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Combined Golgi and Electron Microscopic Study on the Synapses Formed by Double Bouquet Cells in the Visual Cortex of the Cat and Monkey

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ABSTRACT The morphology of certain Golgi-stained cells was examined in the striate and peristriate cortex of the cat and in the striate cortex of the rhesus monkey. Neurons in layer III were selected on the basis of their characteristic vertical axon bundles, which are 20–150 μm in diameter and traverse layers II–V.

Selected neurons were examined under the electron microscope to characterize their synapses and to establish their postsynaptic targets. It was found that double bouquet cells form symmetrical or type II synapses. In the cat the postsynaptic membrane specialization was more extensive than in the monkey. After removing the Golgi precipitate from boutons of two cells in the cat, small pleomorphic and flattened vesicles were found in the boutons.

Earlier suggestions that double bouquet cells make synapses preferentially with spines of apical dendrites could not be confirmed. Out of 66 boutons in area 17 of the cat, 86.4% formed synapses with dendritic shafts, many of them belonging to nonpyramidal cells, 9% with perikarya of nonpyramidal cells, and only 4.6% with spines. Out of 19 synapses examined in area 18, 74% were contacting dendritic shafts and the rest contacted spines. In the monkey 60% of a total of 35 double bouquet cell synapses made synapses with dendritic shafts. A different type of double bouquet cell with densely spiny dendrites is also described in layer IV of the monkey striate cortex. This neuron formed asymmetrical synapses.

It is suggested that layer III double bouquet cells with vertical axon bundles are probably inhibitory and act on other nonpyramidal cells and certain parts of pyramidal cells.

One of the most interesting yet poorly understood cortical local circuit interneurons is Ramón y Cajal's "cellule à double bouquet dendritique," first described in various cortical areas from man (Ramón y Cajal, '11) but subsequently found in the somatosensory cortex of the cat (Szentágothai, '73) and monkey (Jones, '75), and in the visual cortex of the monkey (Szentágothai, '73; Valverde, '78). Valverde described these neurons in area 18 of the monkey but did not find them in the striate cortex (Valverde, '71), nor were they mentioned in another Golgi study (Lund, '73). Although the cell is called "double bouquet" or "double tufted" after its dendritic arborization, a far more characteristic and unique feature of this cell is its narrow, columnar axon bundle passing radially through several layers.

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Previous Golgi studies (Ramón y Cajal, '11; Colonnier, 66; Jones, '75; Szentágothai, '73, '75, '78) agree in describing the vertical axon bundles of double bouquet cells as following apical dendrites, which are known to receive asymmetrical or type-I synapses on their spines (LeVay, '73; Fairén et al., '77; Somogyi, '78). The idea that the axon bundles provide the synapses on the apical dendritic spines is based on the respective courses of apical dendrites and the tight fascicles of the double bouquet cells. However, the Golgi method, though successful in revealing individual neurons, has obvious limitations in identifying

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synaptic connections at the light microscopic level. Recently efforts have been made at the electron microscopic level to trace synaptic connections of cortical neurons characterized by Golgi staining (LeVay, '73; Fairén et al. '77; Parnavelas et al., '77; Somogyi, '77, '78; White, '78; Peters et al., '79). This approach has been very informative in attributing the postsynaptic structures to different types of local circuit interneurons (Somogyi, '77, '78, '79; Peters and Fairén, '78; Somogyi et al., '79).

In the present study we have used the Golgi method to characterize double bouquet cells in striate and peristriate areas of the cat and in the striate cortex of the Macaque monkey. Selected cells were processed subsequently for electron microscopy to identify the type of synapses formed by double bouquet cells. We also sought to determine the nature of postsynaptic structures and the types of postsynaptic cells.

MATERIALS AND METHODS

Two normal adult cats and two adult monkeys (*Macaca mulatta*) were used. In both monkeys an electrolytic lesion was made in the left lateral geniculate nucleus 4 days before death and for reasons unconnected with the present experiment. The cells described in the present study were in the striate cortex ipsilateral to the lesion, but it was not the aim of the present study to trace degenerating boutons to these cells. Following a lethal intravenous dose of anaesthetic (Nembutal) the animals were perfused through the heart with 0.9% saline, followed by an aldehyde mixture containing 2% paraformaldehyde and 1% glutaraldehyde in 0.1 M sodium phosphate buffer at pH 7.4. After 30 minutes continuous perfusion the skull was opened and small blocks of the cortex were dissected and placed in the same fixative for several hours or overnight, at 4° C. Thereafter the blocks were processed for Golgi staining and electron microscopy essentially as described previously (Somogyi, '78). Some of the blocks, however, were fixed in 1% OsO_4 instead of 2% as used previously. Some of the Golgi sections were rinsed in 5% ethanol for 5–45 minutes before dehydration. This partially removed the silver chromate precipitate from the most superficial structures in the section and thereby uncovered some of the internal detail within the impregnated elements. Although the extent and depth of silver chromate removal varies from section to section and is difficult to control, it was possible to examine two cells in this way in area 17 of the cat.

Cells were drawn using a 100 × oil immersion objective and a Leitz camera lucida. In the striate cortex of the monkey drawings were made of one cell in layer III and two cells in layer IV complete with their impregnated axons. In the cat, one cell in area 18 and four cells in area 17 with their axons, and two further cells in area 17 without their axons, were also drawn and photographed. All were in layer III. All quantitative data given relate to the Golgi sections. No correction was made for shrinkage. In the monkey the layering scheme of Lund ('73) was used.

Some of the cells (Table 1) were reembedded for electron microscopy. To improve contrast for electron microscopy Golgi sections were stained with uranyl acetate and ultrathin sections with lead citrate as described previously (Somogyi, '78). Electron micrographs were taken on Philips 201 and JEOL 100B electron microscopes using 20-μm objective apertures.

RESULTS

Light microscopy of Golgi-stained double bouquet cells in layer III

Cells in layer III were selected on the basis of their vertical axon bundle descending through layers III–V as well as on the basis of dendritic morphology. Nonpyramidal cells with very different axon arborizations may possess similar dendritic arborizations.

In both striate and peristriate cortex cells with radial axon bundles were found throughout layer III (Figs. 1, 2, 13) except its most ventral part. The perikaryon is typically fusiform, 10–18 μm in diameter. Smooth or sparsely thorny dendrites originate from the upper and lower pole of the perikaryon, follow a vertical course, and branch infrequently. Sometimes all the dendrites originate from two or three main shafts (Fig. 1), giving rise to the characteristic double bouquet appearance. Dendrites ascend to layer I, but rarely penetrate it and they descend toward layer IV, reaching its most superficial part (Figs. 1, 2, 13). In the monkey the whole dendritic arborization remains within layers II–III (Fig. 15). The dendritic field is always elongated at right angles to the pia mater and is about 90 μm (Fig. 1) to 200 μm wide. Occasional dendrites may deviate from the major configuration by travelling horizontally (Figs. 2, 13). Thus the double bouquet arrangement is not always strictly maintained.

The axon usually emerges from the main shaft of the lower dendritic bouquet (Figs. 1, 2) or from the perikaryon (Fig. 15) and takes a descending course. Rarely it ascends imme-

TABLE 1. Distribution of structures postsynaptic to double bouquet cells

Species		Cell No.	Layers examined by electron microscope	Number of identified boutons contacting: Spine	Dendritic shaft	Perikaryon of nonpyramidal neuron	Total
Cat	area 17	1 (Figs. 1, 4–9)	III–V	1	47	5	53
		2 (Fig. 10A,B, 11)	III	1	7	1	9
		3 (Fig. 12A,B)	III	1	3	–	4
		Total		3 (4.6%)	57 (86.4%)	6 (9%)	66 (100%)
	area 18	4 (Figs. 13, 14)	III	5 (26%)	14 (74%)	–	19 (100%)
Monkey	area 17	5 (Figs. 15–17)	III–IV	14 (40%)	21 (60%)	–	35 (100%)
	area 17	6[1] (Figs. 18–20)	IV	2	7	–	9

[1] This is a different type of cell found in layer IV. All the other cells are from layer III.

diately (Fig. 13). From 30–50 μm from its origin it divides into collaterals which themselves divide and form five to ten main collaterals, ascending and descending radially. A distinct feature of the axon is that the main collaterals increase in diameter as they pass radially.

In the cat the radial fibers ascend to layer II and descend to upper layer V (Figs. 1, 2, 13). They may course in a loose plexus 100–150 μm in diameter (Fig. 2) or some of the collaterals may form interwoven fascicles, in which case the entire bundle is only 20–50 μm in diameter (Figs. 1, 4A, 13). The main radial collaterals have swellings throughout their length and bear small branches crowded with bulbous enlargements (Figs. 1, 2, 4A, 13). These branches are most numerous in layer V and especially in layer III, and as a result the axonal field increases in these layers (Figs. 2, 13).

In the monkey striate cortex the radial fibers always form small fasciculi about 20–50 μm in diameter (Figs. 15, 16). They have more swellings and fewer side branches. The number of bulbous enlargements decreases toward the bottom of layer V where the radial fibers terminate and give off a few short collaterals (Fig. 15).

In both species the tight axon fasciculi provide very high bouton density per tissue volume.

Electron microscopy of Golgi-stained boutons of layer III double bouquet cells

The Golgi-stained axon collaterals were followed in electron microscopic serial sections for fine structural characterization of the synapses and to identify the postsynaptic structures. It has been extensively demonstrated in two previous papers (Somogyi, '78; Somogyi et al., '79) that with our method any stained bouton can be correctly identified and recovered for electron microscopy; therefore, to save space the light and electron microscopic correlation is shown only in one example (Fig. 4). The synapses formed by the impregnated boutons were compared on electron micrographs to synapses established by unstained boutons with the same postsynaptic structure (Figs. 5, 6, 7, 17B,D) as well as to synapses nearby in the same section (Figs. 3, 12A).

In the cat the synapses formed by axon collaterals of double bouquet cells have symmetrical membrane specializations (Figs. 4C,D, 5D, 6, 7, 8, 10A,B, 11, 12A,B, 14A). However, as compared to the symmetrical axosomatic synapses received by pyramidal cells, the postsynaptic membrane thickening was more pronounced (Figs. 5D, 6, 10A,B). Depending on the plane of the section this postsynaptic membrane specialization varied in extent from synapse to synapse but never reached the thickness of asymmetrical axospinous or axodendritic postsynaptic specializations (Figs. 3, 6, 7, 12A). In a few cases symmetrical attachment plaques were also found between boutons of double bouquet cells and dendrites (Fig. 9). Within the synaptic cleft material an electron-dense line could occasionally be observed (Fig. 10A,B).

In the monkey all 35 synapses were of the symmetrical type with very little postsynaptic membrane specialization (Figs. 17A-C).

In the boutons of the two cells which received 5% ethanol treatment the silver chromate precipitate had been partially removed. This enabled us to study the synaptic vesicles.

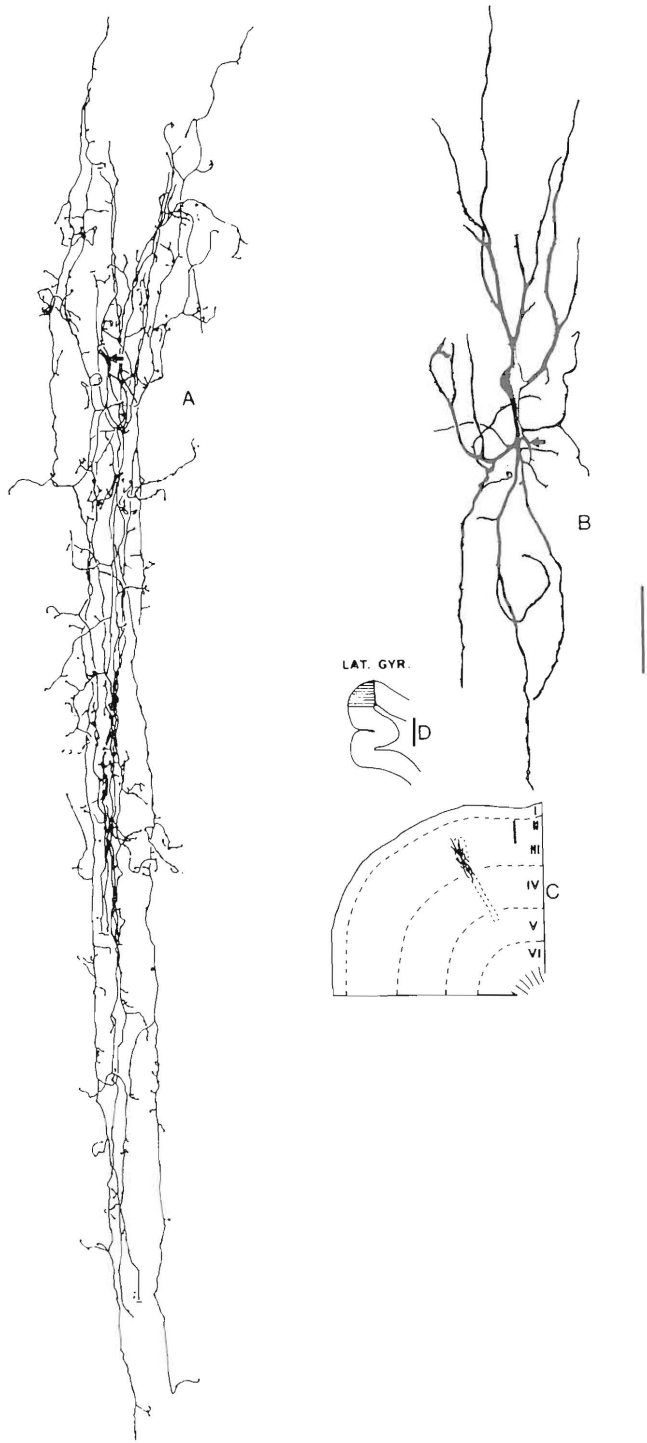


Fig. 1. Double bouquet cell with very narrow axonal (A) and dendritic (B) field in the lateral gyrus of the cat. Arrow indicates the axon initial segment, (C) position of the cell in the cortex, and this area is shaded in the lateral gyrus (D). Scale A, B: 50 μm; C: 200 μm; D: 2 mm. See electron microscopic data in Table 1.

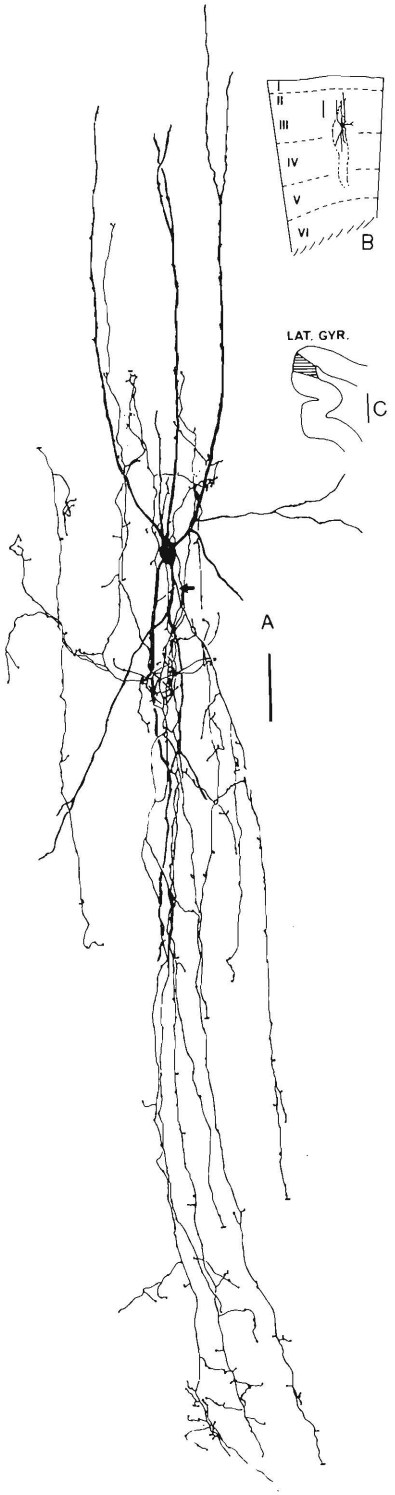


Fig. 2. Double bouquet cell in the medial wall of the lateral gyrus in the cat. Note wider dendritic and axonal field than in Figure 1. Scales and labelling as in Figure 1.

The boutons contained flattened or small pleomorphic vesicles (Figs. 10–12) with occasional large dense-core vesicles also present. This corresponds well to the vesicle types in unstained boutons in the same material, where symmetrical membrane specialization and pleomorphic vesicles are found together (Fig. 3).

Electron microscopy of postsynaptic structures

The nature and distribution of postsynaptic structures is summarized in Table 1. It can be seen that there is a difference between the two species and also between areas 17 and 18 in the cat. However, in view of the small number of cells examined and the large variance within the group of three cells in area 17 of the cat it is not our intention to attach any special significance to these differences.

In area 17 of the cat 86.4% of the boutons terminated on small and medium size dendritic shafts (Figs. 4B-D, 6, 7, 10, 11, 12A) which show no particular orientation. Some of these dendrites were followed in long section series but no spines could be detected on their surface. Many (64%) of the postsynaptic dendrites received synapses from one or more nonimpregnated synaptic boutons in the same section which included the impregnated bouton (Figs. 6, 7). The majority of nonimpregnated boutons established asymmetrical contacts. Main shafts of pyramidal cell apical dendrites were not encountered among the postsynaptic dendrites. Six axosomatic synapses (Figs. 5, 8) were identified between the Golgi-stained boutons and four nonpyramidal neurons, three of which were fusiform with dendrites originating from the upper and lower pole of the perikaryon. One of the synapses was in fact on the emerging main shaft, but was classified as axosomatic (Fig. 5). These neurons could be identified as being nonpyramidal since they received both asymmetrical and symmetrical synapses from unstained boutons on their perikaryon (Colonnier, '68; Parnavelas et al., '77). No pyramidal cell perikaryon or axon initial segment was ever found among the postsynaptic structures. The origin of the three spines postsynaptic to the stained neurons in area 17 of the cat could not be determined.

One neuron was studied under the electron microscope in area 18 (Figs. 13, 14). Spines were more frequently (26%) found postsynaptic to the axon of this neuron (Fig. 14A) but the majority of the synapses were still established with dendritic shafts. One of these shafts could be an apical dendrite since it had a radial course and was about 2 μm in diameter. It received only symmetrical synapses on

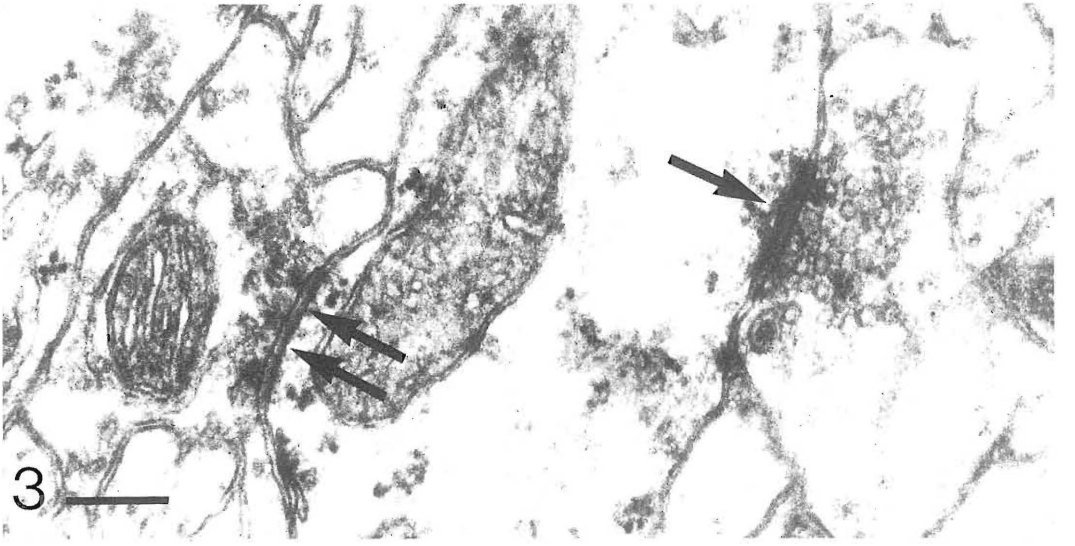


Fig. 3. A bouton containing spherical vesicles forms an asymmetrical or type I synapse (single arrow) with a dendrite in the striate cortex of the cat. Another bouton containing smaller pleomorphic vesicles forms a symmetrical or type II synapse (double arrows) with the same dendrite. Note the extensive postsynaptic membrane specialization at the type I synapse. Scale = 0.2 μm.

the shaft and two spines receiving asymmetrical synapses were seen to emerge from its surface. Another shaft in synaptic contact with an impregnated bouton emitted two spines, both of which received asymmetrical synapses (Fig. 14B). The other dendrites were small or medium size without particular orientation. Only a small number of unstained boutons were found to terminate on the postsynaptic dendrites, indicating that some of them are different from those in area 17. Synapses were seen only in four cases in the plane of the impregnated bouton.

One layer III double bouquet neuron in the monkey striate cortex was reprocessed for electron microscopy. Many more spines were found to be postsynaptic to this neuron than in the cat (Table 1). The proportion of spines was somewhat greater in layer III than in layer IV. All these spines received one asymmetrical synapse from boutons containing round synaptic vesicles (Fig. 17A) in addition to the symmetrical synapse established with the impregnated bouton. The spines had very long but thin stalks which made it impossible to trace back to the parent dendrites. Postsynaptic dendritic shafts (Fig. 17B,C) were small to medium diameter and 19% of them were in asymmetrical synaptic contact with nonimpregnated boutons (Fig. 17C). They had no particular orientation.

We were particularly interested to see whether one postsynaptic structure receives multiple synapses from one double bouquet cell. In each species two reconstructions were made from serial sections. It was found that neighboring or closely situated boutons usually form synapses with different structures. Two neighboring synapses on the same dendrite were only occasionally observed (Fig. 6). However, two of the postsynaptic perikarya received two adjacent synapses from a double bouquet cell.

Layer IV spiny double bouquet cells in the monkey

The lack of specific synaptic relationship between apical dendrites of pyramidal cells and the axons of double bouquet cells found in layer III leaves the question of the origin of presynaptic boutons on apical dendritic spines open.

Double bouquet cells have also been described in layer IV (Szentágothai, '73, '78). Therefore we thoroughly studied this layer, but so far have failed to find similar cells to those in layer III. However, we encountered a new type of small spiny cell with a remarkably narrow vertical dendritic field (Figs. 18, 19). The fusiform perikaryon was about 7–10 μm in diameter and was always in layer IV$C\alpha$. The radial dendrites formed two narrow col-

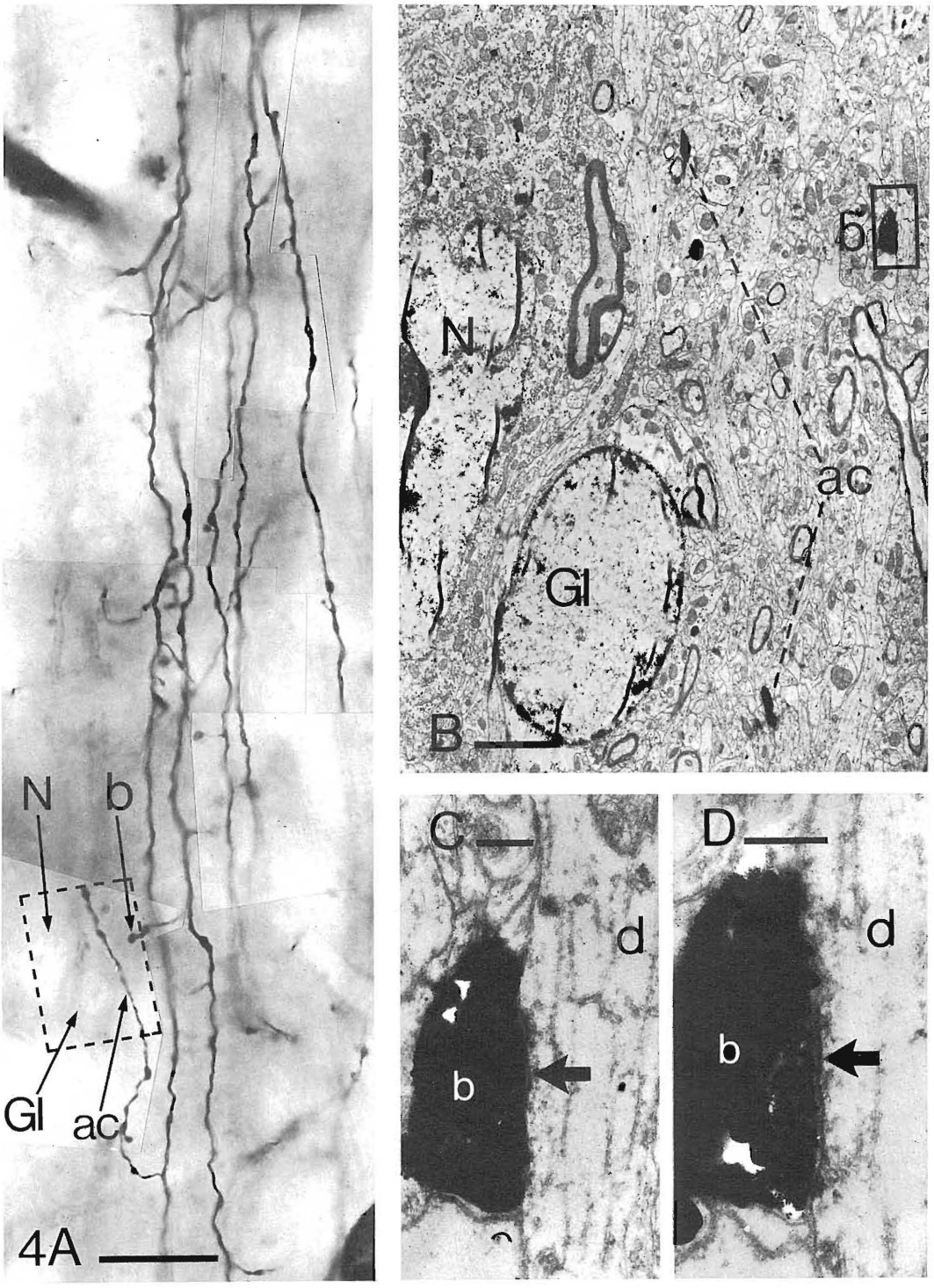


Fig. 4. (A) Photomontage of part of the vertical axon bundle of the neuron shown in Figure 1. Framed area is shown at the electron microscopic level in (B). Structures common to A and B are a neuron (N), a glial cell (Gl), the Golgi-stained axon collateral (ac), and a Golgi-stained bouton (b) from another collateral. This bouton is shown at higher magnification (C) forming a synapse (arrow) with a dendritic shaft (d). The same bouton (b) is seen three sections away at the largest extension of the synaptic contact (arrow). Scales = A: 20 μm; B: 2 μm; C and D: 0.2 μm.

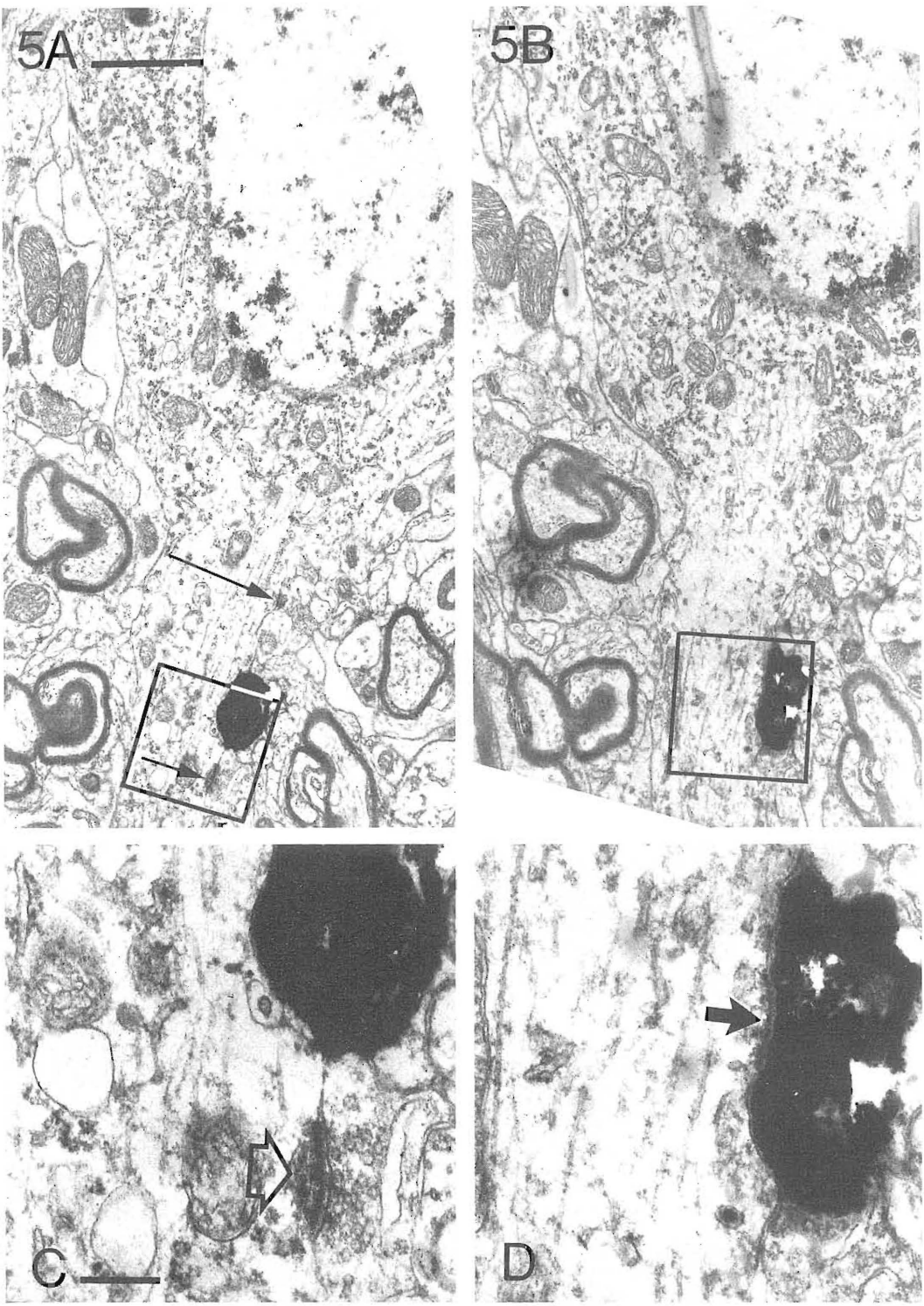


Fig. 5. A and B. Serial sections of a nonpyramidal cell (NC) which received two synapses from the Golgi-stained boutons of the double bouquet neuron in Figure 1. In addition to the impregnated bouton (framed area) two boutons (arrows) are seen to make type I synapses with the same neuron. One of these (open arrow) is shown at higher magnification in (C). In (D) the framed area in (B) is shown at higher magnification where the impregnated bouton establishes a type II synaptic contact (arrow). Note the more extensive postsynaptic density at the type I contact in (C). Scales = A and B: 1 μm; C and D: 0.2 μm.

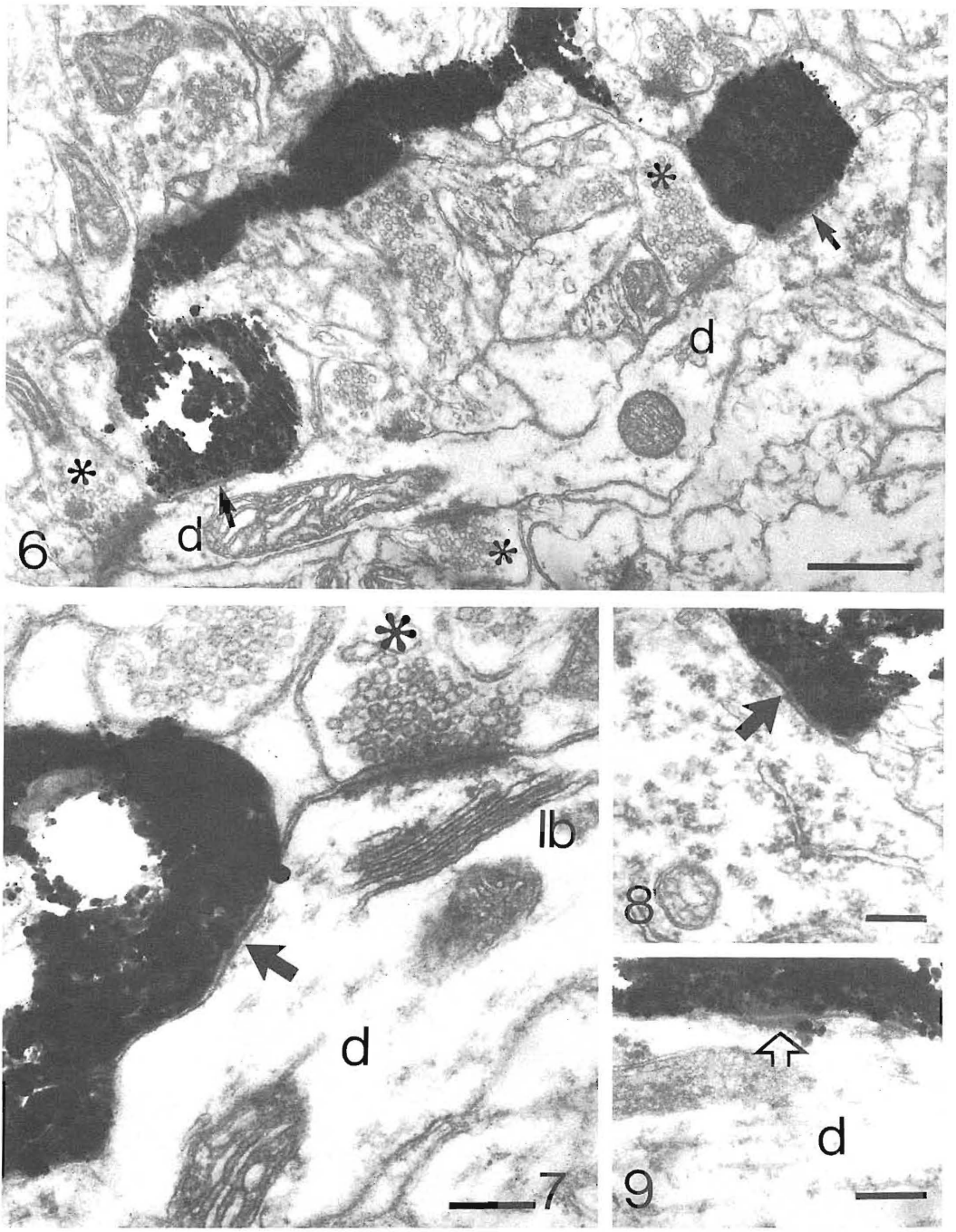
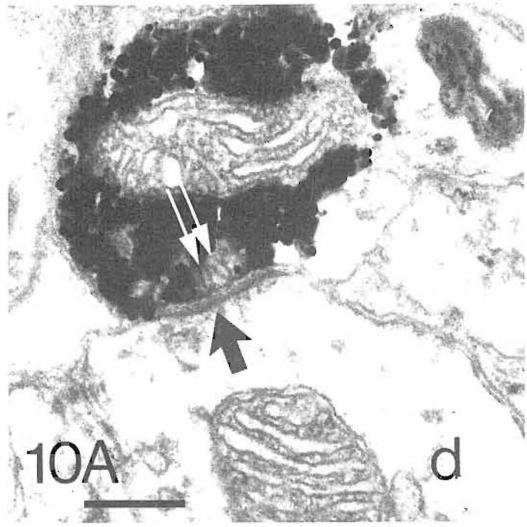


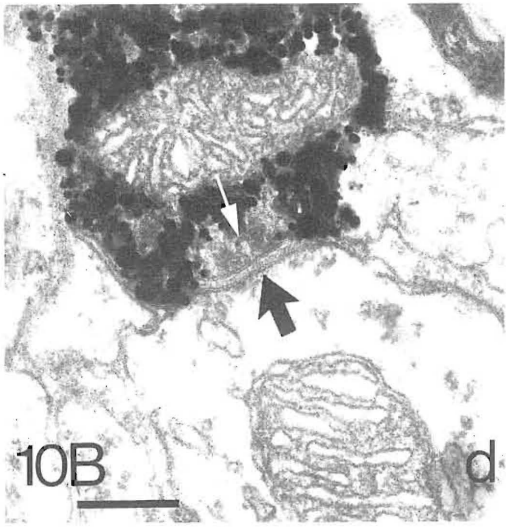
Fig. 6. Two boutons of the same axon collateral from the axon plexus of the cell in Figure 1 make type II synapses (arrows) with a nonpyramidal cell dendrite (d), which also receives three type I synapses from boutons containing spherical vesicles (asterisks). Scale = 0.5 μm.

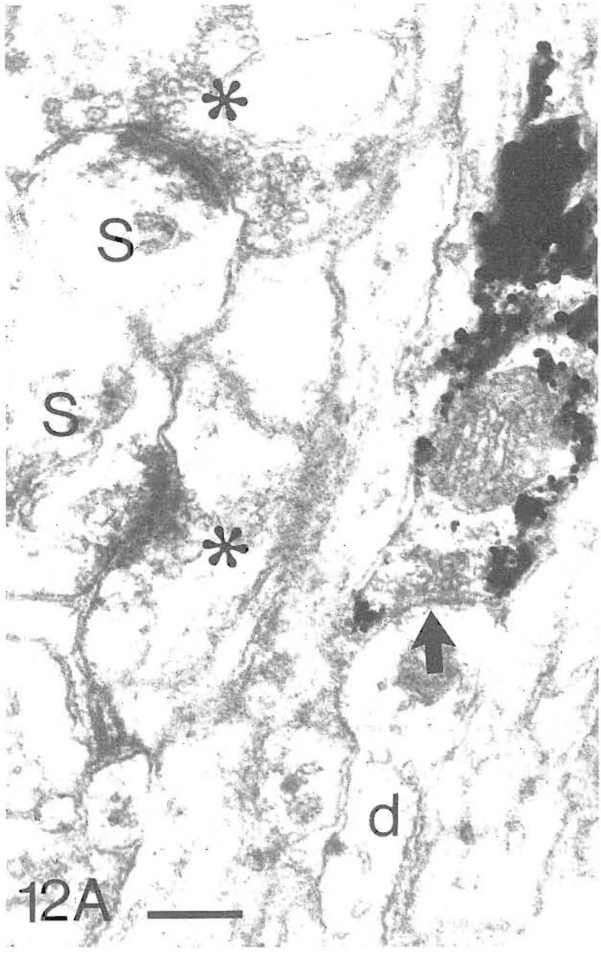
Fig. 7. A dendritic shaft (d) containing a lamellar body (lb) receives a type II synapse (arrow) from the bouton of the cell in Figure 1 and a type I synapse from a bouton with spherical vesicles (asterisk). Scale = 0.2 μm.

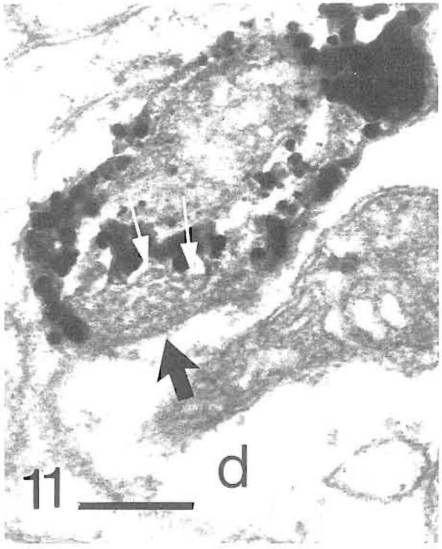
Fig. 8. Axosomatic synapse (arrow) established by the impregnated bouton of the cell in Figure 1 with the perikaryon of a nonpyramidal cell. Scale = 0.2 μm.

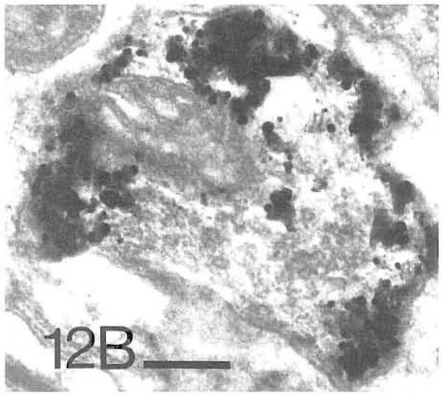
Fig. 9. Desmosomoid symmetrical attachment plaque (open arrow) between the impregnated bouton and a dendrite (d) with dense material accumulation on both sides. Scale = 0.2 μm.











Figs. 10–12. Golgi-impregnated boutons of double bouquet cells. The Golgi precipitate was partially removed from the boutons. Scales = 0.2 μm.

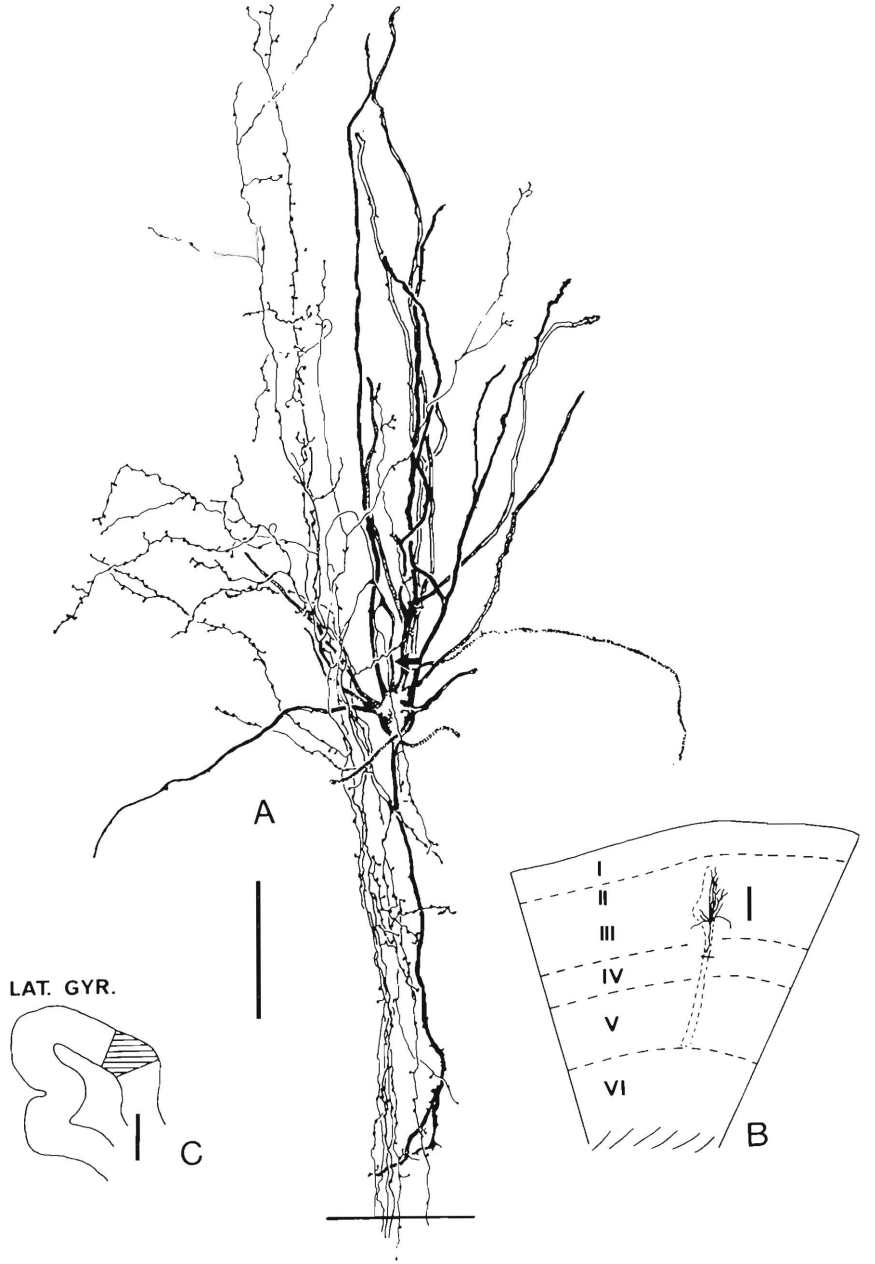


Fig. 13. Drawing of a cell with vertical axon bundle in layer III of area 18 in the cat's lateral gyrus. The axon is only partially drawn, as indicated with a horizontal line in A and B. Scales and labelling as in Figure 1. See electron microscopic data in Table 1.

Fig. 10. (A,B) Serial sections of an axodendritic synapse (large arrow) established by neuron No. 2. in Table 1. Note flattened synaptic vesicles (white arrows) and an electron-dense line in the synaptic cleft.

Fig. 11. A cluster of pleomorphic vesicles (white arrows) in a bouton of the same cell as in Figure 10. The bouton makes a synapse (arrow) with a dendritic shaft (d).

Fig. 12. (A,B) Two boutons of neuron No. 3. in Table 1. (A) One bouton contains small pleomorphic vesicles and establishes a type II synapse (arrow) with a dendrite (d). Two other boutons (asterisks) containing larger vesicles establish type I synapses with spines (S). (B) Small pleomorphic vesicles are revealed in a bouton of the same neuron after removal of the Golgi precipitate. Scales = 0.2 μm.

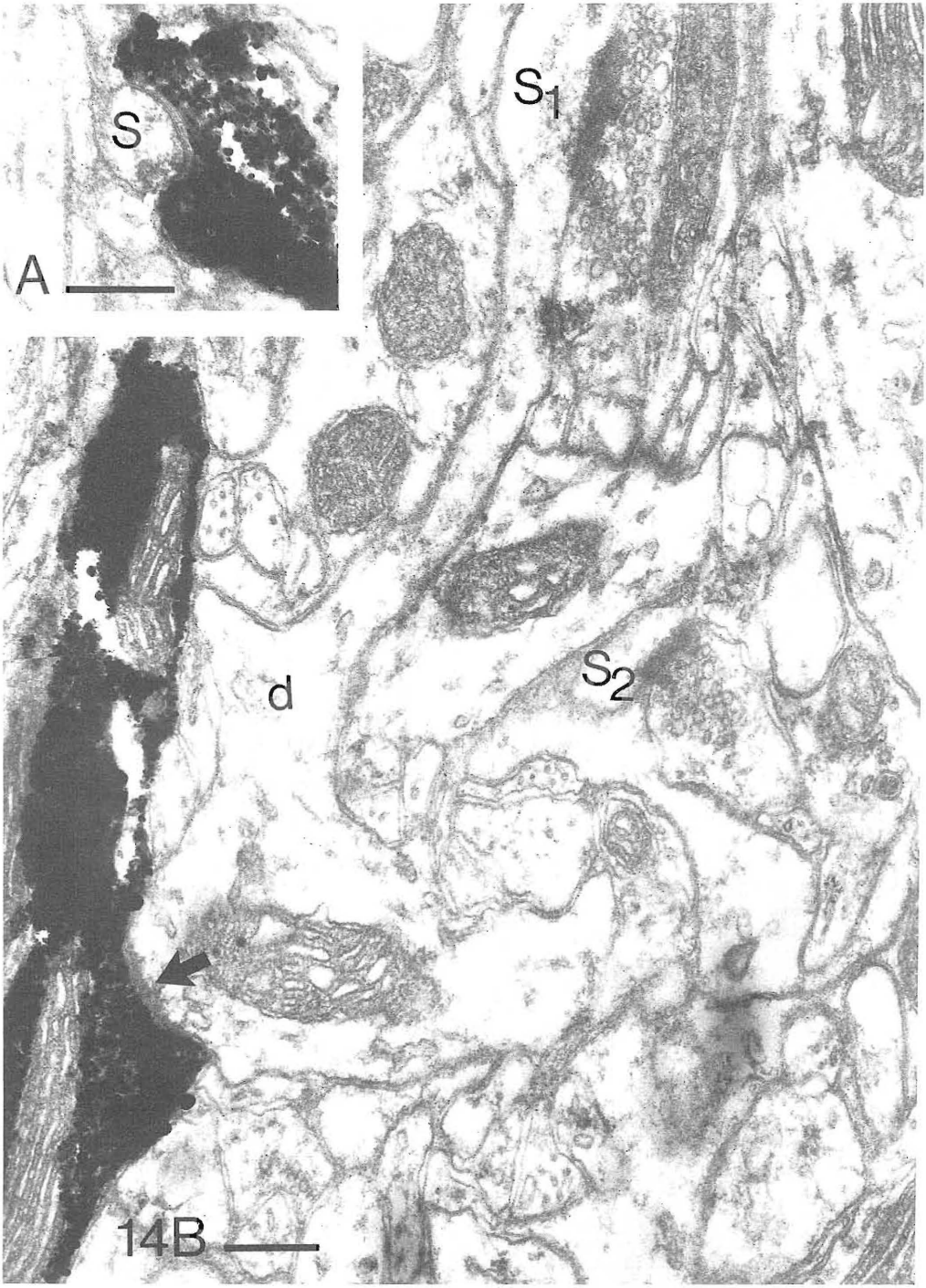


Fig. 14. (A) Type II synapse established by the cell in Figure 13 with the neck of a spine (S). In (B) a dendrite (d) emitting a spine (S_1) receives a synapse (arrow) from the impregnated bouton of the same cell. Because of the tangential plane of the section, the synaptic cleft is not seen here. Another spine (S_2) was traced in serial sections to the same dendrite. Both spines received synapses from boutons containing round vesicles. Scale = 0.2 μm.

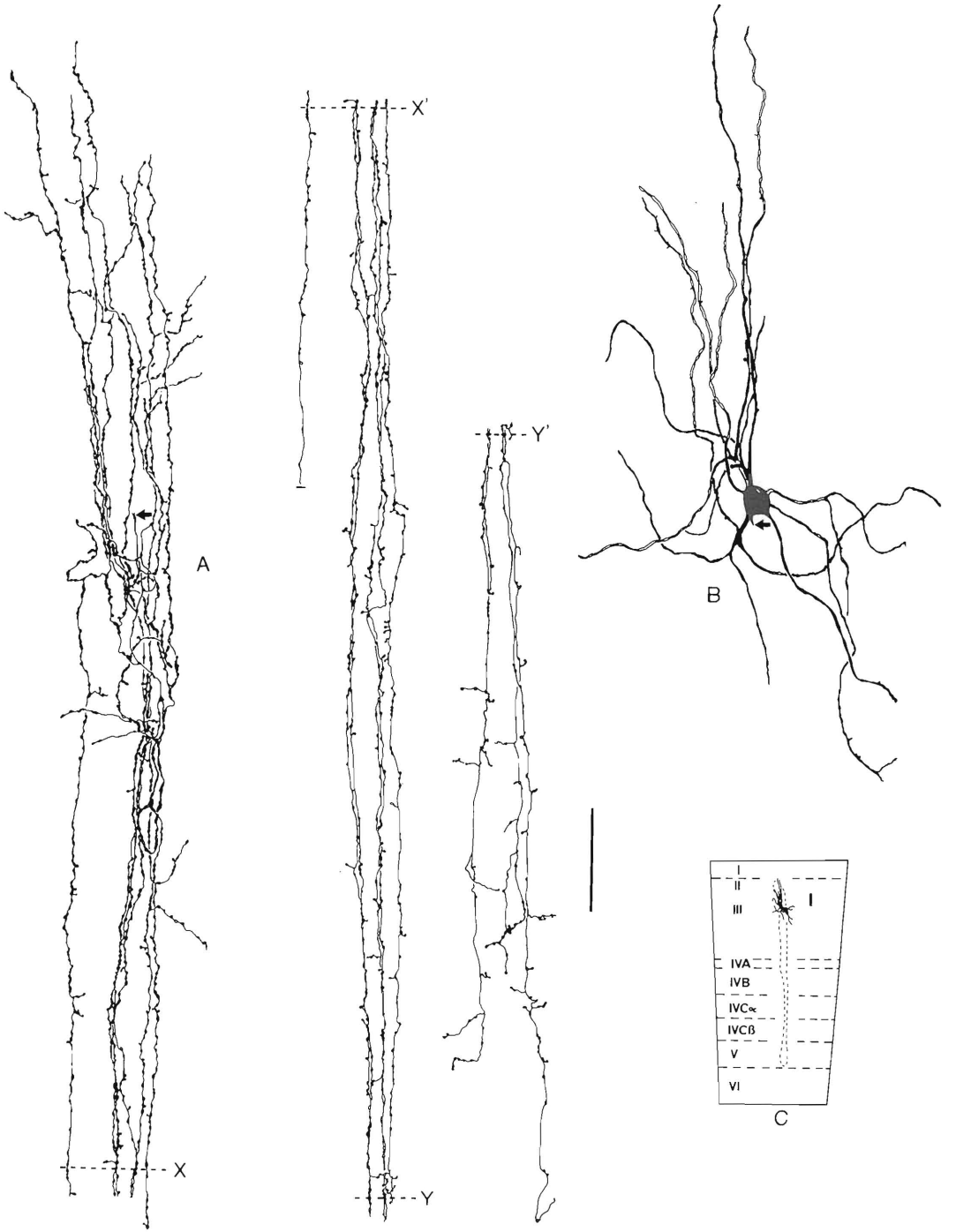
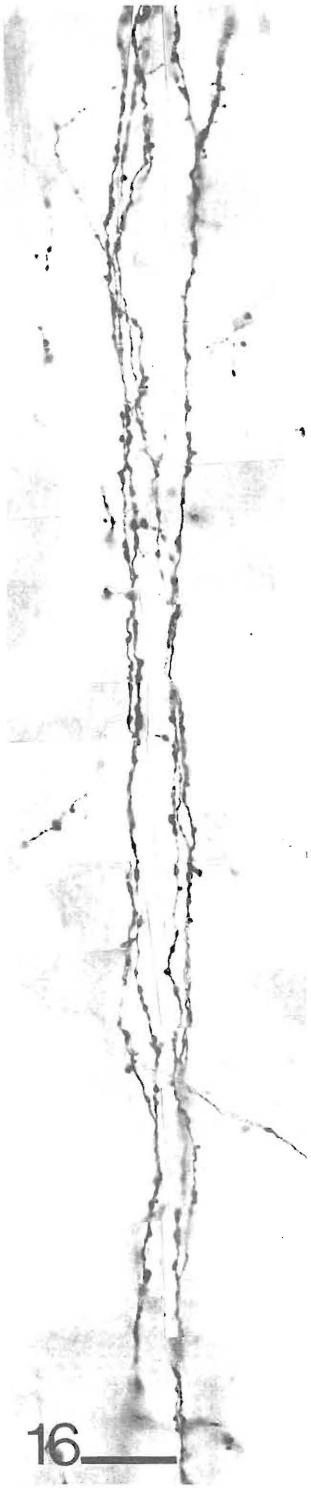
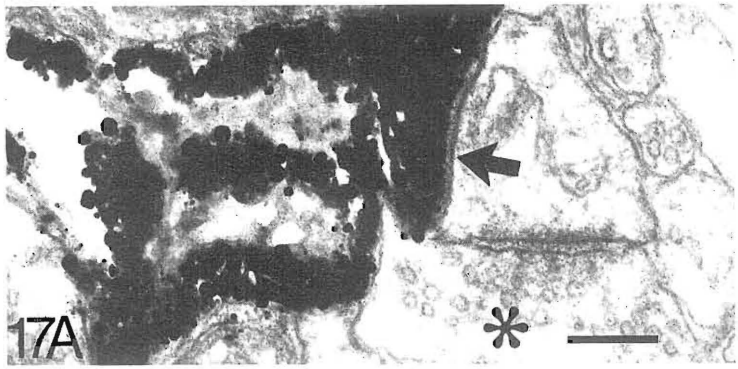
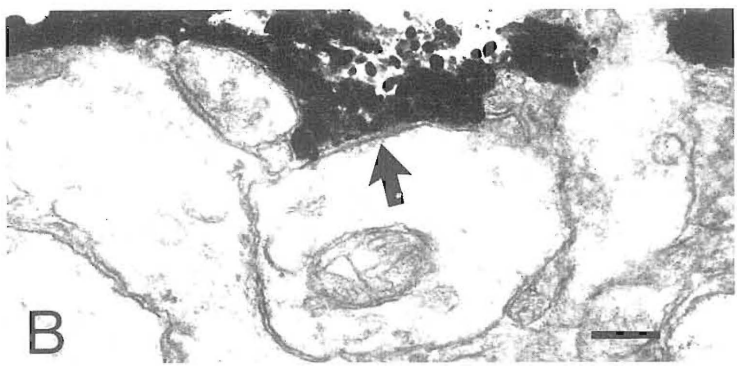


Fig. 15. Drawing of a Golgi-impregnated cell (B) with vertical axon bundle (A) in the striate cortex of the monkey. The axon bundle has been separated into three parts which should be continuous at the points marked by broken lines at X, X^1, and Y, Y^1. Arrow indicates the axon hillock. The position of the neuron in the cortex is shown in (C). See electron microscopic data in Table 1. Scales = A and B: 50 μm; C: 100 μm.







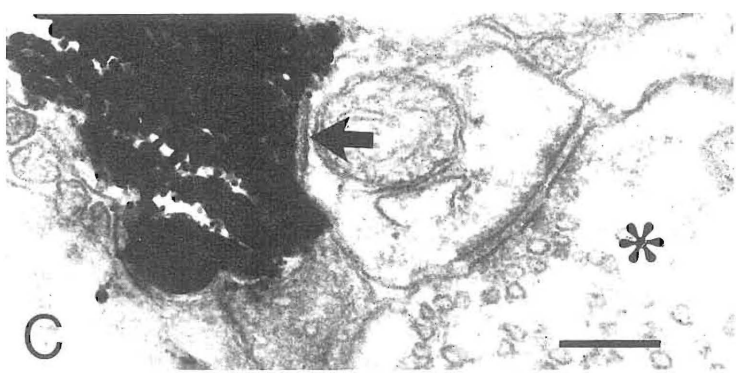


Fig. 16. Photomontage of part of the vertical axon bundle of the cell shown in Figure 15. Scale = 20 μm.

Fig. 17. Boutons from the axon plexus seen in Figure 16 are shown to make type II synapses (arrows) with a spine (A) and with dendritic shafts (B and C). The spine and the shaft in C receive a type I synapse from boutons containing round synaptic vesicles (asterisks). Scale = 0.2 μm.

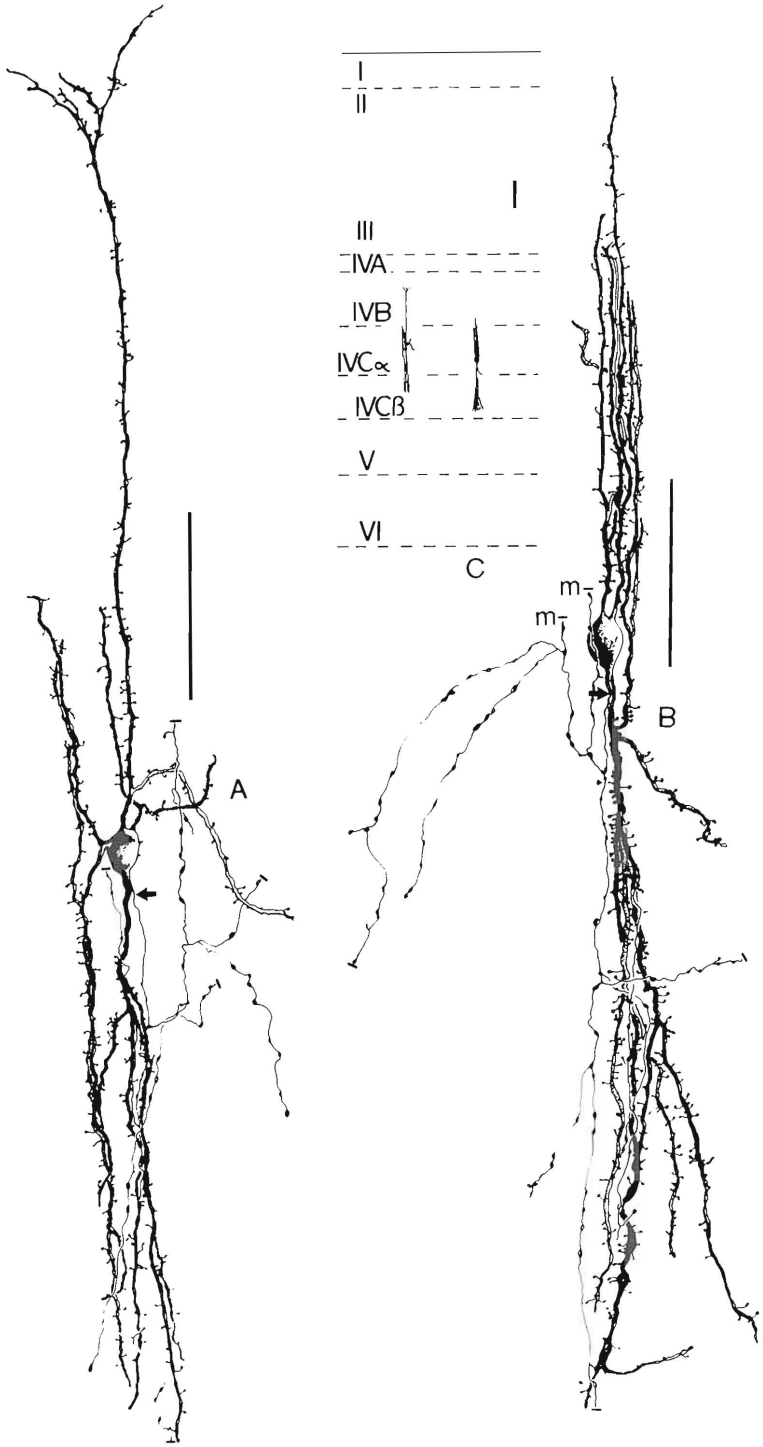


Fig. 18. Drawing of two spiny double bouquet cells in layer IV of the striate cortex of the monkey. The axon hillock is indicated by an arrow. The position of the cells is shown in C. Cell B was studied under the electron microscope (see Table 1), and two of its collaterals were found to become myelinated (m). Scale = A and B: 50 μm; C: 100 μm.

umns of about 20–50 μm in diameter, descending and ascending in layers IVB–C. They branch once or twice but the branches preserve a radial direction. The largest dendrites may terminate in a small tuft. Shortly after leaving the soma the dendrites become encrusted with typical long-stalked spines (Figs. 18, 19).

The axon usually originates from the lower pole of the perikaryon, takes a descending course, and emits several recurrent collaterals. So far we have been able to reveal only small parts of the axonal field. One reason for this, as later electron microscopic studies showed, is that many of the collaterals become myelinated.

Nine of the boutons of the impregnated part of the axon were studied under the electron microscope (Table 1). All made asymmetrical synaptic contacts with pronounced postsynaptic membrane specializations. Spines (Fig. 20A) and dendritic shafts (Fig. 20B) were the postsynaptic elements, the latter being more numerous.

DISCUSSION

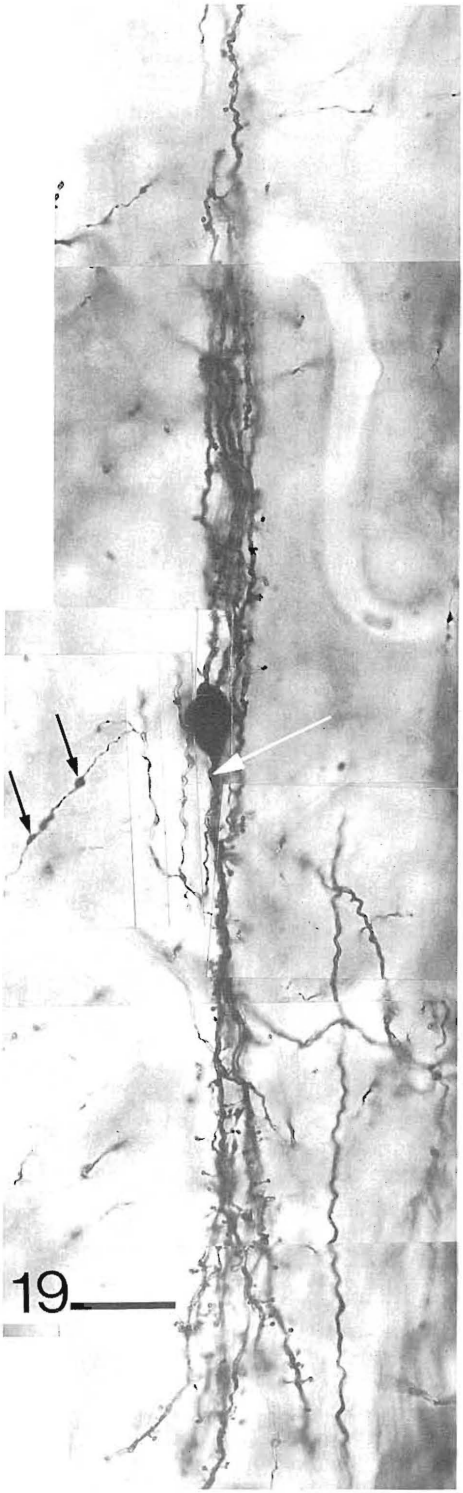
The neurons described in layer III of cat or monkey are identical to Ramón y Cajal's "cellules à double bouquet dendritique" (Ramón y Cajal, '11, Figs. 345, 348, 351), to some of the "cells with horse tail shape axon" described by Szentágothai ('73, '75, Szentágothai and Arbib '74), to type 3 cells of Jones ('75), and to Valverde's "cells with tight vertical bundles" (Valverde, '78). It is astonishing how the description given by Jones ('75) of cells in the monkey somatosensory cortex accurately depicts the features of cells found in our study in different cortical areas and in different species, which may indicate the ubiquitous role played by these neurons in cortical circuitry.

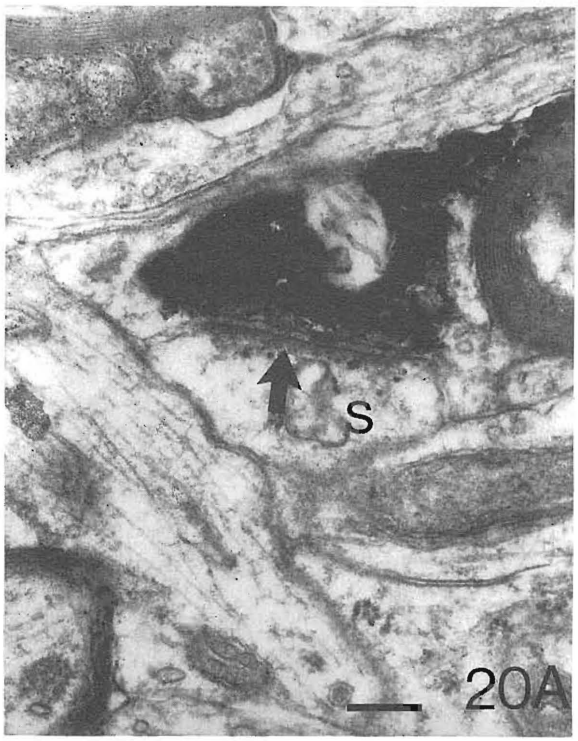
The naming of cortical neurons is a matter needing clarification (Mann, '79). The essential difference between neuron classes is the difference in input and output. As efforts to characterize the afferent and efferent connections of identified cortical neurons started only recently (LeVay, '73; Kelly and Van Essen, '74; Somogyi, '77, '78, '79; Parnavelas et al., '77; Fairén et al., '77; White, '78; Peters and Fairén, '78; Peters et al., '79; Gilbert and Wiesel, '79; Lin et al., '79), the data do not allow a comprehensive differentiation. Consequently classifications based either on light microscopic single neuron staining, such as the Golgi method, or on purely electron microscopic analysis remain in use. To avoid introducing yet another term in the present study we frequently use the name "double bouquet dendritic neuron" coined by Ramón y Cajal. It must be emphasized, however, that other neurons with totally different axon arborizations may have similar double bouquet dendritic fields. Fusiform neurons with similar smooth or sparsely spinous dendrites have been described in layer III of the visual cortex of the rat, but their axons form specialized terminal bouton rows synapsing on the axon initial segment of pyramidal cells (Somogyi, '77).

In the present study another double bouquet cell was described in layer IVC of the monkey's striate cortex. Although possessing a narrow dendritic cylinder like that of the layer III cells, it differed sharply from the latter by having densely spiny dendrites and making asymmetrical synaptic contacts. This means that its connections are different from the layer III cells, yet it is properly described as a double bouquet cell. This neuron is probably a variety of spiny stellate cell, since the latter also form asymmetrical synaptic contacts (LeVay, '73; Somogyi, '78; Somogyi and Cowey, unpublished observations in the monkey). The relatively small number of identified synapses established by this cell type does not yet allow the characterization of the postsynaptic neurons. The nine identified synaptic contacts do not indicate the involvement of apical dendrites among the postsynaptic structures. In previous Golgi studies (Valverde, '71; Szentágothai, '73; Lund, '73) on the monkey striate cortex no similar neuron was described, which shows the limitations of this method in sampling neuronal populations.

As the dendritic morphology is not entirely unique to the neuron type the vertical axon bundle was used as an additional criterion in this study, and the synapses and postsynaptic structures were also characterized to obtain information on the connectivity of the neurons.

Interest has been focused on this type of neuron because its morphology is well suited to distributing information in the vertical direction through layers II–V. This idea fits well with the results of physiological studies demonstrating a columnar organization of the visual (Hubel and Wiesel, '77) as well as other cortical areas (Mountcastle, '57; Asanuma, '75) in which the initial input is confined predominantly to layer IV and is distributed by local circuit interneurons to other layers in a narrow column. Such a scheme is supported by proposals that the vertical axon bundles of layer III double bouquet cells ter-





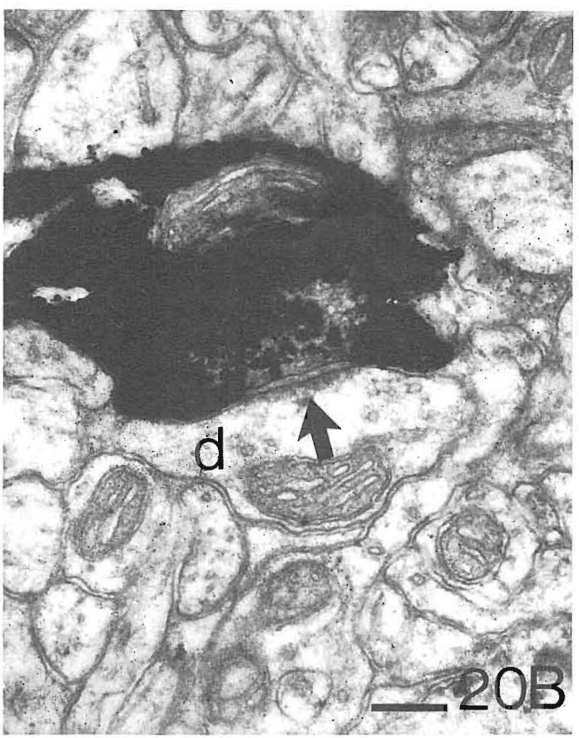


Fig. 19. Photomontage of neuron B in Figure 18. White arrow indicates the axon hillock. Boutons of this cell labelled by arrows are shown in Figure 20. Scale = 20 μm.

Fig. 20. Asymmetrical or type I synapses (arrow) formed by the Golgi-stained boutons labelled in Figure 19. In (A) the synapse is on a spine (S) and in (B) it is on a dendritic shaft (d). Scale = 0.2 μm.

minate on the spines of apical dendrites (Ramón y Cajal, '11; Colonnier, '66; Szentágothai, '73, '75, '78). This proposal could not be confirmed in the present study. In neither species were spines of apical dendrites a major synaptic target of double bouquet cells. On the basis of Golgi studies Valverde ('78) has also questioned whether the apical dendrites could be the postsynaptic targets.

Whether a cell exerts mainly inhibitory or excitatory action through its synapses is not possible to determine by morphological methods. However, there is converging evidence that in the cerebral cortex at least some of the boutons which form symmetrical synapses and contain pleomorphic vesicles are inhibitory. Glutamic acid decarboxylase, the synthetizing enzyme of γ-amino butyric acid (GABA), has been localized in these boutons in the cortex of both rat (Ribak, '78) and monkey (Ribak et al., '79). Furthermore, there is increasing evidence that GABA is an inhibitory transmitter in the cerebral cortex (Iversen et al., '71; Rose and Blakemore, '74; Sillito, '77; Tsumoto et al., '79). It is even more pertinent to the present study that fusiform cells with vertical dendrites have been shown to contain glutamic acid decarboxylase (Ribak, '78) and to accumulate H^3-GABA in layer III (see Emson and Lindvall, '79). This indicates, although it does not prove, that GABA is a transmitter in these neurons, and also possibly in the neurons described here in layer III.

In the monkey the synapses of the cell with vertical axon bundles were unambiguously and typically symmetrical or type II (Gray, '59). However, in the cat the synapses had more pronounced postsynaptic membrane specializations than the axosomatic synapses of pyramidal cells, or the synapses found on pyramidal cell axon initial segments in the same material. This indicates that synapses classified as type II or symmetrical in conventional electron microscopic material may not be homogeneous. When a subpopulation of synapses is separated, as in the present study through the Golgi staining, subtle differences can be repetitively observed. Compared to asymmetrical axospinous or axodendritic synapses the identified synapses with impregnated boutons had much smaller postsynaptic densities. After the partial removal of the Golgi precipitate the presence of pleomorphic and predominantly elongated synaptic vesicles provides further evidence that the boutons of layer III cells form type II synapses.

All nonpyramidal cells with smooth or sparsely spiny dendrites examined so far have been found to establish symmetrical or type II synapses (LeVay, '73; Parnavelas et al., '77; Somogyi, '77, 79; Somogyi et al., '79; Peters and Fairén, '78). It was suggested furthermore that all these neurons might be inhibitory (Peters and Fairén, '78). It follows that any synaptic interaction between them results in the inhibition of an inhibitory interneuron. Such interaction has been proposed to occur in the striate cortex of the cat to explain the discharge characteristics of cortical neurons excited by stimulation of the retina (Toyama et al., '77). It was found that for all cells an initial excitation was followed by a depression, probably mediated by ISPSs, which was rapidly succeeded by a rebound discharge, possibly as a result of inhibition of the first-order inhibitory interneuron. The interneuron with vertical axon bundles described in area 17 of the cat ideally fits the role of the second-order inhibitory neuron because: (i) the characteristics of its synapses suggest it is inhibitory; (ii) it frequently makes synapses with perikarya and dendrites of other nonpyramidal cells which could also be inhibitory; (iii) it is situated in upper layer III, and is thus unlikely to receive input from specific afferents to cause the first-order inhibition.

To provide more direct evidence for this hypothesis it will be necessary to reveal the postsynaptic neurons in more detail together with their axons, perhaps through a method which makes it possible to localize intracellular HRP in cells postsynaptic to Golgi-stained interneurons in the cortex (Somogyi et al., '79).

Some of the dendritic shafts postsynaptic to layer III double bouquet cells could be the basal dendrites of pyramidal cells or side branches of apical dendrites. In two cases in area 18 of the cat, spiny dendrites, very likely of pyramidal cell origin, were the postsynaptic target. Spines which in layer III probably belong to pyramidal cells are a significant postsynaptic structure in area 18. In the monkey the proportion of spines was greater than in the cat and the number increased in layer III, where the majority can belong only to pyramidal cells. In conclusion, besides nonpyramidal neurons demonstrated in area 17 of the cat, certain parts of pyramidal cells also receive synapses from the descending axons of double bouquet cells.

It is difficult to assess whether the differences in the nature of postsynaptic structures between the two species and between the two cortical areas of the cat are significant. The number of boutons studied is not sufficient for

a thorough statistical analysis. Nevertheless for the two most extensively studied neurons in area 17, the difference between the cat and the monkey probably indicate a difference in the efferent synaptic relationships of these particular neurons in the two species. This implies that caution is necessary in extrapolating from one species to another when synaptic relationships of morphologically similar neurons are concerned.

The idea of apical dendrites being the postsynaptic target of double bouquet cells has been attractive since it seemed to explain the narrow, strictly radial course of the axon. It suggested a climbing type of interaction. This could not be confirmed in the present study since the radial beaded axons do not seem to follow any particular postsynaptic structure. Multiple synapses on the same dendrite were rarely observed. However, since the vertical axon plexus has a very high bouton density it is likely that the axon of one double bouquet cell encounters the dendrites or perikaryon of the same postsynaptic neuron several times.

Instead of the climbing type of connection other factors could also explain the small diameter of the axon cylinder. If one thinks of not one neuron but assemblies of double bouquet cells with vertical axon bundles, these axons together form dense "curtains" from layer II down to layer V. The shape of these axon curtains, as viewed from the surface of the cortex, may vary according to which double bouquet cells are active but the edges would be extremely sharp because of the small lateral spread of the axons. Such axonal assemblies may contribute to causing differences in the activity of neighboring neuron populations in a slablike fashion.

Cortical interneurons with smooth or sparsely spiny dendrites have been studied to establish their postsynaptic targets. Peters and Fairén ('78) have reported six neurons in the rat striate cortex which established synapses with practically all structures known to receive symmetrical synapses. Thus pyramidal cell perikarya, axon initial segments, apical dendritic shafts, as well as nonpyramidal cell dendrites and soma were found among the postsynaptic structures. In contrast to this diverse collection of terminal sites another local circuit interneuron, the axo-axonic cell, was found to make synapses exclusively with the axon initial segments of pyramidal cells in the rat, cat, and monkey (Somogyi, '77, '79; Somogyi et al., '79). This is a very high degree of specificity with regard to the postsynaptic targets, which is echoed by the spatial specificity of layer III double bouquet cell, whose overall specific geometry is unsurpassed among known cortical local circuit interneurons. Especially in the monkey but also in the cat the axon bundles occupy a narrow cylinder between layers I and VI. Regarding the postsynaptic structures these neurons are not absolutely specific but neither pyramidal cell perikarya nor axon initial segments were found among the 120 synapses examined so far and an apical dendritic shaft was possibly found in only one case. This clearly shows selectivity and preference for certain postsynaptic elements and emphasizes yet again the intricate wiring of the cerebral cortex.

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