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SYNAPTIC CONNECTIONS OF MORPHOLOGICALLY IDENTIFIED AND PHYSIOLOGICALLY CHARACTERIZED LARGE BASKET CELLS IN THE STRIATE CORTEX OF CAT

P. SOMOGYI*‡§, Z. F. KISVÁRDAY*, K. A. C. MARTIN† and D. WHITTERIDGE†

*First Department of Anatomy, Semmelweis University Medical School, Budapest, Tüzoltó u. 58, H-1450 Hungary. †Department of Experimental Psychology, Oxford University, South Parks Road, Oxford OX1 3UD, U.K. ‡Department of Pharmacology, Oxford University, South Parks Road, Oxford, OX1, 3QT, U.K.

Abstract—Neurons were studied in the striate cortex of the cat following intracellular recording and iontophoresis of horseradish peroxidase. The three selected neurons were identified as large basket cells on the basis that (i) the horizontal extent of their axonal arborization was three times or more than the extent of the dendritic arborization; (ii) some of their varicose terminal segments surrounded the perikarya of other neurons. The large elongated perikarya of the first two basket cells were located around the border of layers III and IV. The radially-elongated dendritic field, composed of beaded dendrites without spines, had a long axis of 300–350 μm, extending into layers III and IV, and a short axis of 200 μm. Only the axon, however, was recovered from the third basket cell. The lateral spread of the axons of the first two basket cells was 900 μm or more in layer III and, for the third cell, was over 1500 μm in the antero-posterior dimension, a value indicating that the latter neuron probably fulfills the first criterion above. The axon collaterals of all three cells often branched at approximately 90° to the parent axon. The first two cells also had axon collaterals which descended to layers IV and V and had less extensive lateral spreads. The axons of all three cells formed clusters of boutons which could extend up a radial column of their target cells.

Electron microscopic examination of the second basket cell showed a large lobulated nucleus and a high density of mitochondria in both the perikarya and dendrites. The soma and dendrites were densely covered by synaptic terminals. The axons of the second and third cells were myelinated up to the terminal segments. A total of 177 postsynaptic elements was analysed, involving 66 boutons of the second cell and 89 boutons of the third cell. The terminals contained pleomorphic vesicles and established symmetrical synapses with their postsynaptic targets. The basket cell axons formed synapses principally on pyramidal cell perikarya (approximately 33% of synapses), spines (20% of synapses) and the apical and basal dendrites of pyramidal cells (24% of synapses). Also contacted were the perikarya and dendrites of non-pyramidal cells, an axon, and an axon initial segment.

A single pyramidal cell may receive input on its soma, apical and basal dendrites and spines from the same large basket cell. Seven pyramidal neurons that were postsynaptic to the second and third identified basket cells received synaptic contacts on their soma (average of 4.4 from one basket cell) and proximal dendrites (average 2.3).

The results provide evidence that large basket cells establish multiple synaptic contacts with the neurons they seem to select as postsynaptic targets. At least 30% or more of their postsynaptic targets are the perikarya of other neurons. In the light of recent immunocytochemical studies on the visual cortex of the cat, large basket cells may use γ-aminobutyrate as their transmitter and they are probably inhibitory in function. Since large basket cells also contact dendrites and spines, their effect cannot be explained by a simple somatic inhibitory mechanism and their functional role must also be considered in relation to other inputs to different parts of the same neuron. The structural features of large basket cells are compared with those of other putative inhibitory interneurons and their role in the functioning of the visual cortex is discussed.

Cortical interneurons differ in their specificity with regard to their postsynaptic targets. In the cat visual cortex, one type of local circuit neuron, the axo-axonic cell, has absolute specificity in that it only forms synapses with the axon initial segments of pyramidal cells.[12,59,62] Another interneuron, the double bouquet cell of layer III, is less selective and forms synapses on the perikarya and dendrites of non-pyramidal cells, as well as some of the dendrites and spines of pyramidal cells.[60] A third interneuron type, the so-called cortical basket cell,[25,28,34,35,36,46,47,66,67,68,69,70,71] appears to contact selectively the perikarya of pyramidal neurons. The basket cell has thus featured prominently in theoretical considerations of cortical circuitry because the perikaryon has been thought to be the main site for inhibitory control of the firing of pyramidal neurons.

The first direct evidence that pyramidal neurons can receive multiple synaptic contacts from the same interneuron came with the demonstration of nine synapses provided by a multipolar stellate cell to a pyramidal cell in the rat visual cortex.[44] Subsequently,

§Present address: Department of Human Physiology, Flinders Medical Centre, Bedford Park, South Australia 5042.

Abbreviations: BC, basket cell; HRP, horseradish peroxidase; GABA, γ-aminobutyrate; GAD, glutamate decarboxylase; Type P/S dendrites, dendrites showing features typical of pyramidal/stellate neurons.

axons thought to derive from basket cells were shown to make a large number of synaptic contacts on the soma of a pyramidal cell.[25] More recent evidence shows that selected parts of the axon of a Golgi-impregnated interneuron classified as a basket cell, make multiple synaptic contacts with the dendrites and perikarya of pyramidal and non-pyramidal cells.[9]

However, because only axonal segments which appeared to terminate on the soma of neurons were selected in these studies, it has not been possible to establish the target specificity of the neurons which provide the synapses to perikarya. Consequently, the evidence that cortical basket cells selectively contact the soma of their target neurons, like cerebellar basket cells,[11,41] is still largely inferential.

The present study was undertaken to establish the identity and distribution of the elements postsynaptic to physiologically identified and intracellularly labelled cortical interneurons which, from light microscopic examination, appeared to contact neuronal perikarya with some of their boutons. The distribution of the axons of the identified interneurons and the characteristics of their synaptic terminals are also described. From the data obtained, criteria have been set up for the identification of large basket cells. An attempt was also made to establish the number of synaptic contacts received by any one pyramidal neuron from a single large basket cell. Some of the results have been presented in preliminary form in previous papers, where we described the physiological properties of large basket cells.[30,37]

EXPERIMENTAL PROCEDURES

Neurons for the present study were selected from three adult cats prepared for intracellular recording as described earlier.[37,38] Following physiological characterisation using the receptive field classification system of Henry, Harvey and Lund[23] and the electrical stimulation methods described previously,[6,38] neurons in the visual cortex were labelled by intracellular iontophoresis of horseradish peroxidase (HRP) using the methods of Lin, Friedlander and Sherman.[33] At the end of the recording session the animals were killed with an overdose of anaesthetic (Sagatal, May and Baker) and perfused through the heart with a fixative containing 2.5% glutaraldehyde (TAAB) and 1% paraformaldehyde (TAAB) dissolved in 0.1 M sodium phosphate buffer (pH 7.2–7.4).

Sections (80 and 100 μm thick) were cut from blocks of the visual cortex containing the injected cells using a Vibratome (Oxford Instruments). The sections were washed extensively in 0.1 M phosphate buffer and reacted to reveal HRP activity using the *p*-phenylenediamine/pyrocatechol procedure[19] supplemented with cobalt/nickel intensification.[1] Some sections containing basket cell No. 3 were processed with a less sensitive procedure using 3,3′-diaminobenzidine tetrahydrochloride (Sigma) as substrate.[65] After the reaction the sections from two of the animals were washed in phosphate buffer, post-fixed for 1 h in 1% OsO_4, dissolved in 0.1 M phosphate buffer, dehydrated and mounted on slides in DURCUPAN ACM (Fluka) resin as described previously.[58] To enhance contrast for electron microscopy, 1% uranyl acetate was added to the 70% ethanol during dehydration. Sections containing basket cell No. 1 were dried onto gelatine coated slides and a few sections were stained with Cresyl Violet for light microscopic examination only.

Neurons were drawn with the help of a microscope and drawing tube attachment. Two areas from sections containing basket cell No. 2 and one area containing processes of basket cell No. 3 were photographed in the light microscope and re-embedded for correlated electron microscopy as described earlier.[58,65] It has been demonstrated extensively that by using our procedure any process, even single boutons identified by Golgi impregnation[60] or immunocytochemical staining[14] can be recovered for electron microscopic analysis of its synaptic connections and therefore the correlation of light and electron microscopic examination is shown only in one example (Figs 13, 14). The re-embedded processes were sectioned serially, mounted on single slot grids, and stained with lead citrate. Every bouton which appeared in the plane of the section was photographed at the level where it made synaptic contact. As the plane of the section was coronal and not adjusted to the course of the axon branches, and because all the boutons were recorded irrespective of their position on the axon or the position of their postsynaptic element, this sampling method ensured a representative sample for the analysis of the postsynaptic structures. In several cases, the postsynaptic structures were followed to identify their origin, or in cases of postsynaptic perikarya, to determine the total number of synapses received by them from the identified basket cell. However, in the majority of cases the postsynaptic element was not followed after recording the synaptic contact.

Analysis of postsynaptic dendrites

Photographs of the dendrites contacted by the basket cell axons show that the dendrites are heterogeneous. Two features were chosen for comparison; the proportion of the area within the dendritic profile occupied by mitochondria, and the proportion of the perimeter of the dendritic profile occupied by synaptic junctions. These were calculated from measurements obtained from electron micrographs by a planimeter. For each dendrite a photograph was chosen in which the HRP-labelled, identified bouton had its largest synaptic active zone. As not all the postsynaptic dendrites were originally photographed at their full extent, only some of them could be included in the sample. The two values for each dendrite were plotted on an X, Y coordinate system. Although mitochondrial density and synaptic density may not be correlated, linear regression lines were calculated and used as an axis for the point cluster to compare the dendritic populations (Fig. 18).

RESULTS

Criteria for the selection of large basket cells

During the course of analysing more than 100 physiologically characterized and intracellularly labelled neurons in the light microscope,[39] it was noticed that the terminal axon segments of some HRP-filled neurons approached the perikarya of other neurons. These terminal segments usually formed several varicosities which apparently contacted the somata and were visible in the osmicated thick sections (Figs 5E–H and 11A–D). Five such neurons have been found, and the extent of the axonal arborization of two of these (BC1, BC2) is several times longer than that of the dendritic arborization. The axon of a third neuron (BC3) was also injected without labelling its perikaryon and dendrites. The size of this axonal arborization is several times the size of any known dendritic arborization in the cortex. Thus, for neurons BC1–BC3, another common feature is that the lateral extent of the axonal arborization is three or more times that of the

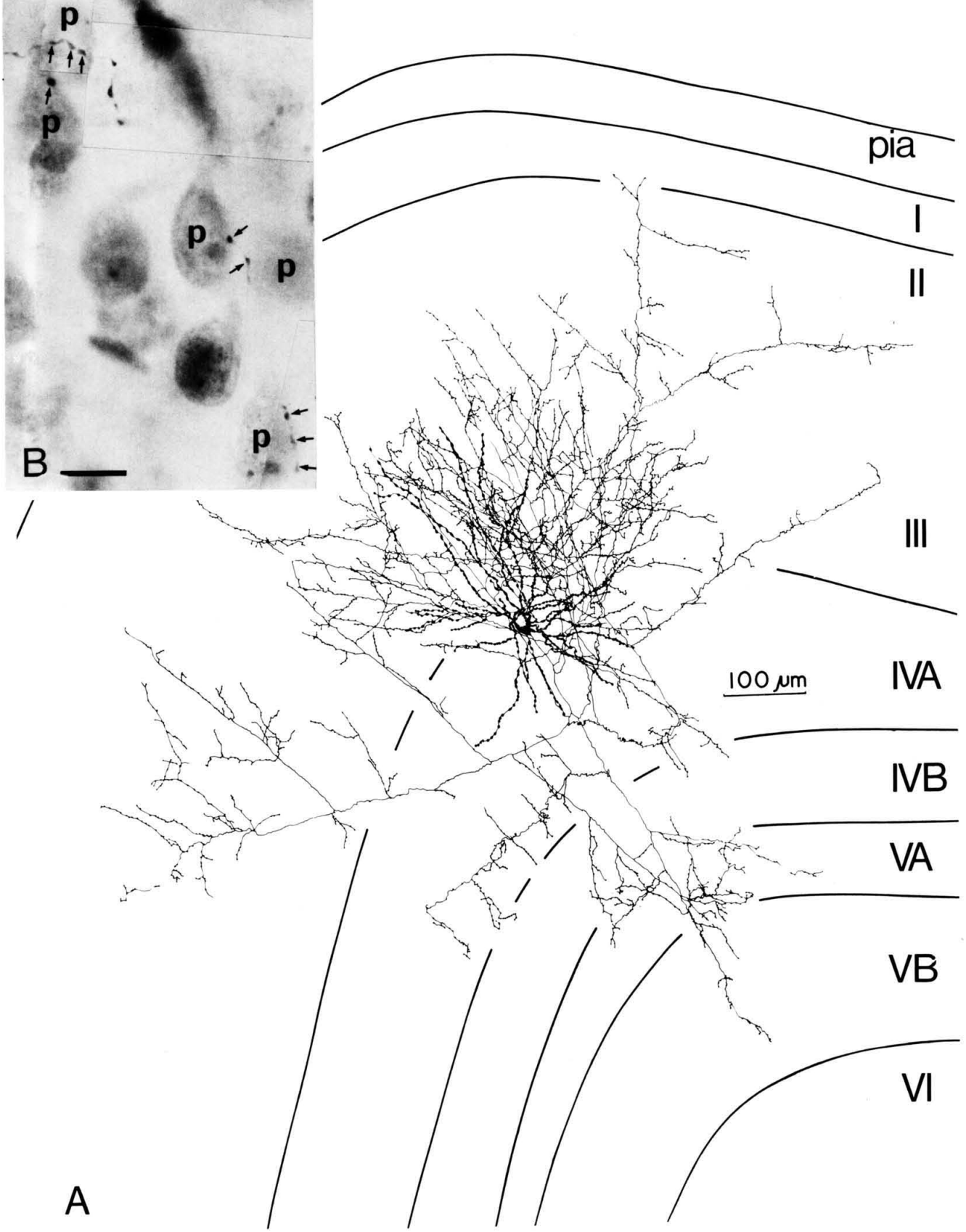


Fig. 1. (A). Camera lucida drawing of basket cell No. 1 in the lateral gyrus (area 17) of the cat. Physiological characteristics: orientation selective, S_2 type receptive field, size $0.25° \times 1.0°$, ocular dominance group 7. This cell showed a directional preference, monosynaptically driven by X-like LGN afferents and monosynaptically driven by callosal fibres.[37] This neuron was completely filled, all the axon branches ended in fine beaded terminal segments. The perikaryon and most of the dendrites were in layer IV but the bulk of the terminal axon segments are in layer III where the axon has its widest distribution. At the antero-posterior level where the soma is situated the axon sends descending collaterals to layer V. Coronal plane. (B). Light micrograph of a terminal axon segment of the same neuron in apparent contact (arrows) with Nissl-stained perikarya of pyramidal cells (P) in lower layer 3. Compare with Figs 5 and 11. Scales: (A) = 100 μm; (B) = 10 μm.

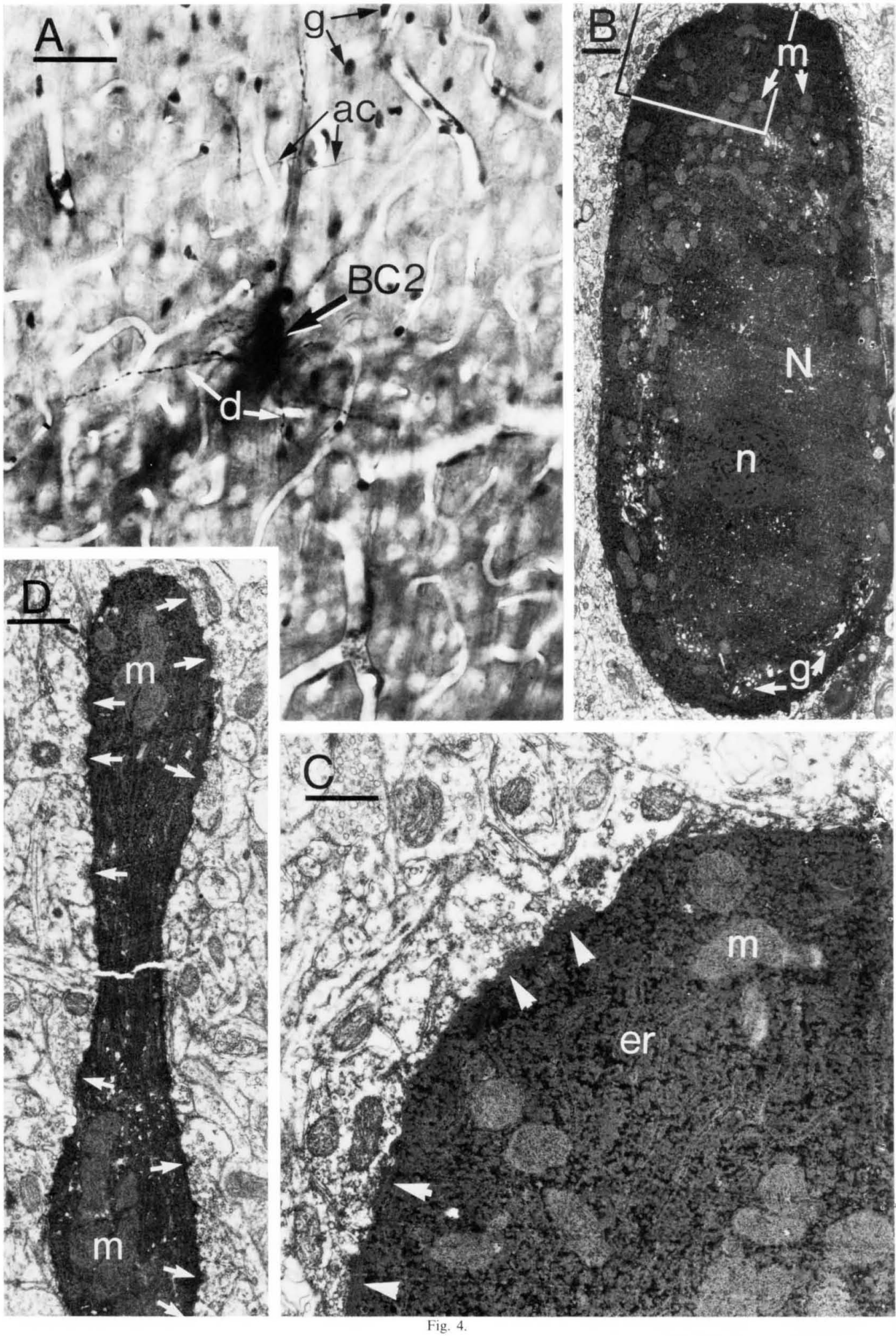


Fig. 4.

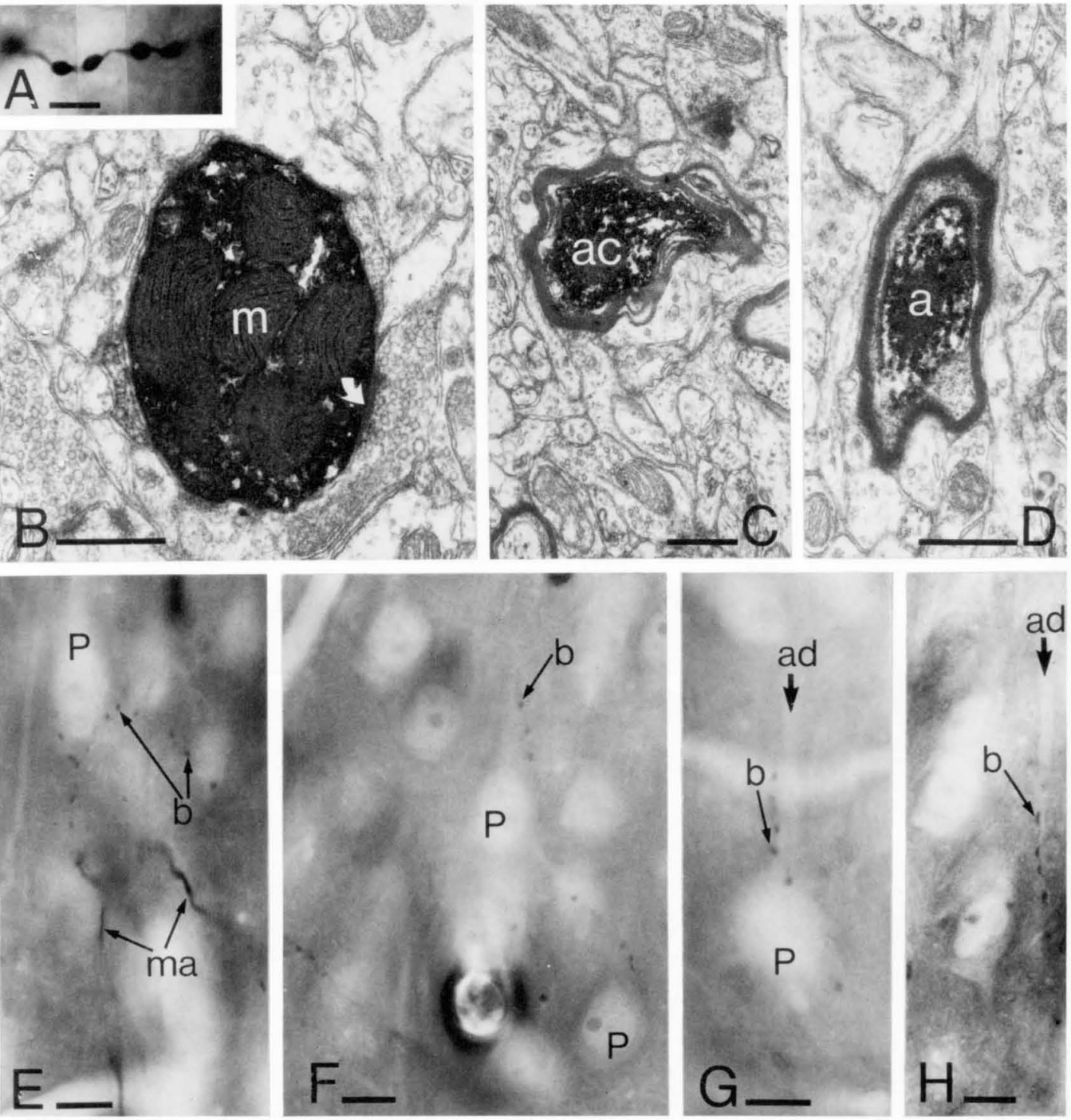


Fig. 5. (A). Light microscopic details of the beaded dendrite of basket cell No. 2. (B). One of the swellings on the dendrite in (A) is shown to contain a high density of mitochondria (m) and to receive a synaptic contact (arrow) from a bouton containing round vesicles. (C). Electron micrograph of one of the laterally running secondary myelinated axon collaterals (ac). (D). Electron micrograph of the main ascending axon (a) just distal to the axon initial segment where it acquired its myelin sheath. (E)–(H). Light micrographs illustrating filled boutons (b) of basket cell No. 2 associated with pyramidal neurons (P), with an emerging apical dendrite (ad) in G, or with a major ascending apical dendrite (ad) in (H). In (E) two myelinated main axon collaterals (ma) are also present. Scales: (A), 5 μm; (B)–(D), 0.5 μm; (E)–(H), 10 μm.

Fig. 4. (A). A light micrograph of basket cell No. 2 with perikaryon at the border of layers III and IV as seen in a thick osmicated section. Some of the beaded dendrites (d) and two of the main axon collaterals (ac) are labelled. Some diffusion of the HRP reaction endproduct around the soma is visible. Granulocytes (g) invading the area of penetration and erythrocytes show up positively with the peroxidase reaction used. (B). Electron micrograph showing the soma of basket cell No. 2. The neuron is heavily filled with reaction endproduct but the nucleus (N), the nucleolus (n), mitochondria (m) and the Golgi apparatus (g) are recognisable. (C). The framed area in (B) is shown at higher magnification illustrating the density of synaptic contacts (arrows) received by the soma. Mitochondria (m) and endoplasmic reticulum (er) are indicated. (D). Proximal dendrite is seen receiving numerous synaptic contacts (arrows). The swellings of the dendrite contain groups of mitochondria (m). Scales: (A), 50 μm; (B), 1 μm; (C) and (D), 0.5 μm.

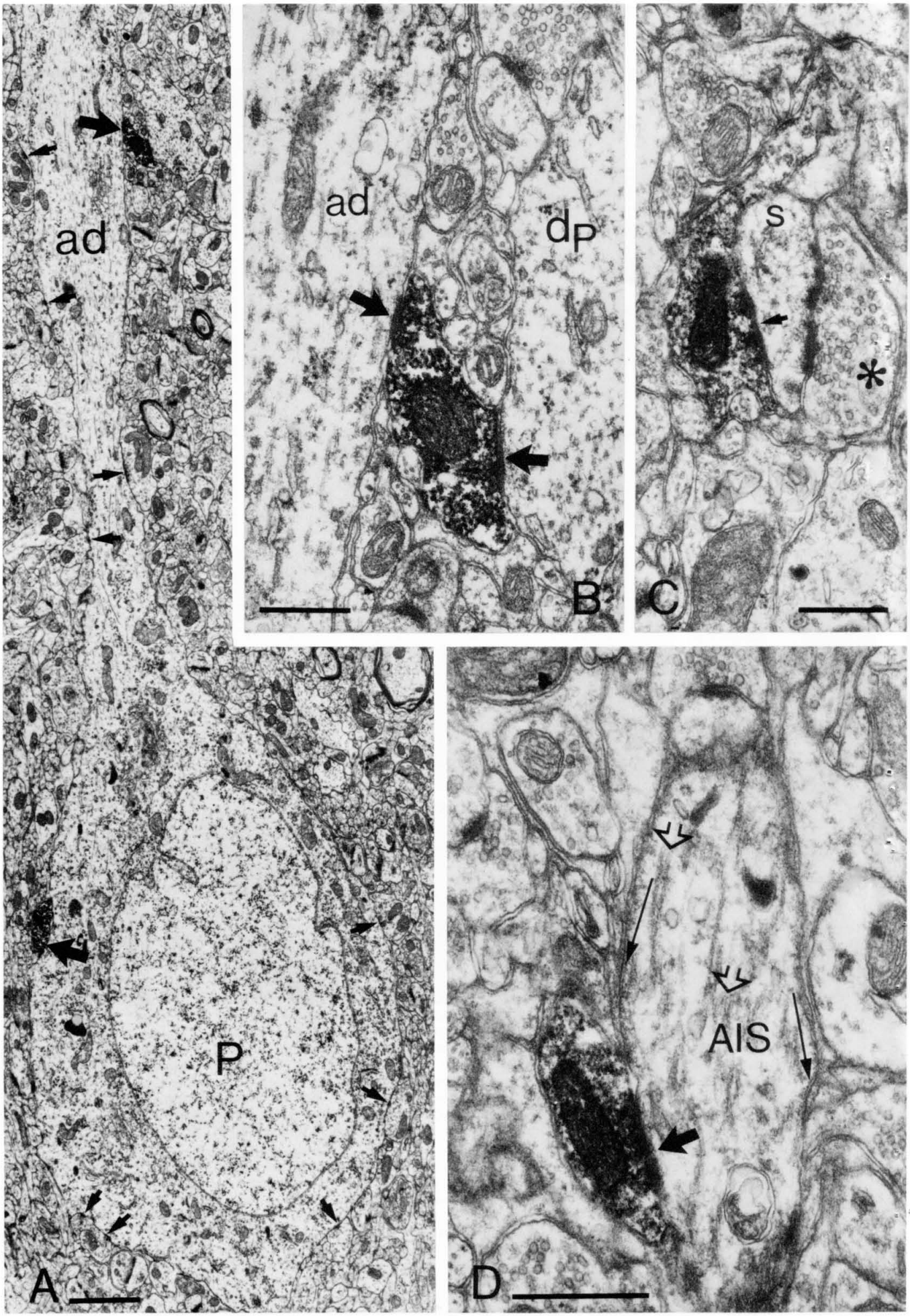


Fig. 6.

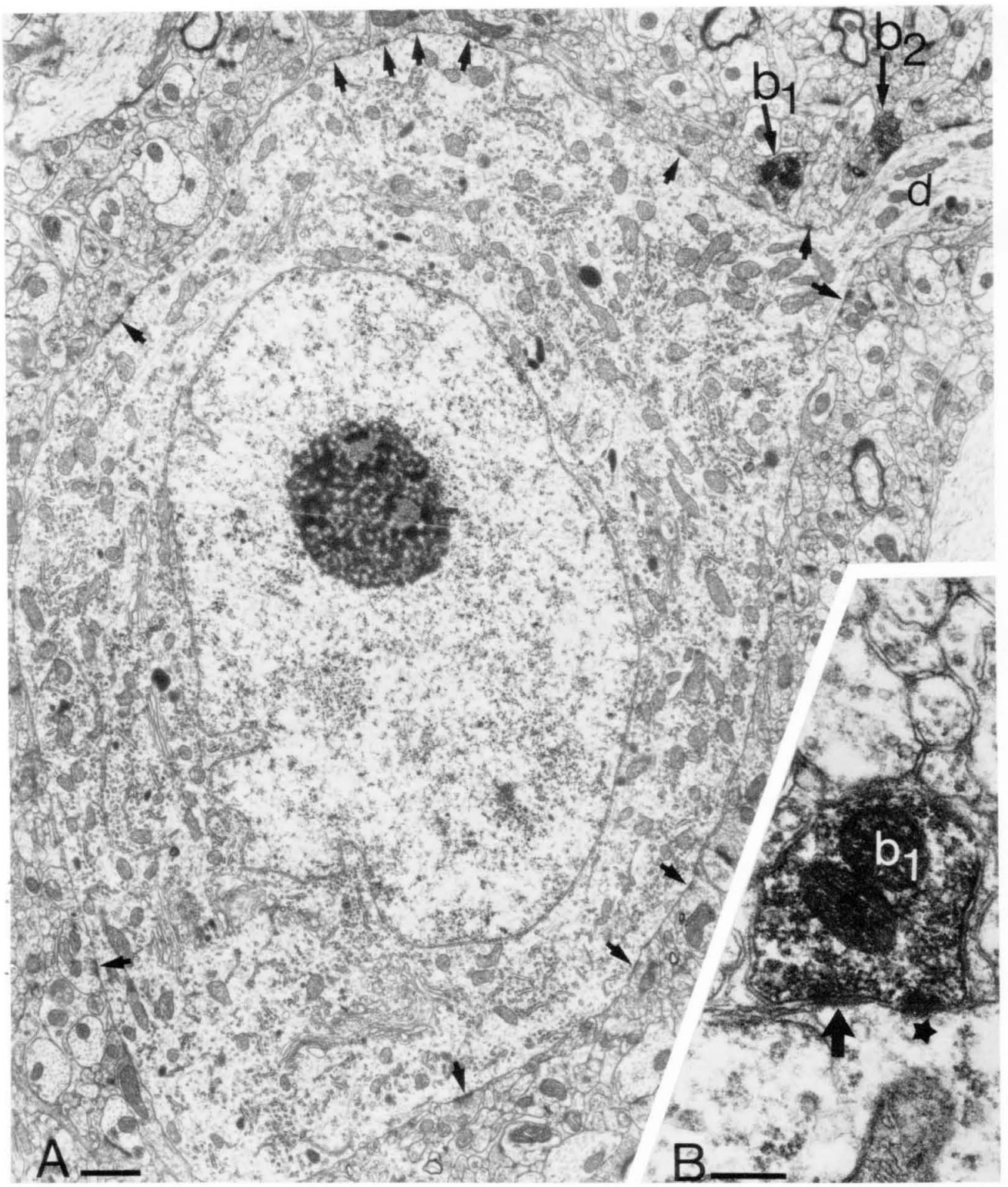


Fig. 7. (A). Two labelled boutons (b_1, b_2) are seen near a large non-pyramidal neuron emitting a dendrite (d). The neuron receives both type II and type I synapses (arrows) on its soma and dendrite. The dendrite is in synaptic contact with one of the basket cell boutons (b_2) and is shown at higher magnification in Figs 8(A) and (B). (B). The other bouton (b_1) is shown in a serial section to make a type II synaptic contact (arrow) and a punctum adherens (star) with the soma of the neuron. Note the small pleomorphic vesicles in the bouton. Scales: (A), 1 μm; (B), 0.2 μm.

Fig. 6. (A)–(D). Electron micrographs of the labelled boutons of basket cell No. 2. (A). A pyramidal neuron (P) receives two synapses (large arrows) from labelled boutons on its soma and apical dendrite (ad) and several more from other unlabelled boutons (arrows). The upper labelled bouton is shown in (B), where it makes synaptic contact (arrows) with the apical dendrite (ad) and another large type P dendrite (dP). (C). A spine (s) receives a symmetrical synaptic contact (arrow) from a labelled bouton and an asymmetrical synapse from another bouton (asterisk) containing round vesicles. (D). An axon initial segment (AIS) which was traced back to a pyramidal neuron in serial sections is seen in synaptic contact (thick arrow) with a labelled bouton. Microtubule fascicles (open arrow) and the membrane undercoating (long arrow) are indicated. Scales: (A) 2 μm; (B)–(D), 0.5 μm.

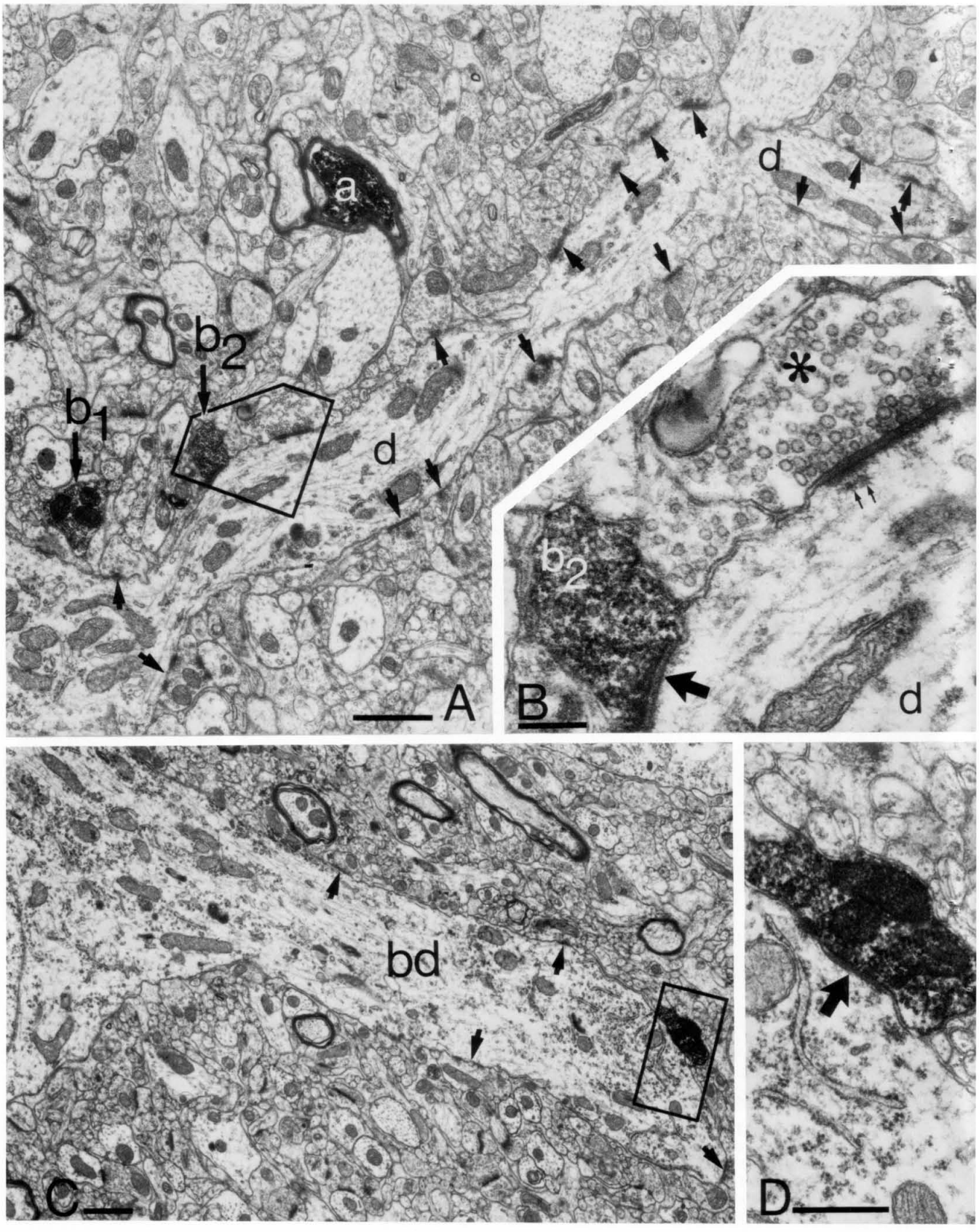


Fig. 8. (A). A dendrite (d) originating from the non-pyramidal cell in Fig. 7(A) is shown to receive a large number of synaptic contacts (arrows). One of the synapses is provided by a bouton (b_2) which originated from a myelinated axon collateral (a) of the basket cell (No. 2). (B). Labelled bouton (b_2) in (A) is shown at higher magnification giving a type II synaptic contact (large arrow) while a neighbouring bouton (asterisk) gives a type I synapse. Small arrows indicate subjunctional dense bodies. The filled bouton contains small pleomorphic vesicles while the other bouton has larger round vesicles. (C) and (D). The basal dendrite (bd) of a pyramidal cell in (C) receives a type II synaptic contact (large arrow) from a labelled bouton of basket cell No. 2 also shown in the framed area and also shown at higher magnification in (D). Similar synaptic contacts (small arrows) are also provided by unlabelled boutons. Scales: (A) and (C), 1 μm; (B), 0.2 μm; (D), 0.5 μm.

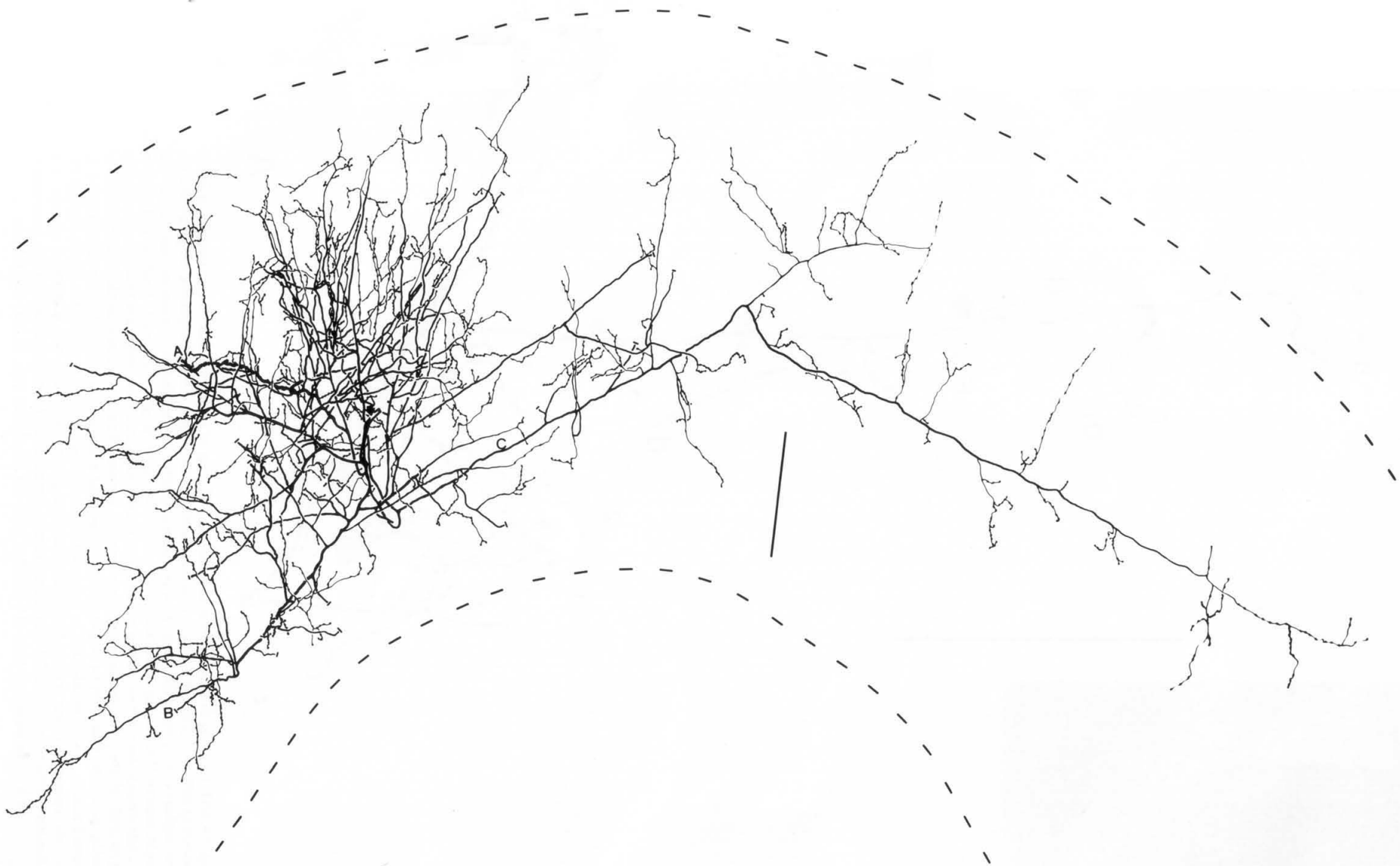
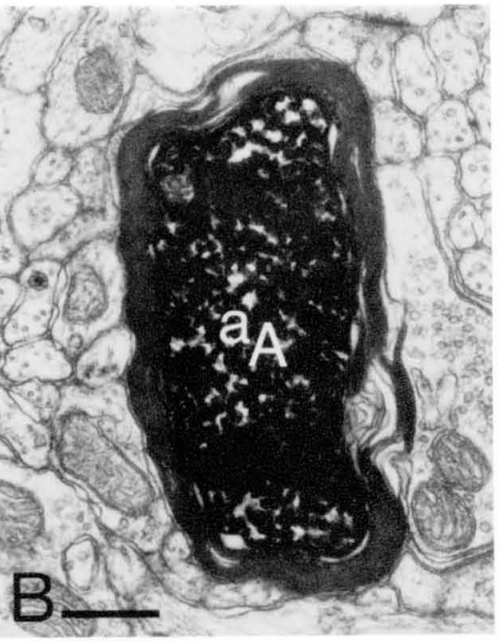


Fig. 9. Camera lucida drawing of a partially reconstructed large basket cell axon (No. 3), as seen in the coronal plane in area 17 (lateral gyrus) of the cat. Physiological characteristics: orientation selective, C type receptive field requiring little length summation, size $2.1^{\circ} \times 3.2^{\circ}$, ocular dominance group 4, directionally selective, polysynaptically driven via Y-like LGN afferents.[37] The main axon was impaled with a microelectrode at the point indicated by arrow. The axon gives rise to collaterals (A) and (B) running rostrally and caudally, respectively, at an oblique angle to the coronal plane. Collateral (C) was within one section and took a lateral course. Upper broken line indicates the border of layers I and II, lower broken line the borders of layers III and IV. Some terminal segments had to be left out in the central region because of the density of overlapping axons in two dimensional reconstruction. Scale: 100 μm.



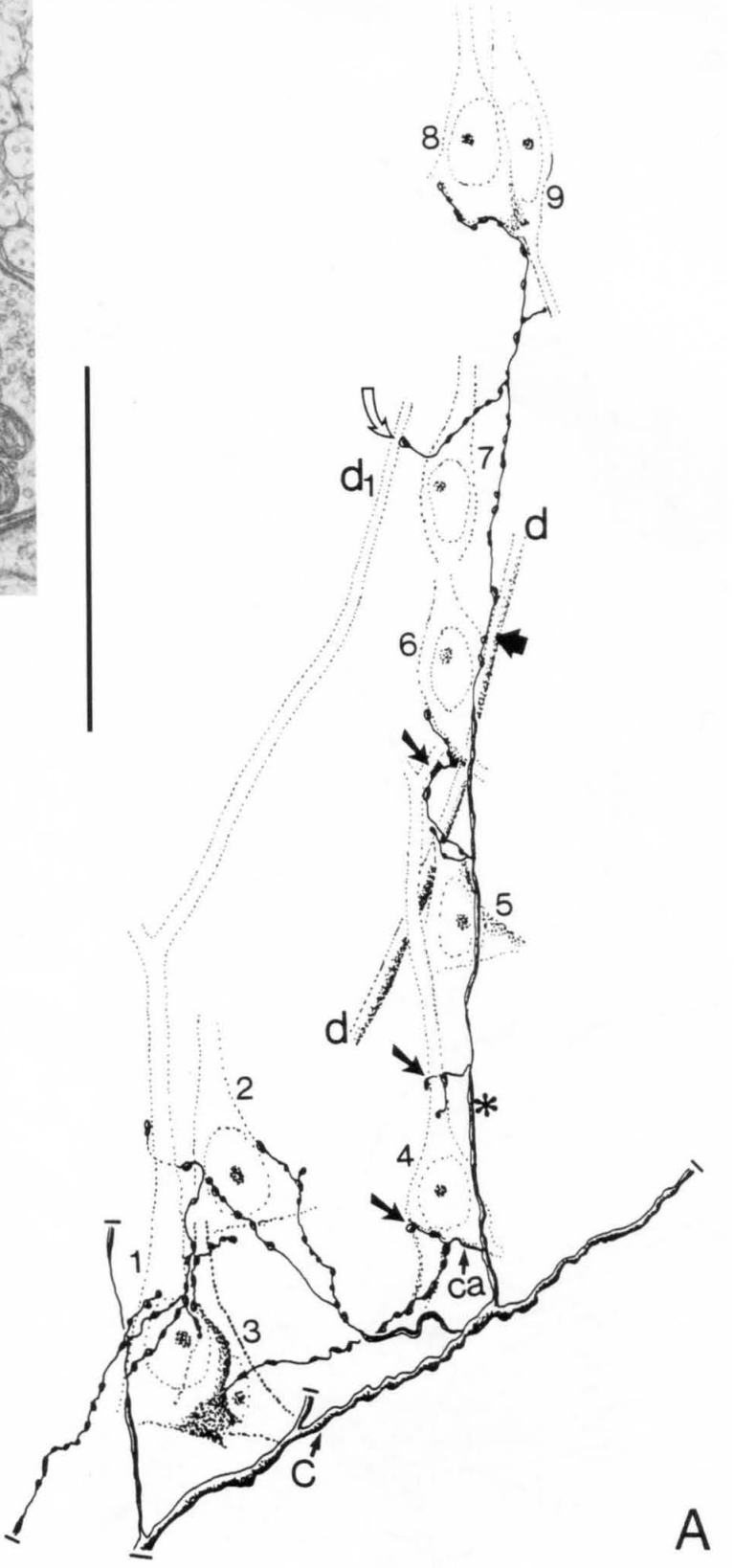


Fig. 10. (A). Drawing of a detail from collateral (C) of axon of basket cell No. 3. One radially oriented ascending collateral (asterisk) is reconstructed complete, some other terminal segments are partly drawn. The outlines of nine pyramidal neurons which were contacted by the labelled varicosities were traced. The largest neuron (No. 1) was contacted on its perikaryon, at the origin of its apical dendrite and receives a single contact (open arrow) further up on one of the side branches of the apical dendrite. Another smaller pyramidal cell (No. 4) is contacted by three terminal segments (oblique arrows) of the same collateral in similar positions. One terminal segment (ca) contacts the soma and the basal dendrite then goes on to contact neuron No. 1. In addition to pyramidal cells and their dendrites a possible apical dendrite (d) was also contacted (thick arrow). Note the radial arrangement of neurons Nos 4–9. Other neurons (not indicated) surrounding the traced cells did not receive contacts. (B). Electron micrograph of a cross section of a main axon collateral (aA labelled A in Fig. 9). Scales: (A), 50 μm; (B), 0.5 μm.

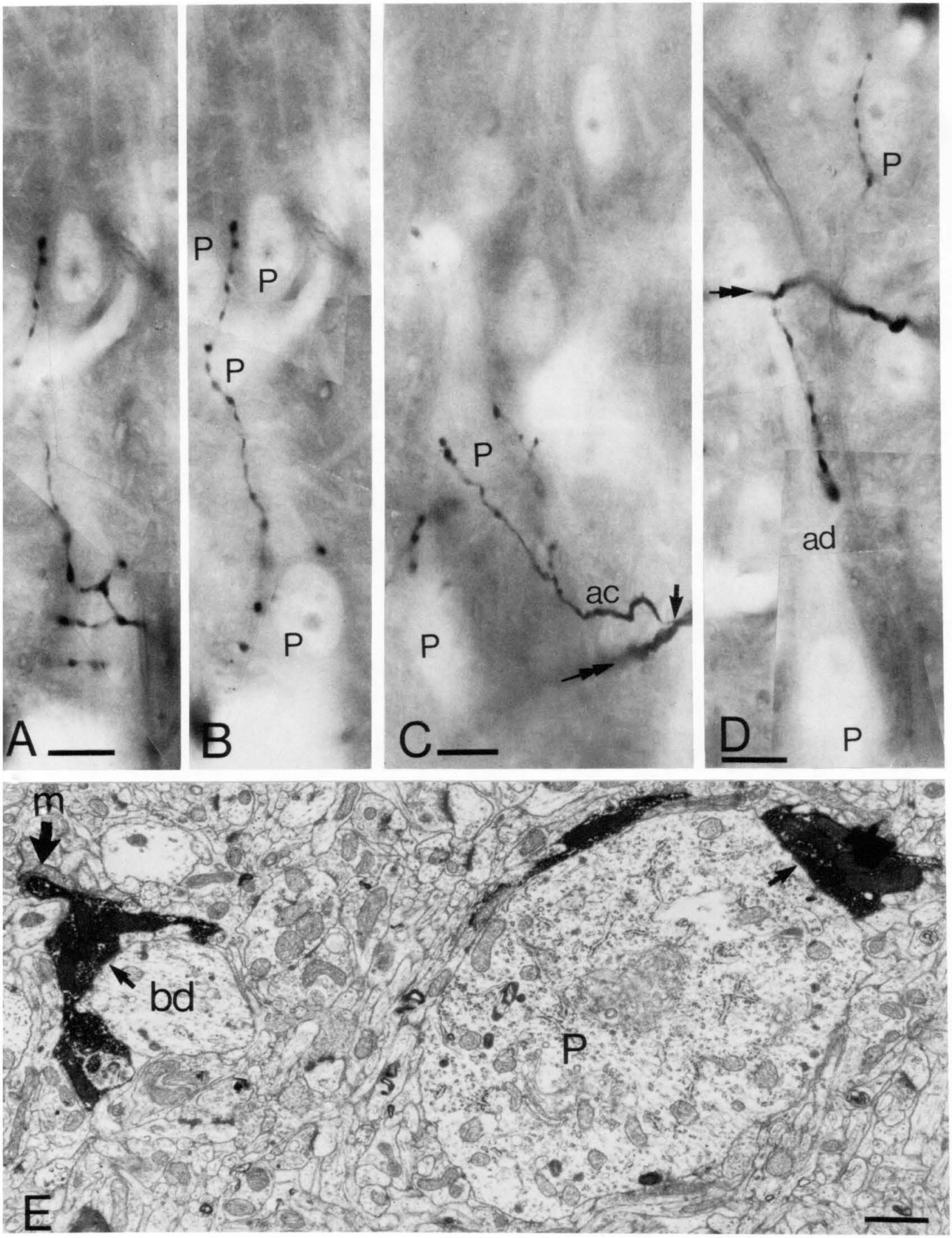


Fig. 11.

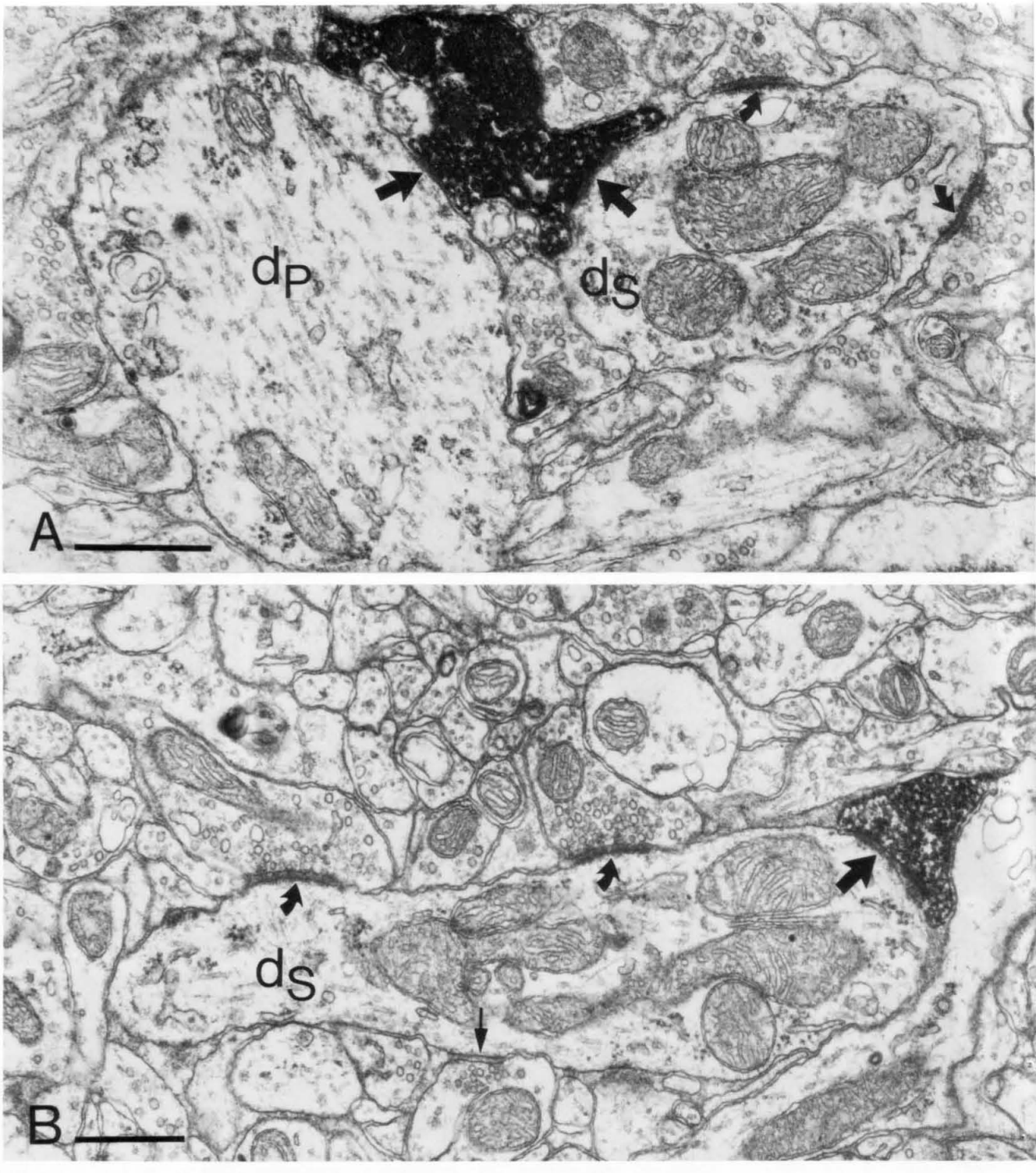


Fig. 12. (A). A bouton of basket cell No. 3 gives synaptic contact (large arrows) to a type P (dp) and a type S (ds) dendrite. The latter contains more mitochrondria and receives additional type I synaptic contacts (curved arrows). (B). A type S dendrite receives type II synaptic contacts from a labelled bouton (large arrow) and from an unlabelled bouton (small arrow) and two additional type I synaptic contacts (curved arrows). Scales: 0.5 μm.

Fig. 11. (A)–(D). Light micrographs of the collaterals of basket cell axon No. 3. (A) and (B) are montages of the same collateral in different planes. The lowermost pyramidal cell (P) is enclosed in a "basket" formed by one collateral which then ascends radially and contacts three more pyramidal neurons (P) with fewer boutons. (C). A secondary myelinated axon collateral (ac) leaves one of the main myelinated axons (double arrow) at a node of Ranvier and gives two collaterals to a large pyramidal cell (P). The lower pyramidal cell (P) is contacted by another collateral at the origin of its apical dendrite. (D). A myelinated horizontal axon (double arrow) gives a short descending collateral to the apical dendrite of a large pyramidal cell (P) at the bottom of layer III and an ascending collateral to the soma of a small pyramid (P). (E). Electron micrograph of a labelled axon emerging from its myelin sheath (m) and contacting with several boutons the soma of a pyramidal cell (P, No. 4 in Table 3) and its basal dendrite (bd) which was traced to the perikaryon in serial sections. Arrows indicate synaptic contacts. Scales: (A)–(D), 10 μm; (E), 1 μm.

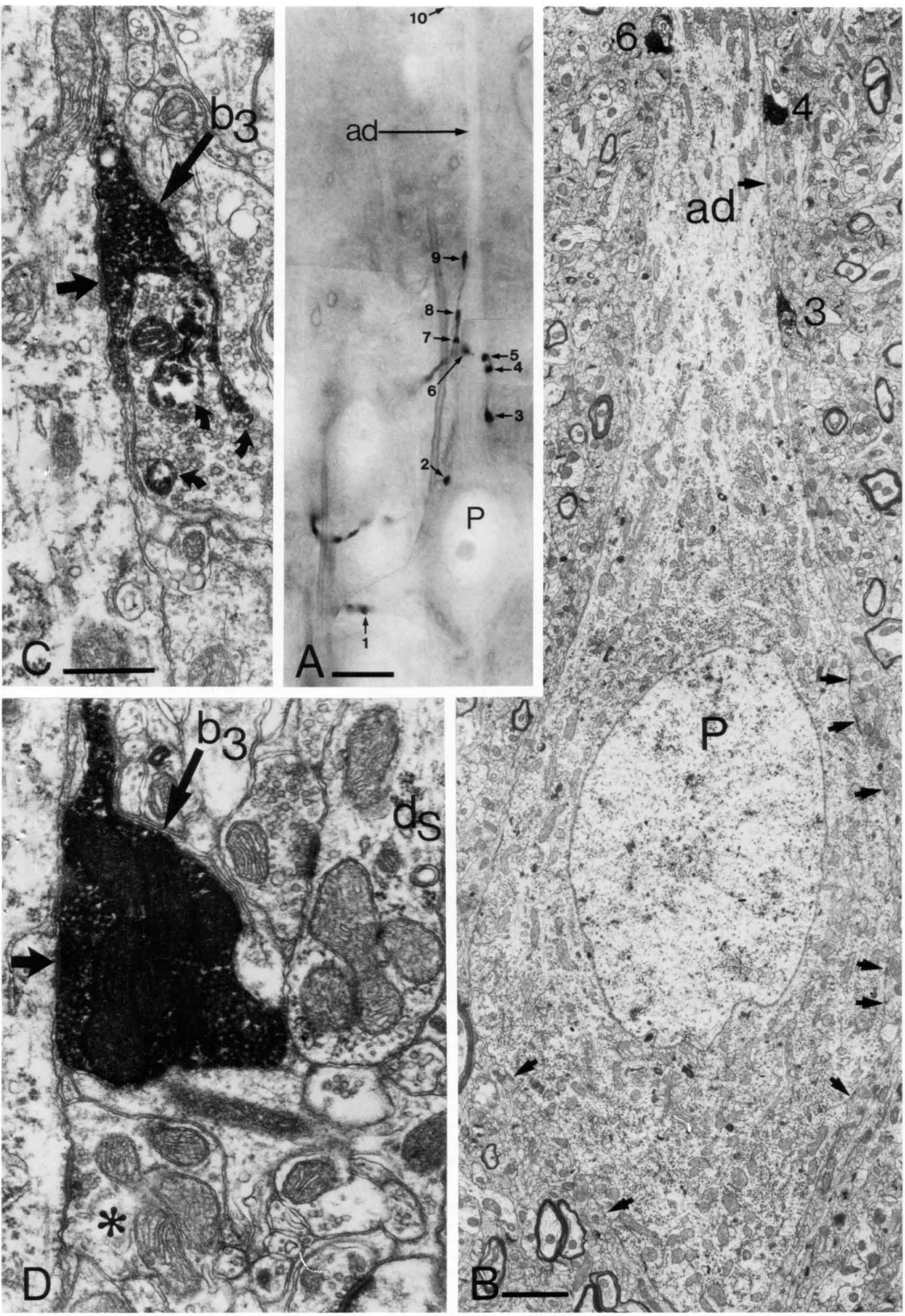


Fig. 13.

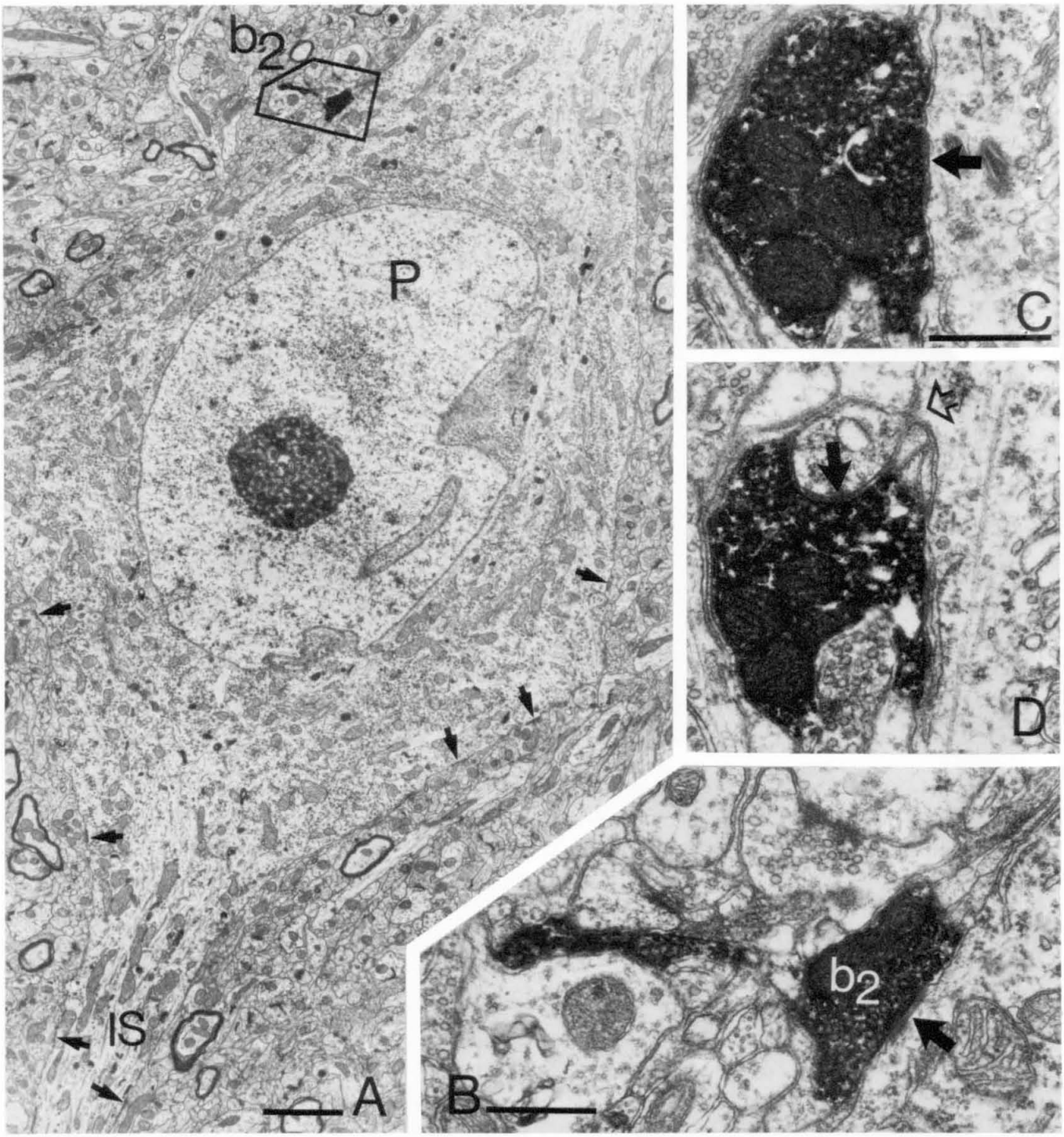


Fig. 14. (A) and (B) show the same neuron (P) as in Fig. 13. Only one labelled terminal (b_2) contacted this perikaryon which received several synapses (arrows) from unlabelled boutons on its soma and axon initial segment (IS). Framed area in (A) is shown at higher magnification in (B) where the synaptic contact is indicated (large arrow). (C) and (D) show serial sections of a bouton contacting the soma of another pyramidal cell, establishing type II synaptic contacts (large arrows) with the soma and with a somatic spine (open arrow). Scales: (A), 2 μm; (B)–(D), 0.5 μm.

Fig. 13. (A). A light micrograph of a large pyramidal cell (P, No. 6 in Table 3) which receives synaptic boutons on its basal dendrite (1), soma (2) and apical dendrite (ad, 3–10) from four different collaterals of the same basket cell axon (No. 3). (B). Electron micrograph of the same neuron (P) at the level of the emerging apical dendrite (ad). It is contacted by three labelled boutons (3, 4 and 6) and numerous unlabelled boutons (arrows). One of the boutons (b3) is shown at higher magnification in serial sections in (C) and (D) to establish a type II synaptic contact (large arrow) with the apical dendrite. In (C) finger-like processes (curved arrows) of the labelled bouton penetrate a neighbouring bouton. In (D) another unlabelled bouton (asterisk) makes a type II synapse with the same apical dendrite. A nearby type S dendrite (dS) was also seen to receive synaptic contact from the labelled bouton in serial sections. Scales: (A), 10 μm; (B), 2 μm; (C) and (D), 0.5 μm.

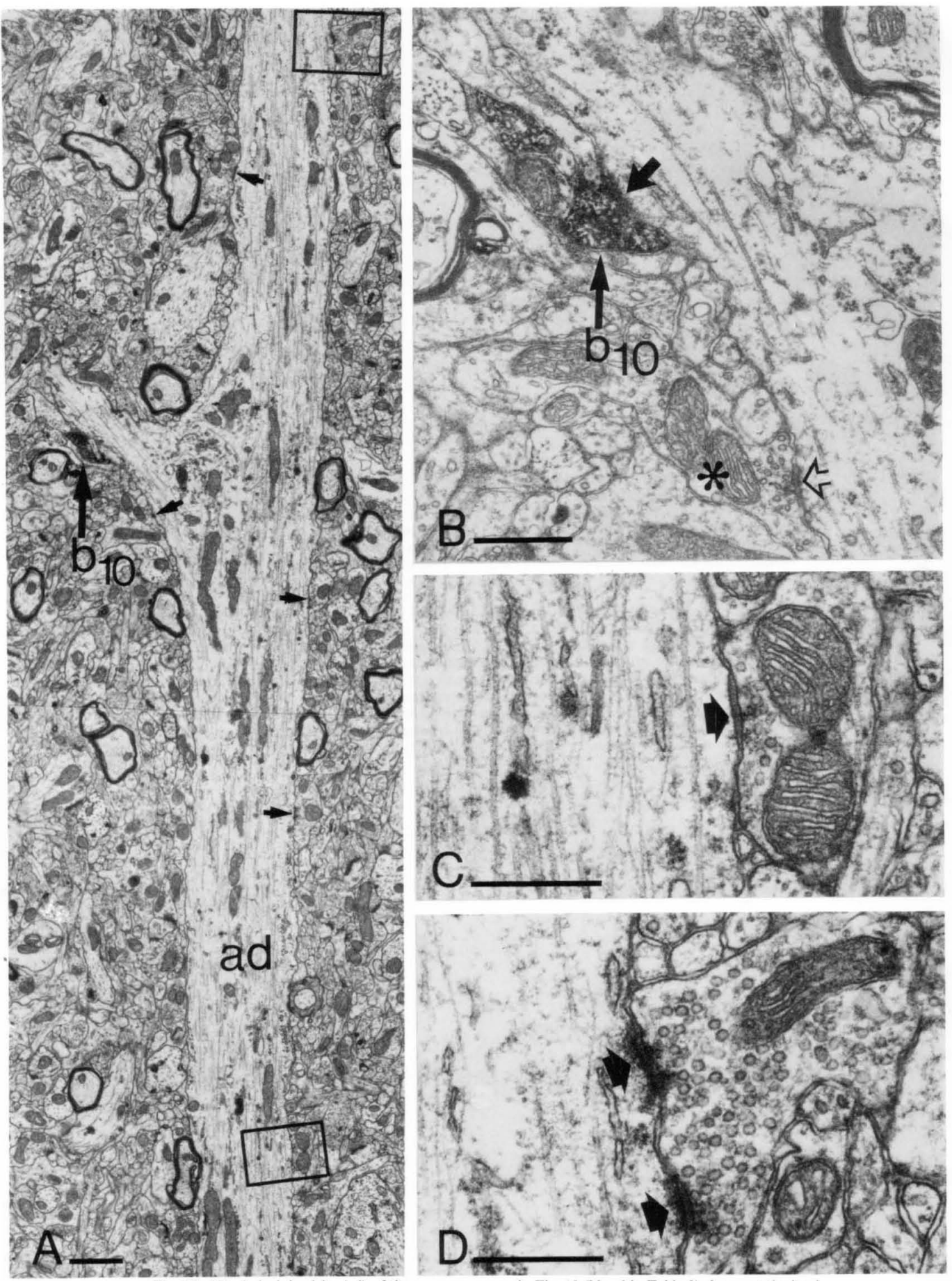


Fig. 15. (A). Apical dendrite (ad) of the same neuron as in Fig. 13 (No. 6 in Table 3) shown at the level where it is contacted by a labelled bouton (b_{10}) at the origin of a side branch. The synaptic contact (large arrow) is shown at higher magnification in a serial section in (B). A similar unlabelled bouton (asterisk) also makes a synapse (open arrow) with the dendrite. The apical dendrite receives numerous synaptic contacts (small arrows in A). One type II (lower framed area) and a type I synapse (upper framed area) are shown at higher magnification in (C) and (D) where thick arrows indicate synaptic contacts. Scales: (A), 1 μm; (B)–(D), 0.5 μm.

Fig. 16. (A)–(C) show representative serial sections of a type P dendrite (dp) emitting two spines (long arrows). Both spines receive a type II synaptic contact (large arrows) from labelled boutons of BC3 at their narrow neck. One of the spines (S) is seen in addition to receive a type I synaptic contact from a bouton containing round vesicles (asterisk). One of the filled boutons (star) also makes a synapse with a dendritic shaft (large arrow in C). (D)–(F): Serial sections of two spines (S) receiving type I synapses from a bouton with round vesicles (asterisk) and type II synapses (arrows) from a labelled bouton. Scales: (A)–(C), 0.5 μm; (D)–(F), 0.2 μm.

Fig. 17. (A). A lightly filled terminal segment of basket cell No. 3 is shown running along a pyramidal neuron (P) (No. 8 in Table 3) and contacting it with three varicosities (white stars) two of which form synaptic contacts (large arrows). An unlabelled bouton also makes a synaptic contact (open arrow). (B). The bouton on far left in (A) is shown at higher magnification in a serial section (asterisk). It originates from the labelled axon (a) collateral (triangles), forms a type II synaptic contact and contains small pleomorphic vesicles. (C)–(F) are from serial sections of a process which was probably an axon (a) as it acquired myelin sheath (m). Just after leaving the myelin it emitted a small collateral (ac) which received a synaptic contact (short arrow in C and D) from the labelled bouton, while the axon itself received two more type II synapses, as indicated in (C) and (D) by short arrows. Scales: 0.5 μm.

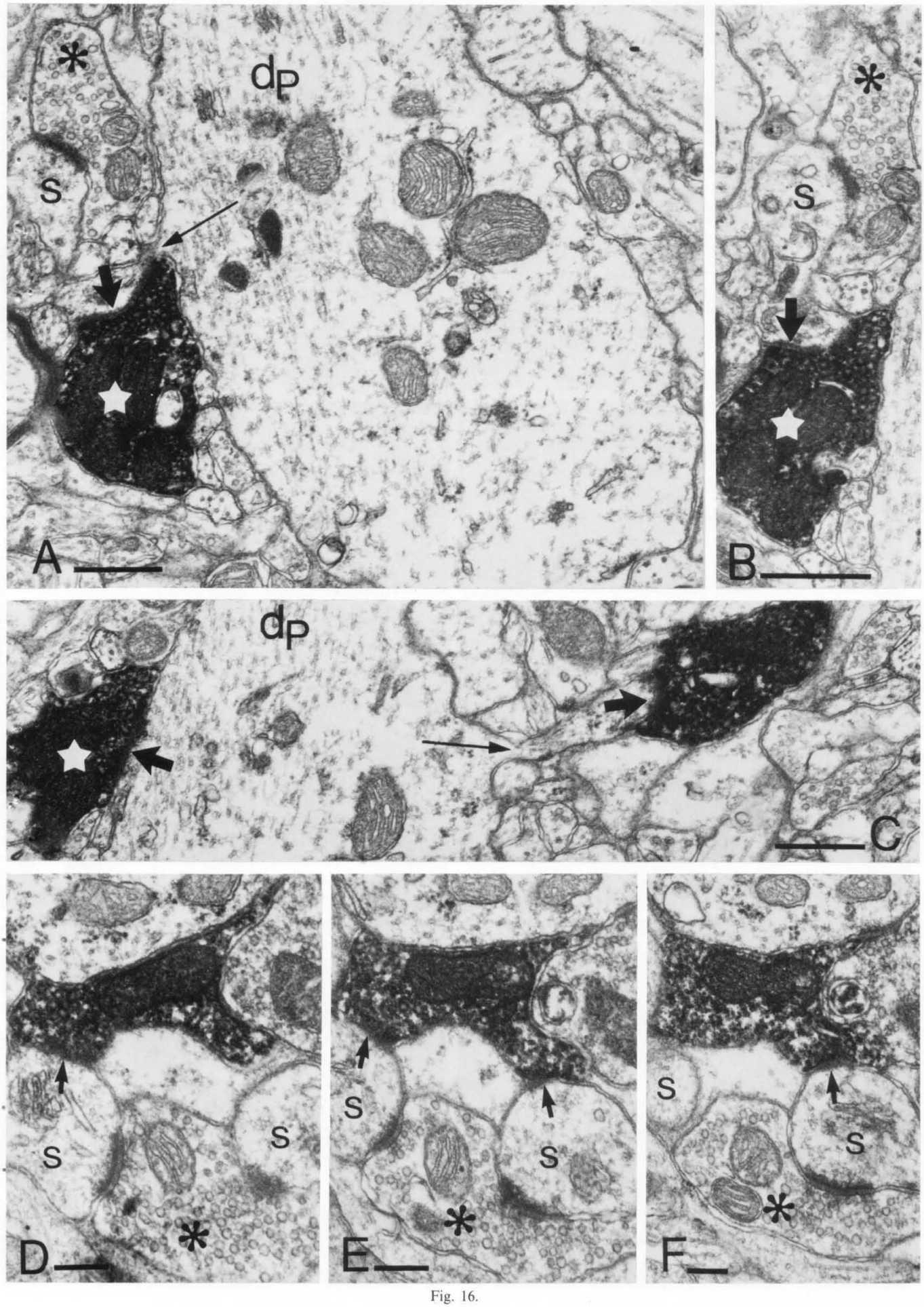


Fig. 16.

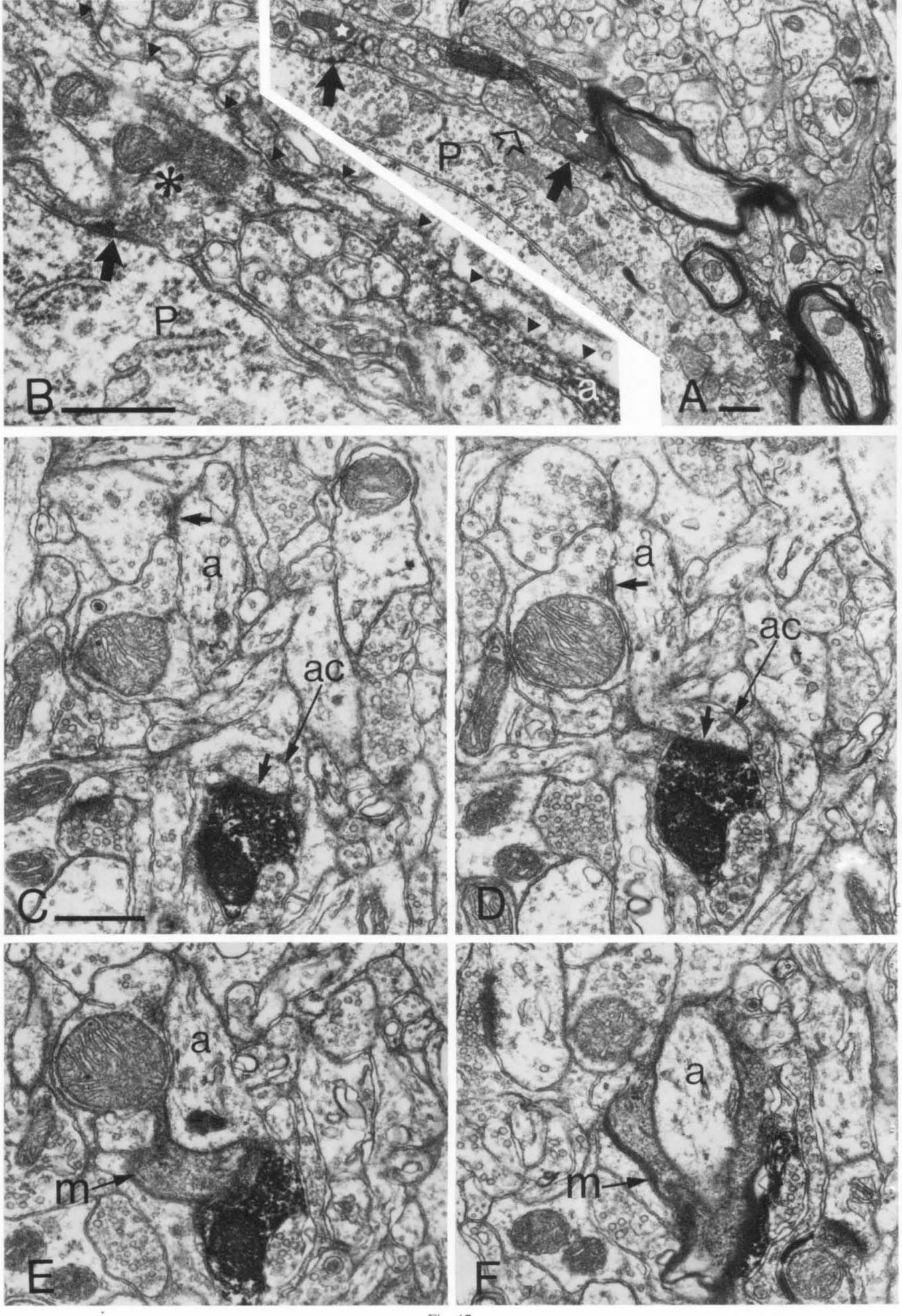


Fig. 17.

dendritic field. The three neurons with axonal arborizations of this size and making multiple contact on the perikarya of other neurons, will be called "large basket cells" and are the subject of this study.

The other two neurons providing axonal varicosities which contacted perikarya had much more limited axonal arborizations: we consider them to be a different type of neuron which will be reported separately.

The position of basket cells in cortex

All three recorded neurons were found on the crown of the lateral gyrus, in area 17. The neurons had receptive fields (RFs) near to the centre of the visual field and their physiological properties have been reported previously,[37] but the main physiological features will be summarised in the legends for the figures showing the two-dimensional reconstructions of each of the cells reported here. Basket cell No. 1 corresponds to cat No. 10/81, basket cell No. 2 to cat No. 20/81, and basket cell No. 3 to cat No. 19/81 in the previous study.[37]

BC1 and BC3 provided very stable intracellular recording (up to 70 mV resting potential for up to 15 min) before the electrode was intentionally withdrawn. In the case of BC2, some RF properties were examined during intracellular recording, but the electrode popped out during iontophoresis of the HRP, which would account for the HRP diffusion reported below.

The perikaryon of BC1 was situated at the top of layer IV, and that of BC2 at a slightly more superficial position at the bottom of layer III. The main axon trunk of BC3 was in the middle of layer III and followed a descending course at the site where it was injected (Fig. 9), suggesting that the parent cell was located in the upper part of layer III.

Light microscopic features : perikarya and dendritic arborizations

The two neurons, BC1 (Fig. 1) and BC2 (Fig. 2) had their soma and dendrites completely filled by HRP reaction end-product. These neurons have an ellipsoid radially elongated soma with a long and short axis of about 30 and 15 μm for BC1 and 18 and 10 μm for BC2. There was diffusion of the peroxidase reaction end-product around the perikaryon of BC2 and a peroxidase-filled microglial cell also surrounded the neuron with its processes. Therefore, in the light microscope the region of the perikaryon (Fig. 4A) appeared very dark. In the electron microscopic sections, however, the soma is well defined and the diffuse end-product, in effect, only gave the surrounding structures slightly more contrast. Primary dendrites radiated in all directions from the soma, but with the majority emerging from the upper and lower poles. Some of the primary dendrites divided close to the perikaryon and this resulted in a 'bouquet' consisting of 3–6 secondary dendrites. Apart from these branching points, the dendrites rarely, if at all, branched more distally, and followed a straight, slightly undulating course. The dendritic field is elongated dorso-ventrally but, because the upper dendrites are about twice as long as the lower ones, there is a dorso-ventral asymmetry. The individual dendrites are strongly beaded (Figs 1, 2, 4A and 5A), composed of varicosities 2–4 μm in diameter and connected by thinner 1–2 μm thick segments.

The size of the dendritic fields is about 300–350 μm in the radial direction, and about 200 μm in the lateral direction. Since BC1 is somewhat deeper in the cortex more of its dendrites are distributed within layer IVA than those of BC2. Most of the dendrites of BC2 are within the confines of layer III (Fig. 2).

Light microscopic features: axonal arborizations of basket cells 1 *and* 2

Basket cell 1. This axon seemed to be completely filled as all its branches ended in fine beaded terminal segments. A partial drawing and a computer-assisted three-dimensional reconstruction of this axon has been published.[37] The dimensions of the axon arborizations are about 1 mm antero-posteriorly and about 1 mm medio-laterally. The axon is distributed approximately symmetrically around the perikaryon as viewed from the pia.[37] The axon initial segment originates at the bottom of the soma and takes a descending course reaching layer VB (Fig. 1). The main axon emits several secondary collaterals almost at 90°, which themselves give rise to tertiary collaterals, most of which are orientated radially. The branching of the axon at angles close to 90° is a characteristic feature of basket cells. The distribution of terminal axon branches is uneven in the different layers. The axon reaches its full extent in the horizontal direction only in layer III and is localized to the lower two-thirds of this layer. In layer IV, the lateral spread of the axon is only about 500 μm, i.e. half of that in layer III. Finally, in layer V, it extends only about 200 μm laterally and it is localized beneath the perikaryon and dendritic field. All collaterals give off beaded terminal segments. The main axonal branches have a soft contour and contain HRP reaction end-product which is lighter than that of the beaded terminal segments, suggesting that the main axon collaterals were myelinated. Sections of this neuron were not treated with osmium tetroxide and thus other neuronal perikarya were not visible in these unstained sections. The varicose terminal segments on their own did not suggest any particular postsynaptic element as they did not form obvious basket-like configurations. Some sections containing part of the axon were counterstained with Cresyl Violet which revealed neuronal perikarya, and it became apparent that some of the beaded terminal axon segments are associated with the perikarya and apical dendrites of pyramidal neurons.

Basket cell 2. In contrast to its soma and dendrites the axon of this neuron was only lightly filled (Figs

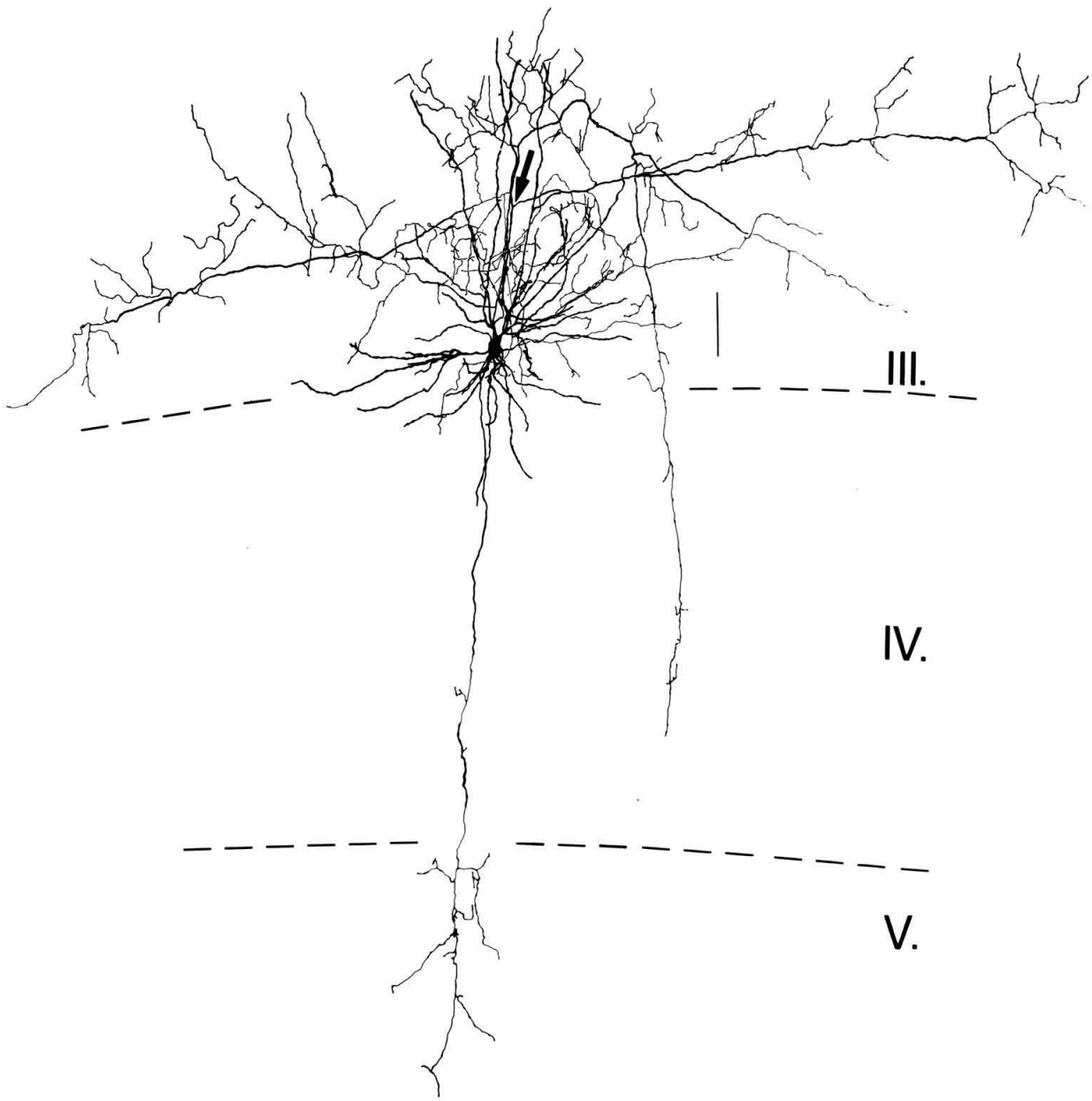


Fig. 2. Camera lucida drawing of basket cell No. 2 in the lateral gyrus (area 17) of the cat. Physiological characteristics: orientation selective, S_2 type receptive field, size $1.4^\circ \times 0.7^\circ$, ocular dominance group 4, monosynaptically driven by X-like LGN fibres, and monosynaptically driven by callosal fibres.[37] This neuron was penetrated with a micropipette at its soma, its dendrites were strongly and completely filled (see also Fig. 4). The axon (large arrow) was lightly filled and while the main axon collaterals were traced completely, only some of the terminal segments could be reconstructed. The perikaryon and the bulk of the dendritic and axonal arborization is in layer III but two axon collaterals descend to layer IV and V. The descending collateral on the right is shown with one of its apparent postsynaptic cells in Fig. 3. Coronal plane. Scale: 50 μm.

4A and 5E-H). Although the main myelinated axon collaterals could be traced in their entirety, many of the fine terminal segments were faint and could not be resolved against the dark brown background of the thick section. Thus, the drawing in Fig. 2 does not represent the whole of the axon of this neuron.

The initial segment of the axon originates from the dorsal pole of the soma and ascends within the dorsal dendritic field (Fig. 2). It breaks up into four major collaterals. The first collateral takes a descending course and arborises in layer V without any detectable terminal branches in layer IV. The second and third collaterals turn lateral and medial and stay in the frontal plane, occupying an area about 900 μm in diameter. The last collateral runs anteriorly about 400 μm and gives rise to a descending collateral at about 250 μm from the plane of the soma. All main collaterals emit varicose terminal segments which

may branch several times. The varicosities often surround pyramidal neurons (Figs 5E–G) or are aligned to their apical dendrites (Figs 5G and H). A single pyramidal neuron may be in apparent contact with 1–8 varicosities, but the usual number of varicosities is about 4–5. The pyramidal neurons and their thick dendrites are recognisable because of the osmium tetroxide treatment. Many of the varicose terminal segments, however, do not seem to be associated with cell bodies, but they travel in the neuropil and their possible postsynaptic targets cannot be predicted from light microscopic examination.

One of the descending collaterals which could not be traced beyond layer IV was associated with a very thick apical dendrite passing through layer IV (Fig. 3). The apical dendrite was contacted by 16 varicosities of BC2 within a 100 μm segment and could be traced back to a giant pyramidal neuron in layer V.

Fine structural characteristics of basket cell 2

Soma and dendrites. Although the neuron was heavily filled with HRP reaction end-product, the internal organelles could be recognised as "negative images" in the highly electron-dense background. The neuron has an eccentrically placed, invaginated nucleus surrounded by dense arrays of Golgi-apparatus (Fig. 4B). The perikaryon contains a very high proportion of mitochondria dispersed among areas occupied by rough endoplasmic reticulum (Fig. 4c). Electron microscopic examination confirmed that all the dendrites are filled to their most distal tips. The enlarged parts of the beaded dendrites contain groups of densely packed mitochondria (Figs 4D and 5A and 5B), while mitochondria were largely absent from the thin segments.

Synaptic input. The soma receives numerous synaptic contacts (Fig. 4B and C) often grouped in patches. Unfortunately, because of the heavy filling and slight diffusion of the reaction end-product, the synaptic boutons are difficult to classify. Classification is easier in the case of the dendrites which are also surrounded by a high density of synaptic boutons (Fig. 4D). Most of the boutons on the dendrites and at least some on the soma contain clear round vesicles (Figs 4D and 5B), similar to those of axo-spinous synaptic boutons in the surrounding neuropil (Fig. 5B).

Axon. The axon initial segment was sectioned serially and the characteristic microtubule fascicles could be identified. The axon hillock received numerous synaptic contacts but no synaptic contact was

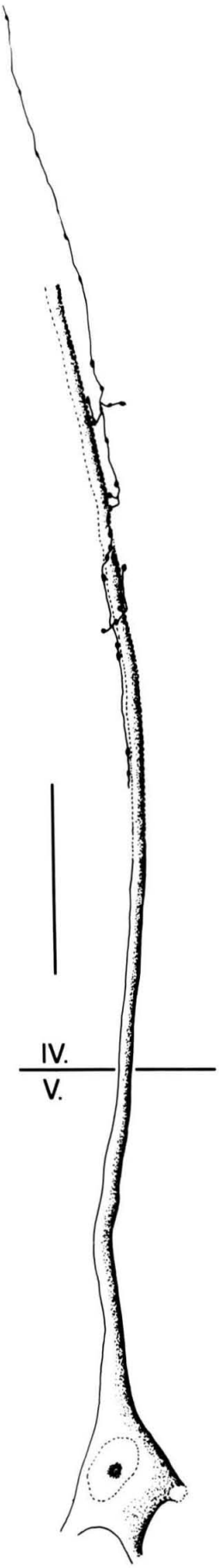


Fig. 3. Camera lucida tracing of one of the descending axon collaterals of basket cell No. 2. The collateral had few varicosities before it approached the ascending apical dendrite of a giant layer V pyramidal cell. Sixteen varicosities of the basket cell axon were directly apposed to the apical dendrite. Scale: 50 μm.

encountered on the initial segment. The axon became myelinated about 20 μm from its origin and gradually thickened on its ascending course, expanding in diameter from 0.4 to 0.5 μm to more than 1 μm (Figs 5C and D). The thickness of the myelin sheath is about the same on the ascending axon and its major collaterals, which are 1–1.5 μm in diameter (Figs 5C and 8A). Electron microscopic examination confirmed that the presence of myelin sheath on parts of the axon can be predicted from light microscopy.

Fine structure of boutons and their synaptic contacts. All synaptic contacts established by BC2 are Gray's type II[18] or symmetrical (Figs 6–8), irrespective of the postsynaptic element. The thickness of the postsynaptic density varies from synapse to synapse but it is always less than those of asymmetrical contacts. In addition to the synaptic contacts, occasional puncta adherentia were observed (Fig. 7B). The boutons were only lightly filled with reaction end-product so that the vesicles could be characterized as the small pleomorphic type (Figs 6B–D, 7B and 8B), rather than the round vesicles in boutons which establish Gray's type I or asymmetrical contacts (Figs 6C and D, and 8B).

The identification and distribution of postsynaptic elements to the boutons of BC2 will be described in comparison with those of BC3 on page 283.

The axon of basket cell 3

Aborization. This axon was the most extensive of the three cells. Its main myelinated axon trunk was about 5 μm in diameter and branched into several thick collaterals, three of which left the main axon arborization and occupied an area about 300 μm in diameter around the main axon. One collateral could be followed 800 μm in the anterior direction, the other one to 700 μm posterior and the third 800 μm laterally in the coronal plane. Neither the first nor the second collateral could be traced to their extreme tips. Some sections of this neuron were processed with a less sensitive method than the rest and, because the axon was not uniformly filled to its most distal tips, this has prevented a complete reconstruction of the arborizations (Fig. 9). Nevertheless, the extent of the axon in the antero-posterior direction certainly exceeds 1500 μm and in the lateral direction it traverses an area about 1000 μm across at its largest extension. The lateral collateral was strongly filled and could be traced to boutons at its tip, thus representing the true extent of the axon (Fig. 9).

The main collaterals are about 2–4 μm in diameter and often run in the radial direction. The branching of the thick axons is close to 90°. All the main branches emit varicose terminal segments which branch themselves. Except for these varicose segments, all the axons seemed to have myelin sheaths, when viewed under the light microscope.

Terminal segments. The varicose terminal segments of BC3 often approach the perikarya of pyramidal cells which then appear to be contacted by 1–8 boutons that often come from several independently originating collaterals (Figs 10A and 11A–D). The same pyramidal neuron also receives such contacts on its apical and basal dendrites. Examples of pyramidal cells receiving differing numbers of contacts are shown in Figs 10A, 11A–D and 13A. The origin of the apical dendrite seems to be a favourite termination site of BC3 (Figs 10A, 11D and 13A). Many of the varicose terminal axons are not associated with cell bodies but, as in BC2, are seen in the neuropil.

As a result of osmium treatment, the outlines of tissue constituents such as myelinated axons, cell bodies and thick dendrites can be recognised remarkably well. It is often seen that boutons of BC3 in the neuropil are aligned along dendrites which are lighter than the background. Interestingly, some of these distal dendrites could be followed back to pyramidal neurons which themselves received multiple contacts from other collaterals of BC3 (Figs 10A and 13A).

Many of the longer collaterals coming from the main branches have a radial course 100–200 μm long. These collaterals contact cells in a radial column (Figs 10A, 11A and B) while neurons outside this group in the surrounding area are not contacted. The largest pyramidal cells in layer III receive a very heavy input (Figs 10A, 11C and D, and 13).

Fine structural features of the axon of basket cell 3. Electron microscopic examination provided evidence for several predictions made at the light microscopic level. Firstly, the entire main axon system of BC3 is myelinated. The major axons have a thicker myelin sheath (Fig. 10B) than the secondary, tertiary collaterals, and this myelin sheath is about twice the thickness of that of BC2. The final collaterals are myelinated to the point where they become varicose and start to give off boutons (Fig. 11E).

Secondly, the assumption that the varicosities are synaptic boutons was also proved, as every varicosity examined made at least one synaptic contact. Thirdly, if it appeared, from light microscopy, that a certain perikaryon or major dendrite was actually contacted and received synaptic contact from a bouton, this would invariably be confirmed when tested by electron microscopy. In the case of the thinner dendrites, however, this relationship becomes less predictable.

The boutons and axons were heavily filled with HRP reaction end-product. However, in some fortuitous cases, lightly filled branches could be identified so that the small, clear, pleomorphic vesicles in the boutons became visible (Figs 17A and B). All boutons established Gray's type II[18] synaptic contacts. The extent of the postsynaptic membrane thickening varied slightly, and was more extensive if seen in tangential sections. In the case of the heavily filled boutons where vesicles were not apparent, the synaptic contact was identified on the basis of electron-dense cleft material, widening of the extracellular space and the postsynaptic density (Figs 12A, 13C and D, and 14B–D).

Table 1. Number of synaptic boutons and postsynaptic elements studied by electron microscopy. Drawings of the two basket cell axons are shown in Figs 2 and 9

No. of basket cell axon	No. of boutons examined in the electron microscope	No. of postsynaptic elements in synaptic contact with a single bouton			
		1	2	3	average
2.	66	61	4	1	1.09
3.	89	77	11	1	1.15

Identification of structures postsynaptic to basket cells 2 and 3

The elements postsynaptic to 66 boutons of BC2 and to 89 boutons of BC3 were analysed from a random sample in layer III (Tables 1 and 2). Several active zones established by one bouton with the same postsynaptic element were considered as one synapse, but two active zones with two postsynaptic elements of common origin, such as a dendrite and its spine (Figs 16A–C) were counted as two postsynaptic structures. As each bouton may establish synaptic contact with up to three different structures (Table 1), a total of 177 postsynaptic elements were encountered (Table 2). The postsynaptic elements were perikarya, dendritic shafts, dendritic spines, and an axon initial segment and a probable axon of unknown origin. When a bouton establishes synaptic contact with more than one structure, any combination of the first three elements can be found. In the following, we briefly describe the criteria used to identify the different structures listed in Table 2.

Pyramidal cell perikarya. These have a triangular, pyramidal or elongated shape, with a major apical dendrite originating at the dorsal pole of the neuron (Figs 6A, 13B and 14A). The axon initial segment originates at the base of the soma (Fig. 14A) and thick basal dendrites leave the neuron as it gradually tapers off conically. The soma and proximal dendrites receive only type II synaptic contacts.

Pyramidal neuron perikarya were one of the main targets for both the basket cells studied, which usually established multiple contacts with them (Fig. 17A). A higher proportion of the boutons of BC3 contacted perikarya as compared to BC2. In order to have an estimate of the total contribution of one basket cell to the pericellular boutons, we serially sectioned 6 pyramidal neurons postsynaptic to BC3 and one postsynaptic to BC2, including all the visible HRP-filled basket cell collaterals around them. The number of axo-somatic synapses on pyramidal cells varied from 1–8 (Table 3) in the case of BC3, and was 3 on one pyramidal cell sectioned serially in the case of BC2, giving an average of 4.4. In any one section, the HRP-labelled boutons provided only a minor fraction of the synaptic contacts received by the pyramidal cells which also received numerous other contacts of similar character (Figs 6A and B, 13B and 14A). One pyramidal neuron besides receiving a synapse on its somatic membrane also received a second synaptic contact on its somatic spine (Figs 14C and D).

Axon initial segment. One example was found postsynaptic to BC2 and was recognisable from the electron-dense membrane undercoating and the microtubule fascicles characteristic of this structure (Fig. 6D). The axon initial segment could be traced back to a pyramidal neuron. Although only one such synapse was found, those pyramidal cells which received somatic synapses from the HRP-filled boutons also had numerous type II synapses on their axon initial segments from unidentified cells.

Basal dendrites. They were found to originate from pyramidal neurons, usually in a lateral or ventral direction (Figs 8C and D). The dendrites gradually decrease in diameter and often branch close to the soma. Their cytoplasm is conspicuously electron-lucent, contains high numbers of parallel-arranged microtubules mainly in the centre, few mitochondria and some endoplasmic reticulum close to the plasma membrane (Figs 8C and D, and 11E). Proximal basal dendrites receive only Type II synaptic contacts, one or two of which are supplied by the identified basket cells (Figs 8C and D, and 11E).

Apical dendrites. They can be recognised as 2–4 μm thick ascending dendrites originating from the dorsal pole of pyramidal neurons (Figs 6A, 13B and 15). They are the thickest dendrites in cortex and because they follow a straight radial course they can be recognised even if not connected to a perikaryon. In the present study, four of them were found in short series of sections and were not traced to perikarya. Apical dendrites usually receive multiple contacts from the same basket cell. One apical dendrite (Table 3 and Fig. 13) received synapses from seven boutons of BC3, close to its origin from a large pyramidal cell. This region is a preferred site of termination for these two basket cells. The postsynaptic apical dendrites are heterogeneous with regard to synaptic input; some of them receive numerous synaptic contacts as well as those filled with HRP and the synapses can be both Gray's Type I and Type II (Fig. 15). Other postsynaptic apical dendrites receive few and only Type II synaptic contacts. The identified basket cell boutons terminate not only on the main shaft, but also sometimes at the origin of side branches of apical dendrites (Figs 15A and B).

Type P dendrites. Most of the dendrites receiving synaptic contacts from the large basket cells could not be traced to perikarya. On the basis of fine structural characteristics two categories were set up. 'Type P' dendrites had an electron-lucent cytoplasm, dense arrays of microtubules, few mitochondria and

Table 2. Distribution of neurons and structures postsynaptic to the basket cell boutons of neurons 2 and 3 identified by electron microscopy

No of basket cell axon	Postsynaptic element																		Total		
	Identified				Pyramidal neuron			Probable				Total	Non-pyramidal neuron					Total			
									Spine				Identified		Probable						
	Soma	Axon initial segment	Basal dendrite	Apical dendrite	Total	Type P dendrite	Origin from P dendrite	Origin unidentif.	Total			Soma	Dendrite	Type S dendrite	Axon	Total		Soma	Other	Total
2.	22 29.4%	1 1.3%	1 1.3%	6 8.0%	30 40.0%	28 37.4%	3 4.0%	12 16.0%	15 20.0%	43 57.3%	73 97.3%	1 1.3%	1 1.3%	—	—	2 2.7%	75 100%	23 30.7%	52 69.3%		
3.	38 37.3%	—	7 6.8%	11 10.8%	56 54.9%	12 11.8%	9 8.8%	11 10.8%	20 19.6%	32 31.4%	88 86.3%	2 1.9%	1 1%	10 9.8%	1 1%	14 13.7%	102 100%	40 39.2%	62 60.8%		

tubules of rough endoplasmic reticulum usually close to the plasma membrane (Figs 6A and B, 12A and 16A–C). The diameter of the dendrites varied from about 1–4 μm. These dendrites received few synaptic contacts, and often the only synapse in the plane of the section was provided by the basket cell. All synapses received by type P dendrites were Type II. The dendrites often emitted spines which were also a target for the identified basket cells (Figs 16A–C). In all respects Type P dendrites are qualitatively identical to those basal dendrites and apical dendrite side branches which were positively identified as belonging to pyramidal neurons. To compare them quantitatively and to see if the population of postsynaptic Type P dendrites contacted by BC2 and BC3 were similar, we measured the proportion of dendritic membrane occupied by synaptic junctions and plotted it against the proportion of dendritic profile occupied by mitochondria (Fig. 18). It is apparent that the Type P dendrites contacted by the two basket cells and analysed here are from the same population. In addition, the identified dendrites of pyramidal neurons are similar to Type P dendrites with regard to the two measured characteristics. "Type S" dendrites (see Fig. 18) had different ultrastructural features and are described below.

Spines. A surprisingly high proportion of the postsynaptic elements were spines, as both basket cells give about 20% of their synapses to this element (Table 2). Some of the postsynaptic spines (34%) could be traced back to their parent dendrites, all of which were P type (Figs 16A–C). The other spines could be identified on the basis of the spine apparatus, their characteristic drumstick shape or their small diameter (Figs 6C and 16). Almost all the spines could be shown to receive a Type I synaptic contact from boutons containing round vesicles (Figs 6A and 16) in addition to the Type II synaptic contact established by the basket cells. More than one spine of the same dendrite may receive synaptic input from the same basket cell (Figs 16A–C) and several spines may receive input within a small area, implying that they may originate from the same dendrite (Figs 16D–F). Interestingly, the Type II contact of the basket cell could often be shown to be more proximal to the parent dendrite of the spine than the Type I contact (Figs 16A–C). The parent dendrites often received synapses from the same basket bouton contacting the spine, or from other identified basket cell boutons (Figs 16A–C).

Summary of postsynaptic elements characteristic of pyramidal cells. In addition to the perikarya, basal and apical dendrites and axon initial segment which have been positively identified as belonging to pyramidal neurons, Type P dendrites and most of the spines can be considered as originating from pyramidal cells. This is because Type P dendrites and spines have similar synaptic input, fine structural characteristics and quantitative parameters to those of identified pyramidal cells (see Fig. 18). It

Table 3. Number of synaptic boutons received by eleven individual pyramidal neurons on their soma and most proximal dendrites from basket cell axon No. 3

Pyramidal neuron No.	No. of synaptic boutons received			
	On soma	On basal dendrites	On apical dendrite	Total
1.	8	—	—	8
2.	6	2	—	8
3.	5	—	—	5
4.	4	3	—	7
5.	4	—	—	4
6.	1	1	7 (+1)†	9 (10)†
7.	3	1	—	*
8.	4	—	—	*
9.	1	—	—	*
10.	1	—	—	*
11.	1	—	—	*

Neurons 1–6 were serially sectioned completely. *Neurons 7–11 were only followed in short section series, thus the total number of boutons does not represent the total contribution of this basket cell axon. †The apical dendrite of neuron No. 6 was found to receive an additional synaptic contact on its distal part.

should be emphasised that electron microscopic examination confirmed the light microscopic observation, that a single pyramidal neuron can receive input on its soma, basal and apical dendrites, and to a lesser extent on its distal dendritic branches and on its spines (Figs 13–15), from the same large basket cell. Basket cell boutons which made synapses on proximal dendrites of the six pyramidal neurons listed in Table 3, and on the one serially-sectioned pyramidal neuron which was postsynaptic to BC2, were counted. This latter pyramidal neuron received two further synapses in addition to the three already described on its perikaryon. Combining data for BC2 with that in Table 3 gives an average of 6.7 synapses from one basket cell found on the perikaryon and proximal dendrites of a single pyramidal cell.

The two basket cells differ in their selectivity for pyramidal neurons as 97.3% of the structures postsynaptic to BC2 are characteristic of pyramidal neurons whilst the corresponding figure for BC3 is only 86.3%. A further difference is the higher proportion of pyramidal dendrites among the structures postsynaptic to BC2 (46.7% as opposed to 29.4% for BC3).

Soma of non-pyramidal neurons. Two such neurons were found to receive synaptic contacts; one received contacts from BC2 and the other from BC3 (Table 2). These non-pyramidal neurons can be identified by the high density of synaptic boutons on the soma (Fig. 7A) and because some of the boutons establish Type I contacts. None of the cells had apical dendrites, and a dendrite originating from one neuron (Figs 7A and 8A) emerged without an initial conical part typical of pyramidal cells. This dendrite was densely covered by synaptic junctions, some of them Type I (Figs 8A and B). The neuron postsynaptic to BC2 received one synapse on its soma and another similar synapse on its dendrite in close proximity, both from the same basket cell collateral (Figs 7 and 8A and B). The neuron postsynaptic to BC3 was smaller, but otherwise similar, and received two synapses on its perikaryon.

Identified dendrites of non-pyramidal neurons. The single dendrite postsynaptic to BC2 is described above. The other dendrite postsynaptic to BC3 was different; it originated from a radially-elongated neuron with few synapses on its perikaryon. The dendrite which received a contact about 15 μm from the soma was a descending main trunk and later gave rise to the axon initial segment. It thus contained fasciculated microtubules but had no membrane undercoating. It emitted a side branch densely covered by synaptic junctions mainly of Type II.

Type S dendrites. Some of the dendrites postsynaptic to BC3 contained numerous large mitochondria, with more electron-dense cytoplasm than dendrites originating from pyramidal neurons, and received several synaptic contacts in addition to the one given by BC3 (Fig. 12). Two thirds of the 33 synaptic contacts received by these dendrites were Type I. Two of the dendrites received two synapses from BC3. Type P dendrites were classified as a separate type and we compare them on the basis of mitochondrial and synaptic density with Type S dendrites and identified pyramidal cell dendrites (Fig. 18). If a linear regression line is used as a centre of gravity for the different dendritic populations, data from Type P and Type S dendrites will fall in different regions of the dot diagram (Fig. 18). We do not imply that the synaptic and mitochondrial densities are related, we merely use the diagram to show that all the measured dendrites show the apparent difference between the P and the S type in the electron micrographs (Figs 6B, 8C, 11E, 12 and 16A–C).

Axon. One postsynaptic structure was found to emerge from a myelin sheath (Figs 17C–F). A thin terminal segment of the basket cell followed the myelin and gave a synaptic contact to a thin process, presumably an axon collateral, just as the collateral branched out from the presumed axon which was leaving the myelin. The axon was about 0.4 μm in diameter and received two more Type II synapses from boutons containing pleomorphic vesicles but with unknown origin (Figs 17C and D). It was followed for about 10 μm dorsally but received no more synapses. Its course was radial and did not have any of the characteristics of an axon initial segment (Figs 17C–F).

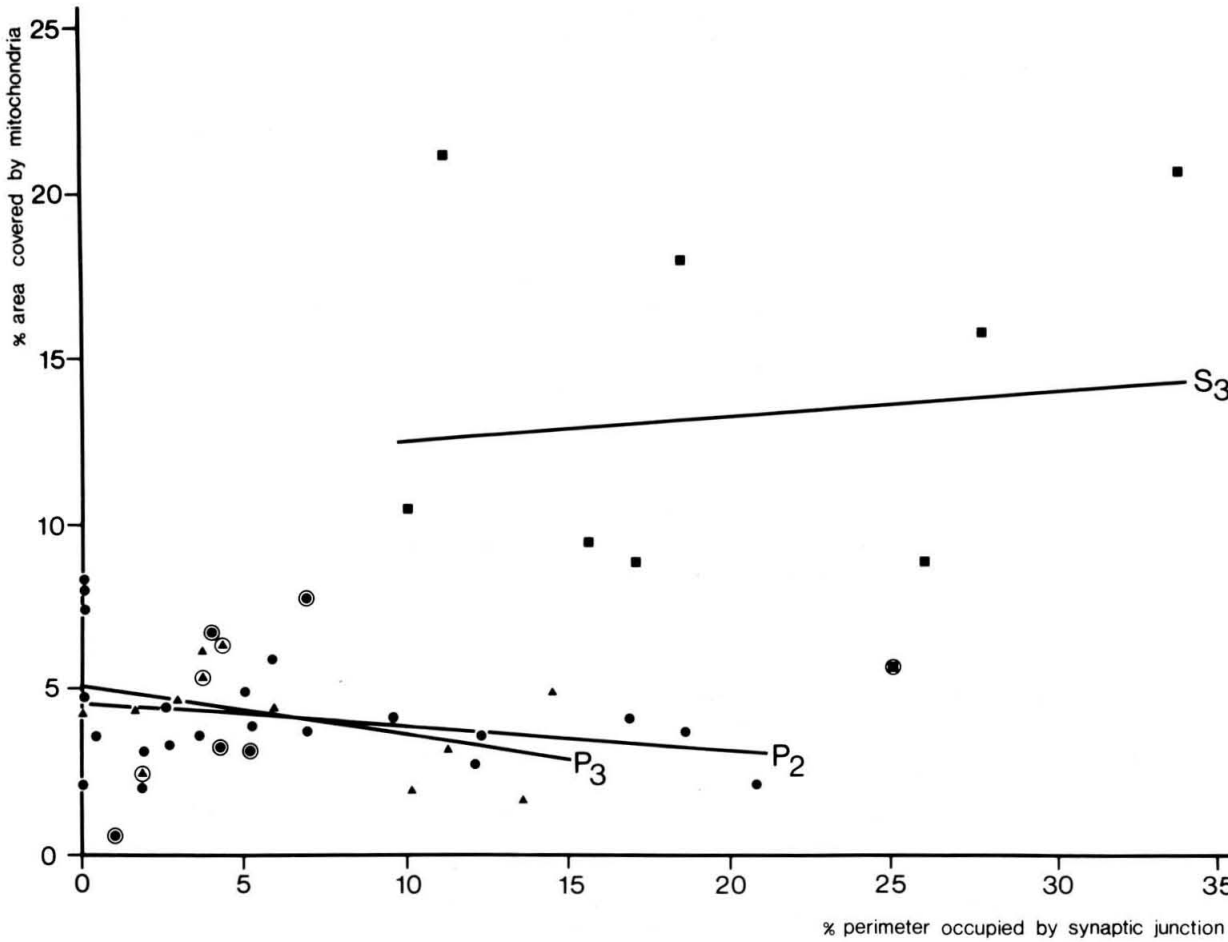


Fig. 18. Characteristics of dendrites postsynaptic to the axon of basket cell No. 2 (●, type P dendrites, line P_2) and No. 3 (▲, type P dendrites; line P_3, ■, type S dendrites, line S_3). Symbols (⊙) and (◉) represent postsynaptic dendrites which were found to originate from pyramidal neurons. One type S dendrite (▣) was found to originate from a non-pyramidal cell. Linear regression lines were calculated and used as an axis of gravity to show that type P dendrites contacted by the two neurons come from the same population (slopes for P_2 and P_3 are -0.076 and -0.138; intercepts 4.705 and 5.010, respectively) as regards the density of synapses and mitochondria, and that they are different from type S dendrites (slope of S_3 is 0.073, intercept 11.18) contacted by basket cell. No. 3.

Summary of the postsynaptic structures to basket cells 2 and 3 (Table 2)

Qualitatively, both basket cells make synapses with similar elements of cortex. However, BC3 establishes more axo-somatic synapses (39.2% opposed to 30.7%) and gives a significant number (13.7%) of its synapses to non-pyramidal neurons in layer III. Both pyramidal and non-pyramidal neurons may receive multiple contacts from large basket cells.

DISCUSSION

Identity of large basket cells

The present study demonstrates that neurons which we classified as large basket cells on the basis of light microscopic examination, give more than 30% of their synapses to perikarya of other cortical neurons. Thus, we can now define large basket cells as local circuit neurons in the cortex which have an axonal field with a lateral extent three or more times that of the dendritic arborization, and which have 30% or more of their efferent synaptic contacts on perikarya of pyramidal and, occasionally, on non-pyramidal cells. The number of elements postsynaptic to the two axons represent the largest sample of any identified neuron type examined at the synaptic level, and they reveal differences between the two neurons. The major differences between the two neurons are that BC2 establishes about 15% more synapses on dendrites of pyramidal neurons, 9% less on perikarya of pyramidal neurons, and 10% less on non-pyramidal cells. These results are based on the ultrastructural identification of 177 postsynaptic elements but even with this extremely demanding approach we cannot be entirely sure whether these differences indicate different cell types as defined by input and output characteristics. It is noteworthy that, as well as the differences in postsynaptic targets, the diameter of the axon, the thickness of the myelin sheath and

the extent of the axon arborization were all substantially larger for BC3. BC1 which was studied only at the light microscopic level was more similar to BC2 in its axon arborization. Thus it is possible that BC1 and BC2 are different from BC3 and may be involved in local interactions leading to different physiological properties.

This seems to be supported by the limited amount of physiological data available at present (described in the legends to Figs 1, 2 and 9) since BC1 and BC2 had S type[23] receptive fields (RFs),[37] and were both activated monosynaptically by X type of geniculate input as well as by callosal input. Also, their RFs were similar in size. In contrast BC3 of the present study had a C type RF[23] requiring little spatial summation, and was polysynaptically driven by Y-type geniculate input, callosal input to this neuron could not be demonstrated and it had a larger receptive field than the other neurons. Thus, although more data are clearly needed, the results suggest that subtypes of large basket cells involved in the processing of different information may exist in the visual cortex.

The problems of defining and comparing putative basket cells in previous studies have been discussed recently.[46] BC1 and BC2 of the present study are similar to the medium size basket cells of Szentágothai,[66] to type H-1 cells in the Clare-Bishop area,[40] and to some of the sparsely spinous or smooth, multipolar neurons with elongate dendritic trees shown in the cat striate cortex.[46] One of the neurons in the latter study (Fig. 5F and Fig. 7 in Ref. 46) shows similarities to BC2. Our large basket cells in the cat visual cortex may also be analogous to the large basket cells in the motor cortex[34,35,36] and to type I cells in the monkey somatosensory cortex.[28] Whilst, on the basis of available data, our large basket cells are different from the bitufted neurons apparently providing pericellular nets,[47] from type 6 cells in the monkey somatosensory cortex, from small basket cells of Szentágothai[66] and Tömböl[71] and from a recently studied basket cell type in superficial layer III of the cat visual cortex.[9] This latter neuron is the only one which was shown by electron microscopy to give multiple synaptic contacts to perikarya of both pyramidal and non-pyramidal cells. Unfortunately, no quantitative data was given concerning the distribution of the elements postsynaptic to this Golgi-impregnated basket cell[9] so its selectivity cannot yet be compared with our large basket cells. It was suggested[9] that this Golgi-impregnated neuron may correspond to the short-range basket cells of Szentágothai.[66] The small basket cells would have a more localized effect than the large basket cells with their widespread axons.

The difficulties in comparing basket neurons in different species and areas arise because of the partial visualization of axons by the Golgi method, the use of young animals in Golgi studies, and because of the lack of quantitative data concerning synaptic connections. This situation will improve with the intracellular labelling of more neurons with known physiological properties and with the obtaining of the synaptic spectrum of more cortical interneurons, although the labour involved in producing a sample of the size in the present study is formidable.

Basket cell input to different parts of pyramidal neurons

Apical dendrite and soma. Our synaptic data are based on samples from layer III. Several collaterals of BC1 and BC2 descend to layer V and varicose terminal segments were also seen in layer IV. Some of these latter terminals are associated with the apical dendrites of layer V pyramidal neurons as demonstrated by an example found in the case of BC2 (Fig. 3). It is noteworthy that the varicosities associated with the apical dendrite of the giant layer V pyramidal neuron were all within a short segment. Similarly, in layer III we demonstrated several examples of apical dendrites receiving multiple synaptic contacts from large basket cells. Some of the terminal bouton rows are somewhat similar to the specialised terminal axon segments of another interneuron, the axo-axonic cell establishing multiple synaptic contacts exclusively on the axon initial segments of pyramidal neurons.[12,45,47,49,62,65] In fact, the axo-axonic or chandelier cell was thought originally to terminate on apical dendrites.[28,67,68,70,71] This hypothesis was based partly on the electron microscopic demonstration of multiple Type II synaptic contacts arranged in groups on apical dendrites.[67,70] However, after the demonstration of the specificity of axo-axonic cells for the axon initial segment, there was no explanation for the origin of the boutons on the apical dendrites. The present study demonstrates that apical dendrites are one of the major postsynaptic targets of large basket cells and they are responsible for the grouped Type II contacts on apical dendrites.

Jack, Noble & Tsien[27] have shown theoretically that in general the optimum position for the location of synapses is on the soma, if the excitation arriving from all the dendrites is to be inhibited. Our study shows that if the basket cell provides substantial input to the apical dendrite of its postsynaptic target, then the input it provides to the soma of the same target cell is weak, and vice versa. These differences could, of course, be due to random variations brought about during development of the connections. Alternatively, they may have some functional significance related to the differences in excitatory input to the apical dendrites.

There is some indirect evidence that the apical dendrites of cortical pyramidal cells may produce a spike, see Ref. 20 and unpublished observations. This would allow a disproportionate weight to be given to the inputs onto the apical dendrite. In such cells which may be prone to generate an action potential which is propagated down the apical dendrite, a more substantial basket cell input may be required on the

base of the dendrite in order to isolate its activity from the activity of other dendrites. Since the apical dendrite's diameter increases as it approaches the soma, and because the soma and basal dendrites would have a short-circuiting effect, an action potential propagated down the apical dendrite may tend to fail as it moves proximally.[27] Thus, the addition of relatively small amounts of inhibition may be sufficient to inhibit a large amount of the activity in the distal portions of the apical dendrite and it may be possible for a small number of basket cells, if not a single basket cell to control effectively the apical dendrite.

The apical dendrite is a structure of particular interest as far as inhibitory control is concerned because, by passing through different cortical layers, it has access to sources of excitation not available to the basal dendrites. It is worth noting that even pyramidal cells in the deep layers can get a substantial input onto their apical dendrites from basket cells in the superficial layers (e.g. Fig. 3). This input can occur in layer IV where the pyramidal cells may receive direct input from lateral geniculate nucleus afferents.

Jack *et al.*[27] have shown theoretically that changes in the conductance of one dendrite due to inhibitory processes will have very little effect on the excitation of another dendrite. Furthermore, if the excitation arriving on the most distal tips of the dendrites is to be specifically inhibited, then this will be done most effectively when the synapses are also located near but proximal to those excitatory synapses rather than on the soma. We have some evidence (e.g. Fig. 13) that basket cells can contact the distal portion of the apical dendrites, but as yet we have no clear evidence as to whether this applies equally to the basal dendrites. Nevertheless, it remains a possibility that basket cells are able to 'prune' the basal dendritic tree and allow only excitation from particular sources to reach the cell.

Spines. The dendritic spines are an intriguing target of the basket cell axon because all of the spines also received a Type I synaptic contact from boutons of unknown origin, but with structural features similar to the boutons of identified pyramidal[32,42,58,80] and spiny stellate cells[32,58,79] which are presumably excitatory. Similar spines with dual input have been described in the cat and monkey visual cortex[60] but there the Type II contacts were provided by another identified interneuron, the double bouquet cell. Interestingly, the Type II contact provided by basket cells or double bouquet cells was usually closer to the neck of the spine while the Type I contact was usually at the head. Also, boutons of the basket cell often formed a contact on the parent dendrite just at the origin of the spine.

It is worth emphasizing the significance of such input. Diamond, Gray & Yasargil[10] have suggested that in terms of the selectivity of inhibition, the inhibitory input to spines could be functionally equivalent to presynaptic inhibition. Jack *et al.*[27] have calculated the possible consequences of spine inhibition and found that indeed it does increase the selectivity of inhibition but not to quite the degree envisaged by Diamond *et al.* Thus, there could be a reduction in up to 10% of the depolarizing activity in the parent dendrite or adjacent spines as a result of inhibitory action on a spine. Nevertheless, given the greater effectiveness of a somatic location, one must suppose that the basket cell's connections to particular spines is an indication that some specific excitatory input is being inhibited. It would be worth identifying the source of the excitatory input as a means of discovering more about the precise role of the basket cells.

The further prediction of Diamond *et al.*,[10] that one would not expect to find only inhibitory input on a spine, because of its relative isolation, seems to be true for those spines contacted by the basket cells. All of those examined had a Type I synapse (presumed excitatory) in addition to the Type II synapse. The arrangement of this pair, with the basket cell synapse located between the Type I synapse and the parent dendrite, is the most effective arrangement for selective inhibition.[27]

Convergence and divergence in the connections of large basket cells

Evidence was obtained in the present study that the most thoroughly studied basket cell, No. 3, establishes on the average 4–5 synaptic contacts on the perikaryon and an additional 2–3 on the proximal dendrites of pyramidal neurons. Even if the average 7 synapses received from a single basket cell on the proximal part of the neuron is an underestimate, because not all the dendrites can be followed, this data means that the terminals of one basket cell are a mere fraction of the population of boutons converging on a single pyramidal cell.

There are good reasons for supposing that a major proportion of the unidentified boutons represent the terminals of other basket cells. The number of cells which could potentially provide such input probably number less than 10% of the total number of cells in the cortex. Of this 10%, at least two types, the axo-axonic cell and the double bouquet cell, were not found to contribute to the synaptic contacts on the soma of pyramidal cells. Some of the remaining possible candidates, the neurogliform cell of Szentágothai[66] or the multipolar cell of Peters & Proskauer [44] have very localized axonal arbors and could only contact a small number of target cells and are thus too few in number to provide the necessary contacts. On the other hand, the large basket cells, with their wide ramifications, are well placed to provide divergent contacts to hundreds of pyramidal cells.

Unfortunately, the total number of synaptic boutons on pyramidal cells which receive input from large basket cells in layer III has not been estimated.

Previous estimates of the density of synaptic contacts on the soma of layer IV pyramidal cells in the cat visual cortex are 10.8 contacts per 100 μm^2,[8] to 7.3 contacts per 100 μm^2 on somata of neurons which would include pyramidal cells in the rat visual cortex[81] and the number of contacts per pyramidal neuron soma in the oppossum somatosensory cortex was estimated to be 48.[7] Assuming that the surface of a pyramidal neuron's soma is 400–1200 μm^2,[81] the density estimates give a total number of 40–140 and 30–90 contacts in the cat and rat, respectively. If a layer III pyramidal cell, which was shown to receive on its soma an average of 4.4 synaptic boutons from the identified large basket cells in the present study, receives roughly the same number of contacts as those pyramidal cells examined in the above studies, then between 10 and 25 basket cells are necessary to provide all the contacts.

This number for convergence is in the same order of magnitude as that obtained from electrophysiological cross-correlation analysis. Toyama, Kimura & Tanaka[73,74] suggested that about 10 neurons account for the simple cell to complex cell inhibitory interaction and this interaction may take place between neurons several hundred μm apart laterally. The large basket cells demonstrated in the present study which had S type receptive fields are a good candidate for mediating this interaction.

More identified basket cells of various types[9,43] and more accurate estimates for the number of boutons on the somata of pyramidal cells which actually receive the identified boutons are necessary before a more reliable number for the convergence of basket cells can be given. It also remains to be established if there is in the visual cortex a basket cell which can by itself provide more elaborate pericellular nets such as those inferred from Golgi studies.[34,35,47] Whatever the final number of converging basket cells, it is likely that they will be more numerous than the number of axo-axonic cells converging onto the axon initial segment of pyramidal cells. Recently, we provided some preliminary estimates, by counting the total number of synapses on three axon initial segments, which suggested that about five axo-axonic cells converge onto layer III pyramidal cells in the cat visual cortex.[62]

It is difficult to estimate the total number of cells receiving boutons from a large basket cell because the latter seems to select only some cells within its axonal field. Our earlier three-dimensional reconstruction already indicated a patchy distribution of the axon.[37] A finer grain of target cell distribution was also revealed by the present study in the form of radially aligned pyramidal cell columns, one or two cells wide, which received input from the radial collaterals of the basket cell (Figs 1 and 10). Furthermore, as discussed above, even large basket cells may differ in the extent of their axonal arborization and number of postsynaptic cells contacted. From the limited amount of information available it seems that the number will be in the order of several hundreds. The only comparable data for other interneurons is our previous count of the number of pyramidal cells receiving input from an intracellularly filled axo-axonic cell in the visual cortex of cat.[14] This interneuron was found to contact 340 pyramidal neurons but these were in a much more limited cortical space than the targets of basket cells.

It is noteworthy that the same pyramidal cell which receives input from identified basket cells on its soma and dendritic arborization also receives heavy input on the axon initial segment. Since only one large basket cell bouton was found in contact with an axon initial segment, most of the boutons on this part of the pyramidal cell come from another interneuron, the axo-axonic cell.[12,45,57,59,62] We have recently discussed the functional implications of this dual, separate and very likely inhibitory input of pyramidal cells.[14] The present study confirms earlier assumptions that basket cells almost completely avoid the axon initial segment. On the other hand, the single basket cell bouton making synapses with a proximal axon initial segment explains the origin of the rarely observed boutons which, in addition to forming contacts with the initial segments, also contact nearby dendrites or spines.[62] An important problem worth experimental investigation, is how this specificity of innervation is brought about.

Non-pyramidal cells postsynaptic to large basket cells

Previous studies on Golgi-impregnated interneurons which gave synaptic contacts to pyramidal cells, showed that the same interneurons also contacted non-pyramidal cells.[9,43,44] Different types of non-pyramidal cells were also found postsynaptic to the two large basket cells in the present study.

One postsynaptic dendrite was traced to an elongated medium size non-pyramidal cell, but its neuronal type could not be identified because fine structural features alone are insufficient to decide among the various categories present in layer III. Another postsynaptic profile was tentatively classified as an axon because it acquired a myelin sheath. We listed this structure among non-pyramidal cells because the only reported example of an axon, other than the specialized initial segment receiving synaptic contact in the cerebral cortex, is that of the axon of the axo-axonic cell in layers II–III.[45] Several symmetrical synaptic contacts were described on the axon of this interneuron beyond the initial segment. As the axon of axo-axonic cells often becomes myelinated,[62] it is possible that the postsynaptic axon in the present study belonged to this neuron implying that large basket cells inhibit axo-axonic cells.

The large non-pyramidal neuron postsynaptic to BC2 in lower layer III was similar to those described in earlier electron microscopic studies as large stellate cells.[8,13,16,29] One characteristic of this neuron is the high density of afferent boutons which give Type I synaptic contacts on its perikaryon and dendrites.

The origin of these boutons is largely unknown but terminals of geniculate afferents establishing Type I contacts are known to make synapses with similar cells. The basket cell terminals demonstrated in the present study could provide an inhibitory input to these large stellate cells.

One non-pyramidal perikaryon contacted by BC3 was similar to the above large stellate cell, and the non-pyramidal dendrites, which represent about 10% of the postsynaptic elements of BC3, had a high density of mitochondria and synaptic contacts. This makes them similar to the dendrites of not only the large stellate cells contacted by BC2 but to the intracellularly labelled perikarya and dendrites of BC2 itself. Thus, it is possible that varieties of basket cells are interconnected and provide inhibitory input (see below) to each other.

More direct evidence for this suggestion could come from the complete visualization of the non-pyramidal cells postsynaptic to large basket cells, by Golgi-impregnation using our recently developed combined HRP-Golgi method.[15] Since even in our large sample of intracellularly labelled neurons only a few basket cells were found, the direct identification of both pre- and postsynaptic neurons may wait for some time. Until then we have to use more indirect evidence for the possible interconnections of basket cells such as that which comes from the immunocytochemical demonstration of the GABA synthesising enzyme, glutamate decarboxylase (GAD).

γ-Aminobutyrate (GABA) as the probable transmitter of large basket cells

It has long been suggested[9,28,30,37,66,67,68,69,70] that basket cells are responsible for GABA-mediated inhibitory processes in the cortex. Although the inhibitory role of GABA[26,31] and its role in the information processing in the visual cortex[50,51,52,53,54,55,56,78] is well documented, the identity and connections of neuron types responsible for the various GABA-mediated events is largely unknown. Earlier suggestions, which still dominate current concepts, that basket cells are the main inhibitory interneurons of the cortex seem simplistic in the light of new data. Thus, studies using [^{3}H]GABA uptake,[22,81] [^{3}H]GABA uptake combined with Golgi-impregnation of the same neuron,[63] GAD immunocytochemistry[21,48] and GAD immunocytochemistry combined with Golgi-impregnation of the same neuron[14,64] demonstrated that GABAergic neurons are heterogeneous in cortex. Furthermore, following the demonstration of GAD-immunoreactive boutons on the axon initial segment of pyramidal cells,[45] the GABAergic nature of an interneuron different from basket cells has now been proved by the immunocytochemical demonstration of GAD in an identified Golgi-impregnated axo-axonic cell.[14] It has also been suggested that another interneuron, the double bouquet cell, uses GABA as its transmitter.[61] The immunocytochemical demonstration of GAD immunoreactive neuronal perikarya in layer I[21,48,64] which contains no basket, axo-axonic or double bouquet cells adds a further type of cell to the list of neurons which could mediate GABAergic inhibition. Considering the heterogeneity of cortical GABAergic neurons we have to examine the indirect structural evidence available for GABA as the transmitter of basket cells.

Firstly, the types of synaptic contacts established by the identified basket cells and the types of synaptic vesicles in the boutons of the present study are the same as those of GAD immunoreactive terminals in the rat,[48] monkey[49] and cat.[14,45] Secondly, our quantitative immunocytochemical study of boutons on layer III pyramidal cells in the cat striate cortex[14] demonstrates that almost all boutons on the perikarya and proximal dendrites are GAD positive. Thus, the boutons of large basket cells shown in the present study to supply synapses to these loci are very likely to be amongst the GAD-positive terminals.

Finally, the immunocytochemical demonstration of the presence of GAD in a conspicuous class of large perikarya[14] of similar size and shape to our identified basket cells at the border of layers III and IV where BC1 and BC2 were situated suggests that these GAD-positive neurons are basket cells. This is further supported by the similarity of large neurons which were both GAD-positive and Golgi-impregnated to the intracellularly labelled basket cells. In conclusion, although the evidence is still indirect there is little doubt that GABA is synthesized in large basket cells and in their terminals.

Role of the basket cells in GABAergic inhibition. The role of GABAergic inhibition in producing receptive field specificity has been investigated principally by iontophoresing GABA antagonists (principally bicuculline) into the extracellular space near the cell being tested. These experiments[51,52,53,54,55,56,78] show that the response specificity of the cell to direction of motion, stimulus orientation and stimulus length can be reduced if not eliminated entirely in many cells after bicuculline administration. There are a number of possible reasons why some cells show no loss or only a reduction in specificity, including the possibility that the inhibitory synapses involved are located on distal dendrites and are thus not being sufficiently blocked by bicuculline, or that there are inhibitory mechanisms which do not use GABA as their transmitter. If the former, then it is possible that some of the more distally placed synapses of the basket cell could be responsible for this residual specificity.

Possible implications for cortical function

From the previous survey we can now conclude that large basket cells are probably GABAergic interneurons with a tangential axonal field of up to 1.5 mm. At least some of them are situated at the border of layers III and IV and spread their axon through layers III–V giving multiple synapses to

neurons, which they seem to select as postsynaptic targets. What can be the functions of such a neuron system?

Serial position of basket cells. From the distribution of basket cell boutons on the postsynaptic sites of the recipient neurons, it is clear that their influence will be integrated with the other inputs converging onto the same neuron. Accordingly, the timing of the activation of basket cell inhibition is critical in relation to the other inputs to the same neurons. Our electrophysiological data shows[37] that the two basket cells at the top of layer IV received monosynaptic X type geniculate input. Since the horizontal spread of X type geniculo-cortical fibers[6,17] in layer IV is very similar to the spread of large basket cell axons, basket cells activated by these fibers could exert a disynaptic inhibitory action to some neurons in the same area activated mono- and disynaptically by the same geniculate fibers. Such a sequence of events has been demonstrated in the cat visual cortex.[75] The other large basket cell, BC3, which could not be shown to receive monosynaptic geniculate input[37] could be responsible for mediating some of the trisynaptic inhibitory effects demonstrated in the cat visual cortex.[72] It has also been suggested that the same local neurons around layer IV mediate inhibitory effects of both specific visual and commissural pathways.[76] The large basket cells identified in the present study are probably responsible for this effect because two of them also received monosynaptic callosal input[37] in addition to the geniculate afferent input.

Horizontal spread of inhibition. Inhibitory input from selected areas surrounding a region of excitation appears to be required to generate much of the receptive field specificity found in the visual cortex.[5,51,52,53,54,55,56,78] There are clearly a number of ways in which this horizontal distribution of inhibition could be brought about. The actual extent of cortex from which this inhibitory input is drawn may vary with the particular receptive field property being specified. Studies[24,74] of the extent of physiologically produced inhibition in the visual cortex suggests that it operates over a range of 100–400 μm. This is consistent with, if an underestimate of, the extent of the putative inhibitory connections revealed by our studies. However, the extracellular recording methods used to determine the extent of inhibitory spread may be relatively insensitive, and intracellular studies may expand this figure.

The principal advantage of having inhibitory cells with long horizontal connections would be to reduce the number of synapses between source and ultimate target, and hence allow rapid inhibition. This rapidity of inhibitory action may be important for cells which are able to rapidly sum their excitatory input.

Effect of myelination. The conduction velocity of the axons of large basket cells is probably fast relative to other intracortical axons as a result of their diameter and heavy myelination. Thus, basket cell inhibition may be responsible for the very short latency IPSPs following the activation of geniculate afferents.[77]

The fact that myelination continues right up to the terminal bouton presumably ensures security of invasion of the action potential into the bouton and a synchronous synaptic activity in any localized region of the basket cell terminations. These factors would reduce the number of convergent basket cells necessary to attenuate or totally inhibit the output from a single cell since simultaneous activation of a number of synapses in a localized area would be more effective than an irregular activation. Since we have now provided a quantitative estimate of the number of synapses formed on the proximal portions of a single pyramidal cell, it should now be possible to model the effectiveness of a single basket cell in inhibiting a single target cell's activity. Hence, we could determine the number of basket cells that need to be active at any one time to inhibit the response of a pyramidal cell. It may turn out that relatively few basket cells are sufficient to hyperpolarise or alter the conductance of the post-synaptic cell sufficiently to prevent initiatiation of an action potential.

One of the observations here which deserves attention is that the same postsynaptic pyramidal cell can receive input on its soma, on proximal parts of apical and basal dendrites as well as on spines and shafts of distal dendrites from the same large basket cell. Only some of the more distal contacts could be unequivocally shown to be on the dendrites of the same pyramidal neurons which received input on their soma and proximal dendrites (Figs 13 and 15) but these examples raise the possibility that all the postsynaptic structures encountered in the neuropil belong to the same sub-population of neurons.

Consequences of basket cell input for the activity of pyramidal cells. On the basis of our data one could envisage three levels of action for the basket cells. Firstly, the generalised inhibition, by means of inhibitory input on the soma, of all the excitation arriving at the soma from the pyramidal cell dendrites. The second level of action would be a selective inhibition of particular dendrites, principally the apical dendrite. The third would be the selective inhibition of excitatory input to particular portions of the dendrite, principally by means of the dendritic spines. Although any single pyramidal cell can receive input at all of these three levels from a single basket cell, there could nonetheless be some independence of control between the different levels. For example, the basket cell input to a particular spine will be sufficient to inhibit the excitation arriving in that localized region but may be insufficient to inhibit the apical dendrite or somatic activity. Again it may be possible to inhibit all of the activity of the apical dendrite but not all of the somatic activity resulting from activity of the basal dendrites.

Further independence of action could arise if there are different types of GABA receptors at different locations. This follows from the observations of

Alger & Nicoll[2,3] on hippocampal pyramidal cells *in vitro*. They found that the presence of barbiturates and sub-physiological temperatures leads to the appearance of a late, bicuculline-sensitive component in the IPSP which is of dendritic origin. They suggest that although the normal dendritic potentials are hyperpolarising, there may be additional extrasynaptic receptors which when activated, give a depolarising response to GABA. Since this depolarising component also involves an increase in conductance, it would have the effect of shunting some of the local excitation while facilitating excitation arriving at more distal sites.[4] It would be of some interest to know whether similar mechanism could be found on cortical pyramids.

Multiple action of basket cells. If the major somatic input to pyramidal cells comes from basket cells and since the soma is generally the optimal site for locating inhibitory synapses, then the simultaneous and coordinated action of these cells must to a large extent determine the net output from the cell. It follows that the basket cells must be responsible for determining a good number of the specific receptive field properties thought to be due to intracortical inhibition.[5,51,52,53,54,55,56,78] It may then be seen that if one basket cell were to provide all the inhibitory input to a single pyramidal cell perikaryon, then it may prove to be impossible to wire up the basket cell in any simple way so as to produce single-handedly all these specific properties. For any single basket cell the wiring diagram required for it to produce end-inhibition may be incompatible with that required to produce orientation selectivity, for example. One solution to this problem would be to divide up all these functions between a number of cells. One set of cells could be responsible for orientation selectivity and callosal inhibition, another for directionality and velocity tuning, and yet another for end-inhibition and disparity sensitivity. This may be the reason why any single pyramid receives a relatively small number of synapses from any one basket cell.

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