

Double Bouquet Cells

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1. Introduction

The name *double bouquet cell* has been used for certain cortical neurons since Ramón y Cajal's vivid description of the "cellule à double bouquet dendritique" in diverse cortical areas of man (Ramón y Cajal, 1911). The term originally embraced several forms of neurons with somewhat different features but which had in common a characteristic bitufted dendritic arborization with its long axis oriented radially, i.e., at right angles to the pia. After being overlooked for several decades, interest in these neurons was rekindled by Colonnier (1966) and Szentágothai (1969, 1971) who called particular attention to the bundles of radially oriented axon collaterals apparent in Ramón y Cajal's drawings.

These neurons deserve attention because their bundles of radially oriented, translaminar axon collaterals are well suited to distributing information in the vertical direction through layers II–V, and such an arrangement could be related to the results of physiological studies which demonstrate a columnar organization of the visual (Hubel and Wiesel, 1977) as well as other cortical areas (Mountcastle, 1957; Asanuma, 1975).

Following Colonnier's investigation (1966), double bouquet cells were reported in many species and in various cortical areas (see below), but the term *double bouquet cell* or *bitufted cell* has been used of neurons which had only the dendritic arborization or the axon resembling the neurons in the original de-

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scription. First of all, therefore, we should attempt to define more precisely the characteristics of neurons we call *double bouquet cells*, especially as the original description may have included several types of neuron (Ramón y Cajal, 1899, 1900, 1911), as pointed out recently (Peters and Regidor, 1981).

2. Characteristics Defining Double Bouquet Cells

The term *double bouquet cell* will be applied to neurons with their perikarya in layers II and III and which have an axon traversing layers II–V, usually in a tight bundle consisting of varicose, radially oriented collaterals. Some of these main collaterals may run outside the bundle and sometimes the bundle is not compact. As the position of the perikaryon may vary considerably within layers II–III, only the axons of deeper double bouquet cells give a prominent ascending plexus reaching layer II to match the descending plexus to layer V. The branching of the main axon into ascending and descending collaterals always takes place within a 50- to 80- μ m stratum about 50–100 μ m from the perikaryon. These axons often but not invariably arise from neurons which have a radially elongated soma and a lower and upper dendritic spray, originating from two or three main shafts as depicted in Ramón y Cajal's drawings (1899, 1900). However, qualitatively similar dendritic features may be possessed by neurons which have a very different type of axon, e.g., the axo-axonic or chandelier cells (Somogyi, 1977; Szentágothai, 1978; Fairén and Valverde, 1980; Somogyi *et al.*, 1982; Peters *et al.*, 1982).

Therefore, in the present chapter we use the axon as a primary identifying feature of double bouquet cells and deal with previously reported cells only briefly if the axon was not sufficiently documented or was clearly of a different type.

Double bouquet cells are also different from bipolar neurons which have sparser, longer dendrites, smaller perikarya, and establish different synaptic contacts (Peters and Kimerer, 1981; also see Chapters 6 and 11).

3. Double Bouquet Cells in Different Species

3.1. Man

It is the historical importance of Ramón y Cajal's description (1899, 1900, 1911) of double bouquet cells in the human cortex which prompts a survey in descending evolutionary order. He illustrated the presence of these neurons in various areas of the very young infant's cortex. The neurons presented in his figures (1899, Figs. 8, 11 E, F; 1900, Fig. 5F) fulfill the criteria proposed above because the axons have a radial course. The axonal features are particularly clear in his Fig. 8, but the vertical bundles are wider than those of neurons described later. He also described the vertical axons as traversing the entire cortex, which is not so in other species, e.g., "Ces filaments sont si longs qu'ils

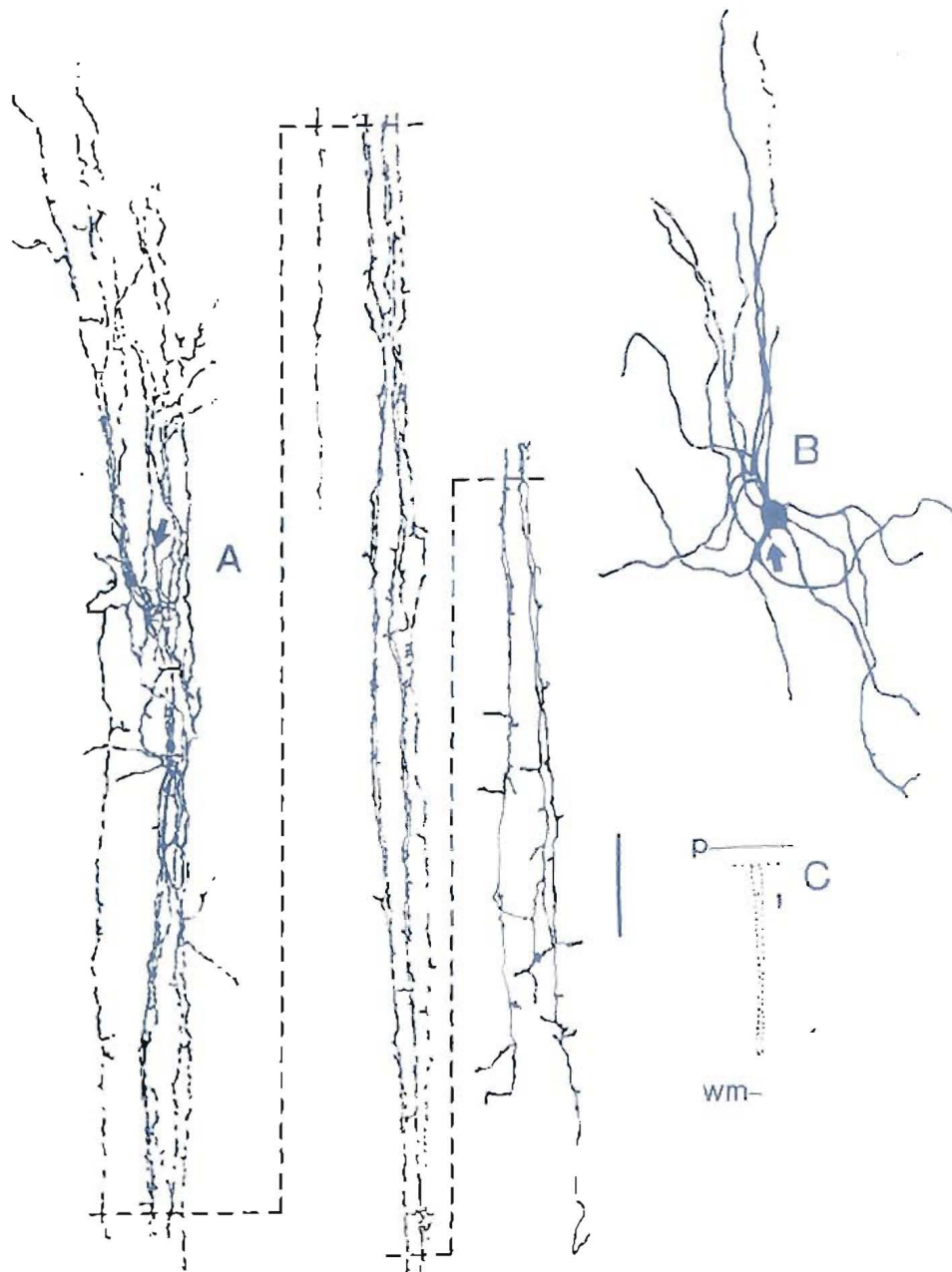


Figure 1. Drawing of the axonal (A) and the dendritic (B) arborization of a double bouquet cell in the striate cortex of the rhesus monkey. Arrows indicate the same axon initial segment. The descending axon plexus is continuous along the dashed line. (C) The position of the perikaryon (dot) and the axon bundle (broken outline) is indicated between the pia (p) and the white matter (wm). Scales: (A, B) 50 μ m; (C) 100 μ m. Modified from Somogyi and Cowey (1981) with permission.

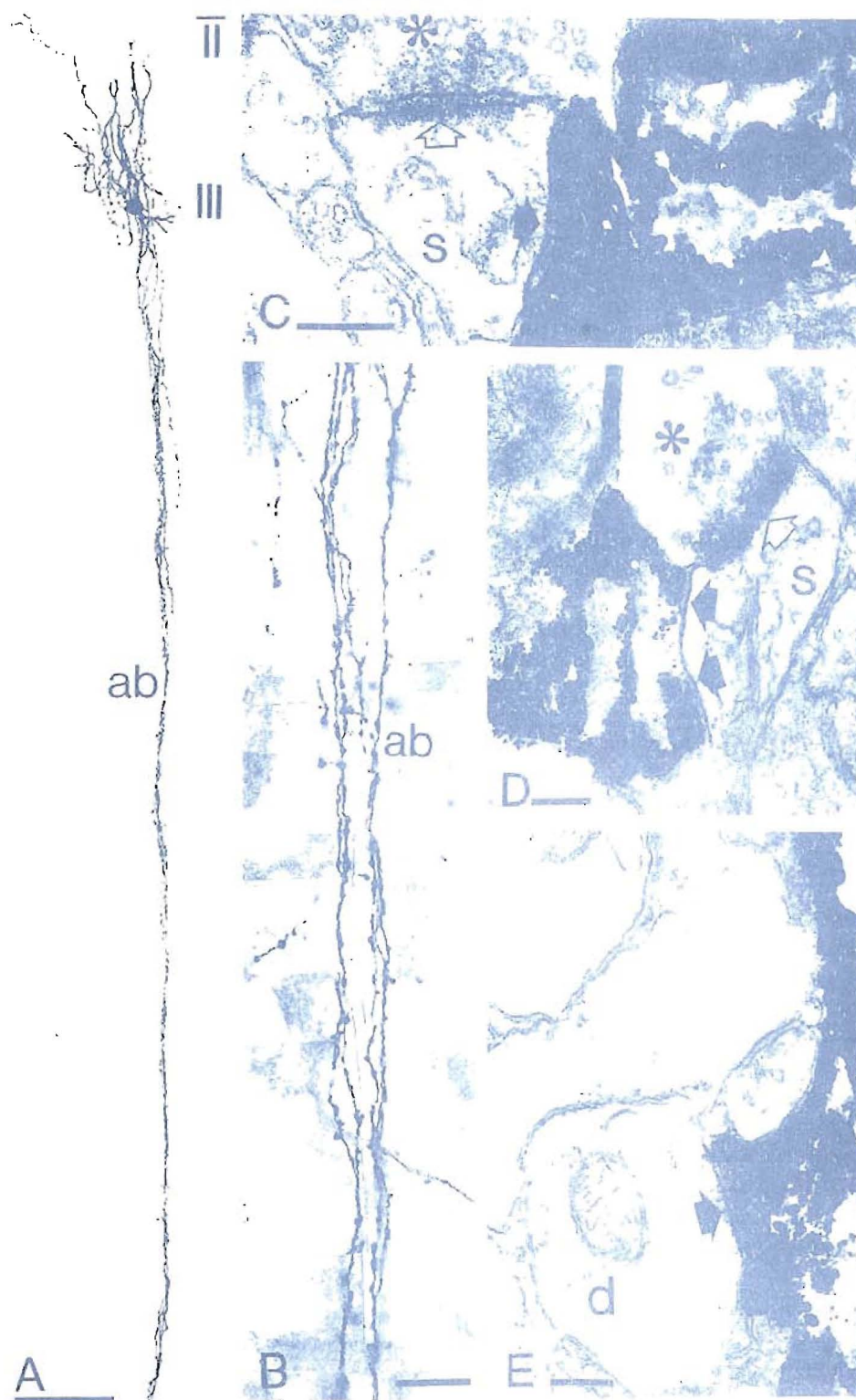
peuvent s'étendre à toute la hauteur de l'écorce" (1911, p. 541). But without further studies on specimens from adults, it is not possible to establish whether the differences are due to the young age of the subjects or other factors. However, age differences will not easily explain why they are so much commoner in man, e.g., "Le nombre des corpuscules à double bouquet dendritique est extraordinairement grand chez l'homme" (1911, p. 539).

3.2. Other Primates

Compared with the number of papers dealing with the morphology of neurons in the cortex of monkeys studied by Golgi methods, descriptions of double bouquet cells with radially disposed axons are rare. They have been described in the striate and prestriate visual cortex (Szentágothai, 1971, 1973, 1978; Valverde, 1978; Tömböl, 1978; Somogyi and Cowey, 1981), in areas 1, 2, and 3 of the somatosensory cortex (Jones, 1975), and in area 5 of the parietal cortex (Jones, 1975). The most detailed account of the axon has been given by Jones (1975) whose description can be applied to these neurons wherever they have been reported. The axon usually emerges from the base of the perikaryon (Fig. 1) or from the lower main dendritic shaft (Fig. 2A), and takes a descending course. Rarely it ascends immediately (Valverde, 1978). At 30–50 μm from its origin, it divides into thin smooth collaterals which themselves branch to form a spray of 3 to 10 main radial branches (Figs. 1, 2A). A distinct features of the axon is that "the thin stem branch which forms each ascending or descending arcade suddenly becomes very much thicker" (Jones, 1975). The thick radial branches are crowded with bulbous enlargements and terminal boutons on the end of thin stalks (Figs. 1, 2B). The radial branches together form tight fasciculi 20–50 μm in diameter (Fig. 2B) resembling a horse tail (Szentágothai, 1973, 1978), but wider axonal fasciculi have also been reported (Valverde, 1978). The main branches give rise to short collaterals in layers III and II, especially in the region of the dendritic arborization, and sometimes a few collaterals in layer V (Figs. 1, 2A).

The soma is 10–18 μm in diameter, round or ovoid, and bears smooth or sparsely thorny dendrites which are also predominantly oriented in a radial direction (Figs. 1, 2A) but which may occasionally take a lateral course. The bitufted character of the dendritic arborization is not always prominent in the monkey (Fig. 1) and more or less round dendritic fields have also been described (Jones, 1975; Valverde, 1978).

Figure 2. (A) Drawing of a double bouquet cell in the striate cortex of the rhesus monkey. The perikaryon and the dendrites are in layer III but the axon bundle covers layers II–V. (B) Photomontage of the axon bundle (ab) of a similar cell in layer III of monkey striate cortex. (C–E) Electron micrographs of symmetrical synaptic contacts (solid arrows) established by Golgi-impregnated boutons of the double bouquet cell shown in (B), with spines (s) and a dendritic shaft (d). The spines receive asymmetrical synaptic contacts (open arrows) from boutons containing round synaptic vesicles. Scales: (A) 100 μm ; (B) 20 μm ; (C–E) 0.2 μm . Drawing courtesy of T. F. Freund. (B), (C), and (E) modified from Somogyi and Cowey (1981) with permission.



Few studies describe cells with long radial axons in this species. Colonnier (1966) mentions them, and although Szentágothai (1973) describes some similar cells in the somatosensory and visual cortex of the cat, his neurons with perikarya in layer IV may be of different types. Double bouquet cells with vertical axons have been described in the suprasylvian gyrus of young cats (Norita and Kawamura, 1981). In a previous study, we provided a detailed description of double bouquet cells in the striate (area 17) and peristriate (area 18) cortex of the cat (Somogyi and Cowey, 1981). The axonal features are similar to those in the monkey, described above, but with some differences. Thus, the main radial branches have fewer bulbous swellings and give out short collaterals especially in layer III and to a lesser extent in layer V (Figs. 3, 6A; see also Figs. 1 and 2 in Somogyi and Cowey, 1981). The radial axon plexus is usually less tightly arranged than in the monkey and may be 50–150 μm in diameter.

In the cat, the perikarya and dendrites are invariably of the characteristic bitufted type (Figs. 3B, E, 4B, 6A) and the main orientation of the dendrites is unambiguously radial. Although some proximal dendrites initially follow a lateral course, they soon bifurcate and divide into ascending and descending branches (Figs. 3, 4B, 6A). In cross-section, the radially disposed dendritic array rarely exceeds 100 μm .

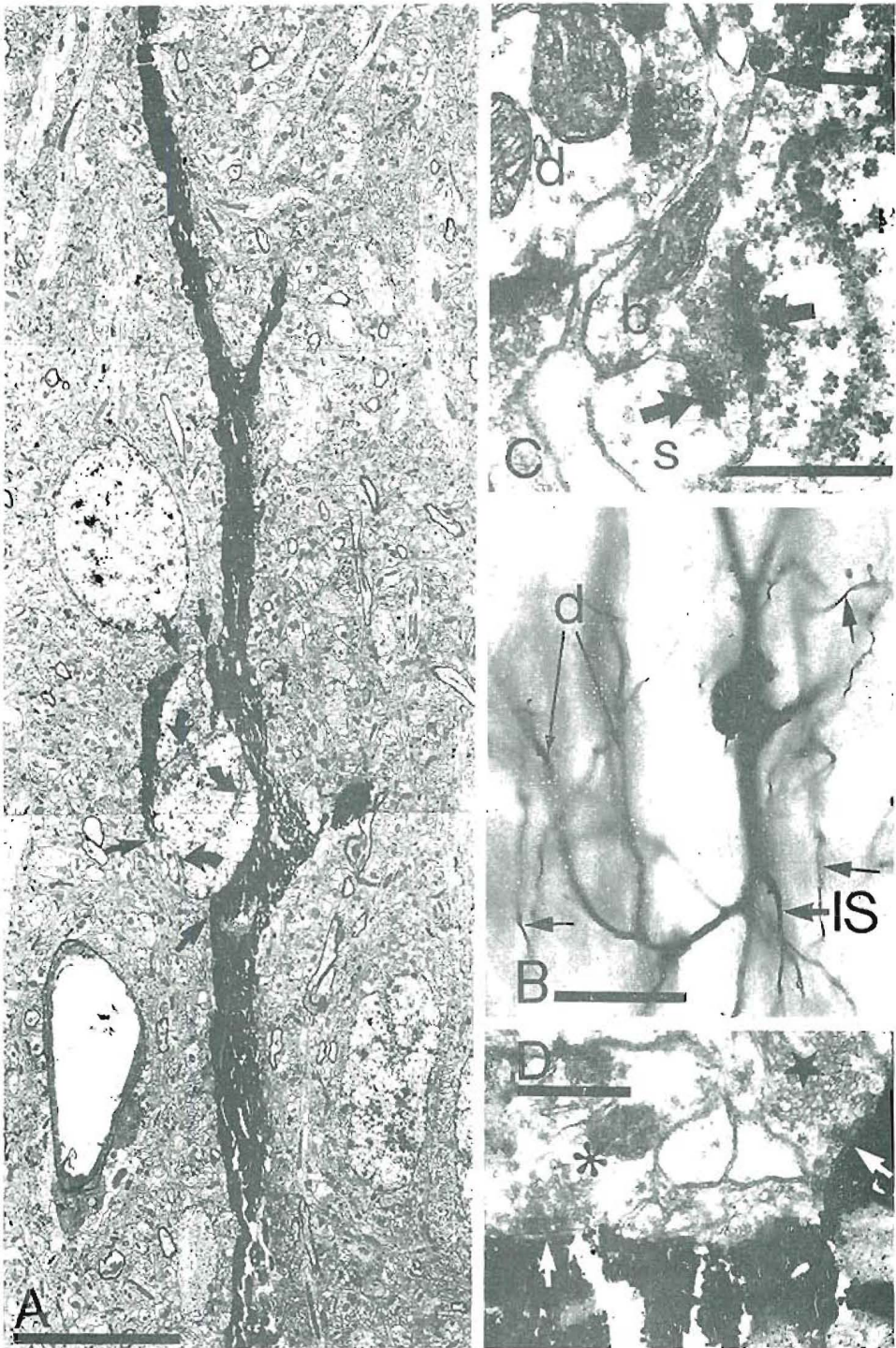
On the basis of dendritic features, similar cells have recently been described in the visual cortex (Peters and Regidor, 1981), but the axons of these neurons seem somewhat different perhaps because of the young ages of the animals.

3.4. Rodents

With regard to axonal features, similar cells have not been described in rodents despite numerous morphological investigations. However, there are several reports of neurons which in either their dendritic features or to a lesser extent their axonal characteristics, may be the forerunners of double bouquet cells in cat and primates. Lorente de Nó (1922) provided drawings of neurons from the parietal cortex of the rat which have their perikarya in layers III or IV and their axon running from layers I to V, with several main vertical collaterals and profuse arborizations in layers III and V (his Figs. 7A, B). The main dendrites are also radially oriented, but both the dendritic and the axonal arborizations are more diffuse than described above in the cat and monkey. In other studies on the visual cortex of mouse (Valverde, 1976) and rat (Peters and

Figure 3. Drawings of Golgi-impregnated double bouquet cells in the striate cortex of cat. The axon drawn in (A) belongs to the perikaryon and dendrites drawn separately in (B). Arrows indicate the axon initial segment. (C) and (F) show the position of the neurons in the cortex, with the territory of the axon outlined. The position of the cortical area in which they are located is shown in the lateral gyrus in (D). The dendritic arborization of a second neuron is shown in (E). Scales: (A, B, E) 50 μm ; (C, F) 200 μm ; (D) 2 mm.





Fairén, 1978), smooth and sparsely spined stellate cells in layer III were shown to send a long vertical axon collateral to layer V, but no axon fascicles have been described (see Chapter 13). The efferent synaptic connections of the neurons in the rat show both similarities and differences to the double bouquet cells of the cat and monkey (Peters and Fairén, 1978; Somogyi and Cowey, 1981).

Bitufted cells have been described in the rat on the basis of dendritic features (Feldman and Peters, 1978) but the majority are outside layers II–III. Thus, they probably belong to other classes of neurons.

In conclusion, the double bouquet cells of cats and monkeys, which have radial axon bundles, most closely resemble and may be related to certain smooth and sparsely spiny stellate (multipolar or nonpyramidal) neurons which are present in rodents in layers II–III and have descending axons. From rodent to primate, there is a clear but still unquantified progression to a tighter axonal plexus, with less branching and more boutons concentrated on the main radial collaterals. There is suggestive but incomplete evidence that the axonal branches traverse all layers in man.

4. The Fine Structure of Double Bouquet Cells

A qualitative description of gross characteristics detectable with the light microscope is a necessary first step in identifying neurons and speculating on their function, but such descriptions have limitations when used to compare neurons in different species or to develop our ideas about their function. Until we can study the physiological and pharmacological properties of individual neurons that are marked and subsequently studied structurally [e.g., the basket cells studied by Martin *et al.* (1983) and Kisvárdy *et al.* (1983)], the best procedure is to analyze the ultrastructure, and in particular the synaptic connections, of Golgi-impregnated cells first identified in the light microscope. This was the procedure adopted for double bouquet cells in the cat and monkey by Somogyi and Cowey (1981), with the following results.

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Figure 4. (A) Electron micrograph of a Golgi-impregnated double bouquet cell in layer III of the striate cortex of the cat, also shown in light micrographs (B) and Fig. 6A. The perikaryon is partially impregnated. Precipitate is not present between straight arrows and is also absent from the deep nuclear invaginations (curved arrows) and from the nucleus. (B) Light micrograph of the same neuron as in (A). Note the fusiform perikaryon with a lower and upper dendritic trunk, the axon initial segment (IS), recurving dendrites (d), and varicose axons (arrows) which ascend toward layer II. (C) Electron micrograph of the perikaryon at the border of the impregnated and nonimpregnated parts (long arrow). The perikaryon receives an asymmetrical synaptic contact (thick arrow) from a bouton (b) containing round vesicles and also making a synapse (thick arrow) with a spine (s). An adjacent dendrite (d) receives similar synaptic contacts. Note the large number of free polysomes in the cytoplasm. (D) Branching point of the dendrite of the same neuron, receiving synapses (arrows) from a bouton with round vesicles (star) and another with small pleomorphic vesicles (asterisk). Scales: (A) 10 μm ; (B) 25 μm ; (C, D) 0.5 μm .

4.1. Characteristics of the Perikarya and Dendrites in the Cat

For the present account, one neuron was examined in the striate cortex of the cat (Fig. 4) using methods described earlier (Somogyi, 1978). The neuron was not densely or uniformly impregnated, which provided an opportunity to study some of the internal detail. The eccentrically placed nucleus had deep invaginations of the nuclear membrane so that it formed several separated profiles (Fig. 4A). Free polysomes were present in conspicuously high density in the thin rim of the cytoplasm (Fig. 4C). Mitochondria were present in moderate numbers, and the Golgi apparatus was located in the perikaryon and proximal dendrites. These are common features of cortical neurons and we have detected nothing unique about the internal features of the perikaryon of the double bouquet cell.

The perikaryon received few synaptic contacts, and mostly from boutons containing flattened, pleomorphic vesicles. But occasionally boutons containing round synaptic vesicles and making asymmetrical synaptic contacts were also found (Fig. 4C). These two basic types of bouton also contacted the dendritic shafts and thorny appendages (Fig. 4D), but on the dendrites more boutons with round vesicles were seen.

The axon initial segment was also studied in this and another neuron of the same type but afferent synaptic contacts on this position of the neuron were never observed.

The above features are similar to those of some other types of identified nonpyramidal neurons with smooth or sparsely spiny dendrites (Peters and Fairén, 1978; Peters *et al.*, 1982; Peters and Kimerer, 1981; Somogyi *et al.*, 1982), and so far no qualitative feature has been found which would make it possible to recognize double bouquet neurons without Golgi impregnation.

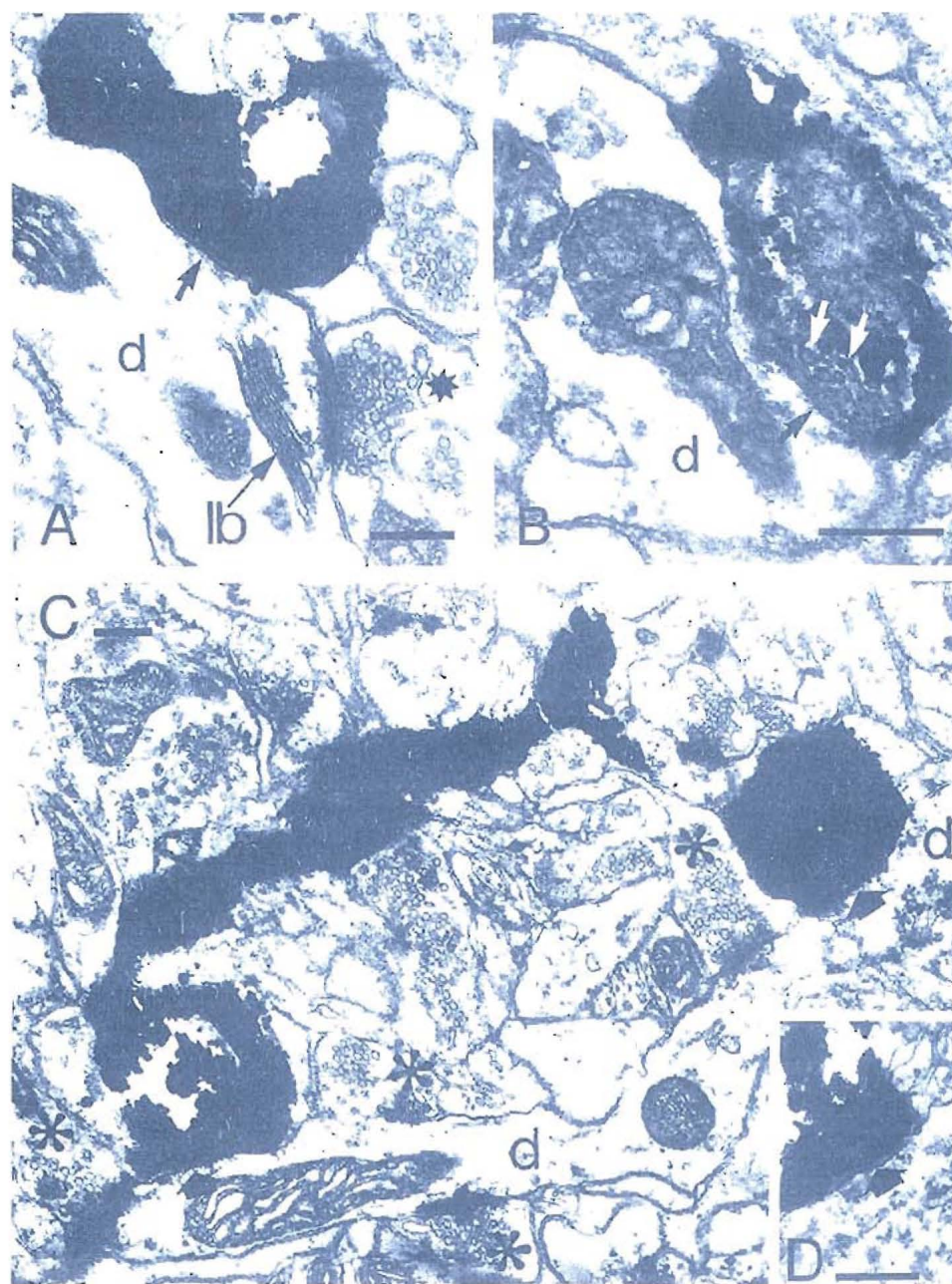
Since there are indications that different types of cortical interneurons differ in their postsynaptic targets, we analyzed the synaptic connections made by axon terminals of double bouquet cells (Somogyi and Cowey, 1981).

4.2. Efferent Synaptic Connections in Cat and Monkey

4.2.1. Characteristics of Double Bouquet Cell Boutons and Synapses

In our previous study (Somogyi and Cowey, 1981), identified Golgi-impregnated axon collaterals were followed in serial sections. In electron micrographs, the synapses formed by the impregnated boutons were compared to

Figure 5. Electron micrographs of Golgi-impregnated synaptic boutons originating from double bouquet cells in cat striate cortex. The boutons shown in (A), (C), and (D) belong to the neuron shown in Figs. 4 and 6A, while the one shown in (B) belongs to the neuron in Figs. 3A and B. (A) A smooth dendrite (d) containing a lamellar body (lb) receives a symmetrical synaptic contact (arrow) from the impregnated bouton and an asymmetrical contact from a bouton containing round vesicles (star). (B) After partial removal of the Golgi precipitate, flattened, pleomorphic vesicles (white arrows) are seen in a bouton making symmetrical synaptic contact (arrow) with a dendrite. (C) Multiple synaptic contacts (arrows) established by a collateral with the same dendrite which also receives numerous asymmetrical synaptic contacts from boutons with round vesicles (asterisk). (D) Symmetrical axosomatic synapse established on the perikaryon of a nonpyramidal neuron. Scales: (A-D) 0.25 μ m. Modified from Somogyi and Cowey (1981) with permission.



synapses established by unstained boutons with the same postsynaptic target (Figs. 2C, D, 5A, C) as well as to adjacent synapses in the same section.

In the cat, double bouquet cells form symmetrical, type II (Gray, 1959), synaptic contacts (Fig. 5). As compared to symmetrical axosomatic synapses received by pyramidal cells, the postsynaptic membrane thickening appears more pronounced in some cases (Fig. 5C). The thickness of the membrane specialization naturally depends on the plane of the section, but it never equaled the thickness of asymmetrical axospinous or axodendritic postsynaptic specializations (Fig. 5). Apart from the criterion of postsynaptic membrane density, synapses were also identified by the electron-dense cleft material which occasionally contained an additional electron-dense line.

In the monkey, one double bouquet cell was stained in a similar way. Seventeen synapses were identified and all were typical symmetrical, type II synapses (Figs. 2C–E).

The silver chromate precipitate had been partially removed from the boutons of two cells in the cat, enabling us to study the synaptic vesicles (Somogyi and Cowey, 1981). The vesicles were flattened or pleomorphic (Fig. 5B). Occasionally, large dense core vesicles were also present. This pattern of vesicles corresponded closely to that found in unstained boutons in the same material, where symmetrical membrane specialization and pleomorphic vesicles occurred together.

4.2.2. Electron Microscopy of Postsynaptic Structures

The form and distribution of postsynaptic structures are summarized in Table I which shows a difference between cat and monkey and also between areas 17 and 18 in the cat. However, it is not clear whether the differences represent anything other than sampling artifacts. Of 66 boutons studied in area 17 of the cat, 57 terminated on small or medium-sized dendritic shafts (Figs. 5A–C) which had no particular orientation. Some of these dendrites were traced in serial sections and they were never found to give rise to spines. In more than half of the sections showing an impregnated bouton from a double bouquet cell, the dendrite also received one or more unimpregnated synaptic boutons (Figs. 5A, C), the majority of which established asymmetrical contacts. Six impregnated axosomatic synapses (Figs. 5D) were identified on four nonpyramidal neurons, three of which were fusiform and had dendrites extending from the upper and lower pole of the perikaryon. These neurons were identified as nonpyramidal since their perikarya received both asymmetrical and symmetrical synapses from unstained boutons (Colonnier, 1968; Parnavelas *et al.*, 1977). The perikarya, axon initial segments, or main dendritic shafts of pyramidal cells were never encountered among the postsynaptic structures. Unfortunately, it was not possible to identify the parent cell of the three spines postsynaptic to double bouquet cells in area 17 of the cat.

One neuron was studied from area 18 of the cat. Although spines postsynaptic to the axon were more frequent (26%), the majority of the synapses were still established with dendritic shafts. One of these shafts was about 2 μm thick and followed a radial course, both characteristics of an apical dendrite. The shaft itself received only symmetrical synapses but two spines bearing asymmetrical synapses emerged from its surface. A further shaft in synaptic contact with an

Table 1. Type and Distribution of Structures Postsynaptic to Double Bouquet Cells

Animal	Cortical region	Cell No.	Layers examined by electron microscopy	Number of identified boutons contacting:			
				Spines	Dendritic shafts	Perikarya of nonpyramidal neurons	Total
Monkey	Area 17	1 (Figs. 1, 2B-E)	III-IV	14 (40%)	21 (60%)	—	35 (100%)
Cat	Area 17	2 (Figs. 4, 5A, C, D, 6A)	III-IV-V	1	47	5	53
		3 (Figs. 3, 5B)	III	1	7	1	9
		4	III	1	3	—	4
			Total	3 (4.6%)	57 (86.4%)	6 (9%)	66 (100%)
Area 18	5	—	III	5 (26%)	14 (74%)	—	19 (100%)

impregnated bouton gave rise to two spines bearing asymmetrical synapses. The remaining dendrites were all small or medium size and had no particular orientation. They received remarkably few unstained boutons, suggesting that some or all of them are different from those described in area 17.

One double bouquet neuron from layer III of the striate cortex of the monkey was reprocessed for electron microscopy. Its boutons innervated many more spines than the boutons in the cat (Table I), and more of the spines were in layer III than in layer IV. Each of these spines received one asymmetrical synapse from an unstained bouton containing round synaptic vesicles (Figs. 2C, D) in addition to the symmetrical synapse established by the impregnated bouton. The spines had long thin stalks which made it impossible to identify their parent dendrites with certainty. As in the cat, the postsynaptic dendritic shafts (Fig. 2E) had no particular orientation, were of small or medium diameter, and about one in five made asymmetrical synaptic contacts with nonimpregnated boutons.

We were particularly interested to see whether one postsynaptic element receives many synapses from one double bouquet cell or whether the innervation is much more frugal. In both cat and monkey, two reconstructions were made from serial sections. It was clear that even the most closely adjacent boutons usually innervate different structures. Neighboring synapses on the same dendrite were rarely observed (Fig. 5C). However, two of the postsynaptic perikarya received two adjacent synapses from the same double bouquet cell.

4.2.3. Discussion of Efferent Synaptic Connections

It has been suggested that the vertical axon bundles of layer III double bouquet cells terminate mainly on the apical dendrites of pyramidal cells (Ramón y Cajal, 1911; Colonnier, 1966; Szentágothai, 1973, 1975, 1978; Jones, 1975). This proposal could not be substantiated by our electron microscopic study (Somogyi and Cowey, 1981), and from an examination of Golgi material Valverde (1978) had previously questioned whether the apical dendrites were the predominant postsynaptic targets.

In our material, many of the postsynaptic dendritic shafts in area 17 of the cat probably belong to nonpyramidal neurons as shown by the fine structural characteristics and synaptic input of the dendrites. All four postsynaptic perikarya also belonged to nonpyramidal cells. However, some of the dendritic shafts postsynaptic to layer III double bouquet cells could be basal dendrites of pyramidal cells or side branches of apical dendrites. In area 18 of the cat, two spiny dendrites were the postsynaptic target. In layer III, the spines probably belonged to pyramidal cells and they may be a significant postsynaptic target in area 18. In the monkey, the proportion of spines receiving input from double bouquet cells was greater than in the cat and there were fewer in layer IV than in layer III, where the majority can belong only to pyramidal cells. So, as well as the nonpyramidal neurons demonstrated in area 17 of the cat, certain parts of pyramidal cells also seem to receive synapses from the descending axons of double bouquet cells.

The idea that apical dendrites are the principal postsynaptic target of double bouquet cells was attractive, since it seemed to explain the narrow, strictly radial course of the axonal branches and suggested a climbing type of interaction. This

could not be confirmed in our investigation, since the radial beaded axons appear not to follow any particular postsynaptic structure. Several synapses on the same dendrite were rarely observed. However, since the double bouquet cell has a dense vertical axon plexus and high bouton density, its axon is likely to encounter different dendrites of the same postsynaptic neuron.

It is probably unwise to stress the differences in the nature of the postsynaptic targets in monkey and cat and the differences between areas 17 and 18 in the cat. Nevertheless, the dissimilarities between cat and monkey for the most extensively studied neurons in area 17 are sufficiently great to suggest that real differences may exist.

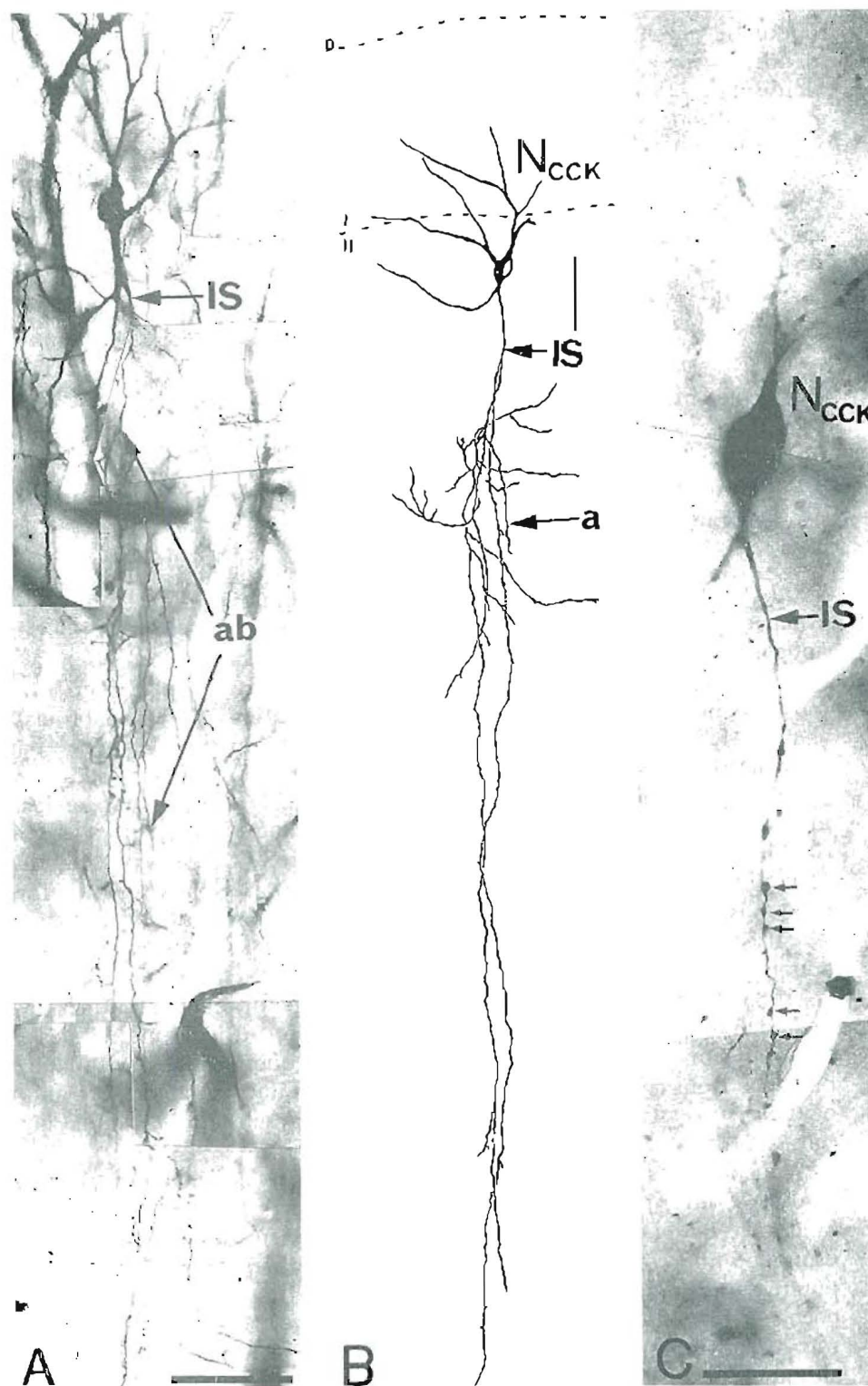
5. Possible Transmitters Used by Double Bouquet Cells

5.1. Comparison of Double Bouquet Cells with Neurons Containing Glutamic Acid Decarboxylase

In our analysis of double bouquet cells from the monkey, all identified synapses were unquestionably symmetrical, i.e., type II. Although the postsynaptic membrane specialization was more pronounced in the cat, it fell far short of the thickening seen at asymmetrical synapses. Consequently, even in the cat too the contacts made by double bouquet cells are also most reasonably classified as type II. Furthermore, the presence of pleomorphic vesicles in the boutons indicates that these boutons form type II synapses. Glutamic acid decarboxylase (GAD), the enzyme synthesizing γ -aminobutyric acid (GABA), has been localized in boutons which form symmetrical synaptic contacts in the cortex of monkey (Ribak *et al.*, 1979) and cat (Somogyi *et al.*, 1983b). Thus, it is possible that the boutons of double bouquet cells also contain GAD and that they therefore use GABA as their transmitter.

It is even more pertinent that neurons of similar size and shape to double bouquet cells in layers II and III of cat visual cortex contain GAD (Somogyi *et al.*, 1983b). Some of these neurons were also Golgi impregnated, which revealed their dendritic arborization (Somogyi *et al.*, 1983b). At least one cell in layer II had very similar features to those of double bouquet cells. Unfortunately, few of the neurons containing GAD have had a Golgi-impregnated axon, and none of those successfully impregnated had axons characteristic of double bouquet cells.

The comparisons described above are necessarily limited and we have therefore used other approaches in an attempt to determine the transmitters used by double bouquet cells and other interneurons. In one line of experiments, using immunocytochemical methods, we have characterized neurons containing putative transmitters, including various peptides. In another approach, we have been studying the selective uptake and transport of [3 H]-GABA in combination with Golgi impregnation. Both procedures have provided information which, although indirect, can help to elucidate the function of these neurons.



5.2. Some Cholecystokinin Immunoreactive Neurons Are Similar to Double Bouquet Cells

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Following pilot experiments which indicated that some nonpyramidal cells in layers II–III contain cholecystokinin (CCK)-immunoreactive material, we studied the striate cortex of cats using the unlabeled antibody enzyme method (Sternberger *et al.*, 1970). An antiserum specific for the COOH terminus of CCK8 (Dockray, 1980) was applied to colchicine-injected striate cortex in a procedure which allows detailed visualization of immunoreactive neurons (Somogyi and Takágyi, 1982). In some fortunate examples, the neurons were revealed almost in their entirety, and one such cell is shown in Figs. 6B and C. The shape of the perikaryon, the disposition of the dendrites, and the origin and course of the axon are similar to those of Golgi-impregnated double bouquet cells described above. The axon descends from layer II to layer V. Although it is far more difficult to follow immunoreactive axon collaterals than Golgi-impregnated ones, two long descending radial branches (Fig. 6B) exhibiting bulbous enlargements (Fig. 6C) and boutons on short stalks could be identified.

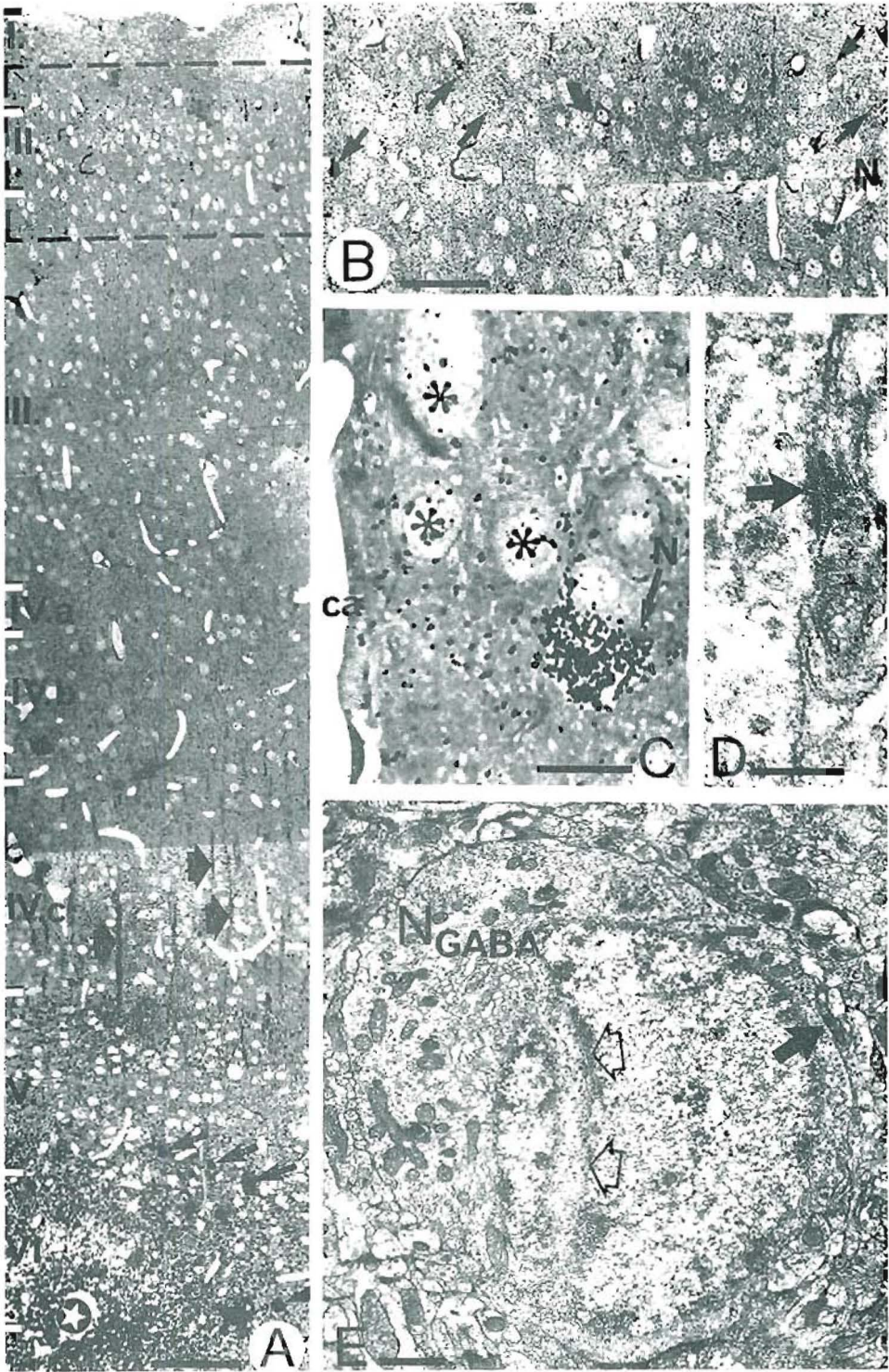
These findings raise the possibility that some double bouquet cells contain CCK. Earlier immunocytochemical studies also showed vertically arranged CCK-immunoreactive dots in the cortex (Emson and Hunt, 1981). However, it is already clear that many CCK-immunoreactive neurons in cat visual cortex are not double bouquet cells. For example, neurons with different axons have been encountered and many CCK-immunoreactive perikarya can be found in layers V–VI, which do not contain double bouquet cells.

Clearly, further studies are necessary to determine the afferent and efferent synaptic relations of CCK-immunoreactive neurons, which appear morphologically similar to double bouquet cells. In addition, more detailed visualization of CCK neurons is necessary using the recently developed combined Golgi impregnation–immunocytochemical staining of the same neuron (Freund and Somogyi, 1983; Somogyi *et al.*, 1983b) before it can be established that some or all double bouquet cells contain CCK.

5.3. [³H]-GABA-Accumulating Neurons in Layers II and Upper III Project to Deeper Layers

From another line of experiments in which we studied the distribution of selectively labeled neuronal perikarya following [³H]-GABA injections into different layers of the visual cortex (Cowey *et al.*, 1981; Somogyi *et al.*, 1981, 1983a), evidence was obtained that GABA may be a transmitter used by double bouquet

Figure 6. (A) Photomontage of a Golgi-impregnated double bouquet cell in the striate cortex of cat, also shown in Fig. 4. The axon initial segment (is) originates from the lower dendritic trunk and gives a descending axon bundle (ab). (B) Drawing of CCK-immunoreactive neuron (N_{CCK}) with a descending axon plexus (a) in the striate cortex of cat. The axon initial segment originates from the lower dendritic trunk. The perikaryon is at the border of layers I and II. (C) Light micrograph of the same neuron as in (B). Note the varicose (small arrows) descending axon. Scales: (A, B) 50 μ m; (C) 25 μ m.



cells. It was noticed in the monkey that when the injection site was in the deep layers (V and VI), in addition to the labeled neurons always present around the injection track, another group of neurons appeared in layers II and upper III (Figs. 7A, B). These neurons lay directly above the injection site but could not have been labeled by local uptake of [^3H]-GABA in the region of their perikarya because there were few or no [^3H]-GABA accumulating neurons below them in layers IV ab and lower III, even though these layers contain numerous labeled neurons when [^3H]-GABA is directly injected into them. The only simple explanation for the heavy labeling of these neurons in layers II and upper III is that they accumulated the labeled substance by retrograde axonal transport from their terminals within the injection site in the deeper layers. This is supported by the simultaneous presence of strongly labeled fiber bundles (Fig. 7A) passing radially through layer IV. The neurons were small to medium in size, and fusiform or elongated (Figs. 7B, C). We studied the fine structural characteristics of these [^3H]-GABA-accumulating neurons (Somogyi *et al.*, 1981) and found that they have an eccentric nucleus with clumps of chromatin, and invaginations of the nuclear membrane (Fig. 7E). The neurons had large numbers of free polysomes and received asymmetrical synapses on the soma (Fig. 7D). These features are characteristic of nonpyramidal cells.

The identity of the [^3H]-GABA-accumulating neurons becomes apparent when they are Golgi impregnated. An example is shown in Fig. 8. We have few examples of such neurons, but all were nonpyramidal cells with smooth dendrites, very similar in dendritic and somatic features to double bouquet cells. Unfortunately, we have still not succeeded in impregnating the axons of the neurons that are Golgi-stained and have accumulated [^3H]-GABA, so that there is not unequivocal evidence that these neurons are double bouquet cells. Nevertheless, the results show that there is a population of aspiny neurons in layers II and upper III, where double bouquet cells occur, which selectively accumulate [^3H]-GABA through their descending axons and which are therefore likely to be GABAergic. The labeled axon bundles suggest that they could be the double bouquet cells. Interestingly, such a population of GABA-accumulating neurons which can be labeled in the upper layers following injections in layer V and VI, has been found in the rat (Cowey *et al.*, 1981), cat (unpublished observation),

Figure 7. (A–C) Light micrographs of semithin sections (1 μm) processed for autoradiography and cut from a Golgi-impregnated, gold-toned thick section of the striate cortex of the monkey. (A) The cortex was injected with [^3H]-GABA through a capillary nearly perpendicular to the plane of the section. Labeled neurons selectively accumulating [^3H]-GABA (small arrows) are present around the injection track (star) which is at the border of the white matter and layer VI. The framed area in layers II and upper III contains another group of labeled neurons and is shown in (B). Thick arrows indicate strongly labeled fiber bundles passing through layer IV. (B) High magnification of framed area in (A) showing neurons that have accumulated [^3H]-GABA (small arrows) and unlabeled gold-toned pyramidal neurons (curved arrows) in layers II and upper III. One labeled neuron (N) is shown at even higher magnification in (C) among unlabeled neurons (asterisk). A capillary (ca) is indicated. (D, E) Electron micrographs of a neuron (N_{GABA}) in layer II from the area shown in (A) and (B), and which was shown by light microscopic autoradiography to accumulate [^3H]-GABA from the deeper layers. The neuron has a deeply indented nucleus (open arrows) and receives an asymmetrical synaptic contact (solid arrows) on the perikaryon. Scales: (A) 100 μm ; (B) 50 μm ; (C) 10 μm ; (D) 0.2 μm ; (E) 1 μm .

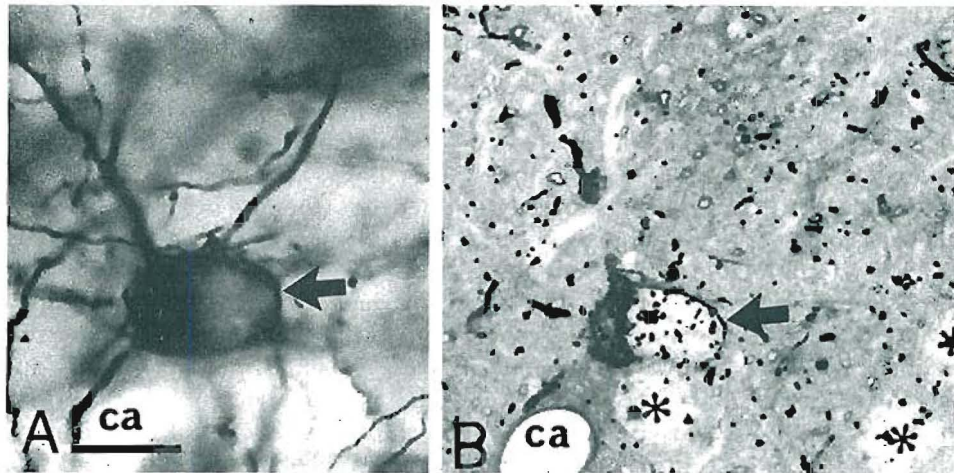


Figure 8. (A) Light micrograph of a Golgi-impregnated gold-toned neuron with smooth dendrites in layer II of monkey prestriate cortex (area 18, V II). This neuron was situated directly above an [^3H]-GABA injection track in layer VI. (B) Semithin section ($1\ \mu\text{m}$) cut from the perikaryon (arrow) of the same neuron and processed for autoradiography. This neuron selectively accumulated [^3H]-GABA when compared to neighboring unlabeled neurons (asterisk). A capillary (ca) serves as reference in the two micrographs. Scales: $10\ \mu\text{m}$. Courtesy of Z. F. Kisvárdy.

and monkey (Somogyi *et al.*, 1981), so they probably form a basic feature of cortical circuitry.

5.4. Conclusions on the Possible Transmitter(s) of Double Bouquet Cells

The types of synapses formed by double bouquet cells and their other morphological features suggest that they use GABA as a transmitter (Somogyi and Cowey, 1981; Somogyi *et al.*, 1981). This idea is compatible with results obtained from the immunocytochemical demonstration of GAD and from the autoradiographic demonstration of [^3H]-GABA following its selective uptake (see Sections 5.1 and 5.3). GABA is an inhibitory neurotransmitter in the cerebral cortex (Krnjevic and Schwartz, 1967; Krnjevic, 1974), which would mean that double bouquet cells with vertical axon bundles are inhibitory. In addition, CCK may be present in some double bouquet cells, either alone or together with other transmitter candidates (see Section 5.2).

6. Functional Implications

Earlier light microscopic studies led to the suggestion (Colonnier, 1966; Szentágothai, 1973) that the vertical disposition of axons of double bouquet cells mediated excitation, and the similarly oriented apical dendrites were considered as the primary postsynaptic targets. In our electron microscopic studies, we could not find a preferential association between apical dendrites of pyramidal neurons

and the axons of double bouquet cells, although more work is necessary especially in the monkey. The idea that apical dendrites receive input from double bouquet cells was attractive, for it seemed to explain the narrow and strictly radial course of the axon, and it suggested a climbing type of interaction. However, the small diameter of the axon cylinder may be the basis of other types of neuronal interaction, and we consider some of them.

It was suggested (Somogyi and Cowey, 1981) that perhaps one should think of not one neuron, but assemblies of double bouquet cells with vertical axons forming dense "curtains" from layer II down to layer V. Viewed from the surface of the cortex, these curtains could be long and narrow, with sharp edges caused by the small lateral spread of the axon. Such axonal assemblies could contribute to differences in the activity of neighboring neuron populations contained within slablike pieces of cortex.

Unfortunately, there is still no evidence about the lateral distribution of double bouquet cells in the cortex, largely because only a small proportion of neurons are impregnated by the Golgi method. Only when it is possible to selectively reveal all or most of the double bouquet cells in a particular region will it be possible to relate them with any confidence to functional groups of neurons such as those contained within the ocular dominance slabs. And even then, an apparently uniform anatomical distribution may conceal physiological specialization that depends on their inputs.

Another likely consequence of the tight radial axon plexus of double bouquet cells is that their action on any particular postsynaptic neuron will be localized to a particular region of that neuron. Thus, while one double bouquet cell may have negligible effect on the postsynaptic neuron as a whole its local effect on a dendrite or spine may be powerful and may interact significantly with other inputs to the same region. It was a striking feature of the postsynaptic spines in the monkey that the type II symmetrical synapse formed by the bouton of a double bouquet cell was invariably accompanied by a type I, asymmetrical synapse from a different bouton, as if the two types of inputs were competing for the same spine.

Another type of interaction was suggested on the basis of results obtained in cat striate cortex (Somogyi and Cowey, 1981), in which a substantial proportion of the elements postsynaptic to double bouquet cells belong to nonpyramidal cells. Many of the latter are GABAergic, as revealed by the presence of GAD immunoreactivity in their perikarya (Somogyi *et al.*, 1983b). If double bouquet cells are also GABAergic and inhibitory, as we suggest, it means that any synaptic interaction between them and other GABAergic neurons would produce disinhibition at the synapses of the latter. In fact, in the striate cortex of the cat, GAD-immunoreactive neurons receive numerous GAD-positive synaptic contacts both on their perikarya and dendrites as revealed by simultaneous Golgi impregnation (Somogyi *et al.*, 1983b), and some of these boutons may originate from double bouquet cells. Disinhibitory interactions have been proposed to explain some of the discharge characteristics of visual cortical neurons excited by retinal stimulation (Toyama *et al.*, 1977). Thus, it has been reported that the initial excitation in all cells is followed by a depression, apparently mediated by IPSPs, which was in turn rapidly succeeded by a rebound excitation possibly as a result of inhibition of the first-order inhibitory interneuron. To a first ap-

proximation, the double bouquet cell described in area 17 of the cat ideally fits the role of the putative second-order inhibitory neuron because (1) its synaptic structure suggests that it is inhibitory; (2) it frequently makes synapses with perikarya and dendrites of other nonpyramidal cells, which may also be inhibitory; (3) its soma and dendrites reside primarily in upper layer III, and are thus unlikely to receive input from specific afferents which could cause the first-order inhibition; (4) it provides the highest bouton density of any cortical interneuron yet described, so that its local effect is probably very powerful.

Finally, it is worth comparing the geometrically specific axon of the double bouquet cell with that of another local-circuit interneuron, the axoaxonic cell, which is now known to make synapses exclusively with the axon initial segments of pyramidal cells in the rat, cat, and monkey (Somogyi, 1977, 1979; Somogyi *et al.*, 1979, 1982; Fairén and Valverde, 1980; Peters *et al.*, 1982). The axo-axonic cell (also known as the chandelier cell, although the two may not always be identical) has a much more dispersed axonal arborization but extraordinary specificity with respect to target structure. This contrasts with the spatial specificity of layer III double bouquet cells, whose radially oriented tightly confined axonal terminal field is unique among known cortical local-circuit interneurons. Their postsynaptic targets are more diverse than those of axo-axonic cells, but nevertheless they appear to exclude the perikarya, axon initial segments, and even the apical dendrites (one possible example found) of pyramidal cells. When contrasted in this way with the different, but still highly specific pattern of connections made by other types of neurons those of the double bouquet cell illustrate the extraordinary specificity and intricacy of the local circuitry of the cerebral cortex.

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7. References

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