Ultrastucture of Synapses From the A-Laminae of the Lateral Geniculate Nucleus in Layer IV of the Cat Striate Cortex

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ABSTRACT
The morphology of synapses in layer IV of the cat striate cortex was studied by electron microscopy (EM) autoradiography of serial sections following injection of tritiated amino acids into the lateral geniculate nucleus. Of the terminals in the neuropil, 28% had 2 or more silver grains in 20 successive sections and were labeled at 8-80 times the background level. These terminals were considered to be specifically labeled and to be derived from the lateral geniculate. Two forms of geniculate synapses were observed. One had medium size, round vesicles and a modest postsynaptic asymmetry (RA); the other had smaller, pleomorphic vesicles and hardly any postsynaptic opacity; that is, it appeared symmetrical (PS). The geniculate RA terminals were presynaptic to dendritic spines, fine processes, and cell bodies; the geniculate PS terminals were presynaptic to dendrites and cell bodies but not to spines. The possible sources of geniculate PS terminals are discussed.

Key words: EM autoradiography, serial reconstruction, lateral geniculate projection

The projection of the A-laminae of the lateral geniculate nucleus to layer IV of the cat striate cortex has been well established by light microscopy (Colonnier and Rospars, '69; Garay, '71; Garay and Powell, '73; Rosenquist et al., '74; LeVay and Gilbert, '76; Winfield and Powell, '76). Studies at the electron microscope level have shown further that boutons from the geniculate A1 fibre about one-quarter of the terminals in the neuropil of layer IV (LeVay and Gilbert, '76) and that most contacts are on dendritic spines, with some on dendritic shafts, and a few on cell bodies (Garay, '71; Garay and Powell, '73; Davis and Sterling, '79; Horning and Garay, '81). Less is known about the normal ultrastructure of the geniculo-cortical synapses because they have been most often observed in the degenerating state (Garay, '71; Garay and Powell, '73; Davis and Sterling, '79; Horning and Garay, '81). The only ultrastructural study of geniculo-cortical synapses in layer IV of normal cats is that of LeVay and Gilbert ('76), who identified the labeled synapses on dendritic spines and shafts following injection of H-proline and H-histidine into the geniculate. These synapses were described as having round vesicles and asymmetric contacts, corresponding to "Gray's Type I terminals as further defined by Colonnier" (Gray, '59; Whittaker and Gray, '62; Colonnier, '69).

While studying geniculo-cortical synapses in layer IV with the same method as LeVay and Gilbert, we sometimes observed labeled synapses with faint, symmetric postsynaptic opacities and vesicles that were small and somewhat pleomorphic. These images could not be discounted as artifacts because of nonspecific labeling, sectioning artifacts, or oblique views of the synapse because they could be observed consistently in serial sections cut perpendicular to the synaptic membranes. When we compared these images to those in Colonnier's study of 100 randomly selected synapses (Colonnier, '69), they resembled those represented within his pleomorphic-symmetric category. Therefore, we have classified our second form of geniculate synapse as PS. In this paper, we describe the range of variation observed in

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the ultrastructure of geniculo-cortical synapses and the bases for our conclusion that there are two morphological types, RA and FS.

MATERIALS AND METHODS

Two cats were used in this study. Each was anesthetized with pentobarbital, and the lateral geniculate nucleus was injected with 0.8 μl of saline containing 12.5 μCi of [3H]papaverine and 12.5 μCi of [3H]leucine. After a 48-hour survival to allow time for transport of the radioactive amino acids to axon terminals in layer IV, the cat was reanesthetized and perfused with a solution of 3% paraformaldehyde and 3% glutaraldehyde in 0.12 M phosphate buffer. The lateral geniculate nucleus was sectioned at 50 μm on a freezing microtome and prepared for light microscope autoradiography.

Preparation of the material for EM

Blocks of striate cortex from the posterior lateral gyrus were sectioned on a Vibratome at 100 μm. Alternate sections were incised, stained en bloc in uranyl acetate, dehydrated, and embedded in Epon 812. The remaining sections were prepared as light-microscope autoradiograms, exposed for 6 weeks, developed in D-19, and stained for Nissl. In the light microscope we identified the sections with the heaviest labeling and selected adjacent Epon-embedded sections for EM autoradiography.

A block was trimmed to include the full depth of layer IV, which was identified by the border pyramidal neurons of layers III and V. The border between layer IVb and IVc was recognized by the disappearance in layer IVc of the large stellate neurons, which are found primarily at the top of layer IV (Okun's and Hausler, 1960). One hundred thirty-eight coronal, ultrathin sections with silver-gold interferences colors were cut and dipped in lidding 1.4 emulsion (Davies et al., 1979). After 12-18 weeks exposure, they were developed in D-19 for 2 minutes. Each section was photographed at a magnification of 4,000 in a JEOL 100B electron microscope at an accelerating voltage of 100 kV. To cover the full depth of layer IV required 36 pictures per section. A series of 50 sections was prepared similarly from a block from the lateral gyrus of the second experimental animal in which essentially the same procedures were carried out. The quantitative analysis presented here is based on the long series of sections from the first experiment. These observations were confirmed qualitatively in the shorter series of sections from the second experiment. Synapses were followed and characterized using a 7X scutar to magnify prints of the same magnification as the one in Figure 3.

RESULTS

Light microscope

Light microscope autoradiograms of the lateral geniculate indicated that in both experiments the amino acid injections were confined to the central parts of the A and C laminae (Fig. 1) representing roughly the central 2-10 de-
Fig. 3. EM autoradiogram, layer IVab. Circled silver grains are associated with presynaptic structures either in the illustrated or in successive sections. Large arrows point to grains overlying processes, which when followed in successive sections here to reveal additional grains. Scale is 2.5um.
grows of the visual field (Chader and Brown, 171; Tanas et al., 78).
Sections of cortex representing the corresponding area (Fig. 25)
showed accumulation of silver grains over layers IV and VI in accordance with previous studies. Grains did not
accumulate over layers I and II, which indicated that few
terminals were present in the C-laminae as described by Reasor and
Talman, 75; LeVay and Gilbert, 76.
Electron microscopy
Distribution of silver grains. The general distribution of
silver grains over the section was extremely sparse. It can
be seen in Figure 3 that there is only one grain over the
cell body and none over the myelinated axons. Eleven of
the 16 grains in the n. superior were clustered directly over
gyriatic terminals, and 3 of the 18 are associated with
small, dense processes resembling unmyelinated axons,
which when followed through successive sections, bore ad-
ditional grains. Four of the 18 grains could not be accounted
for in these ways, and they were assigned to the back-
gound.

The background grain density was computed by counting
the grains and measuring the areas in 10 successive sec-
tions through an oligodendrocyte (0.6 grains/μm²), a blood
vessel (0.3 grains/μm²), and a neuron (0.1 grains/μm²). The
greatness of these was taken as a measure of nonspecific label-
ing. In a similar manner we computed the grain densities
of all the axon terminals with silver zones in a small region
in the middle of layer IV. Sixty out of 86 terminals had no
graains; 8 had 1 grain, 4 had 2 grains, and 14 had 2 or more
graains (Fig. 4). The grain densities of terminals with 2
grains over 10 sections were 0.2 to 4 grains/μm² (0 to 6.6
times the nonspecific level); those with 3 or more grains over 10
sections had grains densities of 5 to 181 grains/μm² (0 to 3000
times the nonspecific level). In the remainder of the study,
terminals with 2 or more grains in 10 successive sections
were accepted as being specifically labeled. By this crite-
ron, the proportion of labeled terminals in the n. superior was
22%, about the same as reported by LeVay and Gilbert (79). In
addition, it closely matched the fraction of termi-
nals in mouse postcommunerellar barre1 field cortex (P3MBSF)
found by White et al. (74) to degenerate synchronously
following a thalamic lesion.

An important question was whether terminals that ex-
hibit specific labeling actually derive from the lateral geniculate or whether some could have been labeled
transnervally. Transnervural transport could conceivably oc-
cur in two ways. A layer IV neuron could accumulate label
shed from geniculate terminals and transport it antero-
gradely to its own axon terminals in layer IV. This would
involve a considerable dilution of the label (Levicht and
Griffin, 73; Wiesel et al., 74), making it unlikely that we
would have detected label arriving at terminals via this
route. Furthermore, it was noted in Figure 9, that the
labeled axons appeared to have been scattered from their true sources in the terminals.

Alternatively, label shed from either a geniculate termi-
nal or pretectal axon might be sequestered directly by
adjacent nonspecific terminals or their pretectal ax-
on. This possibility could also involve a considerable
dilution of the label. If it did occur, one would expect to
observe heavily labeled axonal processes surrounded by
halos of more lightly labeled ones. We looked systematically
for such a pattern in serial sections but never observed it.

As an example, Figure 8 shows serial sections of single
terminal axons taken through cell body. Heavily labeled terminals were present, but they were not surrounded by
heavily labeled terminals, nor were heavily labeled presum-
mental axons adjacent to lightly labeled pretectal terminals
(Fig. 3). This was also the case for neurons in layer IV. We
concluded, therefore, that transnervural transport was not
a significant source of error and that the specifically labeled
terminals were actually derived from neurons in the lateral
geniculate nucleus. In one case (Fig. 8, B, C), a terminal
borne 2 silver grains that appeared to have been scattered

Fig. 5. Six slices through a large solitary pattern in layer IV (each slice was reconstructed from 30 unstained EM sections). Terminals contacting the cell body are shaded, and those that are not contacting the cell body are gray. Silver grains are also indicated by small dots. Note that many heavily labeled terminals are flanked by unlabeled terminals. Lightly labeled terminal shafts appear from the same process but not for the most and adjacent to the lightly labeled ones. Large dark neurons in the left center are postcommuneral barre1 field neurons. These are presumably parasigmate (P9) pretectal neurons in the terminal morphology. Scale is 10 μm.
Figure 6
from an adjacent heavily labeled terminal. Therefore, the terminal with 2 grains was not included in the labeled population.

Synaptic morphology

Nonnongeniculate terminals. It was easy to find in our material nongeniculate terminals that exhibited the features of Collonier's dichotomous classification: round vesicle-asymmetric (RA)/pleomorphic vesicle-symmetric (PS) in its extreme form. Figure 6 shows, for example, 2 RA terminals on a fine dendritic process in which the postsynaptic opacity is thick and dark. The synaptic vesicles were relatively large and homogeneously spherical. In contrast, the PS terminal in Figure 7 has essentially no postsynaptic opacity and only a mild darkening of the postsynaptic membrane itself. The vesicles in this terminal are markedly smaller than in the RA terminal and are pleomorphic.

To quantify the observed differences in the vesicle populations of these 2 forms of nongeniculate terminal, we measured the size and shape of a total of 491 vesicles in 10 terminals (5 terminals of each form). Serial sections through each terminal were projected at a magnification of 57,000X. The outlines of all vesicles within 0.1 μm of the active zone were digitized with a bit pad, and the areas and maximum and minimum diameters were calculated. The area of a vesicle (mean plus standard deviation) in the RA terminals was 0.50 ± 0.13 × 10⁻⁵ μm², the ratio of maximum to minimum diameter (mean plus standard deviation) was 1.18 ± 0.11. The corresponding values for the PS terminals were 0.35 ± 0.13 × 10⁻⁵ μm² and 1.90 ± 0.52. Thus, the area of a vesicle in RA terminals averaged twice that of a vesicle in the PS terminals. The vesicles in RA terminals were almost round and were extremely regular in shape (as indicated by a value of 10.5 for the ratio of mean standard deviation for the measure of shape). The vesicles in PS terminals deviated considerably from roundness and were much less regular in shape (indicated by a value of 3.7 for the mean standard deviation for the measure of shape). These measurements are not offered as a systematic sample of all nongeniculate terminals in layer IV but rather to provide a context for our subsequent observations of the geniculate terminals.

Geniculate terminals. We studied 298 geniculate terminals contacting spines and fine processes in a strip of neuropil spanning the full depth of layer IV. An additional 292 terminals contacting cell bodies (Einstein et al., '81) were also studied. Many of these terminals displayed a definite postsynaptic opacity (Fig. 8A,B), but it was invariably modest and never as pronounced as in the nongeniculate RA terminals illustrated in Figure 6. The vesicles in such terminals appeared homogeneously round but slightly smaller than those in the nongeniculate RA terminals. These observations were
Fig. 8. Serial EM autoradiograms (A,1-3; B,1-3) of 3 granulated terminals (PS) with small, pleomorphic vesicles (small arrows) and synaptic contacts (large arrows) on a cell body. Numerous varicosities, RA terminal in A at upper right. Scale is 0.1 μm.
supported by measurements of 144 vesicles in 5 terminals, performed in the same manner as for the nongenulate terminals. The area in these genulate terminals was 0.42 ± 0.10 μm², a difference of 36% from those in the nongenulate RA terminals. The ratio of their maximum and minimum diameters were 1.19 ± 0.16, the ratio of mean and standard deviation of this measurement was 7.4. Thrice, vesicles in these genulate terminals were almost identical in shape to those in the nongenulate RA terminal and only slightly less regular. Genulate terminals with this morphology have been described previously (LeVay and Gilbert, '78) and are hereafter referred to as genulate RA terminals.

Genulate terminals with a different morphology, not previously described, were also observed (Figs. 9 and 10). These terminals, when cut exactly perpendicular to the synaptosomal membranes, displayed hardly any postsynaptic opacity at the active zone. Some of these terminals, being fairly large, had several active zones; each displayed only a minor and symmetrical densification of the pre- and postsynaptic membranes, and essentially no postsynaptic density. One terminal, making such a contact on a cell body, made an additional contact on a nearby dendrite. This contact (Fig. 10B,5-7) had a definite postsynaptic opacity, but an equal opacity was present presynaptically, so the contact was still symmetrical and distinctly different from what was observed in RA terminals. The vesicles in these terminals appeared smaller than in the RA terminals (both genulate and nongenulate) and were pleomorphic. The vesicles appeared slightly larger and less dramatically flattened than those in nongenulate PS terminals. These observations were supported by measurements of 103 vesicles in 5 genulate terminals, including the one illustrated in Figure 9A. The area of a vesicle in these terminals was 0.25 ± 0.10 μm², the ratio of maximum to minimum diameters was 1.45 ± 0.30, thus the vesicles were about 24% smaller than those in genulate RA terminals and 28% larger than those in nongenulate PS terminals. Further, their derivation from roundness, although in the same range as in genulate terminals, was distinctly less than for nongenulate PS terminals. Members of this second morphological class of genulate terminal, termed hereafter "genulate PS", were labeled at about the same intensity as the genulate RA terminals. In the next set of experiments, we counted the grains over 10 successive sections for all classifiable genulate terminals in a small region in the middle of layer IV. In the first experiment, 39 RA terminals had 37 ± 25 grains/100 μm² and 16 PS terminals had 40 ± 33 grains/100 μm². In the second experiment, 15 RA terminals had 12.5 ± 8.8 grains/100 μm² and 10 PS terminals had 10 ± 5.3 grains/100 μm². That the PS as well as the RA terminals were sometimes intensely labeled in single sections can be seen in Figure 9B. This provided additional evidence that PS terminals were not always labeled by transneuronal distribution. Distribution of the cao morphological forms. We won- dered whether there were differences in the postsynaptic β-spectra and laminar distribution of the genulate RA and PS terminals. The RA terminals were observed, as previ- ously described on spines, dendrites, and cell bodies. The PS terminals were rarely observed on spines but were found on dendrites and cell bodies. The genulate PS terminals were most easily recognized on the cell bodies because these the radius of curvature of synaptic membranes was the largest, and thus the chances of viewing the synaptic cut perpendicular to the pre- and postsynaptic membranes were greatest. In the neuropil, contacts on spines and fine proces- ses tended to have a smaller radius of curvature, and the chances of finding the labeled synapses perpendicular to the membranes were minimized. This, in conjunction with the relatively subtle differences between the two forms and the low magnification of the electron micrographs (re- quired to cover the full depth of layer IV in serial sections) often made it difficult to distinguish between the two forms within the neuropil. Our impression, however, was that the RA terminals were evenly distributed in both subdivisions of layer IV, whereas the PS terminals appeared to be evenly distributed in IV and are more sparse in V and VI but more sparse in the upper two thirds of VI.

DISCUSSION

The main finding of the present study is that there are different forms of genulate synapses (RA and PS) in layer IV. We have chosen to classify these differences dichoto- mously and have called the 2 forms RA and PS according to the criteria established by Colonier et al. ('88). In the present study, we have presented evidence that: 1) the terminals studied were specifically labeled, 2) the labeling of the terminals was by direct anterograde transport of radioactivity from the genulate and not by transneuronal transport, 3) the morphological features that defined a terminal as RA or PS were consistently present at multiple active zones through the same terminal, and 4) the qualitative differences in vesicle size and shape observed between the 2 forms could be supported by quantitative measurements. The question naturally arises as to why this distinction between RA and PS forms of genulate terminal has not been previously reported. In recent electron microscope stud- ies, the geniculo-cortical terminals have been labeled by anterograde degeneration (Garey, '71; Garey and Powell, '71; Davis and Sterling, '79; Hornung and Garey, '81). The synaptosomal vesicles in late degenerating terminals (4-5 days survival) tend to be obscured by electron-dense material that accumulates as part of the pathological process, while those seen 2 days after labeling are still swollen and colosehensive of the vesicles (Garey and Powell, '71). As a result, vesicle morphology cannot be used as a criterion for classifying the terminals. The postsynaptic densities of degenerating terminals are commonly quin- qued (Mamlin et al. '80) and extremely electron dense. How- ever, none of the several hundred labeled terminals exam- ined in the present study showed such thick and intensely synaptoidal densities (although many nongenulate terminals did, Fig. 6). This suggests that the marked asymmetry in degenerating genulate synapses reflects the pathological process. For these reasons one might not expect studies of degenerating geniculo-cortical terminals to have re- vealed the PS form.

Froud et al. ('85a) studied the ultrastructure of the syn- apses formed in layer IV by physiologically identified genula- te axons injected intra-axially with horseradish peroxidase (HRP) into X and Y axons. They observed, with one possible exception, asymmetric contacts. The de- gree of asymmetry and the size and shape of the vesicles are difficult to judge from their published electron micro- graphs because the HRP reaction product tends to obscure the vesicles and to spill into and across the synaptic cleft. However, the main reason why they might not have ob- served genulate PS terminals is that the injected X and Y axons were relatively thick (1.3-5.4 μm), whereas, for exa-
Fig. 10. 3 page serial EM autoradiograms. Sections 1-3 show silver grains over axon (A). Bouton (B) starts to form from axon in section 4 and expands in successive sections to form, in section 14, a large terminal with perikaryal vesicles small arrow and a symmetrical contact (large arrow). Large dots in sections 1-6 indicate corresponding points in each section for the purpose of orientation. In sections 5-7, the bouton contains a fine densecote. This contact has perikaryal vesicles small arrow and an unusually wide cleft. There is a considerable accumulation of postsynaptic electron dense material, but the density, best seen in sections 5 and 6 large arrow, is symmetrical. Scale is 0.1 μm.
Lavay and Gilbert (76) labeled geniculate-ventralterminals in layer IV using essentially the same method as employed here and observed that RA terminals pierce dense spines and shafts. Their 4 published electron micrographs of terminals in layer IV (their Figure 3 reveal substantial agreement with most aspects of our description. That is, 3 of the 4 labeled terminals contain medium-size, round vesicles and, where cut normal to the active zone, are seen to have modest postsynaptic sparcities. The fourth terminal (their Figure 3d, upper left) has only a faint postsynaptic sparsity and contains vesicles that are smaller and more varied in size than the 0.6-µm labeled terminal on the same dendrite (upper right, same figure). This profile bears some resemblance to the one illustrated in our Figure 9b. The main difference between the Lavay and Gilbert study and the present one may be merely in the use of single sections versus our use of serial slices. Subtle differences when observed in a single section may tend to be discounted, whereas it is harder to do that when the same difference is noted in successive sections. Also, use of serial sections allowed us to follow many labeled terminals on cell bodies. This was the population among which we found the greatest number and clearest examples of PS terminals, and this population was not studied by Lavay and Gilbert. Tsumoto’s study (84) of radioabeled geniculate terminals in single sections of nondepolarized cats was based mainly on the neuropil, and thus mainly on geniculate-synapsing contacts, which are agreed to be of the RA form. Terminals from the nondepolarized eye were not illustrated, thus making comparison to the present study difficult.

Functional implications

Terminals in layer IV of the PS form have been labeled by accumulation of 3H-gamma-aminobutyric acid (GABA) (Hasbani et al., 1983). The antiauxonal processes of the geniculate cell dendrocyte (GAD) (Rihak, 78; Somogyi et al., 83; Ruskvlay et al., 88), a strong indication that they are GABAergic. The ultrastructural similarity between glutinated GABA-ergic axon terminals and the geniculate PS terminals suggests that the latter might also be GABAergic. More recent examples of GABA-ergic projection neurons from other nuclei of the mammalian brain (Vincent et al., 85; Blaton and Rakic, 86), and there is a possible source in the lateral geniculate for such a projection to the striate cortex. Roughly 20% of the neurons of laminae A and A1 of the lateral geniculate are thought to be GABAergic (Sterling and Davis, 65; Fujita et al., 84; Zempel and Montequin, 85).

The GABAergic populations of geniculate neurons include the small stellate neurons in the laminae A laminae (Sterling and Davis, 65; Fujita et al., 84; Zempel and Montequin, 85). It was doubtless for a time that a small geniculate nucleus projecting to the cortex, because numerous attempts to label them retrogradely with HRP have failed (Lavay and Versmuyt, 79; Weber and Kuhl, 82): Fujita et al., 84, Recently, however, certain small geniculate neurons have been activated antidromically from the subcollicular white matter and filled by intracellular injection of HRP (Friedlander et al., 81; Weller and Humphrey, 85). The latter study emphasizes that these neurons receive tonic as well as dynamic (less than 1 ms) with long conduction times to cortex (2.5-5.1 ms). They correspond to a newly identified physiological class of geniculate neuron, the “labeled cell” (83). Thus, it may be that the geniculate-ventral PS terminals are the terminal boutons of the labeled X-cells. The fineness of their axons may explain why the cell bodies have not been labeled retrogradely by horseradish peroxidase.

GABA has inhibitory postsynaptic actions in the visual cortex (Silitte, 74, 75ab, 77). As has been demonstrated in the hippocampus, as well as the visual cortex, its effects are hyperpolarizing when its action is on cell bodies and proximal dendrites (Anderson et al., 80; Kemp, 84). If it were shown that the PS population of geniculate terminals were, indeed, GABAergic, it would lead to the speculation that, inhibition as well as excitation, could be directly relayed from the geniculate to the striate cortex. Ferster and Lindsberg (83) concluded that IPSPs recorded intracellularly following electrical stimulation of the geniculate, were all at least dysynaptic. Their conclusion rested, however, on an extrapolation of PSP latencies from their measured latencies to stimulation at the geniculate and at a point two-thirds of the way along the optic radiation. The authors pointed out that the extrapolation underestimated the ocular conduction time to the terminals by more than 0.2 ms for the slowly conducting axons. The fine axons of labeled X-cells are indeed slowly conducting (Mastronarde, 84, 85; Weller and Humphrey, 85). Figure 4 of Ferster and Lindsberg indicates that an allowance of more than 0.25 ms for slower conduction velocity would bring the latencies of some of their IPSPs into the monosynaptic range. Thus, the long latency IPSPs evoked by stimulus of geniculate neurones may actually be mono-synaptic. Further discussion of the possible functions of this pathway is deferred until the next paper (Einstein et al., 87), which describes the distribution of geniculate RA and PS terminals on cell bodies.

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