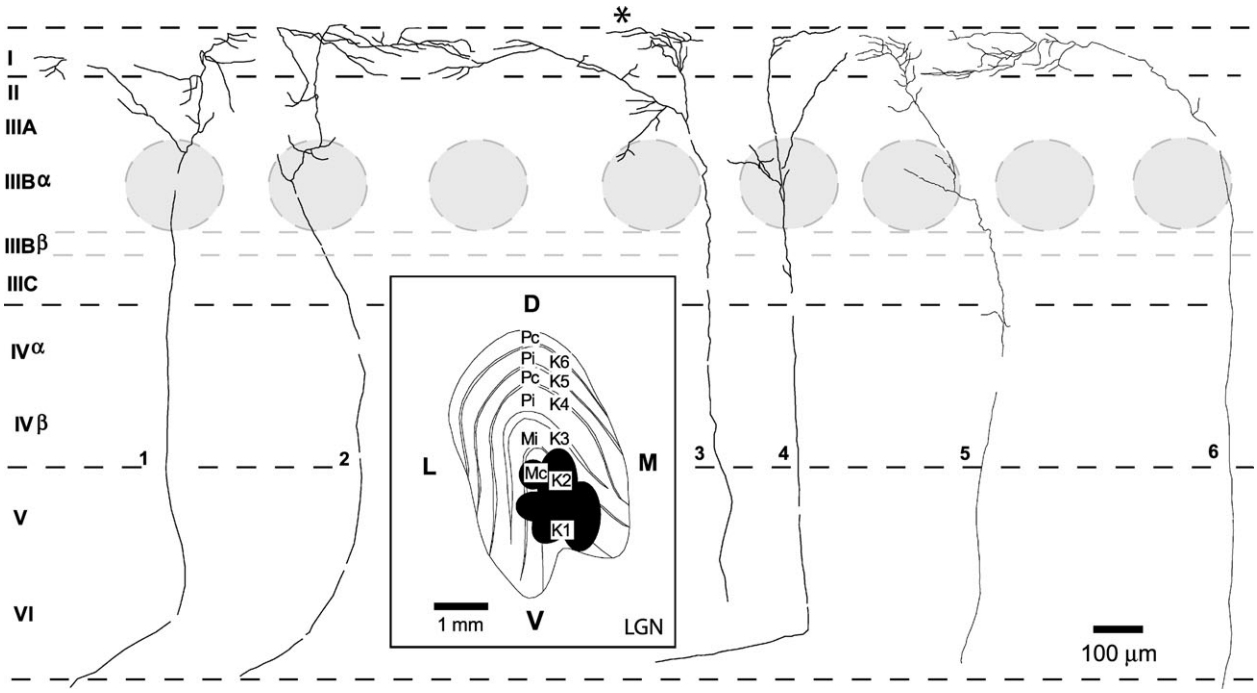


Figure 6. Examples of reconstructed axons from an injection in K1–K2. *Indicates axon pictured in Figure 5(*B*). Other conventions as in Figure 4.

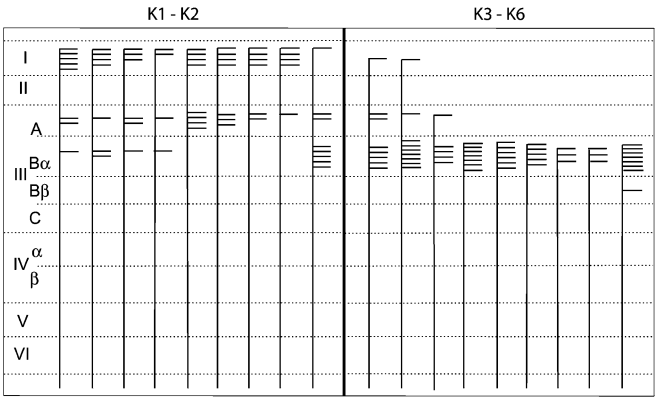


Figure 7. Summary of primary branching patterns for all completely reconstructed K1–K2 and K3–K6 axons from case #1. Bar number and length are not intended to represent actual branch length or number but to give a general idea of branch density in each cortical layer. Roman numerals indicate cortical layers.

distribution of K1–K2 versus K3 axon segments, however, was different than that expected due to chance in the 3Bα population analyzed by itself, χ^2 (N = 65) = 9.62, P < 0.01. That is, in the 3Bα axon segment population, significantly more axons originate potentially in geniculate layer K3 than would be expected by chance (see Fig. 10).

A second set of chi-square analyses was performed to test whether layer 1 and layer 3Bα axon segments were more likely to be associated with continuous label in layer 4α or 4β. For this test, only nonpatchy label patterns restricted to 4α or 4β were used. Recall that label in 4α could arise from axons in the M layers and neighboring K layers, K1 and K2, whereas continuous label in 4β is most likely associated with the P layers and neighboring K layers excluding K3 (K4–K6). In the analyses above we specifically focused on patterns of label that would be predicted to involve K3; K3 axons were identified based on ocular dominance patches in *both* 4α and 4β. There is only a slight chance that a K3 axon would correlate with continuous label pattern restricted to either 4α or 4β. A chi-square test indicated that the likelihood of having label only in 4α or only in 4β associated with axon segments labeled in layer 1 versus layer 3Bα axons was significantly different, χ^2 (N = 88) = 9.17, P < 0.004, indicating an association between axon type and the layer 4 label pattern. Follow-up analysis of the distribution within each type of axon segment population in layer 1 showed that layer 1 axon segments are significantly more likely to exist with coincident label in 4α and less likely to exist with label in 4β relative to what would be expected by chance (χ^2 (N = 45) = 13.89, P < 0.001). In the 3Bα axon segment population, however, the probability of associating with labeled axons in 4α versus 4β was not significantly different from chance (χ^2(N= 43) = 0.21, P < 0.64) (see Fig. 11), suggesting that axons arising from K4 to K6 were no more likely than those arising from K1 and K2 to terminate in 3Bα.

Taken together these results suggest that cortical K axons exhibiting branches in layer 1 are a distinct subpopulation of K axons that have a higher than normal probability of originating from LGN layers K1–K2 and that axon segments found in layer 3Bα have a higher than normal probability of originating in LGN layer K3.

Discussion

Our chief finding is that macaque K LGN axons in V1, although morphologically heterogeneous, are divisible into several classes. Those axons that arise from the ventral K LGN layers and lie close to the M layers appear to be distinct from those that arise from the dorsal K LGN layers and lie adjacent to the P LGN layers. Axons from LGN layers K1/K2 terminate preferentially in cortical layers 1 and 3A, whereas axons from the dorsal LGN layers K3–K6 terminate preferentially in the CO blobs of cortical

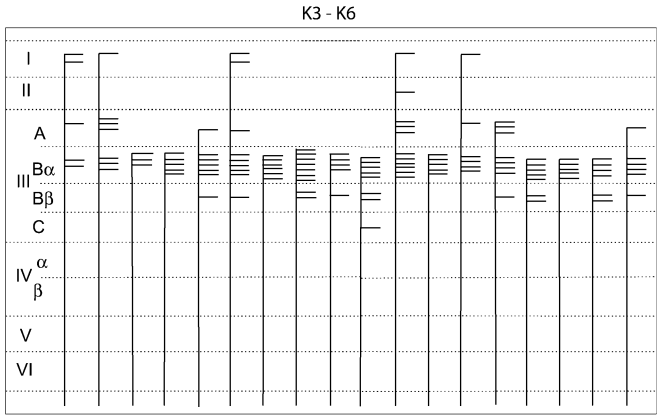


Figure 8. Summary of primary branching patterns for completely reconstructed K3–K6 axons from case #2. Other conventions as in Figure 6.

Table 1
K1–K2 axons: distribution of boutons by layer

Layers										Boutons/layer
1	70	100	75	95	70	45	25	75	15	570 (47%)
2	5	5	5			5		10	5	35 (3%)
3A	40	20	75	40	15	30	15	55	165	455 (38%)
3Bα				20	25	15	15		70	145 (12%)
3Bβ										
3C										
4α, 4β										
5, 6										
Boutons/axon	115	125	155	155	110	95	55	140	255	1205
Axon no.	a	b	c	d	e	f	g	h	i	

Note: Bouton number per layer of cortex for completed reconstructed axons from case #1. Axons arising from LGN injections involving layers K1–K2 have the highest percentage of boutons in cortical layer 1. Roman numerals indicate cortical layers and letters indicate the axons. See text for details.

Table 2
K3–K6 axons: distribution of boutons by layer

Layers										Boutons/layer
1						25	10			35 (2%)
2										
3A		10				5	50			65 (3%)
3Bα	90	145	105	260	390	260	180	215	170	1815 (93%)
3Bβ					40					40 (2%)
3C										
4α, 4β										
5, 6										
Boutons/axon	90	155	105	260	430	290	240	215	170	1955
Axon no.	a	b	c	d	e	f	g	h	i	

Note: Bouton number per layer of cortex for completed reconstructed axons from case #1. Axons arising from LGN injections involving layers K3–K6 have the highest percentage of boutons in cortical layer 3Bα. Roman numerals indicate cortical layers and letters. See text for details.

layer 3Bα. Statistical analysis of the distribution of axon segments in relationship to the labeling patterns in cortical layer 4 suggests further that axons arising in K3 tend to terminate in the CO blobs in 3Bα and are less likely then those arising from K layers 4–6 to send a branch to cortical layer 1. In the discussion below we consider how well these data fit with conclusions from other studies on the K pathway in macaque monkeys and other primates and what these data might mean for the potential function of K cells.

How Many K cell Classes are There in Macaque Monkeys?

The present study demonstrated that K axons projecting to the superficial cortical layers can be quite diverse but follow a general pattern with those from the ventral LGN K layers projecting more dorsally in cortex and dorsal K layers projecting more ventrally in cortex. This conclusion is opposite to the conclusion reached by Hendry and Yoshioka (1994). They concluded, based on retrograde labeling from the superficial cortical layers in macaque monkey, that the dorsal LGN K layers project to cortical layer 1 and that the ventral LGN K layers project to the CO blobs in layer 3Bα. It was specifically because of this result that we conducted a statistical analysis of axon segments to increase our sample of axons labeled in layers 1 and 3Bα. Our axon segment analyses support our single axon reconstruction data, and, we believe, offer a partial explanation for the conflicting conclusion reached by Hendry and Yoshioka (1994). In their study, they compared retrograde labeling in LGN K layers following injections restricted with the superficial cortical layers (layers 1–3A) and those that were larger (cortical layers 1–3Bα). As shown in their Figure 3, with a superficial cortical injection they found only a few retrogradely labeled cells restricted to LGN layers K4–K6 and with a larger injection they show many labeled cells in all K layers (K1–K6). Because K3 is the only K layer, based on our axon segment statistical analysis, that does not appear to send many collateral branches above 3Bα, Hendry and Yoshioka (1994) would only have seen label in K3 after the larger cortical injection concluding, therefore, that the ventral K layers project to the deeper cortical layers. This, of course, does not explain why they saw no K1/K2 LGN cells labeled following their cortical layer 1 injection. One possibility is that the size of their smaller injections was simply too small to label enough of the fine branches in cortical layer 1. In this regard, it is noteworthy that published data on K layer projections to cortex in both squirrel monkeys and owl monkeys support our conclusion shown here in macaque monkey (Fitzpatrick et al. 1983; Diamond et al. 1985; Ding and Casagrande 1997), not the conclusion reached by Hendry and Yoshioka (1994). It also is important to realize that there are virtually no K LGN cells above K3 in either of the latter New World primates and in both of these simian species LGN layer K3 was found to send projections to the CO blobs in 3Bα and LGN layers K1/K2 were found to project to cortical layer 1.

Although there is considerable within group heterogeneity, K axon classes seem distinct from P and M axons because (with minor exceptions) none provide collateral branches to cortical layer 4. In a separate study (Yazar et al. 2004) we identified 3 additional classes of potential K axons in macaque monkey whose main target layer was cortical layer 3Bβ. Of the latter, 2 axon classes did exhibit branches that extended above 3Bβ and so could be considered a subtype of one of the K axon classes identified in this study. The reason we argue for treating axons that terminate primarily in cortical layer 3Bβ as a separate group is that a number of primates including owl monkeys, bush babies, chimpanzees, and humans lack any LGN input to layer 3Bβ (Kaas et al. 1976; Tigges and Tigges 1979; Horton and Hedley-Whyte 1984; Lachica and Casagrande 1992). This means that LGN input to 3Bβ was under separate evolutionary pressure

from LGN input to the other layers. Regardless, such significant species differences argue that K cells that terminate in 3Bβ may, in some way, be functionally unique. Additionally, Yazar et al. 2004 reconstructed a fourth class of axon terminating in layer 3Bβ that sent a major collateral to layer 4β suggesting that these axons could be either a class of P axons or another class of K axons. In fact, it had been proposed earlier that axons that innervate layer 3Bβ in macaque monkeys could either be from LGN P or K layers (see Lund 1988 and below). Evidence is strong that at least some P cells project to 3Bβ in both macaque monkeys based on retrograde labeling from cortex and LGN immunocytochemistry (Hendry and Yoshioka 1994) and in squirrel monkeys (Fitzpatrick et al. 1983) based on restricted anterograde tracer injections of LGN P layers (with no involvement of K cells) and labeling in layer 3Bβ in cortex. Additionally, in squirrel monkeys injections restricted to K3 in some cases clearly labeled axons in layer 3Bβ without any associated label in layer 4 supporting the idea that K axons also project to layer 3Bβ. If all of these classifications are correct, these data argue for at least 5–6 morphological classes of K LGN axons in V1. Conservatively, one could make a strong case for at least 3 K types, those projecting solely to cortical layer 1, those that project to the CO blobs in layer 3Bα, and those that terminate solely in 3Bβ.

Of course, the bulk of the input from the LGN to V1 in macaque monkeys comes from P cells (80%) with an additional 10% arising from M cells (Connolly and Van Essen 1984). Because of the difficulty involved in proving that an axon arises from a K layer and not a neighboring M or P LGN layer, it is difficult to be positive that some of the axons we have identified as K axons are not subclasses of P or M axons. We would argue against this view based on the following observations. Both Carey et al. (1979) and Hendry and Yoshioka (1994) made retrograde tracer injections that were restricted to the superficial V1 cortical layers (3Bα and above) and showed that virtually all retrogradely labeled LGN cells were in the K LGN layers or in occasional thin "bridges" of cells crossing between P LGN layers. More important, Hendry and Yoshioka (1994) showed that these retrogradely labeled cells in macaque monkey were double labeled for αCAMKII, a marker for LGN K cells. In addition, as mentioned earlier, the sizes of the axons we identified as K axons in cortex tended to be much finer than those of P or M cells. We also have shown that K LGN cells identified by immunolabeling for calbindin are smaller in size, on average, than P or M LGN cells (Hendry and Casagrande 1996). This observation fits with their much finer axonal processes in V1.

How Similar are K cells Across Primates?

K axons share several definitive characteristics across primates. Reconstructions of K axons in V1 in bush babies (Lachica and Casagrande 1992), New World simian owl monkeys (Ding and Casagrande 1997), and macaque monkeys (current study) and bulk labeling studies in squirrel monkeys, owl monkeys, and bush babies (Fitzpatrick et al. 1983; Diamond et al. 1985; Lachica and Casagrande 1992; Ding and Casagrande 1997) all show that K axons are distinct from M and P axons in V1. K axons in all of these distantly related species are thin (suggesting that they conduct slowly), branch less densely and have lower numbers of boutons than either P or M axons. Almost without exception, K axons avoid terminating in layers 4 and 6, which are the primary targets of P and M LGN axons. In all studied primates where axon reconstructions or bulk labeling studies have been done, it has been shown that K axons terminate principally in the superficial cortical layers above layer 4 (Livingstone and Hubel 1982; Fitzpatrick et al. 1983; Weber et al. 1983; Lachica and Casagrande 1992; Hendry and Yoshioka 1994; Ding and Casagrande 1997). These morphological features actually make K axons more distinct from P and M axons than the latter are from each other because P and M axons basically target the same cortical layers (4 and 6) even though in different subtiers.

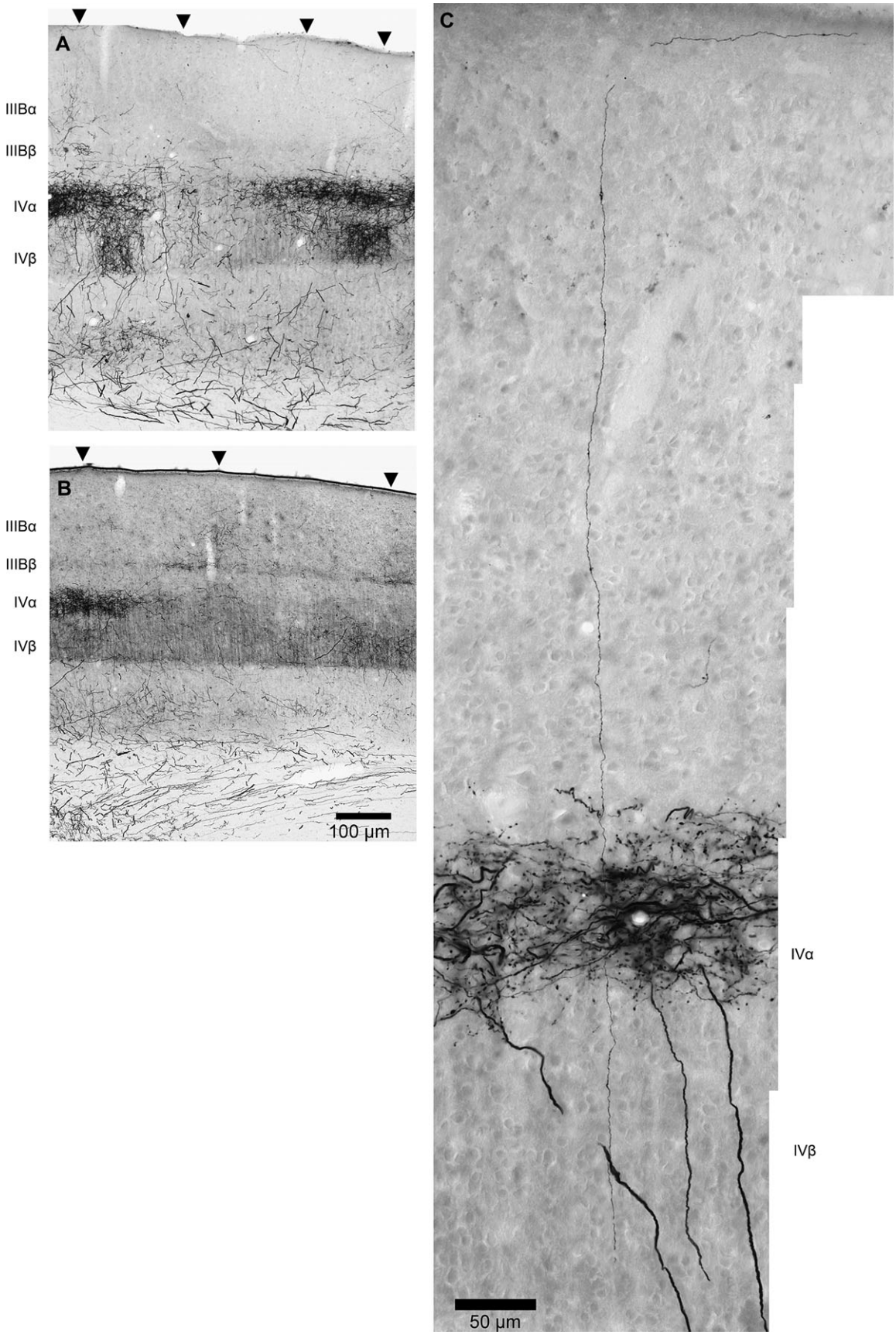
In spite of these overall similarities, the within-group morphological heterogeneity of LGN K cells, their axonal morphology, and their distribution makes it difficult to conclude that all K cell subtypes are represented in all primates (Fitzpatrick et al. 1983; Weber et al. 1983; Lachica and Casagrande 1992; Ding and Casagrande 1997, 1998; Shostak et al. 2003). Although it does appear that that owl monkeys and macaque monkeys have at least 2 main classes of K axon that arise, respectively, from layers K1, K2 versus K3–K6 that are similar, it is not clear whether the same is true in the prosimian bush baby where all K axons reconstructed appeared to terminate both in the CO blobs and in cortical layer 1 (Lachica and Casagrande 1992; Ding and Casagrande 1997). Of course, only the equivalent of macaque layer K4 was injected in bush baby LGN so it still may be that bush babies have other classes of K axons especially given the fact that calbindin positive, presumed K cells located in and below the M layers in bush baby can be double-labeled from injections of tracers in V1 (Johnson and Casagrande 1995).

Regardless, different primates appear to show big differences in the relative size of different K LGN layers. In bush babies what would be the equivalent of K4 in macaque monkeys is best developed, whereas in owl monkeys and macaque monkeys layers K3 and K1 appear to contain the greatest number of cells (Hendry and Yoshioka 1994; Johnson and Casagrande 1995; Hendry and Casagrande 1996; Xu et al. 2001). In fact, in owl monkeys, calbindin immunolabeling suggests that there are almost no K cells in K4 and in squirrel monkeys layers K4–K6 do not appear to exist (Hendry and Casagrande 1996; Xu et al. 2001).

Functional Considerations

Understanding the function of K cells has been hindered by several factors. First, as discussed above for morphological types, it is unclear how many physiological types of K LGN cells exist. It is evident from studies that have recorded from K cells in a variety of primates that they have physiologically diverse receptive field properties measured with achromatic stimuli (Norton and Casagrande 1982; Irvin et al. 1986; Martin et al. 1997; White et al. 1998; Xu et al. 2001). The only single functional "signature" that now appears to be associated with some K cells in diurnal primates is input from S cones. In marmosets, for example, approximately 20% of K cells identified by

Figure 9. Examples of the patterns of label in layer 4 in areas used to analyze the distributions of axon segments. (*A*) Different patterns of label in 4α and 4β with axon segments labeled in layer 1 and 3Bα in the same column (arrowheads). Note that axon segments are labeled even where few axons terminate in 4 itself in the same column. (*B*) Instances of very little label in layer 4 (middle) and a strip of label in 4α (left) and light label in 4β. Labeled axon segments (below arrowheads) can be seen in both 3Bα and 3Bβ. Labeled axon segments in layer 1 cannot be seen at this power. (*C*) This photomontage shows an axon segment in layer 1 above label restricted to the upper third of layer 4. Axon segments in layer 1 were often visible only at higher power. In this example, the axon trunk can be seen ascending to layer 1. No labeled axon segments were labeled in this column in layer 3Bα. Laminar designations as in Figure 2.



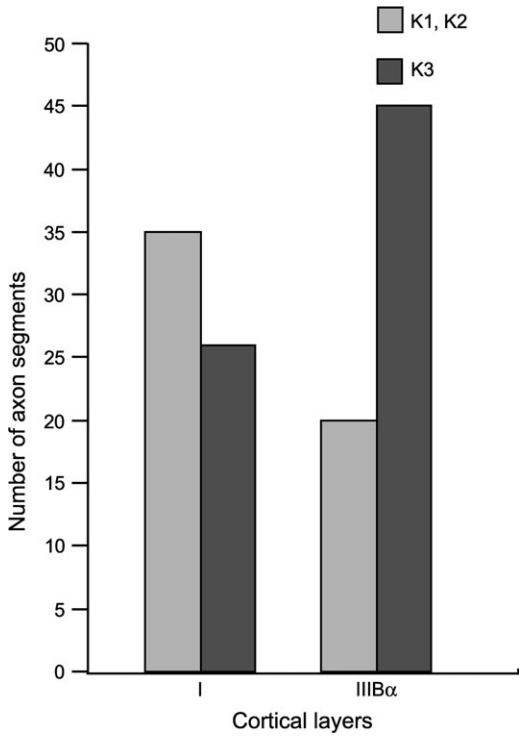


Figure 10. Distribution of labeled axon segments based on predicted K LGN layer involvement in the injection site. Following an injection in LGN layer K3, axons segments are significantly more numerous in cortical layer 3Bα than following an injection in LGN layers K1/K2. This pattern was not found for axon segments in layer 1 although there was a trend for more labeled axon segments to be found following layer K1/K2 injections. See also Figure 9 and text for details.

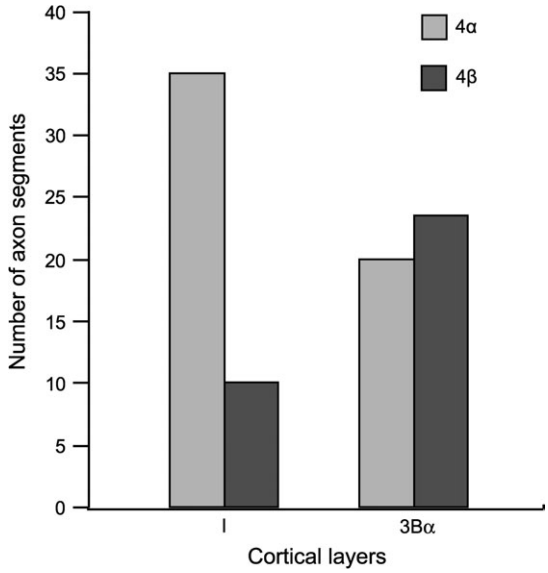


Figure 11. Distribution of labeled axon segments in cortical layers 1 and 3Bα overlying label in layer 4 restricted to either 4α (M LGN layer target) or 4β (P LGN layer target). In cortical layer 1, more axon segments were found above label restricted to 4α (M input) than 4β (P input), suggesting that axons arose from either K1 or K2. Only a weak trend was seen for axon segments found in 3Bα that favored associated label in 4β, suggesting they arose from dorsal K layers. See text for details.

immunostaining for calbindin carry S cone signals (White et al. 1998). The remaining K cells represent a diverse population in terms of temporal and spatial frequency selectivity and other measures, and share features in common with cat W-like LGN cells and bush baby K cells described earlier (Irvin et al. 1986; see Casagrande 1994; Hendry and Reid 2000 for review; White et al. 2001; Xu et al. 2001). In macaque monkey, the axons carrying S cone signals (blue ON) from K cells appear to terminate principally in layer 3Bβ with some evidence of terminations in lower 3Bα (Chatterjee and Callaway 2003). A single axon terminating in both cortical layers 1 and 6 and physiologically classified as blue ON also was previously identified by Blasdel and Lund (1983). The exact origin of the latter axon, however, was unclear given that it was filled in the white matter and therefore could easily have represented a feedback axon for a higher cortical area not one from the LGN. Chatterjee and Callaway (2003) also recorded from a second class of LGN axon in layer 3Bβ carrying blue-OFF S cone signals. The latter were located below axons carrying blue-ON signals in the same layer in macaque monkey. Whether these axons arose from P or K LGN cells remain controversial because some investigators have found evidence of S cone input to midget (P) ganglion cells (Klug et al. 2003) and some have found no evidence for such input (Lee et al. 2005) at the retinal level in primates. Because the axons reconstructed in the current study have the majority of their boutons above layer 3Bβ they may represent a separate population, especially because so few blue-ON cells were identified in layer 3Bα proper by Chatterjee and Callaway (2003). Moreover, because owl monkeys and bush babies lack S cones entirely, have only a single cone type and no color vision, yet have a very similar pattern of input from their K pathway to layer 3Bα these K cells must do something besides transmitting chromatic signals (Jacobs et al. 1996). One possibility is that K channels modulate or reinforce visual information that has arrived in advance via signals from the faster and more robust P and M pathways. We know from our own work that the onset latency of K LGN cells, following stimulation, is slower than that of P or M cells in macaque monkeys (Casagrande et al. 2005). Because K cells send their slower input directly to the superficial cortical layers, however, they may be in a position to add their signals to those that have already been processed through several synapses from the faster P and M pathways. Also, given that K LGN cells receive more extraretinal input from a variety of sources than do P or M LGN cells it is possible that different K LGN cells provide modulatory signals to V1 about several aspects of either stimulus quality or the state of the animal (Casagrande 1994). Exact details of what the majority of K LGN cells signal still remain a mystery and likely will require a better understanding of the functional properties of retinal ganglion cells that provide input to K cells in different K layers and how these K LGN cells are modulated by extraretinal inputs in the awake state.

Notes

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References

Blasdel GG, Lund JS. 1983. Termination of afferent axons in macaque striate cortex. J Neurosci. 3 (7):1389–1413.

Boyd JD, Matsubara JA. 1996. Laminar and columnar patterns of geniculocortical projections in the cat: relationship to cytochrome oxidase. J Comp Neurol. 365(4):659–682.

Brodmann K. 1909. Vergleichende Lokalisationslehre der Grosshirnrinde in ihren Prinzipien dargestellt auf Grund des Zellenbaues. Leipzig: J.A. Barth.

Carey RG, Fitzpatrick D, Diamond IT. 1979. Thalamic projections to layer I of striate cortex shown by retrograde transport of horseradish peroxidase. Science. 203(4380):556–559.

Casagrande VA. 1994. A third parallel visual pathway to primate area V1. Trends Neurosci. 17(7):305–310.

Casagrande, VA, Ding Y, Boyd JD. 1997. The morphology of LGN axons from different K layers in V1 of macaque monkey. Soc Neurosci Abstr. 23:2361.

Casagrande VA, Kaas JH. 1994. The afferent, intrinsic, and efferent connections of primary visual cortex in primates. In: Peters A, Rockland KS, editors. Primary visual cortex in primates. New York: Plenum Press. p. 201–259.

Casagrande, VA, Royal DW, Sáry Gy. 2005. Extraretinal inputs and feedback mechanisms to the lateral geniculate nucleus (LGN). In: Kremers J, editor. The primate visual system: a comparative approach. Hoboken, New Jersey: John Wiley and Sons. p. 191–206.

Casagrande VA, Xu X. 2004. Parallel visual pathways: a comparative perspective. In: Chalupa L, Werner JS, editors. The visual neurosciences. Cambridge, MA: MIT Press. p. 494–506.

Chatterjee S, Callaway EM. 2003. Parallel colour-opponent pathways to primary visual cortex. Nature. 426(6967):668–671.

Connolly M, Van Essen D. 1984. The representation of the visual field in parvicellular and magnocellular layers of the lateral geniculate nucleus in the macaque monkey. J Comp Neurol. 226(4):544–564.

Dacey DM, Peterson BB, Robinson FR, Gamlin PD. 2003. Fireworks in the primate retina: in vitro photodynamics reveals diverse LGN-projecting ganglion cell types. Neuron. 37(1):15–27.

Diamond IT, Conley M, Itoh K, Fitzpatrick D. 1985 Laminar organization of geniculocortical projections in *Galago senegalensis* and *Aotus trivirgatus*. J Comp Neurol. 242:584–610.

Ding Y, Casagrande VA. 1997. The distribution and morphology of LGN K pathway axons within the layers and CO blobs of owl monkey V1. Vis Neurosci. 14(4):691–704.

Fitzpatrick D, Itoh K, Diamond IT. 1983. The laminar organization of the lateral geniculate body and the striate cortex in the squirrel monkey (*Saimiri sciureus*). J Neurosci. 3(4):673–702.

Goodchild AK, Martin PR. 1998. The distribution of calcium-binding proteins in the lateral geniculate nucleus and visual cortex of a New World monkey, the marmoset, *Callithrix jacchus*. Vis Neurosci. 15(4):625–642.

Hässler R. 1967. Comparative anatomy of the central visual systems in day- and night-active primates. In: Hässler R, Stephen S, editors. Evolution of the forebrain. Stuttgart, Germany: Thieme. p. 419–434.

Hendry SH, Casagrande VA. 1996. A common pattern for a third visual channel in the primate LGN. Soc Neurosci Abstr. 22:1605.

Hendry SH, Reid RC. 2000. The koniocellular pathway in primate vision. Annu Rev Neurosci. 23:127–153.

Hendry SH, Yoshioka T. 1994. A neurochemically distinct third channel in the macaque dorsal lateral geniculate nucleus. Science. 264(5158):575–577.

Horton JC, Hedley-Whyte ET. 1984. Mapping of cytochrome oxidase patches and ocular dominance columns in human visual cortex. Philos Trans R Soc Lond B Biol Sci. 304(1119):255–272.

Irvin GE, Norton TT, Sesma MA, Casagrande VA. 1986. W-like response properties of interlaminar zone cells in the lateral geniculate nucleus of a primate (*Galago crassicaudatus*). Brain Res. 362(2):254–270.

Jacobs GH, Neitz M, Neitz J. 1996. Mutations in S-cone pigment genes and the absence of colour vision in two species of nocturnal primate. Proc Biol Sci. 263(1371):705–710.

Johnson JK, Casagrande VA. 1995. Distribution of calcium-binding proteins within the parallel visual pathways of a primate (*Galago crassicaudatus*). J Comp Neurol. 356(2):238–260.

Kaas JH, Lin CS, Casagrande VA. 1976. The relay of ipsilateral and contralateral retinal input from the lateral geniculate nucleus to striate cortex in the owl monkey: a transneuronal transport study. Brain Res. 106(2):371–378.

Klug K, Herr S, Ngo IT, Sterling P, Schein S. 2003. Macaque retina contains an S-cone OFF midget pathway. J Neurosci. 23(30):9881–9887.

Lachica EA, Casagrande VA. 1992. Direct W-like geniculate projections to the cytochrome oxidase (CO) blobs in primate visual cortex: axon morphology. J Comp Neurol. 319(1):141–158.

Lee SC, Telkes I, Grunert U. 2005. S-cones do not contribute to the OFF-midget pathway in the retina of the marmoset, Callithrix jacchus. Eur J Neurosci. 22(2):437–447.

Livingstone MS, Hubel DH. 1982. Thalamic inputs to cytochrome oxidase-rich regions in monkey visual cortex. Proc Natl Acad Sci USA. 79(19):6098–6101.

Lund JS. 1988. Anatomical organization of macaque monkey striate visual cortex. Annu Rev Neurosci. 11:253–288.

Martin PR, White AJ, Goodchild AK, Wilder HD, Sefton AE. 1997. Evidence that blue-on cells are part of the third geniculocortical pathway in primates. Eur J Neurosci. 9(7):1536–1541.

Norton TT, Casagrande VA. 1982. Laminar organization of receptive-field properties in lateral geniculate nucleus of bush baby (*Galago crassicaudatus*). J Neurophysiol. 47(4):715–741.

Rodieck RW, Watanabe M. 1993. Survey of the morphology of macaque retinal ganglion cells that project to the pretectum, superior colliculus, and parvicellular laminae of the lateral geniculate nucleus. J Comp Neurol. 338(2):289–303.

Shostak Y, Ding Y, Casagrande VA. 2003. Neurochemical comparison of synaptic arrangements of parvocellular, magnocellular, and koniocellular geniculate pathways in owl monkey (*Aotus trivirgatus*) visual cortex. J Comp Neurol. 456:12–28.

Tago H, Reiner PB, McGeer EG. 1987. Coupled intracellular horseradish peroxidase-monoamine oxidase histochemistry: description of the technique and its application to the study of physiologically identified tuberomammillary neurons. J Neurosci Methods. 20(4): 271–281.

Tigges J, Tigges M. 1979. Ocular dominance columns in the striate cortex of chimpanzee (*Pan troglodytes*). Brain Res. 166(2):386–390.

Watanabe M, Rodieck RW. 1989. Parasol and midget ganglion cells of the primate retina. J Comp Neurol. 289(3):434–454.

Weber JT, Huerta MF, Kaas JH, Harting JK. 1983. The projections of the lateral geniculate nucleus of the squirrel monkey: studies of the interlaminar zones and the S layers. J Comp Neurol. 213(2): 135–145.

White AJ, Solomon SG, Martin PR. 2001. Spatial properties of koniocellular cells in the lateral geniculate nucleus of the marmoset *Callithrix jacchus*. J Physiol. 533(Pt 2):519–535.

White AJ, Wilder HD, Goodchild AK, Sefton AJ, Martin PR. 1998. Segregation of receptive field properties in the lateral geniculate nucleus of a New-World monkey, the marmoset *Callithrix jacchus*. J Neurophysiol. 80(4):2063–2076.

Xu X, Ichida JM, Allison JD, Boyd JD, Bonds AB. 2001. A comparison of koniocellular, magnocellular and parvocellular receptive field properties in the lateral geniculate nucleus of the owl monkey (*Aotus trivirgatus*). J Physiol. 531(Pt 1):203–218.

Yazar F, Mavity-Hudson JA, Ding Y, Oztas E, Casagrande VA. 2004. Layer IIIBb (IVA) of primary visual cortex (V1) and its relationship to the koniocellular (K) pathway in macaque monkeys. Soc Neurosci Abstr Program No. 300.17.