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Evidence for Interlaminar Inhibitory Circuits in the Striate Cortex of the Cat

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ABSTRACT

An interlaminar, ascending, and GABAergic projection is demonstrated in the striate cortex of the cat. We have examined a basket cell, with soma and smooth dendrites in layers V and VI, that was injected intracellularly with HRP in the kitten. Three-dimensional reconstruction of its axon revealed (1) a horizontal plexus in layer V and upper VI, extending about 1.8 mm anteroposteriorly and 0.8 mm mediolaterally; (2) a dense termination in the vicinity of the soma in layers V and VI; and (3) an ascending tuft terminating in layers II and III in register above the soma and about 250 μm in diameter. Many boutons of this cell contacted neuronal somata and apical dendrites of pyramidal cells and subsequent electron microscopy showed that these boutons formed type II synaptic contacts with these structures. A random sample of postsynaptic targets (n=199) in layers III, V, and VI showed that somata (20.1%), dendritic shafts (38.2%), and dendritic spines (41.2%) were contacted. The fine structural characteristics of postsynaptic elements indicated that the majority originated from pyramidal cells. Direct identification of postsynaptic neurons was achieved by Golgi impregnation of four large pyramidal cells in layer V, which were contacted on their somata and apical dendrites by between three and 34 boutons of the HRP-filled basket cell. Layer IV neurons were not contacted. Golgi-impregnated neurons similar to the HRP-filled basket cell were also found in the deep layers. The axonal boutons of one of them were studied; it also formed type II synapses with somata and apical dendrites of pyramidal cells.

Boutons of the HRP-filled neuron were shown to be GABA-immunoreactive by the immunogold method. This is direct evidence in favour of the GABAergic nature of deep layer basket cells with ascending projections. The existence of an ascending GABAergic pathway was also demonstrated by injecting [^{3}H]GABA into layers II and III. The labelled amino acid was transported retrogradely by a subpopulation of GABA-immunoreactive cells in layers V and VI, in addition to cells around the injection site.

The axonal pattern and mode of termination of deep basket cells make them a candidate for producing or enhancing directional selectivity, a characteristic of layer V cells. The results together with our previous data on basket cells terminating mainly either in layer IV or layers II and III demonstrate great specificity in the origin and laminar distribution of axosomatic, GABAergic, and presumably inhibitory synaptic terminals. The laminar specificity suggests that those functional properties that are brought about by basket cells have to be generated and/or reinforced at each level of cortical processing.

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Key words: visual cortex, GABA, inhibition, local circuit neurons, intracellular recording, immunocytochemistry

Neurons in the visual cortex are more selective for spatio-temporal aspects of the stimulus than the thalamic cells that provide the major input to the visual cortex. This selectivity, for the orientation and direction of movement and dimensions of the stimulus, is thought to be produced or at least enhanced by GABAergic inhibitory mechanisms within the cortex (Sillito, '75, '77; Tsumoto et al., '79; for review see Sillito, '84; but see Ferster, '86). The precise location and origin of the inhibitory synapses that mediate particular aspects of the inhibition leading to the response selectivity is not known.

Blomfield ('74) has suggested on theoretical grounds that dendritic and somatic inhibition may have different effects. His model predicted that inhibition occurring at the dendrites produces a subtractive change in firing rate of the neurons, whereas inhibition at the soma leads to divisive changes in firing. Divisive inhibition has been implicated in both orientation (Rose, '77; Morrone et al., '82) and direction (Dean et al., '80) selectivity.

The distribution and projection pattern of neurons that provide a putative inhibitory axosomatic input is thus of considerable interest. It has long been thought that axosomatic boutons in the cortex originate from local circuit neurons, the so-called basket cells (Ramón y Cajal, 1899; Szentágothai, '65, '73, '75; Marin-Padilla, '69; Marin-Padilla and Stibitz, '74; Jones, '75; Meyer, '83). The first evidence for the intracortical origin of most axosomatic boutons was provided by Szentágothai ('65) by using isolated cortical slabs in the cat. Our previous studies suggested that one of the putative inhibitory neurons of the cat's visual cortex that provides axosomatic synapses is the large basket cell of layer III (Martin et al., '83; Somogyi et al., '83). Although this neuron has long horizontal axons in layer III, it also provides a columnar descending projection through layer IV to layer V. Another putative inhibitory neuron that makes axosomatic contacts is the clutch cell, whose axon and target cells are largely restricted to layer IV—but it also has a restricted projection to the infragranular layers (Kisvarday et al., '85b). At present little is known of such basket-type cells in other layers of the cortex and the extent of their interlaminar connections. In the present study we have analysed the connectivity and neurochemistry of basket cells located in layer V of the cat striate cortex. In addition we have used a combination of high-affinity uptake and immunocytochemistry to reveal the extent of the interlaminar connections of GABAergic cells of the deep layers. The results demonstrate an extensive ascending interlaminar system of GABAergic projection neurons, amongst which is a class of cortical basket cell that provides a rich input to the soma and apical dendrites of pyramidal cells. Preliminary results of some of the findings have been published (Kisvarday et al., '85a; Somogyi, '86).

MATERIALS AND METHODS

Animals

One normal kitten (28 days old) and two adult cats were used. The kitten was part of a larger sample used for intracellular recording and marking of cells in area 17. The two adult cats were used for the study of interlaminar [^{3}H]GABA transport.

Intracellular recording and HRP labelling

Surgical, anaesthetic, and recording techniques for the kitten were the same as reported previously (Friedlander et al., '85). The intracellular recording micropipette was filled with 0.2 M KCl, 0.05 M TRIS (Sigma), and 4.0% HRP (Sigma, type VI) with a final pH of 7.6. The receptive field properties were first evaluated extracellularly. The cell membrane was then penetrated and the receptive fields were revalidated while recording intracellularly. The neuron was then filled iontophoretically with HRP by passing depolarising pulses. The neuron reported here was orientation selective and had an S-type receptive field. It was not tested quantitatively for directional selectivity. The kitten was deeply anaesthetized with additional Nembutal 10 hours after the HRP injection and perfused transcardially with a fixative containing 2.5% glutaraldehyde and 1% paraformaldehyde dissolved in 0.1 M phosphate buffer at pH 7.4.

Combination of intracellular HRP filling and Golgi impregnation

The lateral gyrus of the kitten was sectioned, coronally, at 80 μm with a Vibratome. The sections were incubated to reveal HRP enzyme activity; the procedure of Hanker et al. ('77) supplemented with cobalt and nickel intensification was used. Two 80-μm-thick sections containing the soma and dense axonal plexus of the intracellularly HRP-filled cell were processed with the section-Golgi method (Freund and Somogyi, '83) followed by gold toning (Fairen et al., '77), dehydration, and embedding as described earlier (Somogyi et al., '83). Golgi-impregnated neurons could easily be distinguished from the HRP-labelled profiles on the basis of their different colour and fine structure apparent at both light and electron microscopic levels. Synaptic contacts of identified processes were localised by using correlated light and electron microscopy. Putative contacts were first drawn and photographed in the light microscope from the 80-μm-thick sections; then the sections were reembedded, and ultrathin sections were cut of the same structure and studied in the electron microscope. The sections were stained with lead citrate.

[^{3}H]GABA injections

To confirm the presence of a GABAergic projection from the deep to the superficial layers in the adult cat, [^{3}H]GABA (0.33 mM, 60 Ci mmol^{-1}, Radiochemical Centre) dissolved in artificial cerebrospinal fluid (Jaffe and Cuello, '80) was injected under Nembutal anaesthesia through a micropipette (tip diameter 10–15 μm) at two sites into the medial bank of the posterior striate cortex of two cats. Each injection was made with a penetration close to vertical and nearly parallel to the medial surface of the lateral gyrus. The capillary was advanced for 6–7 mm from the point of entry into the brain and then gradually withdrawn by a stepping motor system while the isotope solution was ejected by pressure in 0.5-mm increments. Each injection track

received about 8–9 μCi of isotope. After a postinjection survival time of about 30–40 minutes the animals were deeply anaesthetized with a further dose of Nembutal and perfused through the heart first with saline followed by the same fixative as used for the kitten.

Tissue processing for [^{3}H]GABA autoradiography and GABA immunocytochemistry

Blocks of the lateral gyrus of the cats were dissected and alternate 40- and 100-μm-thick sections were cut perpendicular (horizontal plane in the brain) to the injection track by using a Slicetome MW-1000 (Polaron). The 40-μm-thick sections at regular intervals were stained with cresyl violet for Nissl substance. Others were processed for autoradiography to see the general pattern and intensity of [^{3}H]GABA labelling. Layering was determined by using Lund et al.'s ('79) modification of O'Leary's ('41) scheme of lamination. The 100-μm sections were postfixed in OsO_4 (1% in 0.1 M phosphate buffer), dehydrated, and embedded into Durcupan ACM resin (Fluka). Adjacent semithin (0.5 μm thick) sections were cut and mounted onto separate gelatine-coated slides. Thus, separate sections of the same neurons could be processed either for autoradiography or for postembedding GABA immunocytochemistry (Kisvarday et al., '86a; Somogyi et al., '85). The antiserum to GABA (code No. GABA 9) has been characterised previously (Hodgson et al., '85). The neurons labelled by either or both methods were compared and evaluated with the same criteria as described earlier (Kisvarday et al., '86a).

GABA immunocytochemistry of HRP-filled boutons

Short series of ultrathin sections containing HRP-filled boutons in layer III were picked up onto Formvar-coated gold grids. Every second grid was reacted for GABA by using procedure I of Somogyi and Hodgson ('85), and the same reagents as for the semithin sections. During this reaction most of the osmium, providing the electron density of HRP reaction end-product, is removed, making the identification of filled boutons difficult. Therefore, synaptic contacts made by the HRP-filled boutons were first identified on the grids that were not reacted for GABA; then the same bouton was photographed on the immunocytochemically reacted grid.

Three-dimensional reconstruction of the HRP-filled cell

The processes of the neurons were recorded by using the X, Y, Z coordinates of representative points fed from the microscope into a PDP 11/34 computer and a Quantimet image analyser system with the software NEURON, NEUMER, NEUROT, and NRN11A as set up at the Department of Anatomy, Semmelweis University Medical School (see Capowski and Rethelyi, '82; Zsuppan, '84). The HRP-filled neuron was reconstructed from twenty-one 80-μm-thick sections. Statistical programmes for the analysis of interbouton and inter-branch-point intervals were kindly provided by Dr. J. Somogyi. The values represent distances traced along the course of the axon collaterals and not the direct point-to-point distances. The smallest distance that can be recorded between two points is 0.5 μm, so the axon's course is approximated with 0.5-μm-long or longer straight lines.

RESULTS

The term "basket cell" will be used throughout the paper to differentiate the neurons studied here from other smooth dendritic cells. Their most striking feature, as observed in the light microscope, is the clustering of axonal boutons around the soma and apical dendrites of other neurons. This feature is present in two other types of basket cell—the large basket cells of layer III (Martin et al., '83; Somogyi et al., '83) and the clutch cells of layer IV (Kisvarday et al., '85b). The axon collaterals of the HRP-filled basket cell, N1, (Fig. 1) were traced over a distance of 1,800 μm to what appeared to be their terminal ends. While analysing Golgi-impregnated sections from the cortex of the same kitten, four cells similar to N1 were found. In contrast to N1, which was traced through 21 sections, the processes of the Golgi-impregnated cells could only be reliably identified in one 80-μm-thick section. Nevertheless, as described below, they had several morphological features in common with N1. One of the Golgi-impregnated cells (N2, Fig. 8A) was also examined with the electron microscope and had fine structural features similar to N1 (see below).

Light microscopy

Soma and dendrites. The large spherical somata of both the HRP-filled and the Golgi-impregnated basket cells were located in layer V or in the uppermost part of layer VI. The slightly elongated somata had short axes of 18–21 μm and long axes of 20–24 μm. Dendrites emerged from three to four main dendritic trunks, which branched shortly after their origin. Tertiary dendritic branches originated from the proximal third of the dendrites but only rarely more distally (Figs. 1A–D, 4A). Distal dendrites tended to be more beaded than proximal ones (Figs. 4A, 8A). The great majority of the dendrites were localised to layers V and VI. Complete three-dimensional reconstruction of neuron N1 (Fig. 1) revealed that the dendritic field occupied a slightly ovoid area elongated radially and superior-inferiorly with longest diameters of 500 and 400 μm, respectively (Fig. 1A–D). Some dendrites of N1 penetrated the white matter (Fig. 1A–C).

Axon of cell N1. From the two-dimensional drawing it was apparent that the axon collaterals of basket cell N1 can be divided into two major divisions: (1) a horizontal plexus in layer V, in which the majority of the axon collaterals were located, and (2) a prominent ascending tuft terminating in layers II and III. Both divisions consisted of straight main axon trunks and frequently branching collaterals with short terminal segments, as seen from the distribution of inter-branch-point distances (Fig. 2). The distribution of interbouton intervals was positively skewed (Fig. 2), indicating that boutons close to each other dominated the population.

It was not possible to visualize the real extent of the axon from the two-dimensional drawing; therefore a computer reconstruction was made and rotated to reveal the distribution of the collaterals in the three principal planes (Fig. 1A–D). All but the principal collaterals were studded with synaptic boutons (Fig. 1). The total number of boutons was 3,773. Viewing the axon in the sagittal plane (Fig. 1B) it is apparent that in the horizontal plexus most of the collaterals and terminals were in layer V; only a few collaterals entered upper layer VI. The top view (Fig. 1D) revealed that the axonal field extended approximately 1.8×0.8 mm in layer V and was elongated anterior-posteriorly. Short

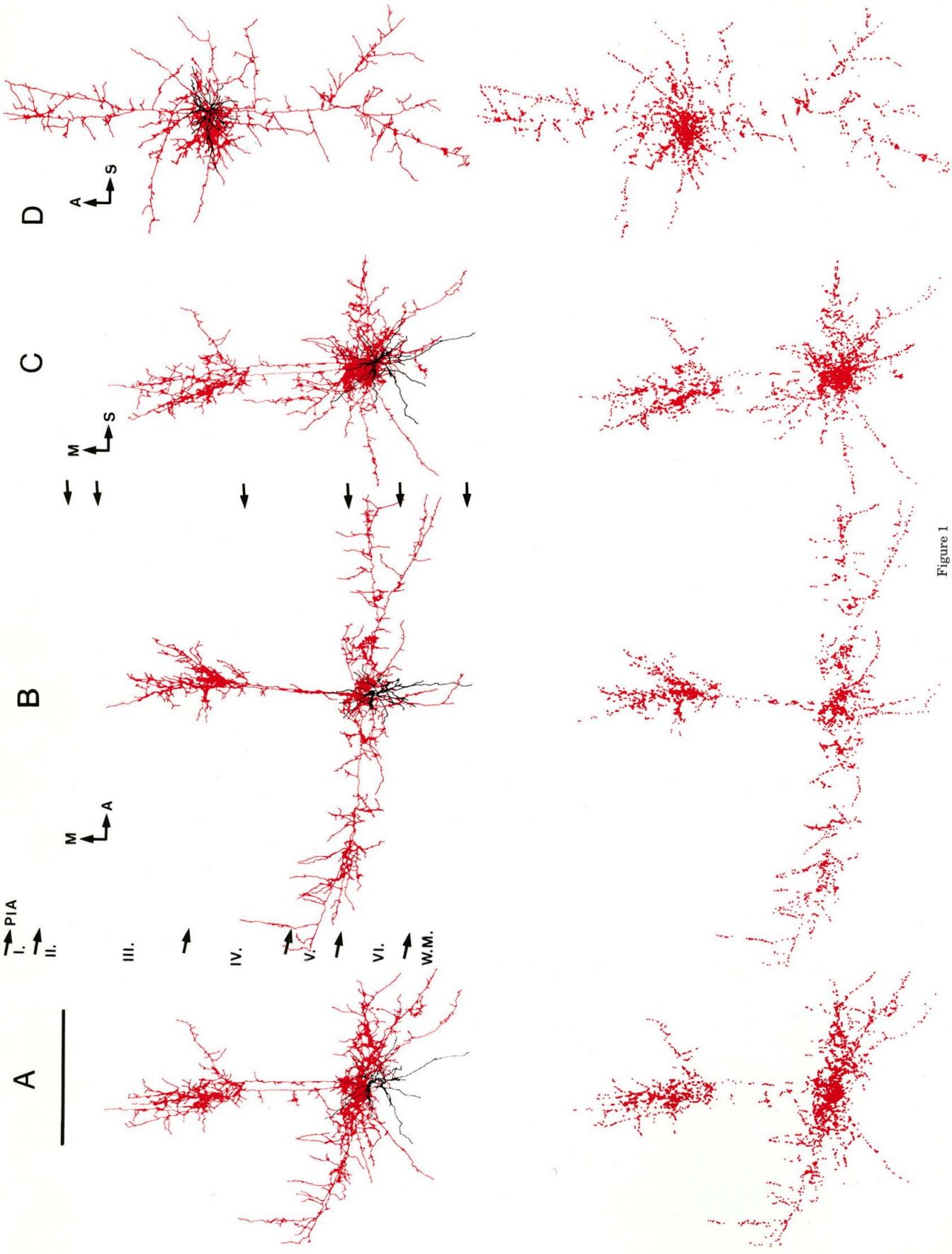

Figure 1

Fig. 1. Complete computer reconstruction and rotations of the axonal (red) and the dendritic (black) arbors of an HRP-filled basket cell in the kitten striate cortex. The lower computer plots show the distribution of axonal boutons in the same plane as the upper rotations. The cell was located in the medial bank of the lateral gyrus so the top of the figure in A, B, and C, where the pia is located, corresponds to the medial surface of the cortex. Planes: **A:** As reconstructed from the coronal sections. **B:** As seen in the sagittal plane. **C:** As seen in the near frontal plane showing the minimum width of the arbor. **D:** As seen from the pia in a plane perpendicular to the apical dendrites. Layers are indicated only in B because the borders between layers bend considerably in the other planes. Note the anteroposteriorly elongated axonal field in layers V and VI and the dense axonal projection to layer III just above the dendritic field. The sum of the length of the axonal segments was about 41.6 mm. Clustering of boutons occurs along the radial axon collaterals in layer V. Very few terminals are in layer IV. Directions as in stereotaxic coordinates: A, anterior; M, medial; S, superior. Scale: 500 μm.

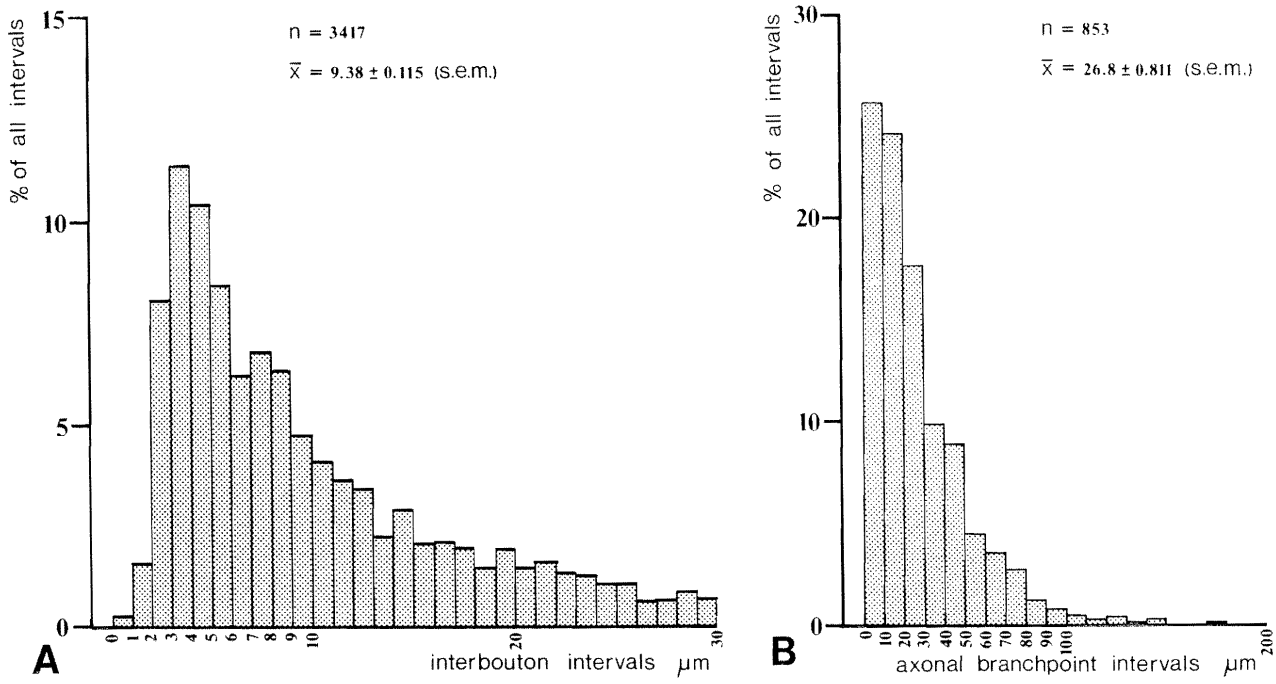

Fig. 2. **A:** Frequency distribution of interbouton intervals along the axon collaterals of HRP-filled basket cell (N1). Interbouton intervals longer than 30 μm and constituting 6.9% of the population are not shown because they are mostly on main axon trunks not on terminal segments. **B:** Frequency distribution of the axonal branching point intervals of the same basket cell (N1).

axonal segments oriented in the radial direction and studded with clusters of boutons often branched from the main horizontal axons at approximately 100-μm intervals (Fig. 1B). Many of their boutons contacted the somata and/or apical dendrite of pyramidal cells of layers V and upper VI.

A most conspicuous feature of neuron N1 was that two main axonal branches ascended to layers II and III where they arborised profusely, providing boutons in a cluster about 200–250 μm in diameter. From the different perspectives afforded by the rotations it could be seen that the cluster of boutons in layers II and III remained within the column of the dendritic field. Boutons in the superficial tuft also surrounded somata of other neurons, most of which were pyramidal cells.

The main axon collaterals ascending to the supragranular layers gave only short terminal segments in layer IV occupying a narrow radial column about 40 μm in diameter.

Axons of Golgi-impregnated cells. As with cell N1, the axons of all four Golgi-impregnated basket cells originated from the soma on the side toward the pia (Fig. 8A). In the case of N2 the main course of the axons was almost identical to that of the axon of the intracellularly labelled basket cell. Large axonal trunks, cut at the surface of the section, were directed horizontally and produced radial terminal branches. These short collaterals were crowded with large boutons often surrounding somata and/or apical dendrites of neighbouring large pyramidal cells (Fig. 8A,B). One thick axon trunk could be followed to layer IV, suggesting that this neuron also had an ascending projection to the superficial layers (Fig. 8A). The axons of the other three cells were less extensive, but the main axonal trunks and several terminal segments contacting pyramidal cell somata were found.

Electron microscopic observations

The fine structural characteristics of dendrites and somata will not be dealt with because they showed no distinctive features that are uniquely characteristic of this type of cell. The analysis concentrated on synaptic terminals and on the identity and distribution of postsynaptic targets. Most of the data were collected from N1. However, a small confirmatory sample was obtained for N2.

Synaptic targets of N1. Since the axonal field spanned almost the entire depth of striate cortex it was necessary to examine all main layers of termination to see if the postsynaptic targets varied in different cortical layers. Electron microscopy was carried out on axon collaterals from layers III–VI. A sample was taken from layer III and another pooled sample from layers V and VI (Table 1). All boutons were followed in serial sections. The sectioning plane was not adjusted to that of the HRP-filled processes, and every bouton (n = 181) in the plane of the section was examined for its postsynaptic target; therefore the sample is considered to be random with regard to the postsynaptic targets. In addition to the above sample, 48 preselected boutons both in contact with and around the Golgi-impregnated cells and a further 13 boutons for GABA immunocytochemistry were examined. The boutons examined (242 in total) made type II (symmetrical) synapses (e.g., Fig. 7C,E).

In the random sample, altogether, 199 postsynaptic elements were encountered for boutons of basket cell N1 (Table 1). One postsynaptic element was an axon initial

TABLE 1. Postsynaptic Targets of Deep-Layer Basket Cells[1]

	Soma	Dendrite shaft	Spine	AIS	Total from cell	Total
Neuron 1 HRP filled						
Random sample						
layers V, VI	9	36	26	1	72	
layer III	31	40	56	—	127	
Total	40 (20.1%)	76 (38.2%)	82 (41.2%)	1 (0.5%)	199 (100%)	
Nonrandom sample						
Golgi imp. targets layers III–V						
P_1*		6 (ad)			6	
P_3*	6	2 (ad)	7 (ad)	—	15	
Nonimp. targets	5	27	11	—	43	
Total	11	35	18	—	64	263
Neuron 2 (Golgi impregnated) layer V						
Nonrandom	9	1	—	—	10	
Total						10

[1]ad, apical dendrite; AIS, axon initial segment.
*Golgi-impregnated layer V pyramidal neurons.

segment in layer VI. Neuronal somata were more frequently contacted in layer III (24.4%) than in deep layers (12.5%). Almost all of the postsynaptic somata were pyramidal cells. They had pyramidal shape and a major apical dendrite could often be seen directed toward the pia (Fig. 7A). In addition to the identified basket cell boutons the perikarya of these pyramidal neurons received only type II synaptic contacts. Dendritic shafts constituted one of the major postsynaptic targets (Table 1) both in layer III (31.5%) and in the deep layers (50%). Examination of serial sections showed that many of the postsynaptic dendrites were spiny, indicating that they originated from pyramidal neurons. Some postsynaptic dendritic shafts were 1–3 μm in diameter (Fig. 7A,B) and oriented radially like the apical dendrites of identified pyramidal neurons (see below).

Dendritic spines were the most frequent (44.1%) postsynaptic elements in the supragranular layers and were major targets in layers V and VI (36.1%) (Table 1). They were recognised on the basis of the spine apparatus and the lack of mitochondria, in addition to their shape and characteristic origin from dendrites as identified from serial sections (Fig. 7C–E). The basket cell synapses were usually located on the proximal part of the spine near the parent dendrite (Fig. 7C). In addition to the synapses established by the basket cell on spines, there were invariably unlabelled boutons forming type I synaptic contacts on the heads of the same spines (Fig. 7D,E). When dendritic spines could be traced back to their parent dendrite, these dendrites showed identical structural features to identified pyramidal cell dendrites postsynaptic to N1 (Fig. 7D).

The random sample suggested that the major postsynaptic targets of N1 were various parts of pyramidal cells. The identity of some of the target neurons was demonstrated directly by tracing the HRP-filled axon collaterals onto identified, Golgi-impregnated pyramidal cells (Figs. 5, 6). Because these contacts were preselected from the light microscope, they do not reflect the overall distribution of axonal varicosities and are shown separately from the random sample (Table 1).

Identified postsynaptic targets. In layer V, four Golgi-impregnated pyramidal cells (P_1–P_4, Figs. 3, 4) were found to receive contacts from the HRP-filled boutons of N1 onto their soma and apical dendrites. These pyramidal cells had large somata and the apical dendrites could be traced through layers IV and III. Three of them were in a group close together (Fig. 3). The numbers of HRP-filled basket cell boutons in contact with individual pyramidal cells were six for P_1, three for P_2, 34 for P_3, and 13 for P_4. Not all boutons were checked in the electron microscope, but those that were in the appropriate plane could be shown to make synaptic contacts with the large pyramidal cells (Figs. 5, 6). Thus, 15 synaptic contacts originating from the HRP-filled boutons of N1 were seen on P_3. All three type of types of postsynaptic elements encountered in the random sample—the soma (Fig. 5A,B), dendritic shaft (Fig. 5C,D), and dendritic spines (Fig. 6A,B) of this single large pyramidal cell—received synapses from the HRP-filled axon (Table 1). It is noteworthy that both the shaft and the spines of the apical dendrite received synapses from the same ascending basket cell collateral that followed the dendrite through layer IV. This is significant because it supports our assumption that the few boutons of N1 in layer IV terminated on ascending dendrites of layer V and not on layer IV cells.

Synaptic targets of Golgi-impregnated basket cell N2. Only a small sample of ten boutons preselected from the light microscope were serially sectioned for electron microscopy from this cell (Table 1). Nine made synapses with somata of layer V pyramidal cells and one with the apical dendrite of one of them (Fig. 8C,D). All boutons formed type II synaptic contacts (Fig. 8D). The synaptic contacts, the size of the boutons, and the fine structural characteristics of the postsynaptic cells were all similar to those described for N1.

Immunocytochemical demonstration of GABA in the terminals of HRP-filled basket cell N1

Basket cells are thought to use GABA as a transmitter. Therefore, we examined the axonal boutons of N1 to see if

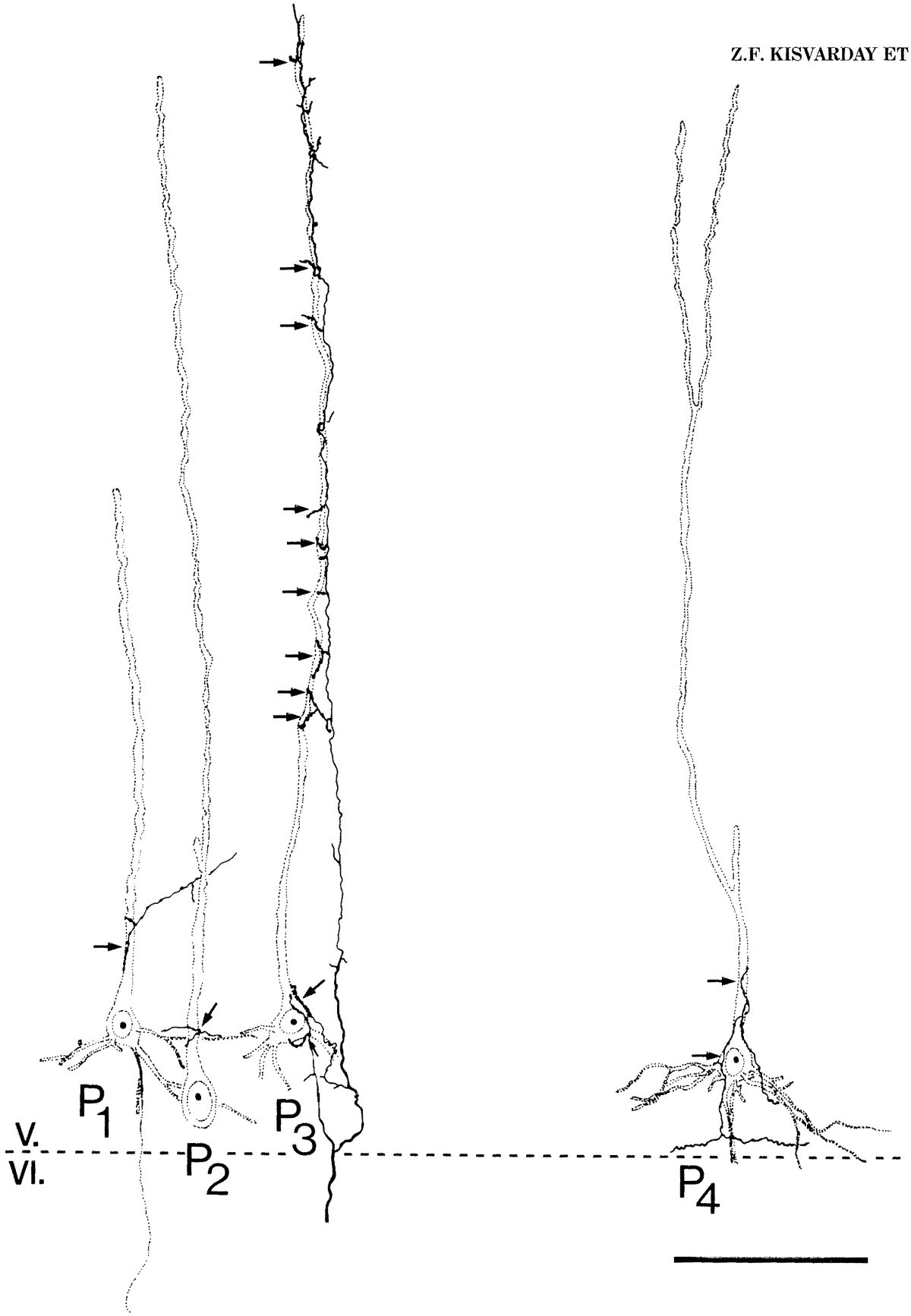

Fig. 3. Axon collaterals (solid lines) of the basket cell, also shown in Figure 1, seem to establish multiple contacts (arrows) with the somata and apical dendrites of large pyramidal cells (P_1–P_4), revealed by Golgi impregnation in layer V. Dendritic spines are not drawn. Pyramidal cell P_3 received numerous contacts on its apical dendrite. Many contacts were confirmed by electron microscopy and were found to have synaptic specialisations (see Figs. 5, 6). Scale: 100 μm.

Fig. 4. **A:** Light micrograph of the HRP-filled basket cell (asterisk) in a section that also contains a Golgi-impregnated gold-toned pyramidal cell—P_3. The cell body and apical dendrite (ad) of P_3 are contacted by basket cell boutons (arrows) that were shown subsequently by electron microscopy to establish synaptic contacts. The dendrites (d), axon collaterals (ac), and the axon initial segment (A.I.S.) of the basket cell are indicated. **B:** Another giant pyramid (P_1) is contacted by HRP-filled basket cell boutons (arrows) on its apical dendrite. Gold-toned axons and boutons of unknown origin are evident throughout the section. Scales: A, 50 μm; B, 10 μm.

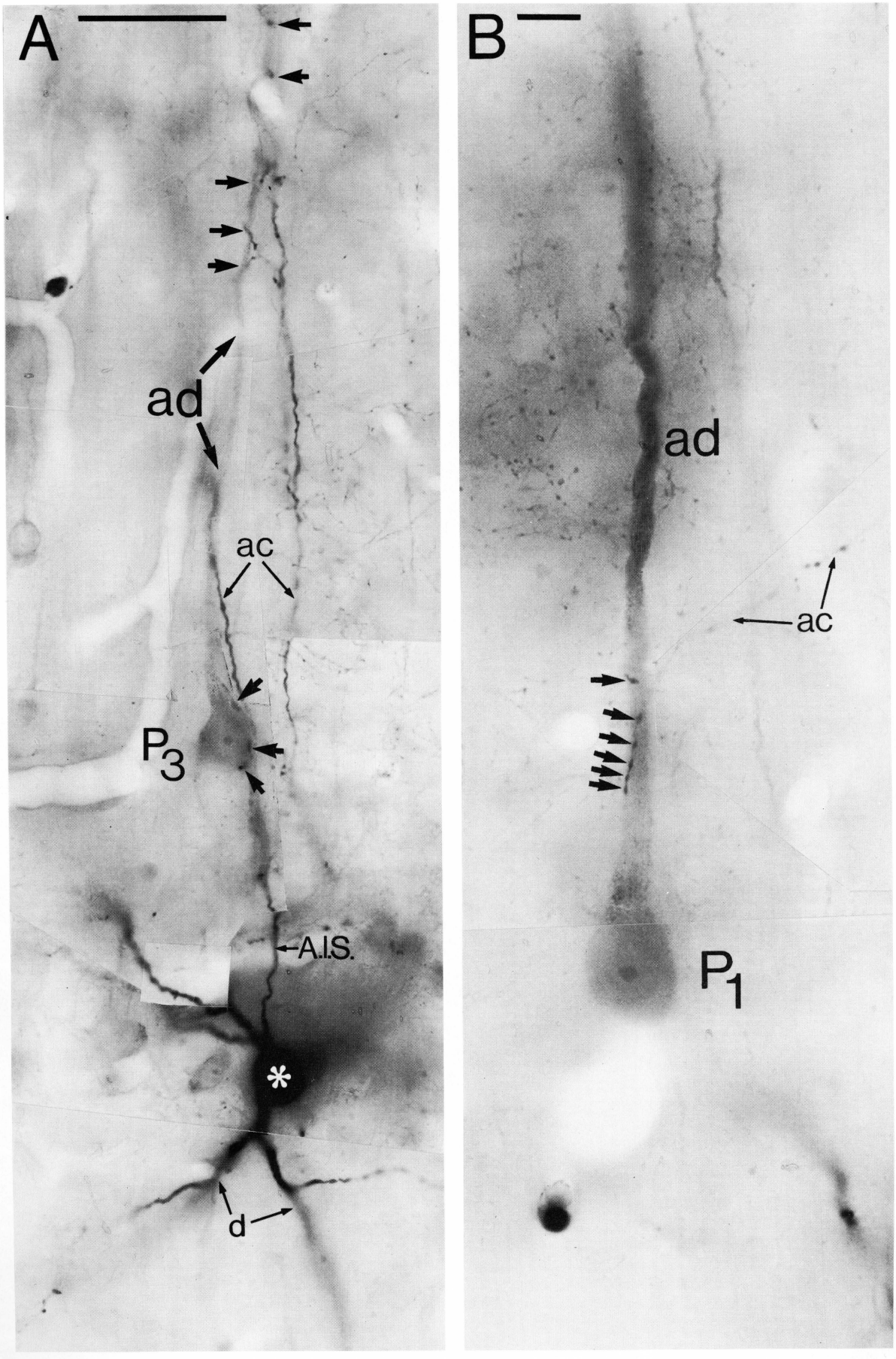

Figure 4

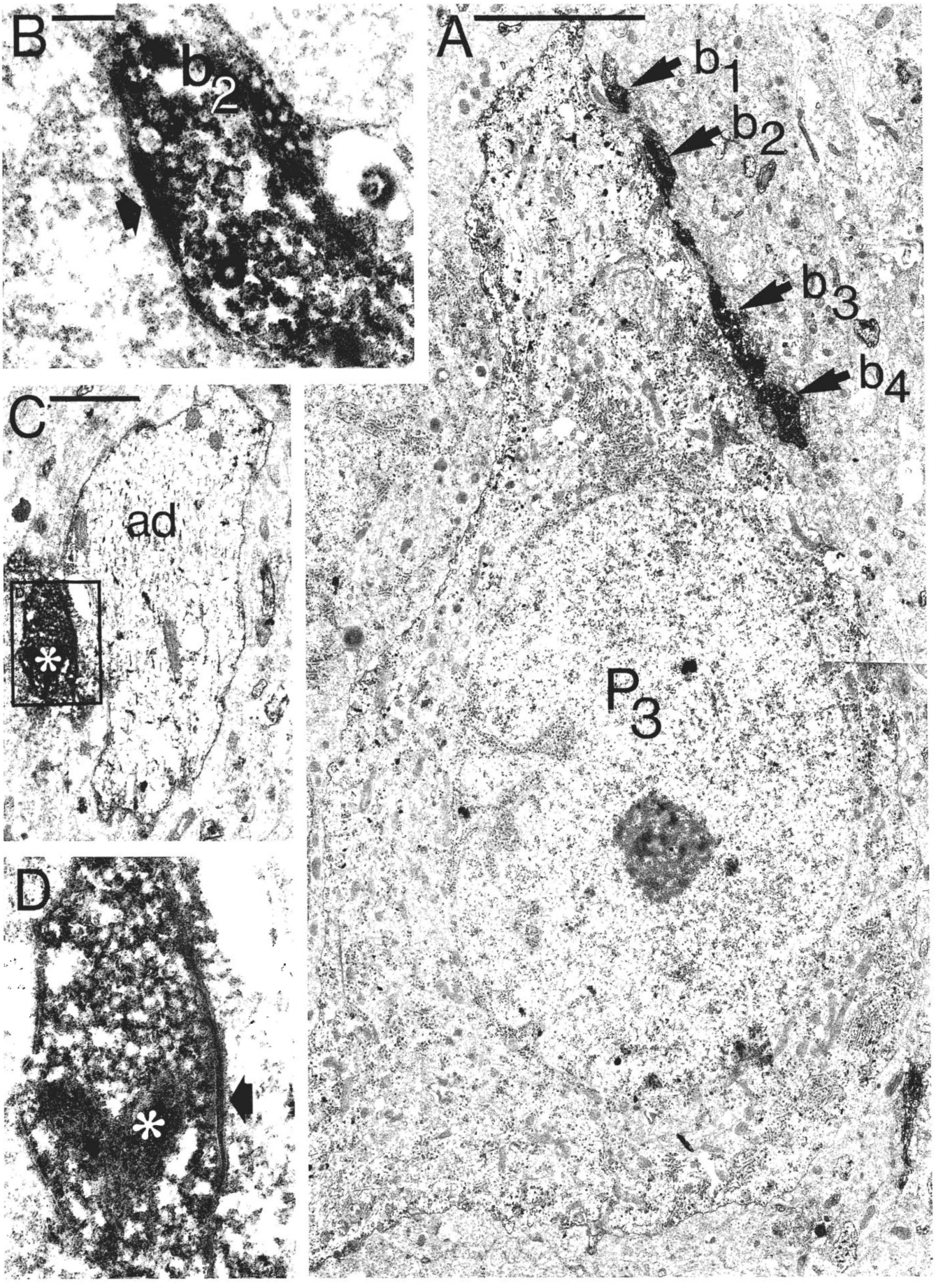

Figure 5

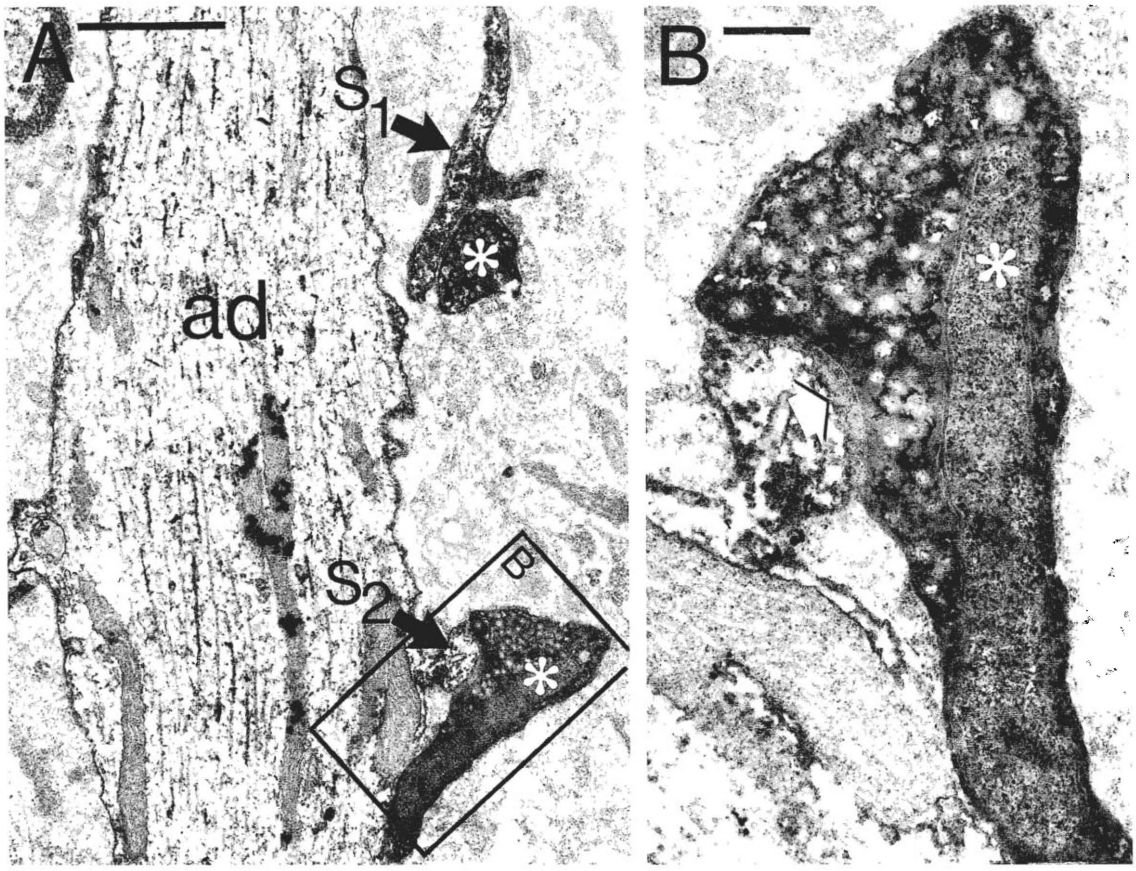

Fig. 6. Electron micrographs showing basket cell boutons identified by HRP filling and the apical dendrite (ad) of a large layer V pyramidal cell (P_3, see also Figs. 2A, 3) identified by Golgi impregnation. **A:** Two spines (s_1, s_2) receive synapses from basket cell boutons (asterisks) on their necks. Framed area (s_2) is seen at higher magnification in **B**, where the type II synaptic contact is indicated by arrow. Scales: A, 1 μm; B, 0.2 μm.

this putative transmitter was present. Thirteen boutons from layer III were serially sectioned and alternate sections were studied either to localise the postsynaptic target (Fig. 9A) or to see if the boutons were reactive for GABA (Fig. 9B). All 13 boutons had selective accumulation of colloidal gold over them, demonstrating the presence of fixed GABA. The accumulation of gold was not due to the presence of HRP reaction end-product because several other types of cells filled with HRP processed with the same procedure showed no GABA immunoreactivity (unpublished observation). The gold particles were distributed very selectively over terminals that made type II contacts and had a heterogeneous population of vesicles (Fig. 9C).

Fig. 5. Electron micrographs of a layer V Golgi-impregnated pyramidal cell (P_3 shown also in Figs. 2 and 3) receiving synapses (arrows) from boutons of the HRP-labelled basket cell. Boutons (b_1–b_4) contacting the soma are seen in **A.** One of them, b_2, shown at higher magnification in a serial section in **B**, establishes a synaptic contact. **C, D:** The apical dendrite (ad) of the same pyramidal cell is shown to receive a type II synaptic contact (arrow) on its dendritic shaft from a basket cell bouton (asterisk). The HRP reaction end-product diffused from the bouton and also filled the synaptic cleft. Scales: A, 4 μm; B, D, 0.25 μm; C, 1 μm.

With regard to the perikaryal localisation of GABA immunoreactivity, only nonpyramidal cells were positive. Six somata postsynaptic to N1 in layers VI and V and two in layer III were tested for GABA but none of them were immunoreactive (Fig. 9), although nearby cells were positively stained.

Demonstration of an ascending GABA pathway

The demonstration of GABA in the terminals of N1 raised the possibility that this neuronal system ascending in a columnar fashion from layers V and VI to the supragranular layers can be demonstrated as a pathway by using the retrograde transport of [^{3}H]GABA (Somogyi et al., '83). When [^{3}H]GABA was injected into layers I–III a large number of neurons became labelled around the injection track (Fig. 10A,B). The labelling was selective to the extent that only some neurons accumulated the labelled amino acid even in the immediate vicinity of the injection track. When the injection site was in layer IV or in lower layer III labelled neurons were distributed in a radially elongated ovoid area from layers II–VI. However, when the injection site was in upper layers II and III the number of labelled

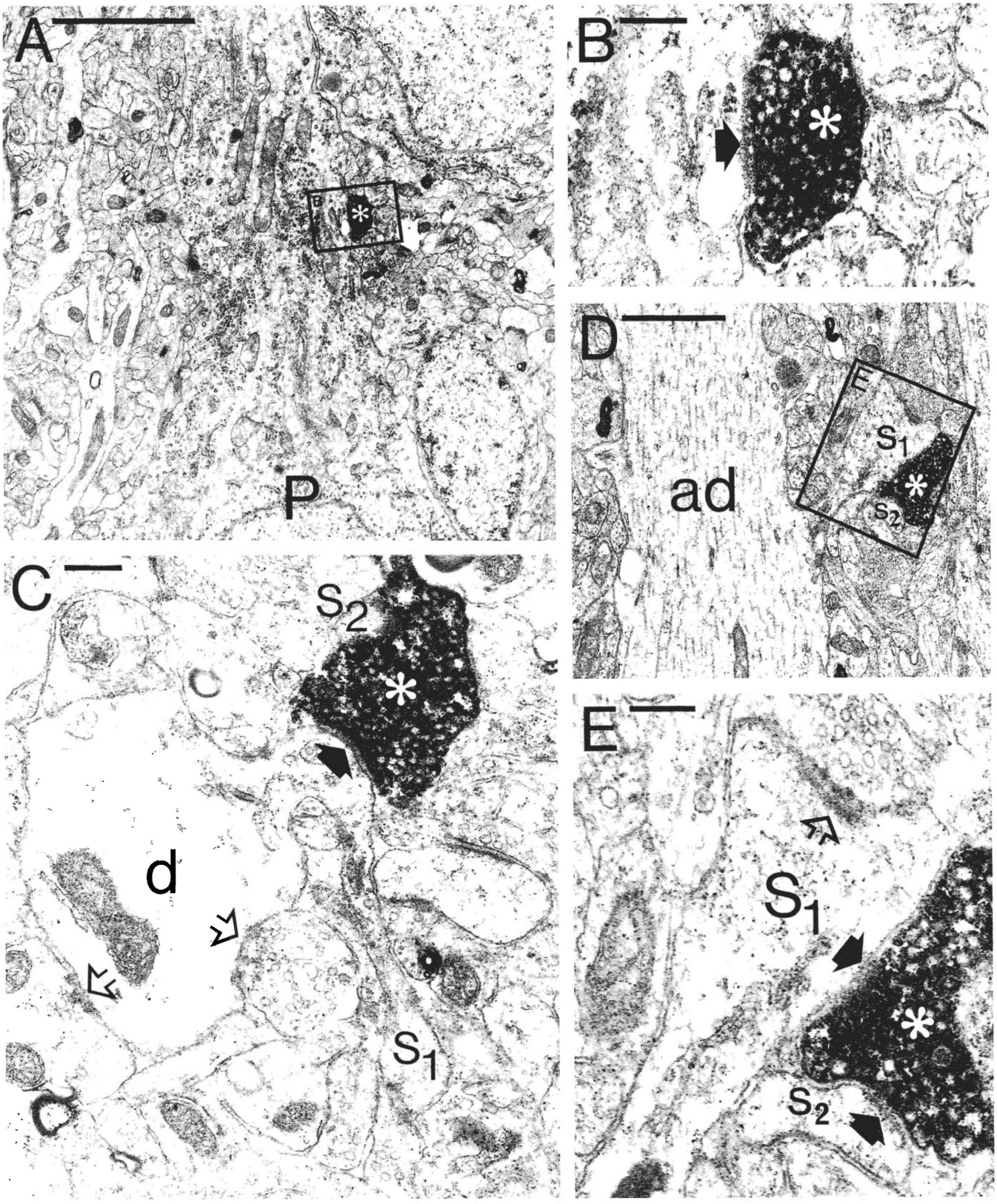

Fig. 7. Postsynaptic targets of the ascending basket cell axon in layer III. **A:** A basket cell bouton (asterisk in the framed area and also shown in **B**) gives synaptic contact (arrow in B) to an apical dendrite emerging from the pyramidal cell soma (P). **C–E:** Most of the targets were dendritic spines. C: Basket cell bouton (asterisk) is shown to establish a type II synapse (arrow) on the neck of a spine (s_1) emerging from a dendrite (d) that also receives synapses (open arrows) from unidentified boutons. A second spine (s_2), cut tangentially in this section, was also found to receive a contact. D: Dendritic spine (s_1) of a pyramidal apical dendrite (ad) is contacted by a basket cell bouton (asterisk). The same bouton as in the framed area is seen at higher magnification in E making synaptic contacts with two spines, (s_1, s_2). Spine s_1 receives an additional synapse from an unlabelled bouton (open arrow). Scales: A, 2 μm; B, C, E, 0.2 μm; D, 1 μm.

cells dramatically decreased at the border of layers III and IV with a few neurons remaining labelled immediately under the injection track (Fig. 10). The GABA-labelled cells became more numerous again in layers V and VI immediately under the injection track. Labelled fiber bundles ran between the two populations of cell (Kisvarday et al., '86a) (Fig. 10A).

In similar experiments on the monkey visual cortex only some of the [^{3}H]GABA labelled neurons were immunopositive for GABA; thus it was concluded that in the monkey not all GABA-accumulating cells may use GABA as transmitter (Kisvarday et al., '86a). Therefore we tested the [^{3}H]GABA-labelled cells in the cats to see if they were immunoreactive for GABA. All labelled cells (n = 236) that we tested were GABA immunopositive (Fig. 10D,E). In layers V and VI only some of the GABA-immunopositive neurons were labelled autoradiographically, indicating that not all GABA-containing cells have ascending projections. The density of GABA-immunoreactive cells was not higher at the GABA injection site than in areas where no radioactivity could be detected, indicating that the injection procedure itself was not producing immunoreactive cells.

DISCUSSION

It is increasingly evident that the cells that provide axosomatic synapses in cortex (DeFelipe and Fairen, '82; Martin et al., '83; Somogyi et al., '83; Kisvarday et al., '85b; DeFelipe et al., '86; Freund et al., '86) do not form elaborate pericellular nets envisaged on the basis of Golgi material (Ramón y Cajal, 1889; Marin-Padilla, '69). Such cells may exist, but the pericellular nets are probably formed by the confluence of axons from several neurons, each giving short collateral segments of boutons that make synapses with the soma as well as other parts of the same cell (Somogyi et al., '83; Kisvarday et al., '85b). The question arises whether it is still justified to maintain the term "basket cell." As before (Martin et al., '83; Somogyi et al., '83), we propose that this name be preserved because it helps to differentiate a distinct family of neurons from the very diverse population of smooth dendritic cells (see, e.g., Szentágothai, '73; Peters and Regidor, '81; Meyer, '83). The features we suggested for the distinction of the basket cell group are (1) the quantitative distribution of postsynaptic elements, (2) the dimensions of the axon, (3) the laminar location, and (4) the biochemical characteristics. These features will be compared below for the various basket cells studied so far.

Target specificity

Previously we found that large basket cells in layer III had betwen 30 and 40% of their efferent synapses on somata (Somogyi et al., '83). Somewhat less, between 20 and 30%, of the efferent contacts of layer IV clutch cells (Kisvarday et al., '85b) were made with somata of other neurons. The cell studied here from layer V is similar to clutch cells in this respect; thus the range emerging for the basket cells studied so far in the cat is between 20 and 40%. The axonal connections of a fourth class of neuron, the "short range basket cell" revealed by cholecystokinin (CCK) immunocytochemistry, was studied quantitatively by Freund et al. ('86). This cell was shown to make about 37% of its synapses with somata. A similar neuron was described by DeFelipe and Fairen ('82) qualitatively. Other smooth cells studied quantitatively make few or no perisomatic synapses (Somogyi and Cowey, '81; Somogyi et al., '82; Freund et al., '86). The cells that make a significant proportion of their synapses with somata invariably contact other parts of the same postsynaptic cells, and this arrangement is probably present in other species (Peters and Proskauer, '80).

Spatial distribution of basket cell axons

The axon of large basket cells in layer III (Somogyi et al., '83) was at least three times the extent of the dendritic field in the lateral dimension, as was the axon of the HRP-filled basket cell described here. The clutch cells in layer IV have much more restricted axons that extend about one quarter as much in the anteroposterior direction (Kisvarday et al., '85b) as the axons of the large basket cells. The lateral extent of "short range basket cell" axons (DeFelipe and Fairen, '82; Freund et al., '86) remains to be established. No doubt further varieties of basket cells exist (Szentágothai, '73, '75). None of the axons of basket cells in the visual cortex have been seen to extend more than 1 mm in any direction from the soma of the cell. Inhibitory influences, however, that could be mediated by basket cells have been shown to act over large areas of the visual field that would correspond to several millimeters in the striate cortex (Nelson and Frost, '85). Since the axons of all large basket cells are myelinated, only intracellular marking will reveal if longer-range basket cells were present in the cortex of cat.

The laminar selectivity of the basket cell axon arborizations provides the best means of differentiation between the different kinds of basket cell. The basket cell described here had an axon that was the mirror image of that shown for the supragranular large basket cells (Martin et al., '83; Somogyi et al., '83). In its infragranular axon plexus there does not seem to be a sharp delineation of collaterals at the layer V/VI border; therefore basket cells of the infragranular layers could be referred to as deep-layer basket cells.

The ascending axonal tuft of the deep basket cell is most intriguing because it is in precise register with the somata situated in the deep layers. The possible circuit that this is related to will be discussed below. It is noteworthy that all basket cells have a radial component in their axonal arborization, but for the superficial cells it is a descending one.

Neurochemical characteristic and transmitter(s) of basket cells

This is the first report of a basket cell in which the presence of GABA has been demonstrated, providing a neurochemical marker that distinguishes these cells from at least 80% of other cortical neurons (Gabbott and Somogyi, '86) that are not GABA positive. This finding was not unexpected since a large body of indirect evidence suggested that basket cells may be GABAergic (see, e.g., Ribak, '78; Freund et al., '83; DeFelipe et al., '86). Our method allows a definitive characterization of physiology, connectivity, and neurochemistry of the same cell. Preliminary evidence suggests that the clutch cells and supragranular basket cells also contain GABA (Somogyi, '86). Some of the short-range basket cells of layers II and III are certainly amongst the cells that are immunoreactive for CCK peptides (Freund et al., '86), but the long-range basket cells are not. Short-range basket cells probably also contain GABA, since all CCK-immunoreactive neurons were also found to react for GABA (Somogyi et al., '84). Thus, all basket cells identified so far probably contain GABA. There is agreement on the inhibitory action of GABA on cortical neurons (Krnjevic and Schwartz, '67; for review, Sillito,

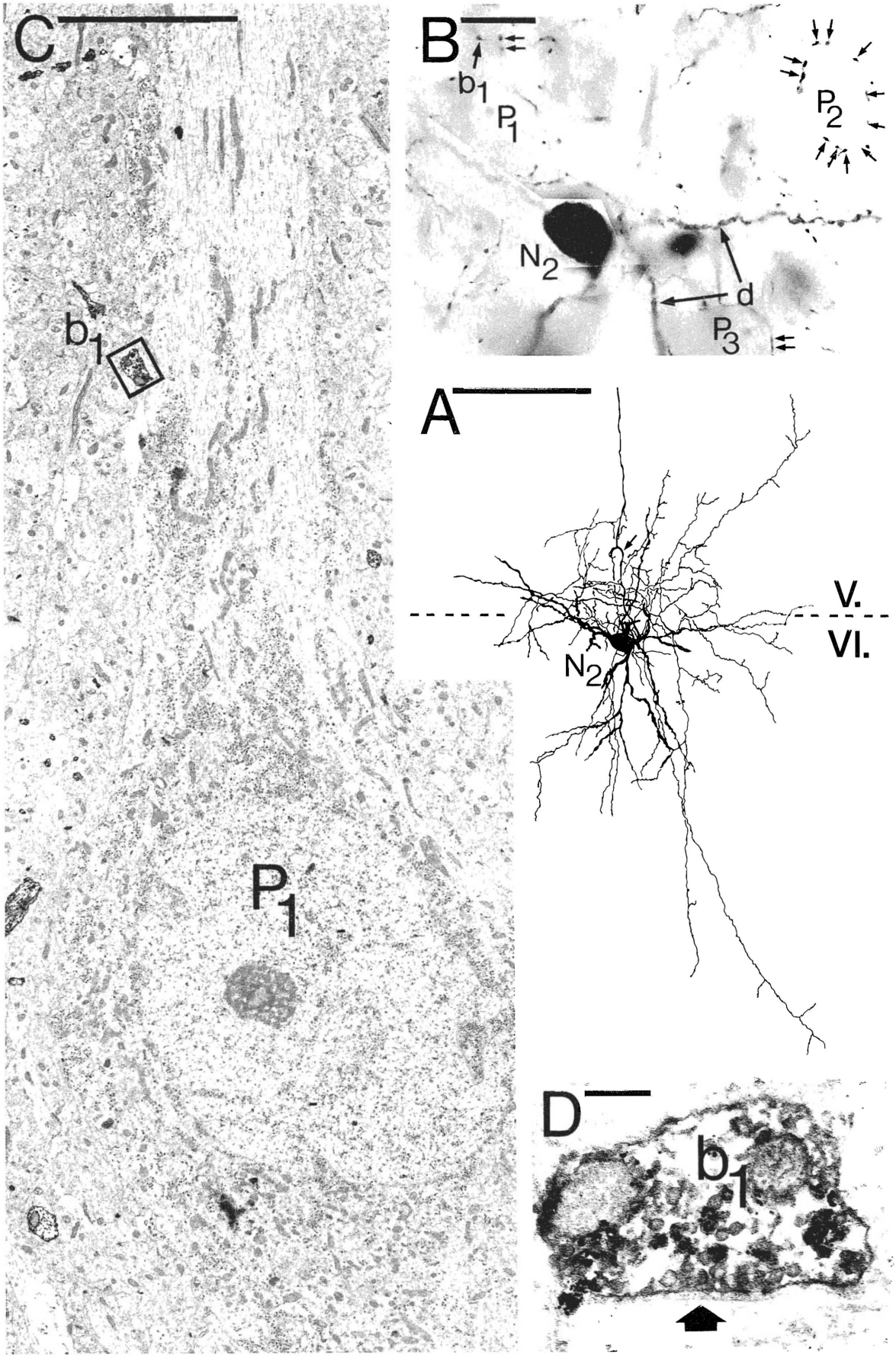

Figure 8

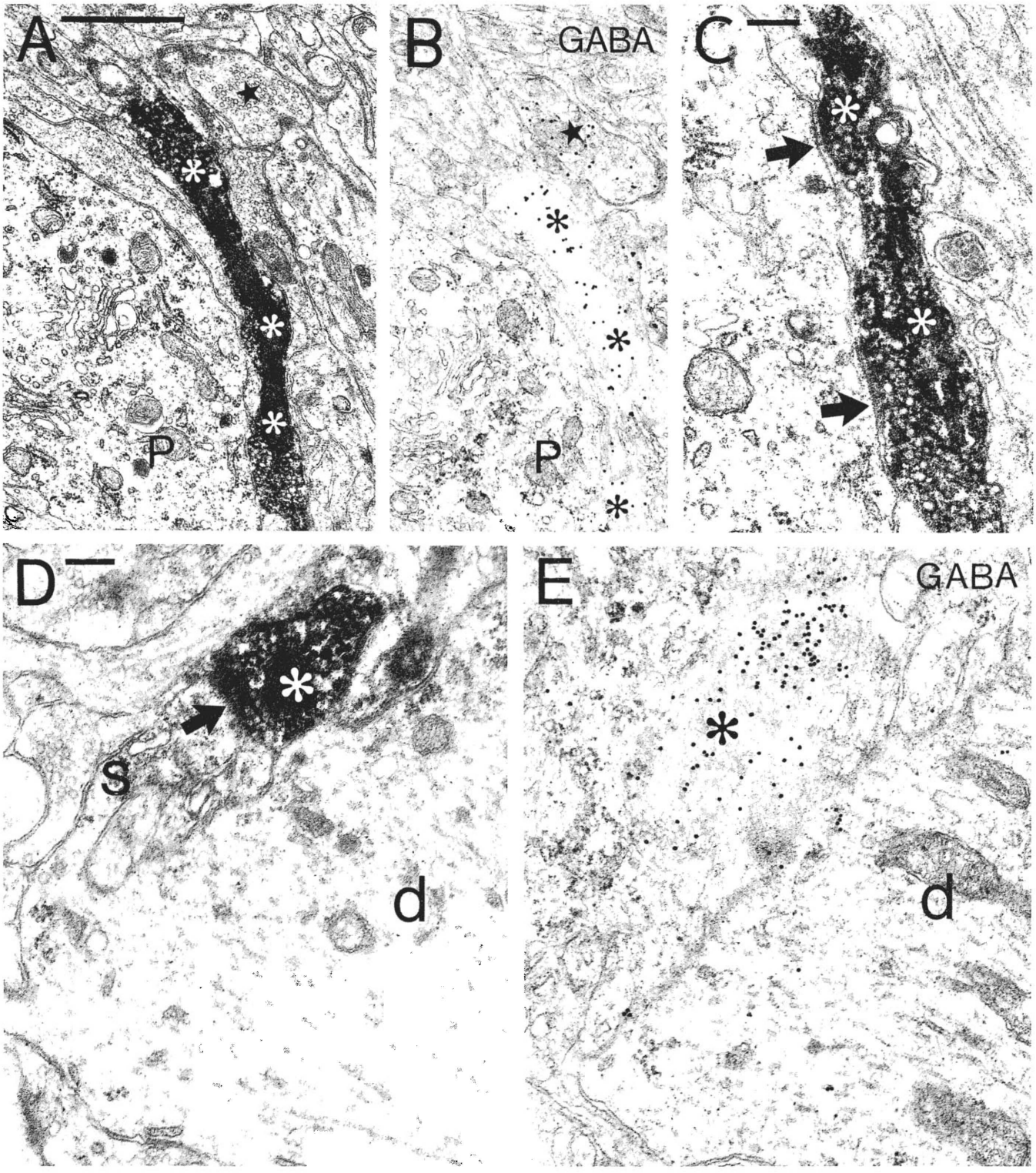

Fig. 9. Immunocytochemical demonstration of GABA in an HRP-filled basket cell terminal. **A–C:** Serial sections of three boutons (asterisks) approaching a pyramidal cell (P) soma. The type II synaptic contacts (arrows) made by two of the boutons are shown in C. The section shown in B was reacted for GABA by the postembedding gold method after the removal of the electron-dense precipitate due to the HRP reaction. The accumulation of electron-dense gold particles indicates that the terminals contain GABA, as does a nearby unlabelled bouton (star). **D–E**: Serial sections of an HRP-filled basket cell terminal (asterisk) making a synapse (arrow) on the stalk of a spine (S) that originates from a pyramidal-type dendrite (d). The bouton is heavily labelled by immunogold in E, demonstrating the presence of GABA. Scales: A and B, 1 μm; C–E, 0.2 μm; D and E same magnification.

Fig. 8. Light and electron micrographs of a Golgi-impregnated basket cell (N2) in deep layers of the kitten visual cortex, area 17. **A:** Drawing of the cell with the somata at the border of layers V and VI and the axon indicated by arrow. **B:** Montage of light micrographs showing the somata (N2) and proximal dendrites (d) of N2 and postsynaptic pyramidal perikarya, (P_1–P_3) surrounded by boutons (arrows) from the axon of the same basket cell. One of them (b_1), shown in **C** and **D**, provides a type II synaptic contact (arrow in D) onto the apical dendritic shaft of pyramidal cell P_1. Scales: A, 100 μm; B, 20 μm; C, 5 μm; D, 0.2 μm.

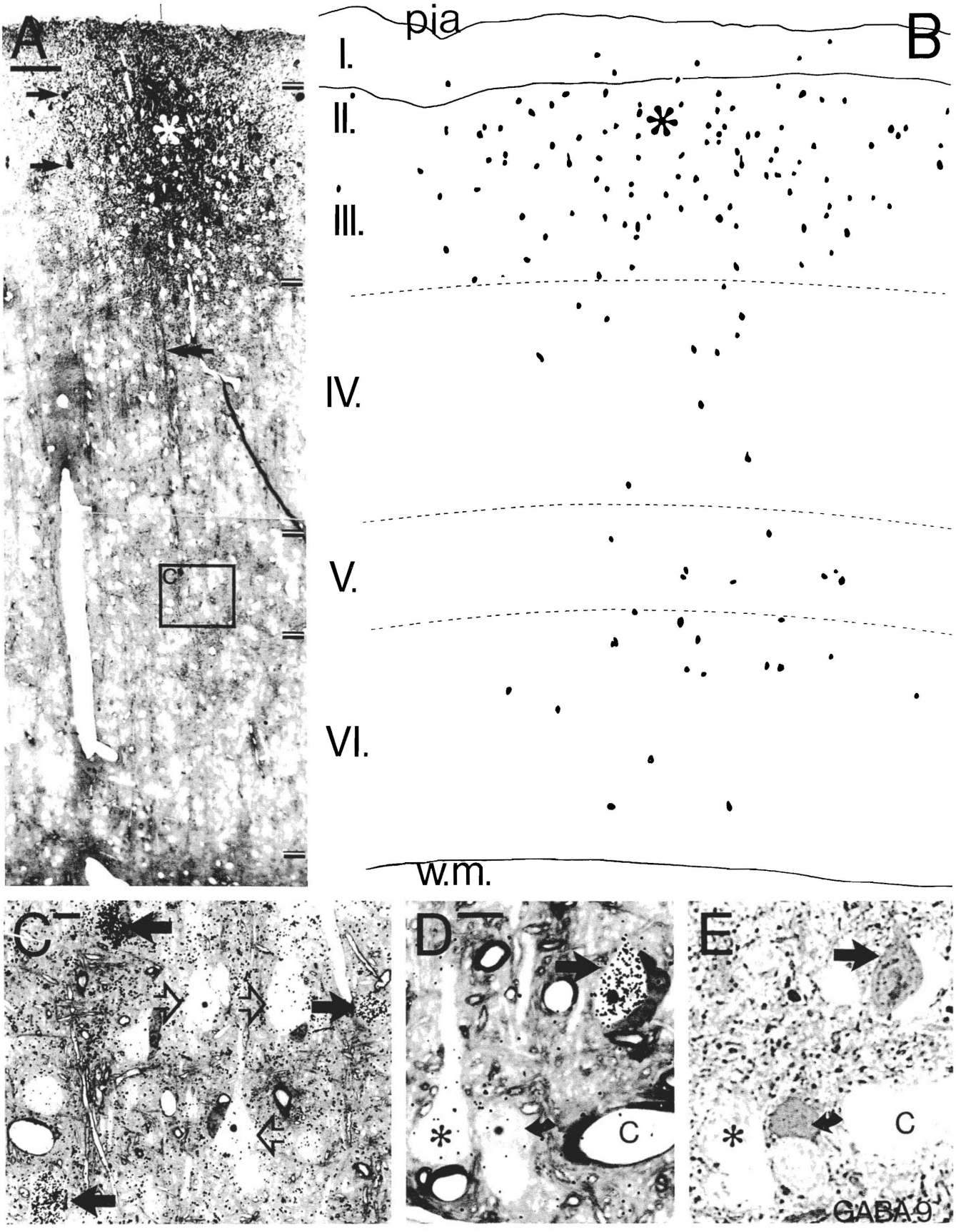

Figure 10

'84), and it is thus reasonable to assume for the present that basket cells inhibit their target neurons. However, we should not be blind to the possibility that GABA plays a more complex role, since any voltage changes it produces may also serve to alter the mode of action potential discharge of the postsynaptic neuron (see Jahnsen and Llinas, '84).

Two other classes of neuron have already been shown to contain GABA (Somogyi et al., '85; Somogyi and Hodgson, '85), and preliminary results show further types that react for GABA (Somogyi, '86). GABA or its metabolic enzymes on their own therefore cannot be used for the identification of basket cells, and further neurochemical markers are needed to establish the distribution and proportion of basket cells among GABAergic neurons, which compose 20% of all neurons in the cat striate cortex (Gabbott and Somogyi, '86).

Target neurons and circuits influenced by deep basket cells

The major postsynaptic targets of the basket cells were the pyramids of layer V. One of the giant pyramidal cells received 34 synaptic contacts from the HRP-filled basket cell. This is the highest number of contacts so far found between two cortical cells and supports our notion that putative inhibitory connections involve a greater number of synapses between individual cells than excitatory ones (Martin and Somogyi, '85). However, the convergent input of many basket cells onto a single pyramidal cell is implied by the observation that many of the type II synapses on the soma were not HRP labelled.

The reconstructions and rotations of the axon revealed the striking columnar organisation of the axon collaterals. Previous work has emphasized the extensive horizontal branches of the basket cell axons (Ramón y Cajal, 1899; Marin-Padilla, '69; Marin-Padilla and Stibitz, '74; Jones, '75; Peters and Regidor, '81; Szentágothai, '73; Martin et al., '83; Somogyi et al., '83; DeFelipe et al., '86). Here that feature is also present, but in addition there are large numbers of boutons provided by the collaterals around and radially above the basket cell soma. On the basis of this observation it is reasonable to suppose that one of the major influences of this cell type is a local one.

The radial, columnar arrangement of the axons of a population of cells in the infragranular layers is also revealed in the distribution of cells in layer V that are labelled by GABA transported from injection sites in the superficial cortical layers. In general the position of cell somata labelled by transported GABA reflects quite well the distribution expected from the known axonal ramifications of morphologically identified GABAergic cells. These include the layer III basket cells (Martin et al., '83), the layer IV clutch cell (Kisvarday et al., '85b), and the basket cells studied here. Similar distributions have also been found through GABA uptake studies in the visual cortex of rat (Somogyi et al., '84) and monkey (Somogyi et al., '83; Kisvarday et al., '86b) and a similar distribution has recently been seen in the somatosensory cortex (DeFelipe and Jones, '85).

This essentially radial organisation of the basket cell axon may provide an important clue to its functional role. In layer V its targets are pyramidal cells of the lower part of layer V, whose axons innervate other pyramidal cells in layers V and VI (Gabbott et al., '87). In layer III the targets are also pyramidal cells, whose predominant descending projection is to pyramidal cells in layer V and possibly layer VI (Gilbert and Wiesel, '83; Martin and Whitteridge, '84; Kisvarday et al., '86b). The main targets of these pyramidal axon systems are the dendritic spine heads (Kisvarday et al., '86b; Gabbott et al., '87). The major targets of the basket cell are the spine necks, the dendritic shafts, and the somas of both layer III and layer V pyramidal cells. Thus, assuming that basket cells are inhibitory in function, they may be controlling two principal sources of excitatory input to the pyramidal cells in layer V, as well as directly controlling these cells themselves.

The role of such an inhibitory influence may also be hinted at by the axon distribution of the basket cells. It is well established that many of the layer V pyramidal cells project subcortically to areas concerned with the control of gaze. A characteristic of layer V cells is their preference for particular directions of motion of objects. Barlow and Levick ('65) proposed a mechanism to explain the direction selectivity of rabbit ganglion cells, and a similar mechanism was proposed by Barlow ('81) to explain motion detection by layer V pyramidal cells in the striate visual cortex. This mechanism involves a spatially specific inhibition so that stimuli moving in the nonoptimal direction would activate the basket cell before activating its target pyramidal cell, while stimuli moving in the optimal direction only activate the inhibitory cells after the pyramidal cell has been activated.

Two points should be noted about this model. Firstly, the inhibitory cells involved in the circuitry underlying motion detection would have to be activated by stimuli very similar to those activating their targets. So, for example, the orientation selectivity, ocular dominance, binocularity, and position in the visual field would be very similar, suggesting that the inhibitory cell and its targets may lie in the same columnar system. Secondly, the inhibitory cells would not necessarily show any evidence of having directional selectivity themselves, since it is the spatial and temporal sequence of excitatory-inhibitory interactions that determine the final output of the pyramidal cell.

One further piece of data may be added in support of the hypothesis that these basket cells are involved in producing direction selectivity. The theoretical account of Blomfield ('74) predicts that the position of the basket cell synapses on the proximal dendrites and soma of pyramidal cell would lead to a divisive type of inhibition if the conductances are large. Physiological experiments have shown that the inhi-

Fig. 10. Columnar retrograde transport of [^{3}H]GABA in cortical area 17 of cat. **A:** Light micrograph and drawing (**B**) of a horizontal (radial) 0.5-μm-thick plastic section through the medial bank of the lateral gyrus showing the [^{3}H]GABA injection site (asterisk) in layers II and upper III. Cells labelled selectively by [^{3}H]GABA were demonstrated by autoradiography (e.g., arrows in A and all black dots in B). Double arrows indicate radial fiber bundles also labelled by [^{3}H]GABA. In addition to neurons around the injection site a group of cells in layers V and VI are also labelled by retrograde transport of [^{3}H]GABA. **C:** Framed area in A is shown at higher magnification demonstrating [^{3}H]GABA-labelled neurons (solid arrows) among unlabelled pyramidal cells (open arrows). All the cells accumulating [^{3}H]GABA drawn in B were also shown to be immunoreactive for GABA. **D, E:** Serial semithin sections processed for autoradiography (D) or GABA immunocytochemistry (E) from layer V below the [^{3}H]GABA injection site. One neuron (straight arrow) labelled by [^{3}H]GABA is also immunoreactive for GABA while another one (curved arrow) is only immunoreactive. A pyramidal cell (asterisk) not labelled by either method and a capillary (c) serve for correlation. Scales: A, B, 100 μm; C, D, 10 μm.

bition involved in directionality (Dean et al., '80) is of the divisive (perisomatic) and not of the subtractive (dendritic) type. However, it should not be assumed that inhibitory processes alone are responsible, since blocking GABA transmission with bicuculline does not always remove directionality (see Sillito, '84). Thus facilitatory mechanisms may also be involved.

Conclusions

Sets of basket cells, as defined here, are found in all the three main tiers of the cortex. Many of the selective physiological properties of cortical cells are generated or augmented by GABAergic inhibitory processes. The direct evidence presented here for the GABAergic nature of the basket cells and the strategic location of their target synapses suggest that they may serve as a general-purpose inhibitory interneuron. The specific properties of cortical cells appear to be generated anew at each cell, and this may account for the presence of the basket cell in each of the three main tiers. Within any single layer particular selectivities may be emphasized according to the output that is required from that layer. For layer V, the basket cells may be particularly responsible for generating "a screen of spatial and temporal inhibitory mechanisms" (Barlow, '81) for the cortical analysis of motion.

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LITERATURE CITED

Barlow, H.B. (1981) Critical limiting factors in the design of the eye and visual cortex. Proc. R. Soc. Lond. [Biol.] *212:*1–34.

Barlow, H.B., and W.R. Levick (1965) The mechanism of directionally selective units in the rabbit's retina. J. Physiol. (Lond.) *178:*477–504.

Blomfield, S. (1974) Arithmetical operations performed by nerve cells. Brain Res. *69:*115–124.

Capowski, J.J., and M. Rethelyi (1982) Neuron reconstruction using a quantimet image analysing computer system. Acta Morphol. Acad. Sci. Hung. *30:*241–249.

Dean, A.F., R.F. Hess, and D.J. Tolhurst (1980) Divisive inhibition involved in directional selectivity. J. Physiol. (Lond.) *308:*84–85P.

DeFelipe, J., and A. Fairen (1982) A type of basket cell in superficial layers of the cat visual cortex. A Golgi-electron microscope study. Brain Res. *244:*9–16.

DeFelipe, J., and E.G. Jones (1985) Vertical organization of γ-aminobutyric acid-accumulating intrinsic neuronal systems in monkey cerebral cortex. J. Neurosci. *5:*3246–3260.

DeFelipe, J., S.H.C. Hendry, and E.G. Jones (1986) A correlative electron microscopic study of basket cells and large GABAergic neurons in the monkey sensory-motor cortex. Neuroscience *17:*991–1009.

Fairen, A., A. Peters, and J. Saldanha (1977) A new procedure for examining Golgi impregnated neurons by light and electron microscopy. J. Neurocytol. *6:*311–338.

Ferster, D. (1986) Orientation selectivity of synaptic potentials in neurons of cat primary visual cortex. J. Neurosci. *6:*1284–1301.

Freund, T.F., and P. Somogyi (1983) The section-Golgi impregnation procedure 1. Description of the method and its combination with histochemistry after intracellular iontophoresis or retrograde transport of horseradish peroxidase. Neuroscience *9:*463–470.

Freund, T.F., Zs Magloczky, I. Soltesz, and P. Somogyi (1986) Synaptic connections, axonal and dendritic patterns of neurons immunoreactive for cholecystokinin in the visual cortex of cat. Neuroscience *19:*1133–1159.

Freund, T.F., K.A.C. Martin, A.D. Smith, and P. Somogyi (1983) Glutamate decarboxylase-immunoreactive terminals of Golgi-impregnated axoaxonic cells and of presumed basket cells in synaptic contact with pyramidal neurons of the cat's visual cortex. J. Comp. Neurol. *221:*263–278.

Friedlander, M.J., K.A.C. Martin, and C. Vahle-Hinz (1985) The structure of the terminal arborizations of physiologically identified retinal ganglion cell Y axons in the kitten. J. Physiol. (Lond.) *359:*293–313.

Gabbott, P.L.A., and P. Somogyi (1986) Quantitative distribution of GABA-immunoreactive neurons in the visual cortex (area 17) of the cat. Exp. Brain Res. *61:*323–331.

Gabbott, P.L.A., K.A.C. Martin, and D. Whitteridge (1987) The connectivity between pyramidal neurons in layer V of cat visual cortex (area 17). J. Comp. Neurol. (in press).

Gilbert, C.D., and T.N. Wiesel (1983) Clustered intrinsic connections in cat visual cortex. J. Neurosci. *3:*1116–1133.

Hanker, J.S., P.E. Yates, C.B. Metz, and A. Rustioni (1977) A new specific, sensitive and non-carcinogenic reagent for the demonstration of horseradish peroxidase. Histochem. J. *9:*789–792.

Hodgson, A.J., B. Penke, A. Erdei, I.W. Chubb, and P. Somogyi (1985) Antiserum to γ-amino-butyric acid. I. Production and characterisation using a new model system. J. Histochem. Cytochem. *33:*229–239.

Jaffe, E.H., and A.C. Cuello (1980) The distribution of catecholamines, glutamate decarboxylase and choline acetyltransferase in layers of rat olfactory bulb. Brain Res. *186:*232–238.

Jahnsen, H., and R. Llinas (1984) Electrophysiological properties of guinea-pig thalamic neurones: An in vitro study. J. Physiol. (Lond.) *349:*205–226.

Jones, E.G. (1975) Varieties and distribution of non-pyramidal cells in the somatic sensory cortex of the squirrel monkey. J. Comp. Neurol. *160:*205–268.

Kisvarday, Z.F., A. Cowey, A.J. Hodgson, and P. Somogyi (1986a) The relationship between GABA immunoreactivity and labelling by local uptake of [3H]GABA in the striate cortex of monkey. Exp. Brain Res. *62:*89–98.

Kisvarday, Z.F., K.A.C. Martin, T.F. Freund, Zs Magloczky, D. Whitteridge, and P. Somogyi (1986b) Synaptic targets of HRP-filled layer III pyramidal cells in the cat striate cortex. Exp. Brain Res. *64:*541–552.

Kisvarday, Z.F., K.A.C. Martin, P. Somogyi, and D. Whitteridge (1985a) Identified basket cells in cat visual cortex: Intracellular filling with HRP reveals distinct types. Neurosci. Lett. *22:*S439.

Kisvarday, Z.F., K.A.C. Martin, D. Whitteridge, and P. Somogyi (1985b) Synaptic connections of intracellularly filled clutch cells, a type of small basket cell in the visual cortex of the cat. J. Comp. Neurol. *241:*111–137.

Krnjevic, K., and S. Schwartz (1967) The action of gamma-aminobutyric acid on cortical neurones. Exp. Brain Res. *3:*320–336.

Lund, J.S., G.H. Henry, C.L. MacQueen, and A.R. Harvey (1979) Anatomical organization of the primary visual cortex (area 17) of the cat. A comparison with area 17 of the macaque monkey. J. Comp. Neurol. *184:*599–618.

Marin-Padilla, M. (1969) Origin of the pericellular baskets of the pyramidal cells of the human motor cortex: A Golgi study. Brain Res. *14:*633–646.

Marin-Padilla, M., and G.R. Stibitz (1974) Three-dimensional reconstruction of the basket cell of the human motor cortex. Brain Res. *70:*511–514.

Martin, K.A.C., and P. Somogyi (1985) Local excitatory circuits in area 17 of the cat. In D. Rose and V.G. Dobson (eds): Models of the Visual Cortex. Chichester: John Wiley & Sons, pp. 504–513.

Martin, K.A.C., and D. Whitteridge (1984) Form, function, and intracortical projections of spiny neurones in the striate visual cortex of the cat. J. Physiol. (Lond.) *353:*463–504.

Martin, K.A.C., P. Somogyi, and D. Whitteridge (1983) Physiological and morphological properties of identified basket cells in the cat's visual cortex. Exp. Brain Res. *500:*193–200.

Meyer, G. (1983) Axonal patterns and topography of short-axon neurons in visual areas 17, 18, and 19 of the cat. J. Comp. Neurol. *220:*405–438.

Morrone, M.C., D.C. Burr, and L. Maffei (1982) Functional implications of cross-orientation inhibition of cortical visual cells. I. Neurophysiological evidence. Proc. R. Soc. Lond. [Biol.] *216:*335–354.

Nelson, J.I., and B.J. Frost (1985) Intracortical facilitation among co-orientated co-axially aligned simple cells in cat striate cortex. Exp. Brain Res. *61:*54–61.

O'Leary, J.L. (1941) Structure of area striata of the cat. J. Comp. Neurol. *75:*131–164.

Peters, A., and C.C. Proskauer (1980) Synaptic relationships between a multipolar stellate cell and a pyramidal neuron in the rat visual cortex. A combined Golgi-electron microscope study. J. Neurocytol. *9:*163–183.

Peters, A., and J. Regidor (1981) A reassessment of the forms of nonpyramidal neurons in area 17 of cat visual cortex. J. Comp. Neurol. *203:*685–716.

Ramón y Cajal, S. (1899) Estudios sobre la corteza cerebral humana. Corteza visual. Rev. Trim. Microgr. *4:*1–63.

Ribak, C.E. (1978) Aspinous and sparsely-spinous stellate neurons in the visual cortex of rats contain glutamic acid decarboxylase. J. Neurocytol. *7:*461–478.

Rose, D. (1977) On the arithmetical operation performed by inhibitory synapses onto the neuronal soma. Exp. Brain Res. *28:*221–223.

Sillito, A.M. (1975) The contribution of inhibitory mechanisms to the receptive field properties of neurons in the striate cortex of the cat. J. Physiol. (Lond.) *250:*305–329.

Sillito, A.M. (1977) Inhibitory processes underlying the directional specificity of simple, complex and hypercomplex cells in the cat's visual cortex. J. Physiol. (Lond.) *271:*699–720.

Sillito, A.M. (1984) Functional considerations of the operations of GABAergic inhibitory processes in the visual cortex. In E.G. Jones and A. Peters (eds): Cerebral Cortex. Functional Properties of Cortical Cells. New York: Plenum Press, pp. 91–117.

Somogyi, P. (1986) Seven distinct types of GABA-immunoreactive neuron in the visual cortex of cat. Neurosci. Abstr. *12:*582.

Somogyi, P., and A. Cowey (1981) Combined Golgi and electron microscopic study on the synapses formed by double bouquet cells in the visual cortex of the cat and monkey. J. Comp. Neurol. *195:*547–566.

Somogyi, P., and A.J. Hodgson (1985) Antiserum to γ-amino-butyric acid. III. Demonstration of GABA in Golgi-impregnated neurons and in conventional electron microscopic sections of cat striate cortex. J. Histochem. Cytochem. *33:*249–257.

Somogyi, P., T.F. Freund, and A. Cowey (1982) The axo-axonic interneuron in the cerebral cortex of the rat, cat and monkey. Neuroscience *7:*2577–2607.

Somogyi, P., T.F. Freund, A.J. Hodgson, J. Somogyi, D. Beroukas, and I.W. Chubb (1985) Identified axo-axonic cells are immunoreactive for GABA in the hippocampus and visual cortex of the cat. Brain Res. *332:*143–149.

Somogyi, P., A.J. Hodgson, A.D. Smith, M.G. Nunzi, A. Gorio, and J-Y. Wu (1984) Different populations of GABAergic neurons in the visual cortex and hippocampus of cat contain somatostatin- or cholecystokinin-immunoreactive material. J. Neurosci. *4:*2590–2603.

Somogyi, P., Z.F. Kisvarday, K.A.C. Martin, and D. Whitteridge (1983) Synaptic connections of morphologically identified and physiologically characterized large basket cells in the striate cortex of cat. Neuroscience *10:*261–294.

Szentágothai, J. (1965) The use of degeneration methods in the investigation of short neuronal connexions. Prog. Brain Res. Singer, M., J.P. Schade, (Eds) Elsevier, Amsterdam *14:*1–32.

Szentágothai, J. (1973) Synaptology of the visual cortex. In R. Jung, H. Autrum, W.R. Loewenstein, D.M. McKay, and H.L. Tenber (eds): Handbook of Sensory Physiology. Vol. VII/3B. Heidelberg, New York: Springer Verlag, pp. 269–324.

Szentágothai, J. (1975) The 'module-concept' in cerebral cortex architecture. Brain Res. *95:*475–496.

Tsumoto, T., W. Eckart, and O.D. Creutzfeldt (1979) Modification of orientation sensitivity of cat visual cortex neurons by removal of GABA-mediated inhibition. Exp. Brain Res. *34:*351–363.

Zsuppan, F. (1984) A new approach to merging neuronal tree segments traced from serial sections. J. Neurosci. Methods *10:*199–204.