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THE LAMINAR ORGANIZATION OF THE LATERAL GENICULATE BODY AND THE STRIATE CORTEX IN THE TREE SHREW (*TUPAIA GLIS*)[1]

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Abstract

The organization of geniculostriate projections in *Tupaia* was studied using three separate methods, anterograde transport from the lateral geniculate, retrograde transport from the striate cortex, and reconstruction of single geniculostriate axons. The results show that each layer of the lateral geniculate body has a unique pattern of projections to the striate cortex, and each pattern consists of a major and a minor target. The two ipsilateral layers project to thin subtiers of layer IV: the major target of geniculate layer 1 is the top of IVa; the major target of geniculate layer 5 is the base of IVb. The minor target of layer 1 is the major target of layer 5.

Two of the contralateral layers can be matched to the ipsilateral layers. Layers 1 and 2 are a matched pair and project to IVa; layers 4 and 5 are a matched pair and project to IVb. Thus, projections of a matched pair overlap.

The remaining two contralateral layers, 3 and 6, project chiefly to cortical layer III. Layer 3 projects to layers IIIb and I and seems to be the counterpart of the parvocellular C layers in the cat and the intercalated layers in primates. Layer 6 projects to the base of IIIc in a zone contiguous with IVa. This contiguity raises the issue of whether the base of IIIc might actually be a part of layer IV. If this were the case, the two tiers of layer IV which are separated by a conspicuous cleft might be considered two subdivisions of layer IVb.

This paper deals with two very old problems: What is the functional significance of the laminar organization of the lateral geniculate body, and what is the functional significance of the laminar organization of the striate cortex? At one time these two problems were unrelated, but now it seems that any step toward a solution of one will shed light on the other. The tree shrew has proved to be a valuable subject in the inquiry because the granular fourth layer of striate cortex is so distinct in this species and because it is divided into two tiers by a conspicuous cleft. This division (what we have called IVa and IVb) permitted us to show for the first time that some geniculate layers project to the tier above the cleft and some layers project to the second tier. Indeed, we found evidence suggesting that *each* geniculate layer projected to a distinct subtier in layer IV of striate cortex (Harting et al., 1973).

We have undertaken the present study for two reasons. The first is directed to the narrow question of resolving the differences between the results of our anterograde degeneration study of geniculostriate projections in *Tupaia* (Harting et al., 1973) and later studies using transneural transport and electrophysiological methods (Casagrande and Harting, 1975; Hubel, 1975; Humphrey et al., 1977). These later studies did not support the conclusion that each geniculate layer projects to a single subtier of striate cortex layer IV.

The second reason concerns the more general question: "What is being segregated by the layers of the lateral geniculate nucleus?" Perhaps the most direct approach to this question would be to try to relate different behavioral functions to different geniculate layers, as was attempted by Le Gros Clark to test his idea that each layer in a set (either the ipsilateral or contralateral set)

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is the target of one of the three "color cells" in the retina (Le Gros Clark and Chacko, 1947; Le Gros Clark, 1949). More recently, anatomical and physiological methods, although providing only indirect evidence of behavioral function, have made significant advances such as the discovery that in the primate the magnocellular and parvocellular layers correspond to the so-called "Y cells" and "X cells" of the retina (Dreher et al., 1976). Now we come to the central issue of the paper: the relation between layers in the lateral geniculate nucleus and layers in the striate cortex. In the primate, dissimilar layers project to *different* layers of the striate cortex, whereas similar geniculate layers project to the *same* cortical layer. However, the organization in cat suggests that it is not the layer per se which is related to cortical termination but *cell type*, since X and Y cells which are mixed in layers A and A1 project to different cortical tiers (Ferster and LeVay, 1978; Bullier and Henry, 1979). Our previous study of tree shrew raises the question: "Does the evidence that each geniculate layer projects to a different cortical layer mean that each layer is functionally different?" To answer this question and to seek confirmation of the earlier results using anterograde degeneration, we decided to reinvestigate the projections of single geniculate layers using more sensitive transport methods—both retrograde and anterograde.

Materials and Methods

Twenty-two tree shrews (*Tupaia glis*) between 150 and 200 gm served as subjects. Animals were anesthetized with 0.5 ml of ketamine hydrochloride (50 mg/ml) and 0.3 ml of diluted Nembutal (5 mg/ml in saline). During the course of the experiments supplementary doses of diluted Nembutal were given as required. Subjects also received a 0.2-ml intraperitoneal injection of atropine sulfate to limit mucus secretions and 0.1 ml of dexamethasone to control edema.

Since the success of these experiments depended upon accurate placement of small injections of wheat germ agglutinin-conjugated horseradish peroxidase (WGA-HRP) into one, or at most, a few geniculate layers, we could not rely entirely upon conventional stereotaxic procedures. Instead, the lateral geniculate was first located with a recording electrode, and, having identified a sequence of ipsilateral and contralateral layers, we tried to retrict HRP to the designated target. The injection of HRP was accomplished in one of two ways. In some instances the same electrode was used both to record responses and to inject HRP (5.0% WGA-HRP (Sigma) in 0.1% poly-L-α-ornithine in saline). But this technique carried with it the possibility of inadvertent leakage of HRP, particularly when multiple penetrations were required to identify the layers of the lateral geniculate body. Alternatively, a saline-filled (0.9%) electrode was used to locate the geniculate layers with respect to the cortical surface. After the layers were identified in terms of our coordinates, the saline-filled electrode was withdrawn and replaced with an HRP-filled electrode which was then lowered to the appropriate location.

All penetrations were made between 30° and 40° from the vertical in order to avoid damaging the striate cortex. Iontophoretic injections of WGA-HRP (electrode positive) were made with a Midgard constant current stimulator model CS3. In all cases injections were made over a 30-min period with alternating current (9 sec on, 9 sec off) at 0.5 to 1.0 μA.

Cortical injections. Injections of HRP into single layers of the striate cortex were made with 20% HRP (Toyobo Co. LTD, Osaka, Japan) dissolved in 0.1% poly-L-α-ornithine in saline. Injecting electrode tip diameters were in all cases 15 μm, driven by a Grass Instruments S4 simulator which generated two square wave pulses per second, each of 200 msec duration. Current was maintained at levels of 0.5 to 1.0 μA for 30 min.

Injections of HRP into the white matter underlying the striate cortex were made using 20% HRP dissolved in 0.9% saline. Except for the electrode tip diameters (40 to 50 μm) and the current level (5.0 μA), the injecting parameters were the same as those for cortical injections.

Eye injections. It is not always easy to identify layers of the lateral geniculate body in *Tupaia*, especially in experimental material. In part, this is because the layers are so similar to each other and, in part, it is because the same layers are not present at different rostrocaudal levels. We found it useful to mark the layers of the lateral geniculate nucleus, and to accomplish this, 5.0 to 20.0 μl of 5.0% WGA-HRP were injected into the vitreous chamber of the left eye of each animal. Of course we recognized that we had to pay some price for this unambiguous identification of layers: cells labeled by *retrograde* transport to the lateral geniculate body from an HRP injection into the cortex could be obscured by the *anterograde* transport of HRP from the eye. In order to avoid this potential difficulty, low volumes (less than 10.0 μl) of WGA-HRP were injected into the eyes of animals used in retrograde transport experiments. Only once in 10 retrograde transport experiments was there any question about whether geniculate cells labeled from cortical injections were obscured by anterograde transport from the retina, and this case was discarded.

Histology. After a 48-hr survival period, animals were perfused through the heart with a solution of 10.0% phosphate-buffered (0.1 M, pH 7.6) formalin which contained 2.0% sucrose. This was followed by a 10.0% phosphate-buffered (0.1 M, pH 7.6) sucrose rinse which contained 1.0% dimethylsulfoxide.

Immediately after the perfusion brains were cut frozen at 50 μm and reacted for peroxidase histochemistry with benzidine dihydrochloride. Every other section was stained for Nissl substance with neutral red; the remaining sections were coverslipped unstained because it was found that staining occasionally obscured anterograde label in the cortex.

For the three animals in which HRP was injected into the white matter underlying the striate cortex, survival times and perfusions were the same as above, but frozen sections were cut at 100 μm and processed for peroxidase histochemistry using cobalt chloride-intensified diaminobenzidine (Adams, 1977), and counterstained with cresyl violet.

Axon reconstruction. Individual axons were reconstructed from camera lucida drawings made under a ×100 oil objective at a final magnification of ×1600. In order to be certain that every collateral branch was identified

from a particular parent stalk, each axon was traced approximately 1 mm into the white matter. In almost all instances this required that axons be traced through two, three, or more consecutive sections. When the axon and all of its terminal branches were drawn, it was then re-examined under lower power (usually ×20 or ×40), and the laminar borders were added.

Measurements of axon diameters were made from ×1600 camera lucida drawings of whole reconstructed axons ($N = 12$) and of axons with known destinations ($N = 29$) of which part only the parent stalk was drawn. In order to get an accurate estimate of fiber diameter, each axon was measured at five randomly chosen points between layer V and the white matter from which was derived a mean axon diameter. There was some evidence for local thinning and thickening of axons which corresponded most probably to changes in myelination, but myelin sheathes were not observed directly (Ferster and LeVay, 1978), and, thus, measurements were *not* made at internodal points.

Results

Retinogeniculate projections. Although our primary aim was not to study the character of retinogeniculate projections, the use of anterograde transport of WGA-HRP from the retina for the purpose of marking geniculate layers revealed some differences between layers 3 and 6 and the other geniculate layers that are worth illustrating. Figure 1, *a* and *b*, shows the distribution of labeled retinal terminals in the ipsilateral and contralateral geniculate layers. The contralateral set includes four layers, two of which (layers 2 and 4) are very dark with terminals, and two of which (layers 3 and 6) are light. This difference, especially between layer 3 and the other layers, is not a new finding and was first reported by Glickstein (1967) in his study of anterograde degeneration after optic tract section.

The present material suggests that layer 6 may be similar to layer 3 in that the terminals from the retina in both layers appear to be less dense and of finer caliber. Studies currently in progress in which the pattern of termination of individual optic tract axons was studied following injections of HRP into the optic tract confirm that both size of terminals and caliber of retinogeniculate axons terminating in layers 3 and 6 are smaller than those in the remaining layers (M. Conley and I. T. Diamond, unpublished studies). The finer caliber of the terminals further suggests the possibility that layers 3 and 6 receive smaller fibers that in turn originate from a distinct class of retinal ganglion cells.

Anterograde transport of WGA-HRP from the lateral geniculate body to the striate cortex. In two experiments large injections of WGA-HRP were made for the purpose of seeing the pattern of projections from *all* geniculate layers. The results of one case (2352R) are shown in Figure 2. The photomicrograph of the injection site shows that WGA-HRP was injected into every geniculate layer and also diffused into the pulvinar nucleus. (The possibility that projections from the pulvinar nucleus contributed to the pattern shown in Fig. 2 can be disregarded for the present; later results with more restricted injections demonstrate that each part of the total pattern is accounted for by a part of the geniculate system.) The most striking feature in the cortex after this injection was the intense band of labeled terminals corresponding to layer IV.

Our initial impression was that the borders of this intense band corresponded precisely with the borders of layer IV, but later results led us to re-examine the dorsal border. Closer study revealed that the intense label extended into layer IIIc, and we will come back to this point. In addition to heavy terminations in layer IV, there was a conspicuous termination in layer IIIb and a sparse projection to the outer portion of layer I. In this and other cases in which large injections of WGA-HRP were made into the lateral geniculate nucleus, cortical layer VI was filled so densely with cells labeled by retro-

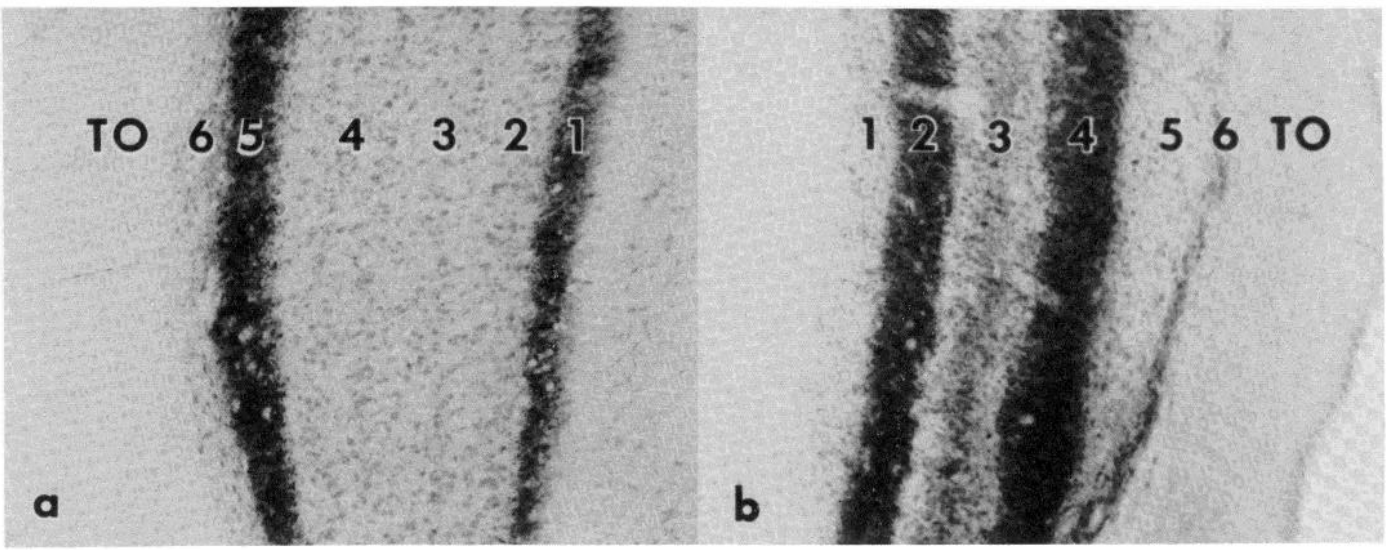


Figure 1. Distribution of HRP-labeled retinal terminals following an injection of WGA-HRP into the left eye. *a*, Ipsilateral layers 1 and 5 contain coarse, darkly labeled terminals; *b*, contralateral layers 2 and 4 contain coarse, darkly labeled terminals whereas layers 3 and 6 contain much finer, lighter label.

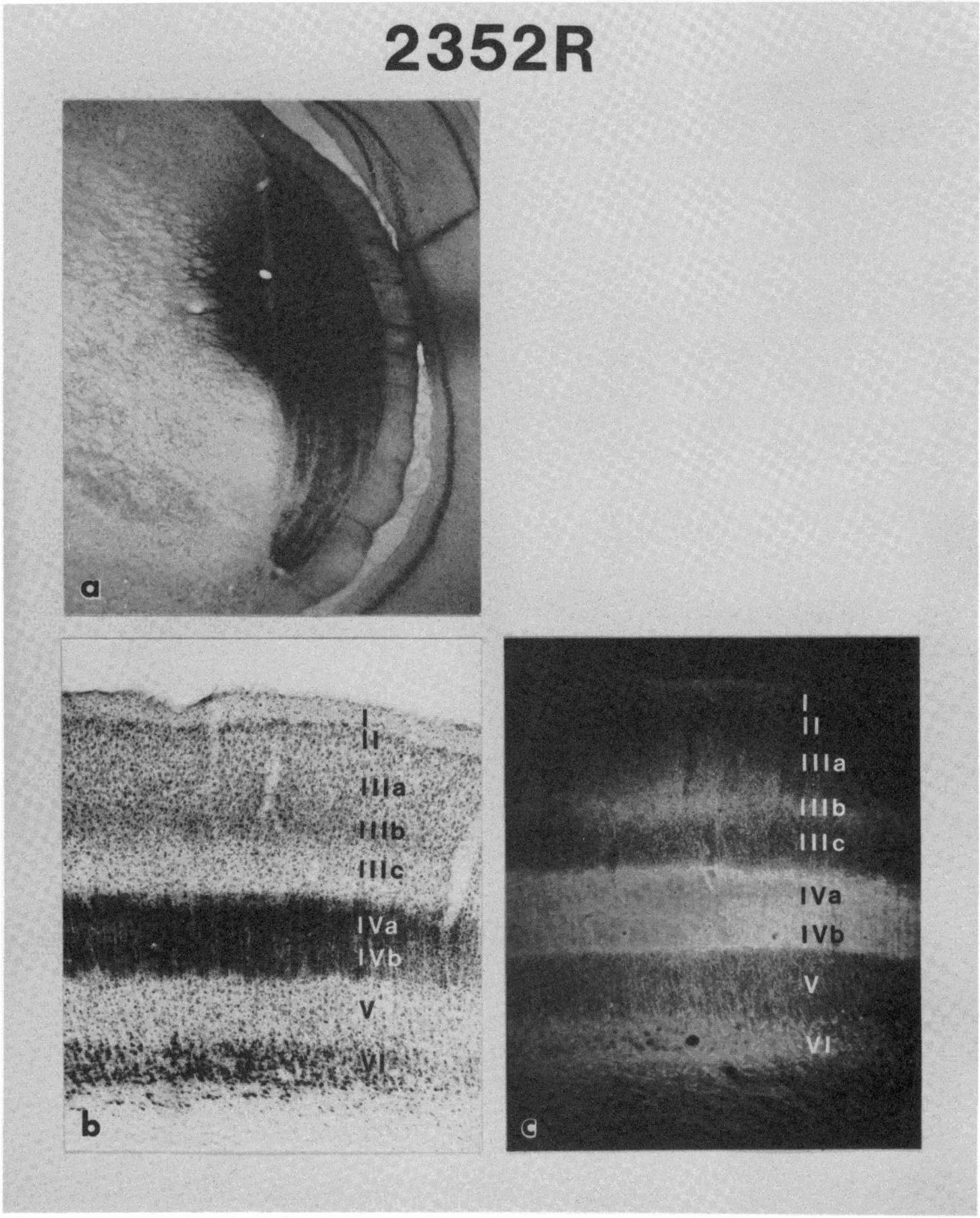


Figure 2. Anterograde transport from the lateral geniculate body to the striate cortex in case 2352R. *a*, Photomicrograph of the injection site which included all layers of the lateral geniculate body and the lateral portion of the pulvinar nucleus; *b* and *c*, light and darkfield photomicrographs of the distributionn of WGA-HRP-labeled terminals and cells in the striate cortex. There are three distinct bands in the cortex: one corresponds to layer VI and consists chiefly or solely of cells labeled by retrograde transport and thus can be ignored; the second band corresponds apparently to layer IVa plus IVb; the third band is in IIIb. Labeled terminals are also present in layer I.

grade transport that it was impossible to determine whether labeled terminals might also be present. However, in cases where small injections of WGA-HRP were made into one or a few geniculate layers, only labeled somata and a few scattered fibers appeared in layer VI. Furthermore, examination of more than 40 geniculostriate axons revealed not a single collateral branch that terminated in layer VI. Thus, *Tupaia* may be different from cat and monkey, in which species the lateral geniculate projects to striate cortex layer VI (see LeVay and Gilbert, 1976; Ferster and LeVay, 1978; Hendrickson et al., 1978).

The contribution of the various layers to this total pattern can be determined by smaller injections of WGA-HRP restricted to a few geniculate layers. But before examining individual protocols, a first approximation of the connections of individual geniculate layers with the striate cortex can be derived from compiling results as shown in Table I. Table I shows that of the seven injections, four produced labeled geniculate terminals in cortical layer IIIc, and these are the only cases which involved geniculate layer 6.

Five experiments involved geniculate layer 3, and all five resulted in labeled terminals in cortical layer IIIb. Two cases did not include layer 3 and neither contained terminals in cortical layer IIIb.

There were three cases with labeled terminals in cortical layer IVa and all three involved geniculate layers 1 and/or 2. Four injections did not produce labeled terminals in cortical layer IVa, and none of the four involved geniculate layer 1 or 2.

Five experiments produced labeled terminals in cortical layer IVb and all involved geniculate layers 4 and/or 5. In neither of the two cases without terminals in IVb was WGA-HRP injected into geniculate layers 4 or 5.

These comparisons and subtractions provide, as a first approximation, the following conclusions: (*1*) geniculate layers 1 and 2 project to cortical layer IVa, (*2*) geniculate layers 4 and 5 project to cortical layer IVb, (*3*) geniculate layer 6 projects to cortical layer IIIc, and (*4*) geniculate layer 3 projects to cortical layer IIIb.

The source of projections to cortical layer I cannot be inferred from Table I, probably because, even with all layers involved in the injection site, the terminals in layer I are very sparse.

Individual protocols provide finer details and further clues about the projections of each layer.

Figure 3 describes case 2346R in which WGA-HRP was injected into geniculate layers 3, 4, 5, and 6. The *upper half* of Figure 3 shows the injection site and the *lower half* shows that labeled terminals are located in three cortical layers: IIIb, a thin band at the base of IIIc, and IVb. Cortical layer IVa is conspicuously free of labeled terminals.

TABLE I

Experiment	Lateral Geniculate Layers in Injection Site						Cortical Layers Containing Terminals				
2352R	1	2	3	4	5	6	IVb	IVa	IIIc	IIIb	I
2346R			3	4	5	6	IVb		IIIc	IIIb	
2355L			3	4	5	6	IVb		IIIc	IIIb	
2355R			3	4	5		IVb			IIIb	
2367L					5	6	IVb		IIIc		
2367R		2	3					IVa		IIIb	
2359R	1							IVa			

The importance of this case is that it reveals the projection to the base of cortical layer IIIc as being separate from the projection to layer IV. This dissection of the projection to layer IIIc from the projection to layer IV would not have been possible had terminals been present in layer IVa, since the band at the base of IIIc is contiguous with the projections to the dense granular layer IV.

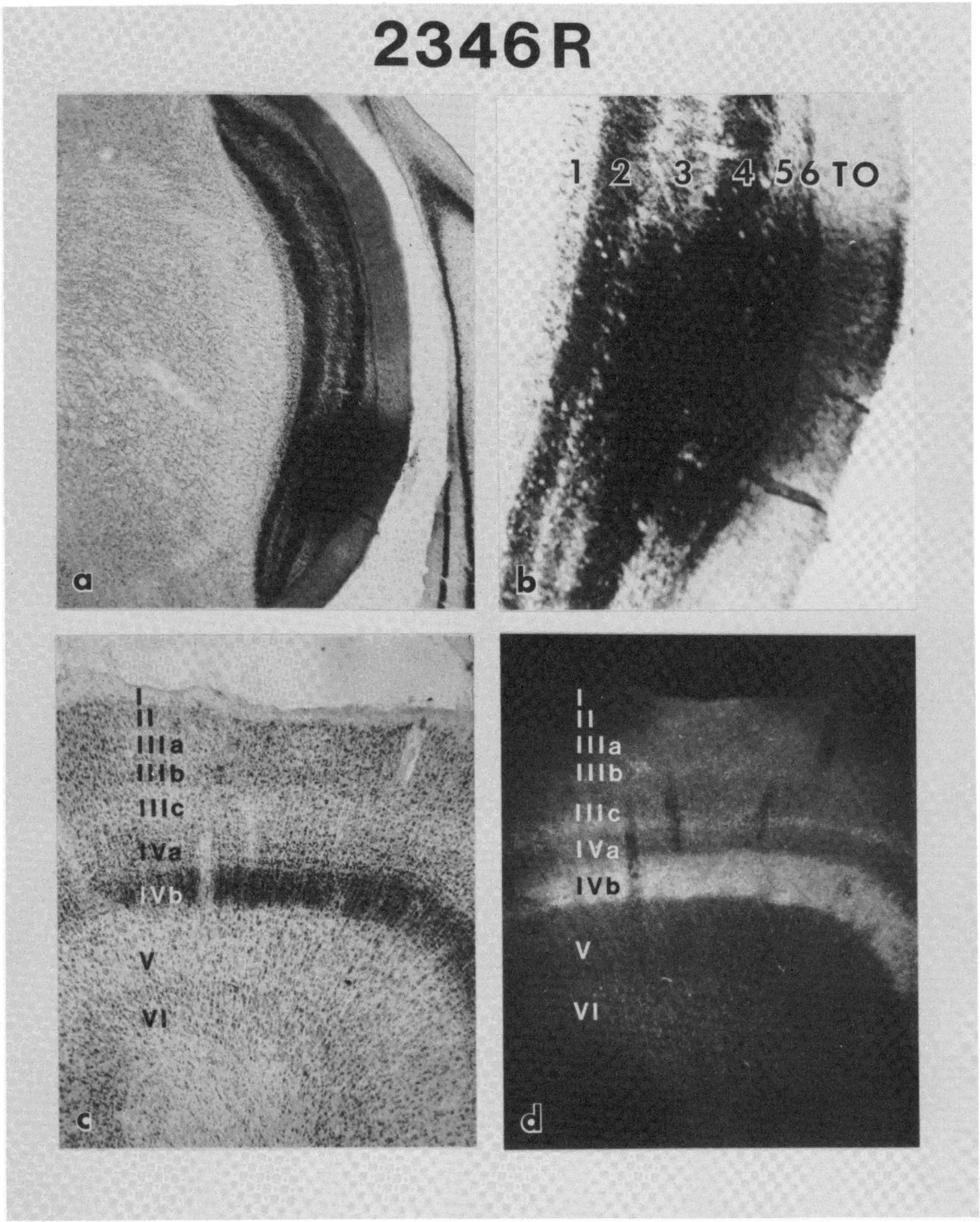
Experiment 2355L was virtually identical to 2346R, and it is illustrated to show at higher magnification the distribution of labeled terminals in cortical layers III and IV (see Fig. 4). Note that terminals densely populate the base of IIIc and IVb, whereas the distribution in layer IIIb is sparse.

Figure 5 illustrates the results of experiment 2355R, which shows that geniculate layer 6 is responsible for the projection to the base of IIIc. The injection site for this case was centered in geniculate layer 4 and included layers 3 and 5, but *not* 6. In the cortex layer IVb is labeled intensely, the base of layer IIIc is free of the dense band of terminals seen in the previous case, and layer IIIb contains a few scattered fibers and terminals. Since this and the two previous cases were identical with the exception of the involvement of geniculate layer 6, we conclude that at least one of the targets of geniculate layer 6 is the base of cortical layer IIIc.

If WGA-HRP is confined chiefly to geniculate layers 5 and 6, labeled terminals are confined to two cortical layers, IVb and IIIc (see case 2367L, Fig. 6). The terminals in layer IIIc can be attributed to the involvement of geniculate layer 6. In cortical layer IVb the density of terminals is clearly less than in cases where *both* geniculate layers 4 and 5 are involved. Furthermore, the terminals are not distributed uniformly—more being found at the base than at the top of the layer. This concentration suggests the possibility that geniculate layer 5 does not project throughout all of cortical layer IVb, or, if it does, it does so in a graded fashion.

In case 2367R, WGA-HRP was restricted principally to layers 2 and 3 (see Fig. 7). In the cortex labeled terminals are most conspicuous in layer IVa; a much sparser distribution of terminals is apparent in cortical layer IIIb. We attribute the labeled terminals in cortical layer IIIb to the involvement of geniculate layer 3 (see Table I). There is also a small patch of terminals at the base of cortical layer IVb, the significance of which cannot be derived from a comparison of individual anterograde transport cases alone. In a later section results will be presented which show that some geniculate axons which terminate principally in cortical layer IVa have a secondary target in layer IVb.

Case 2359R supports the idea that layers 1 and 2 project not only to cortical layer IVa, but also to layer IVb. Figure 8 shows that the injection site for this case was centered in geniculate layer 1 and might have encroached marginally on layer 2. In the cortex layer IVa is densely populated with terminals, less concentrated at


2346R
a
1 2 3 4 56 TO
b
I
II
IIIa
IIIb
IIIc
IVa
IVb
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VI
c
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IIIb
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Figure 3

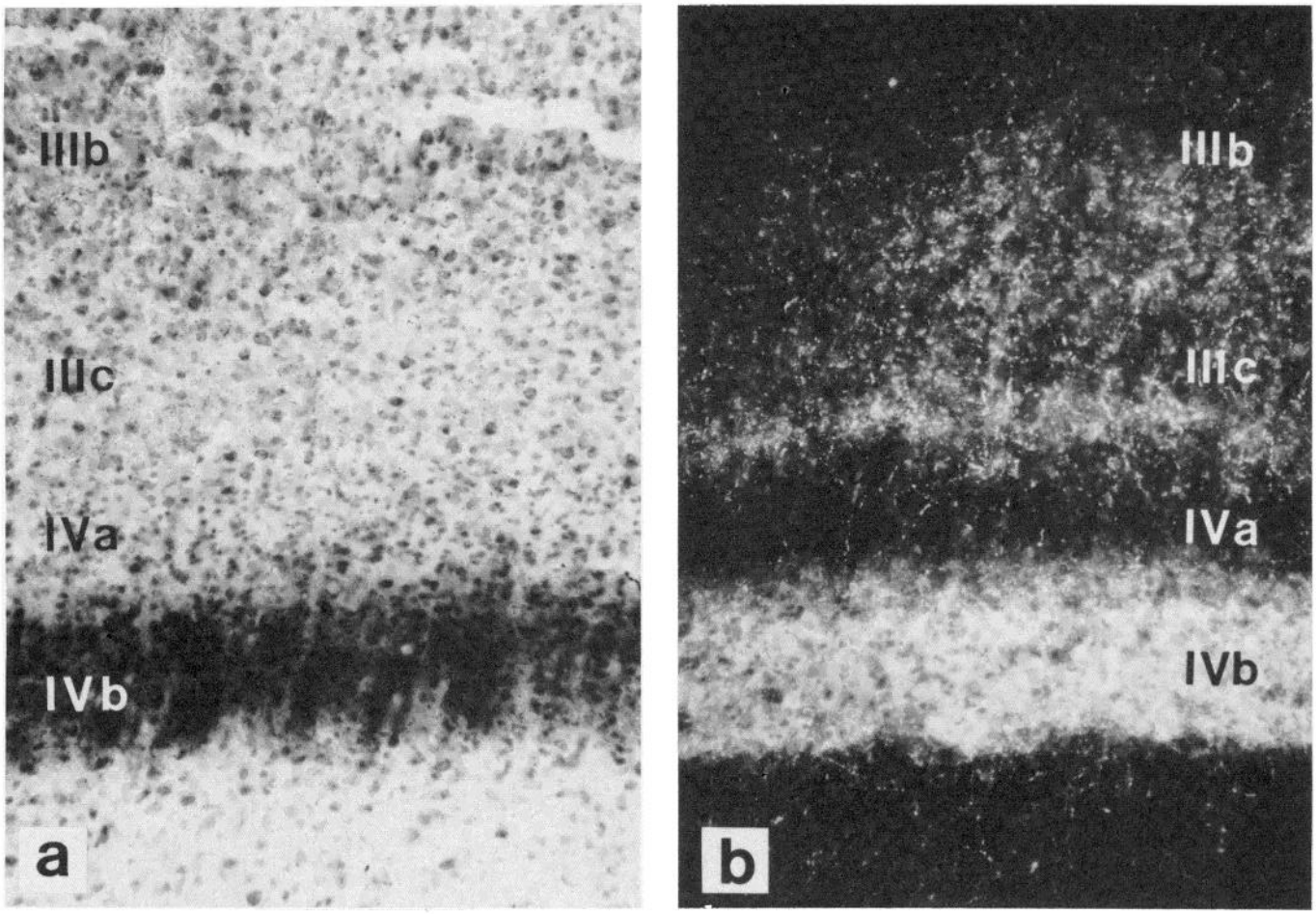

Figure 4. a and *b*, High power light and darkfield photomicrographs showing the distribution of labeled terminals in the striate cortex of case 2355L. The layer labeled most densely is IVb; terminals also fill the cleft. The label in layer IIIc forms a discrete band which straddles the IIIc/IVa border. Other terminals are scattered throughout layer IIIc and there are regularly spaced patches of terminals in layer IIIb.

the base than at the top of the layer. A few terminals are also present at the base of layer IVb. In some sections labeled terminals were confined just to the top half of layer IVa (see higher power photomicrograph in Fig. 9). This distribution is of special interest because similar thin strips of degenerated terminals were found by Harting et al. (1973). Our conclusion is that geniculate layer 1 projects principally to a thin strip at the very top of cortical layer IVa and to a lesser extent to the base of IVb. Sections in which terminals filled more of layer IVa might reflect the involvement of geniculate layer 2 as well as layer 1.

In sum, these anterograde transport results suggest that only four geniculate layers project to layer IV of the striate cortex: geniculate layers 1 and 2 project principally to cortical layer IVa, and geniculate layers 4 and 5 project principally to cortical layer IVb. There is some evidence which suggests that layers 1 and 5 do not project uniformly throughout IVa or IVb. Geniculate layers 3 and 6 project to separate targets above the granular fourth layer, layer 6 to the base of IIIc and layer 3 to layer IIIb. These conclusions can be tested directly with retrograde transport of HRP restricted to different layers of the striate cortex.

Retrograde transport of HRP from striate cortex to the lateral geniculate body. In general, the results of experiments using retrograde transport of HRP from cortex to thalamus support and complement the results just described. For example, a small injection restricted to cortical layers IIIc and IVa (see case 2315L, Fig. 10; see also Fig. 14*c*) resulted in 44 labeled geniculate cells, and all but one were in geniculate layers 1, 2, and 6. This

Figure 3. Anterograde transport from the lateral geniculate body to the striate cortex in case 2346R. *a*, Low power photomicrograph of the injection site in the lateral geniculate body. *b*, Higher power photomicrograph of the injection site. Note that layers 2, 3, 4, and 6 are marked with HRP reaction product from orthograde transport of WGA-HRP injected into the left (contralateral) eye. The injection site in the lateral geniculate body includes all of layers 6, 5, 4, and 3; layers 1 and 2 were not involved. *c* and *d*, Light and darkfield photomicrographs showing the distribution of HRP-labeled terminals and cells on the medial wall of the striate cortex. Labeled terminals are concentrated in layer IVb and the cleft but do not encroach upon layer IVa. The base of layer IIIc is covered by a thin, dense band of labeled terminals. Some light, patchy label occurs in layer IIIb.

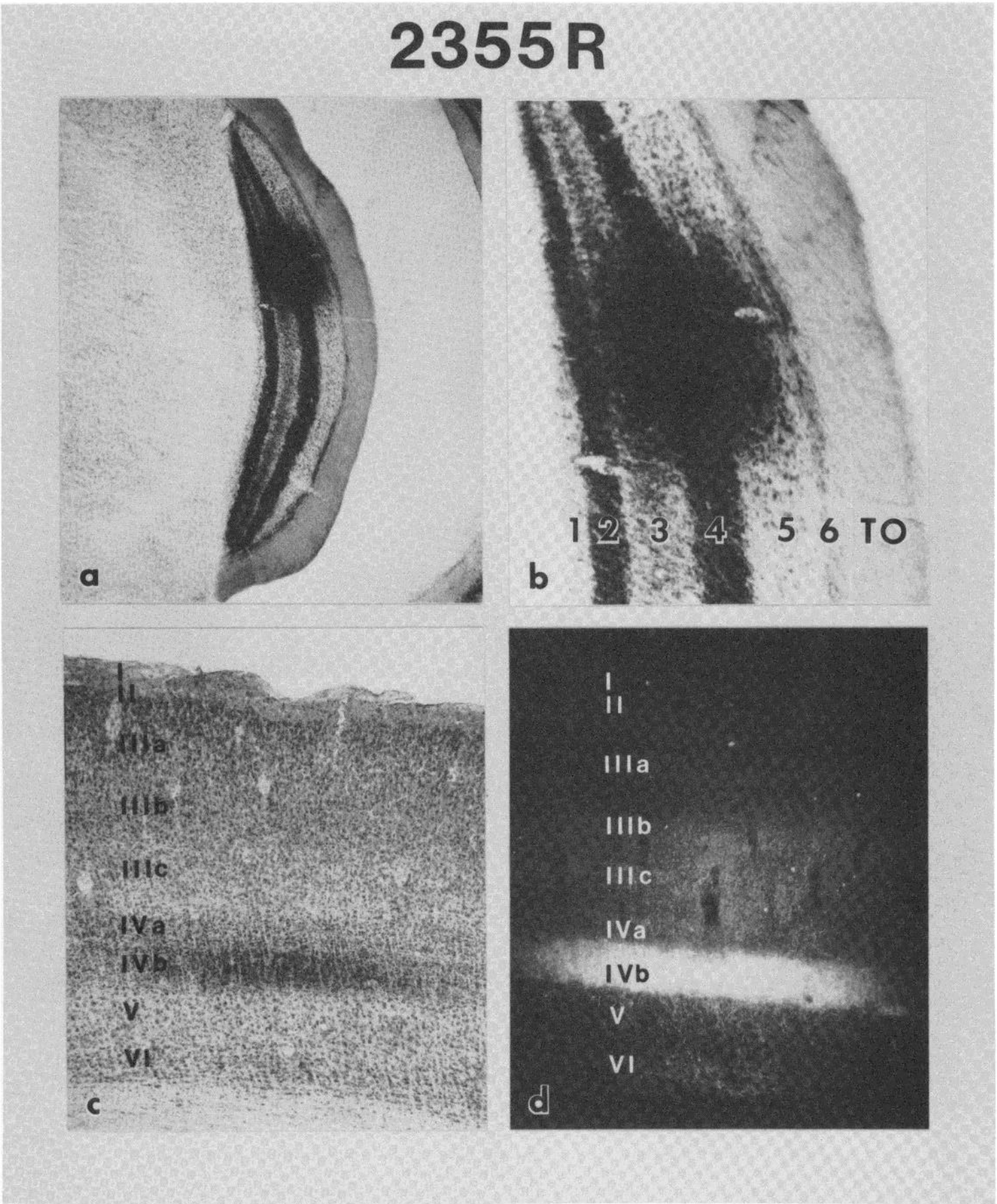

Figure 5. Anterograde transport from the lateral geniculate body to the striate cortex in case 2355R. *a*, Low power photomicrograph of the injection site in the lateral geniculate body. *b*, Higher power photomicrograph of the injection site which includes geniculate layers 3, 4, and 5, but *not* layer 6. *c* and *d*, Light and darkfield photomicrographs showing the distribution of HRP-labeled terminals and cells in the striate cortex. Layer IVb and the cleft labeled heavily, but the base of IIIc is free of the dense band seen in cases 2355L and 2346R. Scattered terminals are seen in layers IIIb and IIIc.

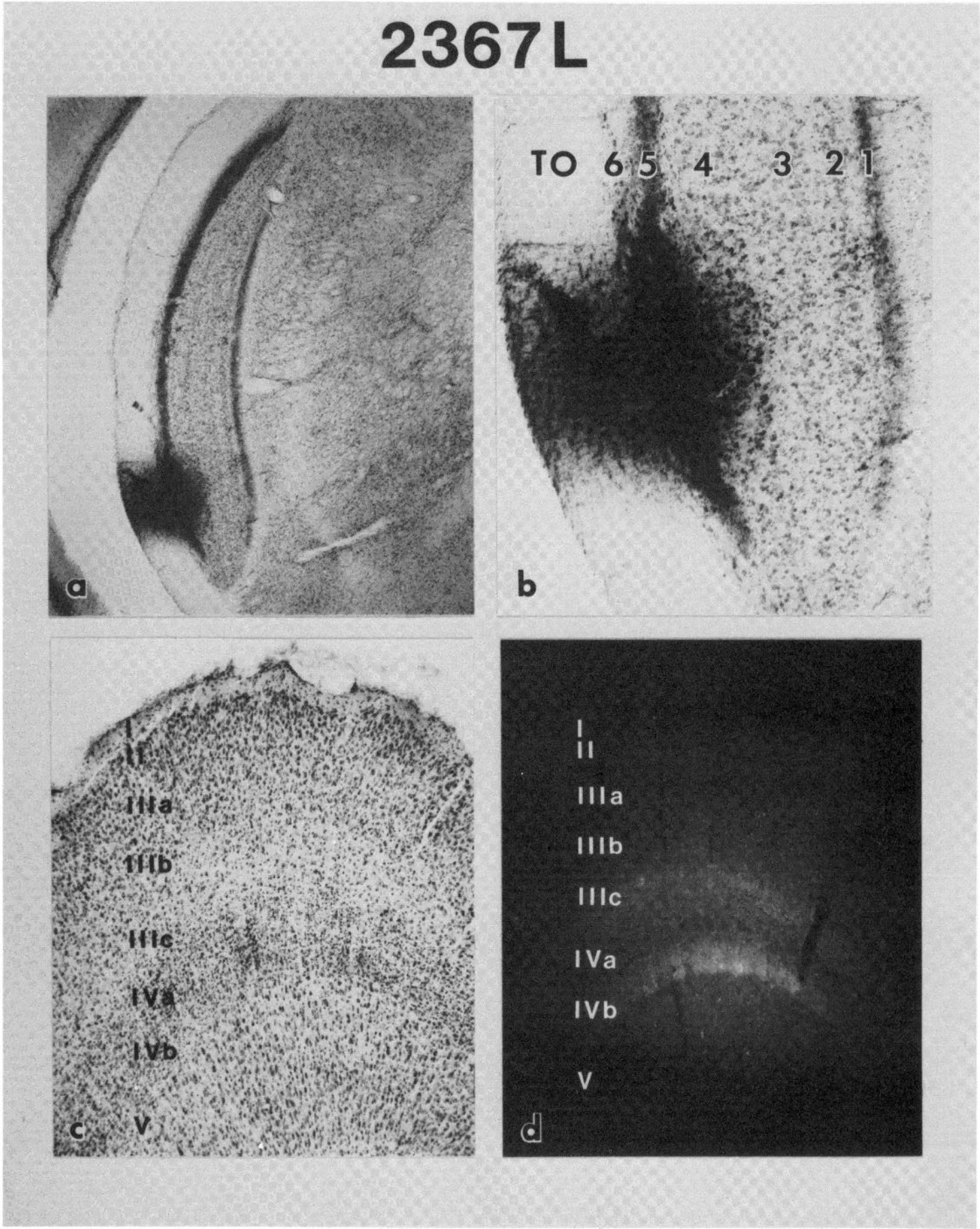

Figure 6. Anterograde transport from the lateral geniculate body to the striate cortex in case 2367L. *a*, Low power photomicrograph of the injection site in the lateral geniculate body. *b*, Higher power photomicrograph of the injection site which shows that all of layers 6 and 5 and a small portion of layer 4 were involved. *c* and *d*, Light and dark photomicrographs showing the distribution of labeled terminals in the striate cortex. Labeled terminals were concentrated in two zones: IVb, where the base of the layer is labeled more heavily than the top, and IIIc where a light band is visible.

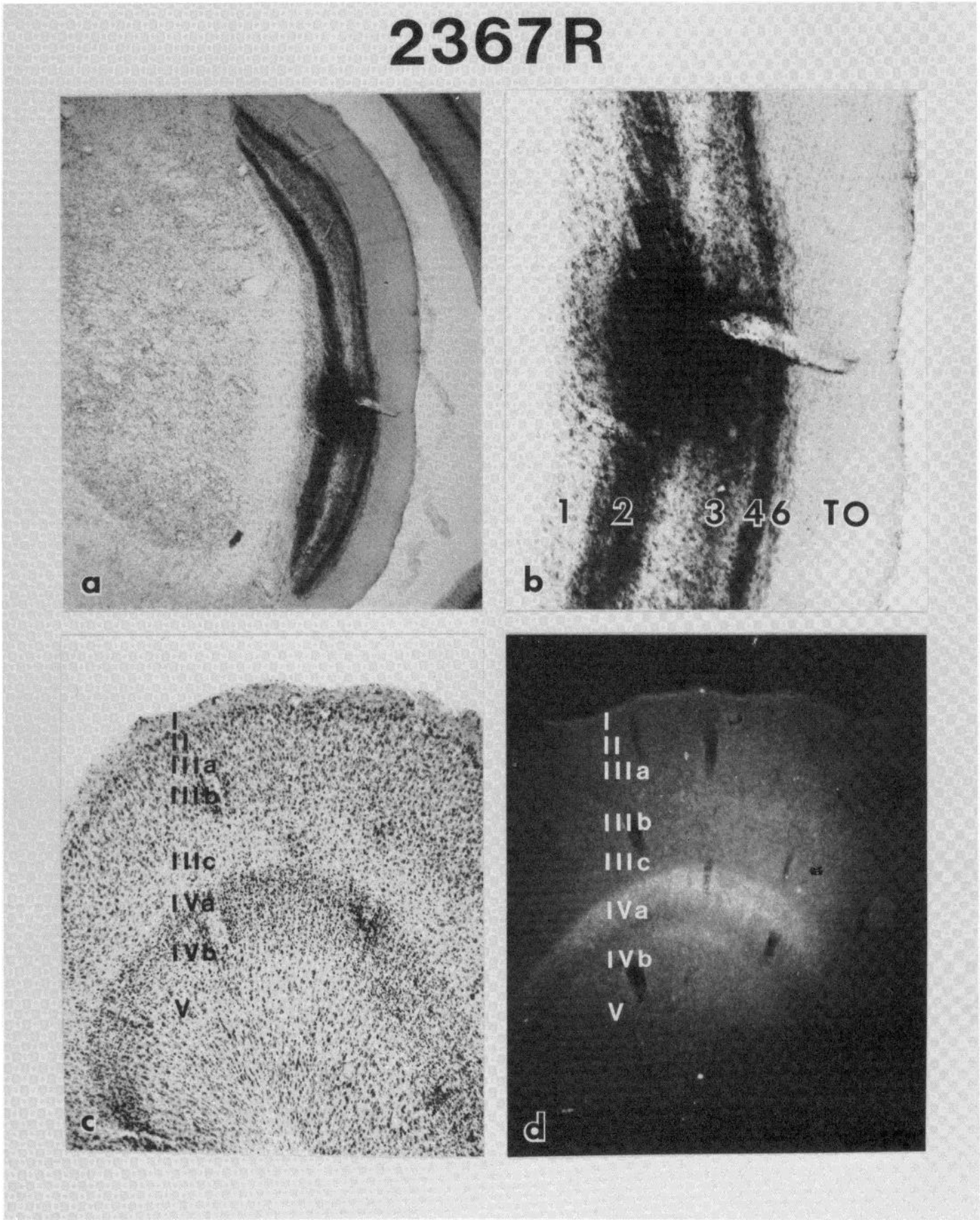

Figure 7. Anterograde transport from the lateral geniculate body to the striate cortex in case 2367R. *a*, Low power photomicrograph of the injection site in the lateral geniculate body. *b*, High power photomicrograph of the injection site which involves all of layers 2 and 3 and, perhaps marginally, layer 1. *c* and *d*, Light and darkfield photomicrographs of the distribution of HRP-labeled terminals in the striate cortex. The labeled terminals cover most of the width of layer IVa as well as a small region in IVb. There is lighter label in layer IIIb.

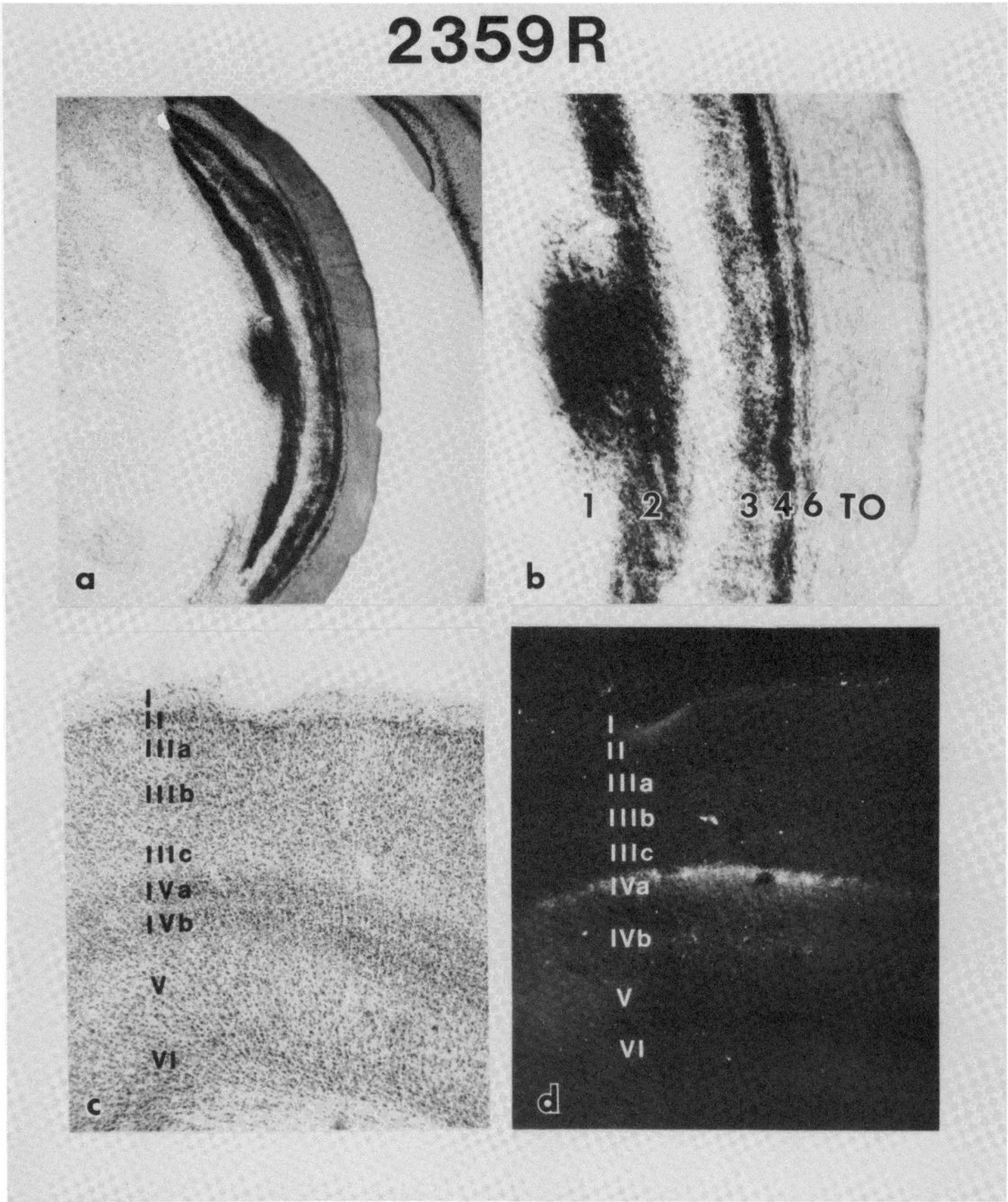

Figure 8. Anterograde transport from the lateral geniculate body to the striate cortex in case 2359R. *a*, Low power photomicrograph of the injection site in the lateral geniculate body. *b*, Higher power photomicrograph of the injection site in a site which involves only the most medial layer, layer 1. *c* and *d*, Light and darkfield photomicrographs showing the distribution of HRP-labeled terminals in the striate cortex. Labeled terminals are distributed mainly in layer IVa, but the distribution is not uniform and the top has more terminals than the base.

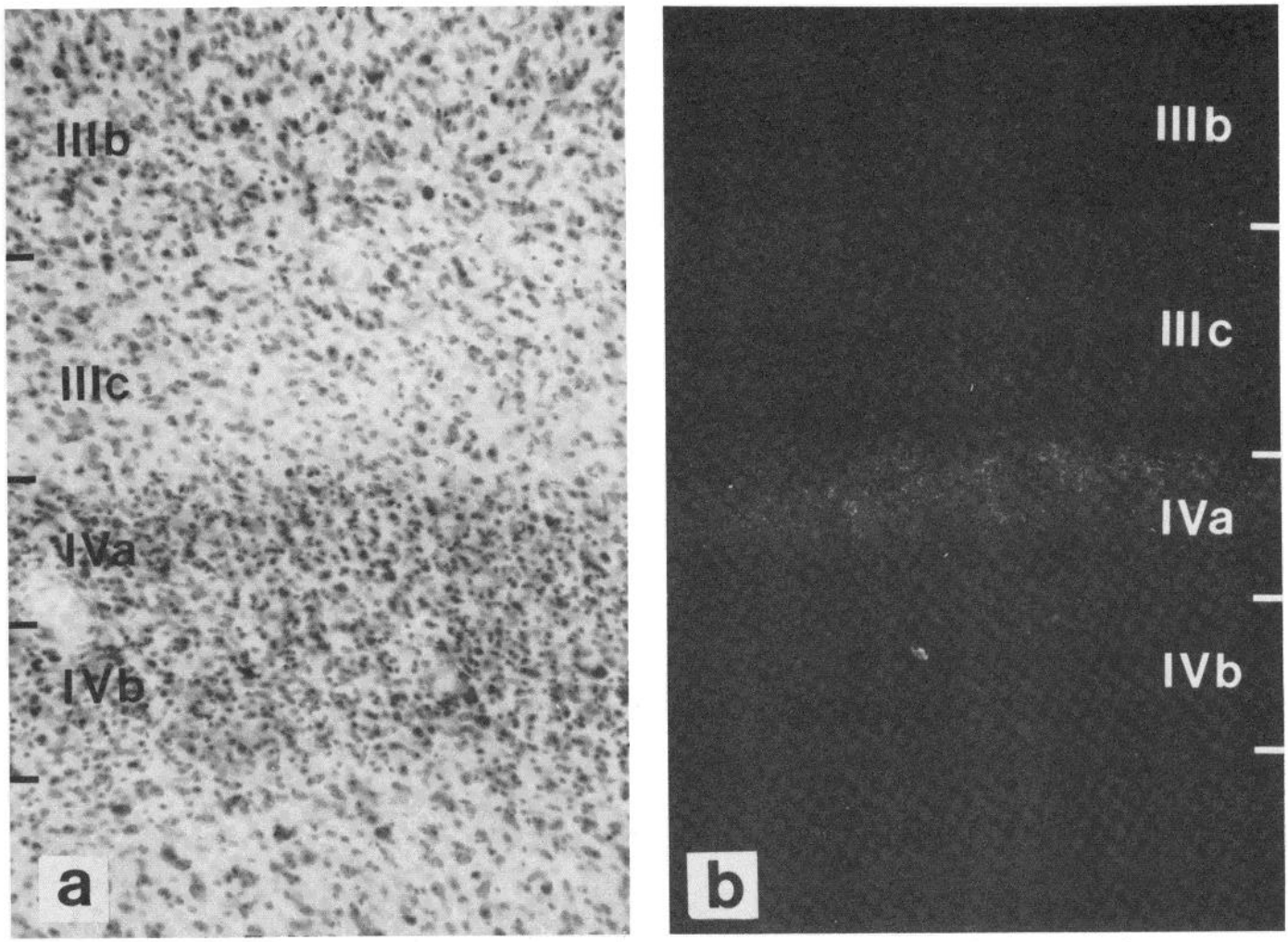

Figure 9. High power light and darkfield photomicrographs showing the distribution of labeled terminals in the striate cortex of case 2359R. Both are photographs of the same section as that shown in Figure 8*d*. Note that labeled terminals are concentrated in the dorsal one-third of layer IVa. The dorsal border of the label corresponds precisely with the layer of dense granule cells (IVa).

distribution is just what would be expected on the basis of anterograde transport results. Even the one "aberrant" cell can ultimately be explained by results to be presented below.

Figure 11 shows the results of a second HRP injection into the striate cortex, this time restricted mainly to layer IVb (see also Fig. 14*d*). The *frequency bar graph* shows that most of the labeled cells were in layers 4 and 5; the three cells in layers 1 and 2 could be explained by the slight diffusion of HRP into cortical layer IVa. (The final section in which individual axons are traced offers another explanation for the few cells in layers 1 and 2: single axons terminating chiefly in IVa send a few collaterals that terminate in IVb.)

The projections to layers IIIb and I are not as dense as those to layer IV; therefore small injections of HRP restricted to the superficial layers of the striate cortex labeled few cells. Still, something can be learned by collating results from several cases, and we show four injections in Figure 12 (see also Fig. 14*a*). Most of the cells labeled from these four experiments were in geniculate layer 3, and this is consistent with the anterograde transport results (see Table I) and also with the retrograde transport results of Carey et al. (1979).

It proved most difficult to inject HRP into cortical layer IIIc without involving the superficial layers or layer IV. Two experiments seemed to meet this aim to a reasonable degree, and a combined distribution from both is shown in Figure 13 (see also Fig. 14*b*). Layer 6 contained the most labeled cells, and this, too, is consistent with the anterograde transport results (Table I). The labeled cells in layers 4 and 5 could not have been anticipated from the results of anterograde transport cases alone, but results to be presented in the next section show that collateral branches of some geniculate layer 4 and 5 axons may terminate in cortical layer IIIc.

Reconstruction of geniculostriate axons. On the basis of the results presented in the two previous sections and on the basis of earlier studies (Harting et al., 1973; Casagrande and Harting, 1975; Hubel, 1975), we support the conclusion that cortical layer IVa is the target of geniculate layers 1 and 2, cortical layer IVb is the target of

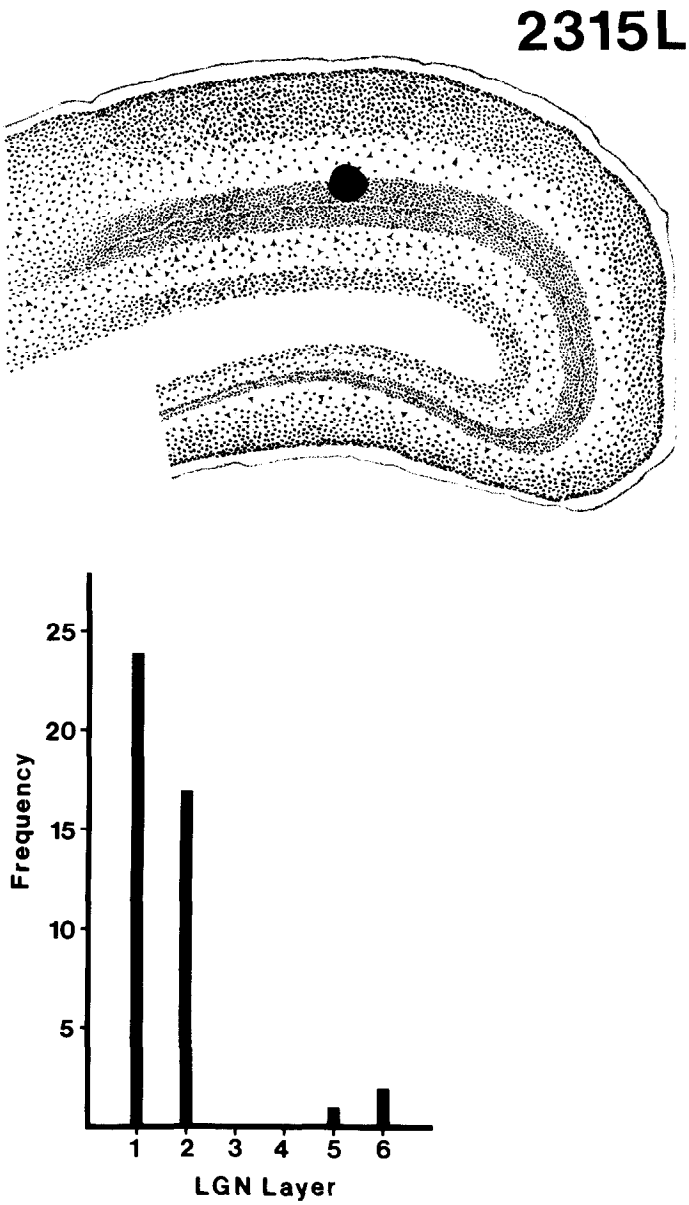

Figure 10. Summary diagram of case 2315L in which HRP was restricted to cortical layer IVa. Almost all of the labeled cells in this case were in layers 1 and 2 supporting the idea that layers 1 and 2 project to layer IVa. The three cells in layers 5 and 6 can be attributed to the slight involvement of layer IIIc.

geniculate layers 4 and 5, cortical layer IIIc is the target of geniculate layer 6, and the superficial cortical layers (IIIb and above) are the targets of geniculate layer 3.

The main questions that remain unanswered are: First, to what extent, if any, do projections from layers 1 and 2 overlap in cortical layer IVa? Similarly, what is the extent of overlap of projections from layers 4 and 5 in cortical layer IVb? Second, do geniculostriate axons have collateral projections to more than one cortical layer? Third, is there any evidence that geniculate axons terminate in thin strips less than the width of IVa or IVb? In order to try to answer these questions we traced the course and pattern of termination of single geniculostriate axons after making large injections of HRP into the white matter underlying the striate cortex. For none of the axons to be described below can we be absolutely certain of the geniculate layer of origin. (Where the axon terminates outside of layer IV, we cannot even be certain that it originates in the lateral geniculate.) However, even with these qualifications, it turned out that some patterns of termination strongly suggested a layer of origin, and all of the results taken together helped answer the questions just raised.

Figure 15 shows an axon that terminates chiefly in the upper half of cortical layer IVa. Such a pattern is consistent with our picture of the projections of geniculate layer 1 (see case 2359R, Figs. 8 and 9). The main feature of this axon's terminal field is its restricted distribution. To be sure, not every terminal is in the upper one-half of layer IVa; a few collateral branches terminate at the base of layer IVb and a single collateral branch ascends

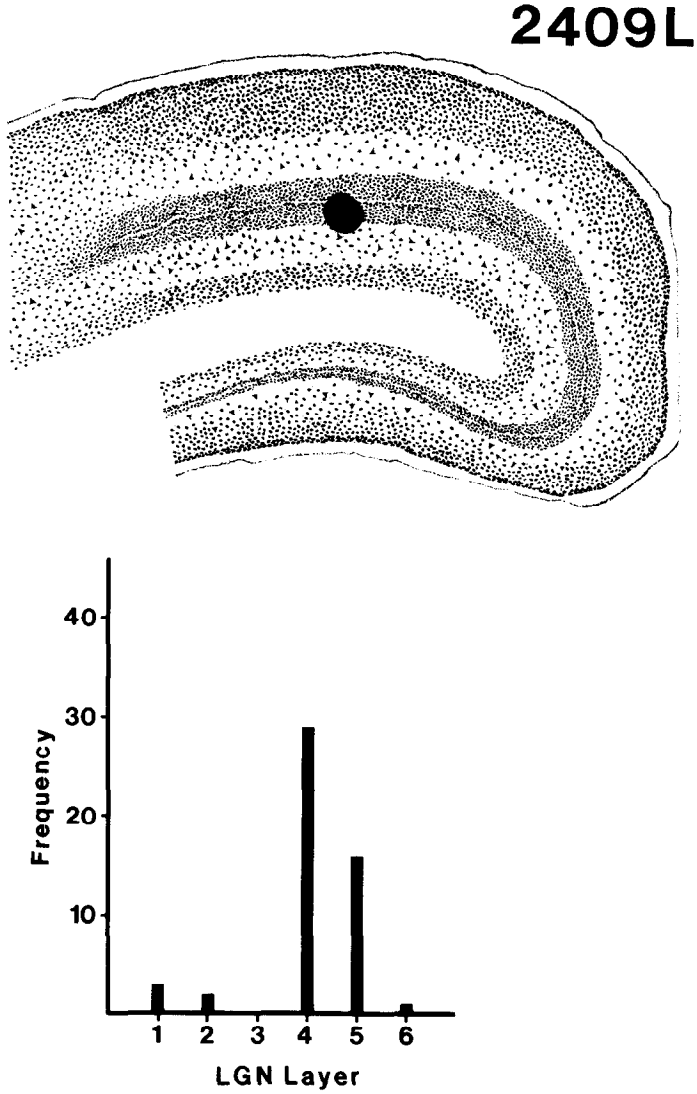

Figure 11. Summary diagram of case 2409L in which HRP was restricted mainly to cortical layer IVb. Most of the labeled cells were found in layers 4 and 5, but several others were seen in layers 1 and 2, most likely because HRP was transported by collateral branches of cells in these two layers which terminate in layer IVb.

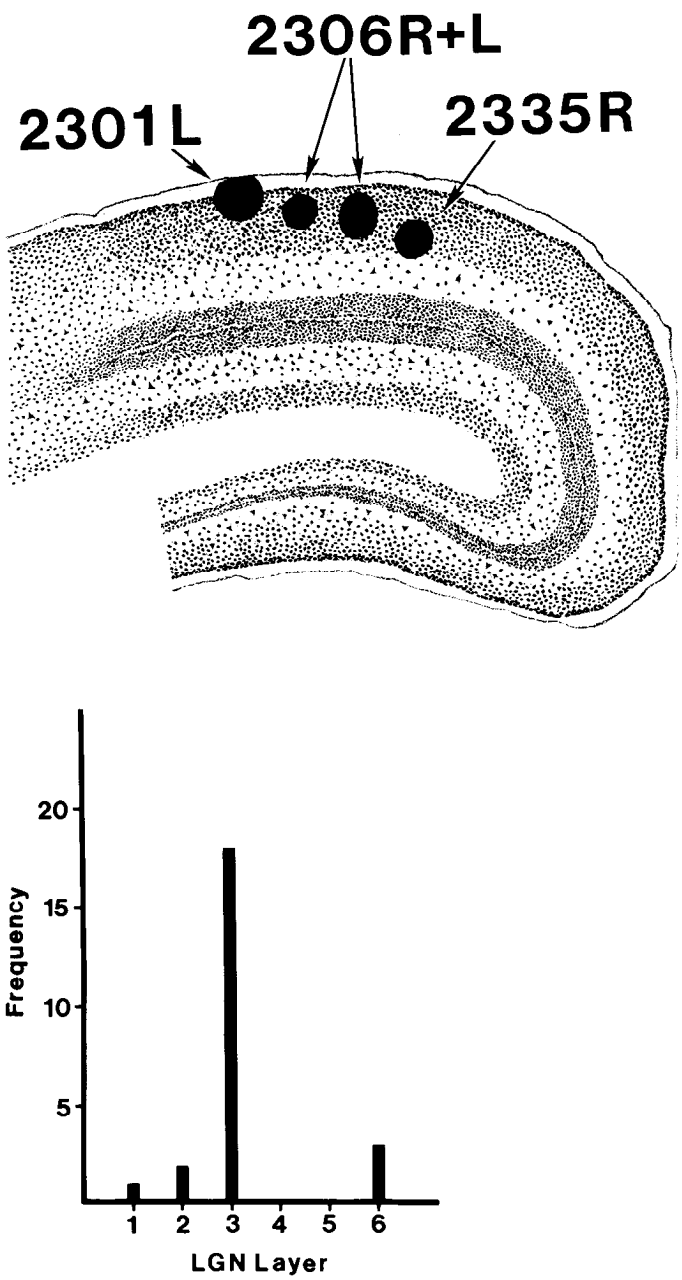

Figure 12. Summary diagram of four cases in which HRP was restricted to the superficial layers of the striate cortex (layers IIIb and above). Almost all of the labeled cells in the lateral geniculate from these cases were in layer 3, but a few were seen in layers 6, 2, and 1.

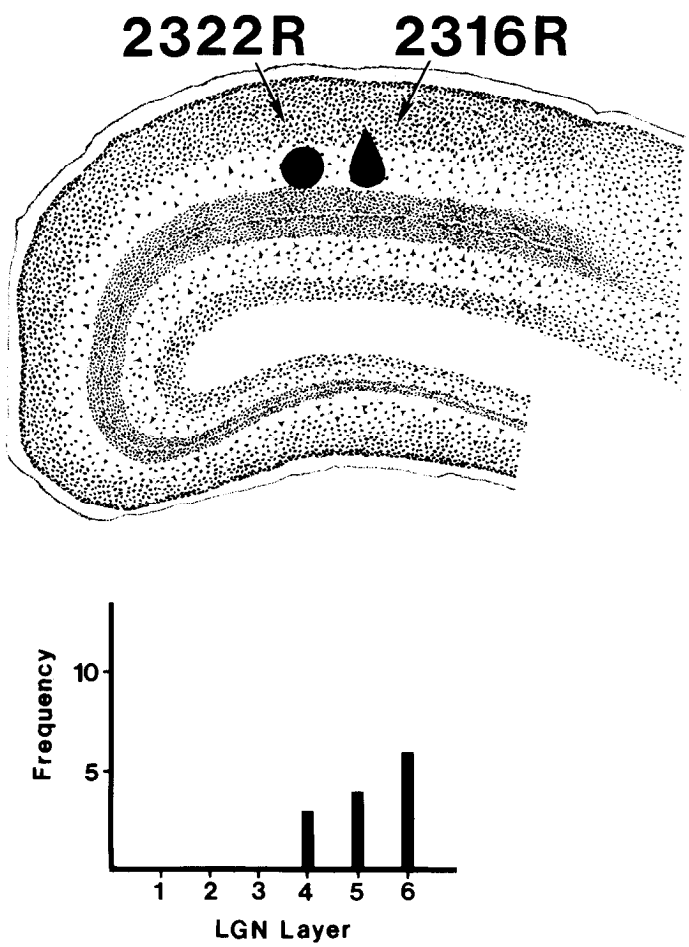

Figure 13. Summary diagram of two cases in which HRP was restricted to cortical layer IIIc. Most of the labeled cells in the lateral geniculate from these two cases were in layer 6, but almost as many were found in layers 4 and 5.

to layer IIIb giving off terminals en route. Similar axons with restricted arborizations at the top of layer IVa but with no collaterals to IVb have been described by Humphrey and Lund (1979). If this axon indeed arose from a cell in geniculate layer 1, then the collateral projection to the base of IVb takes on special significance since it must overlap to some extent the projections of geniculate layers 4 and/or 5 which are the principal sources of input to cortical layer IVb.

Figure 16 shows another axon whose terminal field is somewhat restricted. It, too, sends a small collateral branch to layer III, but the majority of its terminals are restricted to the base of layer IVb. This distribution, it could be argued, could fit either a geniculate layer 4 or geniculate layer 5 pattern, but layer 5 is the better choice in light of experiment 2367L (see Fig. 6).

If we are correct in assessing the layer of origin of these two axons just described, then some geniculate layer 1 axons send a minor projection to the major target of geniculate layer 5. The significance of this result is that both layers 1 and 5 are innervated by the ipsilateral eye.

We found other axons whose terminal fields were not so restricted, and these fell into two groups, those which filled all of cortical layer IVa or IVb (see Figs. 17 and 18), and those which filled the majority of layer IVa or IVb but which had collateral projections outside the main target (see Figs. 19 and 20). Since we have argued that the projections of geniculate layers 1 and 5 may be restricted to thin zones in the upper and lower extremes of layers IVa and IVb, respectively, it is tempting to conclude that the axons in Figures 17 and 19 may originate from cells in geniculate layer 2, whereas those in Figures 18 and 20 may originate from cells in geniculate layer 4.

We also reconstructed a number of axons which ter-

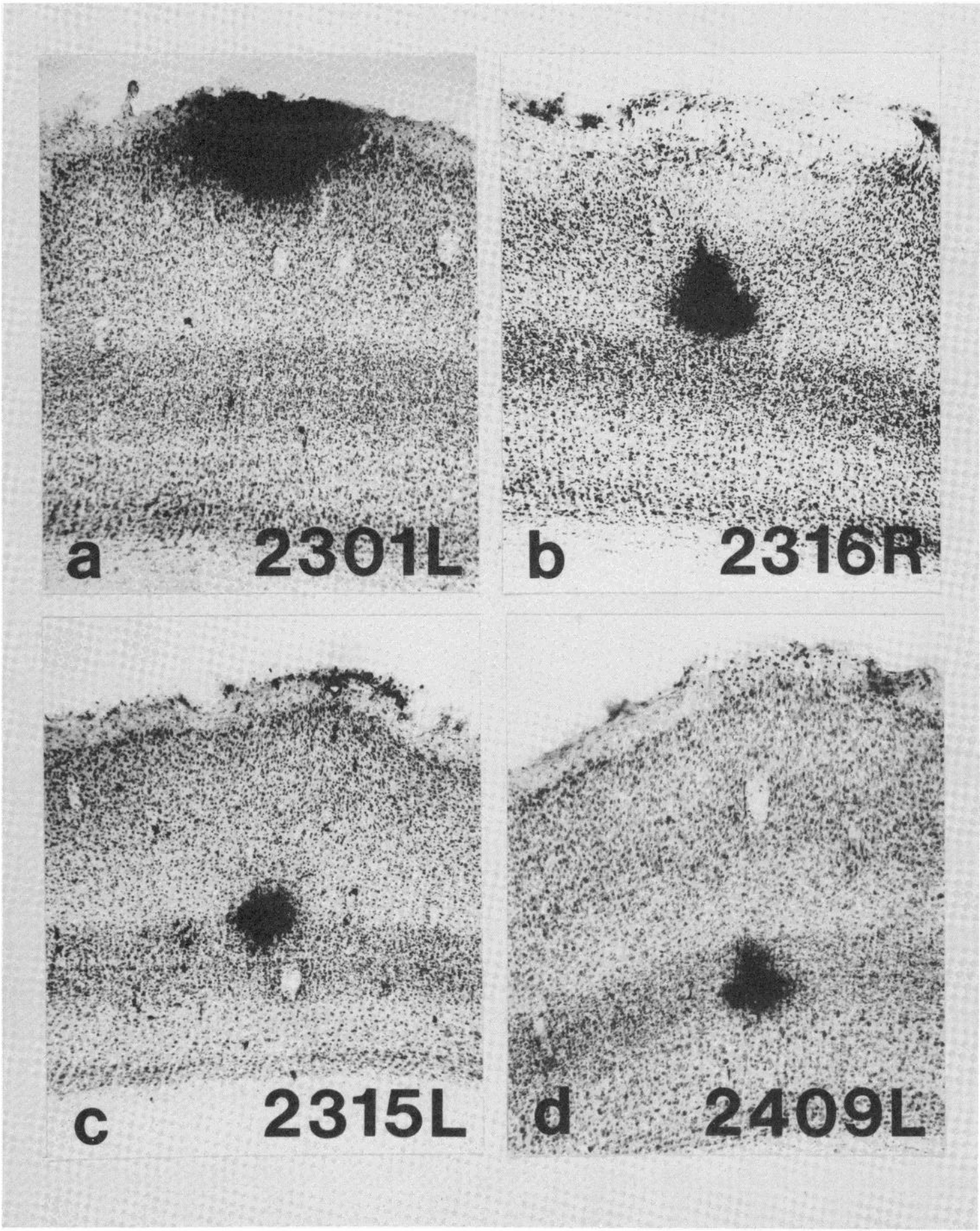

Figure 14. Representative cortical injection sites of (*a*) the superficial layers, (*b*) layer IIIc, (*c*) layer IVa, and (*d*) layer IVb.

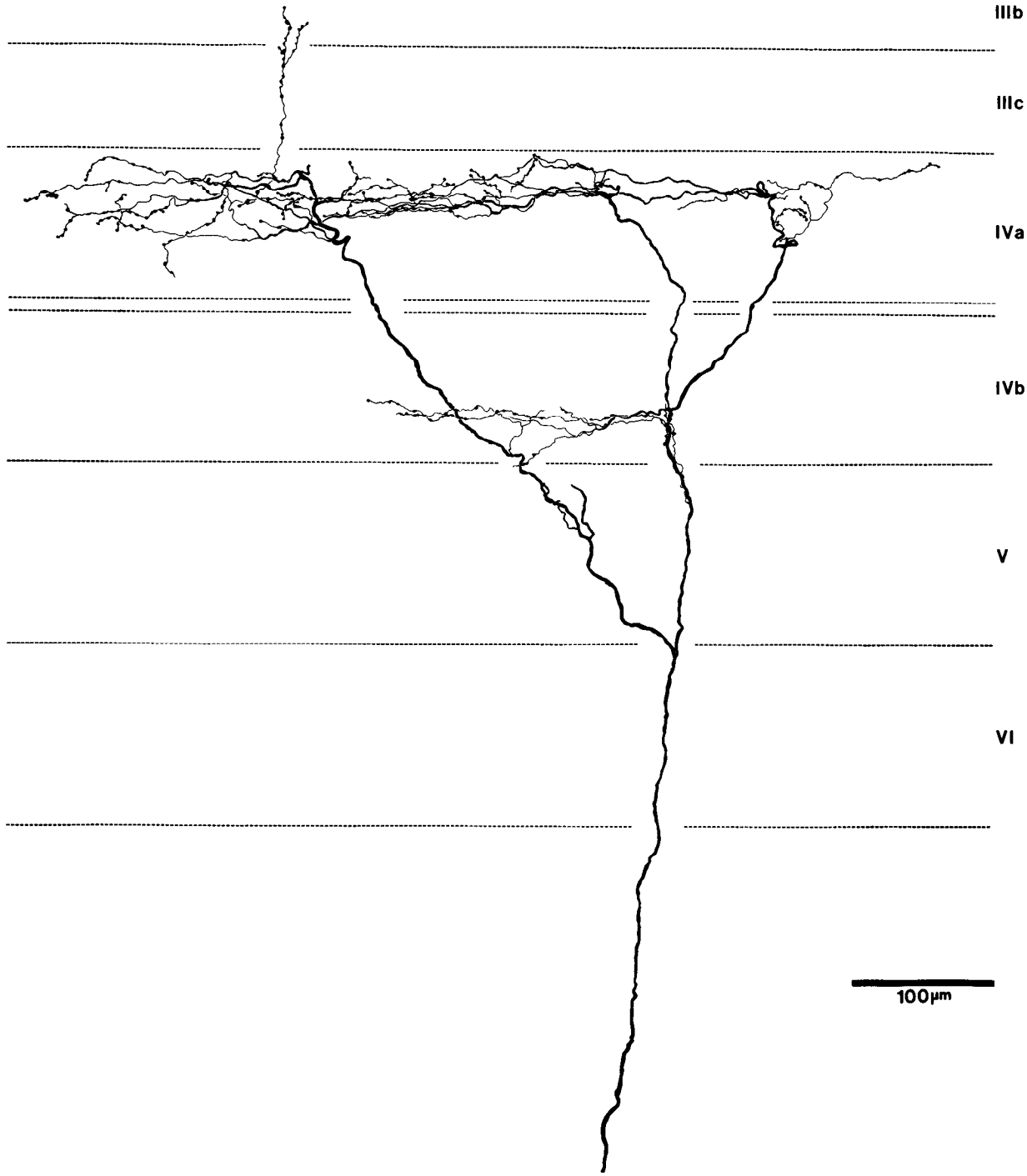

Figure 15. Reconstruction of a putative lateral geniculate layer 1 axon as it terminates in the striate cortex. The parent stalk divides once as it enters layer V; one branch ascends to layer IVa where it arborizes into a dense terminal plexus; a second branch divides again in layer IVb at which point emerge several thin, filamentous collaterals. The remaining branches ascend to layer IVa where they too arborize in a dense terminal plexus that covers approximately one-half of the upper tier. A single fine branch ascends from layer IVa to terminate in layer IIIb.

minated above cortical layer IV, many of which seemed to fit what we would call layer 3 or layer 6 patterns. One such axon which terminates at the base of cortical layer IIIc is shown in Figure 21. Again, it is tempting to equate this pattern with the projection of geniculate layer 6, but since extrageniculate sources project upon layer III, we can only form a hypothesis about its exact origin. Figure 22 is a summary diagram showing our interpretations of the projections of each of the six lateral geniculate layers.

Axon measurements. In both the cat and the monkey, lateral geniculate axons which terminate in the upper tier of layer IV are larger than the axons which terminate in the lower tier of IV (Hubel and Wiesel, 1972; Lund, 1973; Ferster and LeVay, 1978; Bullier and Henry, 1979). This difference in size presumably reflects the difference between Y or Y-like cells and X or X-like cells. The special significance for our purpose of finding that X and Y cells project to different cortical layers is that X and

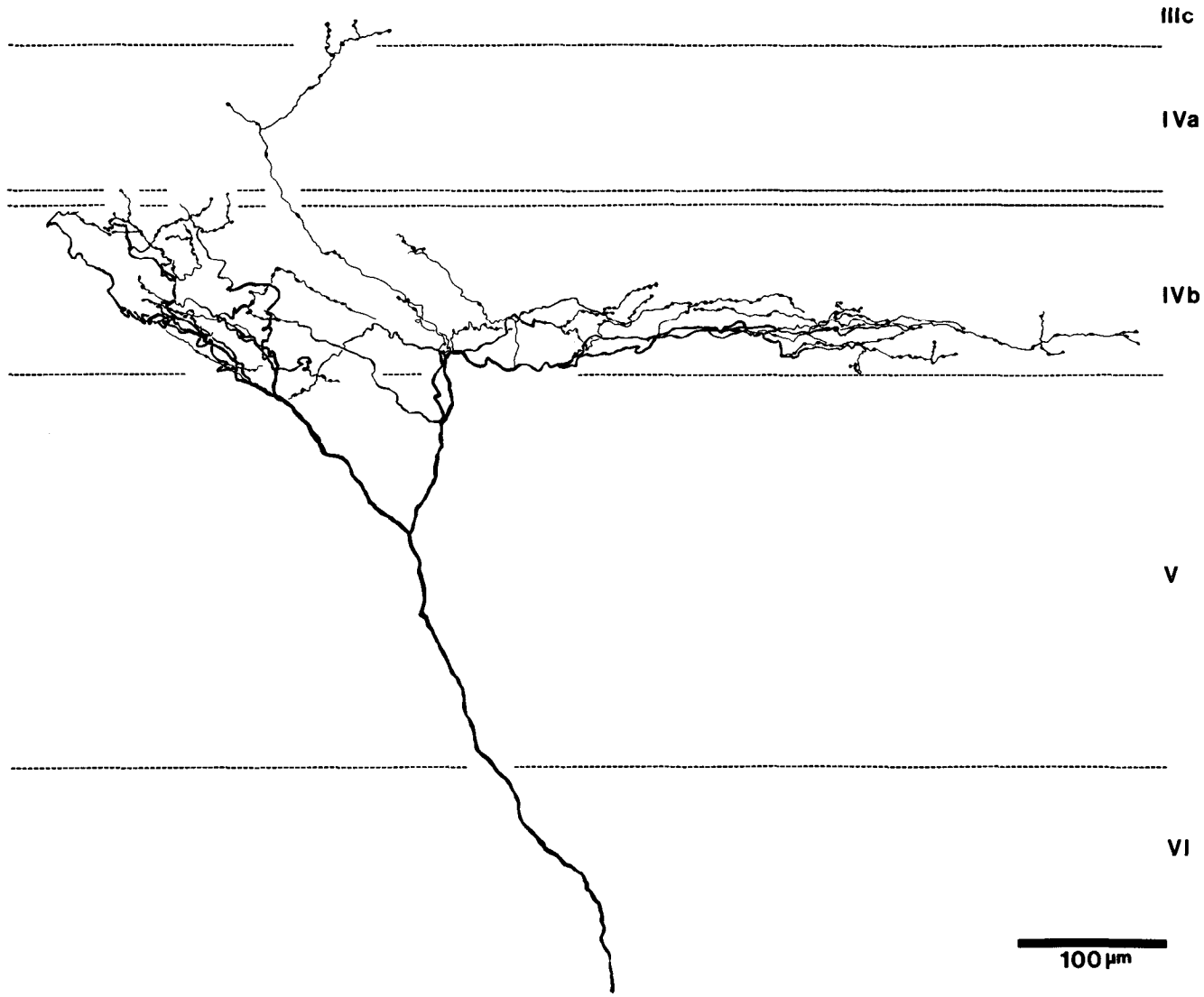

Figure 16. Reconstruction of a putative lateral geniculate layer 5 axon as it terminates in the striate cortex. This axon bifurcates in the middle of cortical layer V. Both branches arborize immediately upon entering layer IVb; the terminals from one branch cover most of the lower tier, including a few in the cleft. The other arborizes in a thin zone in the lower one-half of IVb. A single collateral branch ascends as far as layer IIIc.

Y cells are mixed in layers A and A1 in the cat (Fukuda and Stone, 1974; Wilson et al., 1976), but are segregated in different layers in monkeys (Dreher et al., 1976; Sherman et al., 1976; but see also Kaplan and Shapley, 1982). In any case, it seemed worthwhile to measure the caliber of fibers terminating in layers IVa and IVb in *Tupaia.*

Overall, we measured 41 axons which terminated in the striate cortex. Our sample sizes were too small to make reliable parametric statistical comparisons between axons which terminated in the same layer but with different patterns, but we did compare diameters of 12 axons which terminated in layer IVa with 13 axons which terminated in layer IVb. There was no significant difference between these groups ($p > 0.064$, two-tailed Wilcoxon-Mann-Whitney Signed Rank Test; for both groups mean = 2.0 μm, range, 1.5 to 2.5 μm). However, we did find a difference in caliber between axons which terminated in layer IV (a plus b) and those which terminated in layer III (b plus c). Our measurements on 16 such axons suggest that axons which terminate in cortical layer III are thinner (mean = 1.5 μm; range, 0.5 to 2.0 μm) than those which terminate in layer IV, and this difference was significant ($p < 0.024$, two-tailed Wilcoxon-Mann-Whitney Signed Rank Test). However, since some axons that terminate in cortical layer III may not be of geniculate origin, it would be wrong to jump to the conclusion that this difference in caliber corresponds to the difference between axons originating from geniculate layers 1, 2, 4, and 5 (which project to layer IV) on the one hand, and geniculate layers 3 and 6 (which project to layer III) on the other.

Discussion

The main goal of the present study was to determine the cortical target of each layer of the lateral geniculate body in *Tupaia.* In an earlier degeneration study we had tantalizing clues that each geniculate layer projected to a thin subtier of striate cortex layer IV (Harting et al., 1973). Subsequent studies of geniculostriate projections in *Tupaia* have not supported this interpretation, sug-

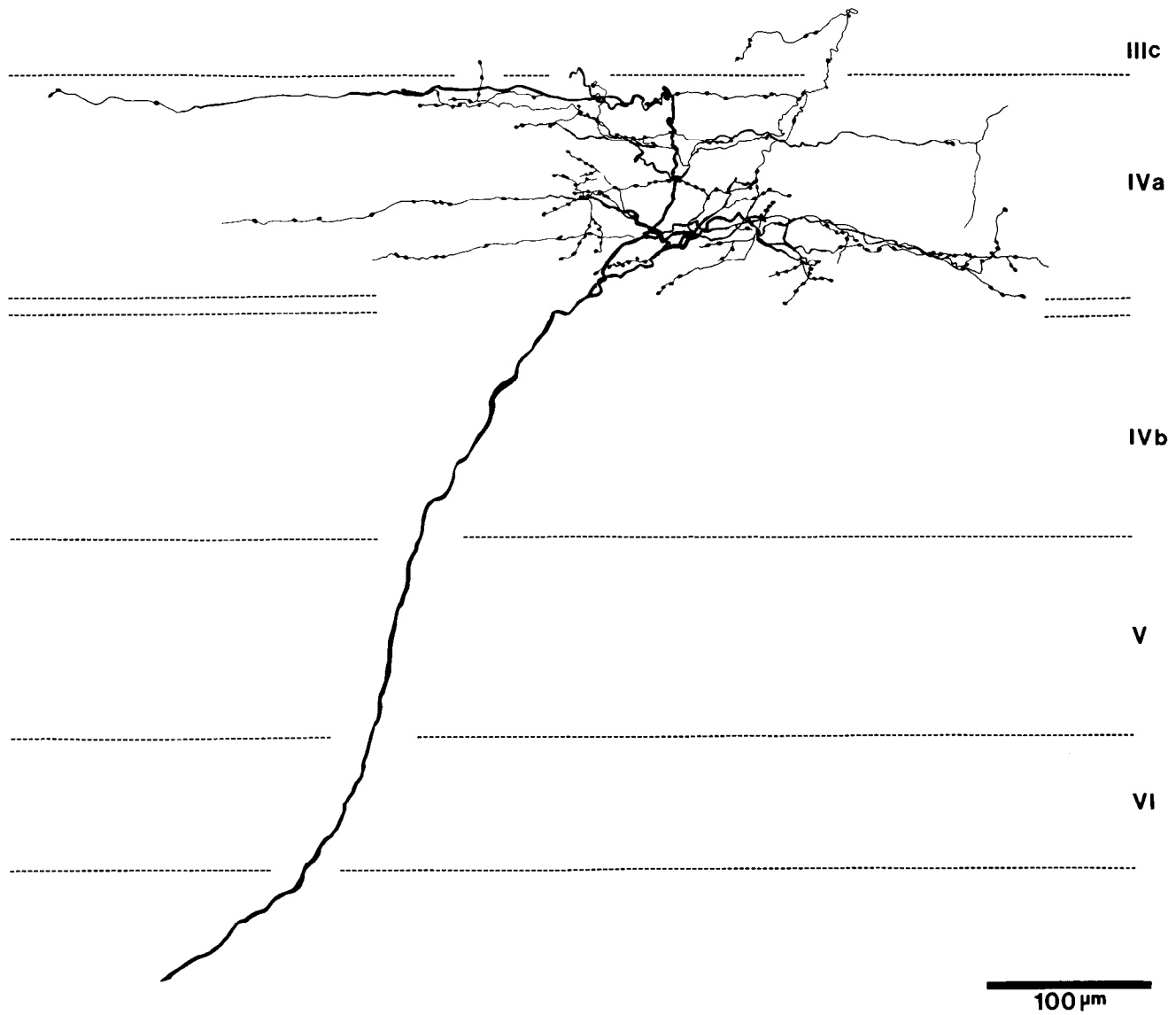

Figure 17. Reconstruction of a putative lateral geniculate layer 2 axon as it terminates in the striate cortex. This axon ascends without branching until it enters layer IVa whereupon it branches repeatedly. Many branches run horizontally giving off terminals along the way. A few branches reach layer IIIc above IVa and the cleft below IVa.

gesting instead that there is overlap between the projections of ipsilateral and contralateral geniculate layers in layer IV (Casagrande and Harting, 1975; Hubel, 1975; Humphrey et al., 1977). Our hope in undertaking the present study was to re-examine this issue using a more sensitive anterograde transport method in conjunction with an effort to determine the pattern of termination of single geniculostriate axons. We did not produce, nor did we expect to produce, injections of WGA-HRP more restricted than the electrolytic lesions in our earlier degeneration study, but the anterograde transport method did indeed prove to be more sensitive: for example, when an injection involved geniculate layer 6, but not geniculate layers 1 and 2, a thin band of labeled terminals could be seen at the base of cortical layer IIIc. This thin band at the base of IIIc originates in geniculate layer 6 and was missed in all previous studies.

The study of terminations of single geniculostriate axons provided a chance to see whether axons terminate in subtiers of cortical layers IVa or IVb, and whether a single axon has collateral projections to more than one cortical layer. Of course, this method alone could tell us nothing about the geniculate layer of origin, but in conjunction with the anterograde and retrograde transport results, the origin of the axon can be inferred, at least as a first approximation. Indeed, the fit between the axonal arborizations and the distribution of terminals was so striking in some cases that the two methods can be said to be complementary. Evidence for the termination of geniculate axons in very thin subtiers within IVa or IVb was obtained, but only the ipsilateral geniculate layers, layers 1 and 5, project in this way. There seems little doubt that these bands of terminals are related to the intrinsic organization of stellate cells in striate cortex layer IV as revealed by the Golgi method (Geisert and Guillery, 1975, 1979). The point of the Golgi study is that most stellate cell dendrites are confined to substrata within layer IVa or layer IVb (Geisert and Guillery, 1979).

Previous studies provided no evidence for alternating

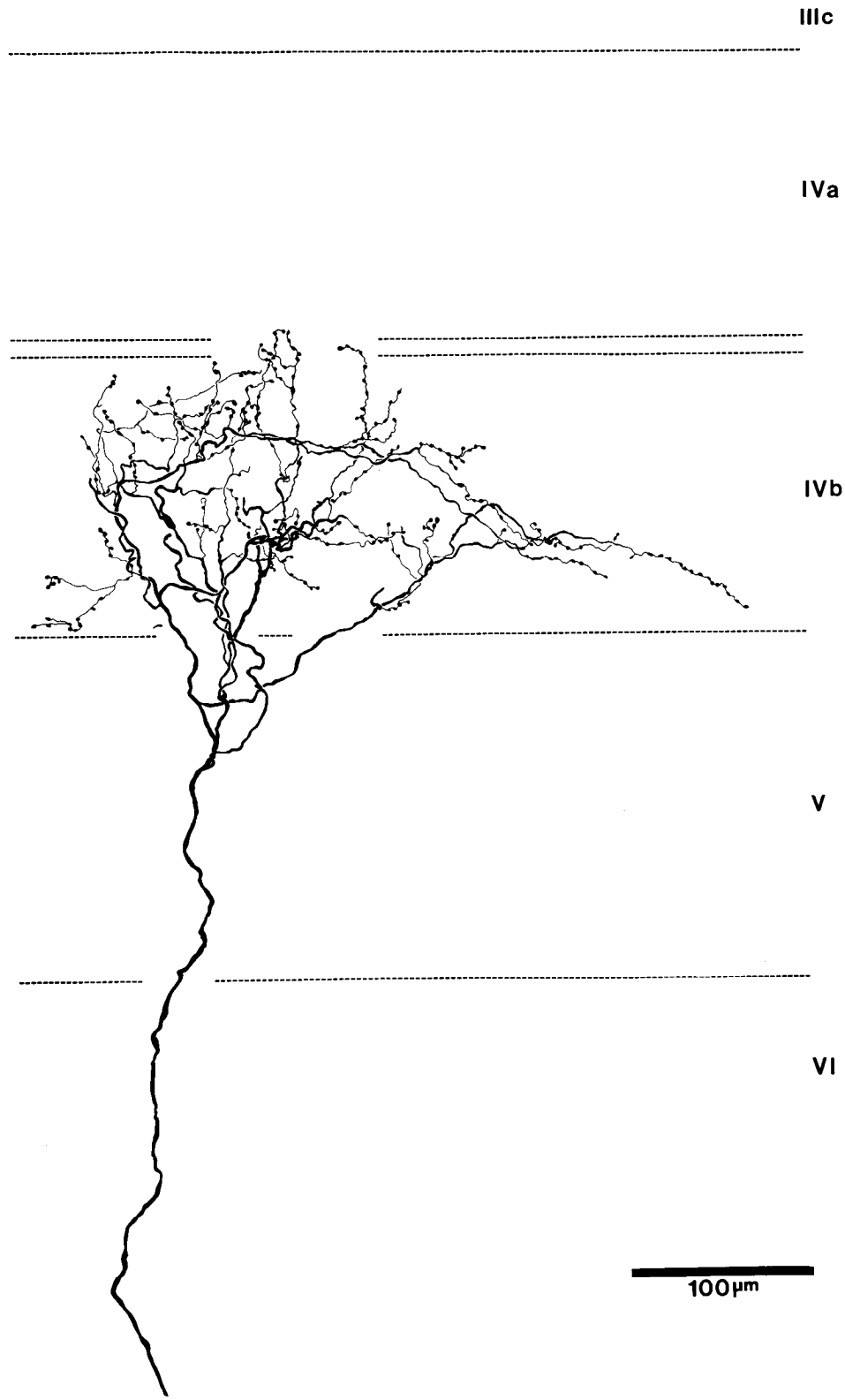

Figure 18. Reconstruction of a putative lateral geniculate layer 4 axon as it terminates in the striate cortex. This axon divides repeatedly in the upper portion of layer V before arborizing into a dense field of fibers and terminals which cover all of layer IVb including the cleft.

ipsilateral and contralateral projection columns in *Tupaia*, and this is so whichever one of the three methods was used: anterograde degeneration (Harting et al., 1973), electrophysiology (Humphrey et al., 1977), and transneuronal transport from the eye to cortex (Casagrande and Harting, 1975; Hubel, 1975). Our present results confirm that geniculostriate fibers in *Tupaia* are not distributed into alternating patches or columns as is

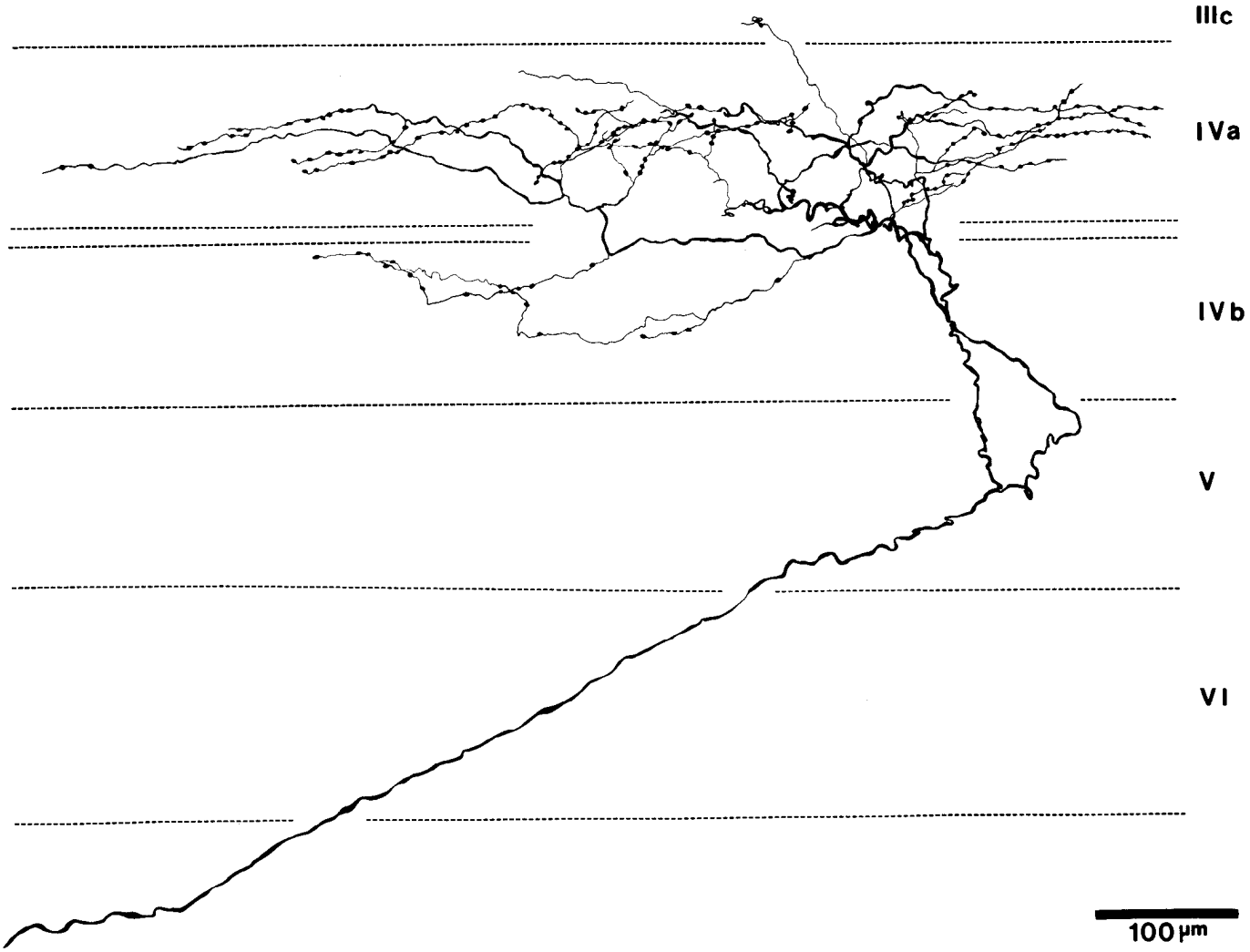

Figure 19. Reconstruction of a second putative lateral geniculate layer 2 axon as it terminates in the striate cortex. This axon bifurcates in layer V and gives rise to two branches which ascend to and arborize in layer IVa. Most of the terminals are confined to the lower two-thirds of layer IVa, but a few terminals are seen in the upper portion of IVb. A single, fine filament reaches layer IIIc.

found in the cat and primate. This species difference between a vertical and horizontal organization raises questions about the neural mechanisms for binocular integration which we will discuss below.

Our chief findings are that only four geniculate layers (1, 2, 4, and 5) project to the granular fourth layer of striate cortex and two layers (3 and 6) project above layer IV. Geniculate layers 1 and 2 project principally to layer IVa, and there is evidence that layer 1 terminates in a subtier at the top of the layer, whereas layer 2 seems to project throughout IVa. Similarly, geniculate layers 4 and 5 project principally to layer IVb, and there is some evidence that layer 5 terminates in a subtier at the bottom of the layer, whereas layer 4 seems to project throughout IVb. Each of these four layers has a minor target as well as a major target: in general, it appears that the minor targets of geniculate layers 1 and 2 are the major targets of geniculate layers 5 and 4, respectively. The minor targets of geniculate layers 4 and 5 are chiefly in layer III (see summary diagram, Fig. 22). The remaining two geniculate layers (3 and 6) project principally to separate zones within layer III: layer 6 to the base of IIIc and layer 3 to IIIb and also to I.

The identification of pairs of layers that match. The study of the laminar organization of the lateral geniculate body began with Minkowski's (1920) discovery that each geniculate layer receives fibers from one eye or the other eye. His discovery suggests that lamination in the lateral geniculate nucleus must be related to binocular integration—certainly, lamination is not just for the sake of segregating fibers of one eye from fibers of the other eye. When the same types of relay cells are found in one ipsilateral and one contralateral layer, we can assume that this matching constitutes part of the substrate for binocular integration. Whether each of the layers in a pair contains one or more than one type of relay cell seems to depend on which species is studied. In *Galago*, for example, there are three pairs of matched layers and each pair can be identified with relay cells of one type—large, medium, and small. These, in turn, correspond roughly to Y-like, X-like, and W-like cells as defined electrophysiologically (Glendenning et al., 1976; Norton

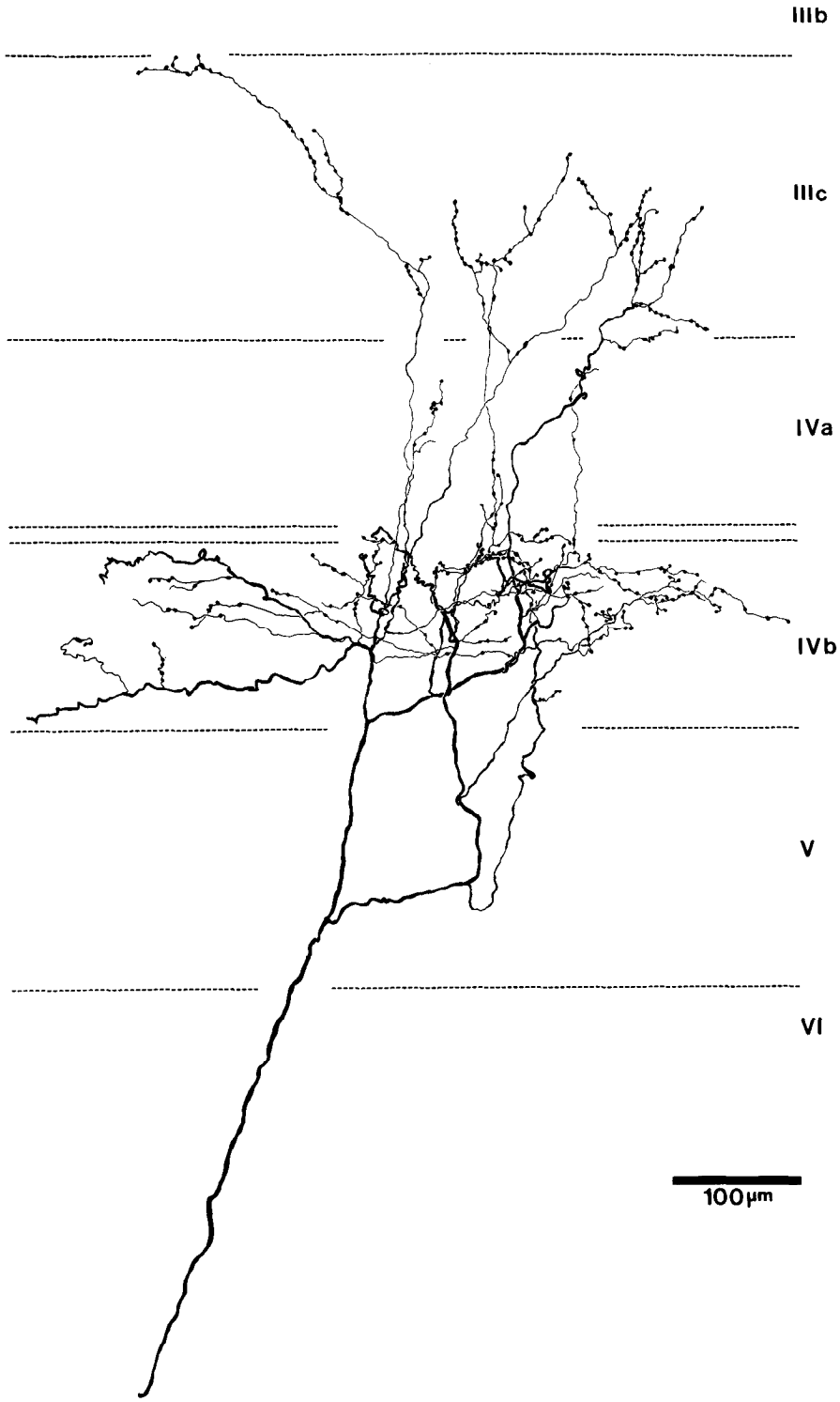

Figure 20. Reconstruction of a second putative lateral geniculate layer 4 axon as it terminates in the striate cortex. Most of the terminals are confined to the upper portion of layer IVb, including the cleft, but several collateral branches ascend through layer IVa to terminate in layer IIIc. A few fibers terminate in layer IVa.

and Casagrande, 1982). In the cat, layers A and A1 are matched layers; yet they contain two or more cell types—for example, type 1 and type 2, or X and Y cells (Guillery, 1966; Ferster and Levay, 1978; Friedlander et al., 1981). In mink there are two A layers and two A1 layers as these terms have been used to describe the lateral geniculate body of the cat (Sanderson, 1974; Guillery and Oberdorfer, 1977). Presumably, all four layers contain a mixture of X and Y cells, yet it is possible to match each A layer with an A1 layer on the basis of the differential

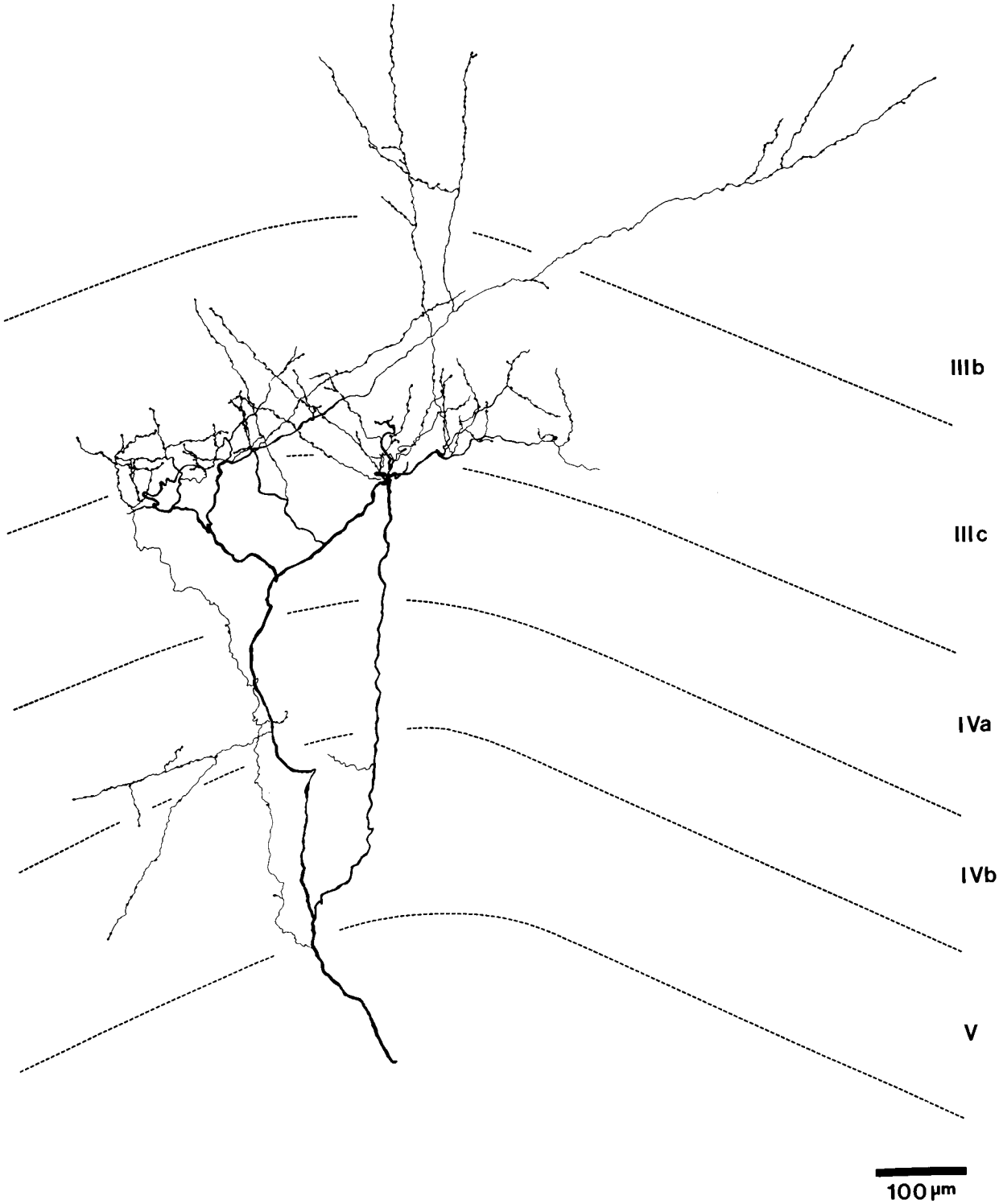

Figure 21. Reconstruction of a single axon presumably originating in lateral geniculate layer 6. This axon branched repeatedly in layer IV before arborizing at the base of layer IIIc; one fine collateral branch with terminals was seen in layer IVb.

distribution of cells with on- and off-center receptive fields (LeVay and McConnell, 1982).

One of the central issues of the present study concerned the identification of matched geniculate layers in *Tupaia*. We knew that no two layers project to alternate columns in the same cortical tier (as do matched layers in primate or cat) (Hubel and Wiesel, 1972; Casagrande and Harting, 1975; Hubel, 1975; LeVay and Gilbert, 1976), but we thought that determining the cortical target of each layer might contribute to the pairing of ipsilateral with contralateral layers. Since there are four contralateral layers and only two ipsilateral geniculate layers in *Tupaia*, obviously not every layer can be a member of a matched pair. All we can hope to find is two contralateral layers that can be matched to the two ipsilateral layers, layers 1 and 5.

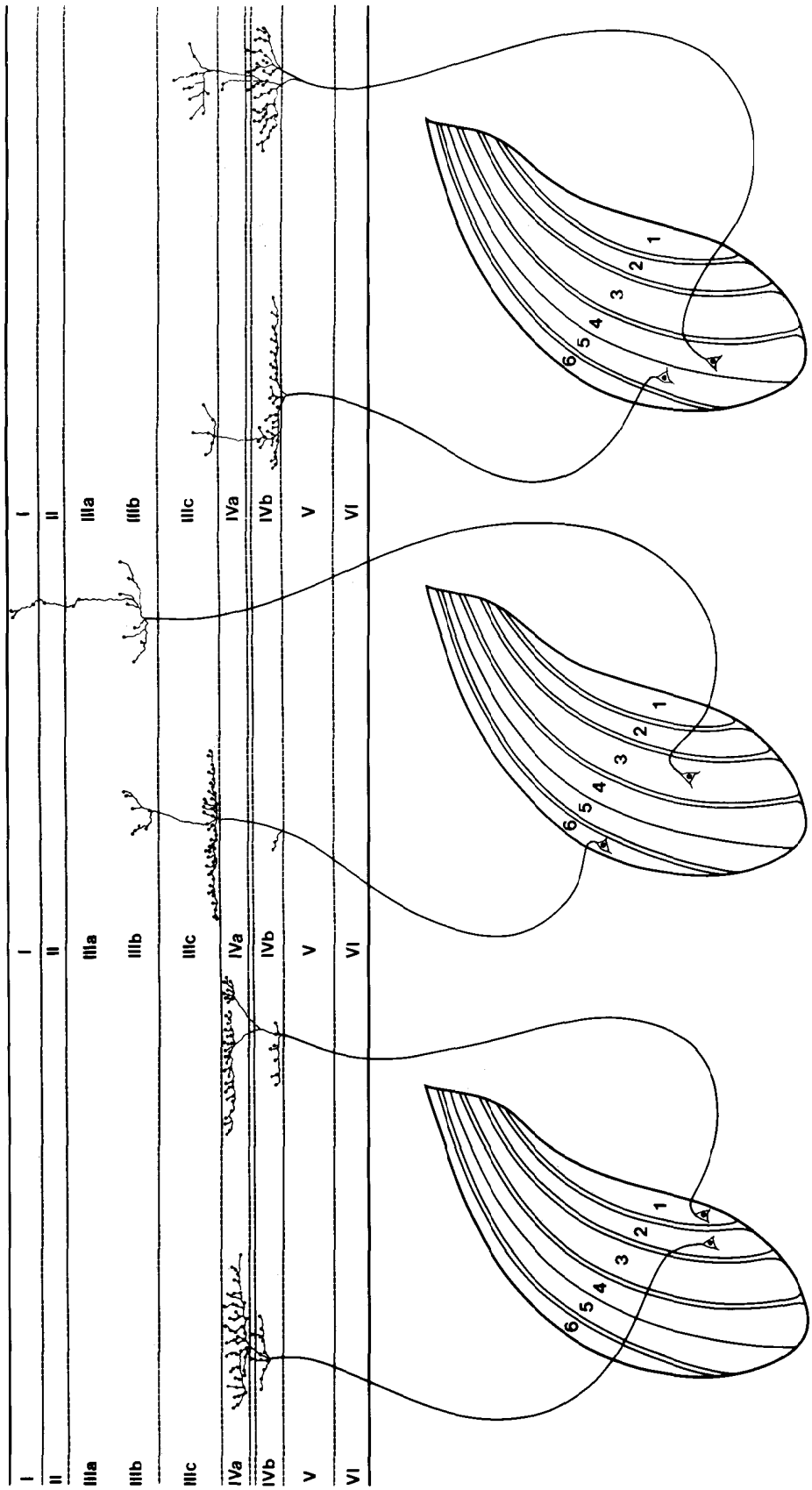

Figure 22. Summary diagram of the major and minor projections of each of the six lateral geniculate layers.

The first suggestion that some geniculate layers in *Tupaia* may be matched was made by Le Gros Clark (1929). In his examination of Nissl-stained sections in *Tupaia minor* he was struck by the similarities between adjacent layers, layers 1 and 2, and layers 4 and 5, on either side of a distinctly different layer containing smaller and paler cells, layer 3 (Le Gros Clark, 1929). Le Gros Clark did not describe layer 6, which is very thin and contains small, lightly staining fusiform cells, many of which are embedded in the optic tract.

Measurements of cell size in the lateral geniculate body support Le Gros Clark's (1929) impression: the mean soma diameter of cells in layer 3 is significantly smaller than those in either layers 1, 2, 4, or 5; layer 6 cells are also smaller than those in layers 1, 2, 4, and 5, but the difference is not statistically significant (Casagrande et al., 1978). The size of cell bodies does not help making pairs among layers 1, 2, 4, and 5 (Casagrande et al., 1978); however, a recent Golgi study of *Tupaia* lateral geniculate nucleus offers evidence that layers 1 and 2 contain larger cells than do layers 4 and 5 (Brauer et al., 1981). Further differences between the two pairs are revealed by dendritic morphology: the dendrites in layers 1 and 2 are chiefly perpendicular to the long axis of the layers and have few protrusions at branching points. In contrast, the dendrites of cells in layers 4 and 5 are tufted and brush-like, with numerous grape-like appendages at dendritic branch points (Brauer et al., 1981).

Anterograde degeneration and anterograde transport studies of retinogeniculate projections also provide important clues for matching. Layers 1, 2, 4, and 5 all receive coarse, large terminals from the optic tract, whereas layers 3 and 6 receive fine, small terminals from the optic tract (Tigges, 1966; Campbell et al., 1967; Glickstein, 1967; Laemle, 1968; see also Fig. 1). Indeed, degeneration in layer 3 was so fine and sparse after optic tract section that, at one stage in the inquiry, there was some doubt that it was a target of the optic tract (Glickstein, 1967).

Given the importance of physiological studies in matching geniculate layers, it is natural to wonder whether layers 1 and 2 or layers 4 and 5 can be matched on the basis of physiological criteria. First, electrophysiological studies of the lateral geniculate body of *Tupaia* do not support the idea that any of the geniculate layers can be identified exclusively with the physiological classes "X" and "Y" (Sherman et al., 1975). However, there is evidence that the receptive fields of cells in both layers 1 and 2 are predominantly "on-center," whereas the receptive fields of cells in both layers 4 and 5 are predominantly "off-center" (Conway et al., 1980; Conway and Schiller, 1983). We might expect, then, that units of striate cortical layer IVa would show on-center response properties, whereas cells of IVb would show off-center responses, and this is just what Norton and his group have found (Norton et al., 1983).

Finding that geniculate layers 1 and 2 project to IVa with overlapping but different distributions of terminals fits the idea that they are matched; that is, their pattern of projection seems to fit the idea that they are closely related functionally. A similar argument can be made for geniculate layers 4 and 5. It remains for further study to determine the nature of the binocular integration achieved by horizontal strips and whether it is equivalent functionally to that achieved by adjacent vertical columns. In any case, given the views of paleontologists about the place of *Tupaia* in phylogeny, the horizontal strips—which in a way repeat the organization found in the lateral geniculate—may reflect an earlier stage of the evolution of the cortical mechanism for binocular integration.

The projections of the two unmatched layers, layers 3 and 6. Geniculate layers 3 and 6, which are members of the contralateral set, are distinct morphologically not only from the matched layers, but from each other as well. The connections of these two unmatched layers provide the answer to a major question of the present inquiry: "Can we identify in *Tupaia* a small cell, small fiber pathway to the superficial layers of the striate cortex similar to pathways found in cat and primate?"

There is little doubt that geniculate layer 3 qualifies for such a pathway. The present results show that geniculate layer 3 projects principally to cortical layer IIIb, but also to all of the superficial layers of the cortex including layer I. The results of our earlier experiments in which HRP was restricted to the superficial layers of the striate cortex are, thus, confirmed (Carey, et al., 1979), but the picture is refined, because before we could not distinguish between projections to cortical layers I, II, and III.

The resemblance between geniculate layer 3 in *Tupaia* and certain layers in distantly related species is remarkable. Layer 3 in *Tupaia* contains the smallest, palest cells, receives the finest caliber fibers from the optic tract, and receives tectal fibers (Le Gros Clark, 1929; Glickstein, 1967; Laemle, 1968; Casagrande et al., 1978; Carey et al., 1979; Fitzpatrick et al., 1980). These features and connections characterize geniculate layers 4 and 5 in *Galago* (Kaas et al., 1978; Fitzpatrick et al., 1980), the intercalated layers in the monkey (Harting et al., 1978; Fitzpatrick et al., 1983), and the parvocellular C layers in the cat (Graham, 1977; Kawamura et al., 1980; Torrealba et al., 1981). Such similarities can hardly be an accident, but at the same time they cannot be ascribed to homology because the lateral geniculate body of the common ancestor could not have been laminated. Our solution to this puzzle is to suggest that the "small" cell type, with all of its connections and characteristics, was present in a common ancestor. This would account for different arrangements of common cell types in different lines and, in fact, would predict slight differences between closely related species as appears to be the case for the intercalated layers of different primates.

The projection of geniculate layer 6 to cortical layer IIIc came as a complete surprise, and the main reason why projections to IIIc were missed in the past is that the strip is so thin that if all geniculate layers were involved in either the lesion or the injection site, the anterograde degeneration or transport would just appear to fill IVa and IVb. It was only when we made an injection of a more sensitive marker into the lateral layers of the lateral geniculate nucleus that two cortical bands, one in

IIIc and one in IVb, were separated by a zone free of terminals (i.e., only when projections from layers 1 and 2 to IVa are unmarked can the projection to the base of IIIc be indentified). Under these circumstances it became apparent that the upper band was, in fact, above IVa.

The question then arises: "Can the pathway in *Tupaia* to the base of IIIc be related to pathways established in other species—notably in cat and primate?" We concede at the outset that if there were no similarities between *Tupaia* and cat or between *Tupaia* and monkey there would be little to discuss. It turns out that this pathway in *Tupaia* is similar to one in the primate, and it is also similar to one in the cat; the trouble is that the pathways in primate and cat which resemble the geniculate layer 6 path to IIIc do not resemble each other—the one in the cat is a so-called "W" path and the one in the primate is a "Y" path. It is this puzzle that is worth discussing.

On the one side, the cortical target of geniculate layer 6 in *Tupaia* shares some features in common with the cortical target of the *magnocellular* layers in primates. The magnocellular geniculate layers project to a sparsely populated stratum lying just above and continuous with the target of the parvocellular layers. The target of the parvocellular layers, in contrast with the cell-sparse zone above, is densely packed with small stellate cells. Thus, on cytoarchitectonic grounds *Tupaia* layer IV (IVa plus IVb) is similar, not to all of layer IV in primate striate cortex, but just to layer IVβ (or IVCβ according to another terminology). Since geniculate layer 6 projects to a sparsely populated stratum continuous with the target of geniculate layers 1, 2, 4, and 5, it is tempting to suggest that the pathway relayed by layer 6 in *Tupaia* may be analogous to the pathway relayed by the magnocellular layers in primates even though the cells of layer 6 are obviously not large. By the same token the pathways relayed by geniculate layers 1, 2, 4, and 5 may be similar in some respects to the pathways relayed by the parvocellular layers in primates. Cortical layer IIIc in *Tupaia*, or at least the base of IIIc, might then be renamed IVα to conform to the similarities between *Tupaia* and primate. What is a more significant implication of this argument is that the cleft in layer IV may be marking some division of the "parvocellular" pathway. We have already cited evidence from electrophysiological studies of *Tupaia* showing that the cells in geniculate layers 4 and 5, and cortical layer IVb as well, are predominantly "off-center," whereas the cells of geniculate layers 1 and 2, and cortical layer IVa, are predominantly "on-center." An analogous segregation of on- and off-center responses has been made in the parvocellular layers of the geniculate in primate (Schiller and Malpeli, 1978).

It only remains to note that the target of the parvocellular layers in the monkey, IVβ (or IVCβ), may consist of two subdivisions which differ in their cell packing density and in their intrinsic cortical projections (Blasdel et al., 1983). In at least one species of monkey (owl monkey) there is even a faint cleft dividing the terminal field of the parvocellular layers into two tiers (Kaas et al., 1976; Fitzpatrick et al., 1983). The significance of the subdivision within IVβ (or IVCβ) remains to be studied.

On the other hand, an argument can be made that geniculate layer 6 shares some features in common with the parvocellular C layers in the cat. First and foremost, of course, the cells of layer 6 are small and fusiform (Brauer et al., 1981; see also Guillery, 1966; Stanford et al., 1981). Furthermore, both layer 6 in *Tupaia* and the parvocellular C layers in the cat receive fibers from the tectum, receive fine caliber terminals from the retina, and are the targets of slowly conducting optic tract fibers. Finally, the parvocellular C layers project to the base of III on the border of layer IV just as the target of layer 6 projections lies on the border between layers IV and III.

It is natural to ask: "What do electrophysiological studies conclude about the response properties of the cells in geniculate layer 6?" We know from Conway and Schiller's (1983) report that layer 6 cells show transient responses, a finding which probably does not settle to everyone's satisfaction the question of how to classify this pathway. For us, the comparison between *Tupaia*, cat, and primate is intriguing, not primarily because it is important and difficult to decide whether the layer 6 pathway is W or Y, and not because it is important to decide whether *Tupaia* is more like a primate than a cat. Instead, our goal is to see species differences as variations on a common theme, and an understanding of that plan of organization seems now to be within reach. This search for the organization common to a group is, of course, in general, the goal of comparative anatomy. It is worth illustrating the power of the method when it succeeds. Take, for example, the organization of the diencephalon and, in particular, the relation between the dorsal and ventral thalamus in mammals, which has been understood since the work of Le Gros Clark (1932). As a result, the enormous differences between species can be viewed as modifications of the same plan. The ventral lateral geniculate is large and conspicuous in all embryos and in primitive mammals such as *Tupaia*, but is reduced to a fraction of that size in the adult human. Without knowing the plan as revealed by the comparative study, the ventral lateral geniculate in adult higher primates would have been misidentified or missed entirely. Indeed, the term "pregeniculate" is evidence for this very point. Surely these differences in the development of the ventral lateral geniculate cast light on the functional relations between the ventral geniculate body and other centers of the visual system.

The plan of organization of the geniculostriate pathways still eludes us, but we feel the study of *Tupaia* has a unique contribution to make, chiefly because the granular fourth layer of striate cortex in that species is divided into two tiers by a conspicuous cell-sparse cleft. There are hints that other species may have a similar division, but in no case is the division so clearly marked. The projection of single geniculate layers to the tiers above and below the cleft suggest that there may be *four* pathways relayed by the lateral geniculate nucleus, three of which terminate in contiguous bands within layer IV, if we count the base of IIIc. Comparison between *Tupaia* and other more commonly studied mammals suggests that this organization may reflect the basic mammalian plan.

Summary and conclusion. The discovery that the optic tract comprises several different pathways was made by George Bishop 50 years ago (Bishop, 1933; Bishop and Clare, 1955). The general question posed by the present paper is: "How many pathways are there and how is 'pathway' related to layers of the lateral geniculate body and the layers of the striate cortex?" A comparison among diverse species shows that there are at least three pathways, but whether they correspond to X, Y, and W cell types is beyond the scope of this discussion. There is one pathway that seems to be the same in all groups including *Tupaia*, cat, and primate: it consists of the smallest optic tract fibers, the small cell layers of the lateral geniculate body (which also are the layers that receive tectal fibers), and the projection to the cortical layers superficial to layer IV, chiefly layer III, and to a lesser extent layer I (Wilson and Stone, 1975; LeVay and Gilbert, 1976; Graham, 1977; Harting et al., 1978; Carey et al., 1979; Levanthal, 1979; Fitzpatrick et al., 1980). The remaining pathways terminate in layer IV, but the question remains whether there are two or more than two of these. In *Tupaia* there are three pathways projecting to layer IV if we include in layer IV the thin strip which is the target of geniculate layer 6. Whereas "pathway" seems to be the fundamental and common principle in the organization of the lateral geniculate nucleus, the number of geniculate layers seems to be the most variable feature (see Guillery, 1978). Closely related species can have a different number of layers showing that further subdivision is an easy achievement of evolution. The general rule seems to be that, whenever there is some segregation in the lateral geniculate body—either between ipsilateral and contralateral input or between cell types—the stage is set for further separation by an interlaminar space. The interlaminar spaces are, of course, rich in geniculate cell dendrites, ascending tectal, and descending cortical projections and may be a significant locus of integration within the lateral geniculate body (Guillery, 1969; Sanderson and Kaas, 1974).

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