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Axonal and dendritic arborization of an intracellularly labeled chandelier cell in the CA1 region of rat hippocampus

X-G. Li[1], P. Somogyi[2], J.M. Tepper[1], and G. Buzsáki[1]

[1] Center for Molecular and Behavioral Neuroscience, Rutgers, The State University of New Jersey, 197 University Avenue, Newark, NJ 07102, USA
[2] MRC Anatomical Neuropharmacology Unit, Mansfield Road, Oxford OX1 3TH, UK

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Summary. During the course of an in vivo intracellular labeling study, a chandelier (axo-axonic) cell was completely filled with biocytin in the CA1 region of the hippocampus. Chandelier cells are known to provide GABAergic terminals exclusively to the axon initial segment of pyramidal cells. The lateral extent and laminar distribution of the dendritic arborization of the chandelier cell was very similar to that of pyramidal cells; the numerous basal and apical dendrites reached the ventricular surface and the hippocampal fissure, respectively. The dendrites, however, had very few spines. The neuron had an asymmetric axonal arbor occupying an elliptical area of 600 by 850 μm in the pyramidal cell layer and stratum oriens, with over three-quarters of the axon projecting to the fimbrial side of the neuron. Counting all clusters of terminals, representing individually innervated axon initial segments, the chandelier cell was estimated to contact 1214 pyramidal cells, a number that exceeds previous estimations, based on Golgi studies, by several-fold. The findings support the view that chandelier cells may control the threshold and/or synchronize large populations of principal cells.

Key words: Interneuron – Axo-axonic cell – Pyramidal cell – Inhibition – Rat

Introduction

Inhibition mediated by gamma-aminobutyric acid (GABA) is an essential element of hippocampal operations. Although some of the GABAergic innervation derives from extrahippocampal sources (Freund and Antal 1988), GABA is mainly released from the terminals of local circuit neurons that exhibit great target specificity in the placement of their synaptic terminals. The most selective of all local circuit neurons is the chandelier or axo-axonic cell, so named because its terminals establish synapses exclusively with the axon initial segment of pyramidal and granule cells (Somogyi 1977). Following the discovery of chandelier cells by Szentágothai in the neocortex (Szentágothai and Arbib 1974), these cells were first described in the CA1 region of the monkey hippocampus (Somogyi et al. 1983), then were subsequently found in the CA1 area of the cat (Somogyi et al. 1985) and in the dentate gyrus of the rat (Kosaka 1983; Soriano et al. 1990; Soriano and Frotscher 1989). However, they have not yet been encountered in the most thoroughly studied area, the CA1 region of the rat hippocampus. Furthermore, all previously described chandelier cells were detected by Golgi impregnation and the processes of the cells, particularly the axon, could only be revealed partially. Therefore, the extent of the axonal arborization and, in particular, the number of pyramidal or granule cells innervated by this specialized type of local circuit neuron has remained unknown.

In the course of in vivo intracellular recording and marking of hippocampal neurons, we filled a chandelier cell whose axonal and dendritic arborization is complete. The axonal arborization of this neuron exceeds all previously reported axons of putative inhibitory interneurons several-fold. We have reconstructed the cell and estimated the number of pyramidal cells influenced by its terminals.

Material and methods

Fifty four adult rats of both sexes were anesthetized with urethane (1.3 g/kg, i.p.) and fixed in a stereotaxic apparatus. The scalp was removed and two small bone windows (1 × 2 mm) were drilled above hippocampus (A: 2–4 mm from bregma, L: 2.5 mm). After the recording electrode was inserted into the brain the bone windows were filled with a mixture of paraffin and paraffin oil, in order to prevent drying and decrease pulsation.

Micropipettes for intracellular recording and biocytin injection (Horikawa and Armstrong 1988) were pulled from 2.0 mm OD capillary glass. They were filled with a solution of 3% biocytin (Sigma, St. Louis, MO, USA) in 1 M potassium-acetate, and possessed in vivo impedances ranging from 50 to 80 MΩ. Once stable intracellular recording was achieved, biocytin was injected through a

Correspondence to: X.G. Li

bridge circuit (Axoprobe-1A), with a 50% duty cycle of 300 ms depolarizing pulses at 8–10 nA for 30–60 min. Neuronal activity was monitored throughout the procedure. Postinjection survival times ranged from 1–2 h. The one CA1 region chandelier cell, reported here, was one of two interneurons from a total of 35 intracellularly filled cells.

Following urethane overdose, animals were perfused intracardially with 100–200 ml normal saline followed by 400 ml of 4% paraformaldehyde and 0.2% glutaraldehyde dissolved in phosphate buffered saline (PBS, pH = 7.3). The brains were removed and stored in the same fixative solution overnight. Sixty μm thick coronal sections were cut on a Vibratome and collected in PBS. After washing for 5 min in 0.5% hydrogen peroxide to inactivate endogenous peroxidases, sections were incubated for 4 h in the avidin-biotin-HRP complex (Vector Lab., Burlingame, CA), diluted 1:200 in 1% triton X-100 dissolved in PBS. Peroxidase enzyme activity was revealed by incubating sections for 30 min in PBS containing 3′-3-diaminobenzidine tetrahydrochloride (0.06%) and hydrogen peroxide (0.003%) for 30 min. Sections were thoroughly rinsed and mounted on gelatin-coated slides, dehydrated, cleared in xylene and embedded in DPX resin under coverslips.

The neuron was drawn using a drawing tube and a 100× oil-immersion objective. For sake of clarity, only the main axon trunks and the terminal segments, showing strings of varicosities, corresponding to synaptic boutons, are represented. The plane of the sections ran at a slight angle to the plane defined by the apical dendrites of pyramidal cells, which is approximately perpendicular to the laminar boundaries. Therefore, the laminar boundaries appearing in consecutive sections shifted gradually when subsequent sections were superimposed in the plane of the sections. For this reason, and also because the axon terminals had very high density in the pyramidal layer and in stratum oriens, the axonal terminal fields are represented separately for most sections. The string of varicosities appearing to follow single initial segments were marked and counted in individual sections, thereby providing an estimate of the number of postsynaptic pyramidal cells. Measurements were not corrected for tissue shrinkage; sections dried onto slides do not shrink during dehydration in the x–y dimensions, however, some shrinkage occurs during fixation.

In order to reconstruct the terminal density in the plane parallel with the pyramidal cell layer the axonal arborization was divided into 60 μm wide bins. Since the sections were 60 μm thick before drying them onto the slides a 60 by 60 μm grid was obtained in the tangential view (Fig. 1C). For this view, the dendritic arborization was realigned so that it is viewed parallel with the main apical dendrite rather than at an angle to it.

Results

Although only one cell was detected in the intracellular electrophysiological records, two neurons, a pyramidal cell and a chandelier cell were filled by biocytin. This is a common observation with biocytin as well as with other intracellular markers and is usually described as dye-coupling (e.g., Kawaguchi et al. 1989).

The cell was located in the most septal segment of the right dorsal hippocampus where the layers bend relative to the stereotaxic coronal plane (Fig. 1). The reconstruction in Fig. 1 shows the cell as it is situated in the brain. In both the septal and temporal directions all axon collaterals terminated in boutons, therefore the axon is regarded as complete.

The neuron was identified as a chandelier or axo-axonic cell based on the following criteria, unique to this type of interneuron (Somogyi et al. 1983, 1985): (a) the terminal axon segments carrying the synaptic boutons are concentrated in the half of the stratum pyramidale bordering stratum oriens and in the half of the stratum oriens adjoining stratum pyramidale (Figs. 1, 2A). This is in contrast to the axonal arborization of intracellularly filled basket cells (Z-S. Han, E. Buhl, P. Somogyi unpublished observation) which provide terminals with equal density to the whole thickness of the pyramidal cell layer. (b) The axonal varicosities are grouped into clusters of 2–10 boutons separated by thin non-varicose segments of the axon. In many cases the varicosities are in a single row parallel with the presumed course of the axon initial segments of pyramidal cells (Fig. 2B).

In addition to the above described and expected features, several further characteristics were revealed as a result of the complete visualization of all neuronal processes. The dendritic arborization matched well the dendritic arbors of pyramidal cells, both in lateral extent and in laminar distribution. However, in contrast to the densely spinous dendrites of the pyramidal cell, the dendrites of the chandelier cell exhibited only a few dendritic spines (Fig. 2C) and some dendrites were completely spine free. Numerous small dendritic branches, many of them reaching the ventricular surface of the hippocampus, were found among the axons of the alveus. The major apical dendrite terminated in a dense tuft in the stratum lacunosum-moleculare (Figs. 1A, 2D) similar to that of pyramidal cells, but more restricted in lateral extent. The other dendrite descending in the stratum radiatum also reached the stratum lacunosum-moleculare with its thin terminal branches. However, as the dendrite coursed temporally, it falsely appears to terminate in the stratum radiatum (Fig. 1A) due to the curvature of the laminar boundaries in three dimensions which could not be represented in a two dimensional reconstruction. The dendritic arborization is located medial and asymmetric to the axonal field and covers an area of about 200 by 300 μm as viewed in the tangential plane (Fig. 1C).

The dendritic branches in the alveus and stratum oriens are in the same position as the local axon collaterals of the pyramidal cells. This could easily be established, since in addition to the chandelier cell, a well-filled pyramidal cell was also present whose axon collaterals mixed with the chandelier cell dendrites. At four positions the pyramidal cell axon was in direct contact with the chandelier cell dendritic branches suggesting synaptic input.

The main axon entered the stratum radiatum, where it emitted secondary branches which re-entered the pyramidal cell layer and travelled horizontally both among pyramidal cells and in stratum oriens. This "weeping willow" arrangement of the main axonal branches (upside down in Fig. 1) has been frequently represented as a characteristic feature of basket cell axons (Ramón y Cajal 1911; Lorente de Nó 1934). All branches in the drawings were traced back to the main axon. The axon of the chandelier cell could also be distinguished from the pyramidal cell axon since the latter emitted more straight, thinner collaterals with fewer and smaller boutons which, in contrast to the chandelier cell collaterals, were mainly located in the deep part of stratum oriens.

The axonal arborization covered an elliptical area of the pyramidal cell layer of about 600 by 850 μm. Within

this area the terminal segments showed an uneven distribution, the highest density occupying an area elongated in the septo-temporal direction. The density of terminal segments, reflecting the density of innervated pyramidal cells, fell laterally (i.e., towards CA3) and towards the edges of the axonal field. Clear 40–50 μm wide groupings of segments can be observed in some individual sections (e.g. 8, 9, in Fig. 1B) producing a banding of the innervated initial segments of pyramidal cells. This is not so obvious in the tangential view because the bin width was too large to reveal it accurately. A smaller bin width could not be chosen because the tissue shrank during processing in the direction perpendicular to the plane of the section.

The approximate number of pyramidal cells innervated by this chandelier cell can be estimated assuming that each cluster of terminals provides input to one pyramidal cell initial segment. The clusters appearing to follow an initial segment as a line of boutons were identified and marked using a 100× oil immersion objective. Previous electron microscopic evidence shows that one cluster usually is associated with one initial segment (Somogyi et al. 1983, 1985), although if the initial segments run close to each other, some terminals may also innervate adjacent initial segments. Counting the terminal segments consisting of varicosities provided a number of 1214 innervated pyramidal cells. A slight overestimation may have resulted from the segments cut on the surface of sections which may have occasionally been included twice. This overestimation would be counterbalanced by a slight underestimation of not counting single varicosities and including them into the nearest cluster.

Discussion

It is somewhat surprising that chandelier cells have not been encountered in the extensively studied rodent hippocampus. This lack of data may be more apparent than real, because cells, like the chandelier cell, providing terminals in and around the pyramidal cell layer have either been grouped collectively as "non-pyramidal" cells (Buzsáki 1984; Kawaguchi and Hama 1987), or described as a general category of so called "basket cells". The latter term derives from Cajal (1911) who suggested that cells with dense terminal arbors around the pyramidal cell layer contacted the somata of pyramidal cells. Undoubtedly, the large number of GABA containing boutons on the somata of pyramidal cells and the presence of numerous GABAergic cell bodies in positions where Cajal's cells were illustrated makes it very likely that these cells supply the somata with inhibitory synapses (Ribak et al. 1981). However, there is a distinct cell type, the chandelier cell, which also terminates in and around the pyramidal cell layer, but never innervates the cell bodies. In the classical Golgi studies (Ramón y Cajal 1911; Lorente de Nó 1934), the cell bodies of many of the so called "basket cells" were in the pyramidal cell layer. Subsequently in many cases it was assumed that non-pyramidal cells with their somata in the pyramidal cell layer were "basket cells", without any information on their axon. It is now clear that chandelier cells with their somata in the pyramidal cell layer and terminals in strata pyramidale and oriens are distinct from the cells that supply inhibitory terminals to the pyramidal cell somata. Therefore, in keeping with Ramón y Cajal's hypothesis (1911), the term "basket cell" should only be used for cells whose axon provides terminals to the somata of pyramidal cells. This distinction is not only semantic, but emphasizes the dual innervation of the soma and axon initial segment by GABAergic terminals (Somogyi et al. 1983, 1985), providing an independent output control at two distinct levels.

Previous estimations of the degree of divergence of chandelier cell-to-pyramidal neurons vary from 330 and 268 in the cat visual cortex (Somogyi et al. 1985; Freund et al. 1983), 166 in the kitten motor cortex (Somogyi et al. 1982), to 323 and 202 in the CA1 region of the cat and monkey hippocampi respectively (Somogyi et al. 1983, 1985). The present estimate of about 1200 innervated pyramidal cells by one chandelier cell in the rat CA1 region supersedes previous estimates based on partially visualized cells several-fold.

It has been calculated from direct counts of synapses in the monkey hippocampus that about 3 chandelier cells converge on one pyramidal cell (Somogyi et al. 1983). Similarly, in the neocortex of the cat a convergence of 5 chandelier cells was estimated (Freund et al. 1983). In the absence of electron microscopic evidence from identified chandelier cells in the rat CA1 region, the degree of convergence is less certain. In a detailed electron microscopic study, between 100 to 200 symmetric synapses were estimated on the axon initial segment of CA3 pyramidal cells (Kosaka 1980). This number was smaller (40–78) in the monkey CA1 region (Somogyi et al. 1983). Since a given chandelier neuron provides 2–10 boutons to a single pyramidal cell as seen here, a pyramidal cell could be under the influence of 4–10 chandelier cells. Because there are about 300,000 pyramidal cells in the CA1 region of the rat (Cassell 1980; Boss et al. 1987) and each chandelier cell innervates approximately 1,200 CA1 pyramidal neurons (assuming the present findings as typical), an estimate of 500 to 2,000 chandelier cells in the CA1 region of the rat hippocampus is obtained.

To date, no information is available on the physiological properties of hippocampal chandelier cells and their functional properties can only be hypothesized from their morphological characteristics and computer simulation studies (Lytton and Sejnowski 1991; Douglas and Martin 1990). A striking feature of our chandelier cell was the paucity of dendritic branches in the stratum radiatum and rich arborizations of the basal dendrites in the stratum oriens and apical dendrites in the stratum lacunosum-moleculare, respectively. This dendritic arrangement suggests that chandelier cells are activated by subcortical, entorhinal and commissural afferents, but only weakly, if at all, by the associational system (Schaffer collaterals). Recurrent activation of chandelier neurons by pyramidal cells is very likely, since there is a rich dendritic plexus in stratum oriens and we found several boutons originating from the simultaneously labeled pyramidal cell contacting the basal dendrites of the chandelier cell.

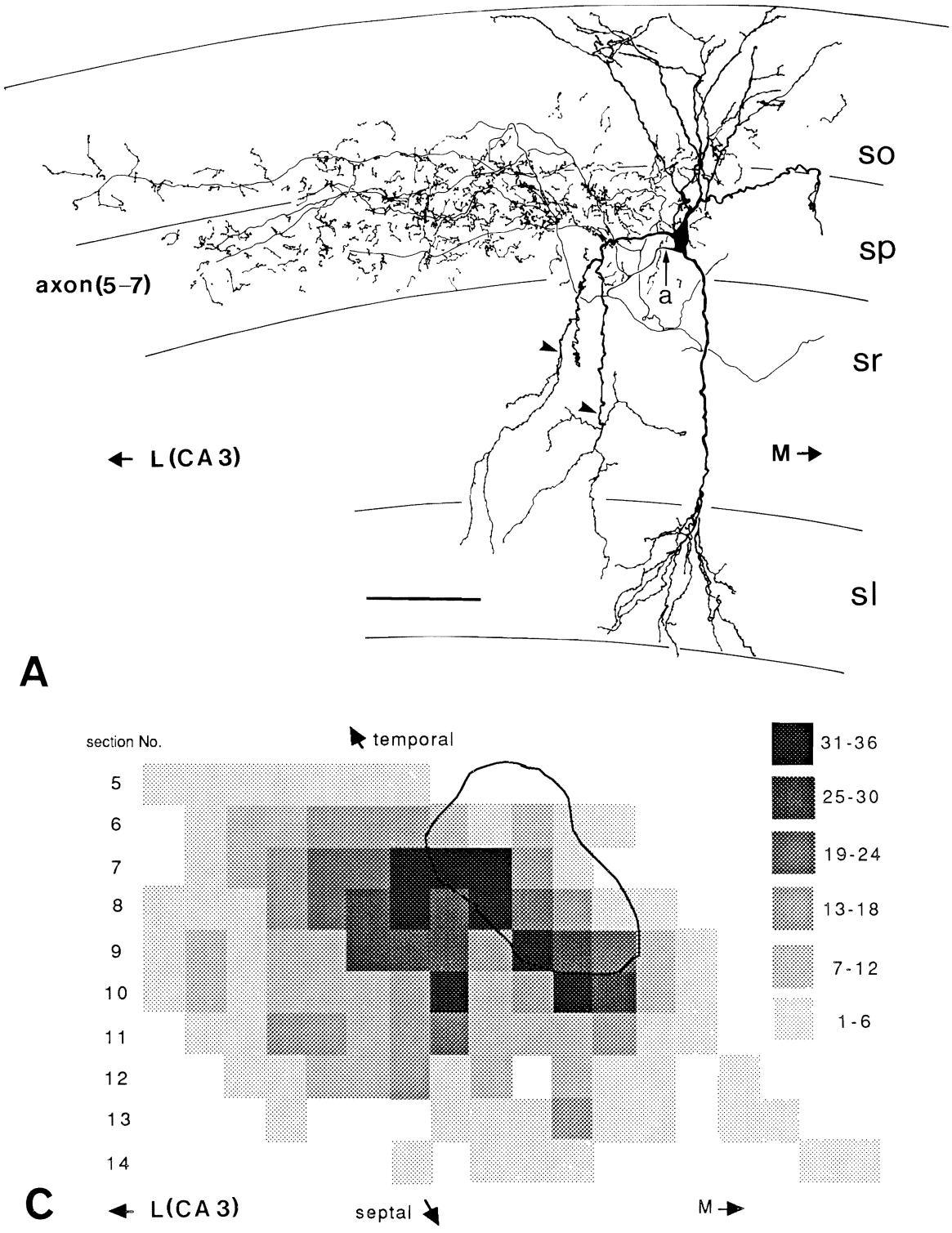

Fig. 1A–C. Axonal and dendritic arborization of a chandelier cell in the CA1 region of the rat dorsal hippocampus intracellularly labeled with biocytin. Dorsal is to the top of the plate. For clarity only the main axon trunks (*long lines*) and the terminal segments connecting boutons are shown. **A** The dendritic arbor was reconstructed from camera lucida drawings of sections 1–10, the axonal arbor (axon initial segment at "**a**") is shown here from the superimposed sections 5–7. Note the asymmetrically situated dendritic field and that all layers are covered by the dendrites. Similarly to pyramidal cells, numerous branches are given in the alveus and in the stratum lacunosum-moleculare. The dendrites marked by arrowheads also branched in lacunosum-moleculare, but falsely appear in stratum radiatum because of the two-dimensional projection of curving layer boundaries. **B** Most of the axon terminal segments are concentrated in the stratum oriens and in the dorsal half of the pyramidal cell layer. The soma and the origin of the axon (**a**) found in sections 7–8 are superimposed on the axonal fields in sections 8 and 9 as reference for alignment with **A**. In the subsequent sections the star first shown in section 9 serve as reference for alignment. The borders of stratum pyramidale are not indicated in the superimposed sections 12–14 where the pyramidal cell layer was cut at a tangential plane. **C** Dorsal view of the axonal (*shaded*) and dendritic (*continuous line*) fields. Numbers on left denote section numbers, on the right the number of innervated pyramidal cells. *L*, lateral (towards CA3); *M*, medial; *sl*, stratum lacunosum-moleculare; *so*, stratum oriens; *sr*, stratum radiatum; *sp*, stratum pyramidale. Scales: **A**, **B** 100 μm; **C** squares 60×60 μm

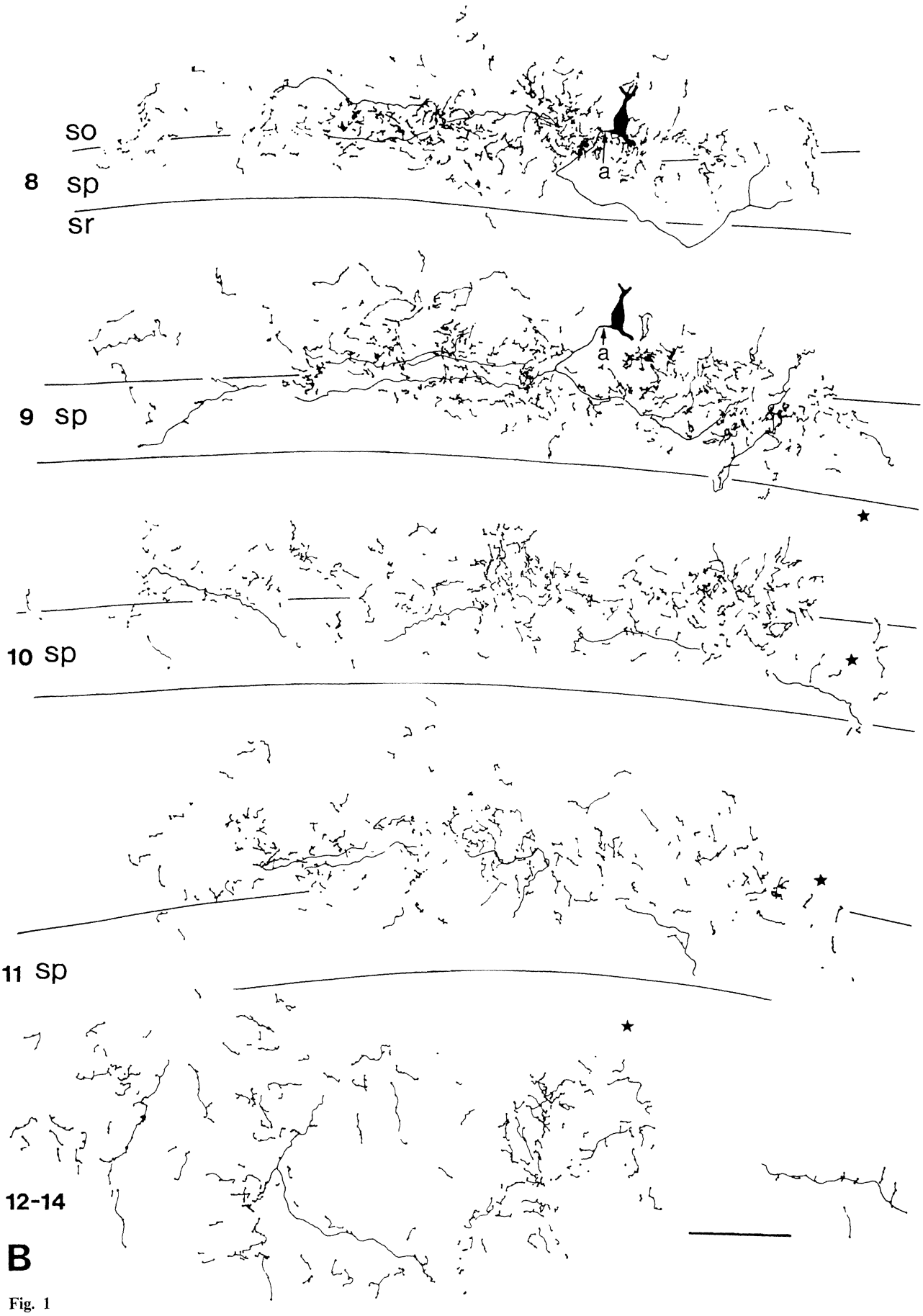

Fig. 1

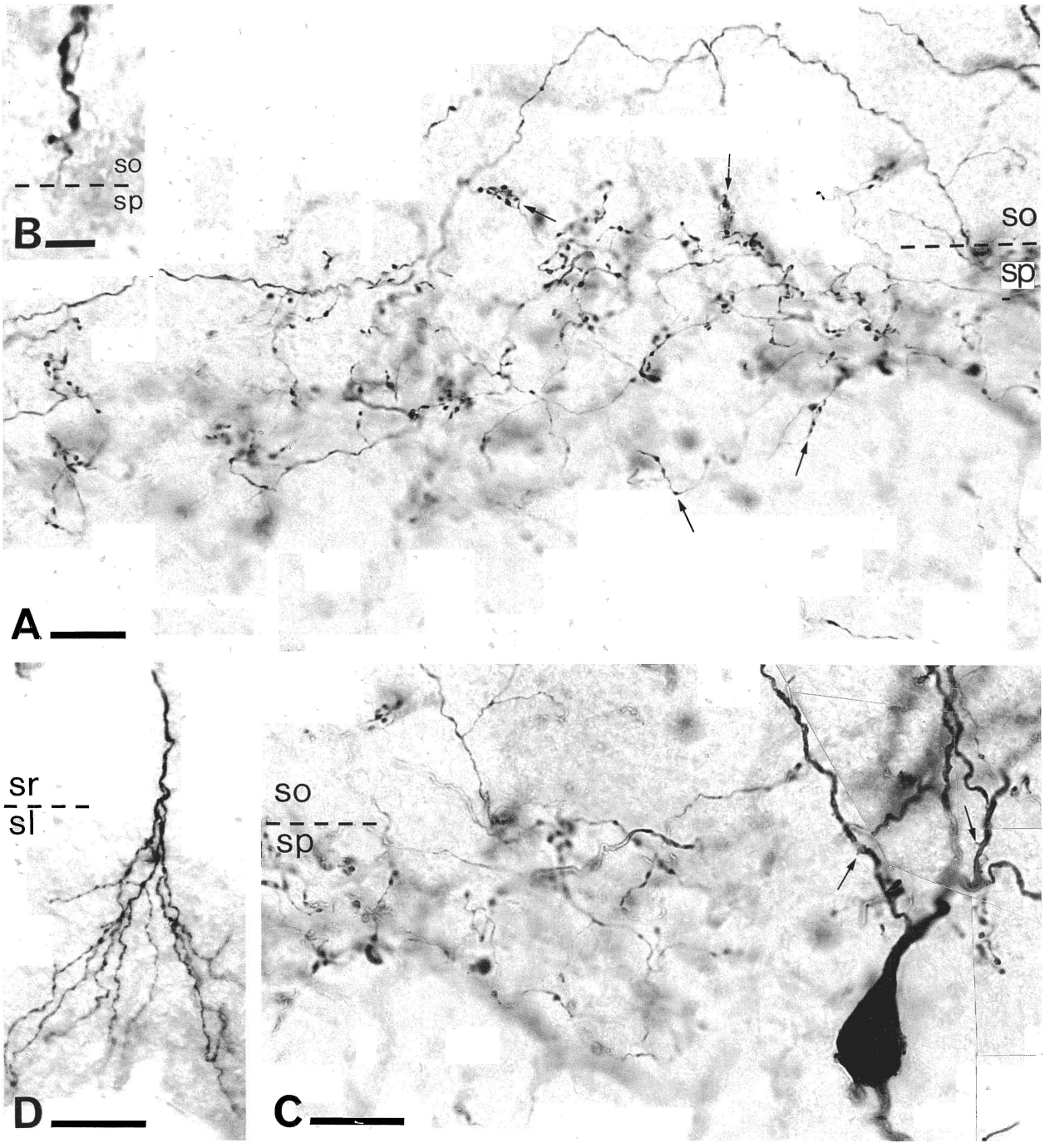

Fig. 2A–D. Light micrographs of different parts of the chandelier cell. **A** The axonal field in section 7, showing numerous clusters of terminals (e.g. arrows) and long horizontal collaterals in stratum oriens (*so*). **B** A single terminal segment with 8 radially arranged boutons immediately dorsal to the stratum pyramidale (*sp*). **C** The dendrites, radiating dorsally from the soma, are mainly smooth, only occasional spines (*arrows*) can be observed. **D** The main dendrite gives a rich terminal tuft in stratum lacunosum-moleculare (*sl*)

The dense dendritic tuft of the chandelier cell in the stratum lacunosum-moleculare may be of special importance since this layer receives direct neocortical (entorhinal) inputs (Steward 1976). These entorhinal afferents densely innervate the pyramidal cells, yet electric stimulation of the same fibers rarely discharges CA1 pyramidal cells (Andersen 1975; Yeckel and Berger 1990; Doller and Weight 1982; Doller and Weight 1985). Feed-forward excitation of chandelier cells and principal cells by the entorhinal input, however, may result in a strong feed-forward inhibition of pyramidal cells (Buzsáki 1984) thereby preventing or delaying discharges of these cells. On the other hand, selective inhibition of chandelier neurons by subcortical inputs may powerfully disinhibit CA1 pyramidal cells and enhance their responsivity to other inputs. This hypothesized feed-forward function of chandelier

cells is compatible with the "threshold control" mechanism originally suggested by Somogyi et al. (Somogyi et al. 1982; Somogyi et al. 1983) and recently supported by computer simulations (Douglas and Martin 1990).

Another notable feature of the chandelier cell was the asymmetric distribution of its axonal arbor. Such asymmetry is clearly recognizable in previously published Golgi-stained chandelier cells in the rat dentate gyrus (Soriano et al. 1990). As illustrated in Fig. 1, over three-quarters of the boutons, occupying an area of 850×600 μm, were found on the fimbrial (CA3) side of the cell body. Assuming an even distribution of CA1 principal cell axons (Knowles and Schwartzkroin 1981; Christian and Dudek 1988), such asymmetric arrangement suggests that recurrent activation of the chandelier neuron by the pyramidal cells does not necessarily inhibit its excitatory source (i.e., pyramidal cells surrounding the chandelier cell) but produces lateral inhibition. The direction of feed-back inhibition therefore is in a direction opposite to the unidirectional excitatory circuitry of the hippocampal formation and can be conceived of as a negative spatial feed-back.

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