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Physiological and Morphological Properties of Identified Basket Cells in the Cat's Visual Cortex*

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Summary. In 87 cells studied physiologically, and filled intracellularly with horseradish peroxidase (HRP), we have found four cells which make multiple contacts with the perikarya of their post-synaptic targets. These cells are all multipolar non-pyramidal neurones with elongated smooth dendrites. Three resemble the classical "basket cells" of Ramón y Cajal (1911), having widely distributed axons which contribute to the "nids pericellulaires" around pyramidal cell perikarya. The fourth cell has a much more restricted axon virtually confined to layer 4 and appears to contact principally small, probably non-pyramidal, cells. Two of the basket cell axons have been examined by electron microscopy and make symmetrical, Gray's type II contacts with the perikarya and apical and basal dendrites of pyramidal cells. Ten percent of the synapses are on dendrites of non-pyramidal cells.

The axon arborizations of all four cells are distributed in a patchy fashion. In two cells examined for the purpose, very few boutons were found within 100 μm of the cell body and a radially aligned cylinder of the same diameter extending from the cell body to the pial surface. The physiological properties of these structurally similar cells are far from uniform. They can be activated mono- or polysynaptically, by X- or Y-type lateral geniculate input, and can have S or C type receptive fields. Two were activated, probably monosynaptically, via callosal afferents. These cells may play an important role in the inhibitory mechanisms of the cortex.

Key words: Basket cells – HRP-filled neurones – Inhibition – Perikaryal synapse – Visual cortex

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Introduction

In an investigation of the relation between structure, function and connectivity of cells in the visual cortex of the cat, we have recovered 87 functionally-identified cells which have been filled intracellulary with HRP. The four cells reported here have been selected because their axons form multiple contacts with the perikarya of their post-synaptic targets. Ramón y Cajal (1911) has described similar cells in Golgi preparations of the visual cortex, where the axons of these cells form "nids pericellulaires" around the perikarya of pyramidal cells, thus giving rise to the name "basket cell" because of their similarity to the cerebellar basket cell (Ramón y Cajal 1911). Cortical basket cells have been described subsequently by Marin-Padilla (1969) in the motor cortex of man, by Szentágothai (1973, 1975, 1978) in the cat and monkey, by Jones (1975) in the somatosensory cortex of the squirrel monkey and by Tömböl (1978), Peters and Regidor (1981) and DeFelipe and Fairén (1982) in the cat's visual cortex. It has been suggested that basket cells demarcate cortical columns by inhibitory effects produced by axonal branches distributed in narrow vertical slabs (Colonnier 1966; Marin-Padilla and Stibitz 1974; Jones 1975, 1981; Szentágothai 1973, 1975, 1978; Peters and Regidor 1981). We have examined this notion in the visual cortex of the cat where the columnar organisation is well attested, by following the axonal distribution of the axons of the HRP-filled basket cells.

Methods

For these experiments adult cats were prepared for recording under halothane/nitrous oxide/oxygen anaesthesia after which they were paralysed and maintained under anaesthesia with small doses of Althesin (Glaxo) or Sagatal (May & Baker) as required. We

have monitored arterial blood pressure and its level and lability have provided our principal indication of anaesthesia level. We have maintained end-tidal CO_2 between 4–5%. Stimulating electrodes were placed in the chiasm (OX), in the optic radiations above the lateral geniculate nucleus (LGN; OR1), in the radiations immediately below the site of recording (OR2) (Bullier and Henry 1979; Martin and Whitteridge 1981) and in the visual cortex of the opposite hemisphere. Plotting the OX-OR1 latency difference for all our cells gives a monomodal distribution. However, it has been shown that the shorter latency differences are consistently associated with Y cells, while the longer latency differences are consistently associated with X cells (Kratz et al. 1978; So and Shapley 1979). We have independently confirmed this observation and thus can determine the type of input (X or Y) to the cell we are studying. The latency of response to stimulation at OR2 gives an estimate of the number of synapses interposed between the LGN afferents and the cell being recorded from Bullier and Henry (1979), Martin and Whitteridge (1981). The methods used for intracellular recording were in all respects identical to those of Lin et al. (1979). Receptive fields (RF's) were analysed and classified using hand-held stimuli (Bullier and Henry 1979; Martin and Whitteridge 1981). At the termination of the experiment the animals were over-anaesthetised and perfused with a buffered solution of 1% paraformaldehyde and 2.5% glutaraldehyde. The blocks of tissue containing the injected cells were cut on a vibratome at 80 or 100 μm. They were then processed for HRP using a method similar to that of Hanker et al. (1977), using cobalt/nickel intensification. Three of the cells were then processed further for a combination of light microscopy (LM) and electron microscopy (EM) using appropriate stages of previously described methods (Somogyi 1978; Somogyi et al. 1979). Section dimensions were measured during processing to allow corrections to be made for differential shrinkage. The schema of Lund et al. (1979) was used for the cortical layering.

Results

Cell Morphology

On the basis of dendritic morphology alone (Fig. 1), three of the cells we report here would be classified as basket cells (cf. Jones 1975; Peters and Regidor 1981). However, because we have some evidence for further subdivisions amongst multipolar neurones with elongate dendritic trees (see below) we, in common with Marin-Padilla (1969) and Szentágothai (1973, 1975, 1978) have based our classification on the characteristics of the axon. This is necessarily the case with one of our cells in which only the axon was recovered. Those cells in which the axon repeatedly forms loops of boutons around the perikarya of pyramidal cells (Fig. 2a) we have called basket cells. By contrast we have injected one multipolar cell with an elongate dendritic tree in layer 4B in which the axon repeatedly forms loops of boutons around the perikarya of small, probably non-pyramidal, cells in layer 4 (Fig. 2b), and has a far more restricted axonal distribution than the other three cells. Because of these differences to the other three cells, we will refer to this cell separately as the multipolar non-pyramidal cell in layer 4B. The non-pyramidal cell illustrated in Fig. 2b of Gilbert and Wiesel (1979) may be another example of such a cell. After examining a large number of non-pyramidal neurones in Golgi preparations and a small sample of similar HRP-filled cells (which include, amongst others, single examples of a chandelier or axo-axonic cell (Szentágothai 1975, 1978), Peters and Proskauer's (1980) multipolar cell and a neurogliform cell (Szentágothai 1973), we conclude that the repeated looping perikaryal contacts of the cells reported here are peculiar to these types of non-pyramidal cells in the cat. The convergence of the axons of a number of basket cells (probably 10–20) would give the appearance of the "nids pericellulaires" seen by Ramón y Cajal (1911).

In order to study the structure of the synapses formed by the boutons of the basket cells we examined 148 synapses in sections of the axons prepared for EM. Fig. 2c shows an EM photomicrograph of one of the boutons filled with HRP which forms symmetric, or Gray's type II, synaptic contacts with a pyramidal cell perikaryon. These synapses are similar to those formed by the boutons in the cat's visual cortex which contain the GABA synthesizing enzyme, glutamic acid decarboxylase (P. Somogyi, T.F. Freund, A.D. Smith and J.Y. Wu, unpubl. observations). Of the synapses examined, 90% were found on pyramidal cells, 40% on the perikarya, 50% divided between their apical and basal dendrites. The remaining 10% of synapses were on dendrites whose parent cell was not positively identified, but were suspected of being other basket cells.

In view of the possible significance of these cells in the columnar organisation of the functional properties of the cortex we examined the distribution of the axon in three dimensions. Drawings of serial thick sections (80 or 100 μm) of the axon arborisations made it clear that the axon was not confined to a single narrow slab of cortex. In two cells examined for the purpose very few boutons were found within 100 μm of the perikaryon of the HRP-filled cells, and they were sparse in a radial column 100 μm in diameter extending from the perikaryon to the pial surface. Long collateral branches, in the case of the basket cells, distributed boutons to localised clusters or radial columns of cells over distances of up to 1.6 mm in the anteroposterior and mediolateral dimensions. This means the basket cells possess amongst the longest intracortical collaterals of any cell type we have so far injected. In two of the basket cells the axon could be traced only in layers 2 and 3, but in the cell shown in Fig. 1 the axon was distributed in all but layer 6. The axon of the multipolar cell in layer 4B was confined to layer 4, with the exception of two unbranched collaterals which descended to layers 5 and 6.

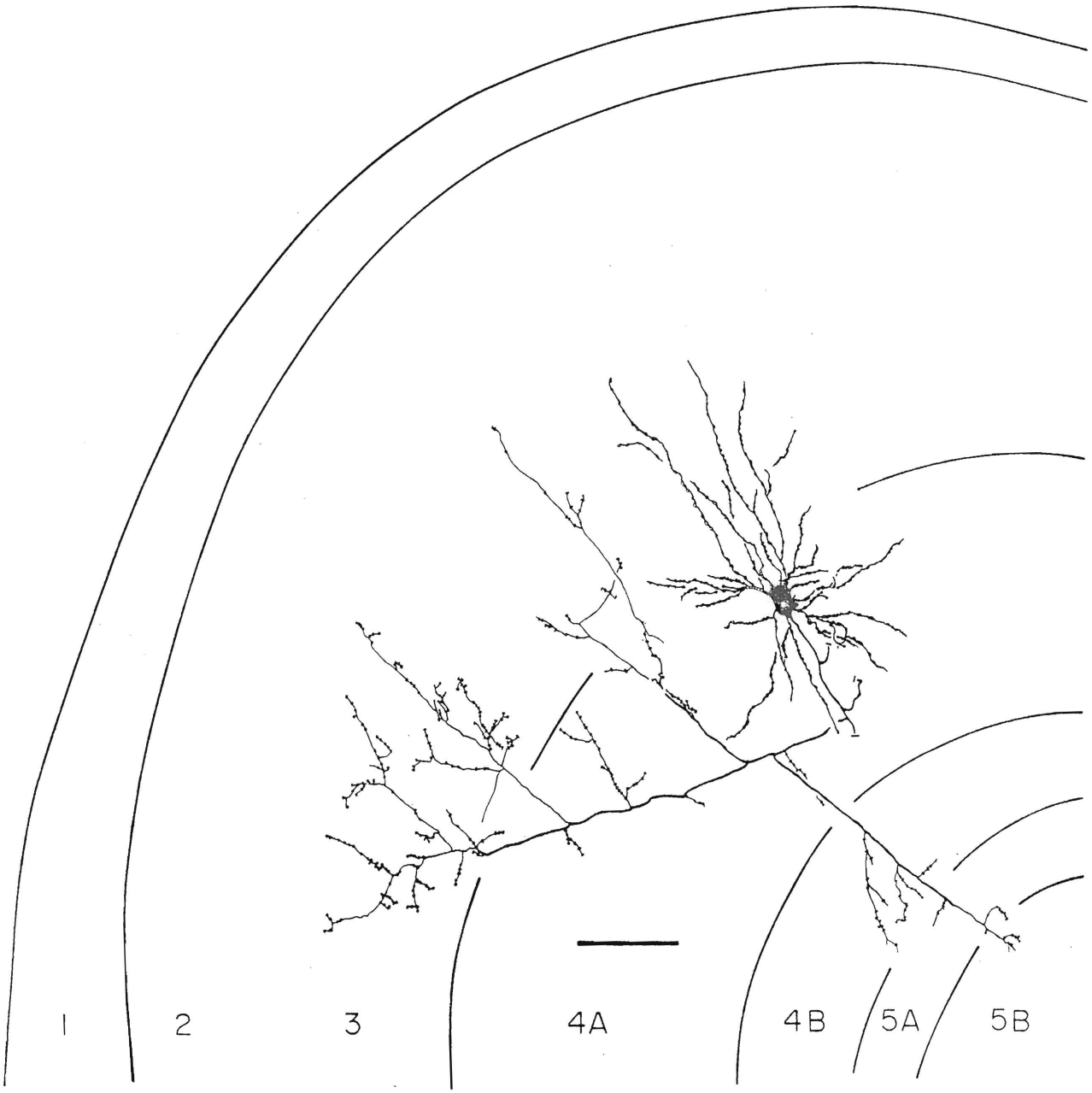

Fig. 1. A partial reconstruction, made with the use of a microscope and drawing tube, of a cortical basket cell in area 17 of the cat. The block containing the cell was sectioned transversely. Only one of the five main branches of the axon is drawn for clarity. This particular branch projected medially giving off radial branches and was completely reconstructed from three serial 100 μm sections. The remaining four branches arborise mainly around and in front and behind the cell and are shown in the complete computer assisted reconstruction of the same cell in Fig. 3. The axonal bouton and dendritic bead size has been exaggerated for clarity. The branching pattern of the axon is characteristic of these cells. The dendritic arbor is completely reconstructed from four serial 100 μm thick sections. Layering after Lund et al. (1979). Scale bar = 100 μm

We investigated the 3-D aspect further by making a complete computer-assisted reconstruction two cells one in layer 4A, the other in layer 4B. Three views of the reconstructed basket cell in layer 4A are given in Fig. 3. The wide ramification of the axon seen in all three views contrasts markedly with previous Golgi reconstructions of apparently similar cells which showed that the axon was confined to a single vertical slab (Marin-Padilla and Stibitz 1974; Jones 1975; Peters and Regidor 1982). Similarly the dendritic tree was not confined to a single narrow slab. The tangential view (Fig. 3c) shows a distinct

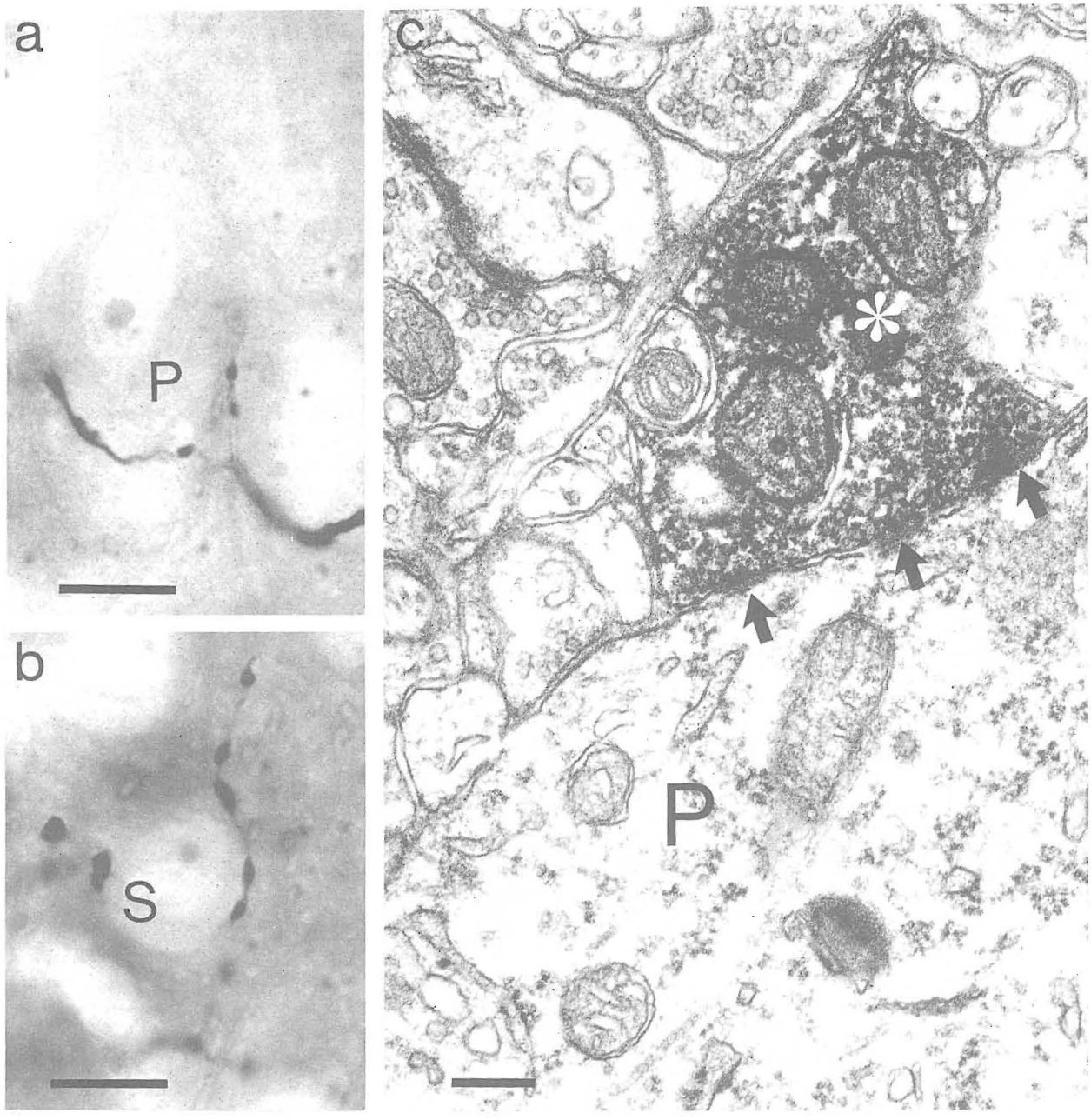

Fig. 2. a Light micrograph of a pyramidal neurone (P) in layer 3 of area 17 in the cat. The HRP-labelled collateral of a basket cell axon approaches the cell from the right, divides into two varicose branches and runs along the perikaryon of the pyramidal cell, suggesting synaptic contacts. **b** Light micrograph of a small non-pyramidal cell (S) in layer 4B of cat area 17 surrounded by the axon terminals of the layer 4B multipolar cell. An HRP-labelled axon collateral with varicosities runs along the right side of the non-pyramidal cell while another collateral contacts the cell from the left. The axon of this cell was almost completely confined to layers 4A and 4B. **c** Electron micrograph of an HRP-labelled bouton (*asterisk*) of the basket cell shown in **a**. The bouton contains small pleomorphic vesicles and makes symmetrical synaptic contacts (*arrows*) with the perikaryon of a pyramidal neuron (P). Scale bars: **a, b** = 10 μm; **c** = 0.2 μm

Fig. 3a–c. A complete computer-assisted reconstruction of the basket cell shown in Fig. 1. Dendrites are shown on the left, axon on the right. The cell body is located where the axes meet. The axes refer to the stereotaxic planes. S: superior; A: anterior; P: posterior; M: medial; L: lateral; **a** The transverse view, where the cell is seen from behind looking forwards. Comparison with Fig. 1 shows that there is a close relationship between the actual bouton distribution and the profuseness of the branching seen in the computer-assisted reconstruction. **b** 90°-rotation of **a** showing the view from the side, looking at the medial surface of the cell. **c** 90°-rotation of **a** showing the tangential view, looking down at the cell from the surface of the cortex. Differential shrinkage of the sections during processing has been corrected for. Scale bar = 200 μm

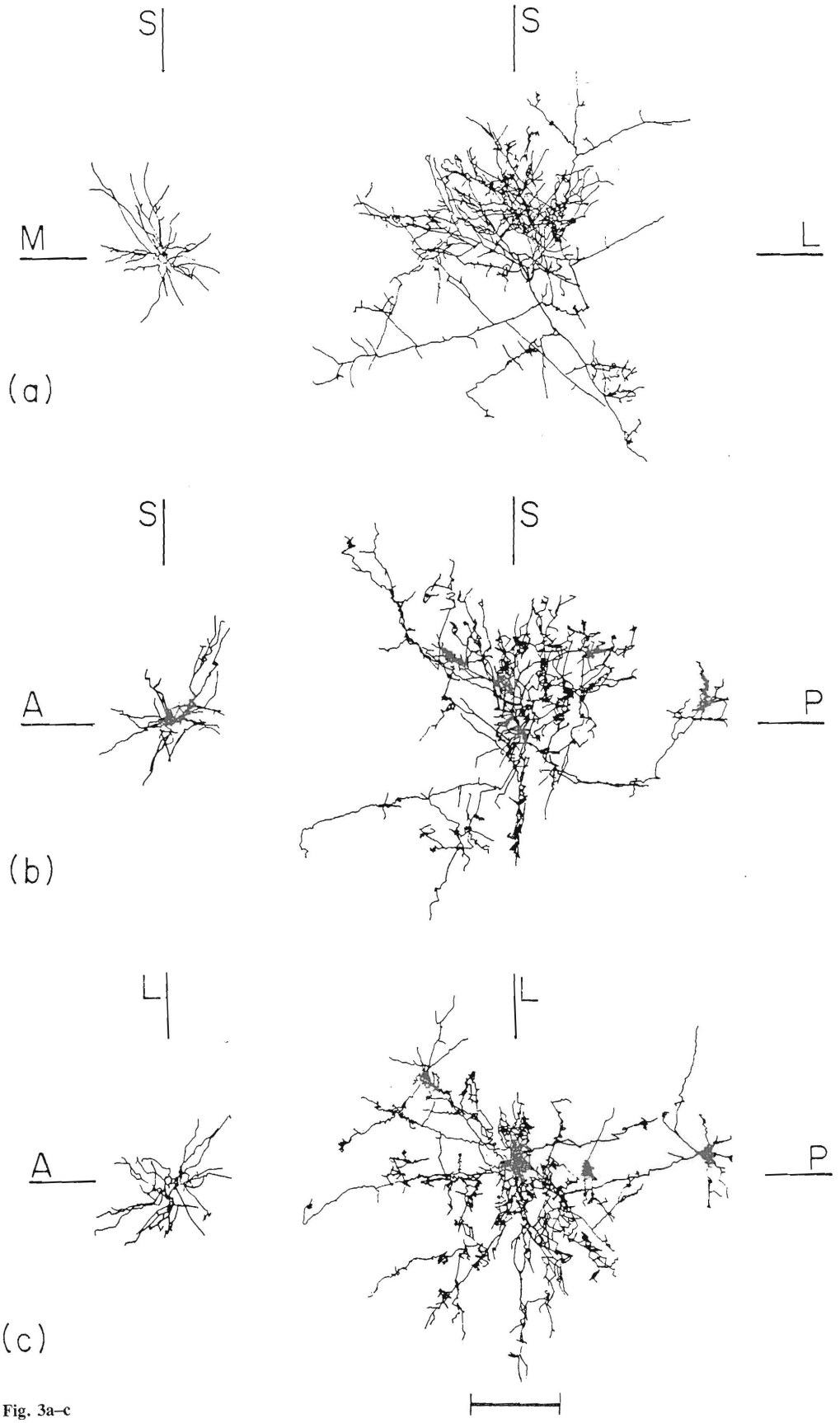

Fig. 3a–c

Table 1. Summary of the physiological data for the HRP-filled cells. The cell body was located in the layer indicated. RF classification after Bullier and Henry (1979). Subscript "2" refers to the number of separate "ON" and "OFF" subfields, "SP" and "ST" defined in text. For binocular cells the RF dimensions of the larger of the two RF's is given. Ocular dominance categories after Hubel and Wiesel (1962). Serial position gives the number of synapses interposed between the LGN afferent input (which could be X or Y in type, see Methods) and the cell being recorded, as determined by electrical stimulation. Call. input: cells could be orthodromically activated by stimulation of the fibres of the corpus callosum. * Estimate. Further details see text

Cat No.	Layer	Morphological type	type	Receptive length	width	azimuth	Field elevation	orientation	Direction	Ocular dom.	LGN input	Serial posit.	Call. input
10/81	4A	Basket cell	S_2	0.25°	1.0°	+0.6°	-2.1°	30°	pref.	7	X	mono.	mono.
19/81	3	Basket cell	C_{SP}	2.1°	3.2°	-1.0°	-7.1°	140°	selective	4	Y	poly.	-
20/81	3	Basket cell	S_2	1.4°	0.7°	+0.6°*	-6.0°*	140°	-	4	X	mono.	mono.
23/81	4B	Multipolar cell	C_{ST}	1.3°	2.9°	0.0°	-11.4°	65°	-	6	Y	mono.	-

non-uniformity in the axon distribution. The reconstructed axon of the cell in layer 4B was much more restricted in extent (0.5 mm diameter in tangential view), but also showed a patchy distribution.

Functional Properties of HRP-Filled Cells

The essential data from the electrophysiological portion of the experiments are summarised in Table 1. Two basket cells had RF's with spatially separate "on" and "off" subregions (S_2-type RF's). The remaining cells had fields with overlapping "on" and "off" subregions (C type RF's), one of which required very little length summation (indicated by subscript "SP"), the other of which required length summation to respond optimally (indicated by subscript "ST"). We were able to drive two of the cells orthodromically by stimulating the opposite hemisphere. The latency of these cells' responses, compared with the antidromic latencies obtained by Harvey (1980), suggested that they were monosynaptically excited by callosal fibres. As can be seen from Table 1 we have not found any distinguishing physiological properties which might enable one to differentiate them from other cells during the recording experiment.

Discussion

In view of previous speculations (Colonnier 1966; Szentágothai 1973, 1975, 1978; Jones 1981, Eccles 1981) it seems appropriate to discuss whether the basket cells we have found could be responsible for the columnar organisation of functional properties in the visual cortex. One of the principal differences between our material and that of previously published reconstructions of the axons of cortical basket cells (Ramón y Cajal 1911, Marin-Padilla 1969; Marin-Padilla and Stibitz 1974; Jones 1975; DeFelipe and Fairén 1982; Peters and Regidor 1981) is that our intracellular injections reveal a far richer axonal arborisation. This is not surprising given that the Golgi material is drawn from juvenile animals and the thick myelin sheaths surrounding the axons, seen in our EM analysis, would prevent complete impregnations in the adult. It is thus probable that previous 3-D reconstructions of cortical basket cell axons (Marin-Padilla and Stibitz 1974; Jones 1975; Peters and Regidor 1981) which show a single slab-like arrangement of the arbor are incomplete. Differences with other studies may also arise because the cells previously reported may not all be the same type.

Although our basket cells are structurally homogenous, even our small sample shows some functional diversity. They can be excited mono- or polysynaptically, by X or Y streams and have either S or C-type RF's. It is very likely that they inhibit the cells on which they synapse and may therefore contribute to those cells' specific RF properties, a number of which are thought to be due to intracortical inhibition (Benevento et al. 1972; Sillito 1975) and are organised in columnar or slab-like fashion (Hubel and Wiesel 1962, 1963, 1974; Shatz et al. 1977; Payne et al. 1980; Tolhurst et al. 1981). It might therefore be expected that the axonal distribution might give some clue to the function of the basket cells. The paucity of boutons immediately surrounding the perikaryon of these cells is to be expected, since cells with similar properties are arranged in columns and are unlikely to inhibit each other. Unfortunately the axons of these cells show no obvious shape or repetition interval which corresponds to the known dimensions of cortical columns or slabs (Hubel and Wiesel 1963, 1974; Shatz et al. 1977). It is possible that the basket cells are responsible for properties which may not be organised in a columnar fashion, such as end-inhibition and binocu-

lar inhibition, but the extensiveness of the axonal arborisation points to a role in intercolumnar inhibition. Obviously, regardless of the basket cell's principal function, it would inevitably sharpen the selectivity of cells it may contact in columns with different stimulus preferences to its own.

Unless a separate subset of neurones is discovered which do have the vertical slab-like arrangement which previously were thought to be the dominant characteristic of cortical basket cells, current theories of the role of basket cells in producing functional columns will have to be reconsidered. Our observation that the basket cell only contacts particular subgroups of neurones within the extensive distribution of its axon is intriguing as it may indicate a finer grain of organisation of the cortical circuitry than has so far been detected.

It seems likely that the major inhibitory input to the perikarya of cortical neurones comes from the cells we have described and thus, together with the axo-axonic cell (Somogyi 1977; Fairén and Valverde 1980; Peters et al. 1982; Somogyi et al. 1982), they are probably responsible for a substantial fraction of the inhibitory mechanisms of the cortex.

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