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Innervation of Cat Visual Areas 17 and 18 by Physiologically Identified X- and Y-Type Thalamic Afferents. I. Arborization Patterns and Quantitative Distribution of Postsynaptic Elements

T.F. FREUND, K.A.C. MARTIN, AND D. WHITTERIDGE

1st Department of Anatomy, Semmelweis University Medical School, Budapest, Hungary; (T.F.F.) Department of Experimental Psychology, Oxford University, Oxford, U.K. (K.A.C.M., D.W.)

ABSTRACT

Specific thalamic afferents to visual areas 17 and 18 were physiologically classified as X or Y type and injected with horseradish peroxidase (HRP). The axons were examined under the light microscope and were then processed for correlated electron microsocpy. X axons arborised in area 17 and in the border between area 17 and 18. The X axons all formed terminals throughout layer 6, but were heterogeneous in their distribution in layer 4. They either occupied the entire width of sublayers 4A and 4B or were strongly biased toward layer 4A. Y axons also arborised in layers 4 and 6, but in area 17 they did not form boutons in sublamina 4B. Some Y axons projected only to area 18; others branched and arborised in both areas 17 and 18. Only the collaterals of one X axon were found to enter area 18; all the others were restricted to area 17. Y axons formed three to four separate patches of boutons about 300–400 μm in diameter, while all but one X axon formed a single elongated patch. Y axons had thicker main branches (3–4 μm) than X axons (1.5–2.5 μm) at their point of entry to the cortex. The main axon trunks and their medium-calibre collaterals were myelinated, but the preterminal segments were unmyelinated and studded with boutons. Each X or Y axon contacted about seven to ten somata, but Y axons made more contacts per soma (three to six) than did X axons (two to three). In addition to somatic synapses, both X and Y axons formed asymmetric (type 1) synapses on dendritic spines and shafts, with spines forming the most frequent targets (80%). Each Y bouton made, on average, 1.64 synapses in area 17 and 1.79 synapses in area 18, whereas each X bouton made only 1.27 synapses on average. Although there are proportionally fewer Y axons than X axons entering area 17, the Y axons provide as many synapses as the X axons because of their larger arbors and multisynaptic boutons.

Key words: visual cortex, intracellular recording, intracellular HRP, postsynaptic targets

The visual cortex of the cat receives a major input from two classes of cells in the dorsal lateral geniculate nucleus (dLGN) of the cat (Friedlander et al., '81; Garey and Powell, '71; Geisert, '80; Holländer and Vanegas, '77; Leventhal, '79; LeVay and Ferster, '77; Singer et al., '75). These cells, the so-called X and Y cells, are physiologically quite distinct (Cleland et al., '71; Hochstein and Shapley, '76; Hoffmann et al., '72; Wilson et al., '76) and are thought to have different roles in visual processing (see reviews by Lennie, '80; Rodieck, '79; Sherman, '85; Sherman and Spear, '82; Stone et al., '79; Stone and Dreher, '73). It has been found that X cells project exclusively to area 17, whereas Y cells project to both area 17 and area 18 (Dreher et al., '80;

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Geisert, '80; LeVay and Ferster, '77; Mitzdorf and Singer, '78).

A number of studies have used anatomical or physiological methods to examine how these X and Y projections are integrated into the circuitry of area 17 (Bullier and Henry, '79a–c; Ferster and LeVay, '78; Ferster and Lindström, '83; Henry et al., '79; Leventhal, '79). All these studies agreed that the X and Y afferents were partially segregated in their terminations in layer 4. Y terminals, and cells driven monosynaptically by them, were found to occupy the upper portion of layer 4 (4A), while the X terminals and cells driven by them occupied the lower portion of layer 4 (4B). The X and Y projection to layer 6 overlapped.

As part of a larger study that combined intracellular injection of horseradish peroxidase with physiological tests, we examined the position and morphology of cells driven monosynaptically by X or Y afferents (Martin and Whitteridge, '84). In contrast to the above studies we found clear evidence that cells in the upper portion of layer 4, including the layer 3/4 border region, could be driven monosynaptically by X-like afferents. The dendritic fields of these neurons did not enter lower layer 4. Two alternative explanations of these results could be offered: (1) it is possible that a class of X afferent, as yet undiscovered, extended up to the 3/4 border; (2) more ominously, the physiological test used to identify the X- and Y-like afferents, the response latency of the retinal ganglion cell axons, was inadequate. If the first option were true then it would have important consequences for cortical circuits driven by the X afferents. In order to decide between these two options it was necessary to reexamine the morphology of the X and Y afferents, and this forms part of the present study.

Studies using degeneration techniques (Garey and Powell, '71; Davis and Sterling, '79; Hornung and Garey, '80,'81; Winfield and Powell, '83) or anterograde transport of labelled amino acids (LeVay and Gilbert, '76) have shown that the LGN afferents make asymmetric synapses, that they synapse mainly on dendritic spines, and that they form about 10–20% of the synapses in layer 4. As the afferent type could not be identified in these studies, it was not known whether there were any differences between the X and Y afferents in the choice of postsynaptic target. The location of the synapses on the neuron may also be important in determining the physiological response of the cell to that input; therefore we sought to determine the precise termination sites of these thalamic boutons.

We were also interested in the relative weighting of the X and Y input to cortex, because we found that more than half of the cortical cells in striate cortex could be activated via Y-like afferents, whereas it has been argued that the Y input has only a "modulatory" role in cortical function (Lennie, '80). This latter view seemed less appealing in the light of evidence suggesting that Y axons made larger terminal arbors (Bullier and Henry, '79c; Ferster and LeVay, '78; Gilbert and Wiesel, '79), and that the individual boutons of Y afferents may make more synapses than the boutons of X afferents (Winfield and Powell, '83). These observations imply that not only is the retinal Y cell input amplified in terms of cell numbers in the LGN, as Friedlander et al. ('81) have found, but that there is a further amplification in the cortex., Thus in the retina the Y cells form only about 5–10% of the ganglion cells (see rev. by Sherman and Spear, '82) but from our estimates, they come to dominate about 50% of the cells in striate cortex.

In the present experiments we attempted to answer the questions raised above by recording from the axons of LGN relay cells, classifying them with a battery of tests, including response latency, and injecting them intra-axonally with HRP. We then examined the terminal arbors in both light and electron microscope. The results indicate that there is a class of X axon projecting to upper layer 4 and support the notion that there is further amplification at a synaptic level of the Y input. A recent extensive study at the light microscope level by Humphrey et al. ('85a,b) agrees substantially with the light microscope observations presented here. Preliminary account of part of this work has appeared (Martin, '84; Martin and Whitteridge, '84).

METHODS

The material presented here was obtained from a total of 18 normal adult cats, four of which were used for electron microscopy. The animals were prepared as described previously (Martin and Whitteridge, '84). Briefly, recordings were performed on cats that were anaesthetised with Althesin (Glaxo; average 0.4 ml/kg·hour) or Sagatal (May and Baker; average 3 mg/kg·hour), paralysed with gallamine triethiodide (13 mg/kg·hour) and tubocurarine (1 mg/kg·hour), and artificially respired. The blood pressure, heart rate, end-tidal CO_2, and rectal temperature were monitored continuously. Any slight rises in blood pressure (from a recorded systolic maximum of 120 mmHg) or heart rate were counteracted with further I.V. doses of anaesthetic. Pairs of stimulating electrodes were placed in the optic chiasm (position "OX") and in the optic radiations immediately above the LGN (position 'OR1'). The recording pipettes were filled with a 4% solution of horseradish peroxidase (HRP; Boehringer Grade 1) in 0.2 M- KCl and 0.05 M- Tris (Sigma) at pH 7.9. The tips were bevelled to an impedance of about 100 MΩ.

The pipettes were advanced through area 18 toward the white matter until axons were encountered. After Friedlander and Stanford ('84) the following parameters were noted for each axon during extra-axonal recording: (1) receptive field (RF) position, (2) eye dominance, (3) RF center and surround sign and size using hand plotting techniques, (4) responsiveness to a large (20°), rapidly moving visual target of contrast sign opposite to that of the RF center, (5) sustained or transient response to a stimulus of appropriate contrast placed in the RF center for 15 seconds, (6) linearity of spatial summation within the RF, determined by using a sinusoidally counterphased sine grating pattern presented at a spatial frequency slightly below the upper cutoff value, (7) regularity of the response to repeated counterphase of the grating at low temporal frequencies, (8) the latency of response to electrical stimulation at the optic chiasm and in the optic radiations, and (9) the regularity and temporal jitter of the response to electrical stimulation.

Axons were classified as X type if they showed linear spatial summation at high spatial frequencies, did not show consistent excitatory response to a fast-moving (>200°/second) large target, and had OX – OR1 latency differences of 1.9 ms or more. Axons were classified as Y type if they showed nonlinear spatial summation (responded at both the fundamental and second harmonic frequency), showed a consistent excitatory response to a large target moved rapidly through their RF, and had OX – OR1 latency differences of 1.7 ms or less.

If recording had been maintained throughout the test, an attempt was made to advance the pipette into the axon. If this was successful, as seen by a drop in the DC potential of 40 mV or more and the appearance of spikes >20 mV, the linearity and latency tests were repeated. HRP was

then iontophoresed into the axon using currents of 2–8 nA for up to 5 minutes. The response to visual stimulation was tested throughout this period, after which the electrode was withdrawn.

After periods ranging from 1–18 hours following the iontophoresis, the animals were perfused through the heart first with saline (2–4 minutes) and then with a fixative containing 2.5% glutaraldehyde and 1% paraformaldehyde in 0.1 M phosphate buffer. Blocks of tissue thought to contain the HRP-filled axons were sectioned in the coronal plane at 80 μm on a Vibratome (Oxford Instruments). For peroxidase histochemistry we used the catechol/p-phenylene reaction (Hanker et al., '77) with colbalt and nickel intensification (Adams '81). Axons in which boutons were clearly visible in wet sections were selected and were postfixed in osmium tetroxide (1% solution in 0.1 M phosphate buffer—PB) for 45–60 minutes depending on the background darkness of the HRP reaction. The sections were dehydrated in ethanol (1% uranyl acetate was included in the 70% ethanol stage for 40 minutes), and mounted on slides in Durcupan ACM resin (FLUKA) under a coverslip and cured for 2 days at 56°C.

The HRP-filled axons were drawn from each section with a microscope with drawing tube attachment and reconstructed as a two-dimensional montage. Areas of interest were drawn under oil-immersion, photographed under the light microscope, and re-embedded for correlated electron microscopy, as described elsewhere (Somogyi et al., '79). Serial ultrathin sections were cut from selected parts of the HRP-filled axons and were mounted on Formvar-coated single slot grids, stained with lead citrate (Reynolds, '63). Electron micrographs were taken at 80 kV with a 20 μm objective aperture.

RESULTS

A total of 15 X axons and 34 Y axons were recorded from and tested with the full battery of tests described in the methods. Many other axons were incompletely analysed because recording contact with the axon was lost. Of those that were fully classified most could be impaled and had HRP iontophoresed into them for lengths of time varying between 5 seconds and 5 minutes. Those in which the iontophoresis was shorter than about 1 minute were either not filled at all or were too faintly filled with the HRP reaction end-product to be of any use. A total of 5 well-filled X and 18 Y axons were studied at the light microscopic level. Of particular interest was the comparison of the classifications of the axon type obtained solely by visual stimulation, and the response latencies obtained through electrical stimulation. For all axons classified as Y, the difference in latency between the responses elicited at the optic chiasm (OX) and those elicited from the optic radiations immediately above the LGN (OR1), was 1.8 ms or less. The latencies for the X axons were 1.9 or more. Thus axons could be classified as X or Y on the basis of stimulation criteria alone. These latencies are consistent with the assumption that we were recording from the axons of cells located in the geniculate complex. The morphology and laminar distribution of the axons also supports this assumption.

For the present study we studied three X axons (Figs. 1, 2A), two Y axons that projected to area 17 (Fig. 2B), and two Y axons that projected to area 18 (Fig. 3). One of the Y axons in area 17 was incomplete and is not illustrated here. The receptive field locations of these axons are given in the figure legends. The three X axons included two of the three main classes of X axon found by Humphrey et al. ('85). One X axon arborised throughout layer 4 with some bias toward layer 4B (Fig. 1A). The other two X axons had almost no terminal arborisation in the lower portion of layer 4B and had a strong bias toward layer 4A (Figs. 1B, 2A). Only one of the three X axons was patchy in its projection to layer 4 (Fig. 2A), and in this respect was similar to all the Y axons recovered in both area 17 and area 18. The other two axons formed a single elongated clump, about 1 mm long and 0.6–0.8 mm wide. Both X and Y axons had a projection to layer 6 and occasionally collaterals entered layer 5. These deep layer projections were considerably smaller than those of layer 4 for both X and Y axons.

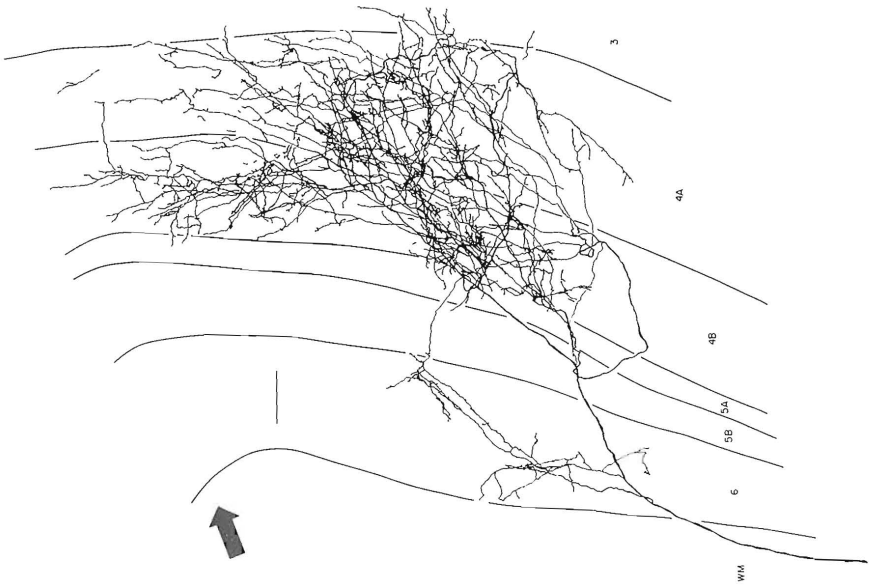
The Y axons had thicker main branches (3–4 μm) at the point of entry to the cortex than the X axons (1.5–2.5 μm). Their terminal arbors in layer 4 were three to four times larger than those of the X axons in the mediolateral and anteroposterior dimensions. In area 17 the main Y arborisation was in layer 4A, but occasional collaterals could extend to the top of layer 3. In area 18 a similar pattern was seen for most Y axons, except that layer 4B was more heavily innervated. All the Y axons formed several patches (three to four) of boutons, each having a diameter of 300–400 μm.

En passant boutons were the most common type of bouton for both X and Y axons. The collaterals occasionally formed small clusters of boutons, about 30–40 μm in diameter, but otherwise the boutons appeared to be uniformly distributed within a patch. As the collaterals of the X axons left the main myelinated branch, they lost their myelin sheath, became studded with boutons and travelled horizontally for several hundred micrometers. This contrasts with the Y axons, where the unmyelinated portions studded with boutons were shorter and more radially orientated. However, the Y axons appeared to produce these unmyelinated portions more frequently than did the X axons.

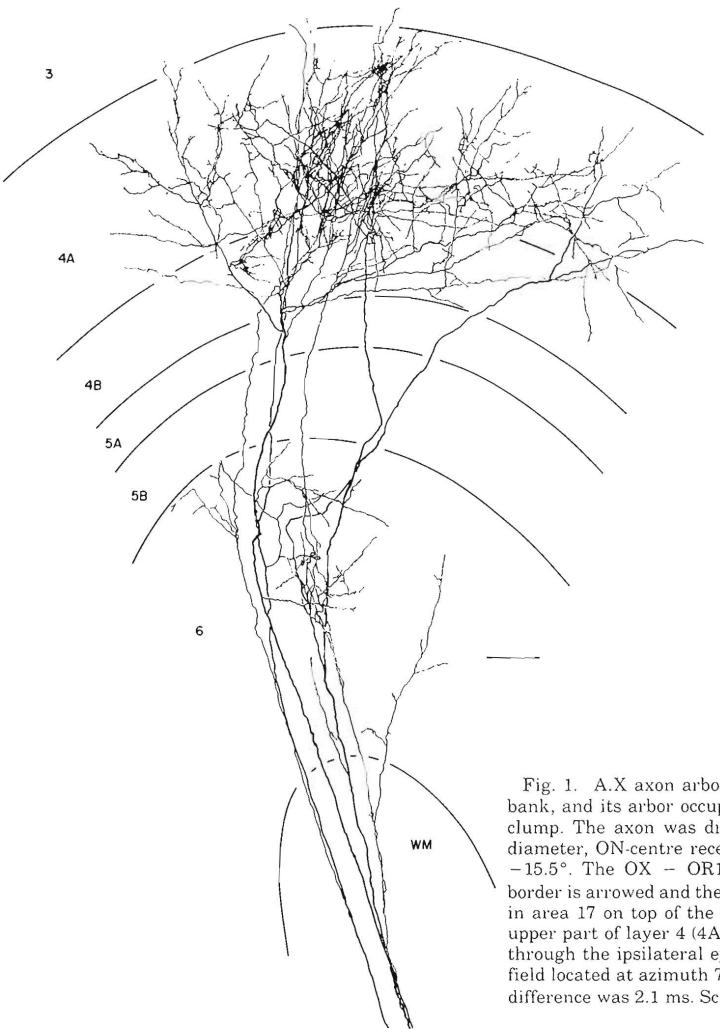
A small proportion of the boutons were in contact with neuronal somata directly. Each axon, whether X or Y, contacted about seven to ten somata (Fig. 4A, but see following paper for detail), mainly in layer 4, occasionally in layer 6. These somata did not appear to be grouped in any obvious way, except that the somata contacted by Y axons in 17 tended to be more superficial in layer 4A than those contacted by the X axons. The Y axons in area 18 contacted perikarya throughout the depth of their arborisation within layer 4. The Y axons made twice as many contacts per soma (three to six) as the X axons (two to three) and contacted larger somata (average diameter 24 μm) than those contacted by the X axons (average diameter 15 μm). In the case of very well-filled axons, there was some transneuronal diffusion or transport of HRP into a small number of postsynaptic structures. This was most evident in some somatic contacts (Fig. 6).

Synaptic connections of X- and Y-type axon terminals

The main axon trunks and their medium-calibre collaterals were always myelinated, but the preterminal axons of bouton-laden collaterals were mostly unmyelinated. Boutons contacting somata were usually near to the end of the myelin sheath. All the varicosities seen in the light microscope and subsequently examined in the electron microscope were found to form synapses. A total number of 320 HRP-filled boutons were examined in the electron microscope; 242 of these (385 synapses) represent a random sam-

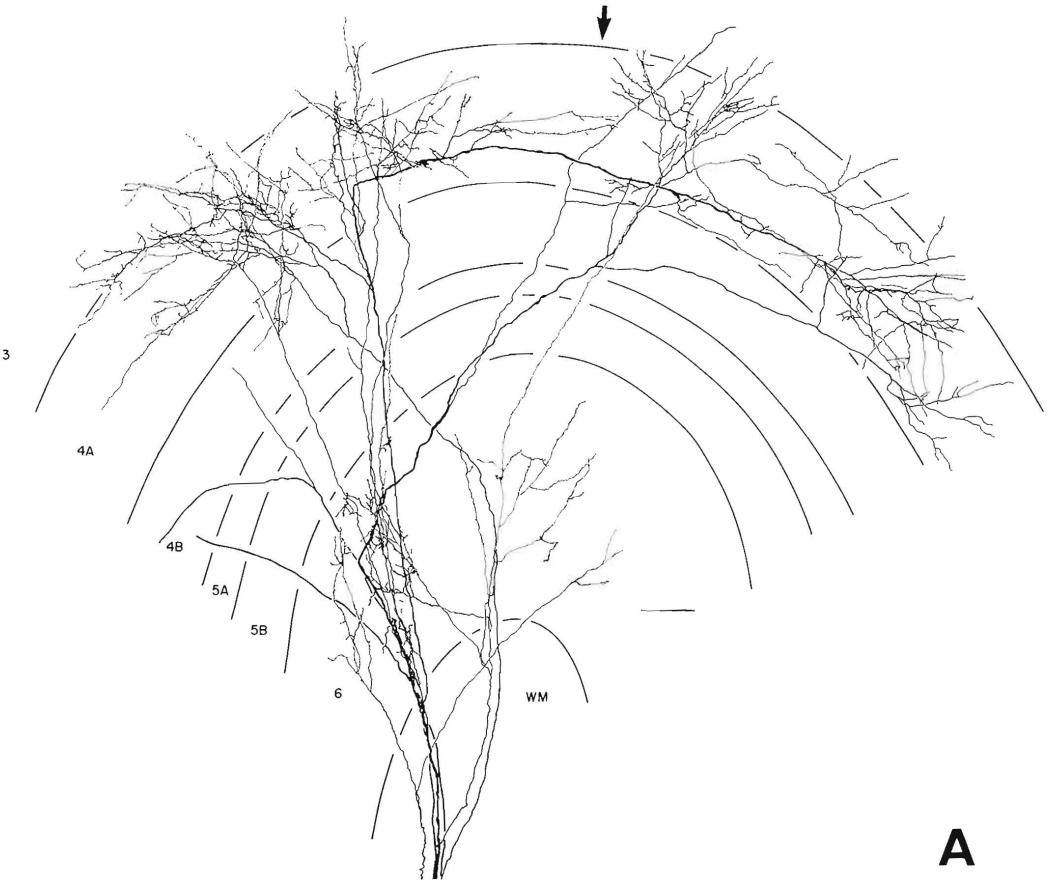


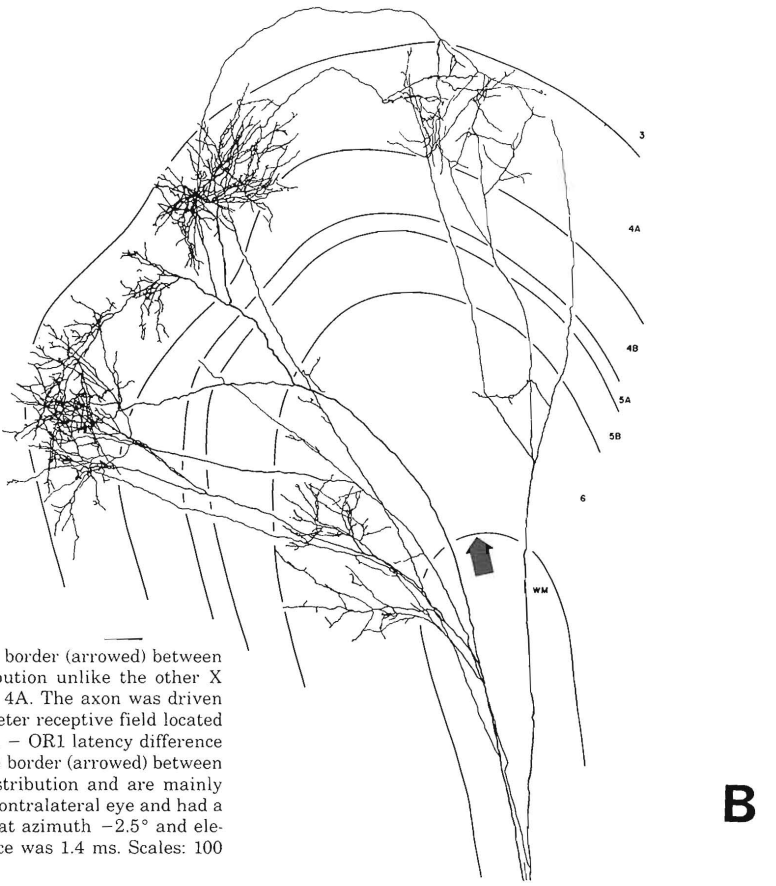
A



B

Fig. 1. A.X axon arborisation in area 17. The axon runs up the medial bank, and its arbor occupies most of the depth of layer 4 forming a single clump. The axon was driven through contralateral eye and had an 0.8° diameter, ON-centre receptive field located at azimuth 0.9° and elevation −15.5°. The OX − OR1 latency difference was 2.1 ms. The area 17/18 border is arrowed and the print has been rotated 90°. B. X axon arborisation in area 17 on top of the lateral gyrus. Its arbor is largely confined to the upper part of layer 4 (4A), and forms a single clump. The axon was driven through the ipsilateral eye and had a 1.4° diameter, OFF-centre receptive field located at azimuth 7.0°, and elevation −17.5°. The OX − OR1 latency difference was 2.1 ms. Scales: 100 μm.





Figs. 2.A. X axon arborisation spanning the border (arrowed) between areas 17 and 18. Its arbor has a patchy distribution unlike the other X axons in Figure 1, and innervates mainly layer 4A. The axon was driven through the ipsilateral eye and had a 0.7° diameter receptive field located at azimuth −2.5° and elevation −13.0°. The OX − OR1 latency difference was 1.9 ms. B. Y axon arborisation spanning the border (arrowed) between areas 17 and 18. Its boutons have a patchy distribution and are mainly confined to layer 4A. It was driven through the contralateral eye and had a 1.4° diameter ON-centre receptive field located at azimuth −2.5° and elevation −13.0°. The OX − OR1 latency difference was 1.4 ms. Scales: 100 μm.

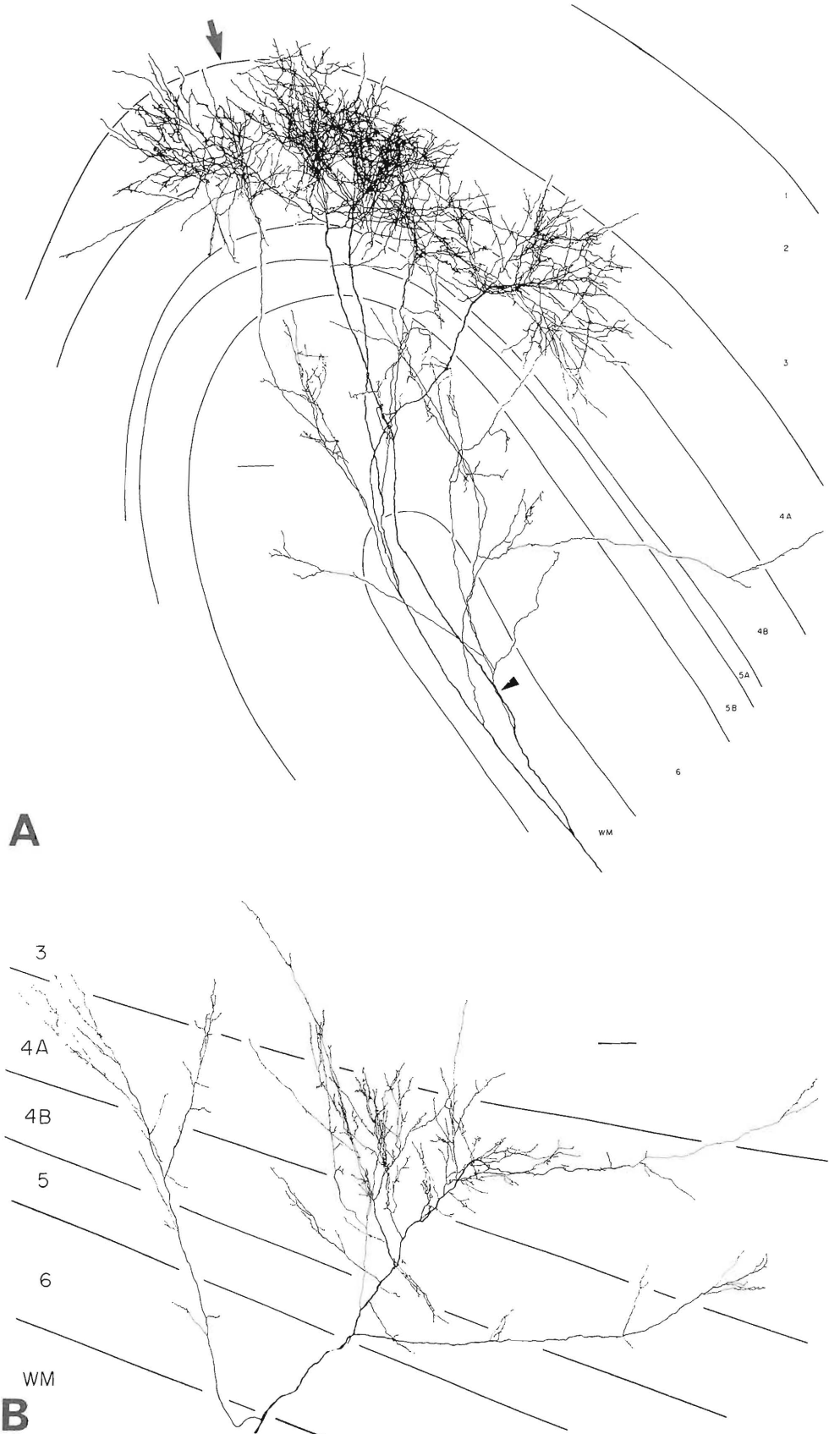


Fig. 3. A. Y axon arborisation in area 18, having a patchy distribution mainly in layer 4A, as all other Y axons. The arrow indicates the area 17/18 border on the lateral gyrus. The axon was driven through the contralateral eye and had a 2.1° OFF-centre receptive field located at −7.0° azimuth and −10.0° elevation. The OX latency was 1.8 ms and no latency was obtained from the OR1 electrodes. Arrowhead indicates the point at which the axon was impaled. B. Y axon in area 18 arborising along the medial bank of the lateral sulcus. The axon was driven through the contralateral eye and had a 2.5° diameter ON-centre receptive field located at azimuth 0.5° and elevation −9.5°. The OX − OR1 latency was 1.6 ms. Scales: 100 μm.

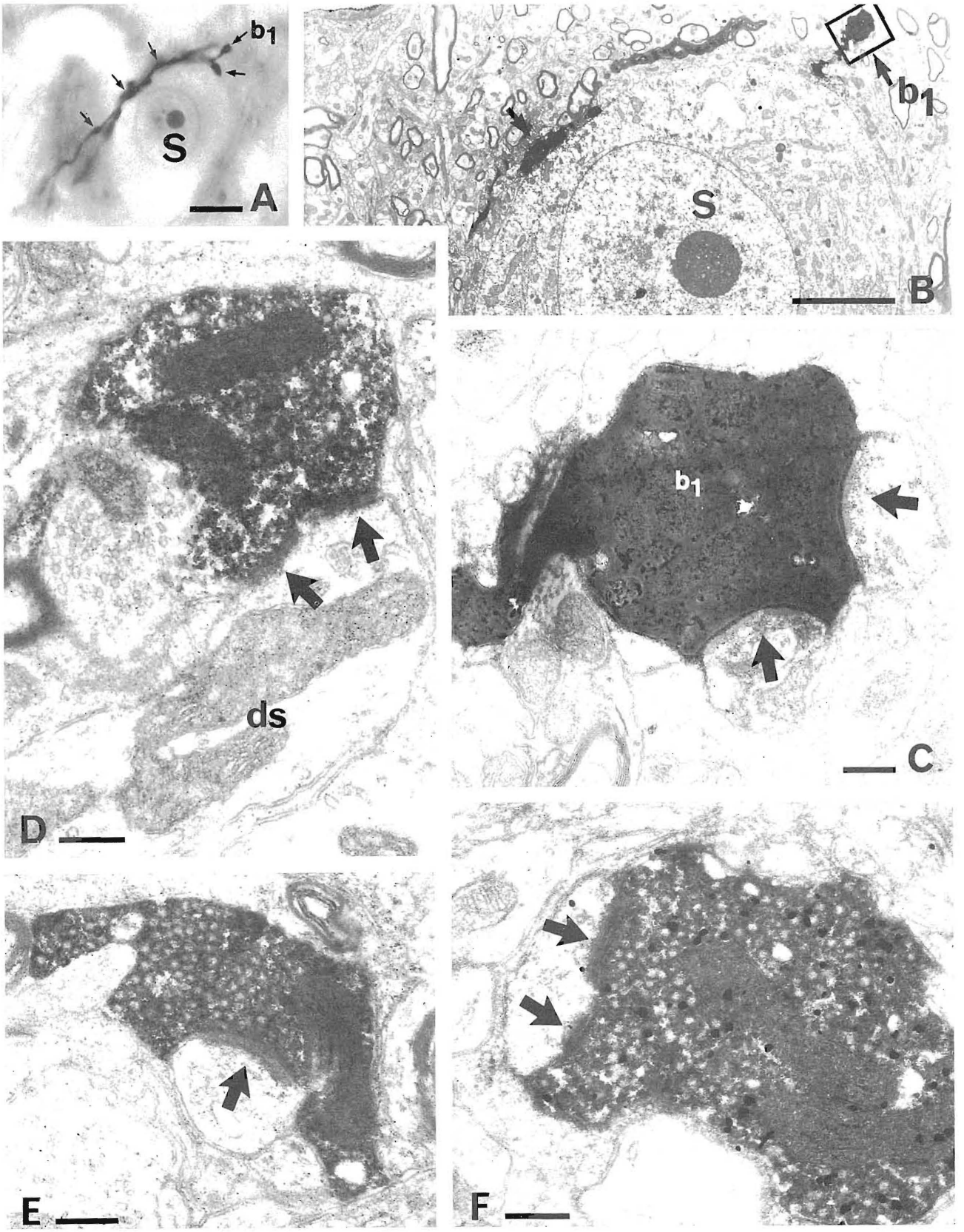


Fig. 4. A–C Correlated light and electron micrographs of a Y-type LGN axon collateral in area 18. The HRP-filled axon is climbing along the membrane of a large perikaryon in upper layer 4 (see in more detail in the following paper) and is myelinated at the preterminal parts (shown in B). One of the boutons (b1) is shown at higher magnification in C, where the asymmetrical synapses (arrows) established with two spines are clearly visible. A spread of HRP end-product was found into the widened synaptic cleft in case of each HRP-filled boutons, but sometimes it also invaded the postsynaptic profile to variable degree. Arrows in A and B indicate some of the varicosities along the HRP-filled collateral. D. An HRP-filled bouton in layer 4 of an X-type LGN axon is in asymmetrical synaptic contact (arrows) with a dendritic shaft (ds) of unidentified origin. E. Another bouton of the same X axon contacts a dendritic spine; the synapse is again of the asymmetrical type (arrow). F. A large bouton of a Y axon in area 17 is in asymmetrical synaptic contact (arrows) with a spine. Scales: A: 10 μm; B: 5 μm; C–F: 0.25 μm.

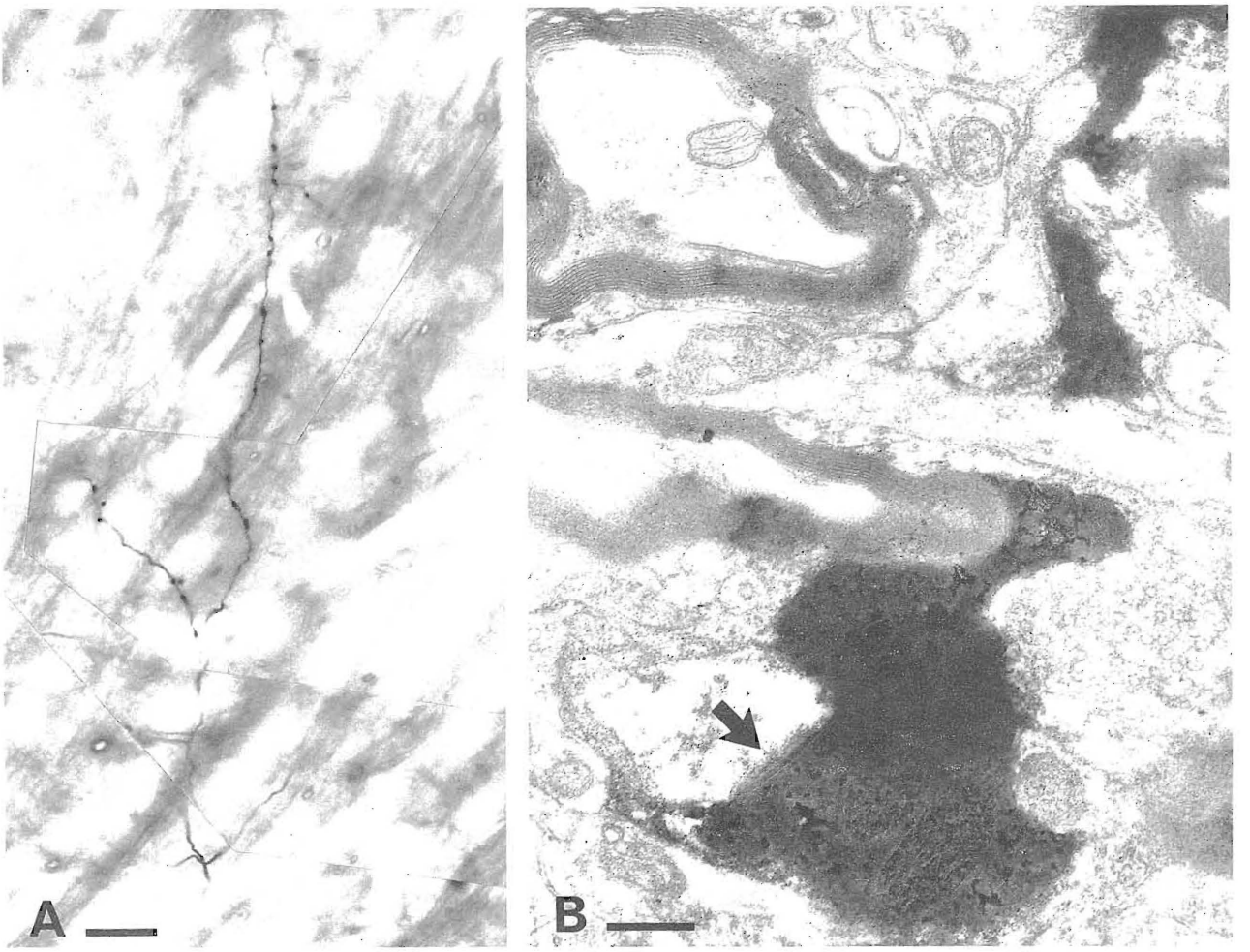


Fig. 5. A. Light micrograph of a layer 6 collateral of an X-type LGN axon. Varicosities indicating possible synaptic contacts frequently occurred along the HRP-filled LGN fibers also in layer 6. B. One of the boutons of the axon collateral shown in A is in asymmetrical synaptic contact (arrow) with a spine. Scales: A: 20 μm; B: 0.25 μm.

ple (184 in layer 4, used in the quantitative studies, and 58 from layer 6) and about 80 boutons were sampled for correlated light and electron microscopy (see the following paper). The postsynaptic elements were dendritic spines or shafts, and somata (Figs. 4–6). The dendritic shafts were not reconstructed to see whether they were smooth or spiny. However, the somata contacted by either X or Y axons had distinctive ultrastructural features, similar to those perikarya, which have in some way been characterized as GABAergic (i.e., large number of mitochondria and free ribosomes in the cytoplasm, indented nuclei, large number of both symmetrical and asymmetrical synaptic inputs, constricted origin of primary dendrites). The immunocytochemical identification of these postsynaptic somata is described in the folowing paper.

Almost all synapses established by LGN afferents were of the asymmetric (type 1) variety (Figs. 4–6). The synaptic vesicles, when visible amongst the HRP reaction end product, were round, but we cannot be sure that the osmotic effects of the HRP injection and/or the reaction endproduct did not produce this form. Unexpectedly, the serially sectioned boutons of one X axon did occasionally form symmetric synaptic specializations on somata and dendritic shafts (see in the following paper), while the rest of its boutons established conventional asymmetric synapses.

X axons. The distribution of the number of postsynaptic elements contacted by individual X boutons is given in Figure 7A, together with the comparable data for the Y axons. All boutons included in Figure 7 have been completely reconstructed from serial sections, and those synapses with a cleft tangential to the plane of sectioning have also been counted. About two-thirds of the X boutons in layer 4 established only one synaptic contact, and the rest two, while in layer 6 a slightly lower proportion (six boutons out of 24) contacted two postsynaptic profiles. The average is 1.27 synapses per bouton (both layers 4 and 6

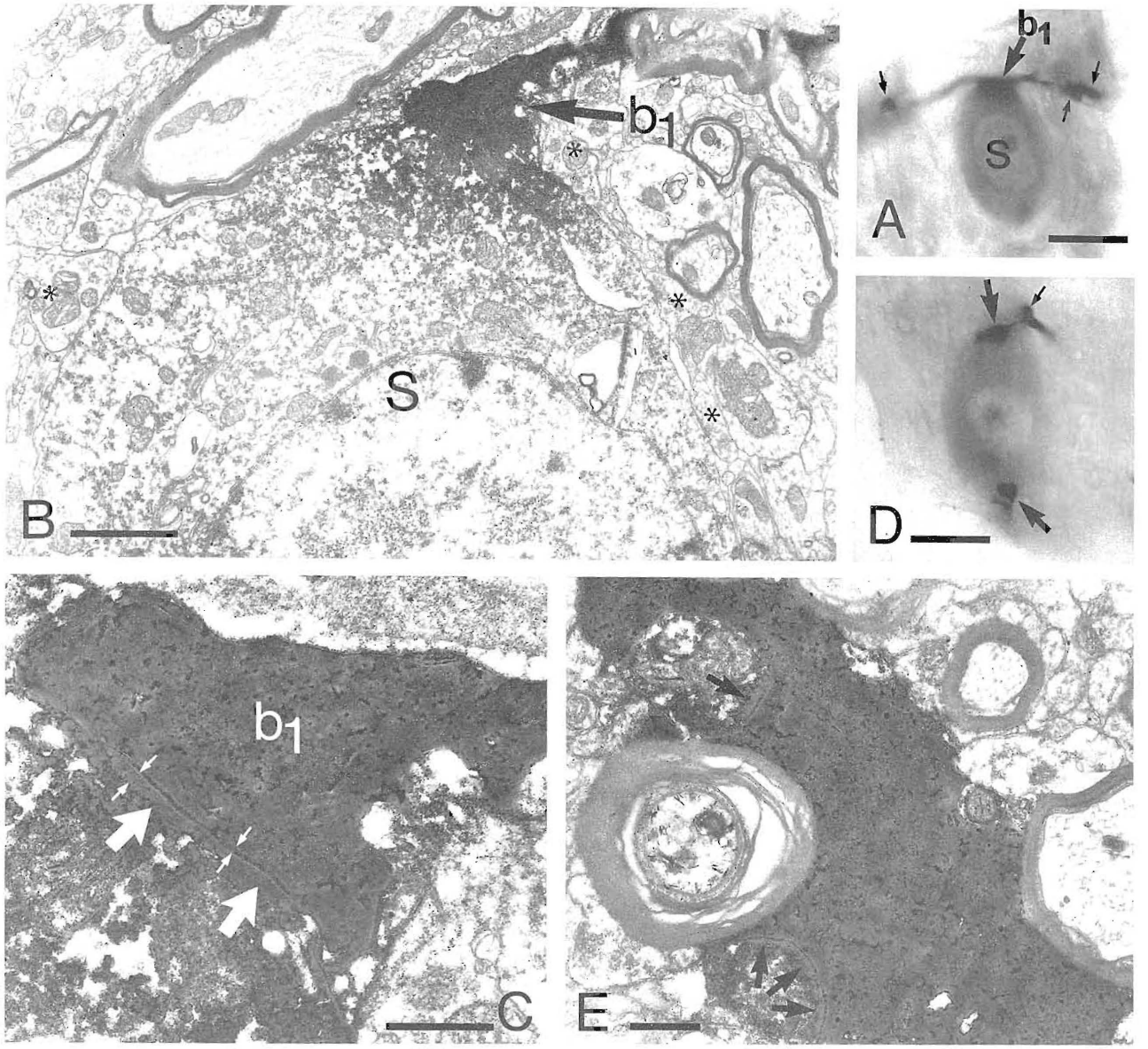


Fig. 6. Transsynaptic HRP labelling is shown at both light and electron microscopic level. A,D. Two collaterals of a Y-type axon in area 18 contacting two cell bodies are shown at the light microscopic level. Both perikarya are labelled by a transsynaptic spread of HRP through the afferent HRP-filled boutons in contact with them (large arrows). Other boutons of the same injected axon are indicated by small arrows. B. Low-power electron micrograph of the perikaryon (s) shown to be labelled by HRP in A. The continuous decrease in intensity of peroxidase end-product on moving away from the HRP-filled afferent bouton. (b1) clearly shows the origin of staining in this cell body. Other unlabelled synaptic boutons (asterisks) were also frequently found in contact with this perikaryon. The HRP-filled afferent bouton (b1) is shown at higher magnification in C, where the asymmetrical synaptic contact (large arrows) is clearly visible. The upper left pair of small arrows label the postsynaptic thickening, which could be recognized on the basis of its electron density being slightly weaker than that of the HRP reaction end-product, and also because they occurred only where the synaptic cleft was typically widened (lower right pair of small arrows). E. A large bouton in layer 6 of the same Y-type LGN afferent heavily filled with HRP is in asymmetrical synaptic contact (arrows) with two spines, both of which are strongly labelled by transsynaptic HRP. Although the magnification is much lower than in C, the widening of the synaptic clefts and the less electron-dense postsynaptic thickenings accompanying them are still clearly visible. Other profiles attached to the HRP-filled boutons without receiving synaptic contacts from them were also found occasionally to contain HRP reaction end-product. Most of these structures appeared to be glial processes, and they were much less intensely labelled than the postsynaptic profiles. Scales: A,D: 10 μm; B: 1 μm; C,E: 0.5 μm.

included). The distribution of different types of postsynaptic profiles is shown in Figure 7B. The major postsynaptic targets of X boutons were dendritic spines (almost 90%), but dendritic shafts (Fig. 4D) and perikarya (see the following paper) were also contacted. The sample was small in layer 6, but it appeared that a higher percentage of synapses were formed on dendritic spines (Fig. 5) than for synapses in layer 4. (The results of correlated light and

electron microscopy were not included in figure 7 in order to avoid non random sampling.)

Y axons. The features of Y axons in area 17 and 18 were fairly similar and will be described together. The distribution of different postsynaptic elements is given in Figure 7B. The Y boutons contacted more dendritic shafts and less spines than the X axons, but the percentage of somatic contacts was very similar for the two axon types (see Figure 7B). The dendritic shafts were contacted more often near the top of layer 4A. About half of the Y boutons were in synaptic contact with two profiles, a third with one, and 10–14% with three or occasionally more postsynaptic elements (Fig. 7A). The average is 1.64 (area 17) and 1.79 (area 18) synapses per bouton (layer 6 included), which is much higher than that for the X axons (see Fig. 7A). In layer 6 only eight out of the 32 tested boutons gave two synapses, while the rest gave only one.

The transneuronal labelling seen in the one Y axon under the light microscope proved to be transsynaptic when examined under the electron microscope (Fig. 6). The postsynaptic profiles were usually somata in layer 4 (their transsynaptic labelling was also visible in the light microscope), and spines in layer 6, where the filling was the heaviest (Fig. 6E). Despite the heavy labelling of HRP at these synapses, the synaptic cleft and the postsynaptic densities were clearly visible, because they had an electron density different than that of the HRP reaction end-product (Fig. 6C,E). Postsynaptically there was a clear gradient of intensity of HRP labelling, from the most dense region around the synapse to more distant regions (Fig. 6B). In layer 6 some HRP was also detected in the extracellular space surrounding the filled boutons, but it was most evident in some postsynaptic elements (Fig. 6E). This "leakage" resulted in a very faint labelling of processes (usually of glial processes) passing by, but they were visible only at the electron microscopic level.

DISCUSSION

Three main findings emerge from this study. Firstly, it was found that the X input to layer 4 is more extensive in the depth of cortex that it occupies than had previously been supposed. Secondly, X and Y afferents provide synapses to a similar variety of postsynaptic elements, spines being the major targets of both types of axons. Thirdly, the Y axons are not only more extensive than the X axons, both in tangential extent and number of boutons, but they make, on average, more synapses per bouton than do the X axons. A recent extensive study at the light microscopic level by Humphrey et al. ('85a,b) using similar techniques has produced data in good agreement with the present light microscopic data. The main differences in axonal morphology is that Humphrey et al. ('85a,b) find a class of X axon more confined to layer 4B, and that, unlike ours, the Y axons in area 18 tend not to innervate layer 4B. The patchy distribution of the axons seen in the present study is typical of the LGN afferents (Ferster and LeVay, '78; Gilbert and Wiesel, '79, '83; Humphrey et al. '85a,b). As Blasdel and Lund ('83) have shown for the monkey, these clumps probably underly the ocular dominance slabs seen in bulk transport experiments (Shatz et al., '77). Those X axons that form only one patch must be running along a single ocular dominance slab.

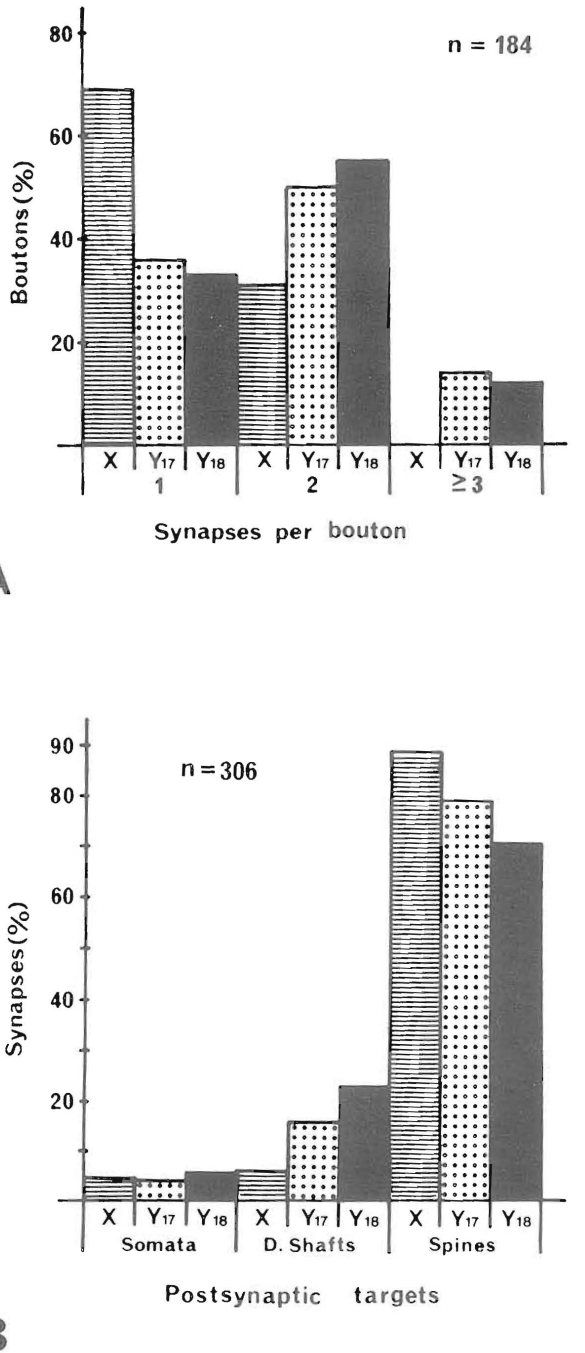


Fig. 7. Histograms, showing (A) the distribution of boutons giving one, two, three, or more synapses per bouton, and (B) the distribution of postsynaptic targets. These termination characteristics of X axons and Y axons in areas 17 and 18 are compared in separate columns. A random sample of 184 HRP-filled boutons (306 synapses) was used for this study (64 boutons of X axons, 64 of Y axons in area 17, and 56 of Y axons in area 18). Each of these boutons has been serially sectioned and reconstructed in the electron microscope.

On the basis of their responses to electrical stimulation, it is reasonable to assume that the X axons shown here are the source of the monosynaptic input to cells in upper layer 4 and lower layer 3. In our earlier studies (Martin and Whitteridge, '82, '84), all the main types of spiny cells were

found to receive monosynaptic input from X or Y afferents. These cells include the border pyramids of layer 3, star pyramids and spiny stellate cells of layer 4, and pyramidal cells in layers 5 and 6. Presumably the dendritic spines, which we confirm as the major target of the LGN afferent synapses, were from these spiny neurones. The spines sampled in the lower portions of layer 6 are most likely to belong to pyramidal cells of layer 6, because the dendrites of layer 5 pyramidal cells only rarely extend as deep.

The somata contacted by the afferents are unlikely to belong to spiny neurones because of their ultrastructural properties (Colonnier, '68; Davis and Sterling, '79; LeVay, '73). The somata of spiny neurones that have been identified at the light microscope level and then examined in the electron microscope have round nuclei with little indentation, a limited number of mitochondria, and a sparse input of symmetrical synapses. The somata seen here were quite different in having large numbers of mitochondria and free ribosomes, and a rich synaptic input of both asymmetric and symmetric synapses. These ultrastructural features are more characteristic of the GABAergic neurones of the cortex that are thought to be involved in inhibitory processes (Freund et al., '83; Ribak, '78; Somogyi et al., '83a). This aspect is analysed further in the companion paper.

Our failure to find a significant input of the Y axons to the lower portions of layer 4 is consistent with our previous finding that cells in this layer are driven mainly by the X afferents (Martin and Whitteridge, '82, '84). A similar trend has been reported by others (Bullier and Henry, '79c; Ferster and Lindström, '83; Mullikin et al., '84), although Humphrey et al. ('85a) found that two out of seven Y axons arborized in both layers 4A and 4B of area 17. As yet our sample of cells is too small to determine whether the X and Y afferents contact different morphological subtypes of spiny neurones in the upper portions of layer 4, but the X afferents may have exclusive access to a group of neurones in lower layer 4 that have axonal projections mainly within layer 4 (Gilbert and Wiesel, '79, '83; Lund et al., '79; Martin and Whitteridge, '84). The postsynaptic targets of these exclusively X-driven cells remain unknown, but from their projections it seems likely that they contact cells that themselves receive monosynaptic input from the LGN. Thus, receptive fields could be elaborated without a strictly hierarchical progession of processing.

Although a few layer 4B neurones do project into layers 2 and 3, these projections are weak compared to these of neurones in layer 4A. The main X projection out of layer 4 must therefore come from those neurones receiving direct X afferent input in layer 4A or be relayed via other cells in layer 4A. Again, the targets of these neurons are not known, but they are probably the large population of spiny neurones in layers 2 and 3, many of which project out of area 17, probably to other cortical areas (Gilbert and Kelly, '75).

In area 18 the situation is less complicated because by far the dominant input to layer 4 comes from Y cells (Mitzdorf and Singer, '78). Here, as in area 17, the major input is onto dendritic spines. Relatively little is known of the different neuronal types in area 18 and almost nothing is known of the morphological types that receive monosynaptic input from the Y afferents, but at least in some respects the processing may be similar to that of the Y projection in area 17. Some evidence for this is given in the companion paper.

Analysis of the number of synapses made by an individual bouton shows that the Y axons, both in area 17 and 18, make more synapses per bouton than the X axons, on average. This supports the earlier evidence of Winfield and Powell ('83), who used degeneration methods to look at the number of synapses made per bouton in layers 4A and 4B in area 17, and in layer 4 in area 18. Their method had two drawbacks. Firstly, on the present evidence and that of Humphrey et al ('85a), their sample of boutons in layer 4A of area 17 must have included both X and Y boutons. Secondly, the approach used involved damaging the claustrum, which also sends a projection to layer 4 (LeVay and Sherk, '81; Olson and Graybiel, '80). Nevertheless, their general conclusion that there is a greater number of synapses per Y bouton is confirmed here, although the average number of synapses per bouton they find for layer 4A is higher than ours. This might imply that the boutons of the claustral afferents also make a number of synapses.

On the basis of results from a number of studies, we can make a preliminary calculation as to the relative proportions of X and Y afferent synapses. In the A laminae of the LGN X cells form 63% of the total and Y cells form 30% (Friedlander and Stanford, '84). Geisert ('80) showed that only about 1% of the projection cells in the A laminae of the LGN do not project to area 17. If one takes averages from the work of Humphrey et al. ('85a,b), it can be calculated that Y arbors in area 17 have 1.62 times more boutons than X axons. Our data show that Y axons have more synapses per bouton than X axons (1.67 vs. 1.24). These ratios balance out the higher number of X cells in the LGN, with the result that there are about equal numbers of X and Y synapses in layer 4. This estimate applies only to the A laminae of the LGN and may underestimate the number of Y synapses because of the minor Y projections from the C laminae of the LGN and the medial interlaminar nucleus (Bullier et al., '84); Geisert, '80; Holländer and Vanegas, '77; LeVay and Ferster, '77; Leventhal, '79).

From these figures it seems likely that the Y afferents dominate at least part of the input/output paths from area 17. In area 18, Y axons appear to be the dominant LGN afferents, since these were the only axons found to project to area 18. These findings are in line with the hypothesis suggested by Friedlander et al. ('81), that there is a progressive expansion of the Y pathway relative to that of the X system, as one moves centrally toward the visual cortex.

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