Exp Brain Res (1986) 64: 541–552

Experimental Brain Research

Synaptic targets of HRP-filled layer III pyramidal cells in the cat striate cortex

Z.F. Kisvárday[1,2], K.A.C. Martin[2], T.F. Freund[1], Zs. Maglóczky[1], D. Whitteridge[2], and P. Somogyi[1,3]

[1] First Department of Anatomy, Semmelweis University Medical School, Tuzolto u. 58, H-1450 Budapest, Hungary
[2] Department of Experimental Psychology, Oxford University, South Parks Road, Oxford OX1 3UD, UK
[3] MRC Anatomical Neuropharmacology Unit, Department of Pharmacology, Oxford University, South Parks Road, Oxford OX1 3QT, UK

Summary. There are numerous hypotheses for the role of the axon collaterals of pyramidal cells. Most hypotheses predict that pyramidal cells activate specific classes of postsynaptic cells. We have studied the postsynaptic targets of two layer III pyramidal cells, that were of special interest because of their clumped axon arborization near, and also 0.4–1.0 mm from the cell body, in register in both layers III and V. 191 terminations from four sites (layers III and V, both in the column of the cell and in distant clumps) were analysed by electron microscopy. Only one bouton contacted a cell body and that was immunoreactive for GABA. The major targets were dendritic spines (84 and 87%), and the remainder were dendritic shafts. Of these 13 were classed as pyramidal-like (P), 8 smooth cell-like (S) and three could not be classified. Four of five S types, but none of the seven P types tested were immunoreactive for GABA, supporting the fine structural classification. The putative inhibitory cells therefore formed not more than 5% of the postsynaptic targets, and their activation could only take place through the convergence of pyramidal cells onto a select population of GABA cells. The results show that the type of pyramidal cells with clumped axons studied here make contacts predominantly with other pyramidal cells. Thus the primary role of both the intra and intercolumnar collateral systems is the activation of other excitatory cells.

Key words: Cortical organization – Pyramidal cells – Intracellular HRP – Synaptic contacts – GABAergic neurons

Present address: T.F. Freund, K.A.C. Martin, and P. Somogyi: MRC Anatomical Neuropharmacology Unit (address see above)
Offprint requests to: Z.F. Kisvárday, Budapest (address see above)

Introduction

The abundance of local axon collaterals originating from pyramidal cells is one of the most impressive and most consistently demonstrated features of the neocortical circuitry (Ramón y Cajal 1899; Lorente de Nó 1922; Sholl 1955; Scheibel and Scheibel 1970; Szentágothai 1965, 1973; Lund 1973). From studies of Golgi impregnated neurons it was recognised that the origin and distribution of collaterals were not random (Lorente de Nó 1922; Lund and Boothe 1975). The lateral spread of the local collaterals, extending often for several millimeters was also emphasized in some Golgi (Scheibel and Scheibel 1970) and degeneration (Fisken et al. 1975; Creutzfeldt et al. 1977) studies. However, the real extent of the intracortical axon arborizations of different pyramidal cells has only recently been revealed by injecting single cells intracellularly with horseradish peroxidase (HRP), (Gilbert and Wiesel 1979, 1983; Landry et al. 1980; Martin and Whitteridge 1984; Noda and Yamamoto 1984).

The various suggestions that have been made for the possible role of the pyramidal axon collaterals can be summarised in three main points.

1. Since the cortex receives input that is often localised to some but not all of the laminae, the collaterals could transfer information vertically to functionally related cell groups in other laminae (Lund and Boothe 1975; Mitzdorf and Singer 1978, 1979; Lund et al. 1979; Gilbert and Wiesel 1979, 1983; Ferster and Lindström 1983; Martin and Whitteridge 1984; Mitzdorf and Singer 1978, 1979).

2. Local collaterals are also present around the dendritic field of the parent cell (Ramón y Cajal 1899) and one of their possible roles may be the activation of inhibitory interneurons thereby providing the structural basis of recurrent inhibition (Phil-

lips 1959; Stefanis and Jasper 1964; Creutzfeldt et al. 1969). In both the above cases the receptive fields of the pyramidal cell and its nearby targets would need to overlap.

3. The role of long intercolumnar collateral systems (Rockland and Lund 1982; Rockland et al. 1982; Gilbert and Wiesel 1983; Martin and Whitteridge 1984) remains much more obscure because the classical receptive fields of the pyramidal cell and its distant targets may not overlap. According to one view their collaterals may connect distant cell groups providing the basis for the summing of receptive fields from different visual field locations (Gilbert and Wiesel 1983; Gilbert 1985), or connect cells with similar response properties reinforcing their common characteristics (Mitchison and Crick 1982; Nelson and Frost 1985; Ts'o et al. 1986). Others suggested that these collaterals may operate to inhibit groups of cells with dissimilar response properties (Matsubara et al. 1985) or produce inhibitory flanks or end zones (Gilbert and Wiesel 1983).

Pyramidal cells are considered to be excitatory (Emson and Lindvall 1979; Baughman and Gilbert 1981; Ferster and Lindström 1985), but through the activation of inhibitory interneurones they could also exert disynaptic inhibition. Putative inhibitory interneurons in the cortex probably use γ-aminobutyrate as transmitter and they differ from putative excitatory neurons in that they have few or no dendritic spines (Ribak 1978; Freund et al. 1983; Somogyi et al. 1985a). Thus, on the one hand, if the main role of the axon collaterals was the activation of the target areas, then one would expect most of the pyramidal axon boutons to give synapses onto dendritic spines of the recipient spiny, presumably also excitatory neurons. On the other hand, if the main role was the inhibition of the target cell groups, then one would expect the pyramidal cell boutons to terminate mainly on dendritic shafts and somata of inhibitory neurons.

Previous studies on the postsynaptic targets of pyramidal cell boutons emphasised the prevalence of dendritic shafts and suggested the activation of putative inhibitory interneurons (Somogyi 1978; Winfield et al. 1981; McGuire et al. 1984), although the biochemical characterization of the dendrites had not been attempted.

To test the hypotheses surveyed above in points 1–3, we examined the postsynaptic targets of layer III pyramidal cells, revealed by intracellular HRP injection, that allowed the sampling of both their proximal and distal axon collaterals in the radial and horizontal directions. In addition to tentative ultrastructural identification of their synaptic targets in some cases we also tested their immunoreactivity for GABA.

Methods

Two neurons analysed in this study were obtained from two adult cats used in a large series of physiological experiments. Animals were anaesthetised and prepared as described previously (Martin and Whitteridge 1984). Blood pressure, heart rate and end-tidal CO_2 were monitored continuously during the experiment. Intracellular recording and iontophoresis of HRP was carried out following the methods of Friedlander et al. (1979). At the end of the experiment the animals were deeply anaesthetised and perfused with a fixative containing 2.5% glutaraldehyde (TAAB) and 1% paraformaldehyde (TAAB) in 0.1 M phosphate buffer solution (pH 7.4).

Tissue processing

Blocks of the area containing the intracellularly HRP filled neurons were dissected and sectioned on a Vibratome (Oxford Instruments), then processed to reveal peroxidase enzyme activity using the p-phenylene/catechol reaction (Hanker et al. 1977) supplemented with cobalt/nickel intensification (Adams 1981). Sections were dehydrated and mounted on slides in Durcupan ACM resin (Fluka). The injected cells were drawn and photographed under light microscope using a 100 × oil immersion objective. Parts of the axonal fields were re-embedded for electron microscopy to obtain a sample of the postsynaptic elements. For this all boutons that fall in the plane of the section were followed in serial sections till the postsynaptic element was identified. Since the plane of the section was not chosen to fit the axon arborisation in any particular way the sample can be considered randomised. The physiological properties and a two dimensional drawing of neuron No 2 was reported earlier (Fig. 6B Martin and Whitteridge 1984).

Postembedding GABA-immunocytochemistry on electron microscopic sections

Dendrites postsynaptic to layer III pyramidal cells (N_1 and N_2) had different ultrastructural features. To see if these structural differences indicated neurochemical heterogeneity we reacted ultrathin sections of some dendrites with an antiserum to GABA (Hodgson et al. 1985; Somogyi et al 1985b) using the postembedding colloidal gold method (Somogyi and Hodgson 1985). Briefly, the sections were mounted on formvar coated single slot grids, treated with 1% sodium periodate and 1% periodic acid for the etching of the resin and removal of the osmium. After washing they were floated sequentially on drops of: 5% normal goat serum (NGS), rabbit anti-GABA serum (code No 9, diluted 1 : 2000), colloidal gold (15 nm) coated with goat anti-rabbit IgG (Janssen Life Sci.Prod., dil 1 : 20 with 0.05% carbovax, Sigma, mw 20000, dissolved in 50 mM tris buffer, pH 7.0). Between these steps the grids were washed in tris (10 mM)-phosphate (10 mM)-buffered saline (TPBS) containing 1% NGS. Following the incubation, grids were washed in distilled water and the contrast increased with lead citrate.

Results

Location of pyramidal cell somata and distribution of their processes

The two pyramidal cells intracellularly filled with HRP were selected on the basis of their characteristic

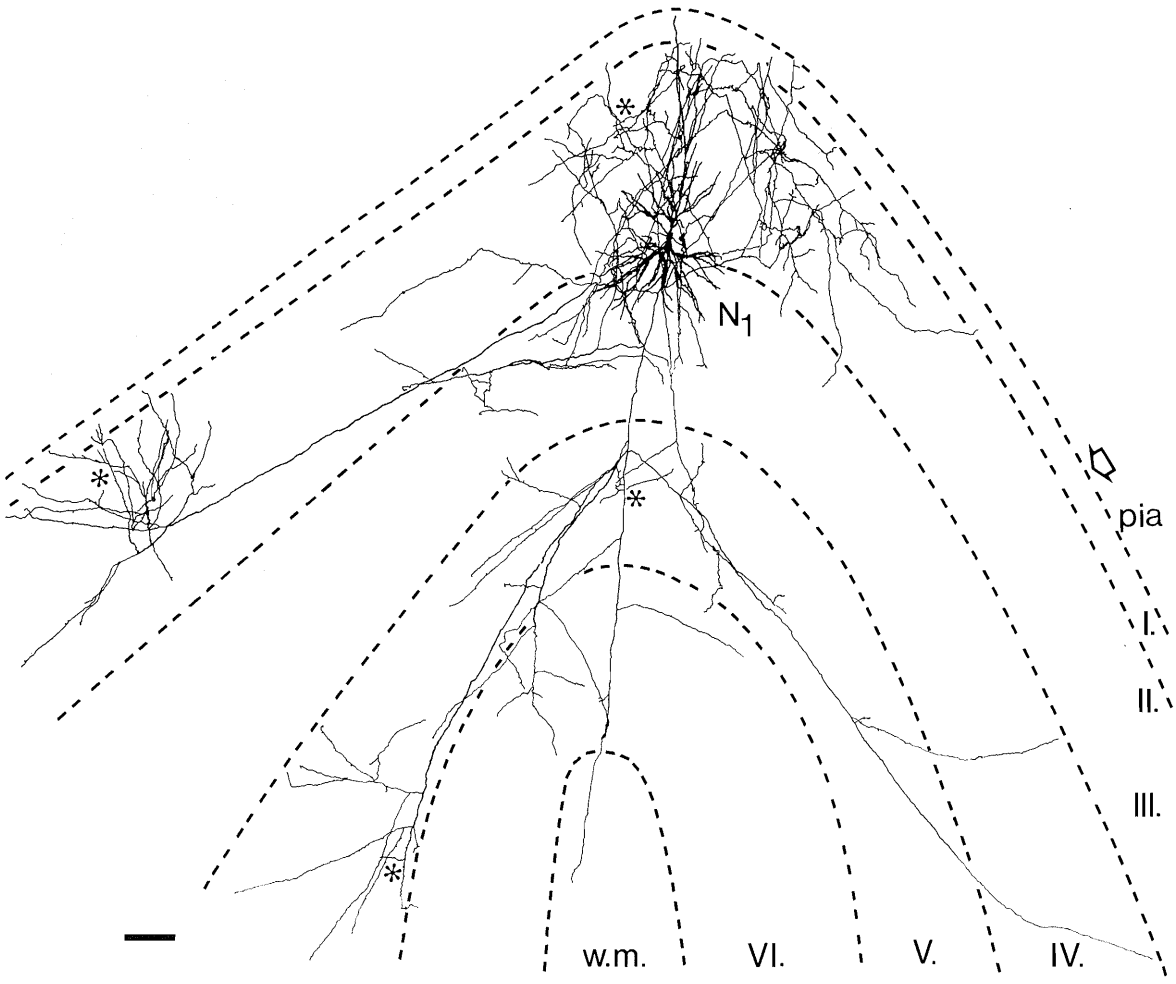

Fig. 1. Drawing of a layer III pyramidal cell (N_1, No 1 in Table 1) in area 17 of the cat's visual cortex that was reconstructed from nineteen 80 μm thick sections. The axon extended 1500 μm anterior-posteriorly and was distributed in patches in both layers III and V. This is partly obscured by the two dimensional image. One collateral projected through the border between areas 17 and 18 (arrow). The postsynaptic targets of the cell were studied in four clusters (asterisks), one near the cells dendritic field in layer III, another one just below the cell in layer V, and in two distal patches in layers III and V, respectively. Scale: 100 μm

axonal distribution. No physiological data were obtained for neuron N_1. The second cell, N_2 (see Martin and Whitteridge 1984, Fig. 6B) was a simple cell with one subfield, had a receptive field 0.75 degrees in width and 1.4 degrees in length, it was monocularly activated and monosynaptically driven from the lateral geniculate nucleus (LGN).

Both pyramidal cells reported here were located at the borders of layers III and IVa. Their basal dendrites penetrated into upper layer IV. The apical dendrite terminated in layer I, and emitted several side branches in lower layer III.

The most characteristic feature of both cells was the distribution of their axon collaterals in different cortical laminae (Fig. 1). The main axons emitted two sets of collaterals before descending radially into the white matter. The first set emerged close to the origin of the axon in upper layer IV and arborised in layers II and III. The second set emerged from the main axon in layer V and arborised mainly in this layer. Both the main axons and the secondary collaterals were myelinated as proved by subsequent electron microscopic examination. The secondary collaterals travelled 0.5–1 mm horizontally emitting terminal branches in a patchy fashion, each patch occupying an area of about 300–400 μm in diameter in layers II–III. The branches of N_2 in layer V were not revealed completely but the collaterals of N_1 in layer V formed a projection similar to that in the supragranular layers. Interestingly, as also shown by Gilbert and Wiesel (1983) the clumps of collaterals in the supra- and infragranular layers were in register radially i.e. they occupied the same "column" (Fig. 1). From the two-dimensional reconstruction of

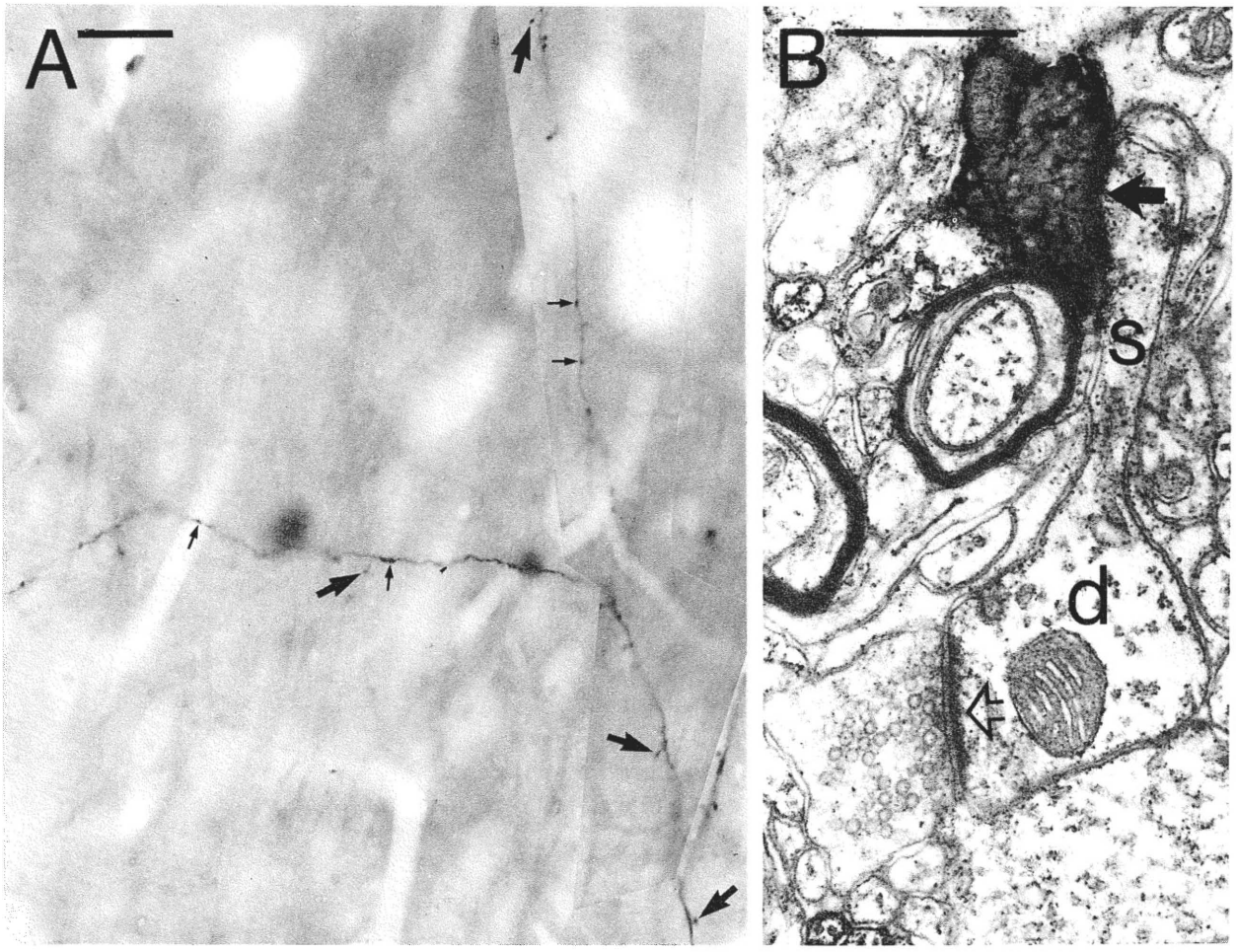

Fig. 2. A Light micrograph showing club-like (large arrows) or en passant boutons (small arrows) on the axon collateral of pyramidal cell No 1 in layer III. **B** Electron micrograph of a spine (s) receiving type I synaptic contact (arrow) from a bouton (asterisk) of pyramidal cell No 1, while its parent dendrite (d) receives a type I contact from another, unlabelled bouton (open arrow). Scales: **A** 20 μm; **B** 0.5 μm

Table 1. Distribution of 191 electron microscopically identified postsynaptic elements to layer III pyramidal cells

Pyr. cell No	Postsynaptic elements									Total			
	In the column of dendritic arbor					In distal axonal clump							
	layer III			layer V		layer III		layer V					
	Soma	Dend. shaft	Spine	Dend. shaft	Spine	Dend. shaft	Spine	Dend. shaft	Spine	Soma	Dend. shaft	Spine	Total
1.	0	2	14	0	3	7	31	2	11	0	11 (4*)	59	70
2.	1	7	48	5	28	2	30	**	**	1	14 (4*)	106	121
										1	25 (8*)	165	191

* Numbers in brackets indicate those dendrites from the total that had smooth cell characteristics (type S)
** No distal axonal clump was filled by HRP in layer V

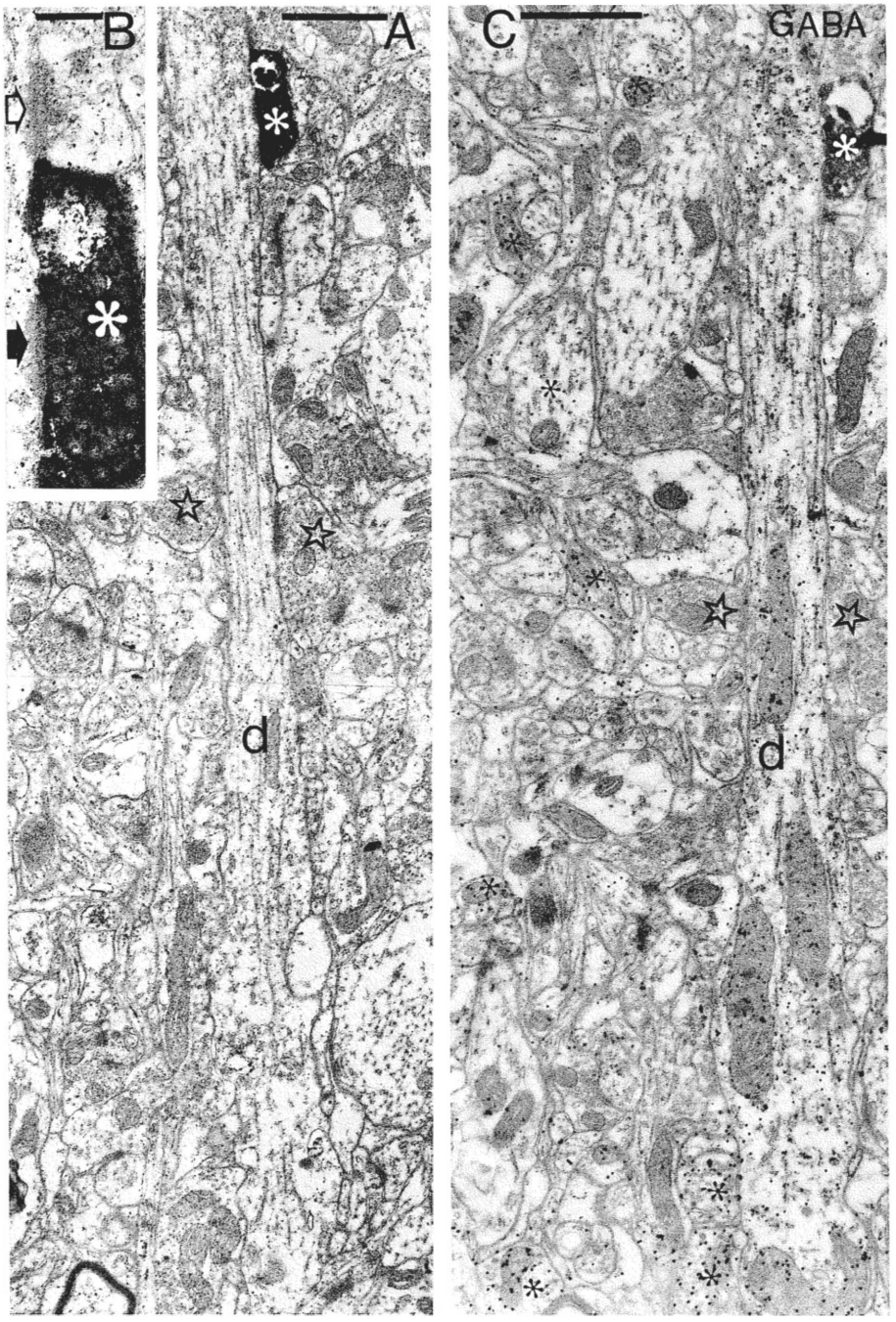

Fig. 3A–C. GABA immunoreactivity in dendrite receiving pyramidal cell input. **A** Electron micrograph of a dendrite (d) postsynaptic to the HRP filled bouton (asterisk) of pyramidal cell No 2. The dendrite was in layer III within the axonal clump in the vicinity of the pyramidal cell soma. **B** The same HRP filled bouton (asterisk) as shown in **A** is seen at higher magnification making a type I synaptic contact (filled arrow), similarly to an unlabelled bouton (open arrow). **C** A serial section of the same dendrite (d) as in **A** was reacted for GABA by the immunogold method. The postsynaptic dendrite as well as other neuronal processes (black asterisks) show GABA immunoreactivity. The dendrite contains large mitochondria and is in synaptic contact with numerous boutons (two of them marked by stars for correlation with **A**, some of which are GABA-positive. Scales: **A, C** 1 μm; **B** 0.2 μm

neuron N_1 and N_2 it is not obvious that most of the axon collaterals seen around the somata in layers II–III formed in fact axonal clusters at some distance from the cell body in the antero-posterior direction similar to those seen for N_1 on the left side in Fig. 1. Many of the collaterals in layers II–III and V emitted fine branches bearing varicosities in the column in which the soma was located. Analysing the axonal distribution of neurons N_1 and N_2 it was evident that the axons were extending for greater distances antero-posteriorly than medio-laterally in agreement with the findings of Gilbert and Wiesel (1983). The two cells differed in the size of their axonal field; N_1 had an axon extending 1.5–2 mm antero-posteriorly while the axon of N_2 extended about 1 mm in the same direction. The unmyelinated terminal branches of the axons were studded with club-like and en passant boutons (Fig. 2A).

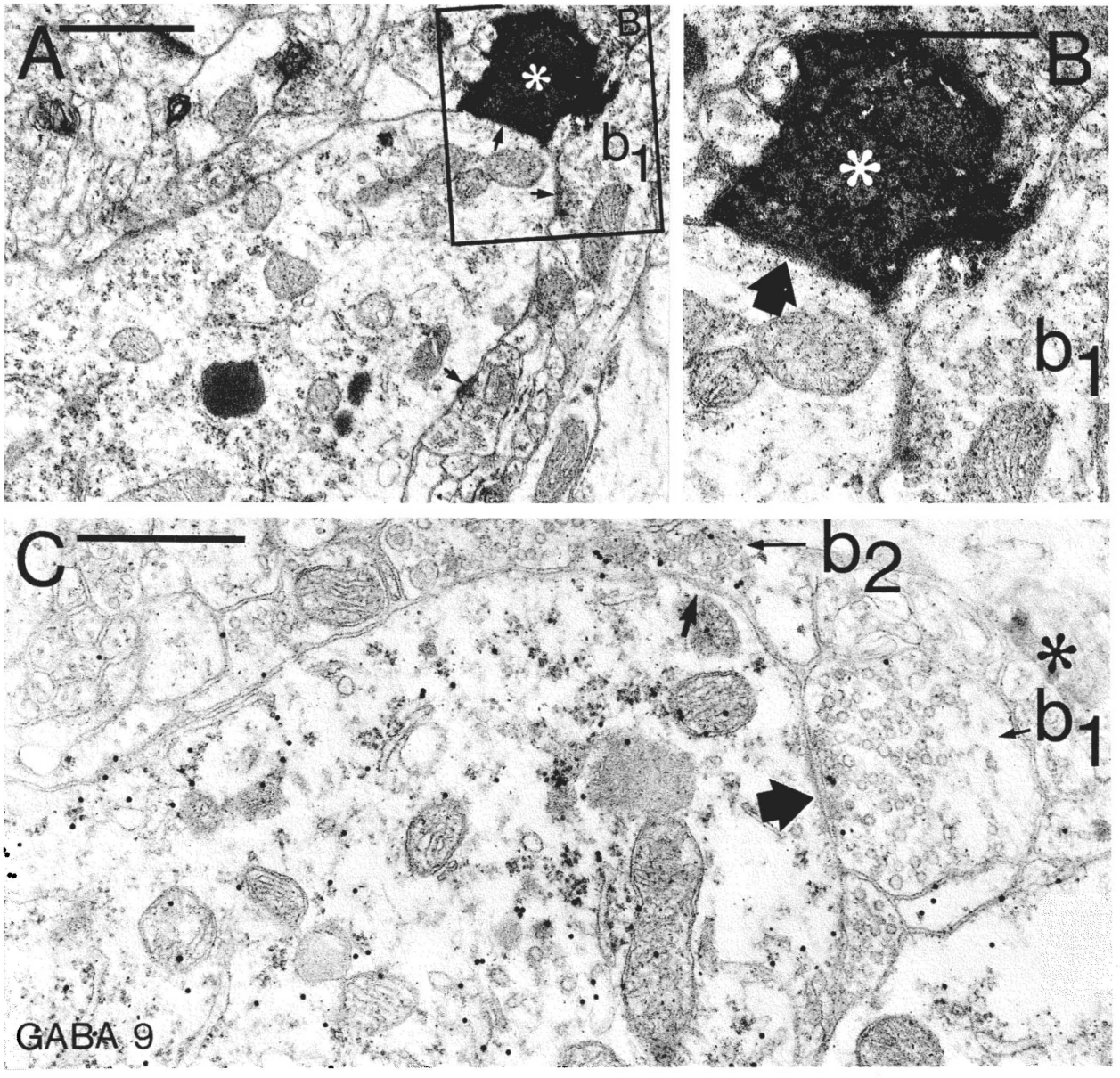

Fig. 4A–C. Electron micrographs of serial sections of a GABA-immunoreactive perikaryon that was postsynaptic to the HRP-filled pyramidal cell No 2. **A** The soma receives several synapses (arrows) one of them provided by the HRP labelled pyramidal cell bouton (asterisk in **A–C**). The HRP filled and an unlabelled bouton (b_1) in the framed area are shown in **B, C** at higher magnification as they make type I synaptic contacts (large arrows). **C** The same perikaryon and a bouton (b_2) that establishes type II synaptic contact with the soma are GABA-immunoreactive as shown by the accumulation of gold particles. Scales: **A** 1 μm; **B, C** 0.6 μm

Electron microscopy of postsynaptic targets

The main aim of the present study was to identify the postsynaptic elements contacted by pyramidal cells with clustered axons. Since different layers of cortex contain different cell populations and since the axon may contact different targets in a distant clump and in the column of the soma, the sample of boutons was collected from four areas. Axon collaterals of both cells were serially sectioned for electron microscopy from the column of the dendritic field separately either in layer II/III or in V. Similarly, axon collaterals were studied in the distant axon clusters separately in layers II/III and in V. Altogether 191 postsynaptic elements were encountered for the two cells (Table 1). Each bouton was found to establish asymmetric, type I synaptic contact with its target element (Figs. 2A, 3B, 4B). All but five boutons made synaptic contact with only one element.

Dendritic spines were the most frequent postsyn-

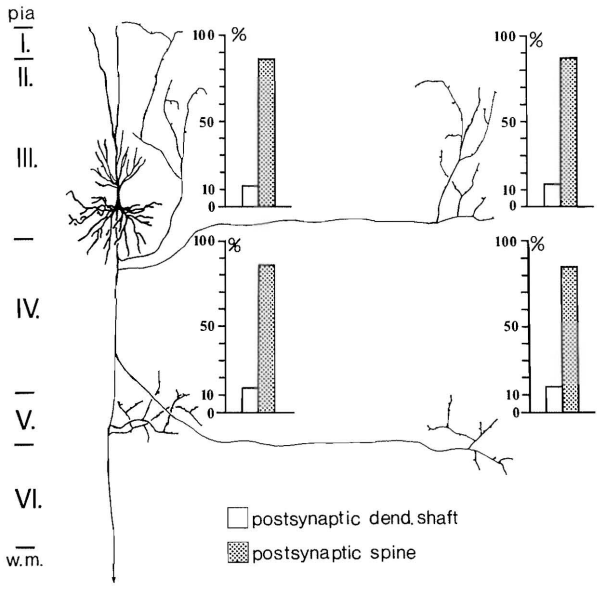

Fig. 5. Cumulative diagram showing the quantitative distribution of postsynaptic elements to pyramidal cells N_1 and N_2 in layers II–III (upper graphs) and V (lower graphs). Data were obtained from axonal clusters either in the same column (graphs on the left) or in clusters distant (graphs on the right) from the perikaryon

aptic targets comprising about 84–87% of all elements (Table 1, and Fig. 5). They were identified in serial sections on the basis of their shape (Fig. 2B), the presence of spine apparatus and by the absence of mitochondria. Postsynaptic spines could often be traced back to their parent dendrite (Fig. 2B).

Dendritic shafts represented 12–15% of the postsynaptic targets in each cluster examined (Table 1, and Fig. 5). They were heterogeneous with regard to their ultrastructural features. Two categories could be recognised, type P (pyramidal-like) and type S (smooth dendritic cell-like) postsynaptic dendrites. Thirteen dendrites, classified as type P, had light cytoplasm, relatively few mitochondria and in the same section received only a few synapses from terminals other than the HRP labelled bouton. They did not have particular orientation. Dendritic spines could sometimes be seen to emerge from postsynaptic type P dendrites. One postsynaptic dendrite showed characteristics similar to the apical dendrites of pyramidal cells. It was 1–2 μm in diameter, oriented radially and rich in parallel running microtubules. Eight postsynaptic dendrites were classified as type S. They were typically filled with mitochondria, which were usually much wider in cross section than those in type P dendrites (Fig. 3C). In addition type S dendrites frequently contained clusters of polyribosomes, and were densely covered by synaptic boutons of different types (Fig. 3). Three dendritic shafts postsynaptic to pyramdial cell boutons, could not be classified as S or P type.

GABA-immunocytochemistry of postsynaptic elements

Type S dendrites are thought to belong to smooth dendritic non-pyramidal cells, some of which have been shown to be immunoreactive for GABA (Somogyi et al. 1985b; Somogyi and Hodgson 1985). This was directly confirmed in the present study by postembedding GABA-immunocytochemistry (Somogyi and Hodgson 1985) using the colloidal gold technique. Once the synaptic contact was identified on the dendrite, further ultrathin sections of the same dendrite were reacted with the GABA antiserum. Five dendrites classified as type S on the basis of fine structural criteria (see above) were tested for GABA immunoreactivity, and four of them were positive for GABA (Fig. 3). In contrast, none of the seven type P dendrites that were postsynaptic to HRP labelled pyramidal cells and incubated with the GABA antiserum showed immunoreactivity. Thus, out of twelve dendritic shafts tested for the presence of GABA one third were positive.

One postsynaptic element was a neuronal soma in layer III (Figs. 4A–C). It was located in the vicinity of the perikaryon of pyramidal cell no 2. The fine structural characteristics and the synaptic input of this cell were similar to cells with smooth dendrites (see Peters and Saint-Marie 1984). The soma received numerous synapses, many forming asymmetrical synapses (Fig. 4A). The cytoplasm was very rich in free polyribosomes (Fig. 4) and granular endoplasmic reticulum. This soma was also immunoreactive for GABA (Fig. 4C).

Discussion

Neuronal elements postsynaptic to pyramidal cells

The results show that dendritic spines form the overwhelming majority of postsynaptic targets for the local collaterals of layer III pyramidal cells having patchy axons. The parent cells of the spines could not be directly identified, but since layers III and V do not contain spiny stellate cells (Lund 1984), and sparsely spinous nonpyramidal cells are rare, we conclude that the majority, if not all of the spines originate from pyramidal cells. This conclusion is also supported by the fine structural features of the parent dendrites when the spine could be traced to them. In addition to the spines more than half (type P) of the

postsynaptic dendritic shafts had characteristics similar to the dendrites of pyramidal cells (see Feldman 1984). Our differentiation of S and P type dendrites on the basis of their ultrastructure is strongly supported by the evidence that most of the S type dendrites tested were also GABAergic, while no P type dendrite was found to be GABAergic. The one S-type dendrite that was immunonegative may contain neurotransmitters other than GABA and technical reasons can not be ruled out for our negative results for P type dendrites. Nevertheless the available evidence strongly suggests that the P-type dendrites originate from pyramidal cells, as shown previously (Somogyi et al. 1983). Thus all in all over 90% of postsynaptic elements probably originate from pyramidal cells. Putative inhibitory cells form not more than 5% of the postsynaptic elements. These results provide strong evidence for previous predictions (Szentágothai 1965, 1978) that the main targets of pyramidal cells are other pyramidal cells in the cortex.

As shown in Fig. 5, there is a striking uniformity in the relative proportion of postsynaptic elements in the four target areas, indicating that layer III pyramidal cells perform the same operational task both in layers III and V, in the vicinity of as well as remote from the parent cell. Our results on the distant targets of layer III cells in the patches agree well with preliminary results published in the primate visual cortex (McGuire et al. 1985) and with the targets of pyramidal cells in layer V of the cat (P.L.A. Gabbott, K.A.C. Martin, D. Whitteridge, submitted for publication). These studies also concluded that the majority of postsynaptic elements were spines and suggested that they originated from pyramidal cells. Thus, it seems that pyramidal cells with patchy termination have a similar range of target cells. These results contrast with the distribution seen for layer VI pyramidal cells that project to layer IV in the cat, where the major targets are dendritic shafts, possibly originating from sparsely spiny cells (McGuire et al. 1984). The differences may be related to the different roles that pyramidal cells in each layer perform.

It has been suggested that paths of different functional significance may end on different locations of cortical cells (Freund et al. 1985). Thus, the role of the layer III pyramidal cells is probably different from that of the layer VI pyramidal cells, which are thought to be involved either in inhibitory functions like end-stopping (McGuire et al. 1984) or in some form of gain control of the geniculocortical transmission (Ferster and Lindström 1985; Martin and Somogyi 1985). In any case, layer III pyramidal cells contact different postsynaptic neurons, and it is worth reassessing previous suggestions for the role of collaterals, given in the Introduction.

Interlaminar connections

The wiring up of the cortex ensures that excitatory inputs to a particular column arrive at restricted laminar locations and are then distributed to other laminae by the radial components of the axonal arbors of the recipient cells, such as spiny stellate and pyramidal neurons. This organisation would form the basis of the hierarchy of visual processing as originally concluded from physiological results (Hubel and Wiesel 1962, 1963). The radial connections would also ensure that cells lying adjacent to or within the same column as the pyramidal cell in layer III would share similar receptive field properties and position in the visual field. The collaterals around the dendrites of these pyramidal cells extend over 300 μm, and they might contribute to the scatter of the excitatory centres observed for the receptive fields of cells in the same functional column (Creutzfeldt et al. 1974a, b; Albus 1975). The projections from layer III to V have been shown to be the main pathway that activates the neurons in layer V (Mitzdorf and Singer 1978; Ferster and Lindström 1983), and our results are compatible with the predominantly activating role.

Intercolumnar interactions

When viewed from the surface most columnar systems appear as stripes. The different columnar systems, e.g. orientation or ocular dominance, appear to be independent and intersect each other at oblique angles. Thus, as Hubel and Wiesel (1977, Fig. 28) have suggested, a long bar presented to one eye activates fragmented patches of striate cortex. They proposed that to link regions of striate cortex activated by such a stimulus would specifically require patchy connections (Hubel and Wiesel 1968, 1977). The increased binocularity of the cells in layer III argues against an ocular dominance column-specific innervation, and leaves the suggested orientation column links (Hubel and Wiesel 1977; Mitchison and Crick 1982; Gilbert and Wiesel 1983) as a strong possibility. Three-dimensional reconstructions indicate that the patches formed by pyramidal cell axons have the required repeat interval (Gilbert and Wiesel 1983) for these columns. This hypothesis requires that the terminals activate cell groups as opposed to inhibiting them. Intracortical facilitation of co-axially aligned co-oriented simple cells has recently been

found over several millemetres of cortex (Nelson and Frost 1985). Pyramidal cell axon collaterals are one of the candidates that may mediate this activation. As most of their targets appear to be pyramidal cells contacted directly without the insertion of an inhibitory interneuron into the pathway, the main effect of the collaterals would be excitation. This may be reinforced by the action of the local collaterals of those cells that reach firing threshold in the target group. Thus pyramidal cell axon collaterals may be responsible for the correlated firing of cell groups with similar orientation preference that has been demonstrated to occur over distances of several millimetres in the cat cortex (Ts'o et al. 1986).

However, orientation is known to depend on inhibition mediated by GABA (see Sillito 1984), that is unlikely to be the transmitter of pyramidal cells. Thus, the GABA antagonist bicuculline applied iontophoretically greatly reduced orientation selectivity (see Sillito 1984), implying that the cells received inputs of all orientation and local GABAergic inhibition rather than excitation by pyramidal cell collaterals is the main factor determining orientation specificity. However, long range pyramidal collaterals may play some role especially for cells activated polysynaptically from the LGN, explaining the residual orientation selectivity remaining in some cells following bicuculline application (Sillito 1979).

The results of Matsubara et al. (1985) are also contrary to the role of long range patchy pyramidal collaterals in connecting cells with similar orientation. They determined the organisation of the ocular dominance and orientation columns in a region of area 18, and then retrogradely labelled cells within this region by small adjacent injection of HRP. Their conclusion was that cells with orthogonal orientation preferences were being connected and that the connections were probably inhibitory. However, application of HRP locally mainly labels pyramidal cells (Rockland and Lund 1982; Rockland et al. 1982), similar to those patchy layer III cells that we studied in area 17. Thus the results of Matsubara et al. (1985) obtained in area 18 can not be reconciled either with the view that iso-orientation columns are connected, or the present evidence that in the striate cortex over 90% of the connections are onto other excitatory cells. Similarly, on the present evidence it now seems unlikely that the pyramidal cells are directly involved in creating, via an inhibitory interneuron, the inhibitory end-zones or flanks of receptive fields (Gilbert and Wiesel 1983). The lateral inhibitory effects on different orientation columns and the inhibitory side bands are more likely mediated by the large basket cells that are probably GABAergic and also have axons comparable in extent to those of layer III pyramidal cells (Martin et al. 1983; Somogyi et al. 1983). It is possible that in the study of Matsubara et al. (1985) basket cells, that could mediate the cross-orientation inhibition, were also labelled retrogradely by HRP, but overshadowed by the large population of pyramidal cells mediating other effects.

Divergence and convergence

Some postsynaptic dendrites were immunoreactive for GABA; all of them being of the S type, probably originating from smooth dendritic, GABAergic non-pyramidal cells. The sparse pyramidal cell input to putative inhibitory neurons could only have significance if a select population of GABAergic cells were contacted. Putative inhibitory neurons may be a more significant target for other types of pyramidal cell (Somogyi 1978; Winfield et al. 1981; McGuire et al. 1984) and the method employed here could be useful in testing this possibility.

It is apparent at first sight that within the axonal arbor of pyramidal cells the number of potential recipient neurons far exceeds the number of boutons in the axonal arborization. We never found two synaptic contacts from one cell onto the same postsynaptic element. More than one of the postsynaptic spines could originate from the same pyramidal cell, but as pointed out by Szentágothai (1978, 1979), the meeting of dendrites and pyramidal axons seems to occur at angles that prevent significant number of multiple contacts. Szentágothai (1975, 1979) predicted on the basis of Golgi studies that in general pyramidal cells were connected by one or very few synapses. With regard to the functional correlate of such sparse connections, Gilbert (1985) suggested that peripheral influences beyond the classical receptive field could be mediated by long horizontal connections. These influences seem to be weak and this is compatible with the weak influence that any pyramidal cell is likely to have on its target cells. Even if, as suggested by theoretical considerations, the termination mainly on spines as opposed to dendritic shafts was to increase the effectiveness of the input (Diamond et al. 1970; Jack et al. 1975; Pongracz 1985), our data suggest that the input from pyramidal cells to other pyramidal cells could become significant only through strong convergence.

Creutzfeldt et al. (1969) pointed out that it was unlikely that the activation of a single synapse on a spine would lead to a detectable EPSP at the soma. Along the same line it has been suggested that at least 100 synapses are needed to bring the cell to threshold (see Martin 1984), implying that the cell will only fire when there is a sufficient 'consensus'

amongst the large number of cells providing its excitatory inputs. This allows for a much more versatile use of the cortical wiring because a single neuron can then be incorporated into a number of different networks involved in different operations.

Cortical output columns

The pyramidal cells that project to different cortical areas are aggregated into independent columnar systems (Gilbert and Kelly 1975; Bullier et al. 1984; Segraves and Innocenti 1985). One characteristic of these systems is that cells from a number of different slabs converge on a localised region of another visual area, e.g. area 18. These same projection cells rarely form collateral projections to an additional cortical area, such as areas 18 and 19. In the cat, as yet there are no known physiological differences of cells projecting to different cortical areas, but their separation into output columns and lack of collateralisation implies that they are functionally different. Because of the strong possibility that one cell contributes only a few synapses to any other cell, a high degree of convergence is needed for a group of cells in area 17 to activate one cell in area 18. This could be achieved with the sort of circuitry found here; pyramidal cells projecting to another visual area could provide activation of other pyramidal cells within the same projection column and of pyramidal cells in other projection columns that innervate the same target visual area. Thus the clumps seen may not correlate particularly with any known functional columnar system, but may be related to the anatomically defined output columns (Gilbert and Wiesel 1983). This does not exclude a role for the intracortical collaterals in forming receptive fields within area 17 and could account for the strikingly similar target specificity of all portions of the axonal arbor of these layer III pyramidal cells.

Acknowledgements. The authors are grateful to Mr. John Anderson, Mrs. Klara Boczko and Ms. Eva Toth for their excellent technical assistance, and to Mrs. Dorothy Rhodes for secretarial help. We thank Dr. I. Benedeczky for participation in the initial phase of this study. This work was supported by the Medical Research Council of the UK and the Hungarian Academy of Sciences.

References

Adams JC (1981) Heavy metal intensification of DAB-based HRP reaction product. J Histochem Cytochem 29: 775

Albus K (1975) A quantitative study of the projection area of the central and the paracentral visual field in area 17 of the cat. I. The precision of the topography. Exp Brain Res 24: 159–179

Baughman RW, Gilbert CD (1981) Aspartate and glutamate as possible neurotransmitters in the visual cortex. J Neurosci 1: 427–439

Bullier J, Kennedy H, Salinger W (1984) Branching and laminar origins of projections between visual cortical areas in the cat. J Comp Neurol 228: 329–341

Creutzfeldt OD, Maekawa K, Hösli L (1969) Forms of spontaneous and evoked postsynaptic potentials of cortical nerve cells. Progr Brain Res 31: 265–273

Creutzfeldt OD, Kuhnt U, Benevento L (1974a) An intracellular analysis of visual cortical neurones to moving stimuli: responses in a co-operative neuronal network. Exp Brain Res 21: 251–274

Creutzfeldt OD, Innocenti GM, Brooks D (1974b) Vertical organization in the visual cortex (area 17). Exp Brain Res 21: 315–336

Creutzfeldt OD, Garey LJ, Kuroda R, Wolff J-R (1977) The distribution of degenerating axons after small lesions in the intact and isolated visual cortex of the cat. Exp Brain Res 27: 419–440

Diamond J, Gray EG, Yasargil GM (1970) The function of the dendritic spine: an hypothesis. In: Anderson P, Jansen JKS (eds) Excitatory synaptic mechanisms. Universitets Forlaget, Oslo, pp 213–222

Emson PC, Lindvall O (1979) Distribution of putative neurotransmitters in the neocortex. Neuroscience 4: 1–30

Feldman ML (1984) Morphology of the neocortical pyramidal neuron. In: Peters A, Jones EG (eds) Cerebral cortex: cellular components of the cerebral cortex, Vol. I. Plenum Press New York London, pp 123–200

Ferster D, Lindström S (1983) An intracellular analysis of geniculo-cortical connectivity in area 17 of the cat. J Physiol 342: 181–215

Ferster D, Lindström S (1985) Synaptic excitation of neurones in area 17 of the cat by intracortical axon collaterals of cortico-geniculate cells. J Physiol 367: 233–252

Fisken RA, Garey LJ, Powell TPS (1975) The intrinsic association and comissural connections of area 17 of the visual cortex. Philos Trans R Soc Lond B 272: 487–536

Freund TF, Martin KAC, Smith AD, Somogyi P (1983) Glutamate decarboxylase-immunoreactive terminals of Golgi-impregnated axo-axonic cells and of presumed basket cells in synaptic contact with pyramidal cells of the cat's visual cortex. J Comp Neurol 221: 263–278

Freund TF, Martin KAC, Somogyi P, Whitteridge D (1985) Innervation of cat visual areas 17 and 18 by physiologically identified X- and Y-type thalamic afferents. II. Identification of postsynaptic targets by GABA immunocytochemistry and Golgi impregnation. J. Comp Neurol 242: 275–291

Friedlander MJ, Lin C-S, Sherman SM (1979) Structure of physiologically identified X and Y cells in the cat's lateral geniculate nucleus. Science 204: 1114–1117

Gilbert CD (1985) Horizontal integration in the neocortex. Trends Neurosci 8: 160–165

Gilbert CD, Kelly JP (1975) The projections of cells in different layers of the cat's visual cortex. J Comp Neurol 163: 81–106

Gilbert CD, Wiesel TN (1979) Morphology and intracortical projections of functionally characterised neurones in the cat visual cortex. Nature 280: 120–125

Gilbert CD, Wiesel TN (1983) Clustered intrinsic connections in cat visual cortex. J Neurosci 3: 1116–1133

Hanker JS, Yates PE, Metz CB, Rustioni A (1977) A new specific, sensitive and non-carcinogenic agent for the demonstration of horseradish peroxidase. Histochem J 9: 789–792

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

Hubel DH, Wiesel TN (1962) Receptive fields, binocular interaction and functional architecture in the cat's visual cortex. J Physiol 160: 106–154

Hubel DH, Wiesel TN (1963) Shape and arrangement of columns in cat's striate cortex. J Physiol 165: 559–568

Hubel DH, Wiesel TN (1968) Receptive fields and functional architecture of monkey striate cortex. J Physiol 195: 215–243

Hubel DH, Wiesel TN (1977) Functional architecture of macaque monkey visual cortex. Proc R Soc Lond B 198: 1–59

Jack JJB, Noble D, Tsien RW (1975) Electric current flow in excitable cells. Oxford University Press, Oxford, pp 197–222

Landry P, Labelle A, Deschenes M (1980) Intracortical distribution of axonal collaterals of pyramidal tract cells in the cat motor cortex. Brain Res 191: 327–336

Lorente de Nó R (1922) La corteza cerebral del raton. I. La corteza acustica. Trab Lab Invest Biol Univ Madrid. 20: 41–78

Lund JS (1973) Organisation of neurons in the visual cortex, area 17, of the monkey (Macaca mulatta). J Comp Neurol 147: 455–496

Lund JS (1984) Spiny stellate neurons. In: Peters A, Jones EG (eds) Cerebral cortex, cellular components of the cerebral cortex, Vol. I. Plenum Press, New York London, pp 255–308

Lund JS, Boothe R (1975) Interlaminar connections and pyramidal neuron organization in the visual cortex, area 17, of the macaque monkey. J Comp Neurol 159: 305–334

Lund JS, Henry GH, Mac Queen CL, Harvey AR (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

Martin KAC (1984) Neuronal circuits in cat striate cortex. In: Jones EG, Peters A (eds) Cerebral cortex, functional properties of cortical cells, Vol II. Plenum Press, New York London, pp 241–284

Martin KAC, Somogyi P (1985) Local excitatory circuits in area 17 of the cat. In: Rose D, Dobson VG (eds) Models of the visual cortex. John Wiley & Sons New York, pp 504–513

Martin KAC, Somogyi P, Whitteridge D (1983) Physiological and morphological properties of identified basket cells in the cat's visual cortex. Exp Brain Res 50: 193–200

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

Matsubara J, Cynader M, Swindale NV, Stryker MP (1985) Intrinsic projections within visual cortex: evidence for orientation-specific local connections. Proc Natl Acad Sci USA 82: 935–939

McGuire BA, Gilbert CD, Wiesel TN (1985) Ultrastructural characterization of long-range clustered horizontal connections in monkey striate cortex. Soc Neurosci Abstr 11: 17

McGuire BA, Hornung J-P, Gilbert CD, Wiesel TN (1984) Patterns of synaptic input to layer 4 of cat striate cortex. J Neurosci 4: 3021–3033

Mitchinson G, Crick F (1982) Long axons within the striate cortex – their distribution, orientation, and patterns of connection. Proc Natl Acad Sci USA 79: 3661–3665

Mitzdorf U, Singer W (1978) Prominent excitatory pathways in the cat visual cortex (A17 and A18): a current source density analysis of electrically evoked potentials. Exp Brain Res 33: 371–394

Mitzdorf J, Singer W (1979) Excitatory synaptic ensemble properties in the visual cortex of the macaque monkey: a current source density analysis of electrically evoked potentials. J Comp Neurol 187: 71–84

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

Noda T, Yamamoto T (1984) Response properties and morphological identification of neurons in the cat motor cortex. Brain Res 306: 197–206

Peters A, Saint-Marie RL (1984) Smooth and sparsely spinous nonpyramidal cells forming local axonal plexuses. In: Peters A, Jones EG (eds) Cerebral cortex: cellular components of the cerebral cortex, Vol I. Plenum Press New York London, pp 419–445

Phillips CG (1959) Actions of antidromic pyramidal volleys on single Betz cells in the cat. QJ Exp Physiol 44: 1–25

Pongracz F (1985) The function of dendritic spines: a theoretical study. Neuroscience 15: 933–946

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

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

Rockland KS, Lund JS (1982) Widespread periodic intrinsic connections in the tree shrew visual cortex. Science 215: 1532–1534

Rockland KS, Lund JS, Humphrey AL (1982) Anatomical banding of intrinsic connections in striate cortex of tree shrews (Tupaia glis). J Comp Neurol 209: 41–58

Scheibel ME, Scheibel AB (1970) Elementary processes in selected thalamic and cortical subsystems – the structural substrates. In: Schmitt FO (ed) The neurosciences second study program. The Rockefeller University Press, New York, pp 443–457

Segraves MA, Innocenti GM (1985) Comparison of the distributions of ipsilaterally and contralaterally projecting corticocortical neurons in cat visual cortex using two fluorescent tracers. J Neurosci 5: 2107–2118

Sholl DA (1955) The organization of the visual cortex in the cat. J Anat 89: 33–46

Sillito AM (1979) Inhibitory mechanisms influencing complex cell orientation selectivity and their modification at high resting discharge levels. J Physiol 289: 33–53

Sillito AM (1984) Functional considerations of the operation of GABAergic inhibitory processes in the visual cortex. In: Jones EG, Peters A (eds) Cerebral cortex, functional properties of cortical cells, Vol II. Plenum Press, New York London, pp 91–117

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

Somogyi P, Kisvárday ZF, Martin KAC, Whitteridge D (1983) Synaptic connections of morphologically identified and physiologically characterized large basket cells in the striate cortex of cat. Neuroscience 10: 261–294

Somogyi P, Freund TF, Hodgson AJ, Somogyi J, Beroukas D, Chubb IW (1985a) Identified axo-axonic cells are immunoreactive for GABA in the hippocampus and visual cortex of the cat. Brain Res 332: 143–149

Somogyi P, Hodgson AJ, Chubb IW, Penke B, Erdei A (1985b) Antiserum to γ-amino-butyric acid. II. Immunocytochemical application to the central nervous system. J Histochem Cytochem 33: 240–248

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

Stefanis C, Jasper H (1964) Recurrent collateral inhibition in pyramidal tract neurones. J Neurophysiol 27: 855–877

Szentágothai J (1965) The use of degeneration methods in the investigation of short neuronal connexions. In: Singer M,

Schade JP (eds) Degeneration patterns in the nervous system. Progress in Brain Research, Vol 14, pp 1–32

Szentágothai J (1973) Synaptology of the visual cortex. In: Jung R (ed) Handbook of sensory physiology, Vol VII. Springer, Berlin Heidelberg New York, pp 270–321

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

Szentágothai J (1978) The neuron network of the cerebral cortex: a functional interpretation. The Ferrier Lecture, 1977. Proc R Soc Lond B 201: 219–248

Szentágothai J (1979) Local neuron circuits of the neocortex. In: Schmitt FO, Worden FG (eds) The Neurosciences Fourth Study Program, pp 399–415

Ts'o DY, Gilbert CD, Wiesel TN (1986) Relationships between horizontal interactions and functional architecture in cat striate cortex as revealed by cross-correlation analysis. J Neurosci 6: 1160–1170

Winfield DA, Brooke RNL, Sloper JJ, Powell TPS (1981) A combined Golgi-electron microscopic study of the synapses made by the proximal axon and recurrent collaterals of a pyramidal cell in the somatic sensory cortex of the monkey. Neuroscience 6: 1217–1230

Received February 25, 1986 / Accepted July 22, 1986