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CELLULAR ORGANIZATION OF RECIPROCAL PATCHY NETWORKS IN LAYER III OF CAT VISUAL CORTEX (AREA 17)

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Abstract—There is no direct information available concerning the exact spatial characteristics of long-range axons and their relationship with the patchy phenomena observed after extracellular injection of retrograde tracers. In the present study, using the recently introduced neuronal tracer biocytin, we demonstrate by detailed three-dimensional reconstruction of 10 pyramidal cells in layer III, that their clustered axonal terminals form a specific patchy network in layers II and III. The reconstructed network occupied an area of 6.5 × 3.5 mm parallel to the cortical surface elongated in an anteroposterior direction. The average centre-to-centre distance between patches within the network was 1.1 mm. On average, the axonal field of each of the 10 pyramidal cells contained a total of 417 boutons at four to eight distinct sites (patches), and in each patch, an average of 79 boutons was provided by the same cell. The identified connections between the patches were predominantly reciprocal. Detailed analyses have shown that many pyramidal cells of the network are directly interconnected so that each of them can receive one to four, chiefly axospinous, contacts onto the distal segment of its apical and basal dendrites from the axon of another pyramidal cell belonging to a different patch labelled from the same injection site.

We hypothesize that the possible functional role of the network is to link remote sites with similar physiological characteristics, such as orientation preference, supporting the model of Mitchison and Crick [(1982) *Proc. natn. Acad. Sci. U.S.A.* **79**, 3661–3665].

Early concepts of cortical organization put major emphasis on the columnar structure of the cerebral cortex where most of the connections were considered local, running predominantly between the laminae perpendicular to the cortical surface.[30,34,42,43] This organization scheme left little room for lateral intracortical interactions; however, it was in good agreement with the notion of columnar arrangement of functionally similar cells.[22–24]

Although some Golgi studies[45] and degeneration experiments[9,13,47] have indicated the existence of lateral intracortical connections spreading over several millimetres, their real extent was not recognized until the introduction of new tracing techniques: (i) intracellular injections of single cells revealed that certain pyramidal neurons possess long axons extending up to several millimetres parallel to the cortical surface, and that the distribution of their axonal branches is uneven, emitting patches of collaterals of 300–400 μm in diameter at regular intervals;[17,18,28,36] and (ii) extracellular injections of neuronal tracers such as horseradish peroxidase (HRP) resulted in labelling of somata and/or fibres in spatially distinct groups, also called patches, up to several millimetres from the injection site.[19,29,32,37,44]

In spite of the remarkable anatomical similarities between the patchy character of axonal arborization that is seen at the single cell level and the patchy distribution of labelling (somata and/or axons) after bulk injections of tracers, their exact relationship is not yet known. Nonetheless, it has generally been assumed that the two phenomena are actually the same.

The functional significance of patchy connections provided by pyramidal cells has been assumed to connect cell groups showing certain similar physiological properties. In this respect, orientation preference is so far the most likely candidate of many of the known physiological attributes. At present there are two concepts predicting the type of interactions for each of which experimental data have been reported. One model predicts that neuronal clusters with like orientation preferences are interconnected and emphasizes findings obtained in area 17 that pyramidal neurons display the structural attributes that could correlate with the pattern of orientation columns.[19,39] The other model suggests connections between sites of unlike orientation preferences with the inclusion of cells of both pyramidal and non-pyramidal types.[37]

In the present study we were interested in answering the following questions. What type of cells with what type of connections are involved in the cellular organization of the overall intrinsic patchy pattern of the visual cortex? Furthermore, which of the models, the like or the unlike orientation, will best suit the connectivity pattern established by these long horizontal projections? We therefore set out to investigate

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Abbreviations: DAB, 3,3′-diaminobenzidine; HRP, horseradish peroxidase; PB, phosphate buffer; RF, receptive field.

in great detail the fine anatomical relationship of labelled cells after small injections of a neuronal tracer into the supragranular division of area 17. Part of the results has appeared in abstract form.[27]

EXPERIMENTAL PROCEDURES

To visualize long-range intracortical connections, the neuronal tracer biocytin[26] (10% in 0.5 M Na-acetate, Sigma) was iontophoresed with + 0.5–1 μA at 1 Hz (1 s ON–OFF duty cycle, for 20 min) through glass micropipettes (10–12 μm tip diameter) near to the central visual representation of area 17 in six hemispheres of four adult cats. In each hemisphere only one biocytin injection was made. In one experiment (case no. 4) negative current was applied, instead of positive; otherwise the same parameters were used.

For initial surgery the animals were anaesthetized with a mixture of ketamine hydrochloride (25 mg/kg, Ketanset) and xylazine (3 mg/kg, Rompun), i.m. Anaesthesia was maintained throughout the experiment using a mixture of 1% halothane (Fluothane) and N_2O/O_2 (70%/30%) for artificial ventilation. Before injecting biocytin, extracellular recordings were made through pipettes containing biocytin in order to determine the retinotopic position of the injection sites. All injections were made within 3–5° from the vertical meridian and at elevations of minus 2–4° in area 17. On completion of the injections the animals were allowed to survive for one day under the same anaesthesia and then given an overdose of anaesthetic and perfused through the heart with Tyrode's solution followed by a fixative containing 1–1.5% glutaraldehyde (Merck) and 1–1.5% paraformaldehyde (Merck) in 0.1 M phosphate buffer (PB) at pH 7.4. Blocks containing the injection sites were dissected and 70-μm-thick consecutive sections were cut parallel to the cortical surface. In order to prevent any distortion in the spatial distribution of the labelled elements, no flattening of the tissue was done. Labelling was revealed by using the avidin–biotin–HRP complex (ABC, Vector, diluted 1:100 in 0.05 M Tris-buffered saline, pH 7.6, overnight) method, and 3,3′-diaminobenzidine 4 HCl (DAB; 0.05%, Sigma) as substrate supplemented with $CoCl_2$ (0.005%) intensification. Following the enzymatic reaction the sections were rinsed in PB, air-dried onto chrome–gelatine-coated slides, dehydrated, and coverslipped under DPX (Serva) mounting media.

In one cat (case no. 2) the axonal and dendritic fields of 10 labelled pyramidal cells were reconstructed from sections as large as 13 × 7 mm, by means of a light microscope fitted with a × 40 dry objective, and attached to a drawing tube. Altogether, 32 adjoining sections were used comprising the total cortical depth. The axons of each labelled cell were drawn from nine to 27 sections by tracing from their soma of origin towards more distal segments. In addition to this, for all 10 pyramidal cells the axonal swellings of either *en passant* or club-like types were registered and counted for each patch of the same cell. Reconstructions of the axonal fields were done by aligning cut ends at either surface of neighbouring sections. Common landmarks such as blood vessels and labelled large axons aided the reconstruction of the network of the 10 pyramidal cells. In the other three animals only the somata of labelled cells were registered in relation to the injection site.

From all four cats some sections at regular intervals were stained for Nissl substance and the laminar boundaries and their distance from the cortical surface were determined on the basis of characteristic landmarks such as difference in cell density and soma size. The exact laminar position of the biocytin-labelled cells and fibres was then determined according to their distance from the cortical surface.

RESULTS

Injection sites

We were interested in the distribution pattern and fine connectivity relationship of those neurons whose long-range patchy axons contribute to the same orientation column in area 17. Therefore, it was of particular importance to produce small tracer injections for the following reasons. Firstly, orientation columns have been shown to comprise a cortical cylinder of some 100 μm in diameter.[22] Secondly, we wanted to label only a reasonably small number of neurons that would enable us to trace their fine processes over long distances. Thirdly, we wanted to analyse the connectivity pattern of layer III cells that have been shown to provide long horizontal patchy axons.[17,36] Accordingly, in one cat (case no. 2) the ejection current for iontophoretic delivery of biocytin was successfully adjusted to produce an injection site in lower layer III with a core diameter of less than 150 μm centring at 450 μm below the cortical surface (Fig. 1B). No backflow of the tracer was observed along the capillary track. In the other three cats, although similar (case nos 1, 4) or identical (case no. 3) conditions (parameters of the applied current and electrode tip size) were used for the delivery of biocytin, the cores of the injection sites were larger (300–500 μm in diameter) and the labelling comprised much of the radial extent of layers III and IV. In these animals, due to the high density of labelled structures, it was not possible to trace and reconstruct individual cells. Nevertheless, the lateral extent and the overall distribution pattern of the labelled cells and fibres did not appreciably differ from those of case no. 2. Thus, these cats with their larger injections served as a control for the distribution pattern of the labelled cells of case no. 2, the subject of the present study.

Morphology and distribution of the labelled cells

The injected tracer produced somata and fibre labelling in layers II and III at a distance of up to 2.6 and 3.3 mm, respectively, from the injection site (Fig. 1A). A few anterogradely labelled axons were found in the deep layers originating exclusively from those labelled neurons in layers III–V whose dendritic field arborized within the injection site. Somata of retrogradely labelled cells outside the column of the injected site were found exclusively in layer III at a level up to 650 μm below the pial surface. They fell into two categories; some scattered neurons were weakly labelled and their axons could not be recovered (dots in Fig. 1A). However, 10 cells at eight distinct sites showed strong labelling, indicating that the injection site considerably overlapped with parts of their axonal termination field. Their somata were found up to 1.8 mm around the injection site, showing an areal distribution elongated in an anteroposterior direction (Fig. 1A). Due to the strong labelling of the 10 pyramidal cells, we were able to

trace their axonal and dendritic fields, and reconstruct in three dimensions. All 10 cells were pyramidal neurons possessing a number of basal dendrites in a radial array (Figs 1B, C, 2A–C), and a major apical dendrite which could be traced up to layer I where it branched profusely. They resembled those physiologically characterized and intracellularly filled pyramidal cells that were shown to possess long horizontal patchy axons in layers II and III.[17,18,28,36] The main axons originated radially from the lower pole of the somata and emitted several recurrent collaterals in layers III and upper IV. The collaterals radiated out in many directions in a plane parallel to the cortical surface individually spanning an overall longitudinal distance up to 4.9 mm, before entering the white matter (e.g. cell no. 8 in Fig. 3C; see also Table 1). The thickness of the main axons and of some of the secondary collaterals was often 1–3 μm in diameter and suggested that they were heavily ensheathed in myelin. Characteristically, the secondary collaterals emitted thinner axonal branches which, in turn, gave rise to patches of bouton-laden fine axons within the column of the dendritic field and at remote sites in layers II and III up to 2.8 mm from the parent soma. In many of the patches, a few collaterals branched in layer I as well. There was considerable difference, however, between different parts of the axon of the same cell with respect to the number and density of fine collaterals and the overall area covered by these collaterals. This difference is exemplified for the axonal field of cell no. 6 (in Fig. 3B) which established a dense axonal plexus within its own dendritic field, and smaller and less dense ones at 1 and 1.5 mm away from the parent soma (Fig. 3B; see legend). Since many of the pyramidal cells often emitted only a few collaterals at certain sites, a definition for the term "axonal patch" was established as follows. All those regions of the axonal field which emitted fine collaterals with terminal boutons of either *en passant* or club-like type, and were spatially distinct from similar collaterals of the same cell by more than 0.5 mm, were considered as an axonal patch. According to this definition, each pyramidal cell established four to eight distinct patches with an average of 5.3 for the 10 cells (Table 1). Each axonal patch covered an area of up to 400 μm in diameter and the centre-to-centre distance between the patches ranged from 0.8 to 1.5 mm with an average of 1.1 mm for the 10 pyramidal axons. In this respect, it is noteworthy that many of the densest axonal patches provided by the same cell were found at extreme distances from the parent soma. This feature is particularly evident for two axonal clusters of cell no. 8 on the extreme left and right in Fig. 3C. The average number of boutons emitted by each of the 10 pyramidal axons was 417, ranging between 69 (cell no. 2) and 1289 (cell no. 3, Table 1). We were interested in comparing the boutonal contribution of a single pyramidal cell at remote (outside the dendritic field of the parent cell) and proximal (within the dendritic field of the parent cell) patches. The results showed that, on average, each pyramidal cell gives off approximately twice as many boutons in a proximal patch (146 boutons) than it does in a remote patch (75 boutons).

Network of patchy axons

It was of particular interest to see whether alignment of the 10 reconstructed pyramidal axons labelled from the same injection site would give rise to a network that is also patchy. Superposition of the 10 axons showed that the patchy distribution we had seen for individual cells was actually preserved for the whole population of cells (Fig. 3A). The network established by the 10 pyramidal cells covered an area of about 6.5 × 3.3 mm elongated in an anteroposterior direction (Fig. 3A). A remarkable property of the network was that many of the patches provided by axons of different pyramidal cells apparently overlapped. These overlapping axonal fields mainly had a round shape, but were occasionally elongated (Fig. 3A). By comparing the distribution of the 10 labelled axons, it became evident that each pyramidal cell shared two to five of its distinct patches, including the one at the injection site, with overlapping axonal patches of one or more other pyramidal cells. Consequently, many of the patches, like the one in the column of cell no. 3, received overlapping axons from up to five pyramidal cells each of which was situated at different remote sites. This highly specific overlapping character of the patchy network is best seen when pairs of labelled cells are compared with each other, as demonstrated in Fig. 3B and C. In an extreme case, pyramidal cell nos 3 and 8 established overlapping patches at five distinct sites, including the injection site (arrowheads and star in Fig. 3B, C). Moreover, a comparison between the axonal distribution of neighbouring cells with complete (cell nos 4 and 5 in Fig. 3A) or partial dendritic overlap (cell nos 7 and 8 at the injection site in Fig. 3A) revealed that their target regions are largely different.

Reciprocity was another interesting feature of the patchy network, as revealed by thorough analysis of those horizontal axons radiating out from cells labelled at the injection site. They were found to terminate primarily in remote regions containing either weakly or strongly labelled somata. Two of those neurons, nos 7 and 8, were reconstructed at the injection site. Their specific reciprocal termination pattern is exemplified in Fig. 3C for cell no. 8 at the injection site and its reciprocal counterpart cell no. 10 at a distance of 1.5 mm caudally. The two cells provided clustered axonal terminals to each other's dendritic field. The largest distance in the network between reciprocally connected patches was 2.7 mm for cell nos 3 and 9 (not shown separately).

Identified targets of the patchy axons

Reciprocity and mutual overlapping of patchy axons raises the question of target selectivity. There-

fore, each of the labelled pyramidal cells was thoroughly examined for possible contacts made by axons of other labelled cells. It was found that six of the 10 pyramidal cells received direct contacts onto the distal segment of their apical and basal dendrites from the overlapping patchy axons of other reconstructed pyramidal cells (Fig. 4). The number of such contacts from one pyramidal cell to another was between one and four. Although the small size of those axon terminals approaching dendrites of other pyramidal cells did not allow us to determine unequivocally whether the contacts were made onto the shaft or spines, in some cases it was possible to visualize contacts made onto the bulging head of a dendritic spine (Fig. 4B, E).

DISCUSSION

Technical considerations

Extracellular application of biocytin[26] was introduced primarily for anterograde tract-tracing in the CNS as a substitute for HRP and lectins. We utilized its concomitant retrograde nature and proved that biocytin is particularly suitable for revealing complete labelling of small populations of cells. Cells lying in close proximity to the injection site became labelled probably via their somata and dendrites. Obviously, many of the labelled cells at remote sites could take up biocytin via their axonal terminals overlapping with the injection site. They then transported the tracer retrogradely towards their somata as well as anterogradely into their distal axonal branches. Interestingly, no marked difference was observed in the intensity of axonal processes of the same labelled cell, indicating that biocytin, at least for a distance up to 3 mm, is equally well transported by either mechanism. The completeness of the strongly labelled cells did match the labelling intensity and quality of those intracellularly HRP-labelled neurons published for example by Martin and Whitteridge.[36] Another indication for the completeness of biocytin-labelled cells

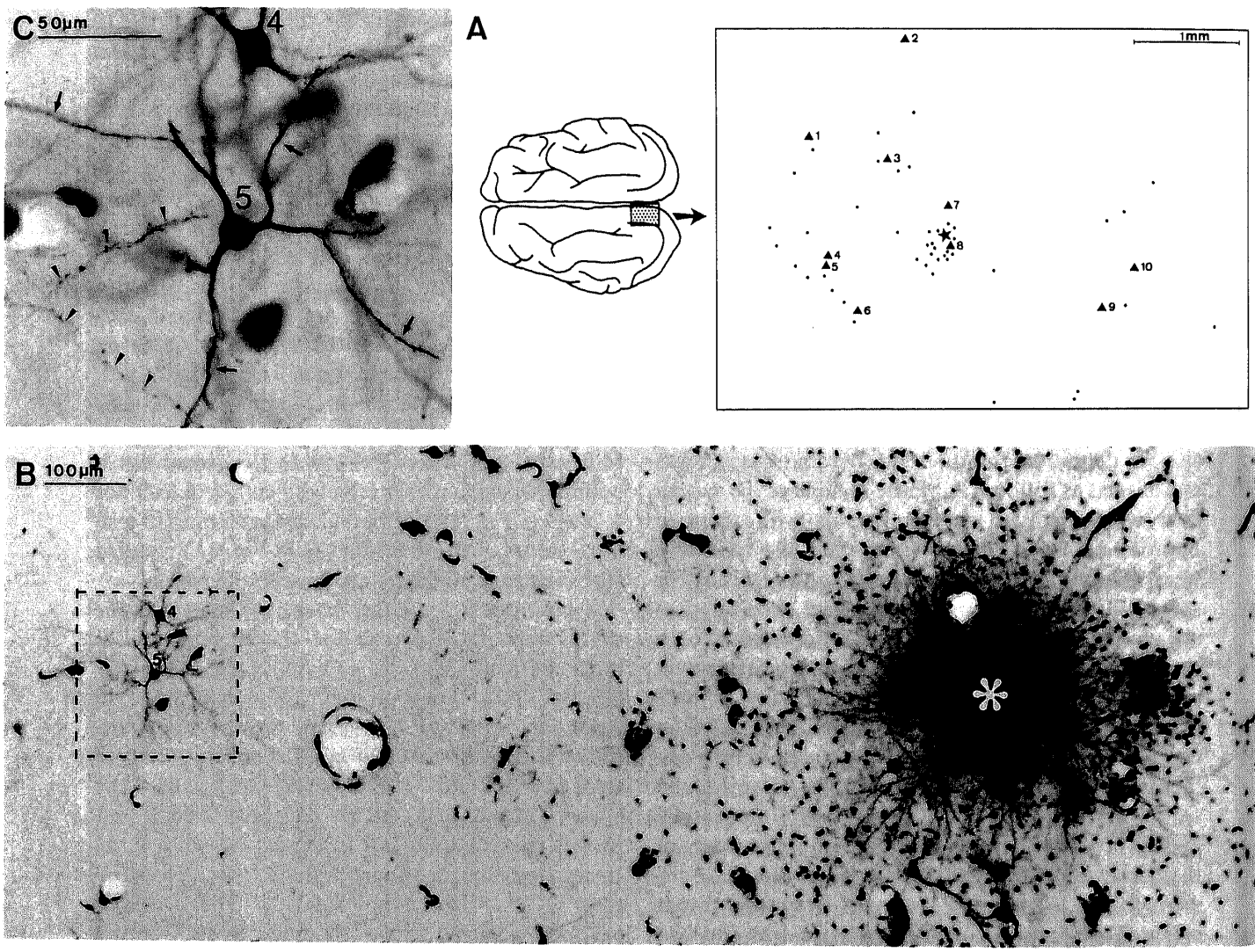

Fig. 1. (A) Outline drawing of the area studied in the cat primary visual cortex (stippled), and an enlarged view (on the right) showing the distribution of retrogradely labelled somata (dots) and the injection site (star). Triangles indicate strongly labelled pyramidal cells (nos 1–10) in layer III that were reconstructed in three dimensions (see also Fig. 3A–C). (B) A horizontal section at the level of lower layer III, containing two retrogradely labelled pyramidal cells (nos 4, 5) in a patch (framed) spaced about 1 mm from the injection site (asterisk). (C) Spine bearing basal dendrites (arrows) and fine axons (arrowheads) of the pyramidal cells framed in B are readily visible. The apical dendrites were present only in more superficial sections.

was that even their finest axons could be traced over considerable distances (e.g. cell no. 8 in Fig. 3C). However, some of the collaterals of a few labelled cells could not be traced as they approached the densely labelled core of the injection site. Many of these axons were, however, giving off side branches towards the injection site, indicating that their zone of termination was at least partially overlapping with the injection site. It is also confirmed here, in agreement with previous findings,[26] that biocytin does not result in labelling of fibres of passage after small injections.

It is worth mentioning that in case nos 2 and 3 the same parameters were used to deliver biocytin. Although the overall patchy pattern of the labelling was similar in both cases, the number and density of the labelled structures differed considerably. It is thus reasonable to assume that, in addition to conventional controllable factors such as parameters of the applied current, and electrode tip size, unknown factors influence the efficacy of the delivery and uptake processes of biocytin.

It should also be noted that the tracer was well deposited and taken up irrespective of polarity of the applied current. This is not surprising since the same phenomenon has been noticed in intracellular experiments.[21] Although only one experiment was done using negative ejection current in the present study (case no. 4), the results extend the intracellular findings in that biocytin can also be extracellularly applied irrespective of polarity.

A further advantage of extracellular applications of biocytin over many of the conventional tracers (e.g. HRP) is that it produces only a narrow rim of halo in the neuropil around the core of the injection site. Therefore, more of the labelled elements can be traced in the vicinity of the injection site and, more importantly the size of the effective uptake region can be assessed more accurately.

Distribution of the patchy axons and the network

An intriguing feature of cortical organization is that apart from the apparent abundance of radial connections many of the fibres, particularly in the

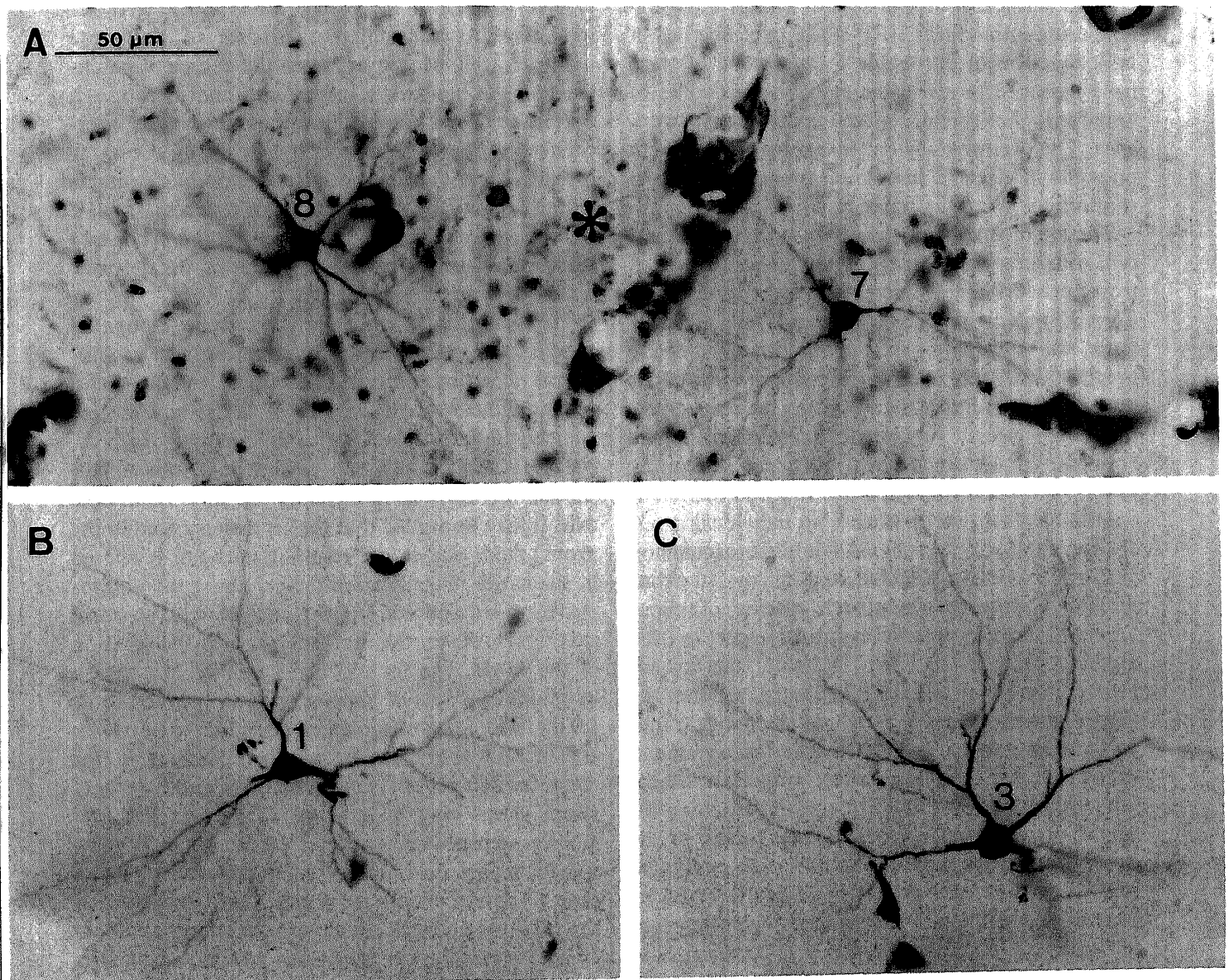

Fig. 2. Light micrographs showing the somata and basal dendrites of four pyramidal cells labelled in layer III. Cell nos 7 and 8 in A at about 150 μm below the centre of the injection site (asterisk). Two other pyramidal cells, no. 1 in B, and no. 3 in C, were at 1.4 and 0.9 mm, respectively, from the core of the injection site. Note that only the somata and basal dendrites are visible at this level.

Table 1. Measurements of the dimensions of the axonal fields, the number of patches, and the number of axonal terminals of the 10 pyramidal cells of case no. 2

Cell no.	Extent of the axonal field (mm) In AP	In LM	No. of axonal patches	Terminals/patch Proximal	Remote	Average	Terminals/cell (Total)
1.	3.2	1.2	5	219	49; 73; 58; 0*	80	399
2.	1.4	1.9	4	30	24; 9; 6*	17	69
3.	3.8	2.2	8	275	272; 194; 147; 140; 106; 82; 73*	161	1289
4.	2.9	1.3	4	57	23; 19; 9*	27	108
5.	2.5	1.0	4	111	199; 188; 0*	125	498
6.	1.8	2.0	4	228	77; 5; 0*	78	310
7.	4.1	1.4	5	68*	72; 71; 32; 31	55	274
8.	4.9	1.9	8	67*	125; 85; 80; 64; 40; 16; 15	62	492
9.	3.9	2.2	5	266	178; 87; 3*; 2	107	536
10.	2.4	2.8	6	130	22; 19; 6; 3; 2*	32	191
Average	3.1	1.8	5.3 (53)	146	63; 75†	79	417 (4166)

Pooled values for the 10 cells are shown in parenthesis. AP, anteroposterior; LM, lateromedial; proximal patch, within the dendritic field of the parent cell. *Injection site; †without values at the injection site.

superficial and the deep layers, run for considerable distances, often several millimetres parallel to the cortical surface. Although several studies are available using intracellular HRP labelling of neurons or bulk injection of retrograde tracers, none of them have managed to reveal the organizational rules of long-range horizontal connections at the network level. The present study has attempted to shed light on some of the fine anatomical properties of these connections using the tracer biocytin.

The major finding of this study is that pyramidal cells with long-range patchy axons establish an anisotropic network that is also patchy and extends up to 6.5 × 3.5 mm in the supragranular layers. Furthermore, the connectivity rules within the network are predominantly reciprocal most likely via connections onto distal segments of the apical and basal dendrites.

The anisotropic structure of connectivity in the network apparently derives from the inherently patchy distribution of each of the 10 pyramidal axons and the fact that the patches from individual axons are not randomly distributed but share a common territory in many cases. Although all the 10 axons showed a consistent interpatch spacing of about 1.1 mm they were quite different with respect to their topographical arrangement. Notably, the axonal patches were not equally distributed around the parent soma resulting in an overall axonal field that is largely asymmetrical. This feature of the pyramidal axons can be appreciated even without statistical assessment (Fig. 3A). One argument questioning the degree of contribution by long-range axons to the patchy phenomenon would be that each pyramidal cell has its own local axonal arbor which would by itself cause a patchy axonal distribution. However, in the light of the present findings this does not hold because the remote axonal patches were often very comparable in size and density to the local axonal arbor of other cells as shown in Fig. 3C for the remote axonal patch of cell no. 8 in comparison with the local axons of cell no. 10. Nevertheless, other remote patches were less dense and contained fewer axonal terminals, suggesting a certain degree of weighting between different patches of the same pyramidal cell. Moreover, when the degree of patchiness of the network is taken into consideration it should be borne in mind that the main axons interconnecting the patches are invariably of the thick myelinated type and do not provide synaptic contacts like their thin collaterals within the patches. Thus, if we consider that the effector sites are the synapsing axon terminals, the patchy character of the network would be even more apparent.

The majority of the cells showed an elongated axonal field chiefly in anteroposterior and mediolateral directions which correspond to the main visuotopic axes at this region of the striate cortex; namely, the vertical and horizontal meridian, respectively (Table 1). Only two of the cells (nos 6, 10) showed no obvious preference for any visuotopic axis. It may be argued that the asymmetry seen in the axonal fields and in the resulting network is related to the larger magnification factor, along the anteroposterior as opposed to the mediolateral cortical axis.[37,51] However, the markedly uneven distribution of the axons, like for cell no. 8 in Fig. 3C, with a longitudinal extent of almost 5 mm as opposed to its 1.9 mm width in a mediolateral direction, rules out an explanation solely based upon a difference in the magnification factor along different visuotopic axes which varies only by a factor of 1.5. As has previously been pointed out[17,18,50] the size of such widespread axonal fields in the supragranular layers of area 17 would enable these cells, and consequently the resulting network, to act over distances of about 10–15° in the visual space, far beyond their own receptive field sizes that have been reported to have an excitatory region of about 1–3°[16] (see below). Such a widespread axonal arborization pattern is, however, not exclusive to pyramidal cells in the upper layers.

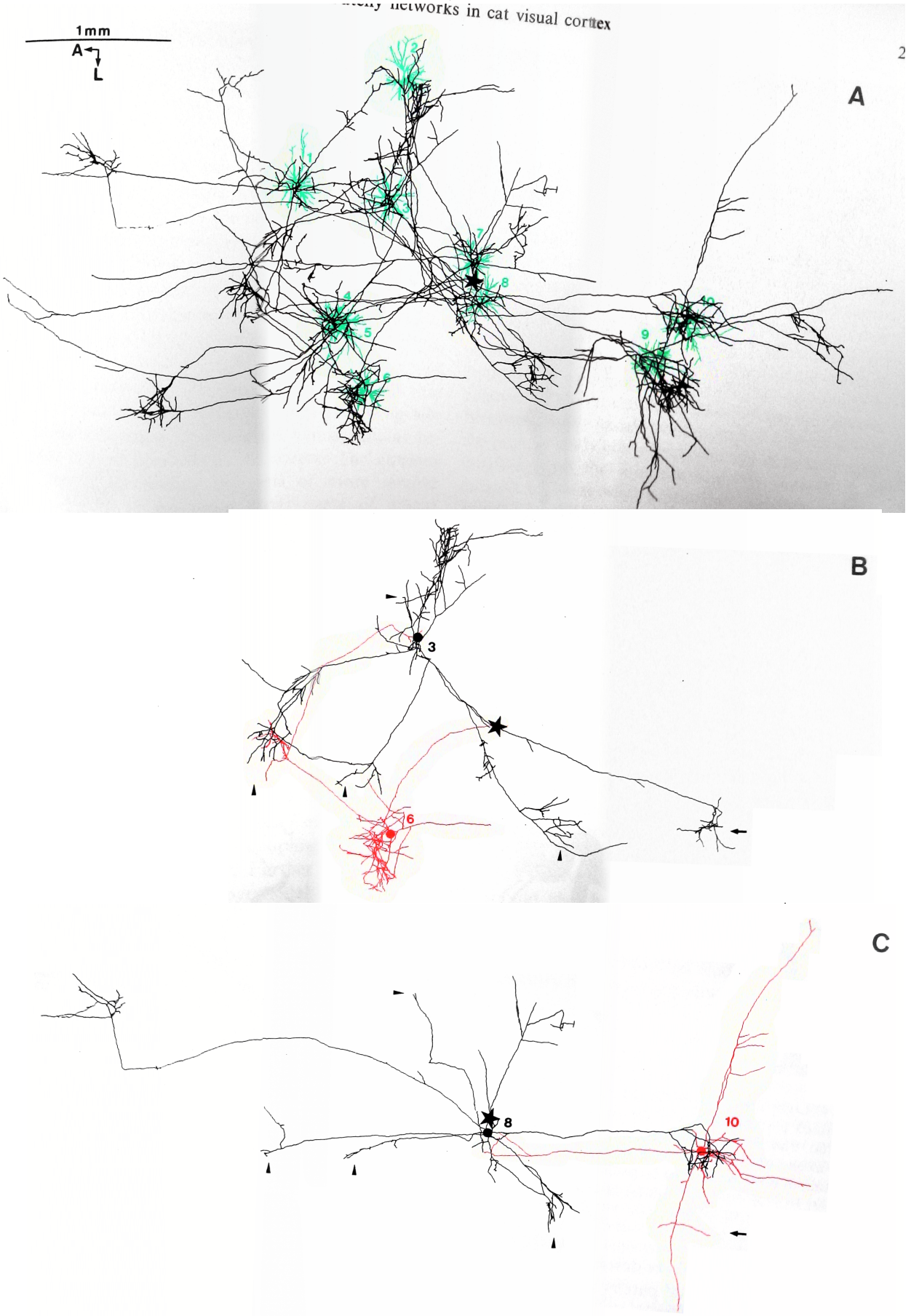

Fig. 3. (A) Composite drawing of the complete axonal (in black) and dendritic (in green) distribution of 10 pyramidal cells (nos 1–10) labelled from the same injection site (black star) also shown in Fig. 1. (B, C) Distribution of the axonal fields for cell nos 3 (in black) and 6 (in red) in B, and for cell nos 8 (in black) and 10 (in red) in C. Additionally, overlapping axonal patches provided by cell nos 3 (in B) and 8 (in C) are indicated by arrowheads. One of the axonal patches (arrowed in B) belonging to cell no. 3 was also in register with axon collaterals (arrowed in C) provided by cell no. 10. Injection site is marked by star in A–C.

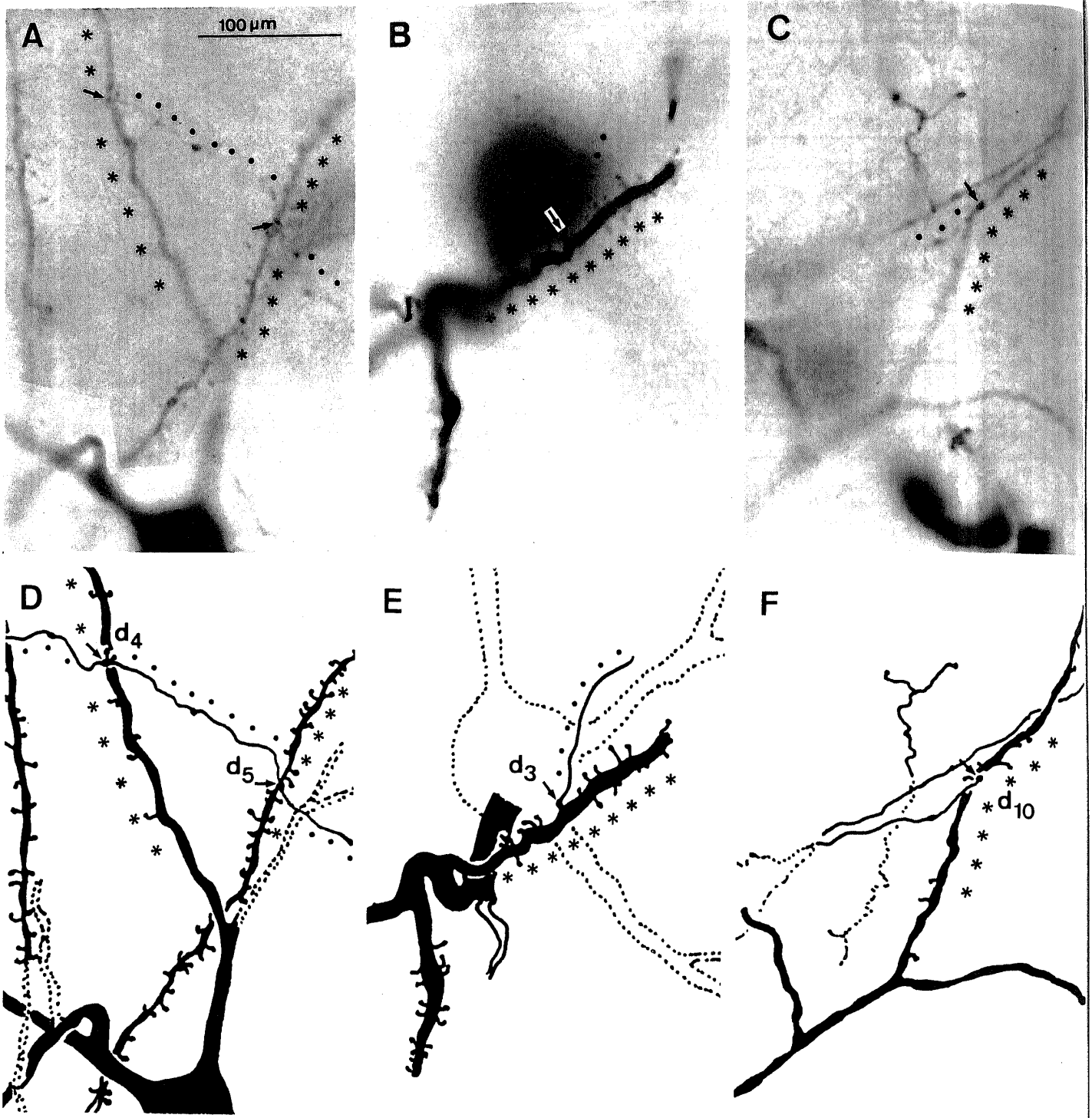

Fig. 4. Light micrographs (A–C) and corresponding drawings (D–F) showing putative contacts between identified pyramidal cells of the network shown in Fig. 3A. Axons of pyramidal cells no. 8 in A and C, and no. 9 in B contact the dendrites (d) of pyramidal cell nos 4 and 5, 3, and 10, respectively. Dendrites and axons out of focus in A–C are shown by dotted contours in D–F. Contacts between the small pyramidal boutons and the targeted dendrites are arrowed. For better visualization the course of the axons (dots) and the dendrites (asterisks) are also marked.

Recent intracellular studies have described pyramidal neurons possessing horizontal patchy axonal fields in the deep layers, chiefly in layer V.[15,17] Because in area 17, layer V receives its main intracortical input from layer III[12,40] it would be interesting to know whether the axonal patches originating from pyramidal cells in the supra- and infragranular layers of the very same cortical column would lie in precisely the same register. The analysis of intracellularly HRP-filled layer III pyramidal cells strongly supports such an hypothesis; notably, their axonal patches in the deeper layer are located under those in the upper layer.[18,28] Interestingly, in the present study, none of the 10 pyramidal cells provided axonal patches in the deep layers. However, given that layer V pyramidal cells send their apical dendrites up to layer I it is conceivable that they can readily sample patchy pyramidal inputs in the upper layers.

Some characteristics of the fine organization of long-range connections have been disclosed by electrophysiological experiments using the current-source density analysis method.[33] It was shown that supragranular responses were recordable up to 2.5 mm from the stimulation site. This is exactly half the distance spanned by the axon of the largest cell (cell no. 8 in Fig. 3C) in the network shown here. On the basis of latency measurements, those connections were considered monosynaptic, for which the present study provides several examples. Furthermore, the amplitude of the responses suggested an inhomogeneous tangential distribution of the connections which is indeed also the case for single cells as well as for the network. The same study indicated that with increasing distance between the recording site and the stimulating electrode the supragranular responses moved upwards in the cortex. The authors interpreted this finding in terms of more remote inputs synapsing on more distal parts of apical dendrites. Although our anatomical findings indicate that patchy axons terminate on distal apical dendrites, the number of contacts does not allow us to draw any further conclusion concerning their fine distribution on the targets as a function of distance from the parent cells.

Convergence and divergence

It has been predicted from Golgi studies that pyramidal cells are interconnected by one or very few contacts.[48,49] A similar conclusion was drawn from recent electrophysiological experiments using cross-correlation technique in area 17 of cat.[50] The results indicated weak excitatory coupling between neurons spaced up to 3 mm apart. Further indication for the sparse input from one pyramidal cell onto another was given by electron microscopic analysis of intracellularly labelled pyramidal cells with patchy axons showing the lack of multiple contacts onto the same target element.[28] The same study showed the quantitative distribution of postsynaptic targets and revealed that the overwhelming majority of the targets was dendritic spines (85%). However, their exact source could not be unequivocally defined.[28] In this respect, the present study provides evidence for the type of cells whose spiny dendrites are targeted and the number of contacts onto them provided by the patchy axons of layer III pyramidal cells. Our data confirm previous assumptions,[48,49] and document that direct connection from one pyramidal cell to another is most often made by one to four contacts, though it is likely that this represents an underestimation. The above electron microscopic study[28] also showed that about 5% of the target elements of layer III pyramidal cells contain GABA. The present study was not able to disclose the type of GABAergic cells receiving long-range excitatory input from layer III pyramidal cells. However, it could be emphasized that any significant amount of long-range excitatory input to those inhibitory neurons as well as pyramidal cells can be made only via strong convergence of a number of patchy axons. This argument is further supported by an estimate* based upon the present findings and available quantitative data for the total number of cells and putative excitatory synapses in the upper layers, suggesting that each patchy axon may contribute only a small proportion, up to 0.1%, of the total excitatory input to its target cells.

Another estimate† was made for the overall influence of the patchy axons on the total cell population of a single patch. The results show that, on average, only about 1–3% of the cells in a given patch are contacted by the same axon originating from a remote site. Furthermore, although our sample is relatively small, it should be remembered that no two or more patchy axons overlapping at a given site in the present study converged onto the same target cell labelled from the injection site. At present, it is difficult to assess what proportion of cells is directly affected by the long-range patchy system. This study and previous studies[19,32,33] clearly indicate that the number of retrogradely labelled cells at remote sites greatly depends on the size and laminar location of the injection site, and the sensitivity of the method applied for tracer detection. Therefore, no exact value can be given as yet. However, the above estimates for the number of contacts and target cells of a patchy axon led us to assume that, most probably, not all pyramidal cells in layers II and III are under the influence of long horizontal patchy axons.

Reciprocity is another fundamental property of the network. Mutually overlapping axons between remote sources indicate that whatever functional signals are transmitted by these connections, they are most likely under feed-back control. Nonetheless, it should also be emphasized that such a reciprocal system does not require monosynaptically transmitted feed-back at the cell-to-cell level. Indeed, directly interconnected feed-back loops between excitatory cells were not observed in our material. Such

*The total number of terminals per neuron is 6000 in the supragranular layers of the binocular region of area 17,[5] about 20% of which are GABA-immunoreactive terminals containing small pleomorphic or large ovoid vesicles.[6] Taking the remaining 4800 terminals per neuron and one to four terminals per pyramidal cell provided by an identified long-range patchy axon onto its target neuron, it is then calculated that each target neuron receives about 0.02–0.08% of its total excitatory input from the same patchy axon.

†The average number of cells in the supragranular layers (I–III) of the binocular region of area 17[4] is 27,800/mm^2, about 20% of which are GABAergic interneurons.[14] The remaining 22,240 are non-GABAergic neurons, mostly pyramidal cells. Considering that the average axonal patch size is 400 μm in diameter, a conservative estimate of 0.13 mm^2 per axonal patch would then cover an area comprising 2891 neurons of non-GABAergic types. Using an average value of 79 terminals per axonal patch and one to four contacts per neuron (see Results) it can be estimated that about 1–3% of all pyramidal cells of the targeted region can be contacted by the same patchy axon.

excitatory feed-back could evoke oscillations or abnormal hyperactivity in a network like this.

Relationship between the patchy network and orientation columns

Long-range fibre systems have been suspected to subserve particular receptive field (RF) characteristics, e.g. the emergence of long RFs by spatially extended excitatory convergence.[8,17,18] The extremely large extent of the patchy axons shown in the present and earlier studies[18] certainly requires the consideration of their functional role in relation to known columnar systems. One possible interpretation is that they establish direct links between certain functional columns comprising clusters of neurons with similar[39] or dissimilar receptive field[37] characteristics, such as orientation selectivity. Importantly, one has to conceive that most of the cells in the supragranular layers of area 17 have relatively small RFs and belong to the complex family with strong side-band inhibition within their RF.[7,16] It seems unlikely that any of these RF attributes require long-range horizontal connections. Furthermore, most cells are binocularly driven, thus linking of ocular dominance is also unlikely as an underlying phenomenon. At present, the most plausible role of long-range connections is to establish links between cells of similar orientation selectivities. There are two alternative models concerning this issue, one suggesting that columns with unlike orientations are interconnected,[37] while the other predicts that columns with like orientation preferences innervate each other.[39] Several lines of evidence support the like-orientation model for our results. Firstly, the patches of pyramidal cells labelled from the same injection site are reciprocally interconnected with each other. If the cross-orientation model applies, these pyramidal cells would show similar orientation preferences both orthogonal to that at the injection site and they would not send overlapping patchy axons to each other's dendritic fields which in turn show iso-orientation tuning. It needs to be added, however, that the results of Matsubara *et al.*[37] were obtained in area 18 and were thought to involve inhibitory cells which may account for the inconsistence with our present findings in area 17. Secondly, cross-correlation experiments have demonstrated excitatory interactions between cells of like orientation preferences recorded in columns separated by up to several millimetres in area 17.[38,41,50] Thirdly, combined anatomical and physiological evidence for the functional specificity of long-range connections was given by the work of Gilbert and Wiesel[19] who demonstrated that after depositing a small amount of retrograde tracer (rhodamine-filled latex beads) into layers II and III in area 17, labelled cells appear in clusters over regions with like orientation preferences shown by 2-deoxyglucose labelling in the same specimen. The patchy distribution of those labelled somata and the overall pattern of the patchy axonal network shown in the present study appear remarkably similar. Further support for the putative functional relationship between the network and the topographical arrangement of orientation columns derives from the quantitative analysis of 2-deoxyglucose labelling in area 17.[31] On the one hand, the centre-to-centre spacing of the iso-orientation bands[31] (1.0–1.1 mm) correlates with the average spatial segregation of neighbouring patches (1.1 mm) within the network formed by the 10 pyramidal axons in this study. On the other hand, the periodic beaded appearance of the iso-orientation bands with an average centre-to-centre bead distance of 0.9–1.2 mm[31] again resembles the spatial parameters of the patchy network. It thus seems very likely that the beaded appearance observed in the 2-deoxyglucose maps is related to the patchy intracortical connectivity system revealed here. It is less clear, however, what type of connections underlie the longitudinal parallel arrangement of iso-orientation slabs which have a major trajectory orthogonal to the area 17/18 border irrespective of the stimulus orientation used.[46] The iso-orientation slabs may well reflect the topographical layout of incoming thalamic afferents, as suggested by Löwel *et al.*,[31] and/or intrinsic connections different from those presented here. In this respect it is interesting to see that many of the cells of the network, e.g. cell no. 8 in Fig. 3C with axons spanning almost 5 mm in an anteroposterior direction, could actually bridge over five iso-orientation slabs.[1,46] It may be speculated that these connections could produce facilitatory effects between neurons whose optimal visual stimuli are co-oriented and co-axially aligned as suggested by Nelson and Frost.[41]

Other possible roles of the network

The reciprocal excitatory connections shown here between patches of pyramidal cells may be involved in a more global functional phenomenon in the visual cortex, namely in synchronized oscillatory responses observed in visual cortical cells with non-overlapping receptive fields and similar orientation preferences.[10,11,20] Synchronization in oscillatory responses within the same cortical area of the cat can occur up to a distance of 7 mm,[10] which is comparable to the 6.5 mm tangential extent of the network shown here. Furthermore, it has been emphasized that synchronized oscillatory events do not have to depend on monosynaptic connections.[10,11,20] Concerning this, the reciprocal network presented here might be a suitable anatomical substrate.

In addition, it is worth noting that the results indicate that virtually any part of area 17 can be reached via myelinated axons and through only a few synapses, permitting rapid integration of information from locations extremely far from each other in visual space. Thus, it is conceivable that successively linked reciprocal patchy networks, such as the one shown here, may represent the cellular substrate for global-linking tasks in the visual cortex,[2,3,25,35,52] e.g. between sites of iso-orientation preference.

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