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Distribution of Synapses in the Lateral Geniculate Nucleus of the Cat: Differences Between Laminae A and A1 and Between Relay Cells and Interneurons

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ABSTRACT

Laminae A and A1 of the lateral geniculate nucleus in the cat are generally considered to be a structurally and functionally matched pair of inputs from two eyes, although there are subtle light microscopic and physiological differences. The present study aims to display ultrastructural differences between these two laminae based on electron microscopic observances on the connectivity patterns of their afferents onto two main cell types: relay cells, and interneurons present in this nucleus. In a design of population measurement from randomized sample areas in laminae A and A1 from six brains, all synaptic contacts made by three terminal types of the geniculate nucleus were identified, and a number of relative distribution properties were analyzed. When the A-laminae were considered as a homogeneous structure, the distribution of the three terminal types on geniculate cells was similar to previously reported results, confirming the validity of the sampling strategies used; RLP (retinal) terminals provided one-fifth of all synapses, whereas RD (from cortex and brainstem) and F (inhibitory) types constituted one-half and one-third, respectively. The relay cells alone received a similar composition of afferents. However, interneurons alone received approximately equal amounts of synapses from the three sources. Similar analyses comparing the distributions in lamina A and A1 revealed that RD and F terminals, but not RLP terminals, innervate these two laminae differently; more RD and fewer F terminals were found in lamina A1. This difference was also present in the distribution of terminals on relay cells alone, but not on interneurons. These results suggest that (1) retinal terminals form a significantly larger fraction of the input to interneurons than to relay cells; correspondingly, cortex and brainstem provide a smaller fraction of all inputs to interneurons than to relay cells; and (2) laminae A and A1 are not strictly equivalent projection sites of the two retinae. The results are discussed in relation to the Y-cell subpopulation in lamina A1 that is involved in corticotectal, as well as corticogeniculate circuits, as opposed to Y-cells of lamina A that are involved in only the latter. J. Comp. Neurol. 390:247–255, 1998.

Indexing terms: thalamus; local circuits; X and Y cells

One of the most intensely studied model systems for local circuitry is the lateral geniculate nucleus of the cat, particularly geniculate laminae A and A1. The functional justification for detailed morphological studies is the relatively recent appreciation that the lateral geniculate nucleus does not provide a simple, machine-like relay of its retinal inputs to cortex (for reviews, see Sherman and Koch, 1990; Sherman, 1993; Sherman and Guillery, 1996). Instead, geniculate relay cells respond to retinal inputs in a variety of ways depending on behavioral state, and this variable is imposed on the relay cells through fairly complex circuitry. A more complete understanding of this dynamically variable relay depends on a more complete anatomical description of the neuropil.

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With few exceptions, electron microscopic studies of the neuropil of the A-laminae have failed to account for possible variations in circuitry contained therein. That is, the A-laminae have generally been treated as a matched pair, differing only in terms of ocular input: lamina A is innervated by the contralateral retina, whereas lamina A1 is innervated ipsilaterally. However, the A-laminae contain at least three different cell types—interneurons, relay X cells, and relay Y cells—with different patterns of synaptic inputs (Montero, 1991; Wilson et al., 1984), and there is evidence of interlaminar differences for at least the Y cells (e.g., Colby 1988; see Discussion section for details). Other subtle interlaminar differences between laminae A and A1 have also been reported (e.g., Wilson et al., 1976; Hickey et al., 1977). Most morphological studies of the neuropil of the A-laminae have not attempted to distinguish between them.

We thus sought to use the electron microscope to describe potentially differing patterns of synaptic terminals in the geniculate neuropil as they relate both to interlaminar differences between laminae A and A1 and to differences in inputs to relay cells and interneurons. We indeed found such differences, and these differences complement and extend previous reports of differences based on physiology and light microscopy. A preliminary report of many of these findings have been presented in abstract form (Van Horn et al., 1996).

MATERIALS AND METHODS

We used brains from six adult cats for this study. All procedures were approved by the institutional animal care committee and comply with National Institutes of Health guidelines. The animals were given a fatal overdose of barbiturate and then perfused transcardially with a mixture of 4% paraformaldehyde and 0.2% glutaraldehyde. The brains were extracted, post-fixed in the same fixative for 2–8 hours, and cut sagittally on a Vibratome to obtain 50- to 60-μm sections. Sections containing the regions of lateral geniculate nucleus that represent the central visual field (i.e., the medial third of the nucleus near its anteroposterior middle) were selected for electron microscopy. Our techniques have been fully described elsewhere (Bickford et al., 1994; Erişir et al., 1997a, 1997b).

Processing for electron microscopy

Embedding. Trimmed geniculate sections were osmicated in 2% osmium tetroxide (OsO_4) in 0.1 M phosphate buffer, pH 7.4, dehydrated in a graded series of ethyl alcohol and treated first with a 1:1, then a 3:1 mixture of resin (Durcupan ACM/Fluka from Electron Microscopy Sciences, Fort Washington, PA) and 100% ethyl alcohol. They were then vacuum infiltrated in pure resin overnight, flat embedded between two pieces of ACLAR (Ted Pella, Inc.; Redding, CA), and placed in a 68°C oven for 48–72 hours. The embedded sections were examined with a light microscope, and laminar borders and landmarks were drawn with the aid of a camera lucida. The two pieces of ACLAR were peeled away from the flat embedded section. An approximate geniculate area of 1 mm × 2 mm was then trimmed to include lamina A and/or lamina A1 and glued onto the end of a blank resin block. Thin sections were cut at approximately 80 nm on a Reichert-Jung Ultracut E ultramicrotome and picked up on Formvar-coated nickel slot grids.

Postembedding immunogold. In order to identify γ-aminobutyric acid (GABA) containing profiles, we stained the ultrathin sections by using a postembedding procedure, which eliminates the antibody penetration problems encountered with preembedding staining procedures. For this, we followed our previously described protocol (Bickford et al., 1994; Erişir et al., 1997a), modified from Phend et al. (1992). Briefly, all solutions except the primary and secondary antibodies were filtered through a 0.22-μm Millipore filter. Thin sections were rinsed in Tris-buffered saline with Triton X (TBST) at pH 7.6 and incubated for 24 hours in anti-GABA primary antibody (Sigma Chemical Co., St. Louis, MO) diluted 1:500 to 1:1,000 in TBST at pH 7.6 at room temperature. The sections were then rinsed, first in TBST at pH 7.6, and then in TBST at pH 8.2 and incubated in goat anti-rabbit IgG conjugated to 15-nm gold particles (Amersham Life Sciences, Arlington Heights, IL) diluted at 1:25 in TBST at pH 8.2 for 1 hour. Finally, the sections were rinsed in TBST at pH 7.6 and deionized water, placed in 2% glutaraldehyde (EM grade) for 10 minutes, rinsed again in deionized water, and counterstained with uranyl acetate and lead citrate to increase contrast.

We used our previously described method (Bickford et al., 1994; Erişir et al., 1997a) for distinguishing significant postembedding levels from background labeling. The control for GABA labeling background were excitatory terminals of extraretinal origin, known as "RD" terminals. These mostly represent terminals of cortical and brainstem origin and are the composite of what was previously described as "RSD" and "RLD" terminals (see Erişir et al., 1997a and also below). We thus determined the gold particle density (number of gold particles divided by the area of terminal) in every RD terminal, and we then computed the frequency distribution of these densities. This frequency distribution was determined separately for every thin section, because the overall amount of gold labeling varied among sections. We chose a 95% confidence level for the labeling. That is, the gold particle density that was equal to or smaller than that seen in 95% of RD terminals was taken as background label, and profiles that displayed such low gold density were deemed GABA-. Any profile within the same thin section that displayed a higher gold particle density was deemed GABA+.

Identification of terminal types

We used a modification of Guillery's (1969a, 1969b) terminology and classification scheme as described in Erişir et al. (1997a,b). The terminal types thus recognized were F (named for flattened vesicles), RLP (named for round vesicles, large profile, and pale mitochondria), and RD (named for round vesicles and dark mitochondria). To identify each of these, we applied five major criteria to the vesicle-filled profiles that displayed a synaptic zone in the plane of sectioning. These criteria were (1) GABA content, (2) dark or pale appearance of mitochondria, (3) round versus flattened or pleomorphic vesicle shape, (4) vesicle packing density, and (5) symmetrical or asymmetrical synaptic zone appearance. A sixth criterion, terminal size, proved unreliable without serial section, and we thus did not rely on it. The GABA staining in the terminals was the primary criterion used to discriminate inhibitory terminals from the excitatory ones. If a terminal was GABA+, it was classified as an F terminal, and these displayed flat or pleomorphic vesicles, dark mitochondria, and symmetric

synaptic contacts. The GABA- terminals were divided into RLP and RD terminal types in the following way. If such a terminal contained loosely packed round vesicles, pale mitochondria, and an asymmetric synaptic contact, it was identified as an RLP terminal. If it displayed tightly packed, round vesicles, dark mitochondria, and an asymmetric synaptic contact, it was identified as an RD terminal. Rare GABA- terminals were encountered that were difficult to subdivide clearly, usually because of an unusual plane of section (see Results).

Sampling of synaptic terminals

For the general distribution of synaptic terminals, we examined nine ultrathin sections from six brains. To determine the laminar borders for each section, we first photographed the whole of the block-face at 200× magnification, and we identified the landmarks (i.e., blood vessels, myelinated axon bundles) and compared them with camera lucida drawings. Then, a region contained well within a single identified lamina (A or A1) was selected for analysis. We sampled from several regions of an ultrathin section in geniculate lamina A or A1, and we treated each sampled region as an individual case for data analysis. Overall, we sampled one region from each of the nine ultrathin sections. Each of these sampled regions contained a sufficiently large number (100–150) of synaptic terminals to permit reliable calculation of relative terminal distributions.

For each selected region, we photographed and analyzed a continuous area of 1,200 μm^2, excluding capillaries and somata. When a terminal was seen with a synaptic contact, its terminal type and postsynaptic profile were identified (see below) and counted. Terminals that did not display a clear synaptic contact in the section examined were ignored. The postsynaptic profiles were identified as relay cell or interneuron dendrites based on their GABA immunoreactivity.

To minimize sampling biases, we used our previously described sampling strategy (Erişir et al., 1997a). By completely examining a continuous 1,200 μm^2 area in lamina A or A1 of each section, we minimized any sampling bias due to small regional fluctuations of terminal density; for example, this density is higher in glomerular than in extraglomerular zones. Furthermore, we sampled every profile that formed a clear synaptic contact. We know from serial reconstructions that virtually all terminals in the geniculate neuropil form synapses (our unpublished results). This strategy ensured that the only major bias involved in sampling terminals was related to the probability of detecting a synaptic contact. Because all sections were from comparable regions of the lateral geniculate nucleus and were all cut in the same sagittal plane, any asymmetries in the shape of these contacts would not contribute to differential sampling biases. Thus, the sampling bias for a given terminal was proportional to the number of thin sections spanned by its synaptic contact (Erişir et al., 1997a). We know that this does confer a bias favoring certain terminals over others (Erişir et al., 1997a), but because the purpose of the present study was to compare the distributions of the same terminal types in lamina A versus lamina A1 and onto relay cells versus interneurons, these biases are unlikely to significantly affect our conclusions (see Discussion section for a more complete treatment of this issue). As part of a related study, we are determining these biases empirically for each terminal type, so eventually we will be able to determine the necessary correction factors, but these are not essential to the present analysis.

Statistics

Unless otherwise indicated, all statistical analyses were based on the χ^2 test.

RESULTS

We sampled a total of 1,191 synaptic terminals in nine regions of lamina A or A1 taken from nine sections of six brains. Of the nine regions sampled, five were from lamina A and four were from lamina A1. Therefore, the data collection strategy, in essence, resembled those in earlier studies, where terminals were sampled through both laminae. As noted in Materials and Methods section, we included every terminal with a visible synaptic contact in the plane of section. A total of 1,191 synaptic terminals were counted, and of these, 1,159 (97%) could be clearly identified as an RLP, F, or RD terminal profiles. We could not classify 32 of the synaptic terminals (3%), usually because of an unusual plane of section, and these are not further considered. Figure 1 shows typical examples of these terminal types. Figure 1 also shows an example of the postembedding GABA label we used to identify processes belonging to relay cells (which are GABA-) versus interneurons (which are GABA+).

Overall synaptic distribution across the geniculate A-laminae

We started with an analysis of the distribution of synaptic terminals encountered in the A-laminae. Figure 2 summarizes these data. In this and subsequent figures, we show the ratio of each terminal type. To calculate these ratios, we first determined the ratios of each terminal type separately for each of nine ultrathin sections, and we then computed the mean and standard error of these nine values. As summarized, RLP terminals constituted 17.9 ± 0.6% of all terminals; F terminals, 29.3 ± 1.2%; and RD terminals, 52.9 ± 1.7%. This distribution is remarkably similar to those reported in several prior studies using different sampling strategies (e.g., Guillery, 1969a; Wilson et al., 1984; Montero, 1991). Also, the low standard errors seen in Figure 2 indicate the reliability of our sampling strategy and further suggest that it is possible to obtain reliable estimates of terminal distributions from single sections.

Innervation of relay cells versus interneurons

The two basic geniculate cell types that receive synaptic input in the geniculate A-laminae are relay cells and interneurons. The former do not contain GABA, whereas the latter do. We could thus distinguish between profiles of relay cells and of interneurons in our material by virtue of the postembedding GABA staining (see Materials and Methods). Thus by evaluating all postsynaptic profiles for their GABA content, we could separately determine the distribution of terminal types onto relay cells and interneurons.

Figure 3 summarizes this analysis. The relative distribution of terminal types contacting relay cells was 14.6 ± 0.6% RLP terminals, 29.6 ± 1.6% F terminals, and 55.8 ±

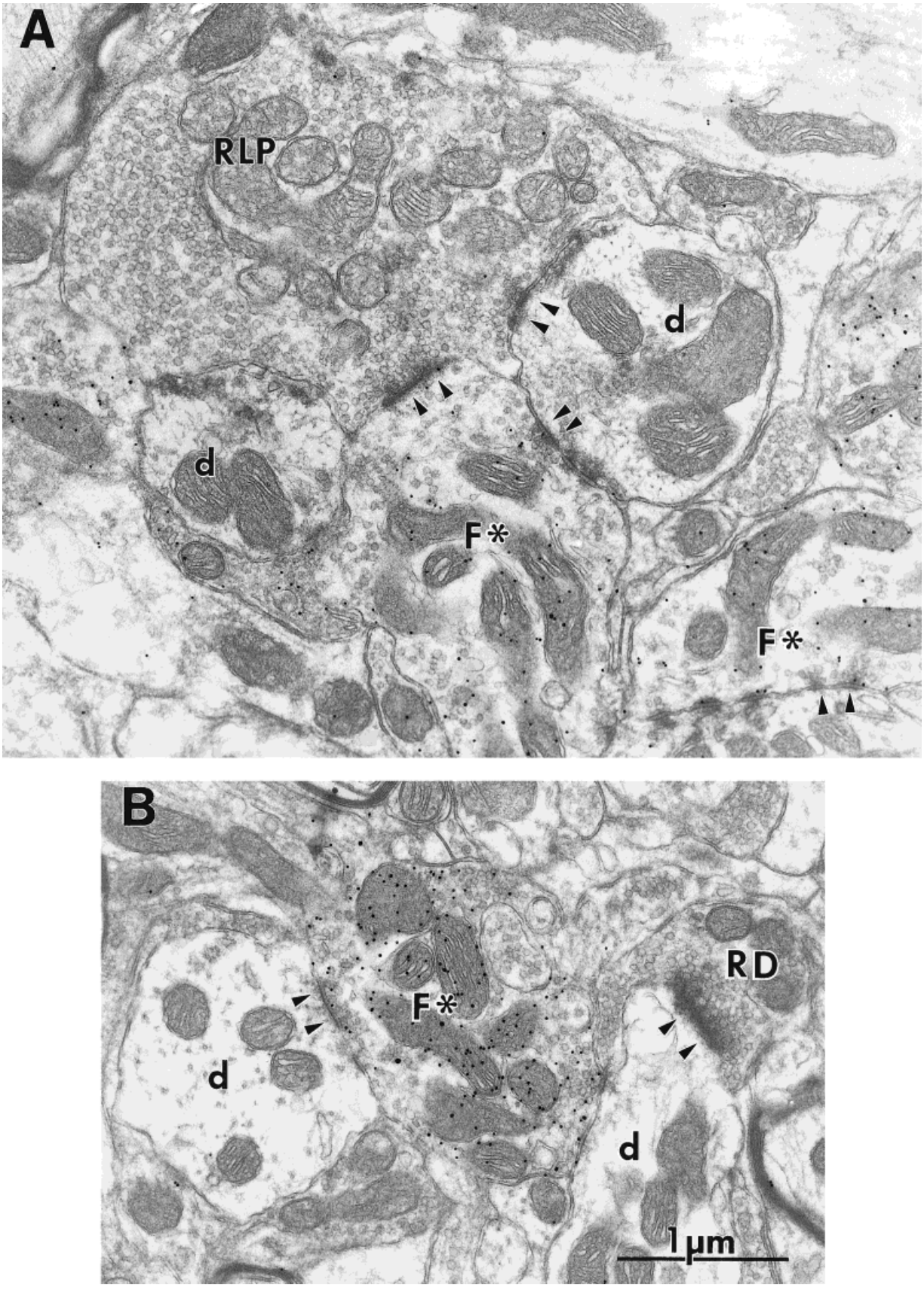

Fig. 1. Electron micrographs of morphologically identified terminal types in geniculate A-laminae. The arrowheads indicate synaptic contacts, and the asterisks indicate GABA+ profiles. Shown are examples of RLP, F, and RD terminals as well as dendritic profiles (d). RLP terminals are GABA-, display round and loosely packed vesicles, have pale mitochondria, and form asymmetric synaptic contacts. F terminals are GABA+, display flat or pleomorphic vesicles, and form symmetric synaptic contacts. RD terminals are GABA-, display round and densely packed vesicles, have dark mitochondria, and form asymmetric synaptic contacts. **A:** Example of RLP and F terminals. **B:** Example of RD and F terminals. For abbreviations, see Identification of Terminal Types section. Scale bar = 1 µm (applies to A,B).

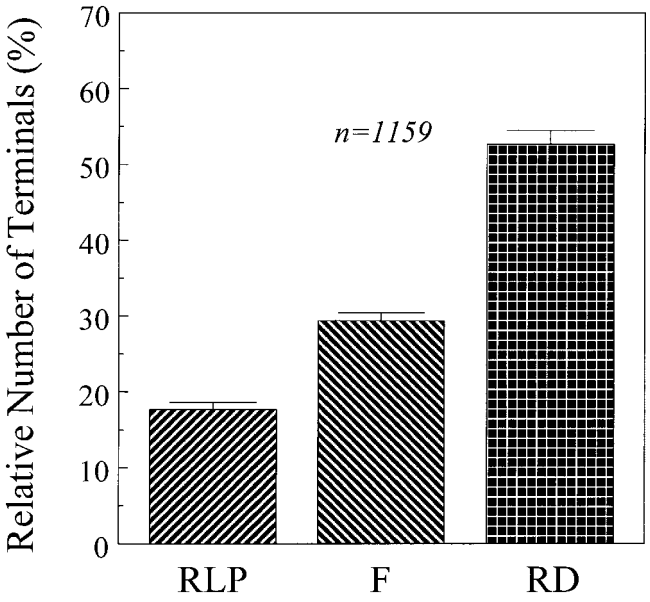

Fig. 2. Relative distributions of 1,159 identified RLP, F, and RD terminals in the geniculate A-laminae. Here and in Figures 3–7, these distributions are expressed as the mean and standard error of the relative percentages recorded separately in each of nine sections. For abbreviations, see Identification of Terminal Types section.

1.7% RD terminals (Fig. 3A). This pattern resembles the total pattern of all terminals sampled in the A-laminae ($P > 0.1$). However, note that the fraction of RLP (that is, retinal) terminals was even smaller for relay cells alone than it was for all cells.

In contrast, the analysis of the total synaptic input onto interneurons revealed quite a different distribution (Fig. 3B). Interneurons received 37.8 ± 1.0% of all synapses from RLP terminals, 26.8 ± 1.5% from F terminals, and 35.4 ± 1.8% from RD terminals (Fig. 3B). This finding differs both from the pattern of inputs to all cells (Fig. 2) and from that to relay cells alone (Fig. 3A). These differences are significant ($P < 0.001$ for either comparison). In particular, RLP terminals form a significantly larger fraction of the input to interneurons than to relay cells, and RD terminals form a correspondingly smaller fraction of this input ($P < 0.001$ for either comparison). There is no difference in the fraction of input from F terminals to either cell type ($P > 0.1$).

Figure 3 shows how the composition of synaptic inputs to relay cells and interneurons is composed of the various terminal types. Figure 4 shows the complement of this: the tendency of terminal types to contact relay cells versus interneurons. We found that within the geniculate A-laminae, relay cells were the target of 85.8 ± 0.6% of all synaptic terminals combined, whereas interneurons were contacted by 14.2 ± 0.6%. We would expect relay cells to be the dominant target, because they are more numerous than interneurons. However, relay cells outnumber interneurons by about 3 to 1 (LeVay and Ferster, 1979; Fitzpatrick et al., 1984), but they dominate as postsynaptic targets by a ratio of more than 6 to 1. This finding suggests that each relay cell must, on average, receive more synaptic inputs than does each interneuron by a ratio of approximately 2 to 1. Figure 4 also shows that the pattern of relay cell dominance as the synaptic target in the geniculate

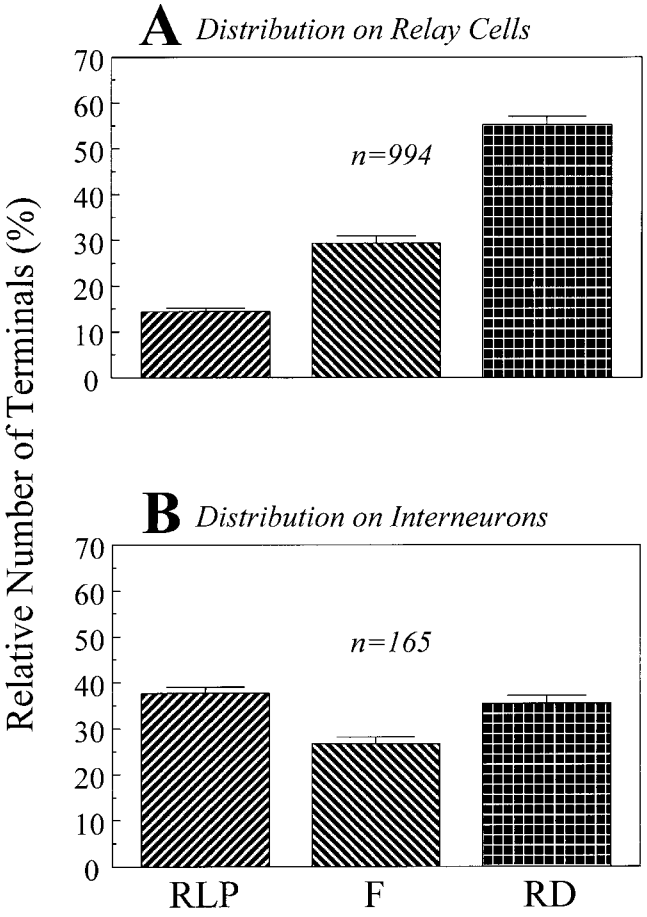

Fig. 3. Data from Figure 2 replotted separately for relay cells and interneurons. **A:** Relative distributions of 994 synaptic terminals contacting relay cells in the A-laminae. **B:** Relative distributions of 165 synaptic terminals contacting interneurons in the A-laminae. For abbreviations, see Identification of Terminal Types section.

A-laminae varied with terminal type. Relay cell profiles were much more dominant as the target for F terminals (87.0 ± 0.6%) and RD terminals (90.5 ± 0.6%) than for RLP terminals (70 ± 1.2%), and this difference was statistically significant ($P < 0.001$).

Synaptic terminal distributions in lamina A versus lamina A1

We further investigated these synaptic terminals by exploring their patterns separately for laminae A and A1. In doing so, we found significant interlaminar differences ($P < 0.01$), and these are summarized in Figure 5. We found that, relative to lamina A1, lamina A contained a lower proportion of RD terminals (50.5 ± 1.7% vs. 57.1 ± 1.8%; $P < 0.01$) and a higher proportion of F terminals (31.0 ± 1.4% vs. 26.1 ± 2.8%; $P < 0.05$). In contrast, the proportion of RLP terminals did not differ significantly between lamina (18.5 ± 0.7% for lamina A vs. 16.7 ± 1.0% for lamina A; $P > 0.1$).

The relative distribution of terminal types that contacted relay cell dendrites provided more evidence of the nature of the differences between laminae A and A1. Figure 6A summarizes this analysis for relay cells, and

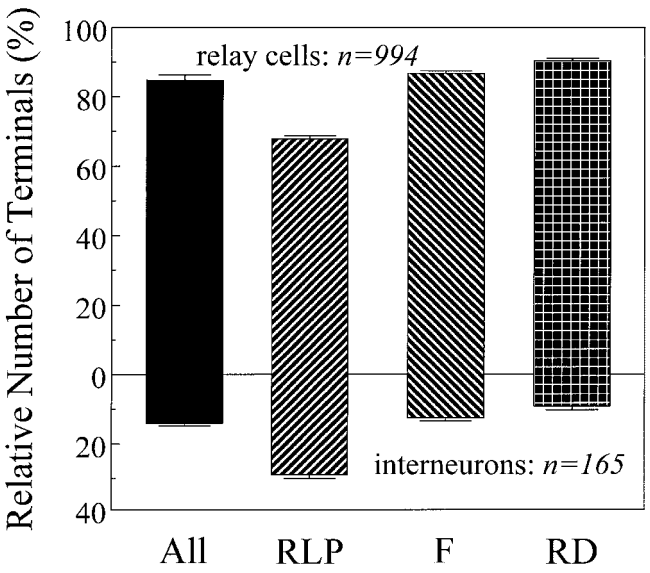

Fig. 4. Relative tendency for each type of synaptic terminal in the A-laminae to contact relay cells (994 terminals) versus interneurons (165 terminals). Shown are all terminals as well as the separate tendencies for RLP, F, and RD terminals. For abbreviations, see Identification of Terminal Types section.

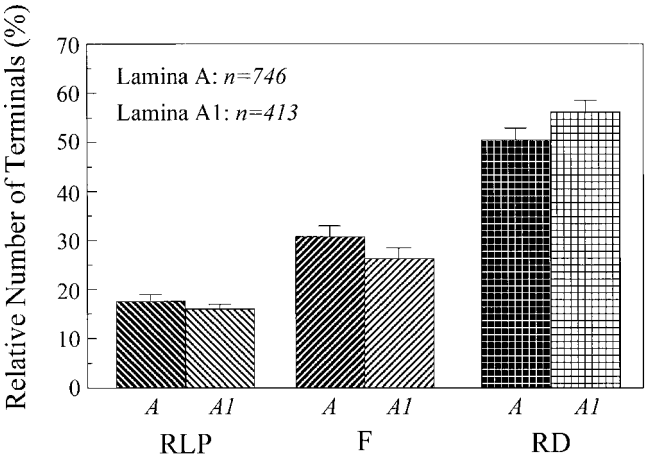

Fig. 5. Data from Figure 2 replotted separately for lamina A (746 terminals) and lamina A1 (413 terminals). For abbreviations, see Identification of Terminal Types section.

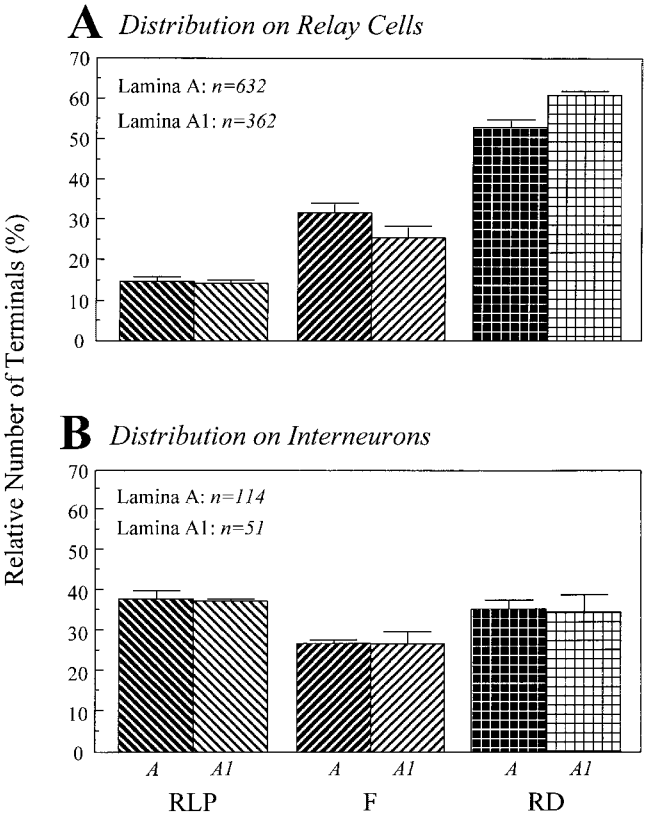

Fig. 6. Relative distribution of terminals on relay cells and interneurons shown separately for laminae A and A1. **A:** Distribution of contacts onto relay cells. Of these, 632 are in lamina A and 362 are in lamina A1. **B:** Distribution of contacts onto interneurons. Of these, 114 are in lamina A and 51 are in lamina A1. For abbreviations, see Identification of Terminal Types section.

subtle interlaminar differences were seen in the pattern of terminals on this cell type ($P < 0.05$). In lamina A, relay cells received 15.0 ± 0.7% of their input from RLP terminals, 32.1 ± 2.5% from F terminals, and 52.9 ± 1.8% from RD terminals. The comparable proportions for lamina A1 were 13.8 ± 1.2%, 25.4 ± 2.5%, and 60.8 ± 1.4%. There was no discernible interlaminar difference in the pattern of RLP inputs onto relay cells ($P > 0.1$); the interlaminar differences derived from relatively fewer RD terminals and more F terminals in lamina A versus lamina A1 ($P < 0.05$ for both comparisons). Figure 6B shows that there were no significant interlaminar differences in the pattern of inputs to interneurons. The pertinent numbers for lamina A are 38.0 ± 1.4% RLP terminals, 26.6 ± 1.3% F terminals, and 35.4 ± 2.0% RD terminals. For lamina A1, these values are 37.2 ± 0.7% RLP terminals, 27.5 ± 4.3% F terminals, and 35.3 ± 4.4% RD terminals.

A similar conclusion of subtle interlaminar differences for relay cells but not interneurons from the analysis of postsynaptic target selectivity. Figure 7 depicts the preference of geniculate terminals for relay and interneuron targets in each lamina. If all terminals are considered, a slightly higher percentage of terminals in lamina A (15.3 ± 0.5%) contacts interneurons than in lamina A1 (12.3 ± 0.4%). Although this difference seems small, it was seen in every case studied, and thus the difference is significant ($P < 0.01$ on a Mann-Whitney U test). However, when the terminal population is broken down into its component types, no significant differences in interlaminar target preferences are seen ($P > 0.1$ on Mann-Whitney U tests for all comparisons. The values are as follows. For RLP terminals in lamina A, 68.8 ± 1.3% target relay cells and 31.2 ± 1.3% target interneurons; in lamina A1, the values are 72.5 ± 1.7% and 27.5 ± 1.7%. For F terminals in lamina A, 87.0 ± 0.9% target relay cells and 13.0 ± 0.9% target interneurons; in lamina A1, the values are 87.0 ± 0.8% and 13.0 ± 0.8%. Finally, for RD terminals in lamina A, 89.4 ± 0.5% target relay cells and 10.6 ± 0.4% target interneurons; in lamina A1, the values are 92.4 ± 0.7% and 7.6 ± 0.7%.

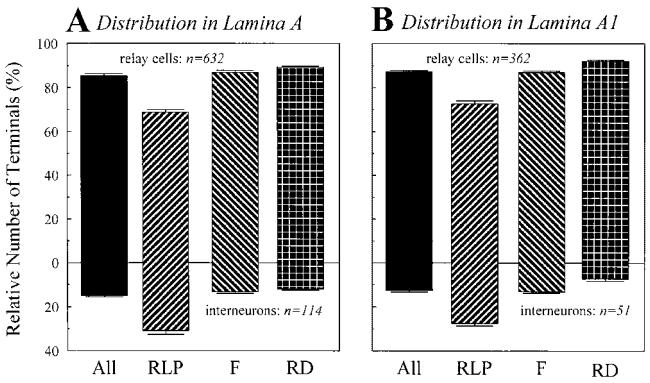

Fig. 7. Relative tendency for each type of synaptic terminal to contact relay cells versus interneurons shown separately for laminae A and A1. Shown are all terminals as well as the separate tendencies for RLP, F, and RD terminals. **A:** Tendency in lamina A. **B:** Tendency in lamina A1. For abbreviations, see Identification of Terminal Types section.

DISCUSSION

We have shown that the pattern of synaptic inputs in the geniculate A-laminae of the cat vary both with respect to postsynaptic cell type (i.e., relay cell vs. interneuron) as well as between laminae A and A1. The former conclusion essentially confirms that made earlier by Montero (1991), who used similar techniques to describe comparable relative numbers of synaptic inputs to relay cells and interneurons, although newer data suggest a subtly different interpretation of the sources of some of these inputs (see below). We also noted a subtle interlaminar difference in the pattern of synaptic inputs to relay cells, but no difference in this pattern for interneurons. We suggest below that all these differences seen may have a single common explanation.

Sampling issues

Before dealing with the different synaptic patterns, we shall discuss more general issues related to sampling biases of the synaptic terminals in the A-laminae. The relative numbers we have reported (e.g., Fig. 2) are in close agreement with many prior reports (Guillery, 1969a, 1969b; Wilson et al., 1984; Montero, 1991). However, whereas there seems to be agreement on the relative number of various terminal types sampled across laboratories and studies, this does not mean that these numbers reflect the actual relative numbers of synaptic terminals present in the geniculate neuropil. This is because different probabilities may exist for sampling each terminal type, and this variable has not been adequately controlled in any study published to date. As one example, in terms of size, RLP terminals are the largest in the neuropil; so if one were sampling terminals simply on the basis of finding profiles identified as such, RLP terminals would be encountered with a frequency that is relatively too high for their actual numbers relative to F and RD terminals. This is because they would occupy more sections in a series than other terminal types, so the probability of finding one in any given section would be relatively high.

In the present study, we sampled terminals in a section if and only if we detected the presence of a synaptic contact. Thus in our case the sampling probability is related to the size of the contact, or the number of serial sections it spans (and this will also be affected by how obliquely contact zones are cut), rather than the size of the terminal per se. We have already shown that differences in contact size between the two major contributors of the RD population, which are corticogeniculate terminals and cholinergic terminals from the brainstem parabrachial region, contribute to biases that favor sampling the former (Erişir et al., 1997b). We are currently extending this to other terminal populations, and it seems clear that significant differential sampling biases exist among the different terminal types. Thus the ratios published here and in other similar studies remain to be corrected for this.

Whereas we emphasize that we have yet to derive the precisely correct ratios of RLP, F, and RD terminals in the A-laminae, this is not the primary focus of the present study. This focus instead is aimed at documenting differences in these ratios between laminae A and A1 and between relay cell and interneuron targets. As long as these biases for sampling different terminals based on synaptic contact size remain constant for the interlaminar and cell type comparisons, they will not affect the conclusions reached here. The possibility that such differential biases may differ for laminar location or target cell type does, however, create one possible qualification to our conclusions.

Differences in inputs to relay cells versus interneurons

Our observations on the differences in synaptic inputs to relay cells and interneurons largely confirm the study of Montero (1991), but there are important differences in interpretation to be considered. Montero (1991) equated retinal terminals with RLP terminals, cortical terminals with RSD terminals, and GABAergic terminals with F terminals. We agree with the identity of RLP and F terminals, but not with that of RSD terminals. As we have previously shown (Erişir et al., 1997b), our RD population includes both RSD and RLD terminals. These were originally distinguished on the basis of size, RLD terminals being larger than RSD, but we have found their size distributions to form a continuum and have argued that there is no basis to divide them arbitrarily into two groups. If, however, we choose to do so and select 1.0 μm^2 as the cut-off in terminal size (cross-sectional area) between RSD and RLD terminals, then 95% of our RD terminal population would be identified as RSD terminals, the RLD terminals representing the top end of the size distribution (see Fig. 2 of Erişir et al., 1997b). Given that, numerically, there seems to be little difference between our RD terminal population and the RSD terminal population identified by Montero (1991), we can reasonably equate our distribution of RD terminals with Montero's RSD terminals. Montero (1991), however, assumed that all of his RSD terminals were of cortical origin, and we have recently shown that roughly half of them are actually cholinergic and NO-containing terminals originating from the parabrachial region of the brainstem (Erişir et al., 1997a, 1997b).

When these differences between RSD and RD terminals and in the interpretation of their origin are taken into account, our distributions are quite similar to those reported by Montero (1991). When we recalculate his values to remove inputs not identified as RLP, RSD, or F, which makes for more straightforward comparison between studies, he found that relay cells receive 13% of their inputs

from retinal (i.e., RLP) terminals 62% from cortical (i.e., RSD, which is roughly equivalent to RD) terminals, and 25% from GABAergic (i.e., F) terminals. The values for interneurons are 28% of the inputs from RLP terminals, 42% from RSD terminals, and 30% from F terminals. Our numbers for relay cells are 14.6% inputs from RLP terminals, 55.8% from RD terminals, and 29.6% from F terminals; for interneurons, they are 37.8% from RLP terminals, 35.4% from RD terminals, and 26.8% from F terminals. The only apparent difference relates to interneurons: we find relatively more inputs from RLP terminals and fewer from RD terminals.

Nonetheless, we can safely conclude from both studies that, compared with interneurons, relay cells receive relatively more inputs from RD terminals and fewer from RLP terminals. Because RD terminals are mostly derived from corticogeniculate and parabrachial axons, both of which are thought to modulate the relay of retinal information through the lateral geniculate nucleus to visual cortex (Sherman, and Koch, 1990; Sherman, 1993; Sherman and Guillery, 1996), and RLP terminals are retinal in origin, this suggests that relay cells are under much stronger or more finely controlled modulatory influence than are interneurons.

Differences in synaptic inputs found between laminae A and A1

As noted in the Introduction section most studies of the lateral geniculate nucleus of the cat treat laminae A and A1 as a matched pair differing chiefly by virtue of the retina that innervates each. The present results make clear that subtle interlaminar differences do exist in the neuropil. We found that lamina A contained fewer RD terminals and more F terminals than did lamina A1, with no discernible difference seen for RLP terminals. Because the difference in input pattern between relay cells and interneurons relates to different terminal types (i.e., RLP and RD) than do the interlaminar differences (i.e., F and RD), it seems unlikely that the interlaminar differences can be simply explained on the basis of a different proportion of interneurons versus relay cells in each lamina. A more detailed analysis reveals that no interlaminar difference exists for input patterns onto interneurons, but relay cells in lamina A receive more F terminals and fewer RD terminals than do those in lamina A1, with no interlaminar difference in terms of RLP inputs onto relay cells. Because this difference is qualitatively similar to the overall pattern of interlaminar differences, some interlaminar difference in the relay cell populations seems to be a likely candidate for the overall interlaminar differences.

The relay cells found in these laminae belong to one of two classes, X and Y, that display different morphological characteristics (see also below). A close inspection of the literature reveals a number of subtle interlaminar differences that are consistent with the conclusion that lamina A contains a lower X to Y ratio among its relay cells than does lamina A1. For instance, compared with lamina A1, lamina A has, on average, smaller somata (Hickey et al., 1977) and a larger X to Y cell ratio (Wilson et al., 1976). Because Y cells tend to have larger somata than do X cells (LeVay and Ferster, 1977; Friedlander et al., 1981), these observations are consistent.

Along the same lines, Colby (1988) has performed an analysis of inputs to the superior colliculus that suggests an extra population of Y cells in lamina A1 that is absent from lamina A. This is schematically diagrammed in Figure 8. Within the main laminae of the lateral geniculate nucleus of the cat, Y cells innervated by the contralateral eye are found in lamina A and the dorsal, magnocellular tier of lamina C (or magno lamina C); Y cells innervated by the ipsilateral eye are found in lamina A1. Although not shown, X cells are located in the A-laminae and are not present in appreciable numbers in magno lamina C. Colby (1988) confirmed earlier studies showing that the retinogeniculo-cortico-collicular pathway is composed essentially exclusively of the Y pathway, with no discernible contribution from the X pathway (Hoffmann, 1973). However, she showed further that this corticocollicular loop from the ipsilateral eye is relayed through lamina A1, whereas that from the contralateral eye is relayed through magno lamina C and not through lamina A. It thus appears that there are two, perhaps partly overlapping, Y pathways: one innervates cortical circuits that do not participate strongly in the corticocollicular projection, and the other does. These Y circuits are separated from the contralateral eye into lamina A and magno lamina C, but from the ipsilateral eye, they both are represented in lamina A1 (see Fig. 8). Thus lamina A1 appears to have an extra Y cell population not present in lamina A. This could explain why lamina A1 contains both larger somata and a higher percentage of Y cells (Wilson et al., 1976; Hickey et al., 1977).

It is tempting to suggest that our observations of subtle interlaminar differences in the pattern of synaptic terminals can be explained by a slightly lower ratio of X to Y relay cells in lamina A than in lamina A1.Although there is not a great deal of evidence on the differential inputs to X and Y cells, the one study that has attempted to reconstruct these inputs onto a small number of identified X and

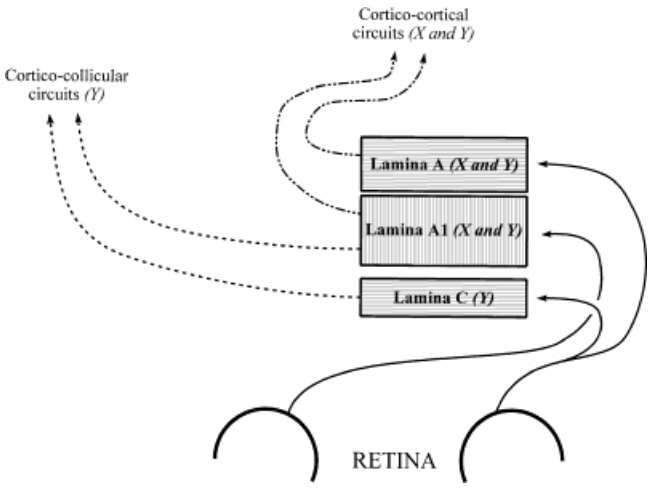

Fig. 8. Schematic view of separate geniculocortical pathways for cortico-cortical and cortico-collicular circuits (see text for details). Laminae A and A1 have both X and Y cells and the magnocellular tier of lamina C has essentially only Y cells. Laminae A and C are innervated by the contralateral retina, and lamina A1, by the ipsilateral retina. The X and Y pathways from lamina A seem to contribute only to cortico-cortical circuits, and the Y pathway from lamina C contributes only to cortico-collicular innervation. Lamina A1 seems to include both pathways, because the X and Y cells from there contribute to cortico-cortical circuits, and the Y pathway contributes to cortico-collicular innervation. For abbreviations, see Identification of Terminal Types section.

Y relay cells (Wilson et al., 1984) has provided evidence that, compared with X cells, Y cells are innervated by a lower proportion of F terminals and a higher proportion of RD terminals. For relay cells, a higher ratio of Y cells in lamina A1 than in lamina A would thus lead to a lower ratio of F terminals and a greater ratio of RD terminals in lamina A1, and this is precisely what we have observed. Thus on the qualitative basis to which we are now limited, our data are consistent with several prior studies indicating that lamina A contains a slightly greater X to Y ratio among relay cells than does lamina A1.

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