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# Electron-Microscopic Analysis of Synaptic Input From the Perigeniculate Nucleus to the A-Laminae of the Lateral Geniculate Nucleus in Cats

JOSEPHINE B. CUCCHIARO, DANIEL J. UHLRICH, AND S. MURRAY SHERMAN
Department of Neurobiology and Behavior, State University of New York, Stony Brook, New York 11794-5230

ABSTRACT

The perigeniculate nucleus of carnivores is thought to be a part of the thalamic reticular nucleus related to visual centers of the thalamus. Physiological studies show that perigeniculate neurons, which are primarily GABAergic, provide feedback inhibition onto neurons in the lateral geniculate nucleus. However, little is known about the anatomical organization of this feedback pathway. To address this, we used two complementary tracing methods to label perigeniculate axons for electron microscopic study in the geniculate A-laminae: intracellular injection of horseradish peroxidase (HRP) to fill an individual perigeniculate cell and its axon; and anterograde transport of *Phaseolus vulgaris* leucoagglutinin to label a population of perigeniculate axons. Labeled perigeniculate terminals display features of F1 terminals in the geniculate neuropil: they are small, contain dark mitochondria, and form symmetric synaptic contacts. We found that most of the perigeniculate terminals (> 90%) contact geniculate cell dendrites in regions that also receive a rich innervation from terminals deriving from visual cortex (e.g., "cortico-recipient" dendrites). The remainder of the perigeniculate synapses (10%) contacted dendrites in regions that also received direct retinal input (e.g., "retino-recipient" dendrites). Serial reconstruction of segments of dendrites postsynaptic to perigeniculate terminals suggests that these terminals contact both classes of relay cell in the A-laminae (X and Y), although our preliminary conclusion is that an individual perigeniculate cell contacts only one class. Finally, our quantitative comparison between labeled perigeniculate terminals and unlabeled F1 terminals indicates that these perigeniculate terminals form a distinct subset of F1 terminals. We quantitatively compared the labeled perigeniculate terminals to unlabeled F1 terminals. Although the parameters of the perigeniculate terminals fell entirely within the range of those for the unlabeled F1 terminals, as populations, we found consistent differences between these two groups. We thus conclude that, as populations, other sources of F1 terminals are morphologically distinct from perigeniculate terminals and innervate different targets.

**Key words: thalamus, electron microscopy, thalamic reticular nucleus**

The perigeniculate nucleus, which is clearly recognizable only in carnivores, is generally thought to be a subdivision of the visual portion of the thalamic reticular nucleus, although certain differences have been noted between the perigeniculate nucleus and visual portion of the thalamic reticular nucleus (Jones, '75, '85; Ahlsén et al., '82; Ide, '82a,b; Oertel et al., '83; Cucchiaro et al., '90). As a subdivision of the thalamic reticular nucleus, the perigeniculate nucleus has homologs in other mammalian species, including rodents and primates. In the cat, the perigeniculate nucleus is a narrow band of neurons lying just dorsal to lamina A of the lateral geniculate nucleus. Perigeniculate neurons share many basic features with thalamic reticular neurons: most or all perigeniculate cells stain positively for antibodies directed against the inhibitory neurotransmitter γ-aminobutyric acid (GABA) or glutamate decarboxylase (GAD), a key biosynthetic enzyme for GABA (Houser et al., '80; Oertel et al., '83; Fitzpatrick et al., '84; Rinvik et al., '87; Rinvik and Ottersen, '88); the perigeniculate nucleus is innervated by collaterals of geniculocortical and corticoge-

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Address reprint requests to Dr. S.M. Sherman, Dept. of Neurobiology and Behavior, State University of New York, Stony Brook, NY 11794-5230.

D.J. Uhlrich is now at the Dept. of Anatomy, University of Wisconsin, 1300 University Ave, Madison, WI 53706.

niculate axons and by axons from various sites in the brainstem reticular core (Jones, '75, '85; Updyke, '77; Friedlander et al., '81; Ahlsén and Lo, '82; Ahlsén and Lindström, '82; Stanford et al., '83; Uhlrich et al., '88; Montero, '89a); and perigeniculate neurons project their axons into the lateral geniculate nucleus where their terminal arbors are essentially confined to the A-laminae (Uhlrich et al., '91). Because of their apparent GABAergic nature and connectivity patterns, perigeniculate cells are thought to provide feedback inhibition onto geniculocortical relay cells (for reviews, see Singer, '77; Sherman and Koch, '86, '90).

Several investigators have used the electron microscope with immunohistochemistry to identify the location of synaptic terminals labeled with antibodies directed against GABA or GAD. Such an approach indicates that, in the A-laminae of the geniculate neuropil, all or nearly all of the terminals containing flattened or pleomorphic vesicles and forming symmetrical synaptic contacts are GABAergic (Fitzpatrick et al., '84; Montero and Singer, '85). Such terminals can be placed in one of two categories known as *F1* and *F2* (Guillery, '69). F2 terminals derive from dendrites of local, GABAergic interneurons and are both presynaptic and postsynaptic (Famiglietti and Peters, '72; Hamos et al., '85; Montero, '86). In contrast, F1 terminals derive from axons and are strictly presynaptic to other profiles, occasionally contacting F2 terminals (Guillery, '69, '71; Famiglietti and Peters, '72; Ohara et al., '80; Montero and Scott, '81).

The source or sources of F1 terminals remain unclear. In the lateral geniculate nucleus of rats, the visual thalamic reticular nucleus is the major source of F1 terminals, and this projection can account for all types of synaptic relationships entered into by F1 terminals (Ohara et al., '80; Montero and Scott, '81). Because of this and also because of the close proximity between the perigeniculate and lateral geniculate nuclei in cats, perigeniculate axons seem a likely source of F1 terminals in the geniculate A-laminae. To address this question, we have labeled perigeniculate axons with electron dense material and used the electron microscope to analyze their terminal arbors in the A-laminae.

## MATERIALS AND METHODS

### Subjects

Our data have been obtained from 5 adult cats. In one cat, we electrophysiologically characterized and intracellularly filled with horseradish peroxidase (HRP) an individual perigeniculate cell and its axon arbor in the geniculate A-laminae. In a second cat, we labeled a population of perigeniculate axons by anterograde axonal transport of *Phaseolus vulgaris* leucoagglutinin (PHAL) after an extracellular injection of PHAL into the perigeniculate nucleus. In a third cat, we labeled cortical terminals by an analogous injection of PHAL into area 17 of its visual cortex. Finally, in each of these cats plus an additional two used for another electron microscopic study, we analyzed an unlabeled population of F1 terminals (see Results for complete description of this and other terminal types).

### Intracellular labeling with HRP

Our methods for electrophysiological recording, intracellular HRP injection, and subsequent preparation of the tissue for electron microscopic analysis have been described previously (Wilson et al., '84; Hamos et al., '85, '87) and are presented here in abbreviated form. We initially anesthetized the cat with 4% halothane in a 1:1 mixture of $N_2O$ and $O_2$. We then performed a tracheotomy, cannulated the femoral vein, and placed the animal in a stereotaxic apparatus. Paralysis was induced with 5 mg of gallamine triethiodide. The cat was artificially respired thereafter and maintained on a continuous infusion of gallamine triethiodide (3.6 mg/hr) and d-tubocurarine (0.7 mg/hr) in 5% lactated Ringer's solution (6 ml/hr). We continuously monitored vital signs, maintained end-tidal $CO_2$ at 4.0 ± 0.2%, and kept rectal temperature at 37.5–38.0°C. During surgical procedures, animals were anesthetized with 1.5–2.5% halothane in a 7/3 mixture of $N_2O/O_2$. We infused all wound margins and pressure points with 2% lidocaine. Following surgery, we discontinued halothane and maintained the cat on the $N_2O/O_2$ mixture with Nembutal added to the infusion solution at 1 mg/kg/hr; this was sufficient to maintain synchronized EEG activity.

We placed bipolar tungsten stimulating electrodes in the optic chiasm for electrical stimulation of retinogeniculate axons. A craniotomy, 1 cm in diameter, was opened over the lateral geniculate nucleus, and a Plexiglas chamber that surrounded the opening was affixed to the skull with dental acrylic. We minimized brain pulsations during recording by filling the chamber with 3% agar in 0.9% saline and sealing it with dental wax.

We first used a 3 M KCl electrode with an impedance (at 100 Hz) of 10 MΩ to locate the lateral geniculate and perigeniculate nuclei. We then switched to a micropipette filled with a solution of 5% HRP (Sigma Type VI) in 0.2 M KCl and 0.05 M Tris at a pH of 7.4; its tip was beveled to achieve an impedance of roughly 75 MΩ. We used this electrode to locate perigeniculate cells, which were identified by their location just dorsal to lamina A and by their characteristic responses that distinguished them from geniculate neurons: large, ill-defined receptive fields with poor visual driving; often binocular receptive fields; and long, variable response latencies from optic chiasm stimulation. Once a perigeniculate cell was characterized, we impaled it by slowly advancing the electrode and passing short pulses of positive current (2–5 nA) or lightly tapping the stereotaxic apparatus. Upon a successful impalement, we iontophoretically injected HRP into the cell by passing 4–15 nA positive current of variable frequency and duty cycle for 1–7 minutes.

### Bulk labeling with PHAL

We made small extracellular injections of PHAL into the perigeniculate nucleus of one cat and into cortical area 17 of another. Our methods for PHAL injection and subsequent preparation of the tissue for electron microscopic analysis have been described previously (Cucchiaro et al., '88; Cucchiaro and Uhlrich, '90), and are presented briefly here. We anesthetized each animal with sodium pentobarbital administered intravenously (initial dose of 15 mg/kg with 5–10 mg supplements as needed), placed it a stereotaxic apparatus, and we used sterile procedures for all surgery. We administered atropine sulphate (0.15–0.20 mg) to minimize salivation, infused all wound margins and pressure points with 2% lidocaine, and covered the corneas with contact lenses. Vital signs were continuously monitored.

For the injection into the perigeniculate nucleus, we first located the lateral geniculate nucleus with a low impedance electrode as described above and then replaced this with a double-barrel pipette in which each of the tips was broken back to a diameter of 2–5 μm. One barrel was filled with a

PHAL solution (2.5% in 0.05 M sodium phosphate buffer, pH 7.4), and the other contained 3 M KCl. The latter barrel permitted electrophysiological recordings that we used to locate the border between geniculate lamina A and the perigeniculate nucleus. We then retracted the electrode to 400 μm dorsal to this border and iontophoretically injected the PHAL through the other barrel (5 μA positive current pulsed on and off at 0.07 Hz for 15 min). For a 48-hour survival period following the PHAL injection, anesthesia was maintained.

In the other cat, similar procedures were used to inject PHAL into area 17 of visual cortex with the following two exceptions. First, we positioned the electrodes under visual and stereotaxic control. Second, because of the lengthy survival period (10 days) needed for transport of PHAL from cortex to the lateral geniculate nucleus, the animal was allowed to recover from anesthesia. The postoperative regimen included analgesics and antibiotics under veterinary supervision.

## Tissue processing

After the variable survival times noted above, each animal was deeply anesthetized with an overdose of sodium pentobarbital and perfused transcardially, first with heparin, then by a brief saline rinse, and finally with aldehyde fixatives. For the HRP labeling, the fixative mix was 1% paraformaldehyde, 1% glutaraldehyde, and 0.1 M $NaPO_4$ buffer at pH 7.4; for the PHAL labeling and for the two cats from which unlabeled F1 terminals were analyzed, we used 4% paraformaldehyde, 0.05% glutaraldehyde, and 0.1 M $NaPO_4$ at pH 7.4. After the perfusion, the brain was removed from the skull and a block of tissue containing the thalamus was placed for 12–18 hours in fixative at 4°C. For the HRP labeling, this fixative was the same mix as used in perfusion; for the remaining cases, the fixative mixture was 4% paraformaldehyde in 0.05 M Na borate buffer at pH 9.5.

The day after the perfusion, we used a Vibratome to cut coronal sections at a thickness of 50 μm. The sections were collected and stored in a buffered saline. For the HRP labeling, we reacted these sections with diaminobenzidine (*DAB*) using $CoCl_2$ intensification. For the PHAL labeling, we passed the sections through an ethyl alcohol series (10%, 20%, 40%, 20%, 10%) for 10 minutes each and back into buffered saline to enhance penetration of subsequent reagents (Eldred et al., '83). These sections were then incubated with gentle agitation in a solution containing primary antibody directed against PHAL (goat anti-PHAL at 1:2000; 2% normal rabbit serum; 0.02 M $KPO_4$ buffer at pH 7.4) for 72 hours at 4°C. We then used the avidin-biotin immunoperoxidase procedure to visualize the antibody (Vectastain ABC kit). Peroxidase was demonstrated using DAB with $CoCl_2$ intensification (Adams, '81).

We mounted the reacted sections (both for HRP and PHAL labeling) onto glass slides with buffer and examined them with the light microscope to select those sections with labeled processes or cells. Selected sections were osmicated, dehydrated, and embedded in plastic (Epon). Labeled cells and processes were examined and drawn with a camera lucida attachment on a light microscope, using oil immersion lenses at 500X and 1000X. Unfortunately, in the PHAL material, we had incomplete penetration of the label through the thickness of the sections. We thus could not determine the number of axon segments included in our analysis, because individual segments could not be reconstructed.

## Electron microscopic analysis

Once blocks were prepared for electron microscopy, serial thin sections were cut, mounted onto formvar-coated, slotted grids, and stained with uranyl acetate and lead citrate. We subsequently examined and photographed selected areas in the geniculate A-laminae. For the HRP material, we examined every fourth section, except at synaptic sites where we photographed every section, and for the PHAL material, we examined every section.

We used Guillery's ('69) classification of synaptic profiles (see Results). In terminals labeled with either PHAL or HRP, the electron-opaque DAB reaction product obscures many features, including presynaptic specializations and cytoplasmic matrix. Although the mitochondria and synaptic vesicles remain unlabeled, vesicle shape, size, and distribution may be affected by the labeling. We have thus relied on the postsynaptic elements to identify the presence and type of synaptic contacts. We identified synaptic contacts by a parallel apposition of the pre- and postsynaptic membranes, some widening of the synaptic cleft, density in the synaptic cleft, a postsynaptic density associated with the contact zone, and the presence of the contact zone in three or more serial sections. We randomly selected for analysis unlabeled F1 terminals according to the criteria established by Guillery ('69). These were sampled from the same blocks as contained HRP- or PHAL-labeled axons or from comparable regions of the A-laminae in the two other cats.

For many terminals, we obtained electron micrographs from a complete series of sections; we printed most of these at various magnifications (see below). These serial micrographs permitted certain other analyses as follows: (1) we confirmed that there were no synapses onto the profile, thereby distinguishing between F1 and F2 terminals; (2) using a magnification of 27,000, we determined the sizes of labeled terminals and unlabeled F1 terminals at sites of synaptic contact by measuring the long and short diameters of each terminal and averaging these values to arrive at a single measure of diameter; (3) with magnifications of 8,200 or 11,800, we used the same method to measure the diameters of postsynaptic dendrites; (4) using magnifications of 8,200 or 11,800, we measured the longest axis of each synaptic contact zone; and (5) using a magnification of 46,000, we assessed the extent of postsynaptic density and the width of the synaptic cleft for each contact that was cut perpendicular to the membranes (Cucchiaro et al., '88).

Finally, these same serial micrographs enabled us to reconstruct limited segments of the dendrites postsynaptic to the terminals under investigation. From this, we identified the types of other synaptic profiles contacting the common target dendrite.

# RESULTS

We used the electron microscope to analyze the synaptic circuitry of labeled perigeniculate axons in lamina A of the cat's lateral geniculate nucleus. To do so, we used two different and complementary methods of axon labeling. One method involved the intracellular iontophoresis of HRP into a single perigeniculate cell. This has the advantage of labeling the entire axon arbor of an individual cell and allows different components to be identified and evaluated. Its disadvantage is the small sample size due to the difficulties with the technique, so that only a single cell was

studied by this method. The complementary method involved the orthograde labeling of a population of perigeniculate cells and their axonal arborizations by placement of the tracer, PHAL, into the perigeniculate nucleus. This greatly increased the sample size of perigeniculate terminals available for study, although it did not permit identification of subregions of terminal arbors from individual cells. We analyzed the PHAL-labeled material to verify and extend observations made from the single, intracellularly labeled perigeniculate neuron.

## QUALITATIVE OBSERVATIONS

### Light microscopic observations

***Intracellular HRP labeling.*** Figure 1a shows a reconstruction of the perigeniculate cell we functionally characterized, intracellularly labeled with HRP, and studied with the electron microscope. The cell had a large receptive field and was driven by the contralateral eye; we found no evidence of a receptive field for the ipsilateral eye. Its well-labeled axon branched and innervated the lateral geniculate nucleus in two main terminal arbors that were offset from each other mediolaterally (Fig. 1a; see also Uhlrich et al., '91). The medial branch was relatively narrow and extended through laminae A and A1. The lateral branches had many more boutons and were limited to lamina A. This sort of mediolateral division of the axon projection into two arbors that differentially innervate the A-laminae seems to be a common feature of perigeniculate cells (Uhlrich et al., '91). Both the medial and the lateral arbors of the perigeniculate axon had numerous en passant swellings that gave the axons a beaded appearance. Stalked appendages were exceedingly rare. As noted below, the synaptic relationships of the medial and lateral arbors were somewhat different from each other.

***PHAL labeling.*** Figure 1b is a composite drawing made from several sections through the site at which we injected PHAL into the perigeniculate nucleus. This shows the injection site in the perigeniculate nucleus and the distribution of labeled boutons in the lateral geniculate nucleus. A dense column of labeled fibers can be seen extending through laminae A and A1, and sparse innervation continues into the C-laminae (Fig. 1b; Uhlrich et al., '91). Nearly all of these PHAL-labeled axons were beaded and looked indistinguishable from the axons labeled by intracellular labeling of individual perigeniculate cells (Fig. 1a and Uhlrich et al., '91). Furthermore, when we serially reconstructed individual, PHAL-labeled axons with the light microscope, we could often see the abovementioned medial and lateral components to the arborizations (Uhlrich et al., '91). These results suggest that most of the axons we labeled with PHAL emanated from perigeniculate cells.

However, among the PHAL-labeled axons in the A-laminae were rare examples of a different morphology, including much finer fibers and boutons appended to short stalks instead of being en passant. The labeled axons in the C-laminae also displayed such morphological features. These axons closely resemble those labeled after injections of PHAL into the visual cortex (Uhlrich et al., '91). As we have noted previously (Uhlrich et al., '91), these probably represent corticogeniculate axons labeled by the PHAL injection as fibers of passage, but they represent such a small sample that they are unlikely to corrupt our interpretation of perigeniculate axon terminals (see also below).

### Electron microscopic observations

***Perigeniculate terminals labeled intracellularly with HRP.*** Ultrastructural analysis of the HRP-labeled axon demonstrated that the en passant swellings seen with the light microscope are indeed the synaptic terminals. Figures 2 and 3 show examples of such terminals. These contained dark mitochondria and made synaptic contacts (see Figs. 2, 3). We have analyzed 75 terminals labeled with HRP that formed synaptic contacts and of these all were exclusively presynaptic in relation to other profiles. A single terminal usually gave rise to a single synaptic contact, although some produced two or occasionally three synapses (see Fig. 3D–F). In our material, when more than one synapse was formed from a single terminal, it always contacted separate dendritic profiles. Every synaptic contact from the labeled terminals had a relatively thin postsynaptic density and was therefore identified as symmetrical (see also below). For these reasons, we have identified these perigeniculate profiles as *F1* terminals based on Guillery's ('69) classification.

***Perigeniculate terminals labeled with PHAL.*** In lamina A, the great majority of axons labeled with PHAL were beaded and formed en passant synapses at swellings. We analyzed 75 such PHAL-labeled terminals; examples are illustrated in Figures 4 and 5. At the electron microscopic level, the PHAL-labeled and HRP-labeled terminals were morphologically indistinguishable, both being identified as F1 terminals. Like those labeled with HRP, the PHAL-labeled terminals contained dark mitochondria and usually formed only a single synaptic contact per swelling, although a single terminal occasionally formed two or more contacts (see Fig. 3D–F, 4A–C, G–I). As with HRP labeling, we found that when more than one synapse was formed from a single terminal, it always contacted separate dendritic profiles. All the labeled terminals that formed synaptic contacts were exclusively presynaptic in relation to other profiles. All of the synapses from PHAL-labeled terminals, except one (Fig. 5I), had thin postsynaptic thickenings associated with the contact zones. We interpret the single asymmetrical contact, which was cut oblique to the membrane and therefore not included in our quantitative measurements of postsynaptic densities (see below), as arising from a labeled cortical axon of passage. Since only one of our 75 labeled terminals had features of a corticogeniculate terminal, we view the level of putative contamination by axons of passage as acceptably low for the purposes of the present study.

***Other terminals.*** Other terminals were included in our assessment of the geniculate neuropil to provide a broader context for analysis of the perigeniculate contribution to synaptic circuitry in the lateral geniculate nucleus. This included unlabeled F1, RSD, RLP, and F2 terminals, based on Guillery's ('69) classification, and corticogeniculate terminals labeled by PHAL injected into the striate cortex. For quantitative measurements, we randomly selected 213 unlabeled F1 and 60 unlabeled RSD terminals from the 5 cats, and we also identified a number of unlabeled RLP and F2 terminals during the course of our serial reconstructions.

Unlabeled terminals were identified as follows on the basis of a series of sections through each profile (Guillery, '69; see also Materials and Methods). F1 terminals displayed a variable size, relatively dark cytoplasmic matrix, inclusion of 1 to 4 dark mitochondria, flattened or pleomor-

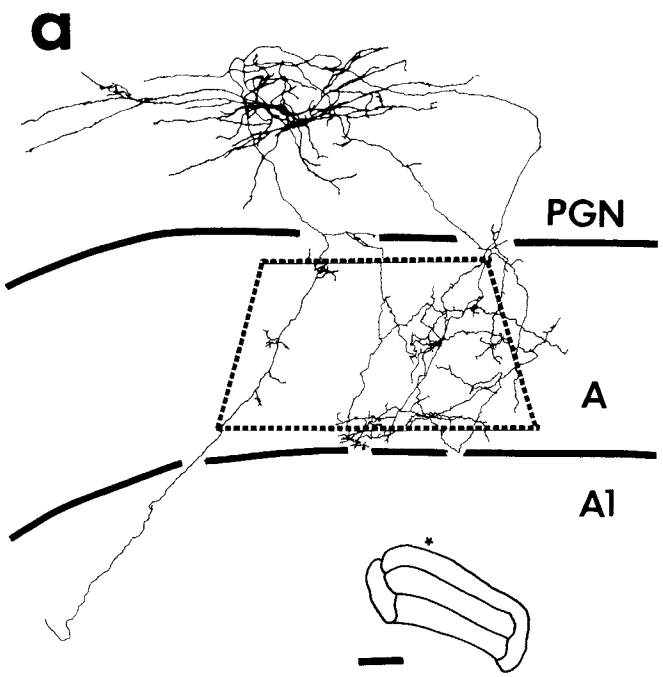



Fig. 1 (this page, left). Camera lucida reconstructions of labeled perigeniculate projections to the lateral geniculate nucleus. Each of the reconstructions was made from several serial sections. Abbreviations: PGN, perigeniculate nucleus; A, lamina A; A1, lamina A1; C, C-laminae. **(a)** HRP-labeled perigeniculate cell and its axonal arbor in laminae A and A1 of the lateral geniculate nucleus. The dashed polygon indicates the location of the block, which was taken from one section, that we used for electron microscopic analysis. The inset shows the location of the labeled soma (star) with respect to the lateral geniculate nucleus. **(b)** PHAL injection site in the perigeniculate nucleus and resultant terminal label in the lateral geniculate nucleus. The injection site is indicated by the filled, irregular blob, and each bouton is indicated by a small dot; for clarity, the axon segments connecting the boutons are not shown. The dashed polygon indicates the location of the block, which was taken from one section, that we used for electron microscopic analysis. Scale bar is 100 μm for the reconstructions and 1 mm for the inset.

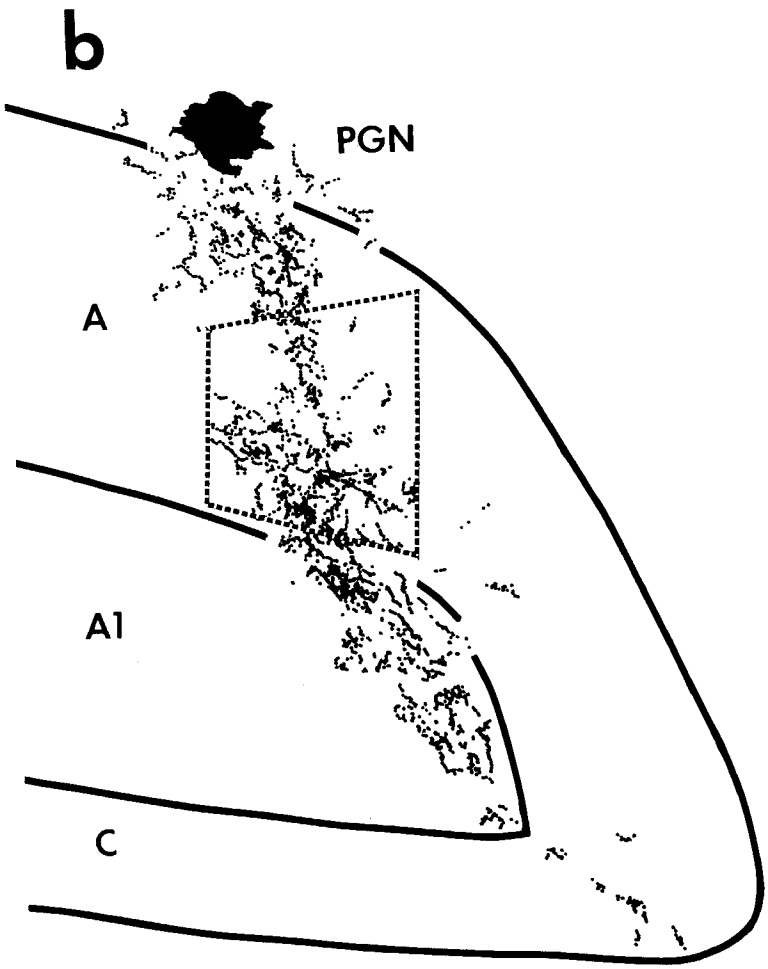



Fig. 2 (opposite page). Electron micrographs showing HRP-labeled perigeniculate terminals forming synapses onto retinorecipient geniculate dendrites. **A–C:** Three sections through a labeled perigeniculate terminal (marked by asterisks) forming a synaptic contact (arrowheads) onto a dendritic appendage (a) of a geniculate neuron. The postsynaptic dendritic segment is reconstructed in Figure 6C. Note that, whereas the perigeniculate terminal is densely labeled, the label is excluded from the mitochondria and the synaptic vesicles. The vesicles are densely distributed throughout the terminal, and the matrix of the single mitochondrion is clearly darker than that in the mitochondria of the nearby retinal terminal (RLP). The appendage of the geniculate cell receives triadic retinal input and is at the edge of a complex synaptic zone known as a glomerulus. The retinal terminal (RLP) is central in the glomerulus. It forms multiple synaptic contacts both onto relay cell appendages and onto the specialized dendritic terminals of interneurons (F2), and these F2 terminals form synaptic contacts onto the same relay cell appendages, thereby forming triads. Two separate F2 terminals are identified as such, because each received a synaptic contact at some level through the series of sections. The F2 terminal closer to the perigeniculate terminal receives a synaptic input from an RSD terminal (arrow in C). Two terminals with pleomorphic or flattened vesicles (F) are not classified further as F1 or F2. **D–F:** Three sections through a labeled perigeniculate terminal (marked by asterisks) forming a synaptic contact (arrowheads) onto a dendritic shaft of a geniculate neuron. The postsynaptic dendritic segment is reconstructed in Figure 6B. The postsynaptic dendrite also receives direct retinal (RLP) synapses (arrows). Scale bar in A is 1 μm and applies to A–F.

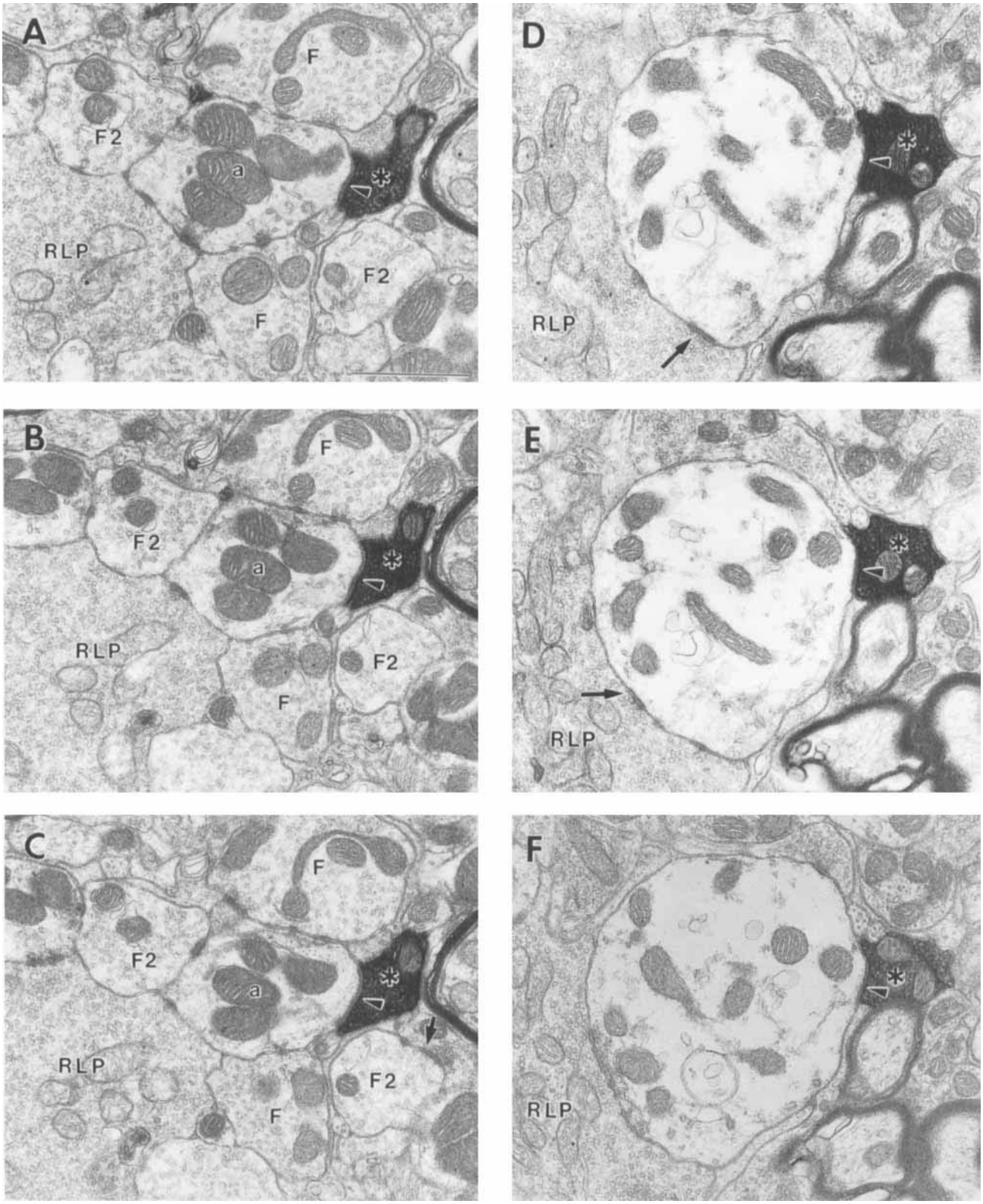



Figure 2

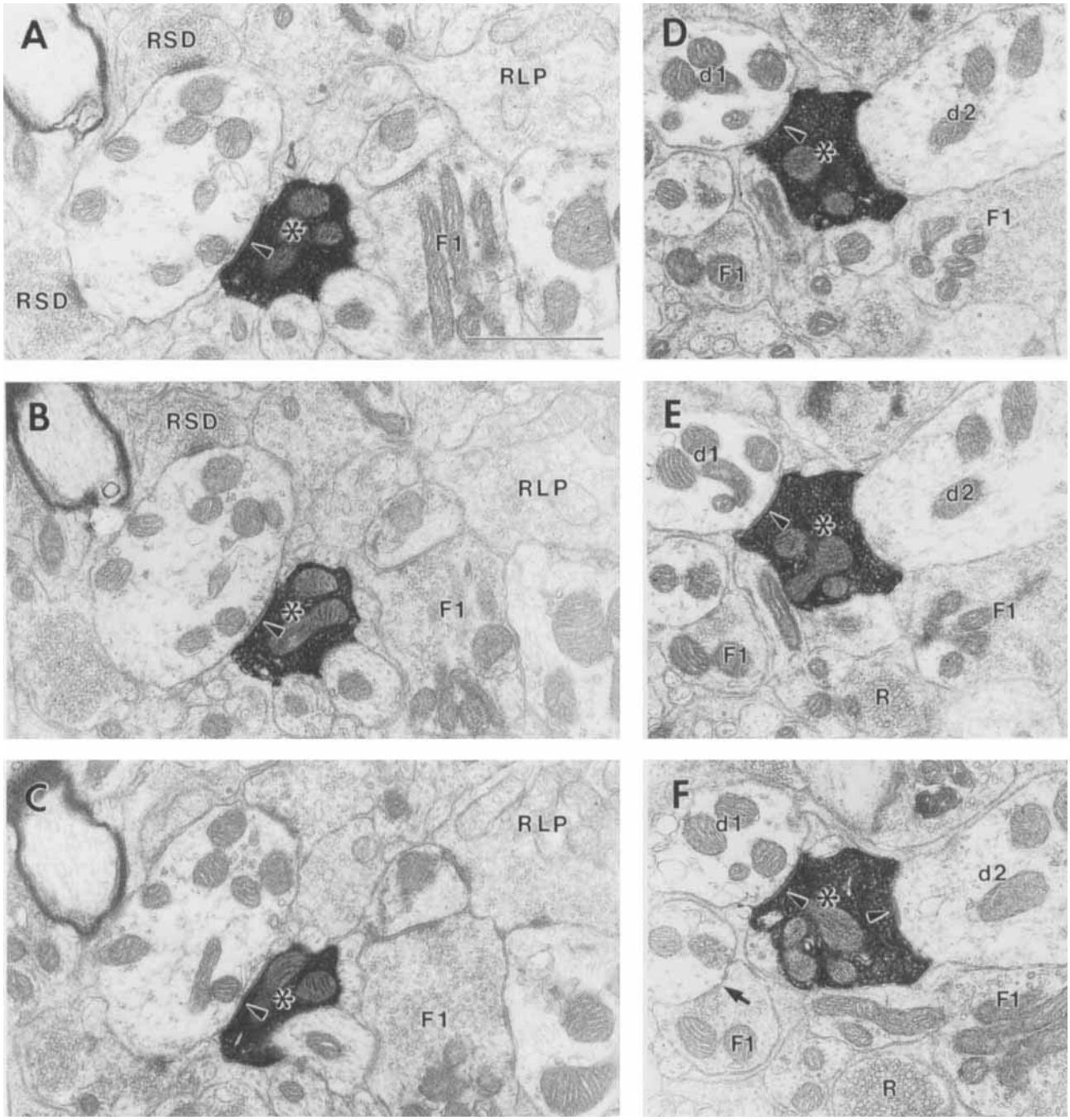



Fig. 3. Electron micrographs showing HRP-labeled perigeniculate terminals forming synapses onto three geniculate dendrites. **A–C.** Three sections through a single synaptic contact site (arrowheads) of a labeled perigeniculate terminal (marked by asterisks). The terminal contains dark mitochondria and is packed with synaptic vesicles. The synapse, which has a thin postsynaptic density, is formed onto a dendritic profile that also receives two synaptic inputs from RSD terminals (RSD). This dendrite is reconstructed in Figure 7F. There is an unlabeled F1 terminal (F1) that makes two synapses in this series, and there is also an unlabeled retinal terminal (RLP) that has pale mitochondria. **D–F:** Three sections through a labeled perigeniculate terminal (marked by asterisks) forming two synaptic contacts (arrowheads) onto two dendrites (d1 and d2). We were unable to determine whether the two dendrites belong to the same or different geniculate neurons. This illustrates a general observation: when a perigeniculate terminal forms two synaptic contacts, they almost always form onto separate dendrites in the geniculate neuropil (see also Fig. 4A–C,G–G–I). The labeled perigeniculate terminal contains dark mitochondria and is densely packed with synaptic vesicles. The synapse onto d1, which is cut in a favorable plane, displays minimal postsynaptic density; the synapse onto d2 is cut obliquely, which makes it difficult to evaluate the extent of postsynaptic density. Serial reconstruction revealed that d1 was a cortico-recipient dendrite, while d2 was retino-recipient. Of the two unlabeled F1 terminals (F1) identified here, the larger one on the right does not form a synapse within this series; the smaller one on the left forms a symmetrical synaptic contact (arrow in F) and contains small, flattened vesicles (compare with the large, round vesicles in the nearby, unidentified profile, R). Scale bar in A is 1 μm and applies to A–F.

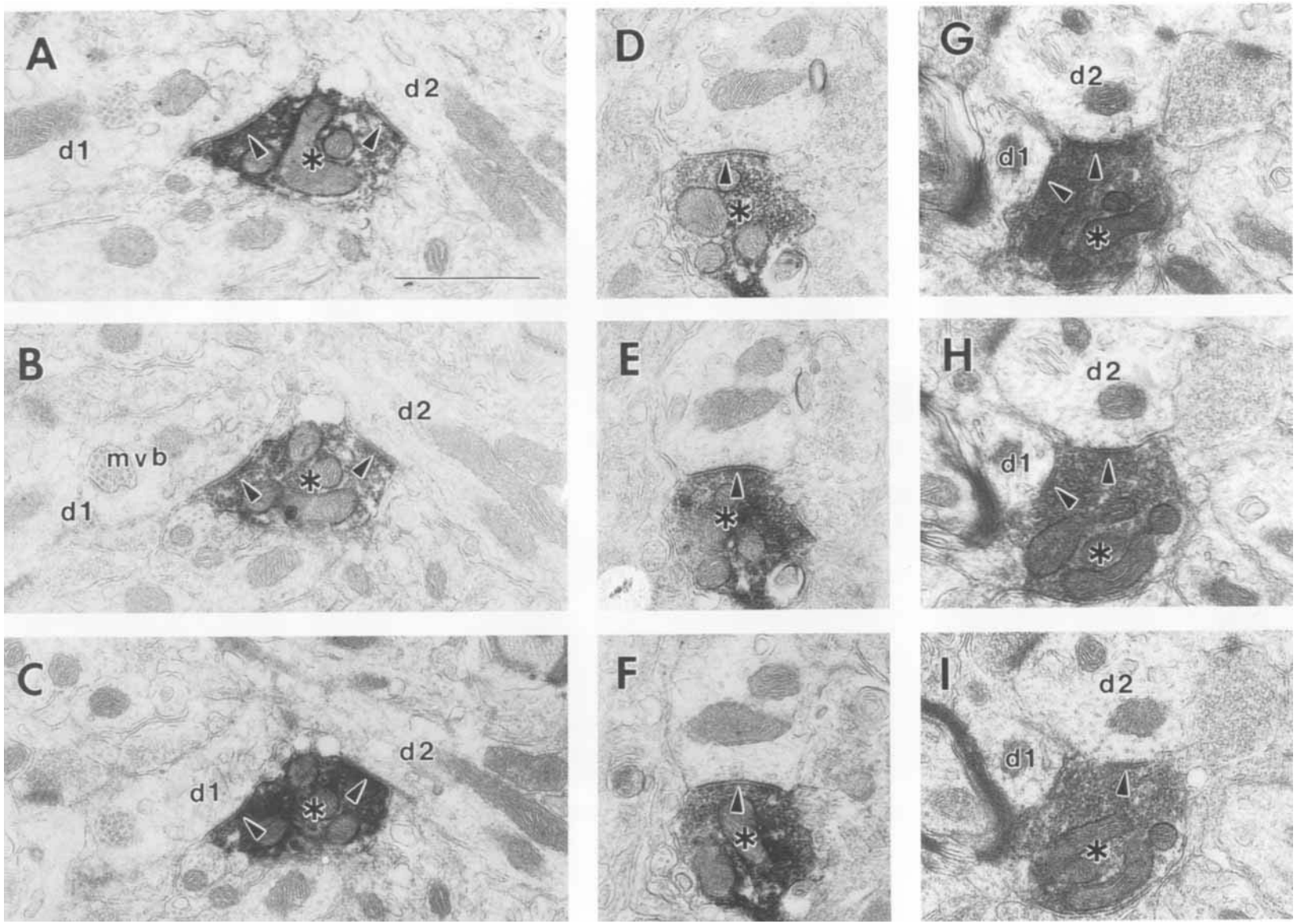



Fig. 4. Electron micrographs of perigeniculate terminals labeled with PHAL. As with the HRP labeling in Figures 2 and 3, the PHAL obscures features of the axonal cytoplasm, but mitochondria and synaptic vesicles exclude the label. Like the terminals labeled by intracellular HRP (see Figs. 2, 3), these contain dark mitochondria, are filled with synaptic vesicles, and form synaptic contacts with thin postsynaptic specializations. **A–C:** Three sections through a labeled perigeniculate terminal (marked by asterisks) that forms two synaptic contacts (arrowheads) onto two dendrites (d1 and d2). Note that d1 contains a multivesicular body (mvb). Figure 9D shows the reconstruction for d1, which we classified as cortico-recipient; Figure 8B shows the reconstruction for d2, which we classified as retino-recipient. As was the case for the example in Figure 3D–F, we were unable to determine whether the two dendrites belong to the same or different geniculate neurons. **D–F:** Three sections through a perigeniculate terminal (marked by asterisks) that forms a synaptic contact (arrowhead) with a thin postsynaptic density onto a cortico-recipient dendrite. **G–I:** Another example of a labeled perigeniculate terminal (marked by asterisks) that forms two synaptic contacts (arrowheads) onto separate, cortico-recipient dendrites (d1 and d2). Again, we were unable to determine whether the two dendrites belong to the same or different geniculate neurons. Scale bar in A is 1 μm and applies to A–I.

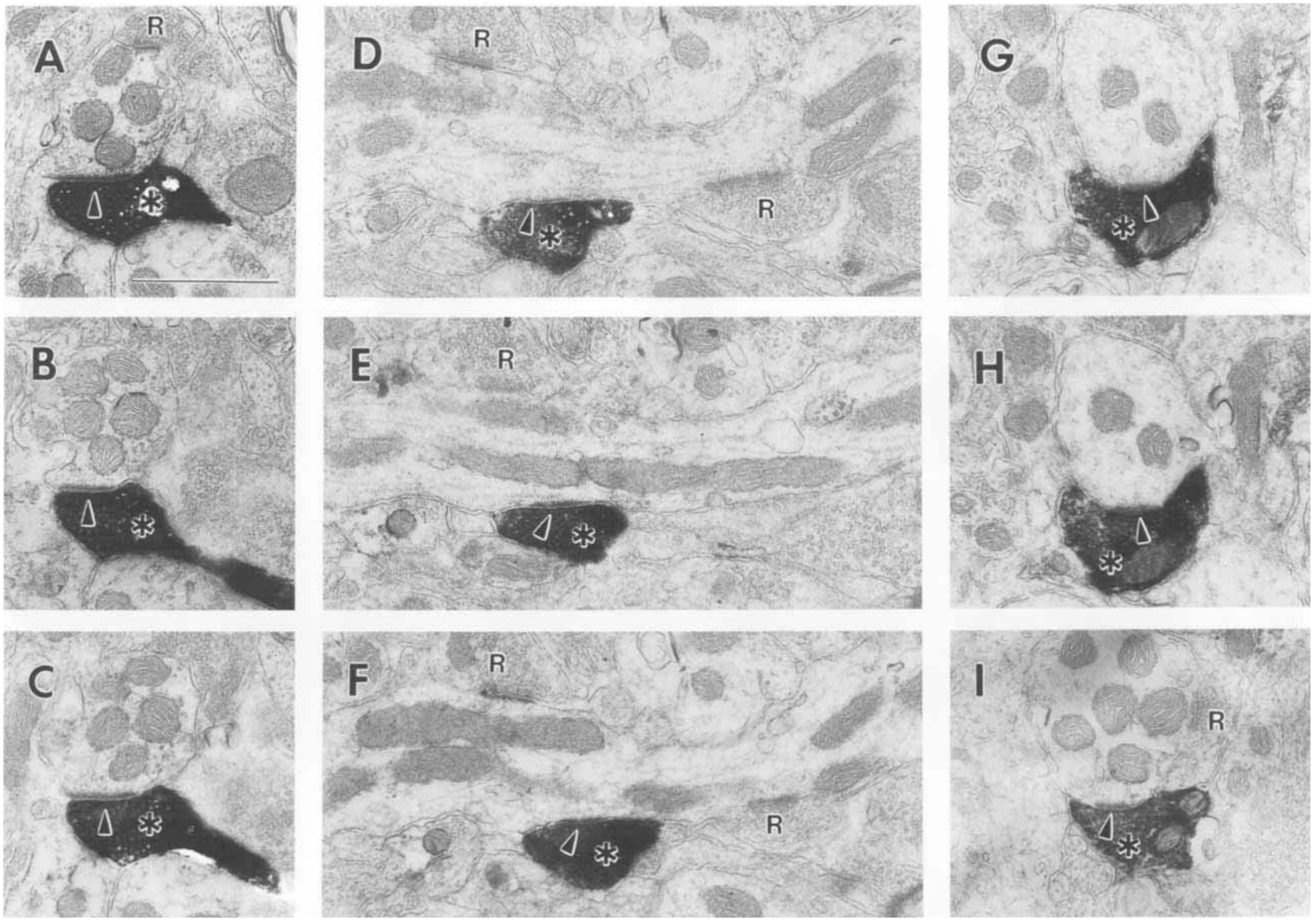



Fig. 5. Electron micrographs of terminals labeled with PHAL transported either from visual cortex (A–H) or from the perigeniculate nucleus (I). Note that the synaptic contacts from the labeled cortical terminals exhibit thick postsynaptic densities, which are clearly distinguishable from the thin densities of perigeniculate terminals. **A–C:** Three sections through a labeled cortical terminal (marked by asterisks) that forms a synaptic contact (arrowheads) onto a cortico-recipient dendrite. Note that the dendrite also receives a synapse from an unlabeled RSD terminal (R) in A. **D–F:** Three sections through another, labeled cortical terminal (marked by asterisks) that forms a synaptic contact (arrowheads) onto a cortico-recipient dendrite. Inputs onto the dendrite from unlabeled RSD terminals (R) are also evident. This pattern of cortical innervation, with one terminal labeled amidst several unlabeled terminals, was common in our material. **G, H:** Two sections through a labeled cortical terminal (marked by asterisks). Although cut obliquely, the synaptic specialization (arrowheads) is evident, and the terminal contains a single dark mitochondrion. **I:** One section through a terminal (marked by asterisk) labeled from the perigeniculate nucleus. Note the thick postsynaptic density (arrowhead), the densely packed vesicles, and the two dark mitochondria, all of which are consistent with RSD morphology. This is the only example from our material of an RSD terminal labeled from the perigeniculate nucleus. Quite likely, this represents a labeled axon of passage (see text for details). There is also an unlabeled RSD terminal (R). Scale bar in A is 1 μm and applies to A–I.

phic vesicles, relatively high density of vesicles, relatively long junctional specializations, relatively thin postsynaptic thickening, and exclusively presynaptic position with respect to other profiles. Whereas most F1 terminals formed only a single synaptic contact, some made two and occasionally three synapses. RSD terminals were chosen on the basis of their small size, relatively dark cytoplasmic matrix, occasional inclusion of one or two dark mitochondria, round synaptic vesicles, high density of synaptic vesicles, short junctional specializations, relatively thick postsynaptic density, and exclusively presynaptic position with respect to other profiles in the neuropil. Each of the RSD terminals made only a single synapse. RLP terminals, which are isomorphic with retinal terminals, are characterized by their round vesicles, large profiles, pale mitochondria, relatively thick postsynaptic density, and exclusively presynaptic position with respect to other profiles in the neuropil. Finally, F2 terminals were identified by their flattened or pleomorphic vesicles, relatively low density of vesicles, pale cytoplasmic matrix, and participation as both presynaptic and postsynaptic elements. Figures 2–5 show examples of these terminals.

We also studied 12 synaptic terminals labeled from PHAL injected into the striate cortex. Figure 5 illustrates examples of such labeled terminals. Each of these corticogeniculate terminals displayed clear evidence of RSD morphology, including small size, densely packed synaptic vesicles, occasional inclusion of one or two dark mitochondria, short junctional specializations, relatively thick postsynaptic thickening, and exclusively presynaptic position with respect to other profiles in the neuropil. Each cortical terminal made only a single synapse. These observations demonstrate that the PHAL labeling permits a clear distinction between different morphological classes of synaptic terminal and thereby provide an important control for this method.

***Identification of postsynaptic profiles.*** Several features of the geniculate neuropil in cats help to identify the postsynaptic profiles. For instance, analysis of individual geniculate cells labeled intracellularly with HRP indicates that the pattern of synaptic inputs onto their dendrites varies systematically with distance from the soma (Mason et al., '84; Wilson et al., '84; Hamos et al., '85, '87). Nearly all of the retinal synapses (i.e., from RLP terminals) are located on larger, more proximal dendrites (i.e., $< 100$ μm from the soma). The smaller, more distal dendrites of geniculate relay cells (i.e., $> 100$ μm from the soma) are dominated by inputs from RSD terminals. Because geniculate relay cells vary in size, there is no established criteria based on diameter to distinguish between distal and proximal dendrites. Furthermore, we cannot distinguish the thin dendrites of interneurons from the distal dendrites of relay cells: both are generally rich in inputs from RSD terminals and small in caliber (Weber et al., '89).

For these reasons, we have classified geniculate dendrites on the basis of whether they have synaptic inputs from RLP terminals or from RSD terminals without RLP input. Furthermore, because the majority of RSD terminals are thought to be cortical in origin (Szentágothai et al., '66; Robson, '83; Weber and Kalil, '87; Weber et al., '89; Montero, '89a), we refer to portions of the dendritic arbor receiving contacts from RSD and no RLP terminals as "cortico-recipient" and those receiving one or more synaptic contacts from RLP terminals as "retino-recipient." In support of this terminology, we observed that all of the terminals labeled with PHAL from the cortical injection contact the RSD-rich, cortico-recipient zones of geniculate dendrites. Geniculate interneurons have specialized dendritic appendages, known as F2 terminals (see above), that represent a major site of synaptic efferents from these cells (Hamos et al., '85; Montero, '86). Thus any contacts onto F2 terminals are contacts onto interneurons. Finally, we identify somata as such and make no attempt to distinguish between somata of interneurons or relay cells.

Figures 6–9 illustrate the sort of reconstructions we have used in our analysis. Figures 6 and 7 show dendrites postsynaptic to perigeniculate terminals visualized from the intracellular HRP labeling, and Figures 8 and 9 show reconstructions based on PHAL labeling. Most of the dendrites in receipt of perigeniculate innervation are thin and have been identified as cortico-recipient (Figs. 7, 9). In most cases, unlabeled F1 terminals contacted the same dendrites amidst labeled perigeniculate terminals. It is possible, even in the PHAL material, that some perigeniculate terminals innervating a given dendrite remained unlabeled. Also, sources of F1 terminals other than the perigeniculate nucleus have been identified (see Discussion).

Figures 6 and 8 illustrate retino-recipient dendrites. Figure 6 shows reconstructions of three dendritic segments that received synaptic inputs from the medial axonal branch of the HRP-filled perigeniculate cell. All three of these dendrites had appendages and all received retinal inputs via synaptic triads. Each had complex glomerular arrangements associated with their retinal inputs (see Fig. 2A–C). Two main relay cell classes exists in the A-laminae, X and Y cells (for review, see Sherman and Koch, '86, '90), and triadic retinal circuitry is most frequently associated with the retinogeniculate X pathway (Wilson et al., '84; Hamos et al., '87). Figure 8 shows reconstructions of five dendritic segments that received synaptic inputs from perigeniculate terminals labeled with PHAL. Unlike the three dendrites in Figure 6, none of the five illustrated in Figure 8 received triadic retinal input. Instead, simple, direct retinal synapses were formed directly onto dendritic shafts (Fig. 8A–C,E) or onto appendages (Fig. 8D). This mode of retinal innervation is most commonly associated with the retinogeniculate Y pathway (Wilson et al., '84). We assume that the apparent difference in postsynaptic targets revealed by the HRP and PHAL labeling represents sampling differences based on our small samples (see Discussion). In any case, these data from HRP and PHAL labeling indicate that perigeniculate terminals innervate both X and Y cells in the lateral geniculate nucleus.

Finally, although we frequently observed unlabeled F1 terminals forming synaptic contacts onto somata and F2 terminals, *none* of the labeled perigeniculate terminals, whether labeled by HRP or PHAL, did so. This suggests that perigeniculate terminals are a subset of F1 terminals. Quantitative data presented below support this conclusion.

## QUANTITATIVE OBSERVATIONS

### Synaptic terminals

***Terminal diameter.*** Table 1 and Figure 10 show that both HRP and PHAL labeled terminals form a similar size spectrum. However, as a population, the HRP-labeled terminals are slightly, but significantly, smaller than their PHAL-labeled counterparts ($p < 0.001$ on a Mann-Whitney U-test). In our companion light microscopic study, for which we measured boutons of a population of both HRP-

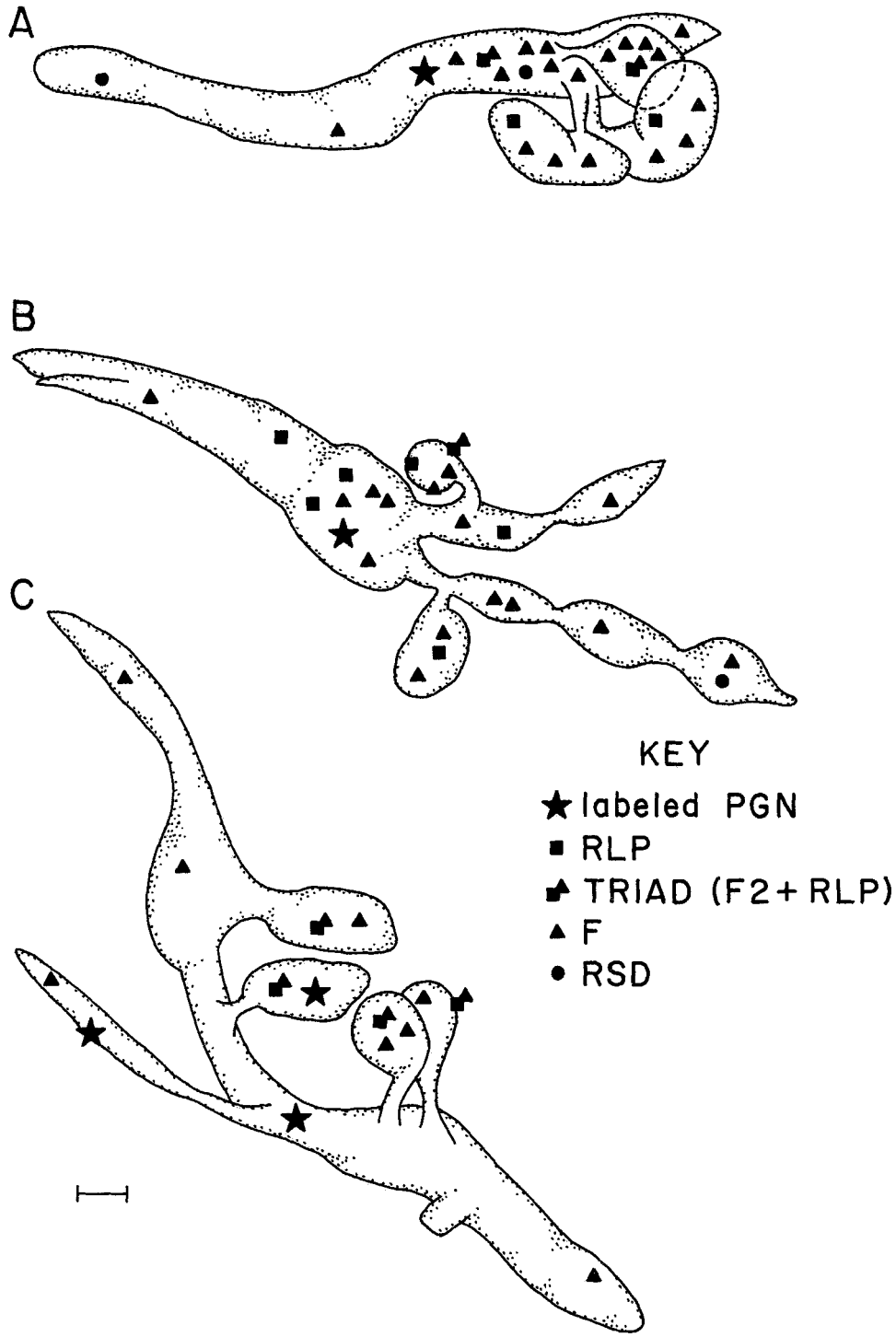



Fig. 6. Reconstructions from the geniculate neuropil of three retino-recipient dendrites postsynaptic to HRP-labeled perigeniculate terminals. Symbols are indicated in the key: star, labeled perigeniculate synapse; square, unlabeled RLP (i.e., retinal) terminals; triangle, unidentified F terminals; circles, unlabeled RSD (mostly cortical) terminals; overlapping square and triangle, unlabeled synaptic triad involving an F2 and an RLP terminal (see text for details). **A:** Dendritic segment reconstructed from 124 serial sections. This segment received one synapse from a labeled perigeniculate terminal (this is the synapse illustrated in Fig. 3D–F). **B:** Dendritic segment reconstructed from 50 serial sections. This segment received one synapse from a labeled perigeniculate terminal (this is the synapse illustrated in Fig. 2D–F). **C:** Dendritic segment reconstructed from 48 serial sections. This segment received synapses from each of three labeled perigeniculate terminals (one of these synapses is illustrated in Fig. 2A–C). Scale bar in C is 1 μm and applies to all three reconstructions.

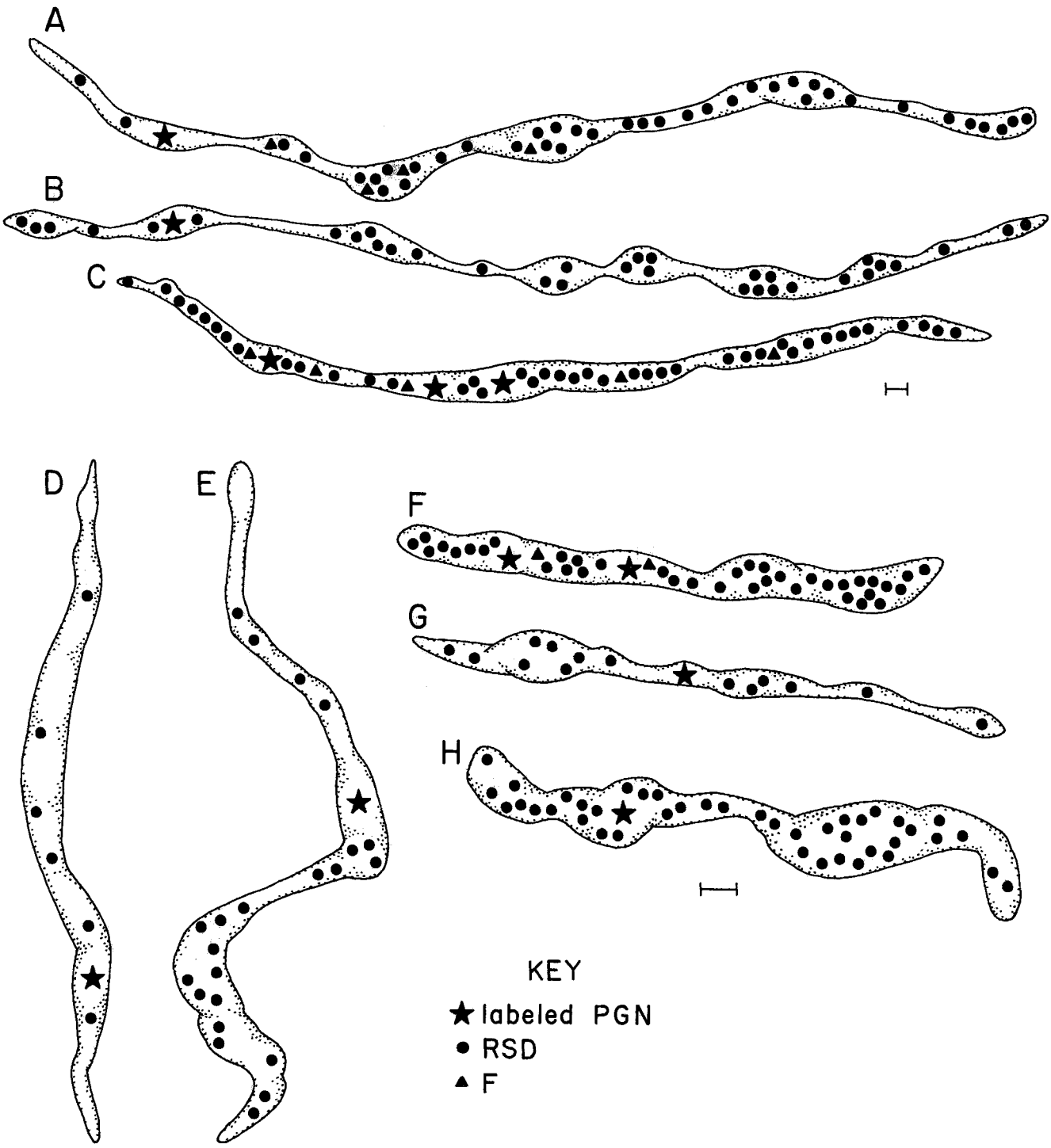



Fig. 7. Reconstructions from the geniculate neuropil of 8 cortico-recipient dendrites postsynaptic to HRP-labeled perigeniculate terminals; conventions and key as in Figure 6. **A:** Dendritic segment reconstructed from 75 serial sections. This segment received one synapse from a labeled perigeniculate terminal. **B:** Dendritic segment reconstructed from 32 serial sections. This segment received one synapse from a labeled perigeniculate terminal. **C:** Dendritic segment reconstructed from 65 serial sections. This segment received synapses from each of three labeled perigeniculate terminals. **D:** Dendritic segment reconstructed from 30 serial sections. This segment received one synapse from a labeled perigeniculate terminal. **E:** Dendritic segment reconstructed from 142 serial sections. This segment received one synapse from a labeled perigeniculate terminal. **F:** Dendritic segment reconstructed from 137 serial sections. This segment received one synapse from a labeled perigeniculate terminal. **G:** Dendritic segment reconstructed from 20 serial sections. This segment received one synapse from a labeled perigeniculate terminal. **H:** Dendritic segment reconstructed from 86 serial sections. This segment received one synapse from a labeled perigeniculate terminal. Scale bar in C is 1 μm long and applies only to **A–C;** scale bar in H is 1 μm and applies to D–H.

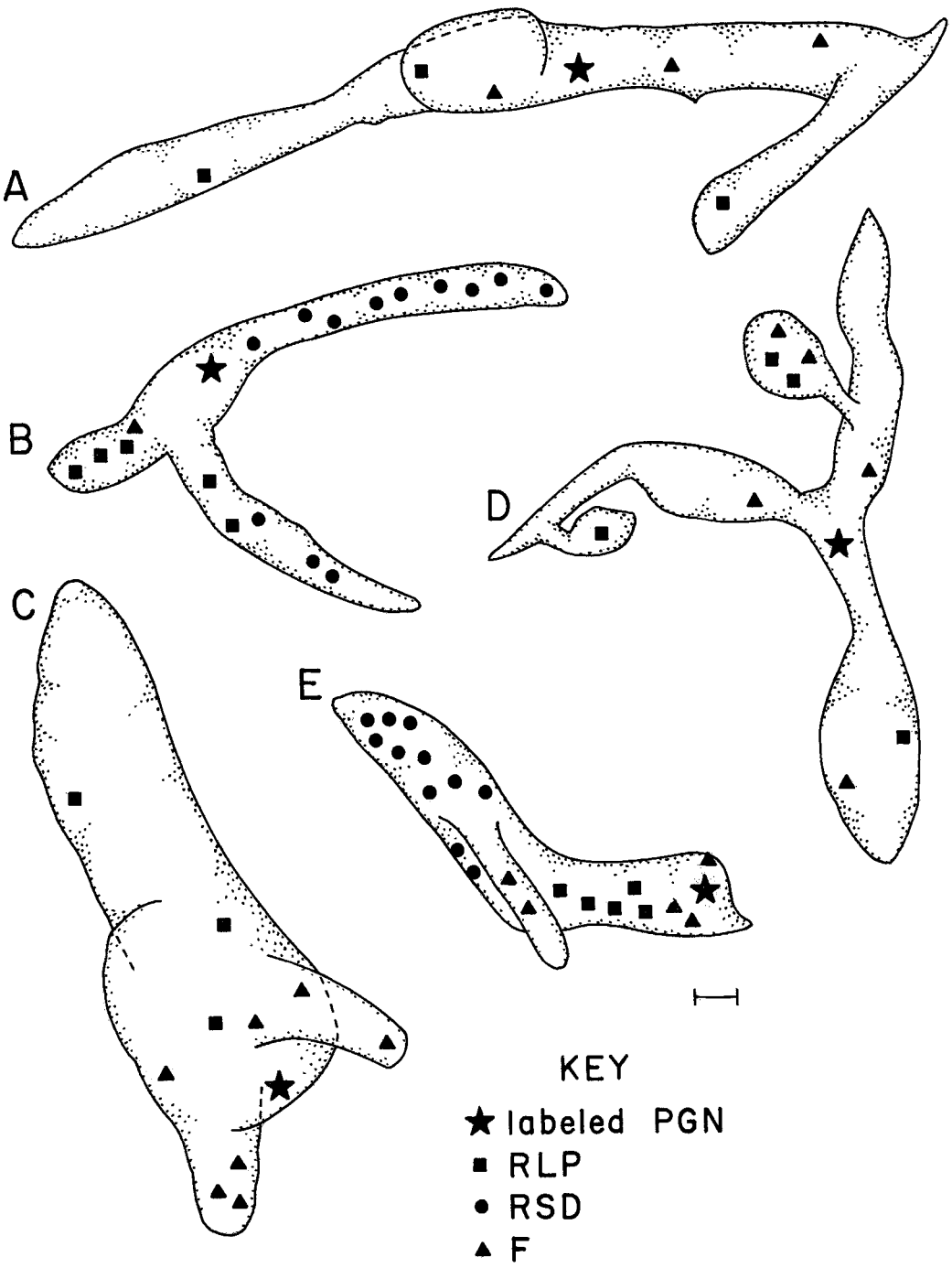



Fig. 8. Reconstructions from the geniculate neuropil of 5 retinorecipient dendrites postsynaptic to PHAL-labeled perigeniculate terminals; conventions and key as in Figure 6. **A:** Dendritic segment reconstructed from 12 serial sections. This segment received one synapse from a labeled perigeniculate terminal. **B:** Dendritic segment reconstructed from 25 serial sections. This segment received one synapse from a labeled perigeniculate terminal. **C:** Dendritic segment reconstructed from 32 serial sections. This segment received one synapse from a labeled perigeniculate terminal. **D:** Dendritic segment reconstructed from 27 serial sections. This segment received one synapse from a labeled perigeniculate terminal. **E:** Dendritic segment reconstructed from 25 serial sections. This segment received one synapse from a labeled perigeniculate terminal. Scale bar in E is 1 μm and applies to all 5 reconstructions.

labeled and PHAL-labeled perigeniculate axons, we found no difference in diameter (Uhlrich et al., '91). Presumably, there is some variability in terminal size among perigeniculate axon arbors, and the single axon labeled with HRP contains slightly smaller terminals than does the average perigeniculate axon labeled with PHAL. In any case, the HRP-labeled and PHAL-labeled terminals from the perigeniculate nucleus are considerably smaller, on average, than are the unlabeled F1 terminals ($p < 0.001$ on a Mann-Whitney U-test for each comparison). However, as is shown

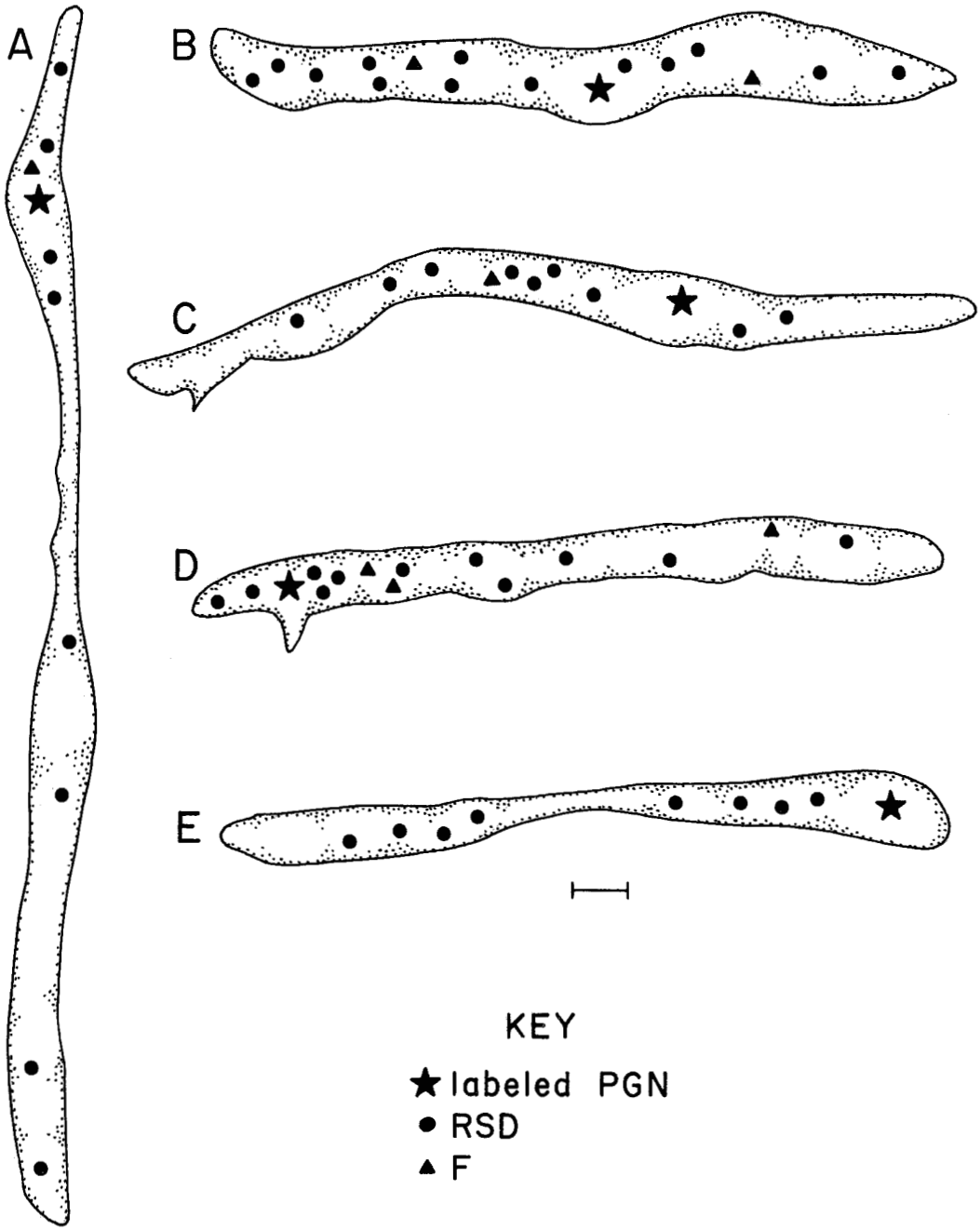



Fig. 9. Reconstructions from the geniculate neuropil of 5 cortico-recipient dendrites postsynaptic to PHAL-labeled perigeniculate terminals; conventions and key as in Figure 6. Serial EM reconstructions of five RSD-rich geniculate dendrites that received synaptic input from perigeniculate terminals labeled with PHAL. **A:** Dendritic segment reconstructed from 12 serial sections. This segment received one synapse from a labeled perigeniculate terminal. **B:** Dendritic segment reconstructed from 36 serial sections. This segment received one synapse from a labeled perigeniculate terminal. **C:** Dendritic segment reconstructed from 32 serial sections. This segment received one synapse from a labeled perigeniculate terminal. **D:** Dendritic segment reconstructed from 34 serial sections. This segment received one synapse from a labeled perigeniculate terminal. **E:** Dendritic segment reconstructed from 12 serial sections. This segment received one synapse from a labeled perigeniculate terminal. Scale bar in E is 1 μm and applies to all 5 reconstructions.

by Figure 10, the labeled perigeniculate terminals fall within the small end of the size range of the unlabeled F1 terminals, suggesting that perigeniculate terminals represent a subset of relatively small F1 terminals. Some of the overlap between C and A,B in Figure 10 probably reflects the fact that the sample in C almost certainly includes unidentified perigeniculate terminals.

***Postsynaptic density.*** Figure 11 shows the distributions of postsynaptic density related to different terminal types we have analyzed (see also Table 1). We found no

difference in these values between the populations of HRP-labeled and PHAL-labeled terminals ($p > 0.1$ on a Mann-Whitney U-test). However, the densities of each of these populations was slightly but reliably greater than those from the unlabeled F1 terminals ($p < 0.05$ for the HRP-labeled population and $p < 0.02$ for the PHAL-labeled, both on a Mann-Whitney U-test). This is consistent with the view that perigeniculate terminals represent a subset of F1 terminals. Figure 11 shows further that corticogeniculate terminals labeled with PHAL (Fig. 11D) have larger postsynaptic densities than any of the F1 populations (Fig. 11A–C; $p < 0.001$ on all pair-wise comparisons on a Mann-Whitney U-test), which is expected, since symmetrical (F1) synaptic contacts have smaller postsynaptic densities than do asymmetric (RSD) synaptic contacts. Finally, there is no significant difference between the corticogeniculate terminals and unlabeled RSD terminals as regards postsynaptic density ($p > 0.05$ on a Mann-Whitney U-test).

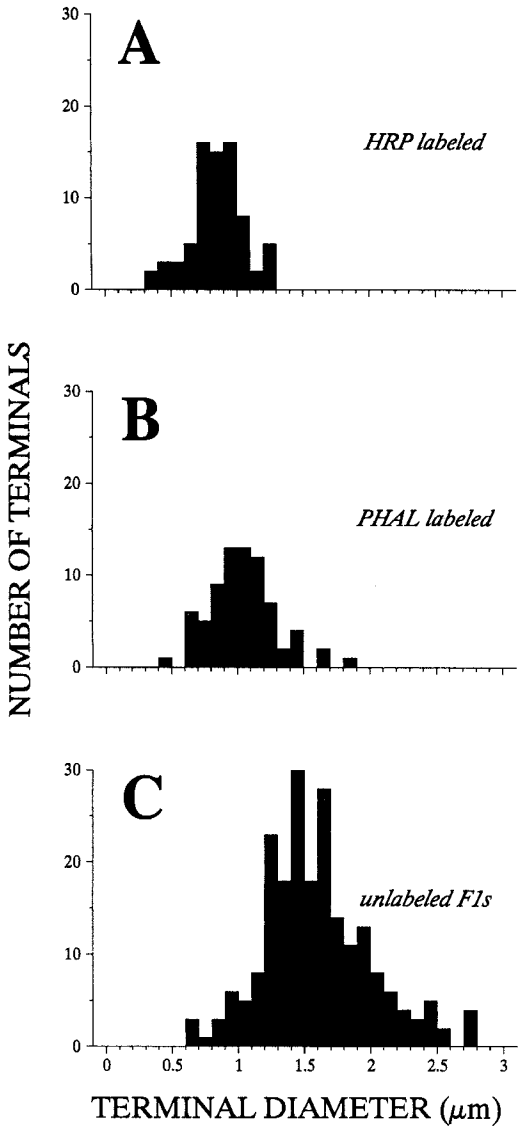



Fig. 10. Histograms showing the size distribution of terminals in the geniculate neuropil. **A:** Perigeniculate terminals labeled by intracellular injection of HRP. **B:** Perigeniculate terminals labeled by anterograde transport of PHAL. **C:** Unlabeled F1 terminals randomly selected from the geniculate neuropil.

***Synaptic contact length.*** A final measure we made for the labeled perigeniculate terminals was the maximum length of the synaptic contact zone (see Table 1 and Figure 12). We found no difference in these values between the HRP-labeled and PHAL-labeled perigeniculate terminals ($p > 0.05$ on a Mann-Whitney U-test). Whereas we also saw no difference in contact length between HRP-labeled perigeniculate terminals and unlabeled F1 terminals ($p > 0.05$ on a Mann-Whitney U-test), lengths of the PHAL-labeled terminals were smaller than those of the unlabeled F1 terminals ($p < 0.001$ on a Mann-Whitney U-test).

## Postsynaptic profiles

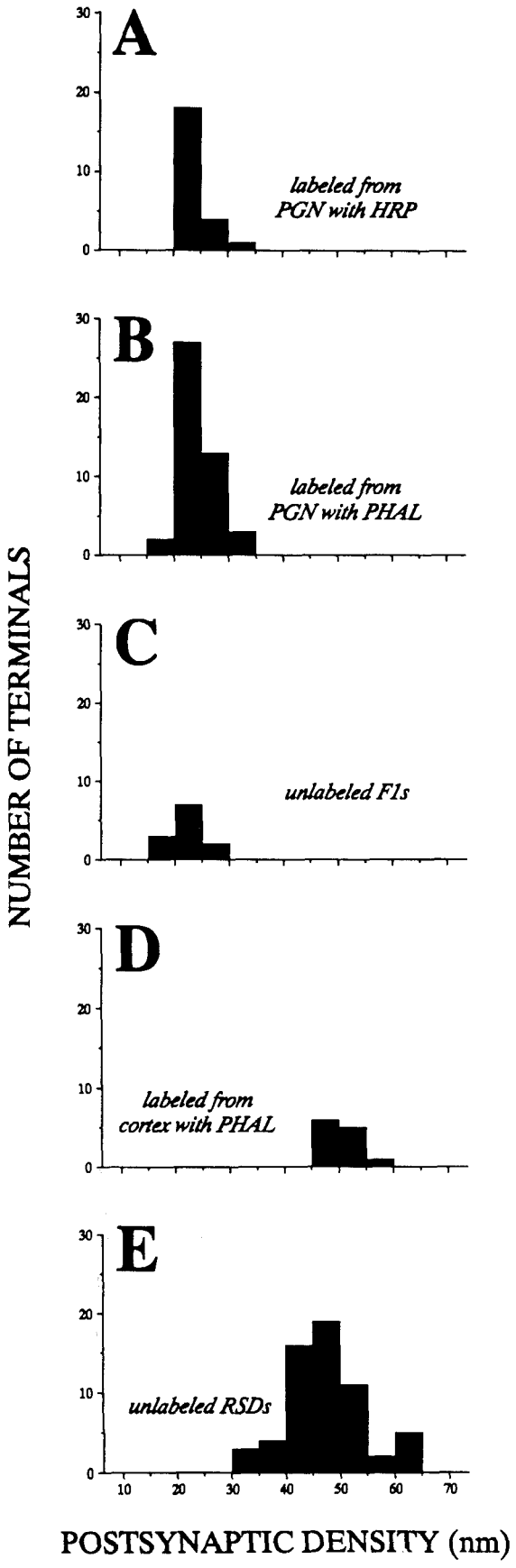
***Types of postsynaptic profile.*** As noted above, the axon arbor of the HRP-labeled perigeniculate cell had medial and lateral components within lamina A. Our analysis included 21 terminals from the medial component and 54 from the lateral (Table 2). We compared terminals from each component on each parameter noted above (i.e., terminal diameter, postsynaptic density, and length of synaptic contact zone), and found no differences between them ($p > 0.1$ on all comparisons on a Mann-Whitney U-test).

We have already noted that labeled perigeniculate terminals do not contact somata or F2 terminals, but contact only cortico-recipient and retino-recipient dendrites. Table 2 shows that each of the 54 terminals from the lateral component of the HRP-labeled axon exclusively contacted cortico-recipient dendrites, whereas 16 of the 21 terminals from the medial component did so. Thus the vast majority (93%) of these perigeniculate terminals contacted cortico-recipient dendrites, and only the medial component of the arbor contacted retino-recipient dendrites. The distribution of postsynaptic targets for the PHAL-labeled terminals is comparable (see Table 2). Of these, 85% contacted cortico-recipient dendrites, the remainder contacted retino-recipient dendrites, and none contacted somata or F2 terminals. We found no difference in postsynaptic targets between the HRP-labeled and PHAL-labeled terminals ($p > 0.1$ on a $\chi^2$-test).

These results for the perigeniculate projection are in contrast to a similar analysis we made for unlabeled F1 terminals (see Table 2). Of these, only 41% contacted cortico-recipient dendrites, 37% contacted retino-recipient

TABLE 1. Parameters of Labeled Perigeniculate Terminals and Unlabeled F1 Terminals in Lamina A

| | HRP labeled | | PHAL labeled | | Unlabeled F1 | |
|---|---|---|---|---|---|---|
| | mean ± s.d. | N | mean ± s.d. | N | mean ± s.d. | N |
| Bouton diameter | 0.85 ± 0.20 μm | 75 | 1.05 ± 0.25 μm | 75 | 1.60 ± 0.41 μm | 213 |
| Postsynaptic density | 24.1 ± 2.2 nm | 23 | 24.2 ± 3.3 nm | 45 | 22.0 ± 2.7 nm | 12 |
| Synaptic contact length | 534 ± 128 nm | 23 | 470 ± 132 nm | 45 | 635 ± 257 nm | 70 |



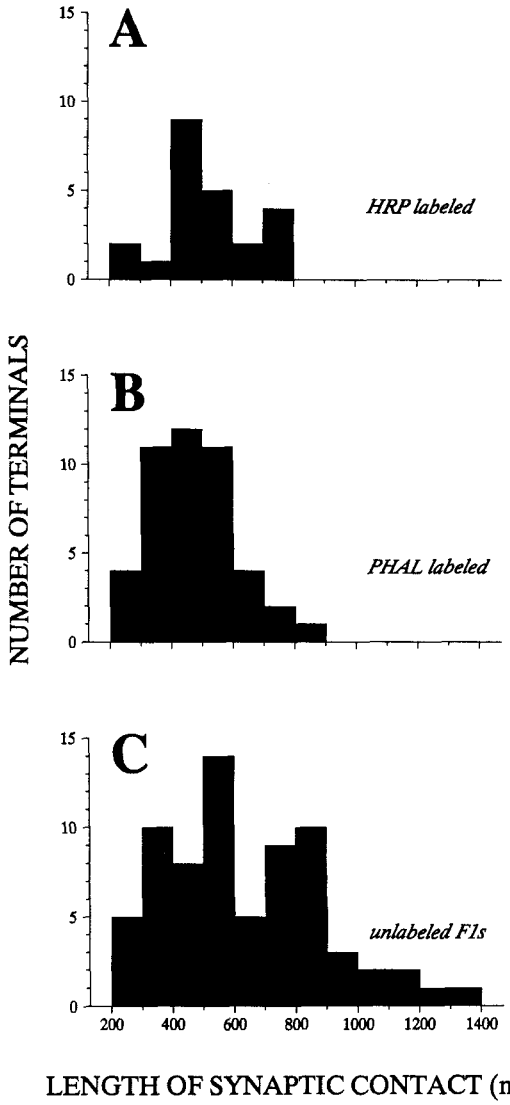




Fig. 12. Histograms showing the distributions of lengths for the synaptic contacts formed from various terminals in the geniculate neuropil. **A:** Perigeniculate terminals labeled with HRP. **B:** Perigeniculate terminals labeled with PHAL. **C:** Unlabeled F1 terminals.

dendrites, 13% contacted F2 terminals, and 9% contacted somata. This pattern of postsynaptic targets differs from either the HRP-labeled or PHAL-labeled pattern ($p < 0.001$ on a $\chi^2$-test for either comparison). This further supports our earlier conclusion that perigeniculate terminals are a subtype of F1 terminal. We also conclude that the major influence of perigeniculate innervation is upon cortico-recipient dendrites, with very limited input to retino-recipient zones.

Fig. 11. Histograms showing the distributions of maximum thickness for the postsynaptic densities associated with synaptic contacts from various terminals in the geniculate neuropil. **A:** Perigeniculate terminals labeled with HRP. **B:** Perigeniculate terminals labeled with PHAL. **C:** Unlabeled F1 terminals. **D:** Cortical terminals labeled with PHAL. **E:** Unlabeled RSD terminals.

TABLE 2. Postsynaptic Targets of Labeled Perigeniculate Terminals and Unlabeled F1 Terminals in Lamina A

| | HRP labeled | | PHAL labeled | | Unlabeled F1 | |
|---|---|---|---|---|---|---|
| | N | (%) | N | (%) | N | (%) |
| Cortico-recipient dendrites | 70 | (93%) | 64 | (85%) | 88 | (41%) |
| Retino-recipient dendrites | 5 | (7%) | 11 | (15%) | 78 | (37%) |
| F2 terminals | 0 | (0%) | 0 | (0%) | 28 | (13%) |
| Somata | 0 | (0%) | 0 | (0%) | 19 | (9%) |

***Diameters of postsynaptic profiles.*** We further analyzed the target dendrites by measuring their diameters as a function of the type of terminal innervating them. Table 3 summarizes these data. Figure 13 shows the distribution of these diameters when innervated by HRP-labeled perigeniculate terminals (Fig. 13A), PHAL-labeled perigeniculate terminals (Fig. 13B), or unlabeled F1 terminals (Fig. 13C). We found no statistical difference between the HRP-labeled and PHAL-labeled terminals in the diameters of dendrites they contacted ($p > 0.1$ on a Mann-Whitney U-test). However, these diameters in each case were smaller than those contacted by unlabeled F1 terminals ($p < 0.001$ for either comparison on a Mann-Whitney U-test).

Because perigeniculate terminals contact cortico-recipient dendrites nearly exclusively, and because the retino-recipient dendrites, which are contacted more frequently by unlabeled F1 terminals (see above) are larger in diameter, we compared these terminal populations separately for the type of dendrite contacted (i.e., retino-recipient or cortico-recipient; see Table 3). Even with this more balanced comparison, we found that the unlabeled F1 terminals contacted larger-caliber dendrites. For the cortico-recipient dendrites, the unlabeled F1 terminals contacted larger dendrites than either population of labeled perigeniculate terminals ($p < 0.001$ for either comparison on a Mann-Whitney U-test). Even for the retino-recipient dendrites, those contacted by unlabeled F1 terminals were larger than those contacted by the PHAL-labeled terminals ($p < 0.02$ on a Mann-Whitney U-test); too few (5) HRP-labeled terminals contacted retino-recipient dendrites to make for a meaningful comparison with this subgroup. These comparisons underscore the conclusion that perigeniculate terminals are a subpopulation of F1 terminals. This also suggests that perigeniculate terminals tend to contact either dendrites of smaller cells (e.g., relay X cells vs. Y cells or interneurons vs. relay cells) or the more distal segments of dendrites than do other F1 terminals.

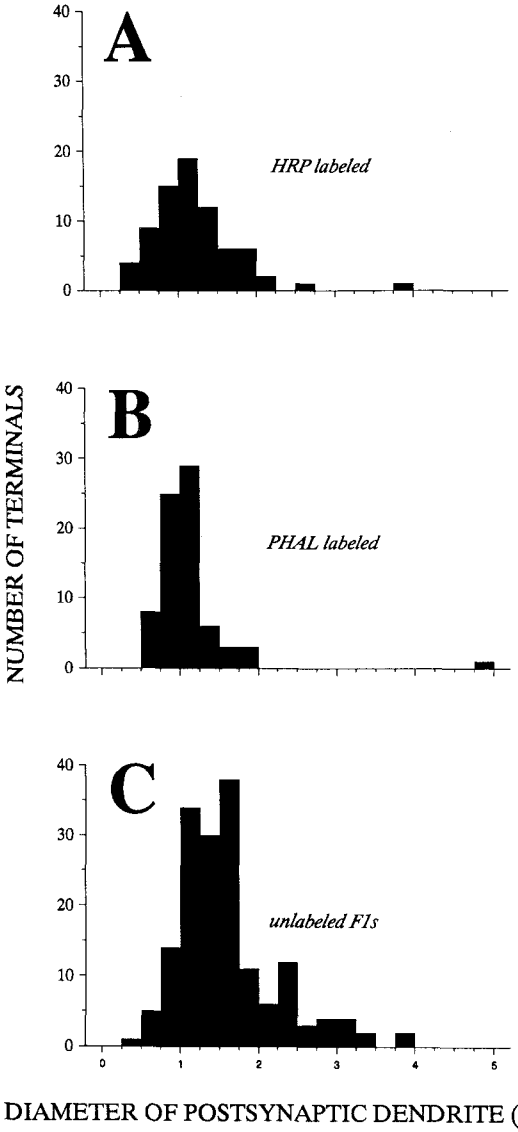



Fig. 13. Histograms showing the size distribution of dendrites postsynaptic to various types of terminals in the geniculate neuropil. **A:** Perigeniculate terminals labeled with HRP. **B:** Perigeniculate terminals labeled with PHAL. **C:** Unlabeled F1 terminals.

## DISCUSSION

We have studied the pattern of synaptic innervation from the perigeniculate nucleus to the A-laminae of the cat's lateral geniculate nucleus. We have found that perigeniculate terminals display the morphology of F1 terminals, and this is consistent with the GABAergic nature of most perigeniculate cells. However, we also found that the perigeniculate terminals seem to be a particular subset of F1 terminals, thereby leaving a large population of F1 terminals undefined in terms of their source. About 80–90% of perigeniculate synapses are onto the cortico-recipient portions of geniculate dendrites, generally thought to be the distal dendrites of geniculate relay cells (see Fig. 14). The remaining 10–20% of the synapses are onto the more proximal, retino-recipient dendrites of geniculate relay cells. When terminals from an individual perigeniculate cell were studied we found that the cell's medial axonal branches formed synapses onto both retino-recipient and cortico-recipient dendrites, whereas all of the lateral branches contacted only cortico-recipient dendrites. We have not found perigeniculate synapses onto somata or onto F2 terminals of geniculate interneurons, and we thus conclude that most or all of the F1 terminals innervating these targets have a source other than the perigeniculate nucleus. This last conclusion comes with a proviso: as noted in Results, we cannot rule out the possibility that some of the unlabeled F1 terminals are also of perigeniculate origin, remaining unlabeled by our methods.

TABLE 3. Dendritic Diameters Postsynaptic to Labeled Perigeniculate Terminals and Unlabeled F1 Terminals in Lamina A

| | HRP labeled | | PHAL labeled | | Unlabeled F1 | |
|---|---|---|---|---|---|---|
| | mean ± s.d. | N. | mean ± s.d. | N | mean ± s.d. | N |
| All dendrites | 1.20 ± 0.53 μm | 75 | 1.11 ± 0.52 μm | 75 | 1.60 ± 0.65 μm | 166 |
| Cortico-recipient | 1.16 ± 0.51 μm | 70 | 1.04 ± 0.26 μm | 64 | 1.43 ± 0.50 μm | 88 |
| Retino-recipient | 1.69 ± 0.55 μm | 5 | 1.52 ± 1.14 μm | 11 | 1.80 ± 0.74 μm | 78 |

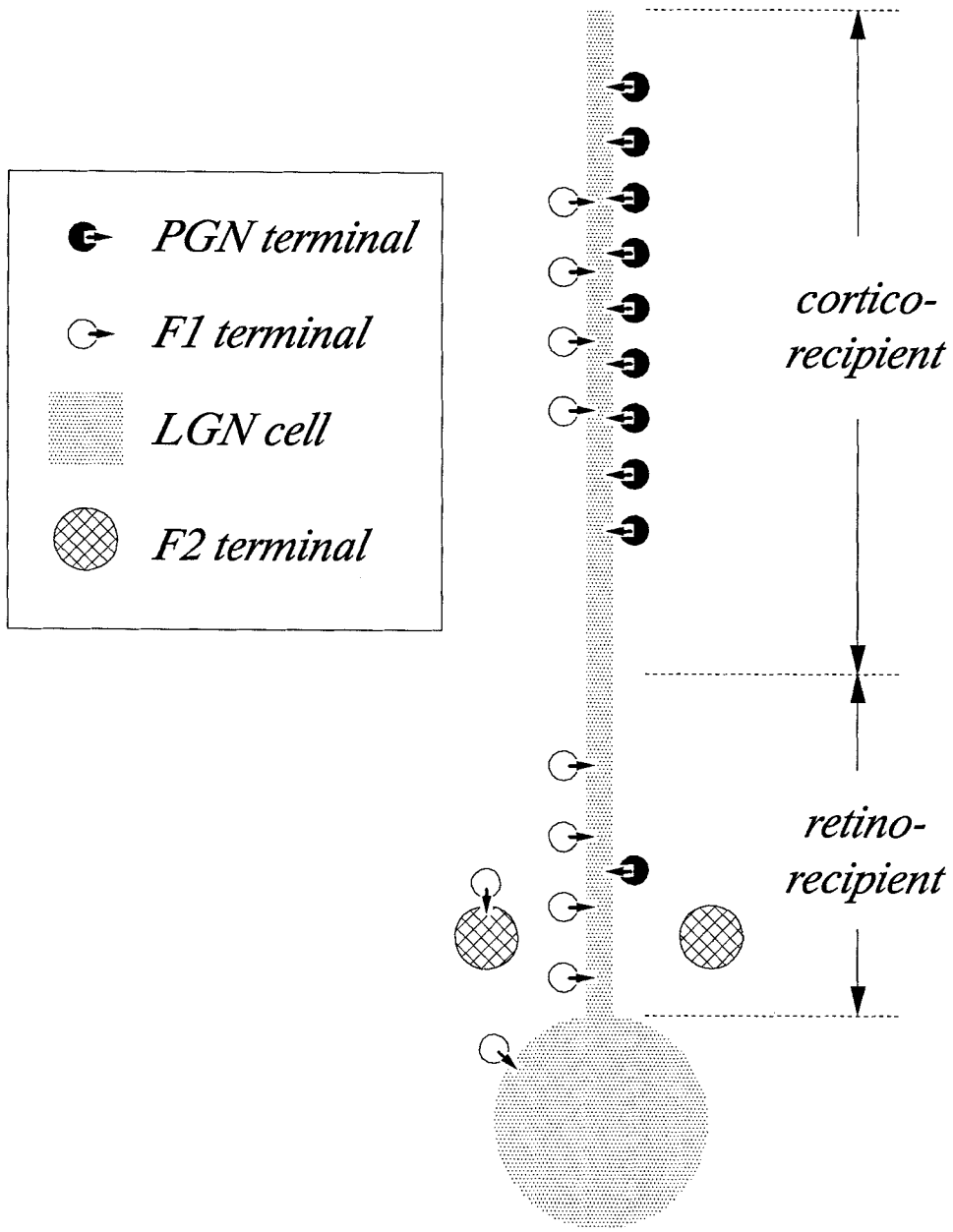



Fig. 14. Schema summarizing pattern of termination for labeled perigeniculate terminals (solid black with arrows) and unlabeled F1 terminals (open circles with arrows) onto geniculate cells (stippled) or F2 terminals from the dendrites of interneurons (cross-hatched). The geniculate cell is divided into cortico-recipient, retino-recipient, and somatic regions. Each symbol represents approximately 10% of the total number for that terminal type.

## Perigeniculate terminals have F1 morphological features

Perigeniculate axons are beaded and form synaptic contacts onto the dendrites of geniculate neurons. These synaptic contacts have thin postsynaptic densities, and their morphological features are consistent with unlabeled F1 terminals. Many geniculate F terminals, whether of the F1 or F2 type, can be labeled with antibodies directed against either GABA or GAD in both rats and cats (Ohara et al., '83; Fitzpatrick et al., '84; Montero and Singer, '85; Montero, '86). In cats, GABA-labeled F1 terminals contact somata and dendritic shafts of relay cells as well as the F2 terminals of interneurons. This is similar to the distribution of unlabeled F1 terminals we found in the geniculate neuropil, but different from the pattern of perigeniculate innervation. We found contacts from PHAL and HRP labeled perigeniculate terminals onto only dendritic shafts and appendages of geniculate cells.

These results suggest that, although the perigeniculate nucleus is a major source of F1 terminals in the geniculate neuropil, this nucleus cannot account for many of the circuits entered into by F1 terminals. Other sources of F1 terminals have been identified: axons of intrinsic interneurons (our unpublished observations, but see Montero, '87); axons from the brainstem, including the parabrachial region (Cucchiaro et al., '88) and the pretectum (Cucchiaro et al., '89); and possibly cells of the A/A1 and A1/C interlaminar region (Montero, '89b). Also, axons from the thalamic reticular nucleus just dorsal to the perigeniculate nucleus innervate the lateral geniculate nucleus (Cucchiaro et al., '90), and these seem a likely additional source of F1 terminals, although this has yet to be confirmed with the electron microscope. Presumably, among these sources derive F1 terminals that innervate F2 terminals and somata in the lateral geniculate nucleus (see also below).

## Geniculate targets receiving perigeniculate contacts

***Cortico-recipient dendrites.*** As noted above, the large majority of perigeniculate terminals formed synaptic contacts onto cortico-recipient regions of geniculate dendrites. This pattern suggests that the perigeniculate feedback to the lateral geniculate nucleus is ideally organized to influence corticogeniculate circuitry more powerfully than retinogeniculate circuitry. Our reconstructions indicate that the number of cortical terminals far outweigh the number of perigeniculate terminals found on a given dendrite, but the perigeniculate inputs are nonetheless well positioned to control cortical access to the cell soma and axonal output. Indeed, it is possible that perigeniculate cells mediate a *feedforward* inhibition of the cortical input to geniculate cells, since cortical axons also provide collateral innervation to the perigeniculate nucleus en route to the lateral geniculate nucleus. The cell-to-cell topography of this innervation, however, remains unknown.

***Retino-recipient dendrites.*** Only 10–20% of the perigeniculate synapses are formed onto proximal, retino-recipient dendrites of geniculate relay cells. However, all of these retino-recipient dendrites postsynaptic to our single, HRP-filled axon received triadic retinal input, the predominant mode of termination for retinogeniculate X axons (Wilson et al., '84; Hamos et al., '87). In contrast, all the retino-recipient dendrites postsynaptic to our PHAL terminals received simple, nontriadic retinal input, the predominant mode of termination for retinogeniculate Y axons (Wilson et al., '84). When a small quantity of PHAL is injected into a cell region, as is the case with our perigeniculate injections, this seems to label a small number of cells and their axons fairly completely (cf. Uhlrich et al., '88). Taken together, these dendritic reconstructions provide evidence that perigeniculate cells are involved in both X and Y retino-geniculo-cortical pathways. However, perhaps many or most individual perigeniculate cells predominately innervate either X or Y relay cells, and the pattern of innervation we observed from our limited sample of axons reflects this specificity of innervation.

Interestingly, all of the synapses onto the retino-recipient geniculate dendrites arose from the medial branch of the perigeniculate axon labeled by intracellular injection of HRP, and in a recent light microscopic study, only the medial branches of these axons innervated both A-laminae (Uhlrich et al., '91). If this pattern holds generally for perigeniculate axons, it suggests that the major impact of the feedback from the perigeniculate nucleus onto retino-recipient dendritic regions might be involved with binocular interactions (Sanderson et al., '71; Singer, '77).

## Is the perigeniculate nucleus part of the thalamic reticular nucleus?

The projection from the perigeniculate nucleus to the cat's lateral geniculate nucleus has long been thought to be the functional equivalent of the projection from the visual segment of the thalamic reticular nucleus in other species. In cats, there is close similarity between perigeniculate cells and those of other regions of the thalamic reticular nucleus: the large majority of these cells use GABA as a neurotransmitter; and their somadendritic morphology and axon projection patterns are similar (Scheibel and Scheibel, '66, '72; Ide, '82a,b; Fitzpatrick et al., '84; Uhlrich et al., '91; Cucchiaro et al., '90). However, there is evidence that the perigeniculate nucleus of cats only partly accounts for the innervation from the thalamic reticular nucleus to the lateral geniculate nucleus, because the perigeniculate nucleus innervates essentially only the A-laminae (Uhlrich et al., '91), whereas more dorsal regions of the thalamic reticular nucleus innervate the remaining geniculate regions (Cucchiaro et al., '90). Thus the perigeniculate nucleus of cats represents only a subregion of the visual segment of the thalamic reticular nucleus found in other species.

This, in turn, may account for our observation that perigeniculate terminals fail to innervate targets, such as F2 terminals and somata, that receive thalamic reticular inputs in other species. In rats, electron microscopic, autoradiographic studies of the projection from the thalamic reticular nucleus to the lateral geniculate nucleus have demonstrated that labeled F terminals make synaptic contacts onto the somata, dendrites, and dendritic appendages of relay cells and onto the dendritic appendages (i.e., F2 terminals) of interneurons (Ohara et al., '80; Montero and Scott, '81). A similar study in Galago showed that labeled terminals are F1 terminals and that, although they primarily contacted cortico-recipient and retino-recipient dendrites, they also formed synapses onto somata (Harting et al., '91). Such comparisons with other species suggest

that in the cat the perigeniculate nucleus is a subregion of the visual portion of the thalamic reticular nucleus. Furthermore, connectivity patterns of perigeniculate axons may be a subset of the input from the visual portion of the thalamic reticular nucleus seen in other species.

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