Neuroscience Vol. 7, No. 11, pp. 2577 to 2607, 1982
Printed in Great Britain

0306-4522/82/112577-31$03.00/0
Pergamon Press Ltd
IBRO

THE AXO-AXONIC INTERNEURON IN THE CEREBRAL CORTEX OF THE RAT, CAT AND MONKEY

P. SOMOGYI, T. F. FREUND and A. COWEY*
1st Department of Anatomy, Semmelweis University Medical School, Budapest, Tüzoltó u. 58, H-1450 Hungary, and *Department of Experimental Psychology, Oxford University, South Parks Rd., Oxford, U.K.

Abstract—The synaptic connections of a specific type of identified cortical interneuron, the axo-axonic cell, were studied using Golgi methods. In the light-microscope axo-axonic cells were demonstrated in certain layers of the primary and secondary visual cortex of rat, cat and monkey, in the motor cortex of cat and in the subiculum and pyriform cortex of rat. The dendrites originating from the oval soma were oriented radially in a lower and upper spray within a cylinder about 100–150 μm wide. Electron-microscopy of Golgi impregnated, gold-toned axo-axonic cells showed predominantly but not exclusively asymmetrical synaptic contacts on their dendrites and spines, few synaptic contacts on the perikarya some of which were asymmetrical, and no synaptic contacts on the axon initial segment. The axon usually arborized within the vicinity of the cell's own dendritic field in an area 100–200 μm in diameter. In the kitten motor cortex the axon of a neuron in layer III descended to layer VI, providing a columnar arborization.

The axon formed specialized, 10–50 μm long terminal segments invariably oriented parallel with the axon initial segment of pyramidal cells. All 85 identified symmetrical-type synaptic contacts, deriving from 31 specialized terminal segments, were found exclusively on the axon initial segment of pyramidal neurons. Rare, lone boutons of axo-axonic cells also made synaptic contact only with axon initial segments, confirming the exclusive target specificity of these cells. In identified gold-toned boutons, flattened pleomorphic vesicles were present. Electron-microscopy showed that axons ending in specialized terminal segments may originate from myelinated fibres, indicating that Golgi impregnation has revealed only part of the axon. Counting of axon terminal segments, each of which was in contact with the axon initial segment of a pyramidal neuron, revealed 166 pyramidal neurons receiving input from a partially reconstructed axo-axonic cell in the motor cortex of the kitten, and 67 from another cell in the visual cortex of the cat. The convergence of five axo-axonic cells onto one pyramidal cell was demonstrated in the striate cortex of the cat by counting all synaptic contacts on three initial segments. Cells from a one-month-old kitten were compared with those of the adult. The axon of the developing neurons was more diverse, having many growth cones and filopodia which made no specialized membrane contacts. However, the developing specific terminal segments formed synapses only with axon initial segments.

It is concluded that the presence of axo-axonic cells in all the species and cortical areas we have examined suggests their association with the structural design of pyramidal cells, wherever the latter occur, and with their participation in the information processing of pyramidal cells. Axo-axonic cells are uniquely endowed with the means of simultaneously influencing the action potential at the site of origin in groups of pyramidal cells. This strategic location may enable them to synchronise the activity of pyramidal neurons, either through inhibitory gating or through changing the threshold of pyramidal cells to certain inputs.

In a correlated light- and electron-microscopic study, a new type of neuronal connection was found in the neocortex:[41] it was demonstrated in the visual cortex of the rat that the axon initial segment of pyramidal cells receives several synapses from a specific interneuron. Synapses involving the axon initial segment had been demonstrated in other parts of the central nervous system[13,17,28,30,48,51] and also in the neocortex,[15,35,63] but the findings in the rat's cortex[41] were unexpected for two reasons. Firstly, the demonstration of innumerable synaptic contacts on a single initial segment showed that, besides the dendrites and the perikaryon, the axon initial segment is a major input zone of the pyramidal neuron. This was confirmed in other cortical areas but the origin of the presynaptic terminals could not be determined in these later studies.[21,40] Secondly, the finding that most of the boutons making synapses on the axon initial segment were provided by an interneuron, the axo-axonic cell, whose terminals established synapses exclusively with the axons of pyramidal cells, revealed an unparalleled target specificity.[41] A preliminary report from our laboratory[43] and a detailed study in the visual cortex of cat[8] confirmed the above findings in other species and led to the conclusion[8,43,49] that the specific axo-axonal interneuron was identical with the chandelier cell discovered earlier by Szentágothai.[56] Although there are several reports on the

presence of axo-axonic or chandelier cells in different species and different cortical areas,[24,25,36,54,61] electron-microscopic confirmation of the specific termination has been done only for the visual cortex of the rat[41] and cat.[8,43]

We therefore studied further the structure and distribution of axo-axonic cells in more detail with the following aims:

(a) identified axo-axonic cells were studied in the cortex of the most frequently used laboratory animals (rat, cat and monkey) to see whether they terminated exclusively on the axon initial segments of pyramidal cells in all species, and whether the structure of the neurons was similar.

(b) As most data on axo-axonic or chandelier cells are from sensory areas and the electron-microscopic data are restricted to granular cortex, we studied whether they are present in the agranular motor cortex. Besides neocortex, the presence of axo-axonic cells was also investigated in paleo- and archi-cortical areas.

(c) Convergence of several axo-axonic cells onto one pyramidal neuron initial segment was predicted in the rat.[41] In the present study, synapses were counted to obtain a numerical estimate of convergence in the visual cortex of cat.

(d) Previous studies dealt only with the terminals of these neurons; here, we studied the structure and synaptic input of the perikaryon, axon initial segment and dendrites of axo-axonic cells.

(e) Hitherto, the identification of these neurons has been made exclusively with Golgi staining, which is frequently carried out in young animals to exploit their poor myelination. However, the morphology and connections of neurons may change during postnatal development. Axo-axonic cells from a kitten and adult cats were therefore compared to see if structural changes occur.

A preliminary report of some of the results has been published.[43]

EXPERIMENTAL PROCEDURES

Six adult male and female CFY-strain albino rats, two adult male Wistar rats, two adult male cats and a one-month-old kitten were used. In addition, blocks of tissue were obtained from two normal, adult, male Rhesus monkeys (*Macaca mulatta*). One of them was used in experiments reported previously,[46] the other was used in behavioural experiments before it was killed for histological study of several brain areas. Rats and cats were anaesthetised with chloral hydrate (350 mg/kg i.p.). Monkeys were sedated with an intramuscular injection of ketamine hydrochloride (10 mg/kg; Ketalar, Parke-Davis) followed by a lethal intravenous dose of sodium pentabarbitone Sagatal, May and Baker). All animals were perfused through the heart with 0.9% saline followed by a fixative containing 0.5–2.5% glutaraldehyde (TAAB) and 1–4% paraformaldehyde (TAAB) dissolved in 0.1 M sodium phosphate buffer (pH 7.4).

Impregnation of any particular cell type cannot be guaranteed with the Golgi method; therefore, it took several years to collect the material for the present report. Accordingly, cortical tissue was processed for combined light- and electron-microscopic study using a method described previously,[42] but with various slight modifications as reported in subsequent studies.[44,45,47,49] Briefly, small blocks of cortical tissue were washed free of aldehydes then treated with OsO_4 either before or together with the potassium dichromate stage of the Golgi procedure. Thereafter, the blocks were immersed in silver nitrate solution, and 80–100 μm thick sections were cut either by hand or using a Sorvall TC-2 tissue chopper. Some of the sections were dehydrated and mounted on slides in Durcupan ACM (Fluka) resin, overlayed with a coverslip, cured and stored. Other sections were mounted in pure glycerol on slides and some were selected and later gold-toned[7] as described previously,[44,49] but employing illumination on a Zeiss (Jena) microscope stage while the sections were still on slides.[47] The gold-toned sections were also dehydrated and stored in polymerized Durcupan ACM resin on slides.

Selected cells were drawn by means of a camera lucida, photographed in detail in the light-microscope and re-embedded for electron-microscopy.[49] Serial ultrathin sections were picked up onto formvar-coated single slot grids and stained with lead citrate.[38] Uranyl acetate stain-

Table 1. Occurrence of axon-axonic cells and/or their axons in the cerebral cortex

Species	Cortical area	Prikaryon + axon in layer	Axon only in layer
Rat	17	II., III.	V., VI.
	18	II., III.	V.
	18a	II., III.	—
	Subiculum	—	Stratum pyramidale
	Piriform cortex	—	II., III.
Cat	17	III., V.*	II., V., VI.
	18	—	II., III.
	4	III.*	V., VI.†
Monkey	17	—	II., III.
	18	III.	III.

* These neurons are from a kitten.

† The specialized terminal axon segments in the deep layers were proven to originate from a cell in layer III.

ing was carried out during dehydration on the Golgi sections. Electron-micrographs were taken at 80 KV.

RESULTS

Terminology

In the present study, we use the term axo-axonic cell to categorize neurons which make synapses exclusively with the axon initial segments of pyramidal neurons, as shown by electron-microscopy. All such neurons were found to have specialized terminal axon segments. In some instances, the specialized, terminal axon segment was so obviously and solely associated with the base of pyramidal neurons, recognizable in the light-microscope, that we did not deem it necessary to carry out electron-microscopic examination. The name axo-axonic neuron is applied to these instances as well (see p. 2601 for discussion of terminology).

Distribution of axo-axonic cells in different cortical areas of the rat, cat and monkey

Axo-axonic cells or their impregnated axon plexi were found in all three species. Because Golgi impregnation is fortuitous it cannot be established that they are absent from certain areas or layers of the cortex, or that they are more numerous in any cortical area. Therefore Table 1 merely lists the observed locations. The precise origin of the material which was used to provide detail and electron-microscopic evidence for specific points considered below, is shown in Table 2.

Light-microscopic appearance of axo-axonic cells

The axon. A unique feature of these neurons is their axon, which branches profusely and terminates with 10–50 μm long terminal segments oriented orthogonally to the pia (Figs 1–16). Such specialized segments can arise from any part of the axon on thin collaterals, but the main collaterals themselves can form vertical segments interconnected by arcadian or horizontal branches (Figs 6, 10, 13, 15). In the adult animal, each terminal segment is usually composed of a single row of varicosities or beads, but less frequently two or three rows originating from one or several collaterals combine to form a terminal segment of parallel or interwoven rows (Figs 9A, 10A). Rarely, two or three single vertical segments from separate collaterals are longitudinally aligned to form a single row of varicosities (Figs 6, 7B). Almost without exception, the varicosities of an axon are in these vertical terminal segments and solitary boutons or varicosities are rarely encountered (Figs 7A, 16). The tendency to form bouton rows is so strong that even individual boutons tend to align into vertical formations (Fig. 7B).

There was no major difference in the morphology of the terminal axon segments of the three species (compare Figs 2, 3, 4, 5D, 6, 10A, 15).

The axons were frequently impregnated without the parent cell body and formed a conspicuous dense meshwork in the supragranular layers (Fig. 1A). Because of the unpredictable nature of the Golgi staining it cannot be determined whether the entire axonal arborization is impregnated in these cases, but in opportune instances it was possible to trace an axon arborization from a main axon trunk (Figs 3A, 10A, 16A). Impregnated axons arising from the white matter were never seen to give rise to specialized terminal segments.

In all three species, axons with specialized terminal segments could be traced back to perikarya in the grey matter of the cortex (Figs 2, 4, 6–8, 11, 13, 15).

The perikaryon and dendritic tree. The cells in all species and areas have some features in common, which will be described together. The soma is oval, radially elongated, fusiform or spindle-shaped, and about 8–10 μm wide by 16–20 μm long.

The dendrites may originate from an upper and lower main dendritic trunk (Figs 2, 4A) or in two bunches from the upper and lower poles of the soma, giving the neuron a bitufted character (Figs 2, 6, 13, 15). Occasional dendrites originate or turn laterally. The predominant orientation of the dendrites is radial, i.e. orthogonal to the pia, and they lie within a cylinder of about 100–150 μm. Some dendrites of the upper radial dendritic arbor invariably reach layer I, and even the pia, if the soma is in the supragranular layers. The main dendrites may branch several times a short distance from their origin but little if any branching occurred further from the perikaryon. The dendrites can be smooth (Fig. 6) or have thorny appendages (Figs 2, 15). Some axo-axonic cells in the rat had sparsely spiny dendrites with drumstick-shaped spines (Fig. 4A). The dendrites in the kitten (Figs 11, 13) are different and will be dealt with below.

In all instances where the main axon was impregnated it originated from the lower main dendritic trunk (Figs 2, 11, 13, 15), sometimes distal to the first branching point (Fig. 6) of the dendrite. It usually descends 50–100 μm before giving off locally arborizing collaterals.

Distribution of axonal fields

Most of the axons were found in the supragranular layers; and all the perikarya, bar one which was in layer V of the kitten visual cortex, were in layers II–III (Tables 1 and 2). The axon arborization could be followed in a cylinder of 100–200 μm. There were indications, however, that because of technical limitations of the Golgi method the axons are incompletely revealed. One such limitation is that myelinated axons are rarely impregnated and even then only in short segments.

From the light-microscopic appearance of impregnated axo-axonic cell axons we suspect that the main axons are partially myelinated. Thus the impregnation of the axon followed from a cell in layer III of the striate cortex of cat stopped abruptly (Fig. 6). Impregnation of another axon in the prestriate cortex of monkey stopped at several main thick branches (Figs

Table 2. Origins of neurons studied

Cell No.	Illustrated in Fig.	Species	Cortical area	Position of perikaryon	Distribution of axon in layers	Electron-microscopic examination: No. of specialized terminal axon segments	Electron-microscopic examination: No. of synaptic bouton on IS	Electron-microscopic examination: Dendrites	Electron-microscopic examination: Perikaryon
1.	4	Rat	Area 17	Upper 1. III.	II–III.	1	3	+	+
2.	1B–D		Area 17	Mid. 1. III.	III.	2	5	+	+
3.	5A–C		Area 17	–	III.	2	4	–	–
4.	2		Area 17	Layer II.	II–III.	–	–	–	–
5.	2		Area 17	Layer II.	II–III.	–	–	–	–
6.	3A–B		Piriform cortex	–	II–III.	–	–	–	–
7.	3C		Subiculum	–		–	–	–	–
8.	13–14	Cat (1 month old)	Area 4	Mid. 1. III.	III–VI.	6	16	–	–
9.	11–12	Cat (1 month old)	Area 17	Layer V.	V.	4	14	–	–
10.	9–10	Cat adult	Area 17	–	II–III.	4	6	–	–
11.	6, 7, 8	Cat adult	Area 17	Mid. 1. III.	III.	3	24	+	+
12.	5D–E	Monkey	Area 17 (V_1)	–	II.	6	8		
13.	15		Area 18 (V_2)	Layer 1. III.	III.	–	–	–	–
14.	16		Area 18 (V_2)	–	III.	3	5		
Total						31	85		

Summary of the data on the distribution of axo-axonic cells in the present study. Although the report is based on the detailed examination of these neurons, in addition, several more axonal arbor' 'tions were studied light-microscopically in the rat and cat. Cells No. 3, 6, 7, 10, 12, 14 represent impregnated axons without the cell of origin. Some dat. not included here on cells No. 1 and 2. have been reported previously,[41] but further examination resulted in additional information. Specialized terminal axon segments of cell No. 11 were studied quantitatively to calculate convergence of axo-axonic cells.

16A, B). Electron-microscopic examination revealed that these main branches were myelinated (Fig. 16C). This may be a general feature of axo-axonic cells, because already in the one-month-old kitten visual cortex the main axon of a cell in visual cortex was impregnated only to a certain point (Fig. 11).

Apart from myelination another problem arises from the difficulty in tracing and joining thin axon collaterals in consecutive sections. This means that the 100–200 μm area occupied by the axon may be an underestimate in the lateral direction, and the vertical extent of the axon is not known. Nevertheless, in one well-impregnated example (Figs 13, 14) in the motor cortex of the kitten, an axon originating from a cell in layer III had several collaterals descending to layers V and VI and giving off specialized terminal axon segments in a column 100–150 μm in diameter in the deep layers and around 400 μm in layer III.

Fine structure of the axo-axonic cell perikarya, dendrites and their synaptic input

Two perikarya in the rat and one in the cat's visual cortex were studied at the electron-microscopic level. The neurons have a large eccentrically-placed nucleus and a thin rim of cytoplasm which is more extensive in the obtuse apices of the neuron, at the origin of the main dendrites (Figs 4B, 7C). The nucleoplasm is electron-lucent, containing dispersed chromatin. In one nucleus an intranuclear rod was observed (Fig. 4B). The nuclear membrane has several, but slight, invaginations (Figs 4B, 7C). The cytoplasm contains all the usual cell organelles; nothing peculiar to this neuron was observed. The endoplasmic reticulum usually does not form Nissl bodies (Figs 4, 7).

The neurons receive very few synaptic contacts on their perikarya. Two at most were present in a single section, but more often none (Fig. 4B). Most of these synaptic contacts are symmetrical, and established by boutons containing flattened or pleomorphic vesicles (Fig. 4C). Rarely, boutons containing ovoid vesicles make asymmetrical contacts on the perikarya (Fig. 7D).

The dendrites and their spines are more richly supplied with synaptic terminals (Figs 4D, E, 7E). The spines are contacted only by boutons containing ovoid vesicles (Fig. 4D). Most of the boutons on the dendritic shafts are also of this type (Fig. 4E) and in the gold-toned dendrites the synaptic active zones are asymmetrical (Fig. 7E). Some, but many fewer, boutons making symmetrical synaptic contacts were also observed on the dendritic shafts.

One axon initial segment in the cat and one in the rat were also studied. They did not receive synaptic contacts.

The fine structure of the pyramidal cell axon initial segment, identified axo-axonic cell boutons and their synaptic contacts

The axon initial segments could be recognized by their unique fine structural features, namely, the membrane undercoating and microtubule fascicles[32] (Figs 1C, D). Additional characteristics are dispersed ribosomes and cisternal organelles (Figs 1B, C) some of them very extensive (Fig. 1B). Numerous coated pits were also observed (Figs 1B, C, 12D), and spines were frequently encountered along the initial segment (Figs 1C, D). Most of the axon initial segments studied were traced back to pyramidal cells, recognised by the characteristic origin of the descending axon either from the base of the perikaryon or from a main basal dendrite. Furthermore, the presence in the electronmicrographs of one apical dendrite and homogeneous, symmetrical axo-somatic synapses also served as criteria to identify the neurons as pyramidal cells.

The identified axo-axonic cell bouton rows climb along the initial segments, usually terminating 10–30 μm below the base of the soma (Figs 3A, B, 7B), but occasionally reaching the perikaryon. The axon initial segments of pyramidal cells bear synapses from impregnated, therefore identified, axo-axonic cell boutons as well as from non-impregnated boutons. All the synaptic contacts are symmetrical, both on the axon shaft and on its spines (Figs 1B–D, 5E, 8, 10B, C, 12D, C, 14E, 16D). The postsynaptic membrane thickening, an electron-dense band of granulated material which starts at the inner electron-dense lamina of the axon membrane, is different from the membrane undercoating of the rest of the initial segment. The latter is interrupted at the synaptic junctions and its densest lamina is separated from the axon membrane by a thin translucent zone (Figs 1B, C). The symmetrical synaptic contacts are very similar to those established by other boutons on dendrites (Fig. 5C), spines and perikarya.

The Golgi-stained and gold-toned axo-axonic cell boutons contain small, clear, flattened vesicles (Figs 5B, 8C–E). Occasional large granulated vesicles were also observed further from the synaptic active zone. The non-impregnated boutons are similar to the impregnated ones in every respect. In the case of Golgi-impregnated boutons without gold-toning, the identification of synaptic contacts is based on the electron-dense cleft material, the slight widening of the synaptic cleft and the postsynaptic membrane thickening (Figs 1B, C, 5E, 10B, C, 12C, D, 14E, 16D).

Rather than being evenly distributed on the surface of the initial segment the synaptic boutons tend to form clusters. The most distal boutons coincide with the origin of the myelin sheath of the pyramidal axon (Fig. 9B).

Specificity in the termination of axo-axonic cells

All 85 boutons, belonging to 31 specialized axo-axonic cell terminal segments, established synaptic contact only with axon initial segments of pyramidal cells. All except two of the several hundred non-impregnated boutons making synapses with axon initial segments were contacting only these axons. One of the two exceptions was in the striate cortex of cat

where the bouton made synaptic contact with a spine (Fig. 10D), the other in the striate cortex of rat contacted a large dendrite in addition to the initial segment. It is conceivable that the specialized axon portions make synapses with initial segments but that other boutons of the axo-axonic cell may contact dendrites, perikarya, etc. Although lone varicosities are rare in the identified axons, we examined some with the electron-microscope. These boutons, too, made synapses solely with axon initial segments (Figs 16A, B, D).

Which pyramidal cells receive input from axo-axonic cells? As the outlines of pyramidal neurons can be seen in osmicated material it was possible to establish that within the axonal field of an impregnated axo-axonic cell not all pyramidal neurons are supplied at their base with specialized terminal axons. Especially at the periphery of the axonal arbor, many pyramidal neurons are not innervated.

As none of the axons could be reconstructed completely, the number of specialized terminal axon segments from each axo-axonic cell and the number of pyramidal cells they innervate are presumably much higher than we could observe. The highest number of pyramidal neurons receiving input from one axo-axonic cell was in the motor cortex of the kitten where 166 terminal segments were counted (Fig. 13). In the visual cortex of the cat 67 terminal segments were traced from one axon (Fig. 10A). The other cells were so incompletely displayed that we did not count the terminal segments.

Convergence of axo-axonic cells on pyramidal neurons

Once a neuronal process is impregnated, the Golgi precipitate usually extends to the limiting membrane and fills it completely. This means that every bouton arising from one terminal axon segment is impregnated and that the non-impregnated boutons in synaptic contact with the same initial segment originate from other neurons. Assuming that all or most boutons on one initial segment are from axo-axonic cells and that only one of the latter will usually be stained, the reciprocal of the fraction innervated should roughly indicate the number of axo-axonic cells converging onto one pyramidal neuron. The data shown in Table 3 are based on serial sections of three pyramidal cell initial segments in the striate cortex of the cat. A gold-toned axo-axonic axon was chosen in order to facilitate the recognition of all synaptic contacts of the impregnated boutons (see Figs 8B, C, E). A further reason for studying this axon was that its origin from a local cell was established (Fig. 6). The three terminal axon segments were chosen because all three could be included in the same section series. The plane of the sections was oblique to the initial segment, which meant cutting more sections but made it easier to identify synaptic contacts above or below the initial segment. It can be seen that a convergence of about five axo-axonic cells occurred. This number is only a first approximation, of course, because it is not known yet whether the converging axo-axonic cells contribute equally to the innervation of the target initial segment. It should be noted that the same axon arborization may provide simple bouton rows consisting of 3–5 varicosities as well as more complex ones built up of as many as 10–12 varicosities.

Table 3. Synapses on axon initial segments of three pyramidal neurons that were derived from a single axo-axonic cell

Pyramidal cell axon initial segment	No. of synapses received from impregnated axo-axonic cell No. 11	Total No. of synapses received	Ratio
1.	8	44	5.5
2.	8	42	5.3
3.	8	44	5.5

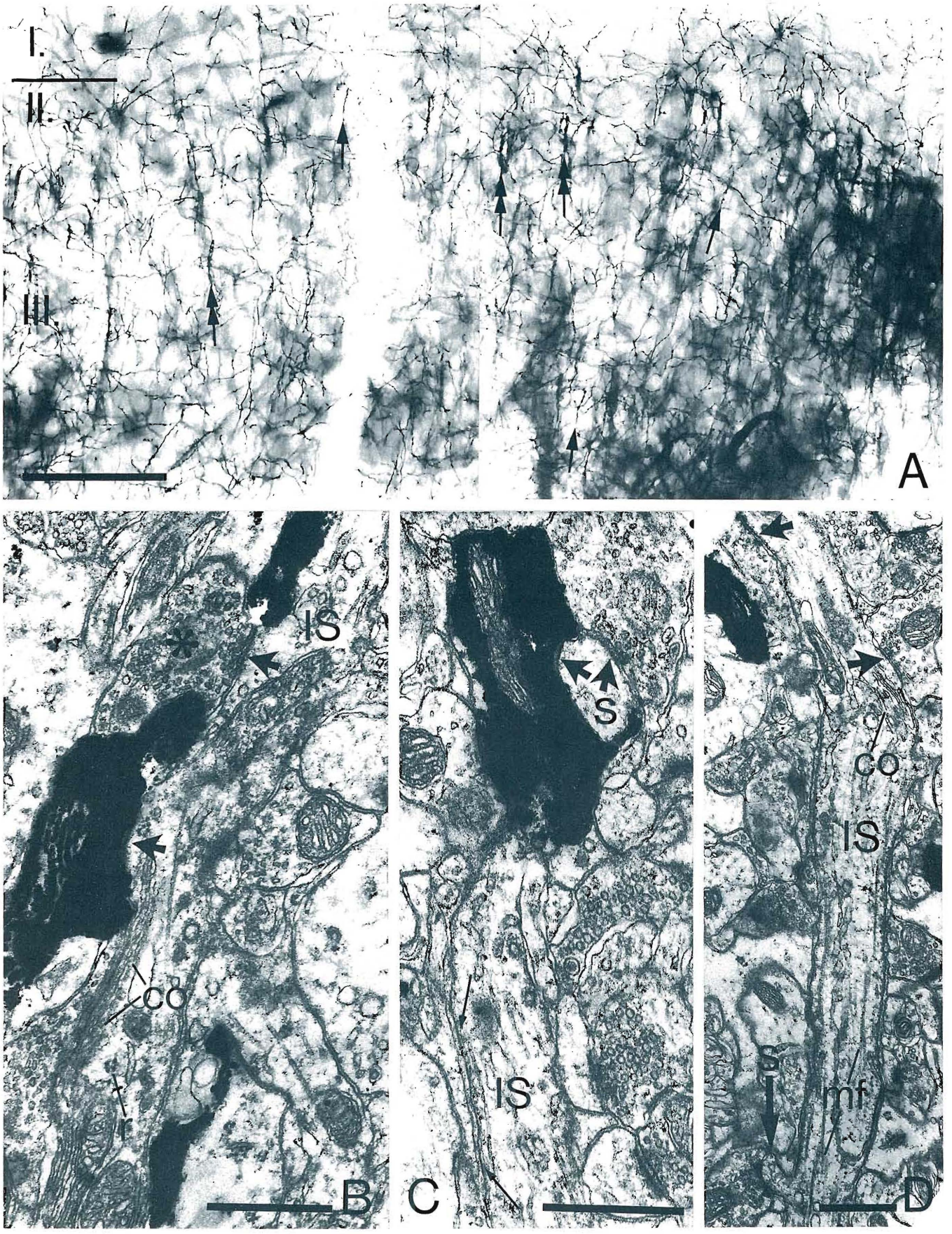
Number of synaptic contacts on the axon initial segments of three layer III pyramidal neurons which received input from a Golgi-impregnated axo-axonic cell (No. 11 in Table 1, see also Figs 6, 7) in the striate cortex of the cat. Synapses were counted in uninterrupted series of electron-microscopic sections.

Other evidence for convergence is that when the axons of several axo-axonic cells are impregnated in the same region the terminal bouton rows on the initial segment of pyramidal cells become more complex and thicker (Fig. 1A).

Differences in the morphology of axo-axonic cells in the kitten and the cat

One cell in the visual cortex (Fig. 11) and another in the motor cortex (Fig. 13) of the same kitten were

Fig. 1. Golgi material from the rat's visual cortex. (A) Light-micrograph of layers I–III, illustrating axo-axonic cell axons which are often impregnated together, without the parent neurons. Some single narrow (arrow) and thickened dual (double arrow) terminal axon segments are labelled. (B–D) Electron-micrographs of pyramidal cell axon initial segments (IS) with their accompanying impregnated (black) axo-axonic cell bouton rows of cell No. 2 in Table 2. (B) Convergence of impregnated and non-impregnated (asterisk) boutons. (C) Convergence of similar boutons onto a spine found in serial sections to originate from the initial segment. (D) An axon spine (S) receives a symmetrical synapse similar to those on the initial segment. co, cisternal organelle; mf, microtubule fascicles; r, ribosomes; small arrows, membrane undercoating; large arrows, synaptic contacts. Scales: A, 100 μm; B–D, 0.5 μm.


I.
II.
III.
A
IS
cc
r
B
S
IS
C
co
IS
s
mf
D

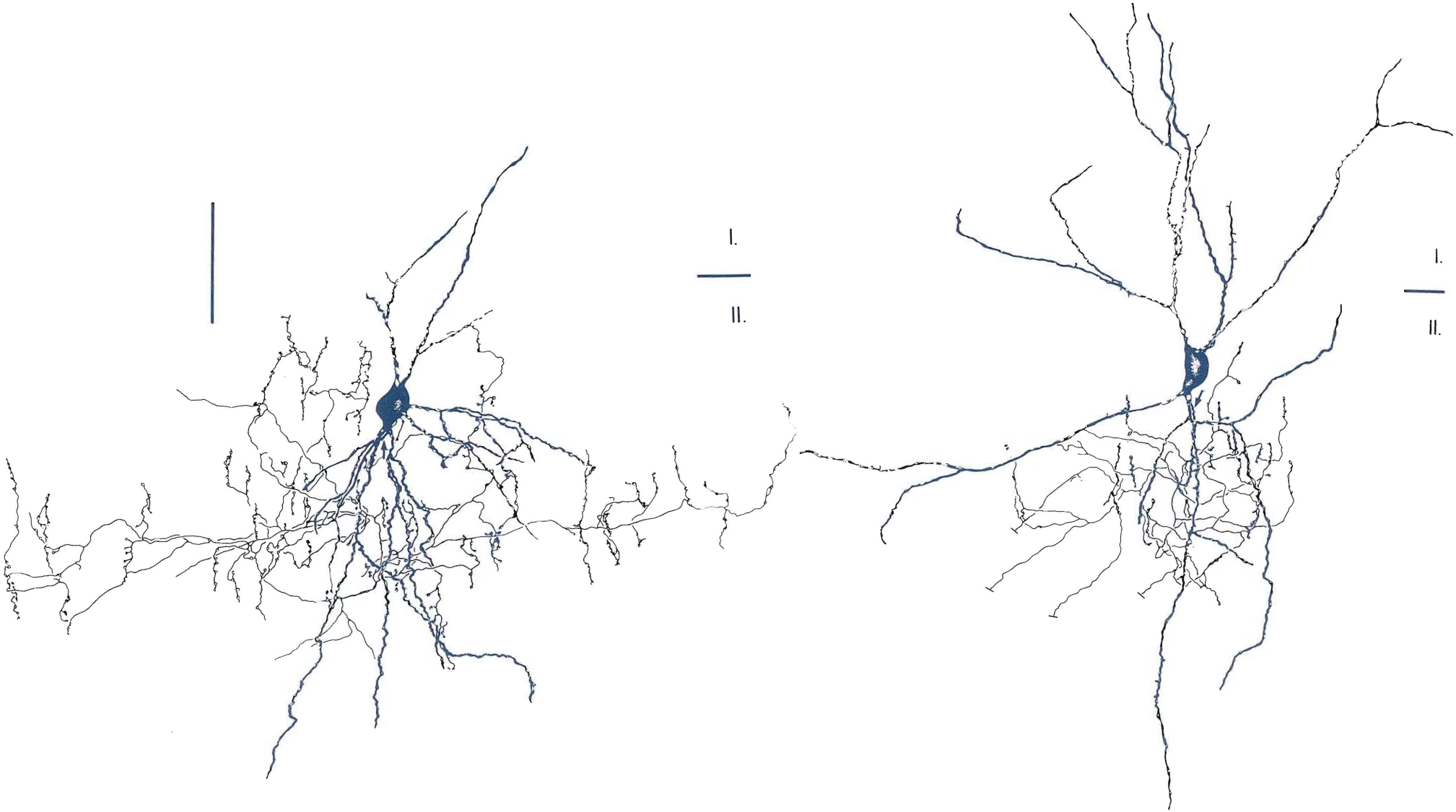


Fig. 2. Camera lucida drawing of Golgi-stained, gold-toned axo-axonic cells (No. 4 and 5 in Table 2) in sagittal sections of the rat's primary visual cortex, with their perikarya in layer II. Arrows indicate the origin of the axons. Scale: 50 μm. Horizontal bar marks boundary between layers I and II.

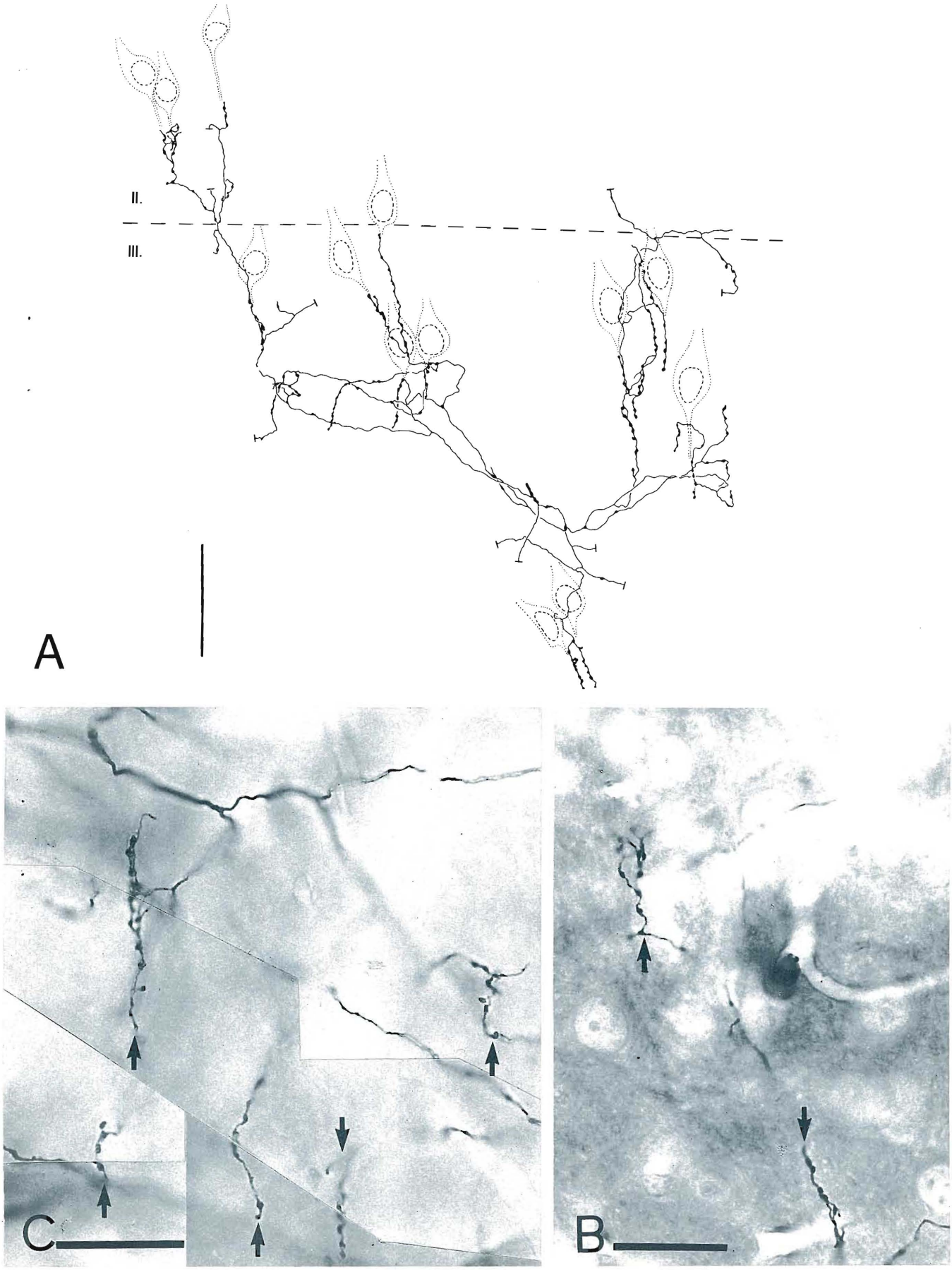


Fig. 3. (A) Camera lucida drawing of part of an axo-axonic cell axonal field in layers II and III of the rat's piriform cortex. Horizontal section, Cell No. 6 in Table 2. The terminal segments are aligned parallel with the axons of pyramidal neurons (dotted). (B) Light-micrograph of the terminal axon segments (arrows) in the piriform cortex. (C) Light-microscopic montage of an axon arbor with the characteristic terminal bouton rows (arrows) in the subiculum (No. 7 in Table 1) of the rat. Scales: A, 50 μm; B and C, 25 μm.

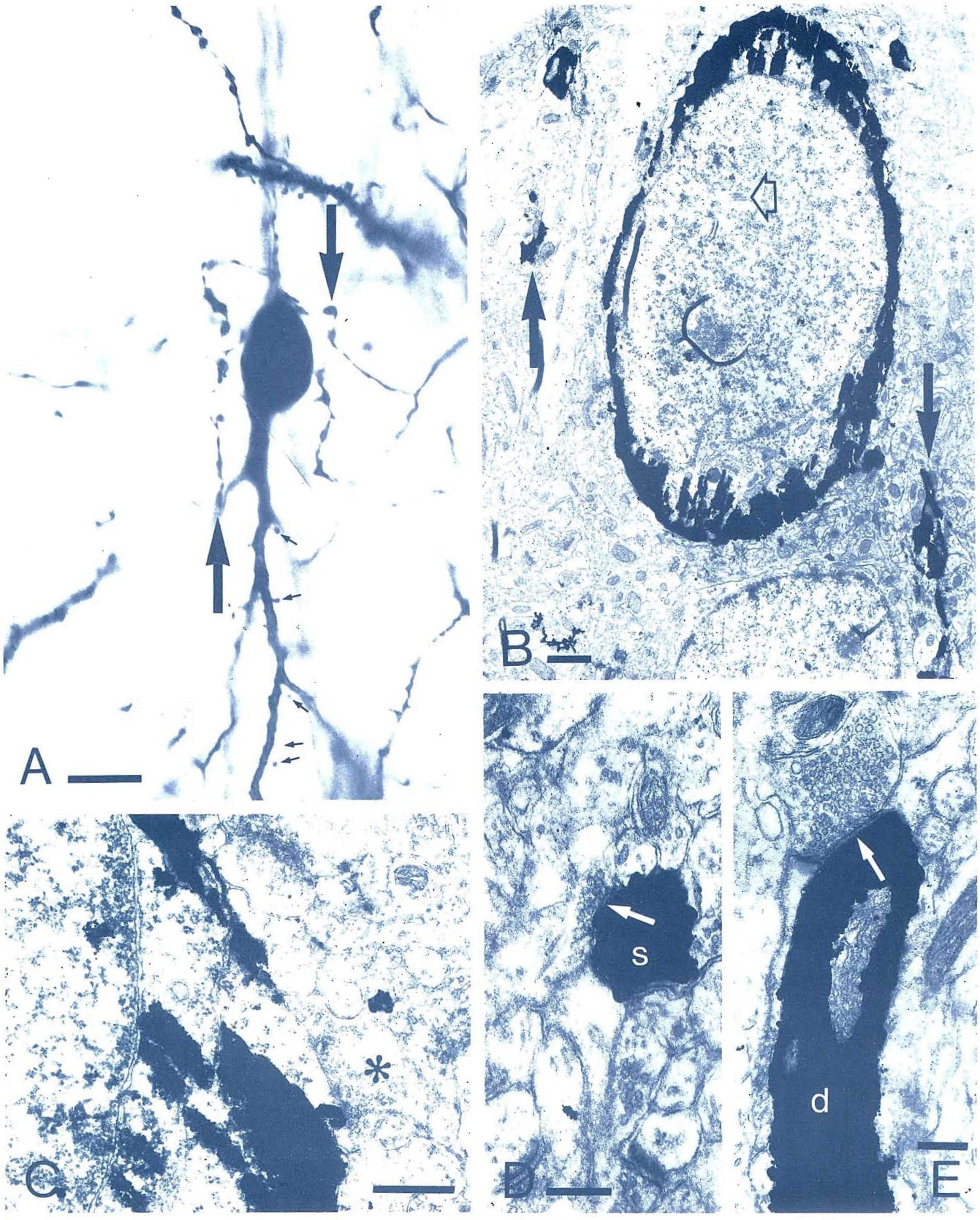


Fig. 4. Axo-axonic cell (No. 1 in Table 2) in layer III of the primary visual cortex of the rat. (A) Light-micrograph of the perikaryon with descending spiny (small arrows) dendrites. Large arrows in (A) and (B) indicate the same terminal axon segments of this neuron. (B) Electron-micrograph of the partially-impregnated perikaryon. Open arrow marks intranuclear rod. (C) Bouton containing pleomorphic vesicles (asterisk) makes symmetrical contact with the perikaryon of the same neuron. (D and E) A spine (s) and a dendritic shaft (d) of the same neuron, both receiving synapses (arrows) from boutons containing predominantly ovoid vesicles. Scales: A, 10 μm; B, 1 μm; C, 0.5 μm; D–E, 0.2 μm.

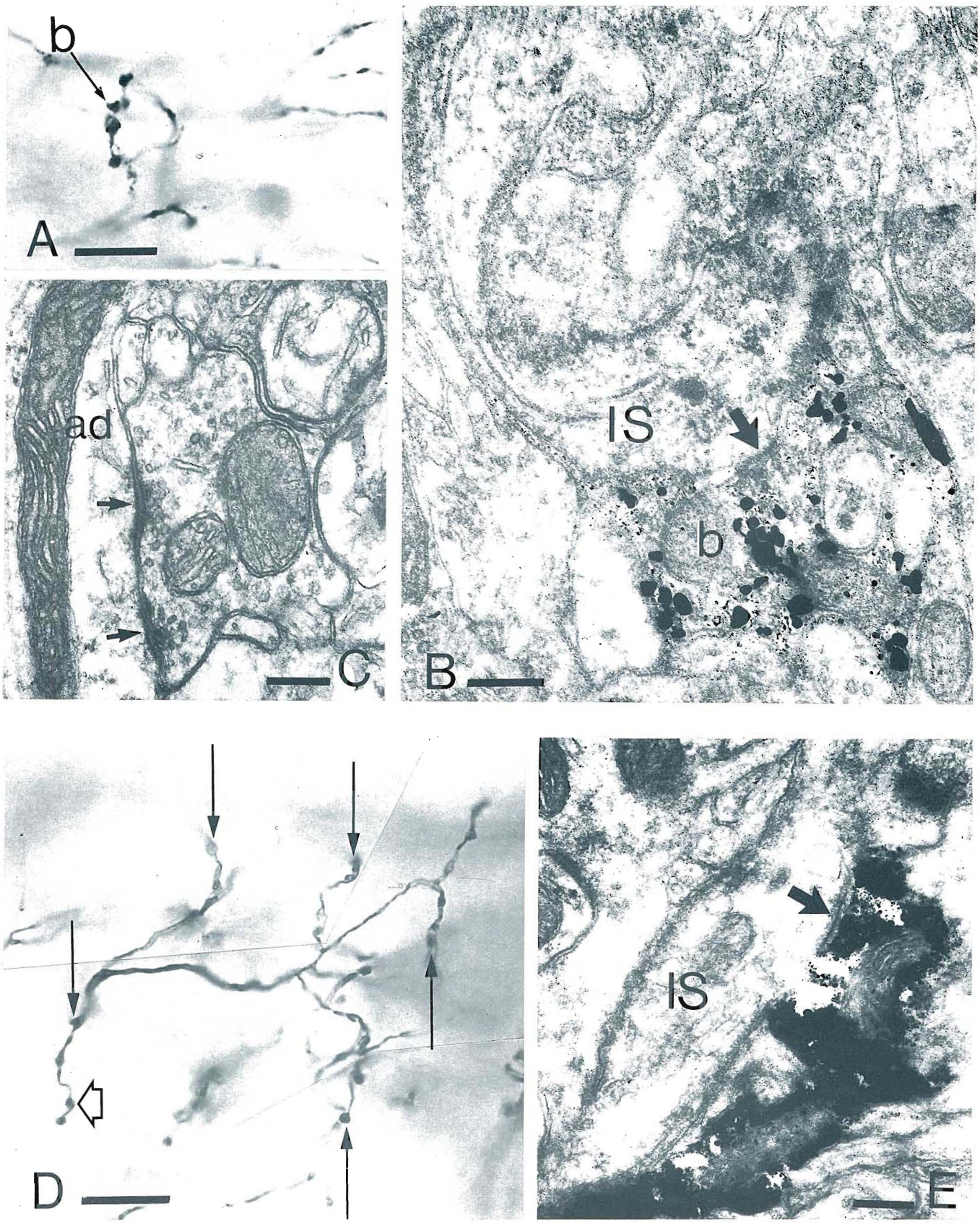


Fig. 5. (A–C) Rat, primary visual cortex. (A) Gold-toned terminal axon segment of an axo-axonic cell in layer III, No. 3 in Table 2. One bouton (b) is seen to make synaptic contact (arrow) with an initial segment (IS) in (C). The identified bouton contains flattened, pleomorphic vesicles similar to those in other boutons making symmetrical synaptic contacts. Such a bouton is shown in (B) to make a synapse (arrows) with an apical dendrite (ad) of a pyramidal neuron. Identified axo-axonic cell boutons made synapses only with initial segments. (D–E) Monkey, striate cortex. (D) Golgi-stained axon (No. 12 in Table 1) in layer II with the specialized terminal axon segments (arrows). Open arrow indicates a bouton shown also in electron-micrograph (E) to make synaptic contact (arrow) with the axon initial segment of a small pyramidal neuron. Scales: A and D, 10 μm; B, C and E, 0.2 μm.

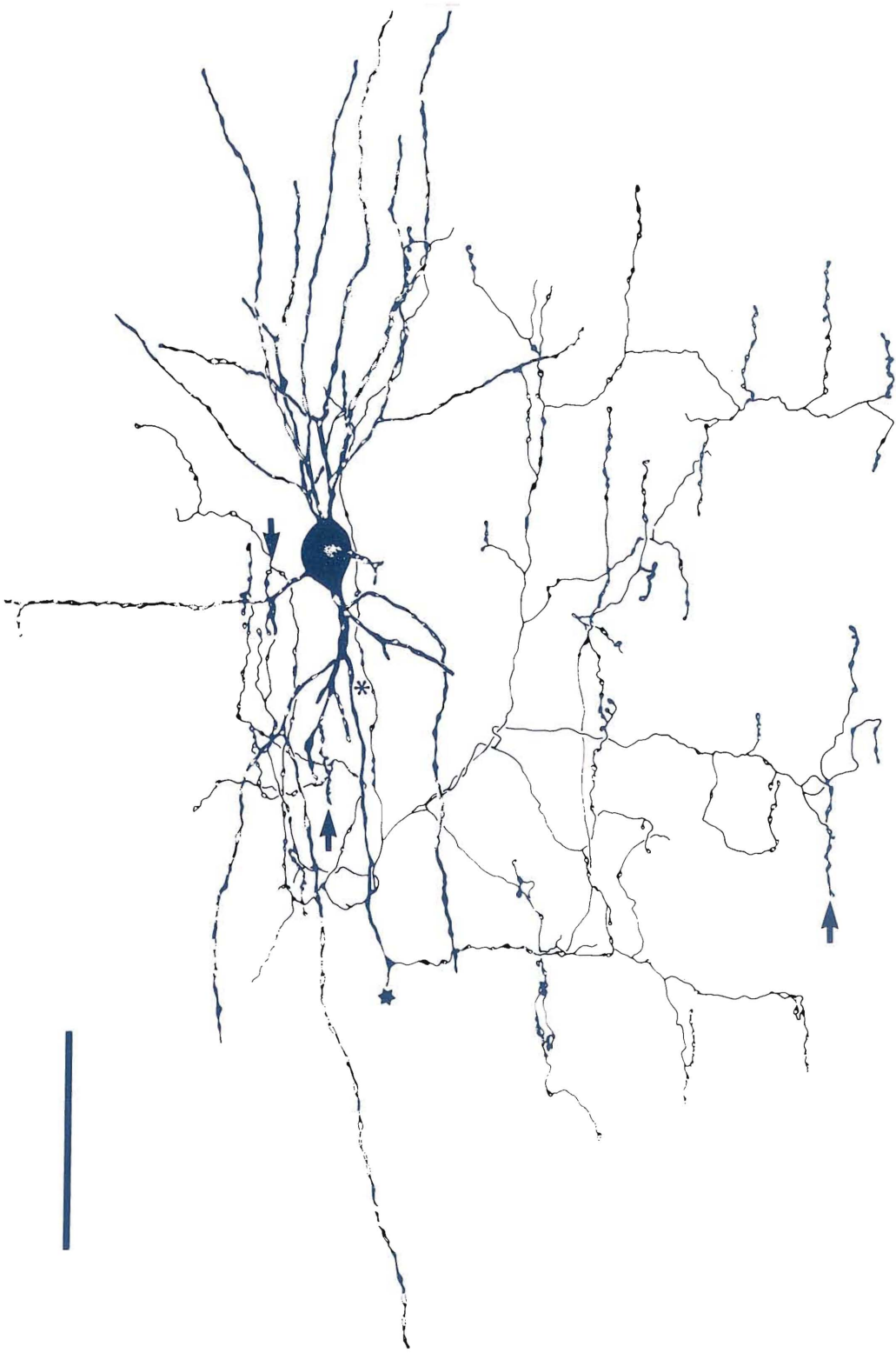


Fig. 6. Camera lucida drawing of a gold-toned axo-axonic cell in the primary visual cortex (area 17, layer III) of the cat (No. 11 in Table 2). The axon, originating (asterisk) from the lower main dendrite ends abruptly (star), suggesting the beginning of mylin sheath. Three terminal axon segments (arrows) were studied quantitatively (Table 3) Scale, 50 μm.

Fig. 7. (A) Light-micrograph of the same neuron as in Fig. 6. The origin of the axon (asterisk), two of the terminal bouton rows (arrows), and a dendrite are labelled. (B) A third specialized terminal portion is built up of two independently originating segments, both converging on the axon of the same pyramidal cell (P). Note that even single boutons of the axo-axonic cell gather into vertical rows (small arrows). (C) Electron-micrograph of the perikaryon. A bouton (b) is shown in (D) at high magnification in a consecutive section. It forms asymmetrical synaptic contact (arrow) and makes another synaptic contact with a spine (s). (E) Electron-micrograph of the dendrite (d) shown in (A). Numerous boutons (asterisk) establish synapses with the dendrite, and they contain predominantly ovoid vesicles. Scales: A and B, 10 μm; C, 1 μm; D and E, 0.5 μm.

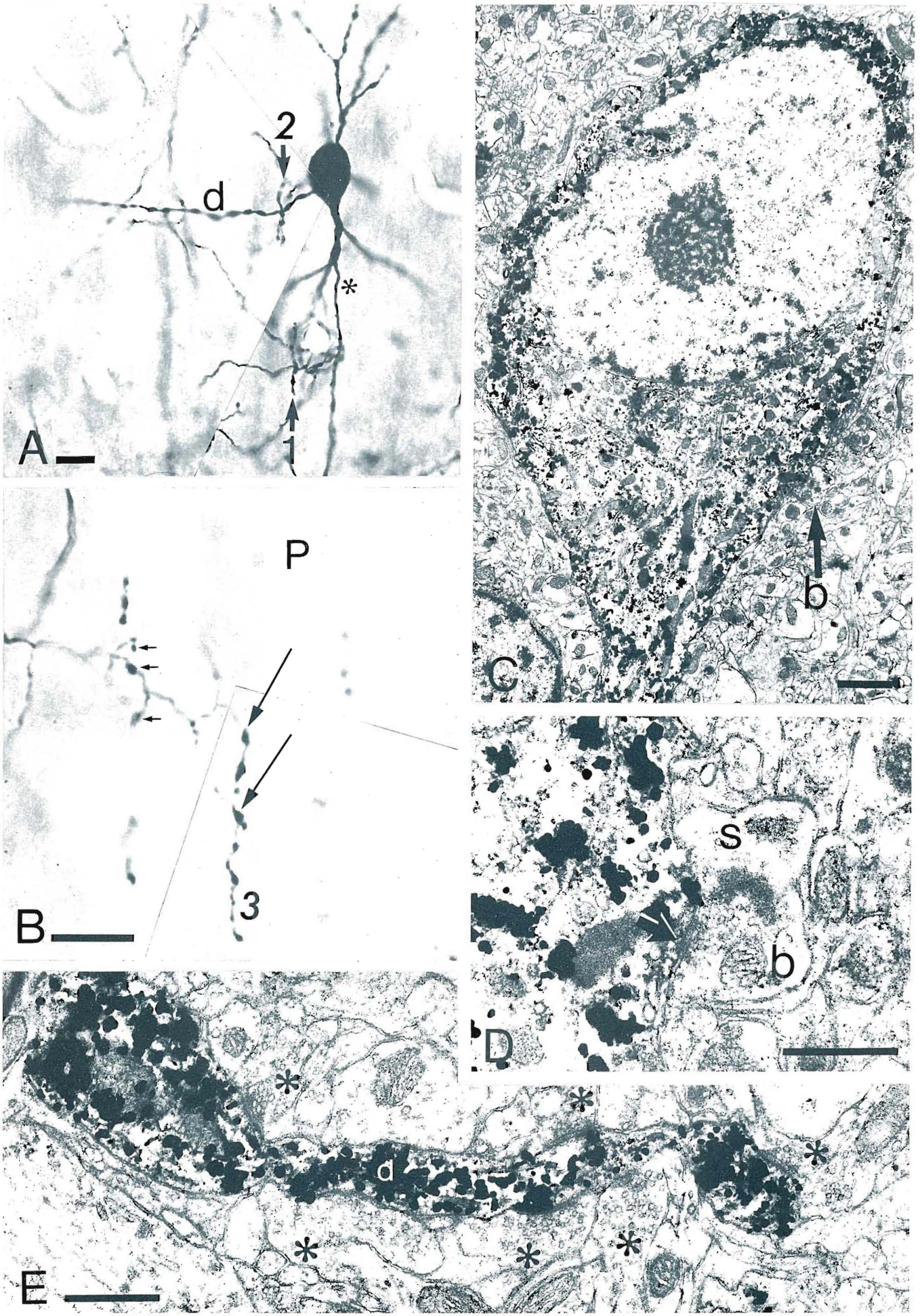
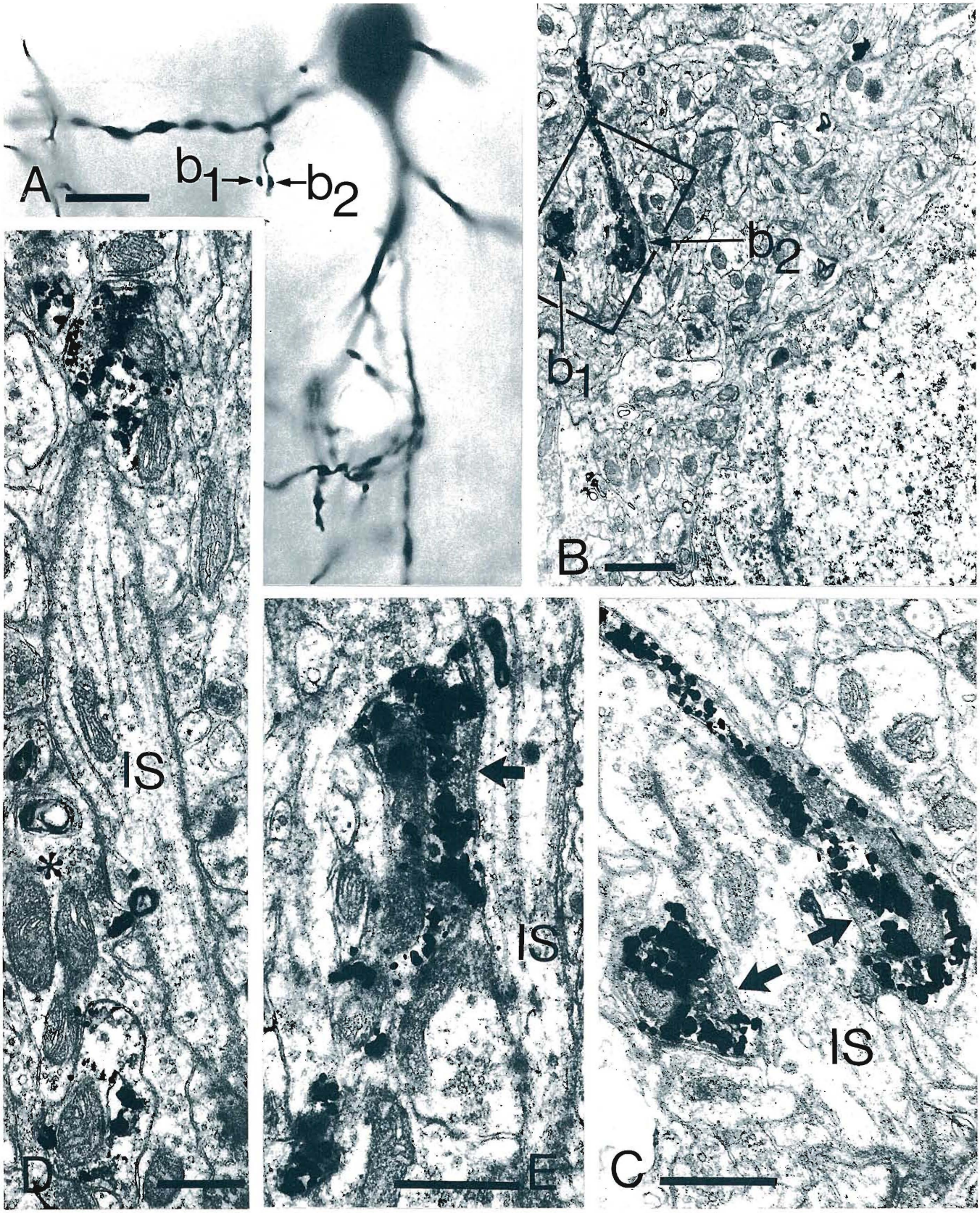
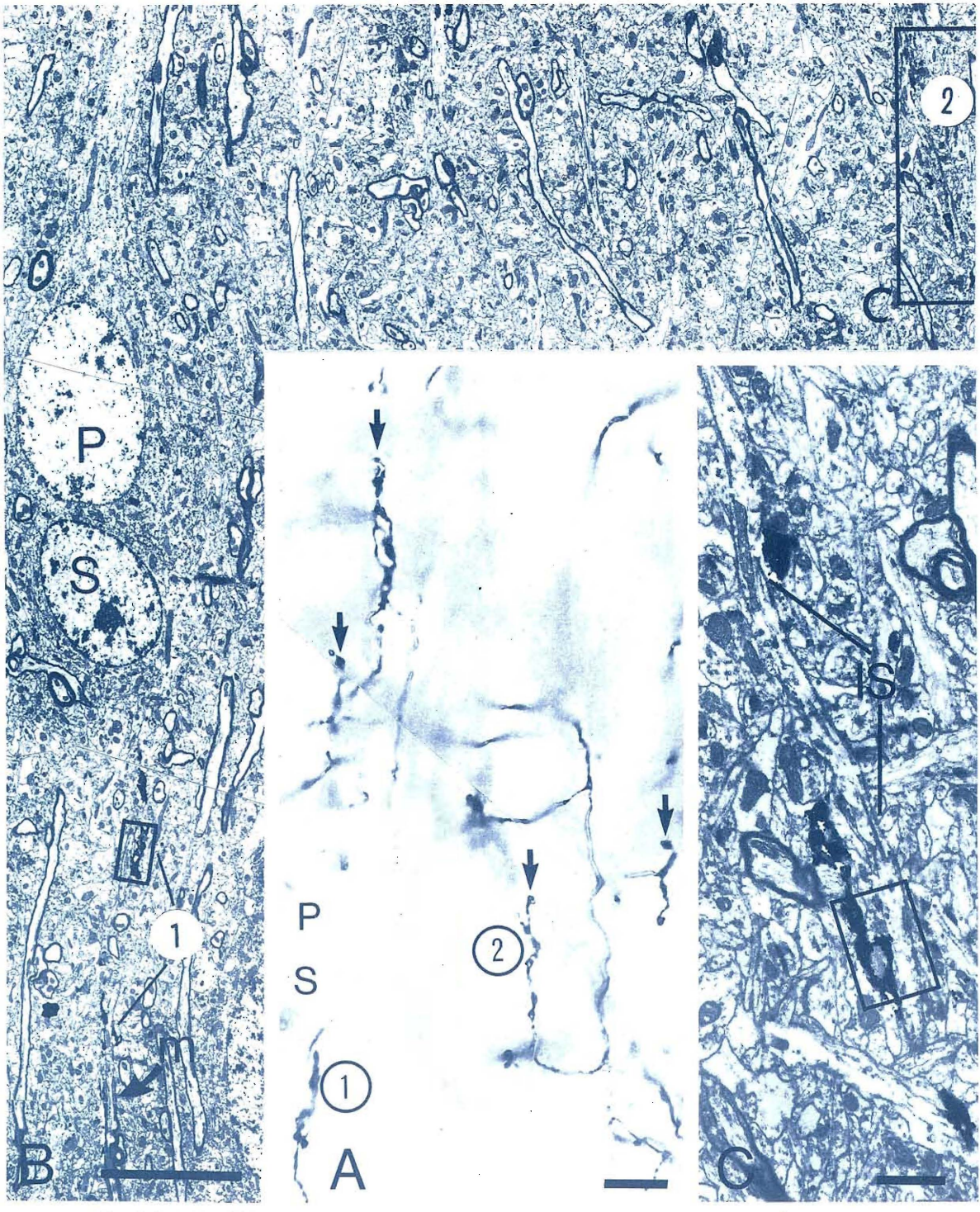
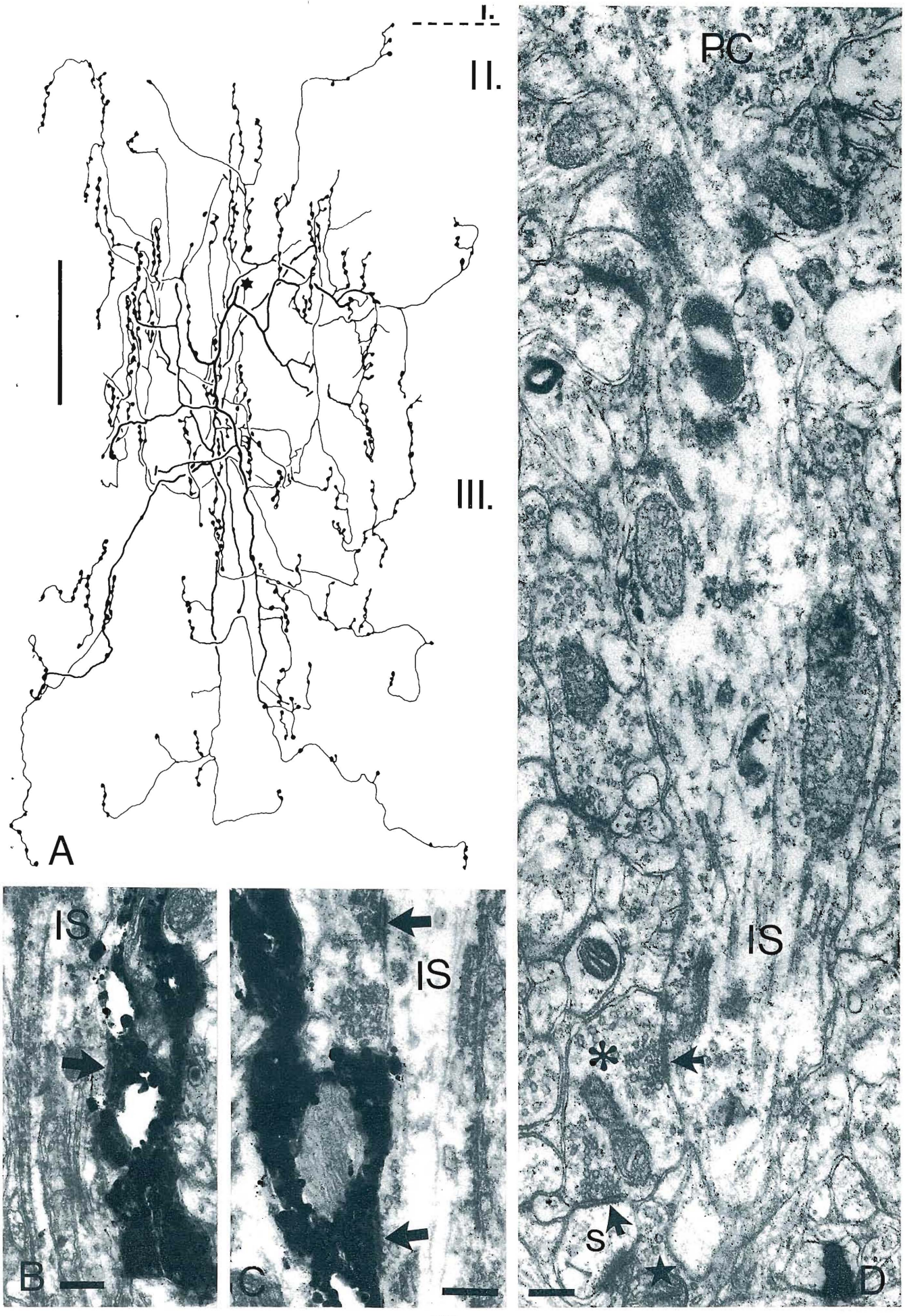

2
d
*
1
A
P
3
B
b
C
s
b
D
*
d
*
*
*
*
*
E

Fig. 8. Same axo-axonic cell as in Figs 6 and 7 in the striate cortex of the cat. Correlated light (A) and electron-micrographs (B and C) of two boutons (b_1 and b_2) from a terminal axon portion making synapses (large arrows) with an axon initial segment (IS). This initial segment received eight synapses from impregnated and 34 synapses from non-impregnated boutons. (D) Convergence of impregnated and non-impregnated (asterisk) boutons onto the same initial segment (IS). (E) Symmetric synaptic contact (arrow) by identified axo-axonic cell bouton. Note small flattened and pleomorphic vesicles in the gold-toned boutons in Figs C–E. Scales: A, 10 μm; B, 1 μm; C–E, 0.5 μm.


A
b1
b2
B
b2
b1
IS
*
D
IS
E
IS
C



Figs 9–10. Cat striate cortex. (9A–10C) Correlated light (9A) and electron-microscopic (9B, C; 10B, C) identification of axo-axonic cell (No. 10 in Table 2) terminal bouton rows. The axon, traced from two sections, is shown in drawing Fig. 10A arborizing in layers II–III. Star indicates the origin of the axon. (9A) Terminal axon segments (arrows), two of which (1, 2) are shown at the electron-microscopic level in (B) where they follow axon initial segments. The initial segment at No. 1 was found to originate from a pyramidal cell (P) and received synapses from the impregnated boutons (framed area shown in 10B) before it entered its myelin sheath (m). (9C) The same initial segment as framed area No. 2 in Fig. 9A, but two sections apart. Synaptic contact in framed area is shown in Fig. 10C. (10B, C) Higher magnification pictures of synaptic contacts (arrows) on pyramidal cell axon initial segments (IS) shown in Fig. 9. (10D) An initial segment originating from a pyramidal cell (PC) receives a symmetrical synapse (arrow) from a bouton (asterisk) making a similar synapse with a spine (s). The spine was not connected to the initial segment and also receives an asymmetrical synapse from another bouton (star). Scales: 9A, 10 μm; 9B, C, 1 μm; 10 A, 50 μm; 10 B–D, 0.2 μm.


I.
II.
III.
A
PC
IS
IS
IS
B
C
D
S

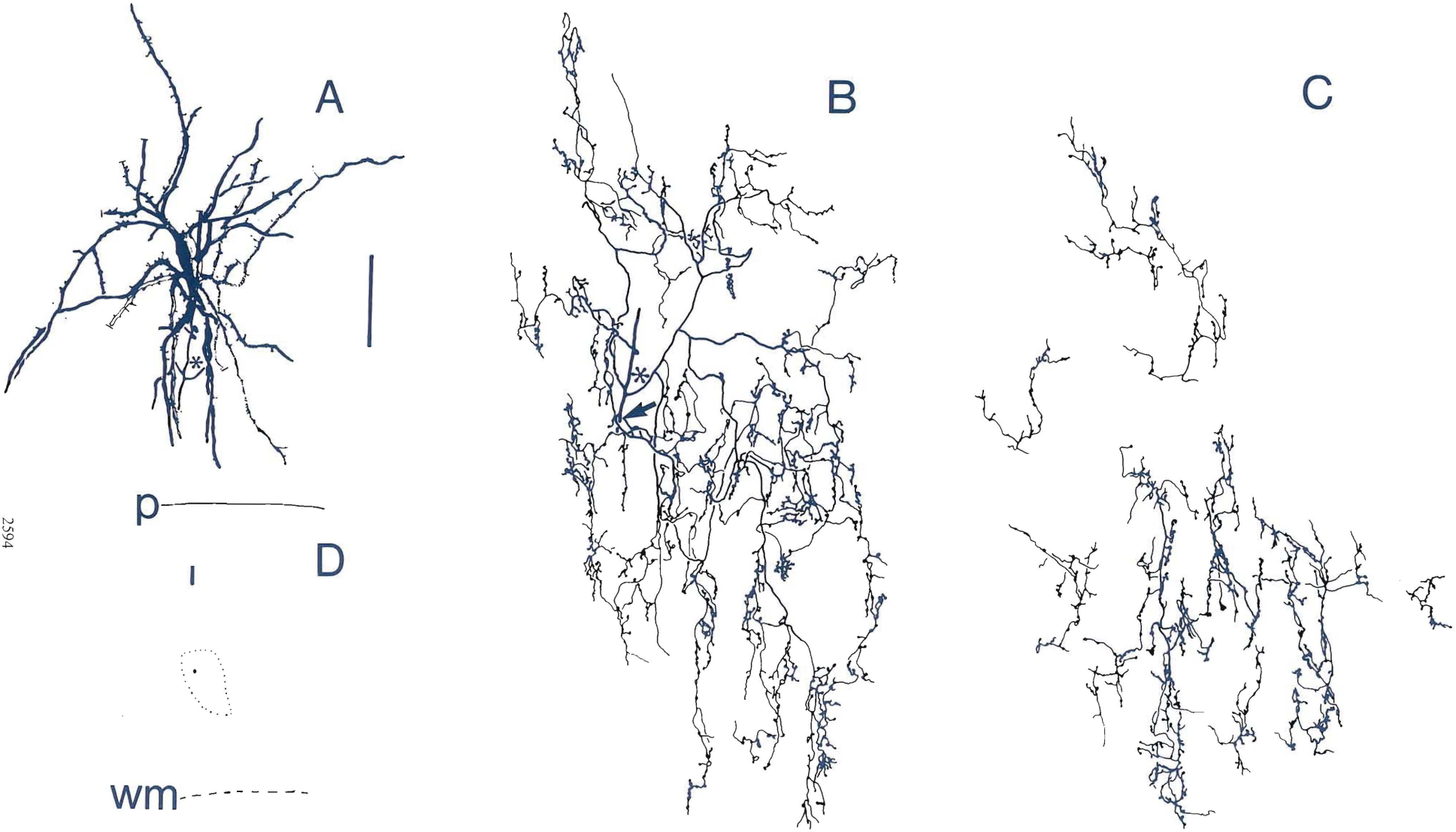
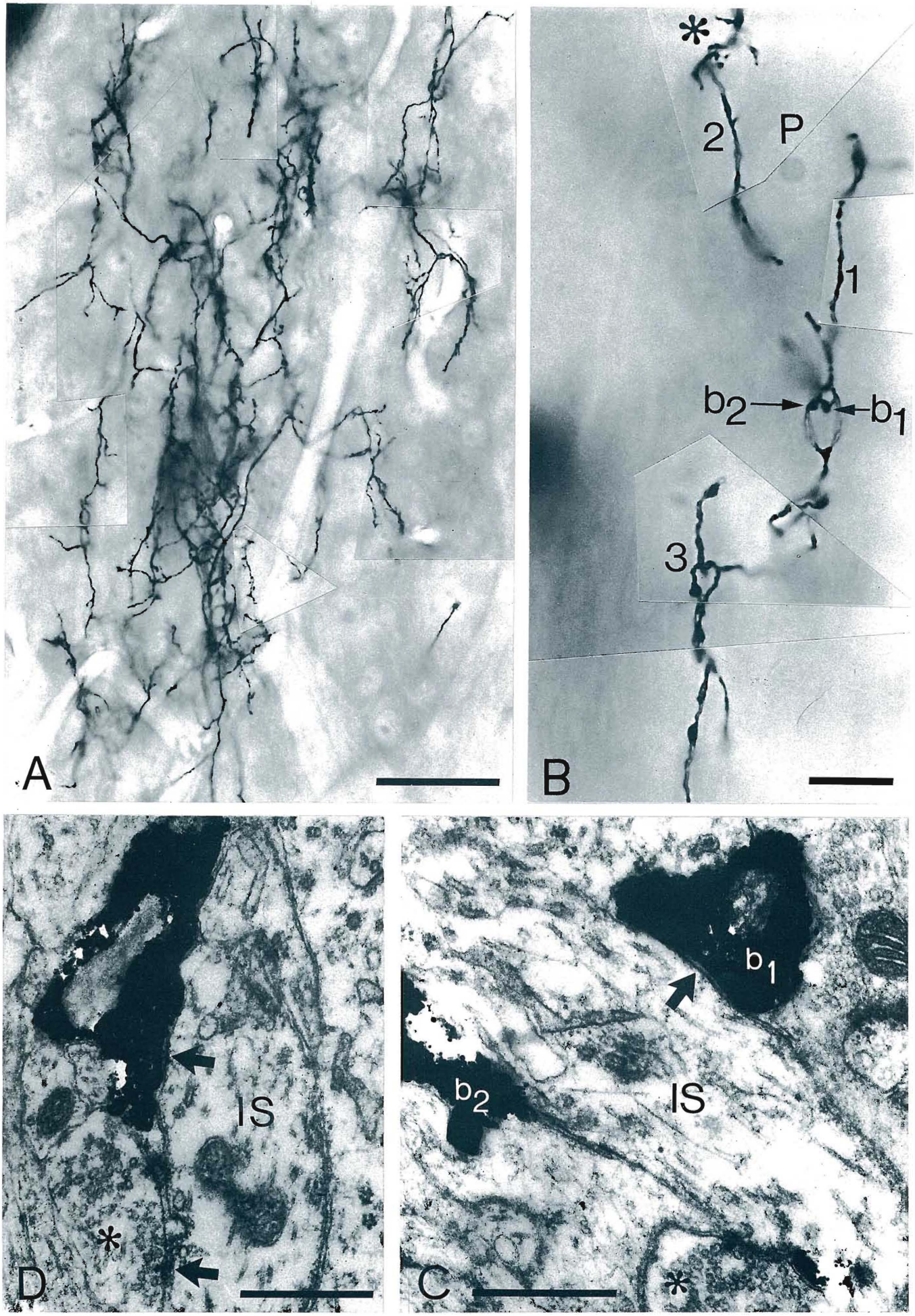
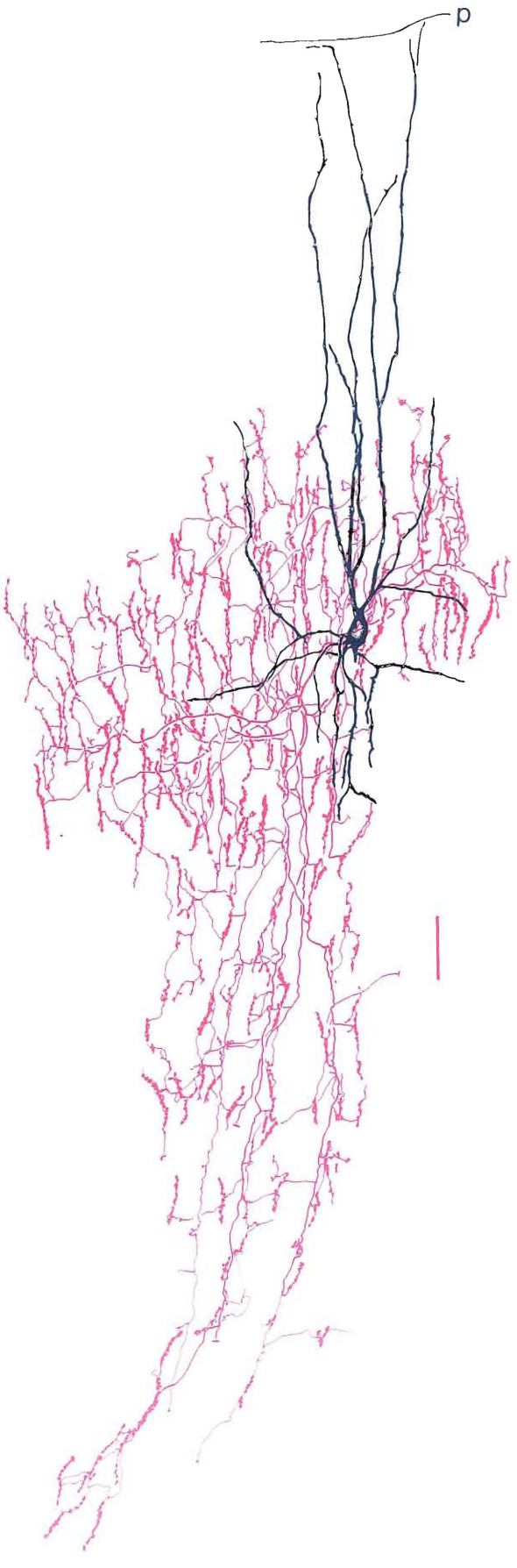


Fig. 11. Drawing of an axo-axonic cell from the visual cortex of the kitten. (No. 9 in Table 2.) The perikaryon with the dendrites (A) is shown separately from the axon (B) and both are from the same section. Asterisk marks the main axon trunk, which ends abruptly (arrow). (C) Part of the axon in the adjacent section. (D) Position of the neuron (dot) and the axonal field (dotted line) between the pia (p) and the white matter (wm). Scales: A–C, 50 μm; D, 100 μm.

Fig. 12. Same neuron as in Fig. 11. (A) Light-micrograph of the axon plexus. Note the profuse branching and the hairy appearance of the terminal segments. (B) Light-micrograph of an axon branch traced at the electron-microscopic level. Two branches (1, 2) climb around a pyramidal neuron (P), No. 1 descends along the initial segment, and was found to make synaptic contacts on it. One of the synapses (arrow) established by bouton b_1 is shown in (C) along with a non-impregnated synaptic bouton (asterisk). The other branch (2) ends in a brush like a growth-cone (asterisk). A third branch (3) forms typical terminal segment. (D) An initial segment (IS) receives synaptic contacts (arrows) from the impregnated bouton of the same neuron and from another bouton (asterisk). Scales: A, 50 μm; B, 10 μm. C and D, 0.5 μm.


A
B
2
P
1
b2
b1
3
D
IS
C
b1
b2
IS


p
wm

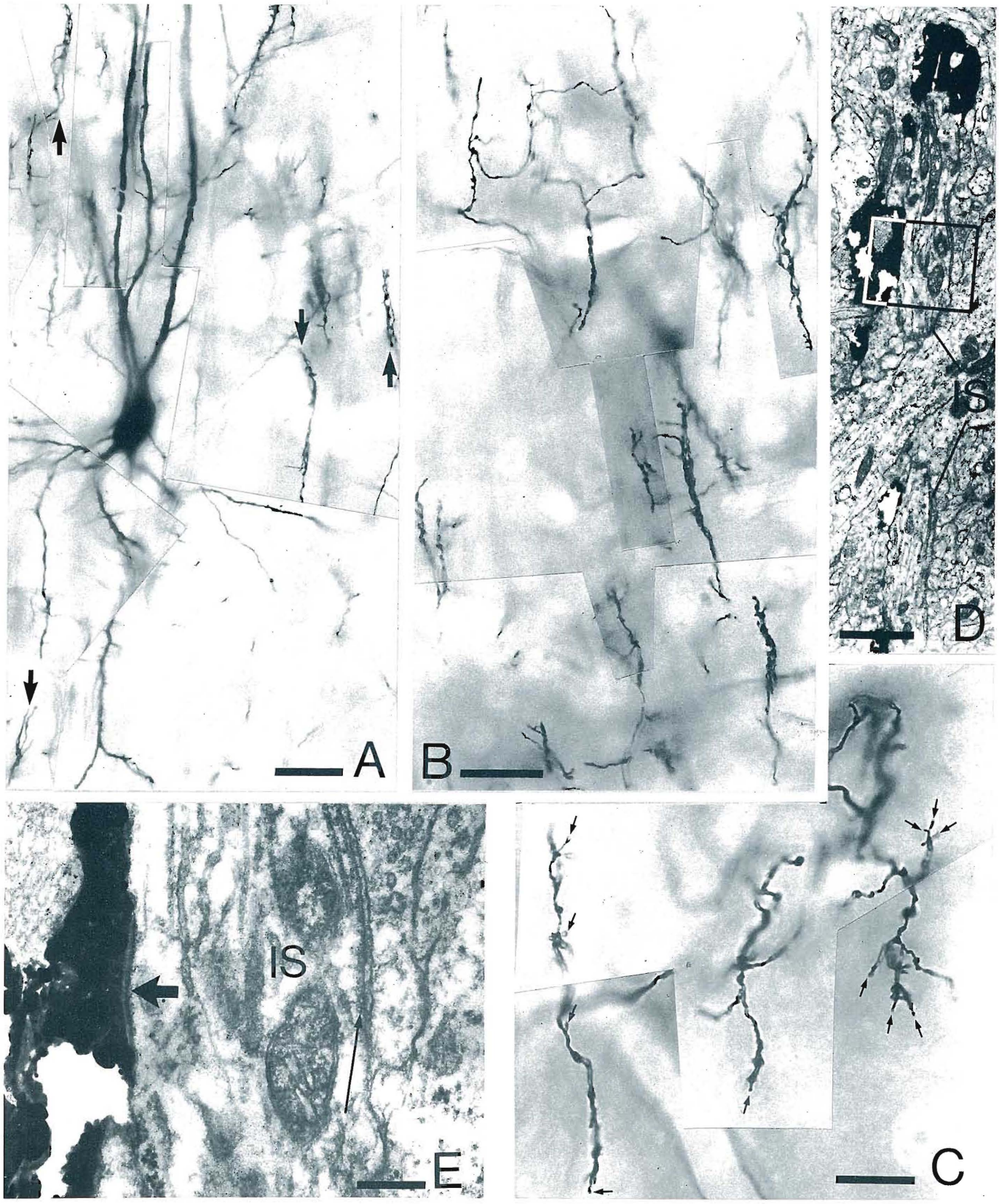
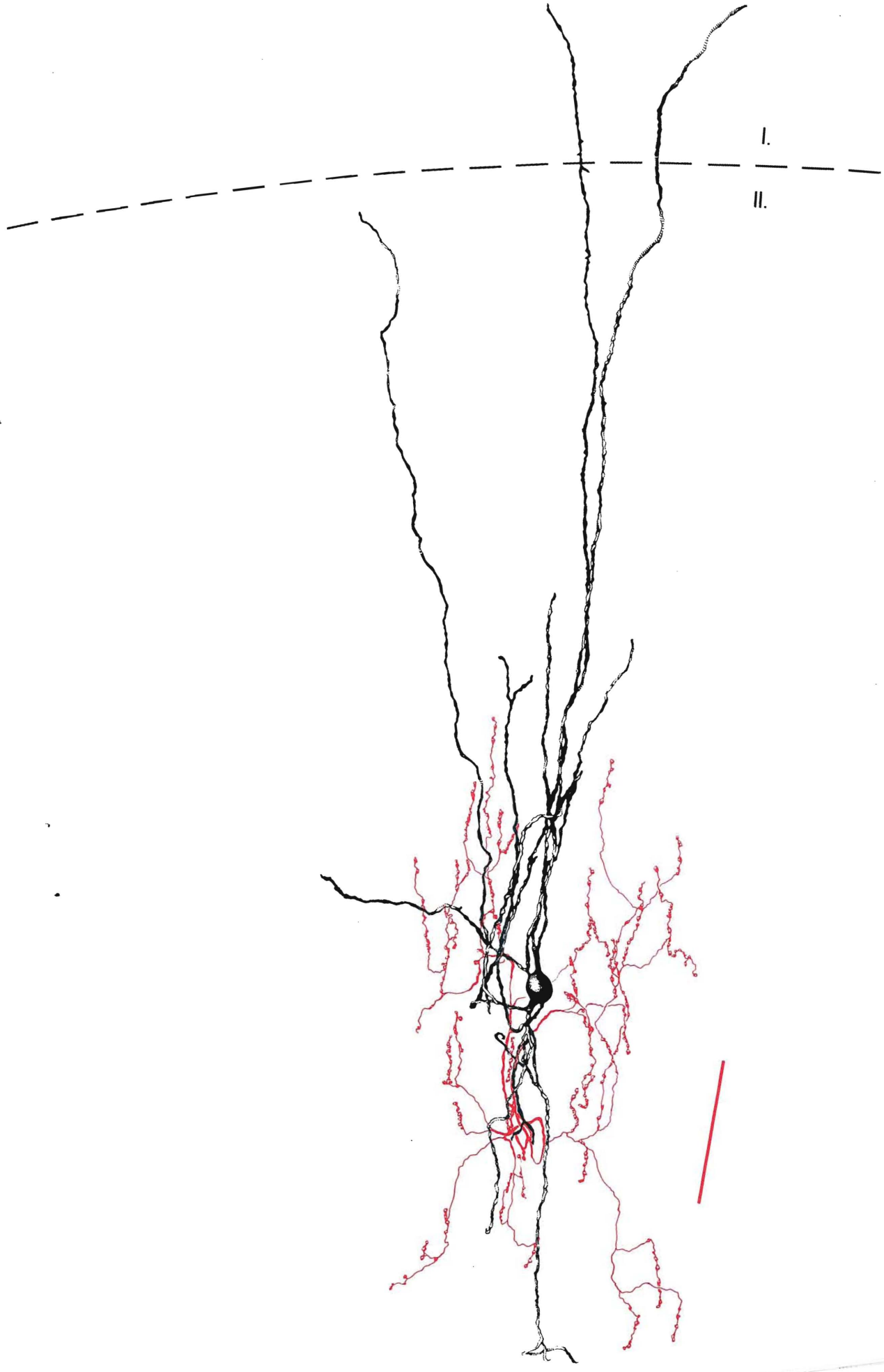


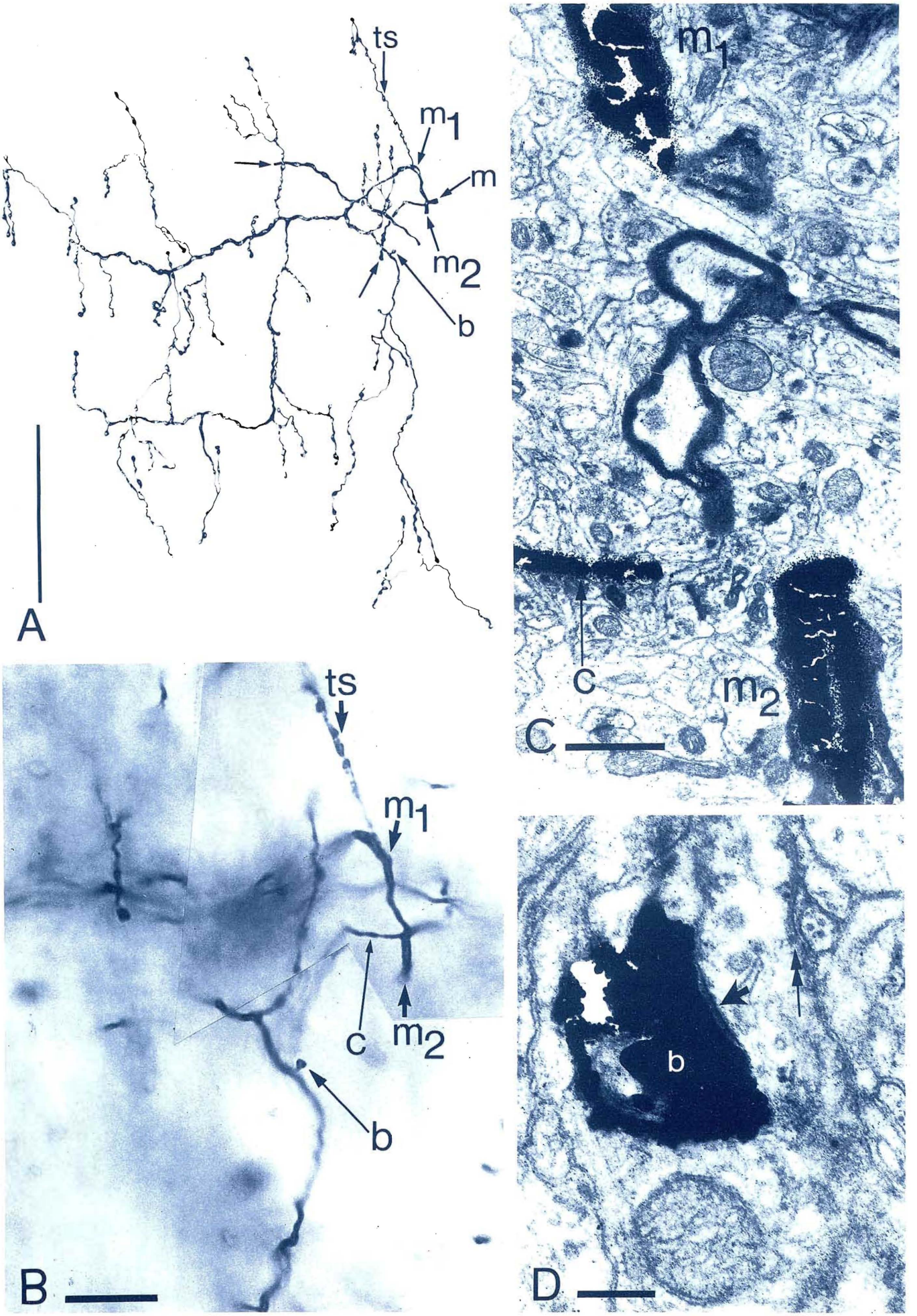
Fig. 14. Same neuron as in Fig. 13. (A) Photomontage of the perikaryon with some of the dendrites and terminal axon segments (arrows) in layer III. (B) Network of terminal axon segments in layer III. Note the fuzzy appearance and complexity of the specialized axon portions. (C) Three specialized axon segments with numerous filopodia and finger-like processes (arrows). (D) Electron-micrograph of a Golgi-stained terminal axon segment establishing synaptic contact with an initial segment (IS) of a pyramidal neuron. Framed area is shown in (E) at higher magnification. Large arrow indicates synaptic contact, small arrow marks membrane undercoating. Scales: A, B, 25 μm; C, 10 μm; D, 1 μm; E, 0.2 μm.

Fig. 13. Drawing of an axo-axonic cell (No. 8 in Table 2) from the motor cortex of the kitten (area 4, sagittal section). The perikaryon and the bulk of the axon (red) is in layer III, but a column of axon with specialized terminal segments descends to layer VI. p = pia; wm = white matter. Scale: 50 μm.

Fig. 15. Drawing of an axo-axonic cell (No. 13 in Table 2) with part of its axon (red) in the posterior lip of the lunate sulcus (area 18, V_2) in the rhesus monkey. The axon (red) originates from the lower main dendrite and terminates in specialized segments in layer III. Scale: 50 μm.

Fig. 16. (A) Drawing of an axo-axonic cell axon (No. 14 in Table 2) in cortical area 18 (V_2) of the monkey. Several main axon branches (arrows) end abruptly. Three of the branches (m) were found in the electron-microscope to possess myelin sheath. (B) Light-micrograph of a part of the same axon as in (A), with the same labelling. One terminal axon segment (ts) a lone bouton (b) and a collateral (c) are indicated. (C) Electron-micrograph of the myelinated main axons (m_1, m_2) and a collateral (c). (D) The lone bouton (b) forms synaptic contact (arrow) with an axon initial segment (IS). Double arrow marks membrane undercoating. Scales: A, 50 μm; B, 10 μm; C, 1 μm; D, 0.2 μm.


I.
II.


ts
m1
m
m2
b
A
m1
c
m2
C
ts
m1
c
m2
b
B
b
D

studied at both light- and electron-microscopic level and compared to cells in the adult visual cortex (Figs 6, 10).

There were conspicuous differences in the number of spines, protrusions and appendages on the dendrites (Figs 11, 13, 14A), which were more numerous in the kitten, and more numerous in the visual cortex (Fig. 11).

Even more striking is the difference in the morphology of the axons. The neuron in the motor cortex has the most complex terminal segments of all cells studied (Figs 14A, B). They are 20–70 μm long, longer than in other areas, and several collaterals from the same axo-axonic cell may contribute to one axon initial segment. The specialized vertical bouton rows emitted finger-like processes and filopodia (Figs 14B, C). Solitary or clustered filopodia also arose from swellings resembling growth cones on collaterals which were not part of the vertical, terminal configurations. Electron-microscopic examination revealed that these filopodia arising from the terminal segment do not form synaptic or other specialized junctions. Normal synaptic contacts were formed by boutons of the specialized vertical axon segments (Figs 14D, E), and the axon initial segments received synapses from non-impregnated boutons as well.

The axon of the neuron in layer V of the visual cortex (Fig. 11) was less precisely defined than either the axon of similar cells in the adult or that of the neuron in the motor cortex. In fact, it was difficult to determine from light-microscopy whether this neuron was an axo-axonic cell. One reason was that few typical terminal segments were discernible, and even those were obscured by the abundant filopodia and growth cones (Fig. 12A). Another feature was that some of the collaterals seemed to surround perikarya of neurons, which was never seen in the adult (Fig. 12B). However, electron-microscopy revealed that this neuron made synaptic contacts only with axon initial segments and only *via* its varicose vertical axon segments (Figs 14C, D). Again, the initial segments also received synapses from non-impregnated boutons. The filopodia, growth cones and finger-like processes did not make any obvious specialized junction. The two processes surrounding the perikaryon of a pyramidal neuron and shown in Fig. 12B were traced in serial sections and they contacted the plasma membrane of the neuron but without giving rise to any specialized junction.

DISCUSSION

Terminology

The specific interneuron which makes synapses with the axon initial segment of pyramidal cells was called the axo-axonic cell.[41,43,49,53,54,55] Subsequently, it was concluded that it is identical with the chandelier cell,[56] previously thought to make synapses with the apical dendrites of pyramidal neurons. In several recent studies, the term chandelier cell was reserved for neurons shown,[6,8,33] or thought[24,25,36,61] to establish synapses with the axon initial segment. But although the name chandelier cell is vividly descriptive, it gives no information on the synaptic connections of the neuron and could even be associated with neurons terminating on the apical dendrites as originally proposed.[56] In retrospect it is not possible to establish which and how many of the so-called chandelier cells reported previously[14,52,53,54,55,56,57,58,62] made synapses with apical dendrites or axon initial segments. Furthermore, it is important to add that the notion of chandelier cells forming synapses with apical dendrites was based not only on their light-microscopic appearance, but also on electron-microscopic demonstration of multiple symmetrical synaptic contacts in close proximity on apical dendrites.[52,56] The origin of these boutons is still unknown, and it thus remains to be established whether interneurons exist which provide multiple synapses to apical dendrites, and whether such interneurons show any selectivity for terminating on apical dendrites. Our preliminary data indicate that such interneurons are present in the visual cortex of the cat, but whether they should be called chandelier cells is questionable because they are not selective for apical dendrites (P. Somogyi, Z. F. Kisvárday, K. A. C. Martin, D. Whitteridge unpublished observations).

In conclusion, we prefer the term axo-axonic cell for neurons which specifically terminate on the axon initial segment of pyramidal cells.

Specificity of axo-axonic cells

The present study confirms and extends earlier reports[8,41,43,49] of the unqualified specificity of the efferent connections of axo-axonic cells by providing quantitative data on the postsynaptic target of 85 identified boutons from several cortical areas of several species. The results demonstrate that not only the specialized terminal axon segments but also the rare single boutons of this neuron make synapses exclusively with the axon initial segment of pyramidal neurons.

This degree of specificity is unparalleled among all the neurons of the cortex that have so far been described and similar examples are rare anywhere in the nervous system, since axo-axonic cells terminate only on a very restricted and specific region of one type of neuron. The other two local interneurons in the cortex examined for their efferent connections in similar detail are the multipolar neurons with smooth and sparsely spinous dendrites in the visual cortex of the rat,[34] and the double-bouquet cells in the visual cortex of the cat and monkey;[45] both form symmetrical synapses. The multipolar neurons made synaptic contact with all postsynaptic sites known to receive symmetrical synapses, and out of 68 synapses, one was on the axon initial segment of a pyramidal neuron.[34] In contrast the double-bouquet cell is less eclectic; of 120 synapses, none contacted the axon initial segment or

perikaryon of pyramidal neurons.[45] Double-bouquet cells made synaptic contacts with spines and dendrites of pyramidal and non-pyramidal neurons and with perikarya of non-pyramidal neurons. The remarkable specificity of axo-axonic cells for the initial segments of pyramidal cells is, therefore, unmatched by the two other types of cortical interneuron that have been similarly examined.

Although all identified boutons of axo-axonic cells make synapses only with axon initial segments, the same initial segments bear synapses from boutons with similar morphological characteristics and which make additional synapses with nearby spines or dendrites. In the present study, two such boutons were found. This may raise doubt about the absolute specificity of axo-axonic cells. However, an alternative explanation is that some boutons on the initial segment originate from other types of interneurons. This explanation is supported by an earlier finding of a bouton originating from an identified multipolar cell which made a symmetrical synaptic contact with an axon initial segment.[34] In the visual cortex of the cat an identified, Golgi-stained interneuron was also found to make two synaptic contacts with the axon initial segment of pyramidal neurons, although most of its synapses were on the perikarya and proximal dendrites of the same neurons (P. Somogyi, unpublished observation). It is, therefore, likely that a small but uncertain proportion of synaptic boutons on the axon initial segment arise from interneurons other than axo-axonic cells.

In connection with the specificity of axo-axonic cells, it should be noted that so far mainly the supragranular layers, and to a lesser extent the infragranular layers, have been studied with the electron-microscope.[8,41,43,49] We cannot, therefore, exclude the possibility that in those cortical areas where spiny stellate cells are present in layer IV, they may also be innervated by axo-axonic cells. In the present study, layer IV was not examined by electron-microscopy and all the initial segments postsynaptic to identified axo-axonic cell terminals, and which could be traced back to the parent cell body, originated from pyramidal neurons.

Distribution and identity of axo-axonic cells

We have provided evidence for the widespread presence of axo-axonic cells among very different species and cortical areas. Furthermore, their presence and similar organization in paleo-, archi- and neocortical areas, and in both motor and sensory cortex, suggests a basic role in information processing. In addition, our recent finding of axo-axonic cells in the hippocampus of the monkey[50] suggests that they may be associated with pyramidal neurons wherever the latter occur.

Axo-axonic cells can be recognised only on the basis of their specialized axons, for neither the dendritic tree nor the perikaryon is sufficiently different from those of other neurons. But on the basis of their dendrites axo-axonic cells can be classified more generally as smooth and sparsely-spiny non-pyramidal neurons. The dendrites are usually organized into a lower and upper bunch oriented more or less perpendicular to the pia. If only the dendrites are considered, they can be called cells with bitufted or double-bouquet dendritic fields, in accordance with recent attempts to classify cortical non-pyramidal cells on the basis of morphological features seen by light-microscopy.[9,36,62] However, the qualitative evaluation of dendritic arborizations does not invariably lead to conclusive identification of a neuron. For example, our finding that axo-axonic cells may have either sparsely-spiny or smooth dendrites indicates that the presence of some spines on the dendrites is not always a reliable criterion of cell type. Furthermore, neurons with very similar double-bouquet dendritic trees may have completely different axonal arborizations. For example, the dendritic tree of a double-bouquet cell reported earlier in layer III of the striate cortex of the cat, and which had a vertically descending tight axon bundle (see Fig. 1 in Ref. 45) is qualitatively indistinguishable from the dendritic tree of the axo-axonic cell described here in the same area and layer (Fig. 6). However, it is possible that subtle differences may be revealed by using quantitative methods such as the product–moment analysis successfully applied to other areas.[60] It should also be mentioned that chandelier cells with approximately spherical dendritic arbors have also been reported.[14,24]

As pointed out earlier,[45] the classification of cortical neurons is ideally based on their input and output characteristics, whether the data are obtained by ultrastructural studies of the synaptic connections of morphologically-identified neurons or by physiological and pharmacological experiments, or a combination of these. Unfortunately, because of the laboriousness of this approach, classification schemes based only on light-microscopic characteristics or physiological response properties will be used for some time, all too often independent of each other.

The specialized terminal axon segment in different species

The present study shows that the organization of the specialized terminal axon segments is remarkably constant both among species and among cortical areas of different evolutionary position. Thus, the same simple rows of boutons are present in the visual cortex of the monkey (both V_1 and V_2) and in the subiculum or piriform cortex of the rat.

This finding is at variance with earlier reports of an evolutionary trend towards more complex terminal axon segments.[6,8] However, the earlier study in the cat[8] used young animals, whereas our neurons in the cat and monkey, with similar terminal segments as those of the rat, were from adult animals. Thus, a likely explanation for more complex terminal segments in previous reports[6,8,25] is the age of the ani-

mals, i.e. the axo-axonic cells were still in the process of retracting terminals during ontogenesis. This is supported by our observation in the one-month-old kitten.

It is likely that the complexity of the terminal axon portions and the number of synaptic contacts established by any one segment depends on the number of segments competing for the available synaptic space on the particular axon initial segment, and much less, or not at all, on the cortical area or species.

Developmental considerations

It is apparent from comparing the neurons found in the kitten with those in the adult animals that axo-axonic cells undergo substantial changes postnatally. The axon still possesses processes like growth-cones, and branches more profusely in the young animal. The illustrations of previous reports[8,25] when compared with our illustrations of the adult suggest that the axon, and probably the connections of the neuron, change up to the age of 7–8 weeks in the visual cortex of the cat. The terminal axon segments become progressively simpler, the boutons appearing more prominent, bulbous and organized into vertical rows instead of interwoven twisting fascicles. The filopodia and finger-like processes have disappeared in the adult.

The difference between the axonal structure of a neuron in the visual cortex and another in the motor cortex of the same kitten indicates that axo-axonic cells or at least their axons may develop earlier in the motor cortex. In addition, at one month of age, the pyramidal neurons in the motor cortex are provided with a higher number of terminals from several collaterals of the same axo-axonic cell.

The greater frequency of spines and protrusions on the dendrites of axo-axonic cells in the young animal is in agreement with previous studies of other cortical neuron types.[23,26,27]

Distribution of axonal fields

Two technical problems restrict the ability of Golgi studies to display axon distribution. One is that myelinated axons are not impregnated, or only partially so. The other is the difficulty of tracing axons across consecutive sections. The first problem is often tackled by using young animals, in which myelination is incomplete. As indicated above, this introduces a further complication because, as shown, the axo-axonic cells continue to develop for several weeks postnatally and the age at which the distribution of their axons reaches its mature form is unknown.

Whenever the axonal arborization could be traced back to the cell body, it was distributed in the vicinity of the cell body and in the same layers as the dendritic tree. The single exceptional neuron was in the motor cortex of the kitten. Its axon descended from layer III to layer IV in a narrow array. This raises the possibility that axo-axonic cells play a role in the functional columnar organization of the cortex. Similar columnar distribution of axons with specialized terminal segments has been reported in the somatosensory cortex of the monkey.[14] It is noteworthy that in the visual cortex of the kitten a similar axon was shown to descend to the infragranular layers from layer III, providing specialized terminal axon segments in both layers III and VI, but not in layers IV and V.[25] Another descending axon together with electron-microscopic evidence for its specific termination was demonstrated in the visual cortex of the kitten.[8] In both of these examples the axon appeared to enter the white matter and originate in layer III, although the perikarya were not impregnated. Our results show that axons without perikarya may be impregnated only up to the myelin sheath, making it difficult to determine where the cell body is located. Nevertheless, if the vertical distribution and descending course of axo-axonic axons proves to be a general feature of these neurons it will be similar to the laminar axon distribution of bouble-bouquet cells. The latter interneuron, situated in layer III, provides vertical axon fascicles[14,37,45,54,59] and was suggested to play a role in vertical inhibitory interactions on the basis of retrograde [^{3}H]GABA transport studies.[46] The two neurons have in common the location of their cell bodies in layer III, and both make symmetrical synaptic contacts. However, the postsynaptic targets of the two neurons could not be more different, as double-bouquet cells do not contact axon initial segments.[46]

It has been suggested that because the descending axons of some axo-axonic cells seem to enter the white matter they may have a distant as well as a local target.[8] However, because the specimens were obtained from young kittens, an alternative explanation is that the descending axon supplied pyramidal neurons deep in layer VI, or that the final pattern of the axon field was not yet fully developed.

With regard to laminar distribution of axo-axonic cells, axons with specialized vertical segments seem to be present in all cortical laminae,[8,24,25,36,41,43,49,52,53,54,55,56,57,58,61] except lamina I, although some discrepancies exist. In a detailed examination, axons were illustrated in all layers except layer I in the visual cortex of kitten and cat.[8] All studies including the present one indicate more axons in the supragranular layers. It is not known, however, whether this is a result of preferential impregnation. Golgi studies do not readily lend themselves to quantitative measures of the frequency of cell types.

In spite of the widespread occurrence of axo-axonic cells it is not known whether all pyramidal cells receive this specific input or only a sub-class of them. In a previous study of the visual cortex of cat, reconstruction of serial semi-thin sections revealed that within the territory of the axon arbor of an axo-axonic cell by no means all pyramidal neurons are contacted.[8] Similar observations were made here. One explanation is that several axo-axonic cells occupy the

same territory and collectively innervate all the pyramidal neurons. Another possibility is that only some pyramidal neurons receive this input. Pyramidal neurons can be classified according to their projection areas. By combining Golgi staining and retrograde transport of horseradish peroxidase it was shown that pyramidal neurons projecting from the visual cortex to the contralateral hemisphere receive input from axo-axonic cells in the rat.[49] Future studies, using the same technique with peroxidase injections in other target areas, could show if pyramidal neurons projecting elsewhere also receive axo-axonic cell input.

Divergence and convergence in the connections of axo-axonic cells

The total number of pyramidal cells contacted by one axo-axonic cell cannot be reliably determined because of technical difficulties with the Golgi method. The 166 and 67 specialized terminal segments on one axon in the motor and visual cortex, respectively, indicate only part of the population of pyramidal neurons reached by these two cells, because many axon collaterals could not be unmistakenly followed in neighbouring sections. Nevertheless, the numbers are of the same order of magnitude as obtained for an axo-axonic cell in the hippocampus of the monkey, where 202 terminal segments were traced from a single neuron.[50] As each terminal segment represents a pyramidal neuron initial segment, one axo-axonic cell may innervate at least 50–200 pyramidal neurons, and this number may well vary among different cortical areas.

It is equally difficult to determine the number of axo-axonic cells converging onto one pyramidal neuron. The figure of five obtained in the present study is merely indicative because it is based on the minute examination of only three initial segments. It would be prohibitively time-consuming to examine a statistically satisfactory sample. The present study and a previous report[34] indicate that neurons other than axo-axonic cells form occasional synaptic contacts with the axon initial segment of pyramidal neurons. Thus, our assumption, in estimating convergence, that all synaptic contacts derive from axo-axonic cells, introduces an error. The true number may be fewer than five in the striate cortex of the cat.

The number of synaptic contacts received by axon initial segments of pyramidal neurons in the visual cortex is comparable to that in the hippocampus.[21] From 25 to 143 terminals were demonstrated on individual axon initial segments of pyramidal neurons. Our recent findings in the hippocampus indicate that most if not all of these boutons are provided by axo-axonic cells.[50]

The number of synaptic terminals has also been estimated on axon initial segments in the somatosensory cortex of the monkey.[40] The sampling procedure was based on the assumption that synaptic contact areas are circular and that the boutons are distributed homogeneously on the initial segment. However, neither of these assumptions has been tested experimentally and in a previous report it was suggested that the number of synaptic boutons increases distalwards.[15] In the striate cortex of the cat we found that synaptic boutons tend to be distributed in groups, an arrangement that also seems to be present in the hippocampus.[21] This indicates that little less than complete reconstruction can give the total number of synaptic terminals.

Axon initial segments of other neurons

There is evidence that the initial segment of axons is not invariably or even usually a postsynaptic element in the CNS. Thus, the axon initial segment of motor neurons,[3] various inhibitory interneurons of the cerebellar cortex,[11] and the axo-axonic cells studied here do not receive synaptic contacts. On the other hand, special neuron types which themselves have both pre- and post-synaptic dendritic elements may have an initial segment which is presynaptic as well as receiving a significant number of contacts. (see Refs 11, 12, 22).

While the functional significance of these local arborizations remains to be established, it is apparent that in at least three disparate parts of the central nervous system a complex apparatus developed independently around the axon initial segment of projection neurons. The three neuron types influenced by these structures are: the Mauthner cell of teleosts receiving the axon cup (see Refs 2, 29); the Purkinje cell of the cerebellum receiving the pinceau (see Refs 5, 31, 37); and the cortical pyramidal neurons receiving the axo-axonic terminal segments. The three structures surrounding the initial segment differ substantially in their configuration, but studies on the function of the first two indicate functional similarity.[5,10,18,20] Thus, the initial segment of the Mauthner cell receives numerous synaptic contacts of the chemical type but in addition is surrounded by a system of axons, the so-called axon cup.[2,29] The Purkinje cell initial segment receives few chemical synapses from basket cell terminals (see Ref. 48) but is also surrounded by a mass of freely terminating interwoven axon terminal plexi.[5,13,31,37] In both cases the non-synaptic axons provide field inhibition of the target neurons[10,18] and at least in the case of the Mauthner cell the synaptic contacts seem to evoke inhibitory postsynaptic potentials.[10,19] This leads to the question: is the function of axo-axonic cells in the cortex similarly inhibitory?

Functional considerations

From the very discovery of the chandelier cell[56] it has been suggested that axo-axonic cells inhibit pyramidal neurons.[8,41,43,49,52,53,54,55] The suggestion also rests on two analogies. One concerns the Purkinje cell and the Mauthner cell, both of which are inhibited by structures surrounding their axon initial segment.[5,10,18,19] However, both structures differ substantially from the axo-axonic cell terminals around

the pyramidal axon. The other analogy has been based on the symmetrical structure of the synaptic contact and on the evidence that axo-axonic cell boutons contain flattened, pleomorphic vesicles.[41] Boutons with these structural features have long been considered to be responsible for inhibition in the cortex.[34,52,54] While the present study conclusively demonstrates that axo-axonic cell boutons contain flattened vesicles and make symmetrical synaptic contacts in all species examined, there is no direct evidence that all such boutons, whatever their origin, exert inhibitory influence.

Further support for the proposed inhibitory role came from the immunocytochemical demonstration of glutamate decarboxylase (GAD), the GABA synthetising enzyme, in boutons containing pleomorphic vesicles and making symmetrical synaptic contacts.[39] Furthermore it was also demonstrated that at least some synaptic boutons on the axon initial segment contain GAD.[39] It was recently suggested that axo-axonic cells do indeed contain GAD and are, therefore, GABA-ergic,[33] as speculated earlier.[41,43,49,54,55] But it is difficult to prove this with present immunocytochemical techniques because of the great difficulty in tracing the stained axon terminals to immunostained neuronal perikarya. It is not sufficient to show that some boutons on the axon initial segment contain GAD; the demonstration of the origin of those boutons from a cell which is specific and provides specialized axon segments is necessary. Until this is accomplished, the possibility remains that the GAD-containing boutons originate from other interneurons establishing occasional terminals on the axon initial segment. Of course, if it could be demonstrated that all or nearly all terminals on the axon initial segment contain GAD, the axo-axonic cell boutons must have contributed. The demonstration of GAD in smooth and sparsely-spiny stellate cells[39] gives equally little information about the transmitter of axo-axonic cells because these stellate cells are heterogeneous with regard to their axon arborization and morphology.

Another means of identifying the neuronal type of GABA-ergic interneurons was introduced recently through the combination of [^{3}H]GABA uptake and Golgi staining in the same material.[47] One of the major aims of this approach was to test whether axo-axonic cells are GABA-ergic. The procedure depends on successfully impregnating axo-axonic cells in an area of the cortex where [^{3}H]GABA has been injected and selectively taken up by GABA-ergic neurons. So far, two identified axo-axonic cells have been found within the area of cell labelling in the visual cortex of the rat. Unfortunately neither of them was labelled on autoradiographs (P. Somogyi, Z. F. Kisvárday, T. F. Freund unpublished observation), although neighbouring neurons were labelled. This may indicate that they did not have the machinery for the selective, high affinity uptake of labelled GABA and thus that they are not GABA-ergic, but another possibility is that the axon of neither neuron reached the area where the concentration of [^{3}H]GABA was high enough to result in significant labelling of the perikarya through retrograde transport.

Lacking direct evidence of the transmitter of axo-axonic cells, the only clue to their function remains their striking specificity for the axon initial segment of pyramidal cells. The strategic location of a large number of synapses on the initial segment, where the action potential is generated,[1,4,16] suggests an influential role. The other important point is that an axo-axonic cell can reach several hundred pyramidal neurons and influence them simultaneously. Thus, through divergence and by terminating at the most commanding post-synaptic point, the axo-axonic cell is in a position to modulate and perhaps even to 'silence' a small patch of cortex.

The simultaneous action of axo-axonic cells on several nearby pyramidal neurons may synchronise the latter, as pointed out earlier.[43,49,50] The presence of axo-axonic cells in the hippocampus, an area especially prone to seizures, may also indicate their role in synchronous discharge activity.[50] This suggestion was based on the assumption that the action of axo-axonic cells provides a gating mechanism for pyramidal neurons which are either inhibited or released from inhibition.

Other influences may be more subtle. Thus axo-axonic cells may serve to change the conductance of the axon membrane and thereby the threshold of groups of pyramidal neurons responding to a particular sensory quality. For example, in the visual cortex they could be involved in determining whether a cell is monocular or binocular, and in its tuned response to orientation, disparity, direction of movement, etc.

At present, there is no firm evidence for these speculations and only direct electrophysiological studies of cells subsequently identified as axo-axonic together with cross-correlation analysis of their target neurons will give a definite answer.

Note. After the manuscript was submitted a detailed study appeared on identified, Golgi-stained chandelier cells in the visual cortex of the rat.[35a] Peters, Proskauer & Ribak[35a] present evidence that glutamic acid decarboxylase is present in boutons making synaptic contact with axon initial segments. The boutons are similar to those of axo-axonic cells in containing pleomorphic vesicles and making symmetrical synaptic contacts.

Acknowledgements—The authors are grateful to Mrs K. Boczkó for technical assistance, to Dr K. A. C. Martin for helpful discussion and to Dr J. Szentágothai for his comments on the manuscript. This work was supported by the EPA Cephalosporin Trust, the Hungarian Academy of Sciences, the International Cultural Institute of Budapest, the Medical Research Council, The Royal Society and the Wellcome Trust. During part of this project P. Somogyi was supported by the Wellcome Trust in the Dept. of Pharmacology, Oxford University. T. F. Freund is an undergraduate student at Lóránd Eötvös University, Budapest.

REFERENCES

1. Araki T. & Otani T. (1955) Response of single motoneurons to direct stimulation in toad's spinal cord. *J. Neurophysiol.* **18,** 472–485.
2. Bodian D. (1952) Introductory survey of neurons. *Cold Spring Harb. Symp. quant. Biol.* **17,** 1–13.
3. Conradi S. (1969) Observations on the ultrastructure of the axon hillock and initial axon segment of lumbosacral motoneurons in the cat. *Acta physiol. scand.* **Suppl. 332,** 65–84.
4. Coombs J. S., Curtis D. R. & Eccles J. C. (1957) The generation of impulses in motoneurons. *J. Physiol., Lond.* **139,** 232–249.
5. Eccles J. C., Ito M. & Szentágothai J. (1967) *The Cerebellum as a Neuronal Machine* Springer Verlag, Berlin.
6. Fairén A., DeFelipe J., Martinez Ruiz R. (1981) The Golgi-EM procedure: a tool to study neocortical interneurons. In *Eleventh International Congress of Anatomy: Glial and Neuronal Cell Biology, Progr. clin. biol. Res.* (eds Acostavidrio E. & Fedoroff S.) pp. 291–301. Alan R. Liss Inc., New York.
7. Fairén A., Peters A. & Saldanha J. (1977) A new procedure for examining Golgi-impregnated neurons by light- and electron-microscopy. *J. Neurocytol.* **6,** 311–337.
8. Fairén A. & Valverde F. (1980) A specialized type of neuron in the visual cortex of cat: a Golgi and electron-microscope study of chandelier cells. *J. comp. Neurol.* **194,** 761–780.
9. Feldman M. L. & Peters A. (1978) The forms of non-pyramidal neurons in the visual cortex of the rat. *J. comp. Neurol.* **179,** 761–793.
10. Furukawa T. & Furshpan E. J. (1963) Two inhibitory mechanisms in the Mauthner neurons of goldfish. *J. Neurophysiol.* **26,** 140–176.
11. Hámori J. (1981) Synaptic input to the axon hillock and initial segment of inhibitory interneurons in the cerebellar cortex of the rat. An electron-microscopic study. *Cell Tissue Res.* **217,** 553–562.
12. Hámori J., Pasik T. & Pasik P. (1978) Electron-microscopic identification of axonal initial segments belonging to interneurons in the dorsal lateral geniculate nucleus of the monkey. *Neuroscience* **3,** 403–412.
13. Hámori J. & Szentágothai J. (1965) The Purkinje cell basket: ultrastructure of an inhibitory synapse. *Acta biol. hung.* **15,** 465–479.
14. Jones E. G. (1975) Varieties and distribution of non-pyramidal cells in the somatic sensory cortex of the squirrel monkey. *J. comp. Neurol.* **160,** 205–268.
15. Jones E. G. & Powell T. P. S. (1969) Synapses on the axon hillocks and initial segments of pyramidal cell axons in the cerebral cortex. *J. Cell Sci.* **5,** 495–507.
16. Kandel E. R. & Spencer W. A. (1961) Electrophysiology of hippocampal neurons—II. After-potentials and repetitive firing. *J. Neurophysiol.* **24,** 243–259.
17. Kemp J. M. & Powell T. P. S. (1971) The synaptic organisation of the caudate nucleus. *Phil. Trans. R. Soc. Ser. B.* **262,** 403–412.
18. Korn H. & Axelrad H. (1980) Electrical inhibition of Purkinje cells in the cerebellum of the rat. *Proc. Nat. Acad. Sci. U.S.A.* **77,** 6244–6247.
19. Korn H. & Faber D. S. (1976) Vertebrate central nervous system: same neurons mediate both electrical and chemical inhibitions. *Science N.Y.* **194,** 1166–1169.
20. Korn H. & Faber D. S. (1979) Electrical interactions between vertebrate neurons: field effects and electrotonic coupling. In *The Neurosciences* Fourth Study Program (eds Schmitt F. O. & Worden F. G.) pp. 333–358. The MIT Press, Cambridge.
21. Kosaka T. (1980) The axon initial segment as a synaptic site: ultrastructure and synaptology of the initial segment of the pyramidal cell in the rat hippocampus (CA3 region). *J. Neurocytol.* **9,** 861–882.
22. Kosaka T. & Hama K. (1979) Pre- and post-synaptic character of the axon initial segment of the mitral cell of the goldfish olfactory bulb. *Brain Res.* **169,** 570–574.
23. Lund J. S., Boothe R. G. & Lund R. D. (1977) Development of neurons in the visual cortex (area 17) of the monkey (Macaca nemestrina): a Golgi study from fetal day 127 to postnatal maturity. *J. comp. Neurol.* **176,** 149–188.
24. Lund J. S., Hendrickson A. E., Ogren M. P. & Tobin E. A. (1981) Anatomical organization of primate visual cortex area VII. *J. comp. Neurol.* **202,** 19–45.
25. Lund J. S., Henry G. H., MacQueen C. L. & Harvey A. R. (1979) Anatomical organization of the primary visual cortex (area 17) of the cat. A comparison with area 17 of the Macaque monkey. *J. comp. Neurol.* **184,** 599–618.
26. Miller M. (1981) Maturation of rat visual cortex—I. A quantitative study of Golgi-impregnated pyramidal neurons. *J. Neurocytol.* **10,** 859–878.
27. Miller M. & Peters A. (1981) Maturation of rat visual cortex—II. A combined Golgi-electron-microscope study of pyramidal neurons. *J. comp. Neurol.* **203,** 555–576.
28. Mugnaini E. (1972) The histology and cytology of the cerebellar cortex. In *The Comparative Anatomy and Histology of the Cerebellum: The Human Cerebellum, Cerebellar Connections and Cerebellar Cortex* (eds Larsel O. & Jansen J.) pp. 201–265. Univ. Minnsota Press, Minneapolis.
29. Nakajima Y. (1974) Fine structure of the synaptic endings on the Mauthner cell of the goldfish. *J. comp. Neurol.* **156,** 375–402.
30. Palay S. L. (1964) The structural basis of neural action. In *Brain Function, RNA and Brain Function: Memory and Learning* (ed. Brazier M. A. B.) Vol. 2. pp. 69–108. Univ. Calif. Press, Los Angeles.
31. Palay S. L. & Chan-Palay V. (1974) *Cerebellar Cortex, Cytology and Organization.* Springer Verlag, Berlin.
32. Palay S. L., Sotelo C., Peters A. & Orkand P. M. (1968) The axon hillock and the initial segment. *J. Cell Biol.* **38,** 193–201.

33. Peters A. (1981) Chandelier cells in rat visual cortex. *Soc. Neurosci. Abs* **7,** 173.
34. Peters A. & Fairén A. (1978) Smooth and sparsely-spined stellate cells in the visual cortex of the rat: a study using a combined Golgi-electron-microscope technique. *J. comp. Neurol.* **181,** 129–172.
35. Peters A., Proskauer C. C. & Kaiserman-Abramof I. R. (1968) The small pyramidal neuron of the rat cerebral cortex: the axon hillock and initial segment. *J. Cell Biol.* **39,** 604–619.
35a. Peters A., Proskauer C. C., Ribak C. E. (1982) Chandelier cells in rat visual cortex. *J. comp. Neurol.* **206,** 397–416.
36. Peters A. & Regidor J. (1981) A reassessment of the forms of nonpyramidal neurons in area 17 of cat visual cortex. *J. comp. Neurol.* **203,** 685–716.
37. Ramón y Cajal S. (1911) *Histologie du Système Nerveux de l'Homme et des Vertébrés.* Vol. 2. Maloine, Paris.
38. Reynolds E. S. (1963) The use of lead citrate at high pH as an electron opaque stain in electron-microscopy. *J. Cell Biol.* **17,** 208–212.
39. Ribak C. E. (1978) Aspinous and sparsely-spinous stellate neurons in the visual cortex of rats contain glutamic acid decarboxylase. *J. Neurocytol.* **7,** 461–479.
40. Sloper J. J. & Powell T. P. S. (1979) A study of the axon initial segment and proximal axon of neurons in the primate motor and somatic sensory cortices. *Phil. Trans. R. Soc. Ser. B.* **285,** 173–199.
41. Somogyi P. (1977) A specific axo-axonal interneuron in the visual cortex of the rat. *Brain Res.* **136,** 345–350.
42. Somogyi P. (1978) The study of Golgi-stained cells and of experimental degeneration under the electron-microscope: a direct method for the identification in the visual cortex of three successive links in a neuron chain. *Neuroscience* **3,** 167–180.
43. Somogyi P. (1979) An interneuron making synapses specifically on the axon initial segment (AIS) of pyramidal cells in the cerebral cortex of the cat. *J. Physiol. Lond.* **296,** 18–19P.
44. Somogyi P., Bolam J. P. & Smith A. D. (1981) Monosynaptic cortical input and local axon collaterals of identified striatonigral neurons. A light- and electron-microscopic study using the Golgi-peroxidase transport–degeneration procedure. *J. comp. Neurol.* **195,** 567–584.
45. Somogyi P. & Cowey A. (1981) Combined Golgi and electron-microscopic study on the synapses formed by double bouquet cells in the visual cortex of the cat and monkey. *J. comp. Neurol.* **195,** 547–566.
46. Somogyi P., Cowey A., Halász N. & Freund T. F. (1981) Vertical organization of neurons accumulating [^{3}H]GABA in the visual cortex of the rhesus monkey. *Nature, Lond.* **294,** 761–763.
47. Somogyi P., Freund T. F., Halász N. & Kisvárday Z. F. (1981) Selectivity of neuronal [^{3}H]GABA accumulation in the visual cortex as revealed by Golgi staining of the labelled neurons. *Brain Res.* **225,** 431–436.
48. Somogyi P. & Hámori J. (1976) A quantitative electron-microscopic study of the Purkinje cell axon initial segment. *Neuroscience* **1,** 361–365.
49. Somogyi P., Hodgson A. J. & Smith A. D. (1979) An approach to tracing neuron networks in the cerebral cortex and basal ganglia. Combination of Golgi-staining, retrograde transport of horseradish peroxidase and anterograde degeneration of synaptic boutons in the same material. *Neuroscience* **4,** 1805–1852.
50. Somogyi P., Nunzi M. G., Gorio A. & Smith A. D. (1982) A new type of specific interneuron in the monkey hippocampus forming synapses exclusively with the axon initial segments of pyramidal cells. *Brain Res.* submitted.
51. Sotelo C. (1969) Ultrastructural aspects of the cerebellar cortex of the frog. In *Neurobiology of Cerebellar Evolution and Development* (ed. Llinás R.) pp. 327–371. AMA-ERF, Chicago.
52. Szentágothai J. (1975) The module-concept in cerebral cortex architecture. *Brain Res.* **95,** 475–496.
53. Szentágothai J. (1978) Specificity versus (quasi) randomness in cortical connectivity. In *Architectonics of the Cerebral Cortex* (eds Brazier M. A. B. & Petsche H.) pp. 77–97. Raven Press, New York.
54. Szentágothai J. (1978) The neuron network of the cerebral cortex: a functional interpretation. Ferrier lecture. *Proc. R. Soc. Ser. B.* **201,** 219–248.
55. Szentágothai J. (1979) Local neuron circuits of the neocortex. In *The Neurosciences* Fourth Study Program (eds Schmitt F. O. & Worden F. G.) pp. 399–409. MIT Press, Cambridge.
56. Szentágothai J. & Arbib M. A. (1974) Conceptual models of neural organization. *Neurosci. Res. Program Bull.* **12,** 307–510.
57. Tömböl T. (1976) Golgi analysis of the internal layers (V–VI) of the cat visual cortex. *Expl Brain Res.* **Suppl. 1,** 292–295.
58. Tömböl T. (1978) Comparative data on the Golgi architecture of interneurons of different cortical areas in cat and rabbit. In *Architectonics of the Cerebral* Cortex (eds Brazier M. A. B. & Petsche H.) pp. 59–76. Raven Press, New York.
59. Valverde F. (1978) The organisation of area 18 in the monkey. A Golgi study. *Anat. Embryol.* **154,** 305–334.
60. Van den Pol A. N. & Cassidy J. R. (1982) The hypothalamic arcuate nucleus of rat. A quantitative Golgi analysis. *J. comp. Neurol.* **204,** 65–98.
61. Vogt B. A. & Peters A. (1981) Form and distribution of neurons in rat cingulate cortex: areas 32, 24 and 29. *J. comp. Neurol.* **195,** 603–625.
62. Werner L., Hedlich A., Winkelmann E. & Brauer K. (1979) Versuch einer Identifizierung von Nervenzellen des visuellen Kortex der Ratte nach Nissl- und Golgi-Kopsch Darstellung. *J. Hirnforsch.* **20,** 121–139.
63. Westrum L. E. (1970) Observations on initial segments of axons in the prepyriform cortex of the rat. *J. comp. Neurol.* **139,** 337–356.

(*Accepted* 14 *July* 1982)