

Synaptic Target Selectivity and Input of GABAergic Basket and Bistratified Interneurons in the CA1 Area of the Rat Hippocampus

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ABSTRACT: To assess the position of interneurons in the hippocampal network, fast spiking cells were recorded intracellularly *in vitro* and filled with biocytin. Sixteen non-principal cells were selected on the basis of 1) cell bodies located in the pyramidal layer and in the middle of the slice, 2) extensive labeling of their axons, and 3) a branching pattern of the axon indicating that they were not axo-axonic cells. Examination of their efferent synapses ($n = 400$) demonstrated that the cells made synapses on cell bodies, dendritic shafts, spines, and axon initial segments (AIS). Statistical analysis of the distribution of different postsynaptic elements, together with published data ($n = 288$) for 12 similar cells, showed that the interneurons were heterogeneous with regard to the frequency of synapses given to different parts of pyramidal cells. When the cells were grouped according to whether they had less or more than 40% somatic synaptic targets, each population appeared homogeneous. The population ($n = 19$) innervating a high proportion of somata ($53 \pm 10\%$, SD) corresponds to *basket cells*. They also form synapses with proximal dendrites ($44 \pm 12\%$) and rarely with AISs and spines. One well-filled basket cell had 8,859 boutons within the slice, covering an area of 0.331 mm² of pyramidal layer tangentially and containing 7,150 pyramidal cells, 933 (13%) of which were calculated to be innervated, assuming that each pyramidal cell received nine to ten synapses. It was extrapolated that the intact axon probably had about 10,800 boutons innervating 1,140 pyramids. The proportion of innervated pyramidal cells decreased from 28% in the middle to 4% at the edge of the axonal field.

The other group of neurons, the *bistratified cells* ($n = 9$), showed a preference for dendritic shafts ($79 \pm 8\%$) and spines ($17 \pm 8\%$) as synaptic targets, rarely terminating on somata ($4 \pm 8\%$). Their axonal field was significantly larger ($1,250 \pm 180 \mu\text{m}$) in the medio-lateral direction than that of basket cells ($760 \pm 130 \mu\text{m}$). The axon terminals of bistratified cells were smaller than those of basket cells. Furthermore, in contrast to bistratified cells, basket cells had a significant proportion of dendrites in stratum lacunosum-moleculare suggesting a direct entorhinal input.

The results define two distinct types of GABAergic neuron innervating pyramidal cells in a spatially segregated manner and predict different functions for the two inputs. The perisomatic termination of basket cells is suited for the synchronization of a subset of pyramidal cells that they select from the population within their axonal field, whereas the termi-

nation of bistratified cells in conjunction with Schaffer collateral/commissural terminals may govern the timing of CA3 input and/or voltage-dependent conductances in the dendrites. © 1996 Wiley-Liss, Inc.

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INTRODUCTION

Approximately 5.8% (Aika et al., 1994) to 11% (Woodson et al., 1989) of neurons in the hippocampal CA1 area are so-called interneurons, also called non-pyramidal cells; the rest are pyramidal (principal) cells. Interneurons play a crucial role in the normal function of the hippocampal network, and they have been implicated also in pathological changes (for reviews, see Schwartzkroin and Mueller, 1987; Lopes da Silva et al., 1990; Buzsáki and Chrobak, 1995). Since many of the "interneurons" project outside the hippocampal area where their cell body is located (e.g., Kunkel et al., 1988; Tóth and Freund, 1992; Sík et al., 1994), the term *non-principal cell* more accurately delineates them in the hippocampal network. Non-principal cells share some connective, intrinsic physiological and neurochemical characteristics. Their dendrites are usually smooth, beaded, or sparsely spiny, their local axonal arbor is very extensive, their terminals, with rare exceptions, form type 2 (symmetrical) synapses, they fire short-duration action potentials, and most of them use γ -aminobutyric acid (GABA) as a transmitter (Ribak et al., 1978). Notwithstanding these similarities, non-principal cells are very diverse in the distribution of their axonal and dendritic fields, some neurochemical characteristics, and

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functional roles. Several attempts, based on different methodological approaches, have been made to define non-principal cell subclasses, but many of the subdivisions are based only on qualitative descriptions.

Non-principal cells were first classified morphologically on the basis of the shape of the soma and the distribution of dendrites and part of the axons, as these were readily visualized by the Golgi method (Schaffer, 1892; Ramón y Cajal, 1893; Lorente de Nó, 1934). Following Schaffer's description of several non-pyramidal cells, Ramón y Cajal reported several types of neuron having cell bodies in stratum oriens and axons richly arborizing in stratum pyramidale, where their terminal axonal branches were depicted as wrapping around the somata of pyramidal cells (Ramón y Cajal, 1893; Figs. 4, 5). Lorente de Nó (1934) distinguished 13 types of non-pyramidal cells in the Ammon's horn of the rodent, including six types of cell having somata in or near stratum pyramidale, of which three types of cell richly innervated the pyramidal cell layer. He therefore referred to them as basket cells. The remaining three types of cell innervated stratum radiatum. Following electron microscopic examination of the somata of pyramidal cells, Blackstad and Flood (1963) reported that the synapses which presumably originated from basket cells were of the symmetrical type, thought to be characteristic of inhibitory action. Indeed, the pioneering experiments of Andersen et al. (1963, 1964), demonstrated a strong inhibitory input to the somatic region of hippocampal pyramidal cells. More recent electron microscopic examination confirmed that some of the interneurons having cell bodies in or close to the pyramidal cell layer make a large proportion of their synapses on the somata and proximal dendrites of pyramidal cells in the hippocampus (Seress and Ribak, 1990; Gulyás et al., 1993a; Buhl et al., 1994a; Sík et al., 1995) and evoke GABA_A receptor-mediated inhibitory postsynaptic potentials (IPSPs) (Buhl et al., 1994a, 1995).

Until recently, most GABAergic cells that have cell bodies in the pyramidal cell layer were thought to terminate on somata and have been called "basket cells." However, a growing number of studies demonstrate that even in such a restricted subregion as the pyramidal cell layer, non-principal cells display distinct characteristics. One subclass, the axo-axonic cell, has been recognized to specialise by making multiple synaptic contacts on the axon initial segment of the principal cells (Somogyi et al., 1983, 1985; Li et al., 1992; Buhl et al., 1994b). Without electron microscopic examination, axo-axonic and basket cells are not always easy to differentiate, and neurons having many terminals in the pyramidal cell layer are often referred to as basket cells (Schwartzkroin and Kunkel, 1985; Kawaguchi and Hama, 1988; Thurbon et al., 1994). Quantitative criteria would therefore be useful to distinguish basket cells from other interneurons.

Other cells seem to innervate the dendritic region of principal cells (Ramón y Cajal, 1893, Fig. 4A; Lorente de Nó, 1934; Kawaguchi and Hama, 1987; Gulyás et al., 1993a; Sík et al., 1995). Based on the co-alignment of its axon with the Schaffer collateral/commissural terminals in stratum radiatum and oriens of the CA1 area, we named one of these cell types *bistratified cell* (Buhl et al., 1994a). Bistratified cells evoke fast GABA_A receptor-mediated IPSPs in pyramidal cells, and the two examples that

have been illustrated had most of their terminals in the dendritic region of pyramidal cells, largely avoiding the cell body region (Buhl et al., 1994a; Sík et al., 1995). However, basket cells also contribute to dendritic innervation, and some of them have a substantial proportion of their axon in stratum radiatum and/or oriens. Since only two bistratified cells have been anatomically documented so far, it has remained unclear to what extent basket and bistratified cell populations have differentiated into distinct neuronal classes. It could be argued that the predominantly somatically or dendritically terminating cells are only the extreme examples of a continuum of non-principal neurons innervating the somato-dendritic domain of pyramidal cells.

The most relevant information for the place and role of the various subtypes of non-principal cells in the hippocampal microcircuitry is their synaptic connections. We have analyzed a relatively large sample of cells in order to examine whether distinct classes can be defined quantitatively according to their connections. The results demonstrate that in the CA1 area, in addition to axo-axonic cells (Buhl et al., 1994b), the pyramidal cell layer contains the cell bodies of basket and bistratified cells, two distinct classes of GABAergic non-principal cell. In the accompanying article, we demonstrate that these two classes of cell possess similar as well as distinct intrinsic physiological properties (Buhl et al., 1996).

MATERIALS AND METHODS

Young adult (7–8 weeks old), female Wistar rats were used. The details of the preparation of hippocampal slices, electrophysiological recording, filling of neurons, the visualization of biocytin-filled cells, and the correlated light and electron microscopic methods were similar to that in previous studies (Han et al., 1993; Buhl et al., 1994a,b).

Tissue Preparation, Electrophysiological Recording, and Histological Processing

The animals were intracardially perfused, under ketamine (100 mg/kg) and xylazine (10 mg/kg) anesthesia, with cold artificial cerebrospinal fluid (ACSF) containing sucrose, and their brains were quickly removed and sectioned. Intracellular recordings were carried out in an interface-type chamber using sharp electrodes filled with 2% biocytin dissolved in 1.5 M KCH₃SO₄. The recording conditions are described in the accompanying article (Buhl et al., 1996). Thirty to 60 min was allowed for the transport of biocytin, then the slices were fixed overnight in a fixative containing 2.5% paraformaldehyde, 1.25% glutaraldehyde, and 15% saturated picric acid dissolved in 0.1 M phosphate buffer (pH 7.4). Slices were cryoprotected in sucrose, freeze-thawed using liquid nitrogen, embedded in gelatin, and 70 μm thick sections were cut on a Vibratome. Avidin-biotinylated peroxidase complex (Vector Laboratories) was used to visualize filled cells. Peroxidase activity was demonstrated using 3,3'-diaminobenzidine tetrahydrochloride (DAB, Sigma) as chromogen. The sections were postfixed in 1% osmium tetroxide for 1 h, block-

stained with 1% uranyl acetate, dehydrated, and flat-embedded on glass slides in Durcupan ACM epoxy resin (Fluka).

Light and Electron Microscopy

Neurons were evaluated in the light microscope and chosen for further analysis on the basis of 1) having cell bodies located in the middle of the recorded slice (~400 μm thick), therefore having a reasonable chance of displaying a substantial part of their axonal and dendritic processes in the slice; and 2) the good quality of the visualization, which was based on the presence of extensive labeling of their axons. Cells were reconstructed from the serial 70- μm -thick sections, using a light microscope with the aid of a drawing tube, at $\times 1,000$ – $1,250$ magnification. Light microscopic photographs were taken in order to document characteristic axonal and dendritic patterns. Based on their characteristic terminal axonal segments (Somogyi et al., 1983), presumed axo-axonic cells were collected for a separate study, and the remaining 16 cells were chosen for further analysis in the present study.

Following light microscopy, axon-rich areas including all layers covered by the axonal field were re-embedded for ultrathin sectioning. Serial sections were cut and mounted on single-slot Formvar-coated copper grids, stained with lead citrate, and used for establishing synaptic connections of the identified neurons in the electron microscope. The sections were scanned, and all biocytin-filled axonal profiles were traced through serial sections until they formed synaptic contacts. Since all labeled profiles were followed and the plane of the section randomly cuts through the axonal arbor, the above procedure ensured an unbiased sample of postsynaptic targets. A total of 400 efferent synapses, ranging from ten to 61 for each cell, were collected, and the proportion of the different postsynaptic elements was established.

Postembedding Immunogold Reaction for GABA

The GABAergic nature of basket cells has been well documented. In this study, two bistratified cells were tested for the presence of GABA in their terminals and, for comparison, two basket cells served as positive controls. Serial sections were mounted alternately on Formvar-coated single-slot copper and nickel grids. Sections on copper grids were used for the localization and identification of biocytin-filled boutons; sections on nickel grids were immunoreacted for GABA (Somogyi, 1988). The reaction was carried out on droplets in humidified Petri dishes. The resin was etched with 1% periodic acid; osmium tetroxide was removed from the sections with 4% sodium metaperiodate. The primary antiserum to GABA (code No. 9; Hodgson et al., 1985) was used at a dilution of 1:1,000 at 4°C, and the secondary antibody, goat anti-rabbit immunoglobulin covalently coupled to 1.4-nm gold particles (Nanoprobes, Stony Brook, NY), was applied for 1 h at a dilution of 1:100 at room temperature. Tris-buffered saline containing 1% normal goat serum was used for washing and dilution of the primary antiserum. The secondary antibody was dissolved in 50 mM Tris buffer (pH 7.4) containing 1% bovine serum albumin and 0.5% Tween 20. After thorough washing and postfixation in 1% glu-

taraldehyde, the bound gold-conjugated antibody was visualized with silver intensification using the HQ Silver kit (Nanoprobes, Stony Brook). The sections were then stained with aqueous solution of uranyl acetate and lead citrate. Boutons that were found to make a synapse were located in adjacent sections immunoreacted for GABA.

In control experiments, the primary antiserum was either omitted or replaced with 1% normal rabbit serum, and the sections containing biocytin-filled pyramidal cell dendrites and axons were incubated as described above. In these sections, silver particles were not accumulated on any biocytin-filled cellular profile.

Quantitative Analysis

In addition to 16 cells analyzed in the present study, data previously published from our laboratory for 12 similar cells was included into the statistical analysis. In two instances (cell Nos. 7 and 16 in Table 1), an additional sample of two and 11 synapses was obtained and included in the analysis. A χ^2 test for heterogeneity was used to compare cells on the frequency of different kinds of elements among their postsynaptic targets.

The dendritic arbors were compared with regard to the proportion of dendritic length in stratum lacunosum-moleculare. The two-dimensionally projected dendritic length in strata radiatum and lacunosum-moleculare of 19 filled interneurons was measured on a digitizing tablet from drawings. The cells were compared on the basis of the relative proportion of dendrites in the two layers.

The area of synaptic boutons was measured from single electron microscopic sections for four basket and two bistratified cells with the best fine structural preservation. For each bouton a section was chosen where the synaptic junction appeared at its maximum extent. The cross-sectional areas of 96 synaptic boutons from four basket cells (13 to 44 per cell) and 43 synaptic boutons from two bistratified cells (20 and 23 boutons, respectively) were measured with the aid of a digitising tablet using MacStereology software. Dendrite and bouton populations were statistically compared using the Mann-Whitney U-test. Data are shown as mean \pm SD.

Results

Identification of interneurons

Table 1 lists all non-principal cells analyzed in this study. Sixteen biocytin-filled cells were included on the basis of criteria described in the Materials and Methods section. Data for 12 similar cells, which have been reported in our previous publications (Buhl et al., 1994a; 1995; Cobb et al., 1995), are included in order to make the statistical comparison more comprehensive. On five of the published cells, additional dendritic measurements were carried out for the present study.

The cell body of all cells was in or very close to the compact pyramidal cell layer. This is partly due to the sampling process for intracellular recording, which was centered on the pyramidal cell layer; consequently our sample may be biased for cells in the pyramidal cell layer. The somata of the same cell types may also

*Synaptic Targets of Non-Pyramidal Cells Having Somata in the Pyramidal Layer of the CA1 Area**

No.	Code	Publication ^a	Illustrated in	Physiology (figures) ¹	No. of boutons studied	No. of synapses studied	Postsynaptic targets								
							AIS		Soma		Dendritic shaft		Spine		Unid. No.
							No.	%	No.	%	No.	%	No.	%	
1	0512941	4	—	—	22	23	0	0	16	70	7	30	0	0	0
2 ^b	1902921	1	Fig. 3 in 1	2C, 7A–D	32	33	0	0	23	70	10	30	0	0	0
3 ^b	2602921	1	—	3A,6A–B,8	42	43	2	5	29	69	11	26	0	0	1
4 ^b	2306922	1	Fig. 4 in 1	2A,3A–C	32	32	4	13	20	67	6	20	0	0	2
5	1609941	4	—	—	12	12	1	8	8	67	3	25	0	0	0
6 ^b	1110931	1	—	—	11	12	0	0	7	58	5	42	0	0	0
7 ^b	2311941	4 ^c	Fig. 1 in 4	—	13	13	0	0	7	54	6	46	0	0	0
8	0809922	1	—	—	32	32	0	0	17	53	15	47	0	0	0
9	2801921	3	Fig. 3 in 3	3C	37	37	0	0	19	51	18	49	0	0	0
10 ^b	1507941	3;4	—	—	10	10	0	0	5	50	5	50	0	0	0
11	1606943	3;4	—	—	10	10	0	0	5	50	5	50	0	0	0
12 ^b	1611934	3	Fig. 2 in 3	1A,B	58	61	0	0	29	48	30	49	2	3	0
13 ^b	0411931	1	—	—	19	19	0	0	9	47	10	53	0	0	0
14	0306942	3;4	—	—	11	11	0	0	5	45	6	55	0	0	0
15 ^b	0602921	2;3	Fig. 1 in 2	—	37	43	0	0	19	44	19	44	5	12	0
16	1905943	3;4 ^c	—	—	20	21	1	5	9	43	11	52	0	0	0
17	2301921	3	—	—	12	12	0	0	5	42	7	58	0	0	0
18 ^b	2606921	1	Fig. 5 in 1	5A–F	33	33	0	0	14	42	19	58	0	0	0
19	2307921	1	—	2B,3A,B,4A–C	43	45	3	6	18	41	24	53	0	0	0
20 ^b	0507934	1	—	2D,3F	24	25	0	0	6	24	17	68	2	8	0
21 ^b	1409922	2	Fig. 2 in 2	—	35	35	0	0	4 ^d	11	27	78	4	11	0
22 ^b	3105931	1	—	3D	19	21	0	0	1	4	18	86	2	10	0
23 ^b	2502922	1	—	1C,D,3D,4F,7E–H	15	19	0	0	0	0	13	68	6	32	0
24 ^b	1902922	1	Fig. 9 in 1	3D–F,4D,E	28	29	0	0	0	0	26	89	3	11	0
25 ^b	2611922	1	Fig. 10 in 1	2E,F	26	26	0	0	0	0	22	85	4	15	0
26 ^b	2905912	1	—	—	10	11	0	0	0	0	8	73	3	27	0
27 ^b	1604921	1	—	—	10	10	0	0	0	0	8	80	2	20	0
28 ^b	3011941	1	—	—	9	10	0	0	0	0	8	80	2	20	0
					Total = 662	Total = 688									

*Interneurons are included if quantitative data, based on random samples, are available of their postsynaptic elements and if axon initial segments represent less than 15% of their targets. Cells whose targets are mainly axon initial segments, i.e., axo-axonic cells, have been reported previously (Buhl et al., 1994b). The table includes 16 cells studied here and data reported by Buhl et al. (1994a, 1995) and Cobb et al. (1995). Figure numbers refer to illustrations presenting a light microscopic reconstruction of the cells. Based on a χ^2 test for heterogeneity applied to the frequency of different synaptic targets, cells 1–19 were classified as *basket cells* and they are distinct from cells Nos. 20–28, classified as *bistratified cells*. Column headed “Physiology (figures)” refers to Buhl et al. (1996).

^a1, This study; 2, Buhl et al. (1994a); 3, Buhl et al. (1995); 4, Cobb et al. (1995).

^bCells represented in Figure 11.

^cSample increased since last publication.

^dOne synapse on pyramidal, three synapses on non-pyramidal cell somata.

be present in other layers, as cells with similar dendritic and/or axonal arborization have been published earlier, although without the identification of their postsynaptic targets (Ramón y Cajal, 1893; Lorente de Nó, 1934; Schwartzkroin and Mathers, 1978; Lacaille and Schwartzkroin, 1988a,b; Kunkel et al., 1988). All selected cells displayed the characteristic morphological features of GABAergic non-principal cells. The majority of cells had an axonal arbor centered on, and most dense in, the pyramidal layer. However, the axons of some other cells extended substantially into strata radiatum and oriens. There was a tendency for the axon to become relatively sparser in the pyramidal layer as it extended further to the distant radial borders of strata oriens and radiatum. Parallel with the differences in the axonal arborization, the distribution of the dendrites appeared heterogeneous as well. A significant proportion of the dendrites of some cells was present in stratum lacunosum-moleculare, whereas the dendrites of other cells appeared to terminate without entering this layer; therefore a quantitative comparison of dendritic trees was also carried out (see below).

The axons and dendrites of the identified cells were easily recognizable in the electron microscope from the presence of the electron-dense peroxidase reaction product. The biocytin-labeled terminal boutons of all cells established type 2 (symmetrical) synapses (see below) with their postsynaptic targets, which were identified on the basis of published criteria (Peters et al., 1991; Halasy and Somogyi, 1993) as either somata, dendritic shafts, somatic or dendritic spines, and axon initial segments. Since the output of the non-principal cells is a functionally important characteristic, we compared the cells first on the basis of their synaptic target preferences (Fig. 1).

Statistical Analysis of Synaptic Target Distribution

Random samples of between ten and 61 synaptic boutons for each cell were collected by electron microscopy in order to establish the synaptic target distribution of each cell (Table 1). Applying a χ^2 test for heterogeneity and testing all cells on the basis of the four possible postsynaptic targets, the 28 neurons were found to be heterogeneous with regard to the frequency of their

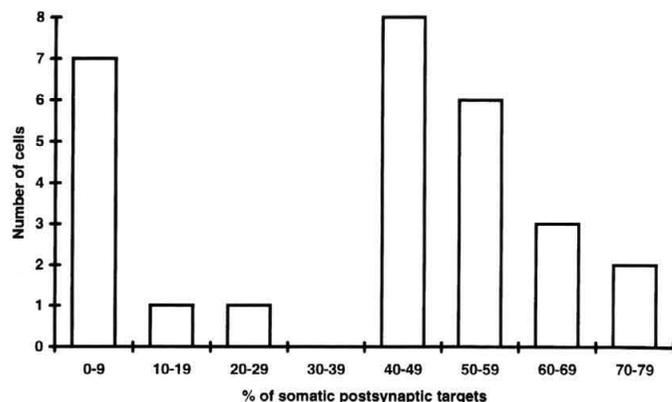


FIGURE 1. The distribution of 28 non-principal cells, according to the proportion of their efferent synapses on cell bodies of pyramidal cells, demonstrates the presence of two populations.

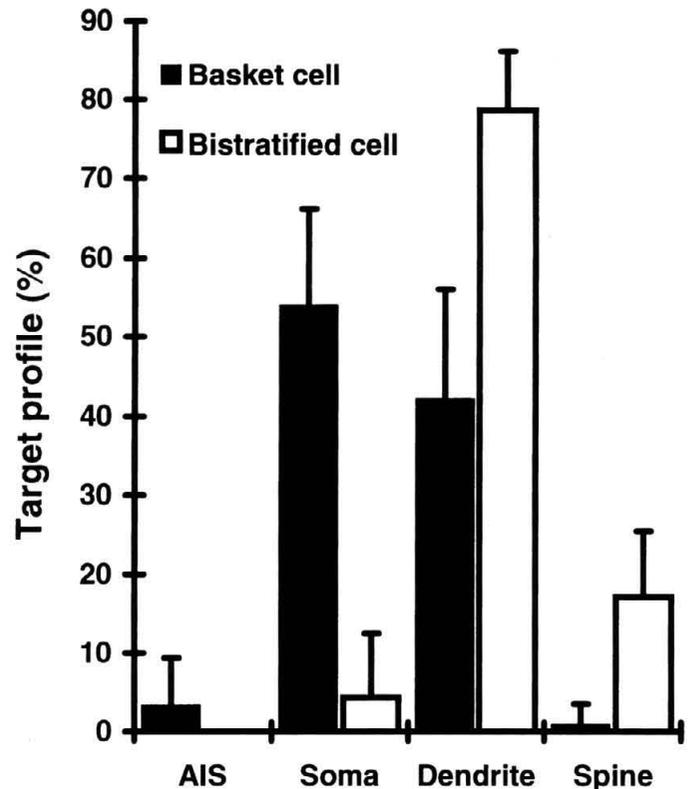


FIGURE 2. Distribution of the postsynaptic targets (mean \pm SD) of basket ($n = 19$ cells, $n = 502$ synapses) and bistratified cells ($n = 9$ cells, $n = 186$ synapses). The two populations of cell significantly differ ($P < 0.005$) in the frequency of postsynaptic somata, dendritic shafts, and spines among their postsynaptic targets. Spines may include somatic spines for basket cells. AIS, axon initial segment.

efferent synapses located on different parts of the postsynaptic pyramidal cells ($\chi^2 = 267.38$, $P < 0.005$). There were cells with a high proportion of somatic targets, whereas others had none (Fig. 1). The cells were therefore divided into two populations, one comprising 19 cells, which included those having more than 40% somatic targets. This population was not significantly heterogeneous ($\chi^2 = 97.29$, $P > 0.05$). The other population included cells with less than 24% somatic targets and was also not significantly heterogeneous ($\chi^2 = 30.09$, $P > 0.05$). Moreover, when any cell from one group was added to the other, the population became significantly heterogeneous.

The proportion of efferent somatic synapses, as established by electron microscopy, correlated with the density of axonal arborization in the pyramidal cell layer. Thus, the population with a high proportion of somatic targets corresponds to *basket cells* ($n = 19$), whereas the population with a low proportion of synapses on cell bodies is hereafter called *bistratified cells* ($n = 9$) (Fig. 2).

Basket Cells

The common features of the 19 non-pyramidal cells belonging to the group of basket cells are the high density of axonal ar-

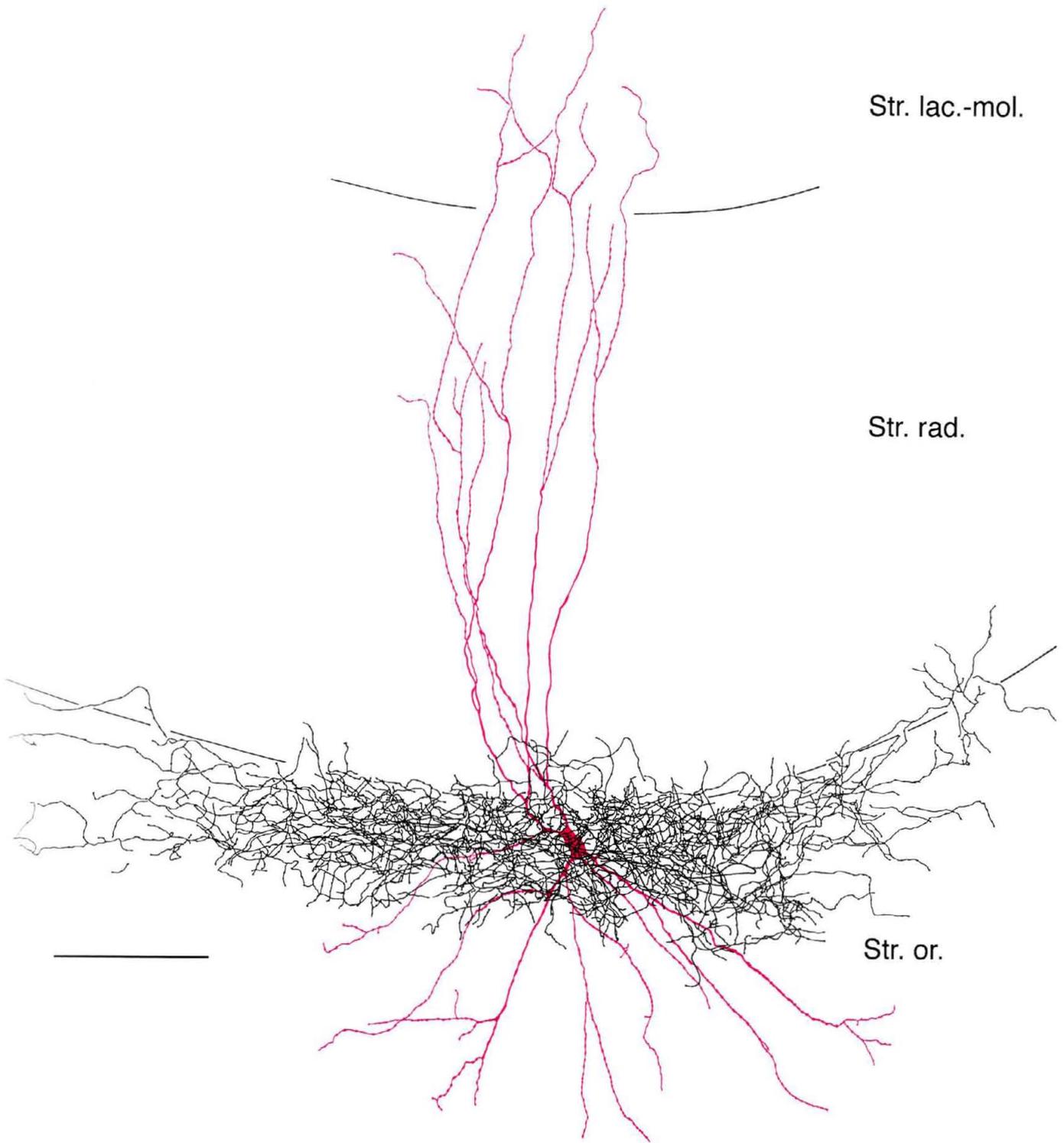


FIGURE 3. Light microscopic reconstruction of the axonal and dendritic arborization of a basket cell visualized by intracellular injection of biocytin. The main axon originates from a proximal basal dendrite and forms a dense axonal plexus (black), ramifying throughout the pyramidal layer and adjacent stratum oriens (Str. or.), but

not in stratum radiatum (Str. rad.). The dendrites (red) extend to all layers and branch in lower stratum lacunosum-moleculare (Str. lac.-mol.). A random sample of labeled axon terminals showed that this cell (No. 2 in Table 1) formed synapses with pyramidal cell somata (70%) and dendrites (30%). Scale bar = 100 μ m.

borization in the pyramidal layer and the presence of a substantial part of the dendritic arbor in each layer of the CA1 area (Figs. 3–6). The shape and size of the somata was variable; it could be multipolar, ovoid, or triangular in shape (Figs. 3–5, 6A). When the recording electrode was in the soma, the penetration might have caused alterations in its shape. Basket cell somata gave rise to four to five main dendritic trunks, often branching further in the vicinity of the cell body. The dendrites ran obliquely toward the alveus or radially in the direction of the hippocampal fissure (Figs. 3–5). They often branched again once or twice toward their end, forming small tufts in stratum lacunosum-moleculare (Figs.

3, 4). However, the dendrites of some basket cells ended without branching (Fig. 5). The dendritic branches in stratum oriens deeply penetrated into the alveus. The dendrites were smooth and/or beaded (Figs. 3–5, 6A), and very rarely a few long spines were observed on proximal dendritic branches (Fig. 4). More often, irregular dendritic appendages could be seen in the alveus.

The main axon of the basket cells emerged either from somata (Fig. 4) or from one of the main proximal dendrites (Figs. 3, 5) and proceeded into stratum radiatum, then turned back and arborized in the pyramidal layer in a “weeping willow” manner (Fig. 5), or ramified immediately among the pyramidal cells (Figs. 3,

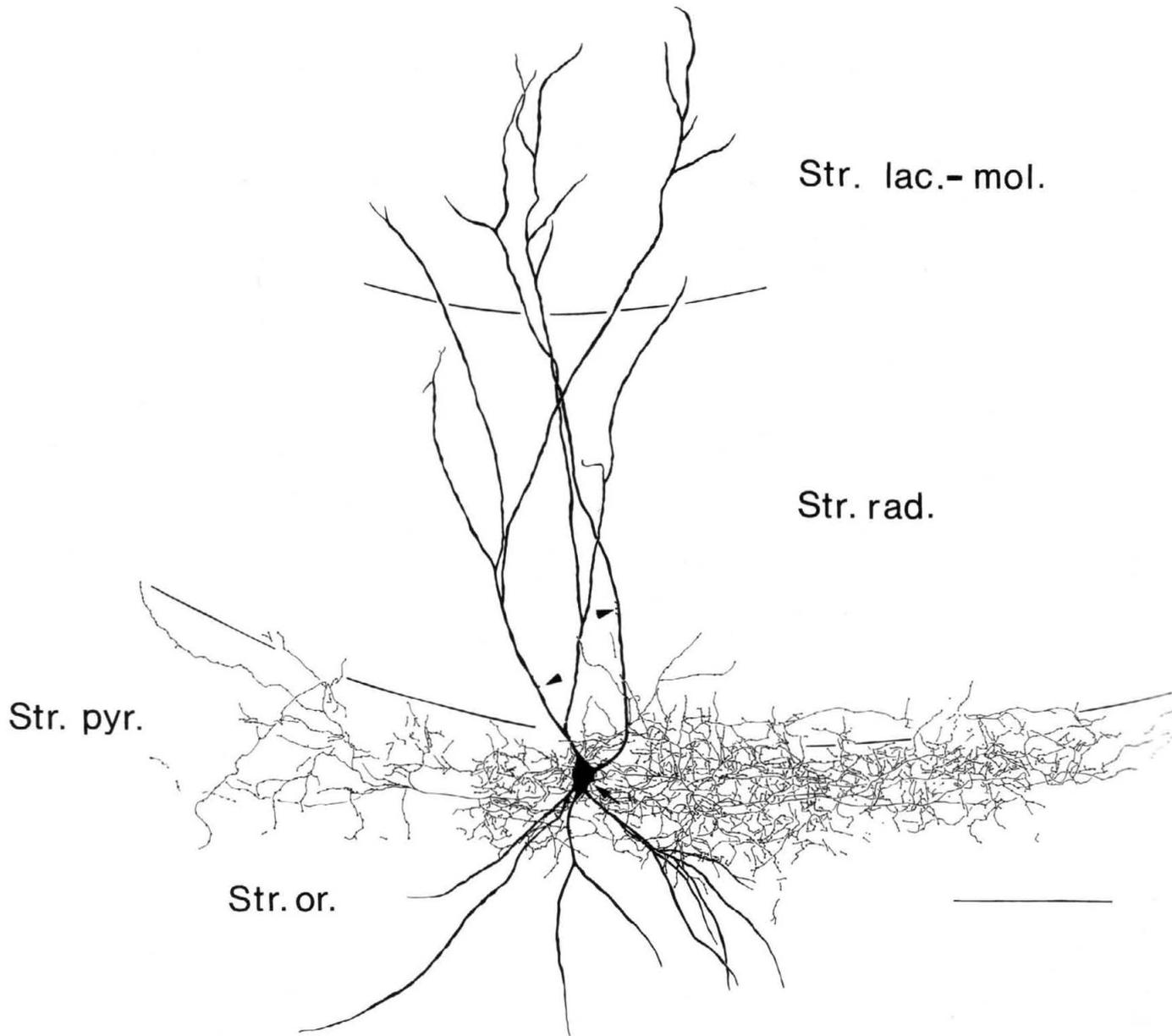


FIGURE 4. Reconstruction of a basket cell (see also Fig. 6B). In this case the main axon originates from the pyramidal shaped soma (arrow). The dendrites emitted occasional spines (arrowheads). There were terminal axon branches in lower stratum radiatum. A random

electron microscopic sample of labeled axon terminals revealed that this basket cell (No. 4 in Table 1) formed synapses with somata (67%), dendrites (20%) and, infrequently, with axon initial segments (13%). Scale bar = 100 μm .

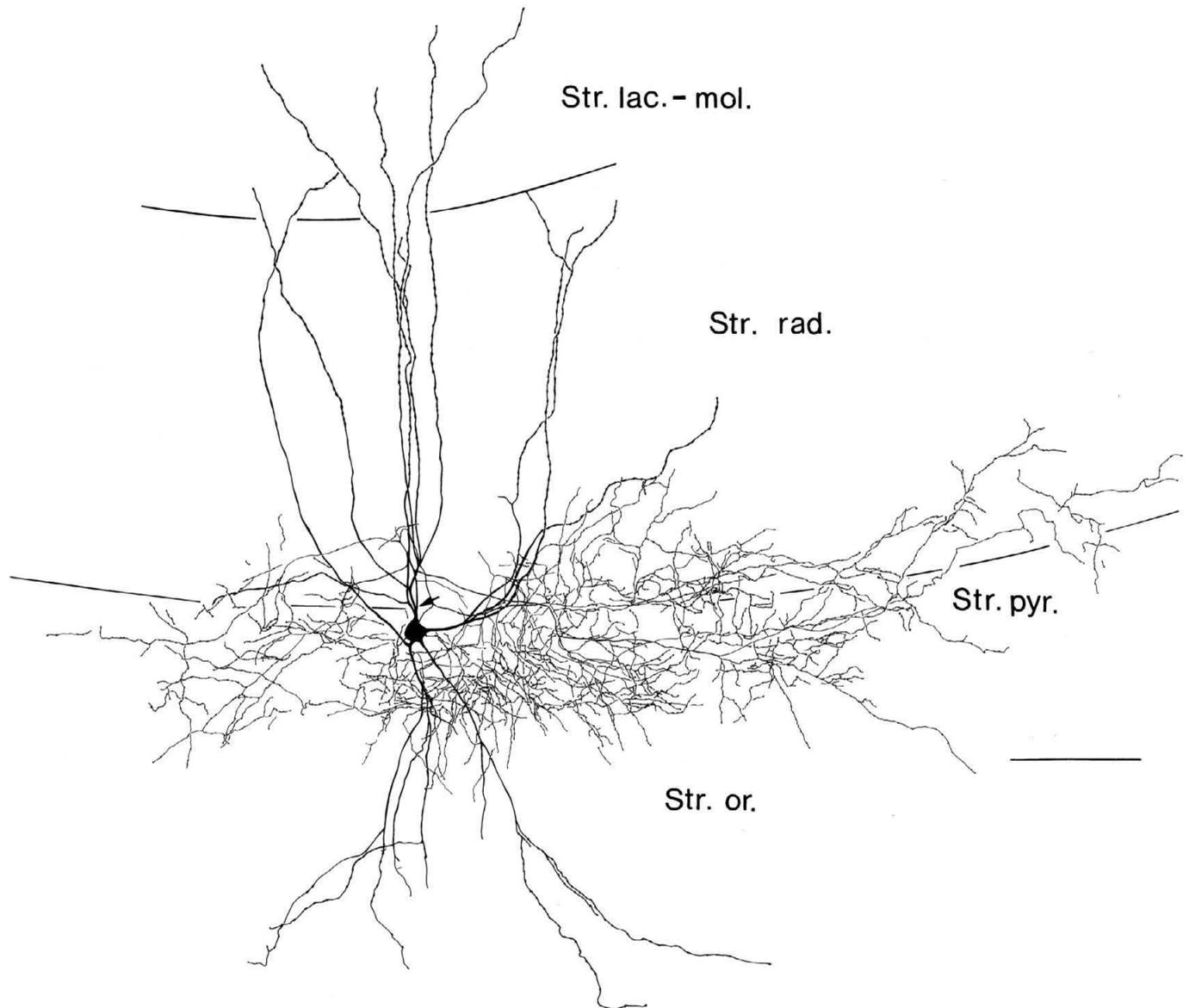


FIGURE 5. Axonal and dendritic arborization of a basket cell with a round soma (see also Fig. 6C). The dendrites were reconstructed from the entire 400- μm -thick slice, but the axon is shown from only two 60- μm -thick sections. The main axon originates from a proximal dendrite (arrow), ascends to the inner stratum radiatum where it branches, and turns back toward the stratum pyramidale. The axon

of this cell spreads further into the adjacent stratum radiatum than that of the cells illustrated in Figures 3 and 4. The dendrites rarely branch at their distal segments. A random electron microscopic sample of labeled boutons showed that this cell (No. 18 in Table 1) gave synapses to pyramidal cell somata (42%) and dendrites (58%). Scale bar = 100 μm .

4). Basket cell axons had a medio-lateral extent of $760 \pm 130 \mu\text{m}$ ($n = 5$, including cells in Figs. 3–5). The initial part of the main axon and secondary branches was usually ensheathed by myelin. The varicose, bouton-laden axon branches run among the pyramidal cells establishing multiple appositions with pyramidal somata and proximal dendrites (Fig. 6B–E). The axonal branches innervating a single pyramidal soma could originate from more than one axon collateral, and up to 15 varicosities were seen to be apposed to individual pyramidal cells (Fig. 6D,E). The dense pericellular baskets described in earlier studies using Golgi impregnation (Lorente de N6, 1934) were not seen in our material, and

they probably result from a superimposition of many simultaneously impregnated basket cell axons.

Divergence and Synaptic Coverage by Basket Cells

In the case of one well-filled basket cell (No. 12 in Table 1; shown in Fig. 2 in Buhl et al., 1995), light microscopic analysis indicated that the total number of axonal varicosities, some of them shown with subsequent electron microscopy to correspond to synaptic boutons, was 8,859. The number of synaptic *boutons*

(i.e., varicosities which were shown to form one or more synaptic junctions) originating from one basket cell and innervating a single pyramidal cell has been established previously in two cases as seven and 12, respectively (Buhl et al., 1994a, 1995). Taking an average of nine to ten boutons per pyramidal cell and assuming that all pyramidal cells receive synapses from similar number of varicosities, this basket cell is expected to innervate 933 pyramidal cells in the confines of the slice. The axon, reconstructed from six sections of 96–70 μm thickness, occupied an ellipsoid-shaped area of the hippocampus measuring $456 \times 904 \mu\text{m}$ along the septo-temporal (slice thickness) and medio-lateral axes, respectively. The number of varicosities in each sequential section was 730, 2,090, 1,737, 1,691, 1,610, and 1,001. The second to fourth sections were sampled for the density of boutons at three positions: close to the parent cell, at the edge and in the middle of the axonal arbor. Moving away from the parent cell, the density of varicosities decreased from $57,000 \pm 6,000/\text{mm}^2$ of cell layer, as viewed perpendicular to the pyramidal layer, to $17,000 \pm$

$8,000/\text{mm}^2$ in the middle, to $8,000 \pm 6,000/\text{mm}^2$ at the edge. From the decreasing number of varicosities and decreasing area occupied by the axon in the two sections at each end of the slice, it can be calculated that the majority of varicosities were within the slice. Assuming that the axon of this cell and the decrease in the density of varicosities was symmetrical in the septo-temporal and medio-lateral directions, as reported for cells labeled *in vivo* (Sik et al., 1995), it can be estimated that 1,969 varicosities corresponding to 207 innervated pyramidal cells were outside the slice. Thus, the revised estimate of the number of varicosities for the total axonal arbor is 10,828, innervating 1,138 pyramidal cells, assuming nine to ten boutons corresponding to about 11 synapses per pyramidal cell (Buhl et al., 1994a, 1995).

From the area of the pyramidal cell layer occupied by the axon in each section, it was calculated that, as viewed perpendicular to the pyramidal cell layer, the axon of this basket cell was distributed over an area 0.331 mm^2 in the slice, and it was estimated that another 0.155 mm^2 (32%) could have been sliced off, giv-

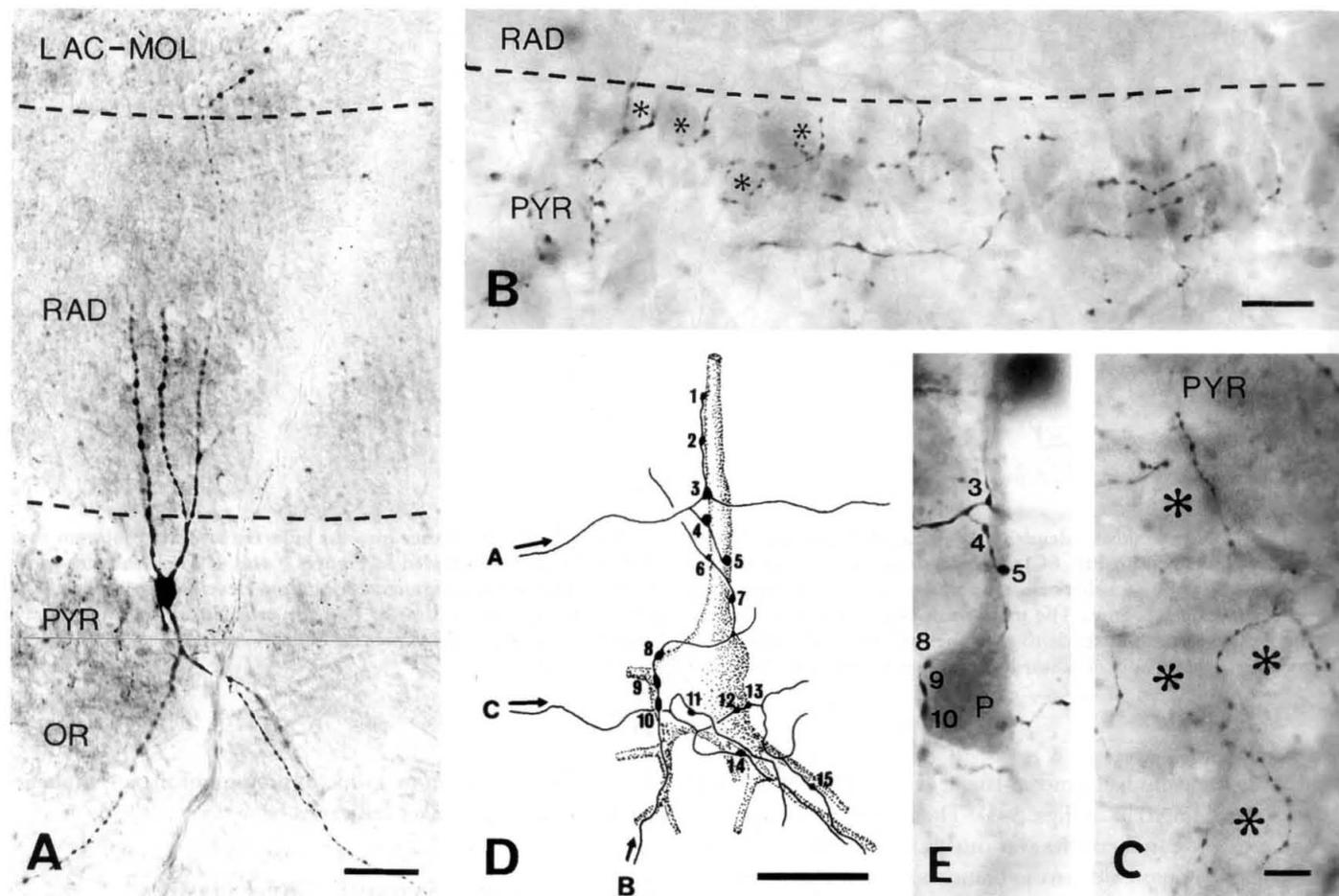


FIGURE 6. Light micrographs of labeled basket cells. **A:** A basket cell (No 8 in Table 1) in the middle of stratum pyramidale (PYR) emits beaded dendrites traversing stratum radiatum (RAD) and entering stratum lacunosum-moleculare (LAC-MOL). The basal dendrites run through stratum oriens (OR) and extend into the alveus. **B:** Axonal pattern of a basket cell also shown in Figure 4. Asterisks mark pyramidal cell somata surrounded by basket cell axons. **C:**

Perisomatic bouton rows, given by the basket cell shown in Figure 5, at higher magnification. **D,E:** Photograph and drawing of the cell body and apical dendrite of a faintly biocytin-labeled pyramidal cell (P) surrounded by three separate collaterals (A–C) of a basket cell. Each collateral makes five perisomatic varicosities. Broken lines denote the border of layers. Scale bars = 50 μm in A; 25 μm in B; 10 μm in C; 20 μm in D,E.

ing an estimated total of 0.486 mm^2 . The thickness of the pyramidal cell layer was $82 \mu\text{m}$ in this area and plane of cutting. Thus, the volume occupied by the pyramidal cell layer overlapping with the axonal arbor was 0.0271 mm^3 in the slice and 0.0399 mm^3 for the whole estimated axon. The density of pyramidal cells in the CA1 pyramidal cell layer was estimated as $264 \times 10^3/\text{mm}^3$ (Aika et al., 1994). Thus, the total number of pyramidal cells in the area covered by the axon is expected to be 7,154 in the slice and 10,520 for the whole estimated axon. Assuming uniform coverage of the area by this basket cell axon, 13.0% of pyramidal cells would be innervated in the slice, or 10.8% for the whole estimated axon. However, as indicated above, the bouton density of the identified basket cell was uneven, decreasing toward the periphery of the axonal arbor. Using the measures of bouton density along the medio-lateral axis, and the same assumptions as above, it can be calculated that the proportion of pyramidal cells innervated by this basket cell changes from $28 \pm 3\%$ near the parent cell body, to $8 \pm 4\%$ in the middle, to $4 \pm 3\%$ at the margin of the axonal arbor.

Postsynaptic Targets of Basket Cells

The synaptic boutons of the basket cells were relatively large (mean area in electron micrographs taken at the largest extent of the synapse, $0.367 \pm 0.028 \mu\text{m}^2$, $n = 96$ boutons of four cells), and they usually contained one or two mitochondrial profiles (Figs. 7, 8). The 486 identified basket cell boutons (255 this study) made a total of 502 synaptic junctions (1.03 synapse per bouton), but not all boutons could be followed completely through in serial sections. Of these, $53 \pm 10\%$ were made with somata (Figs. 7A, 8C,D), including a few somatic spines (Fig. 7C,D). The majority ($44 \pm 12\%$) of the remaining basket cell synapses were made with the shafts of either basal or apical dendrites (Fig. 8A,B). Spines ($n = 7$, 2 cells) of unknown origin were rare postsynaptic targets. Five of the 19 basket cells also gave a few synapses ($7 \pm 3\%$ of their targets, $2 \pm 4\%$ of total targets) to axon initial segments (Fig. 7B). The overall synaptic target distribution of this cell population was similar to that of a Golgi impregnated cell reported from a 10-day-old rat (Seress and Ribak, 1990). The position and fine structural characteristics of the postsynaptic targets showed that the vast majority belonged to pyramidal cells. None of the postsynaptic somata or dendrites could be unequivocally identified as belonging to a non-pyramidal cell. Therefore, it is concluded that the basket cells examined here very rarely innervate other GABAergic neurons (but see Sík et al., 1995).

Bistratified Cells

The somata of this cell population ($n = 9$) were ovoid or triangular, often elongated in the radial direction. Usually five to six main dendritic branches emerged from the soma, some of them branching repeatedly close to their origin, but not further along their course (Fig. 9). The dendrites were organized into two bouquets, one arborizing in stratum radiatum and the other in stratum oriens (Figs. 9, 10). The dendrites deeply penetrated the

alveus, turning laterally among the myelinated axon bundles. The dendrites in radiatum could give off a few tapering branches toward their end, which rarely entered stratum lacunosum-moleculare. Apart from a few long thin spines on the proximal dendrites (Fig. 9), the dendrites were smooth and considerably less beaded than those originating from basket cells (Figs. 9, 10, 12A).

The most striking difference between the dendrites of basket and bistratified cells was the proportion of dendrites in stratum lacunosum-moleculare (Fig. 11). The mean length of the two-dimensionally projected dendrites in stratum lacunosum-moleculare was $74 \pm 53 \mu\text{m}$ ($n = 9$) for bistratified cells, which was significantly shorter (Mann-Whitney U-test, $Z = -3.67$, $P < 0.0002$) than the dendritic length of basket cells ($824 \pm 260 \mu\text{m}$, $n = 10$) in the same layer. For this measurement, basket cells with few dendritic truncations in stratum radiatum were selected. The two-dimensionally projected dendritic lengths in stratum radiatum were not significantly different between basket and bistratified cells ($Z = -1.388$, $P > 0.05$). Since the dendrites in stratum lacunosum-moleculare are the continuation of dendrites in radiatum, the two-dimensionally projected dendritic lengths in lacunosum-moleculare were normalized to the lengths in stratum radiatum for each cell and plotted against the proportion of their somatic efferent synapses. This procedure clearly delineated basket and bistratified cells as two distinct populations (Fig. 11).

The main axon of bistratified cells emerged from the soma (Figs. 9, 10) and often was partially covered by myelin sheet. The primary branches could be followed to the inner one-third of stratum radiatum where they densely arborized in a characteristic bifurcating manner (Fig. 12B–D). The axon had a medio-lateral extent of $1,250 \pm 180 \mu\text{m}$ ($n = 4$, including the cells in Figs. 9 and 10), which was wider than the axon of basket cells (Mann-Whitney U-test, $Z = -2.45$, $P < 0.02$). Several branches descended among the pyramidal cells to stratum oriens where they formed another dense arborization (Figs. 9, 10), hence the name "bistratified cell." The axonal arborization either covered the whole depth of both strata radiatum and oriens (Fig. 10), or it was more concentrated in the areas close to stratum pyramidale (Fig. 9). Some long, bouton-laden axon branches ran parallel with the border of the pyramidal layer over long distances (Fig. 9). The axonal branches were very densely covered by varicosities (Fig. 12B–D). Drumstick-like boutons "en terminaux" with short connecting stalks (Fig. 12D) were more often observed than on basket cell axons.

The total number of boutons within the slice for the cell that had the most complete axon was 8,752 (No. 21 in Table 1, shown in Fig. 2; Buhl et al., 1994a). In contrast to basket cells, only 10% of the boutons were in stratum pyramidale, whereas stratum radiatum contained 41% and stratum oriens contained 49%. Because bistratified cells have wider axon arborization than basket cells, probably a more significant part of the axon was lost during the preparation of the slices.

Postsynaptic Targets of Bistratified Cells

The synaptic boutons of bistratified cells (Figs. 13, 14) contained mitochondria, but less frequently than those of basket cells.

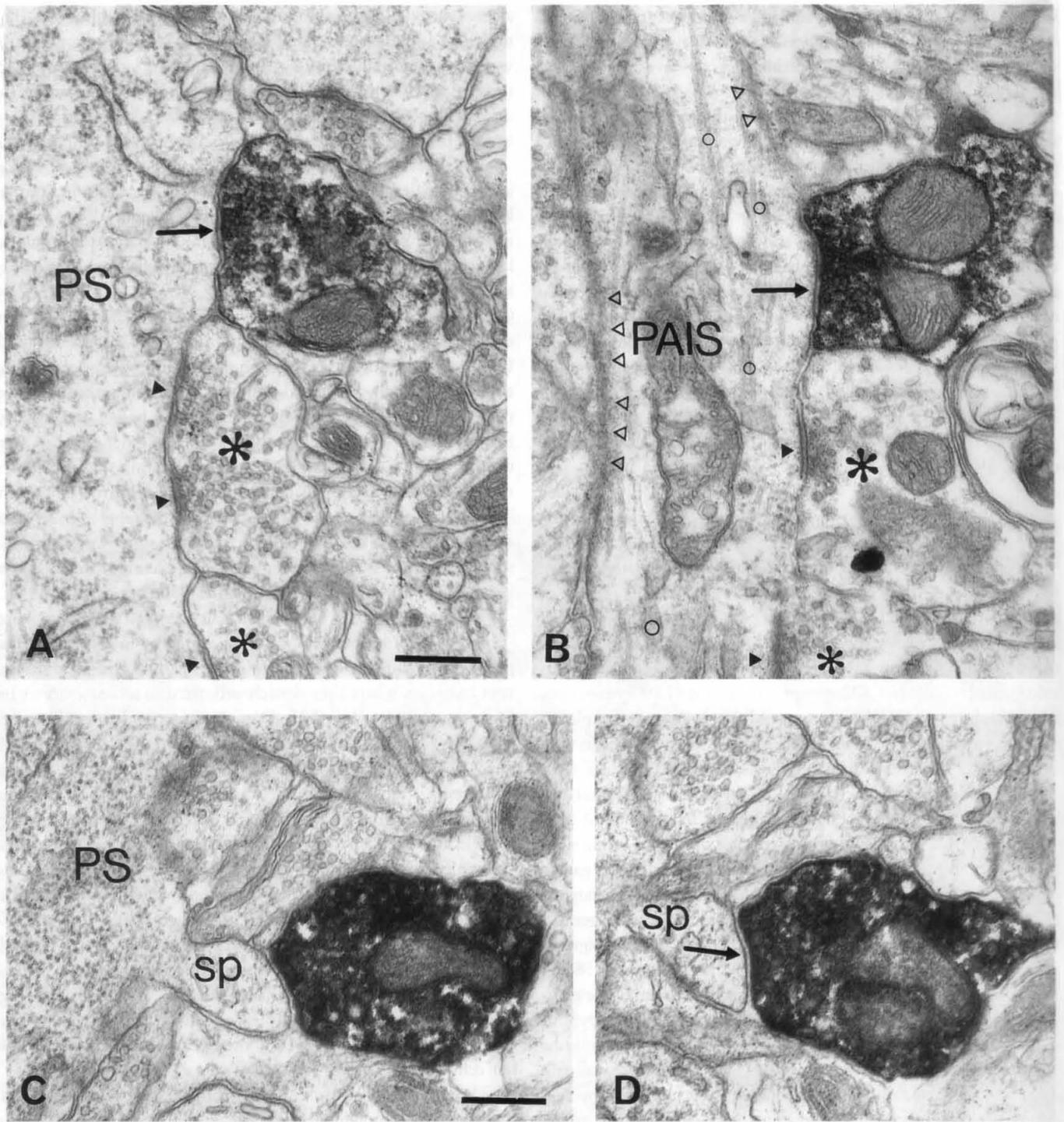
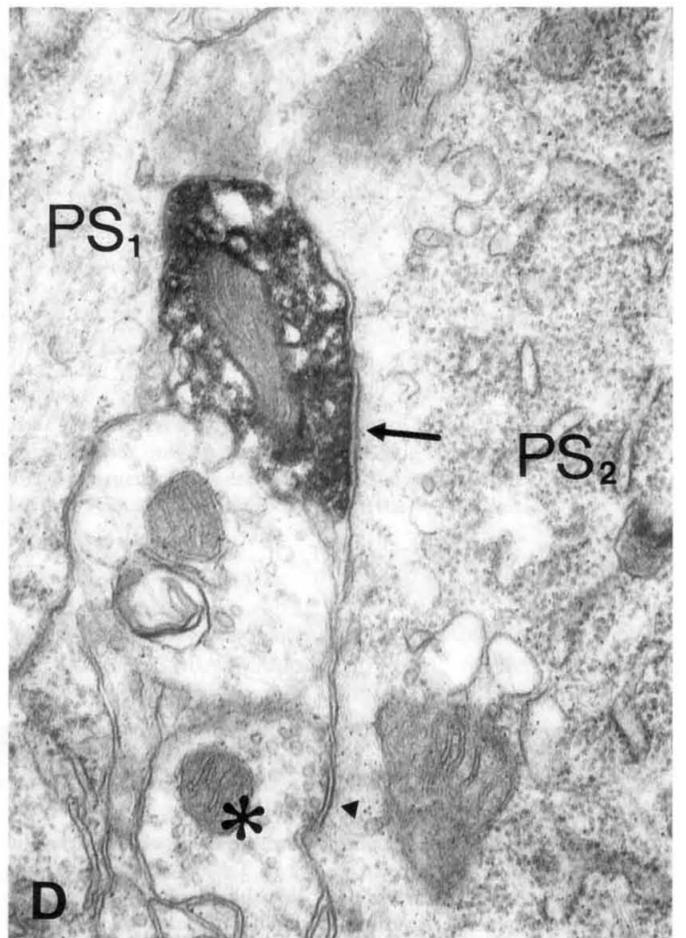
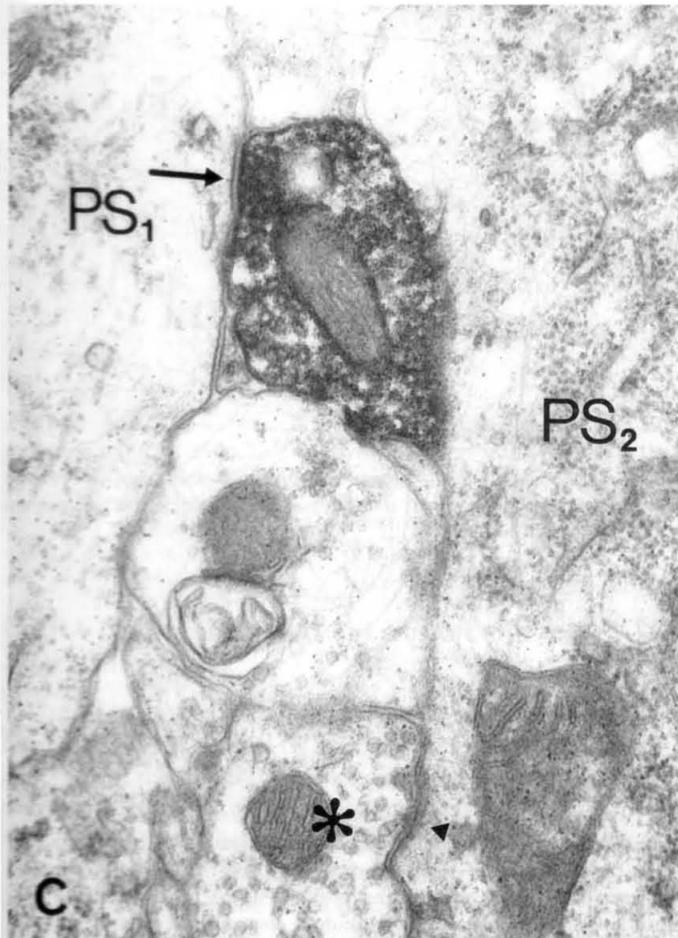
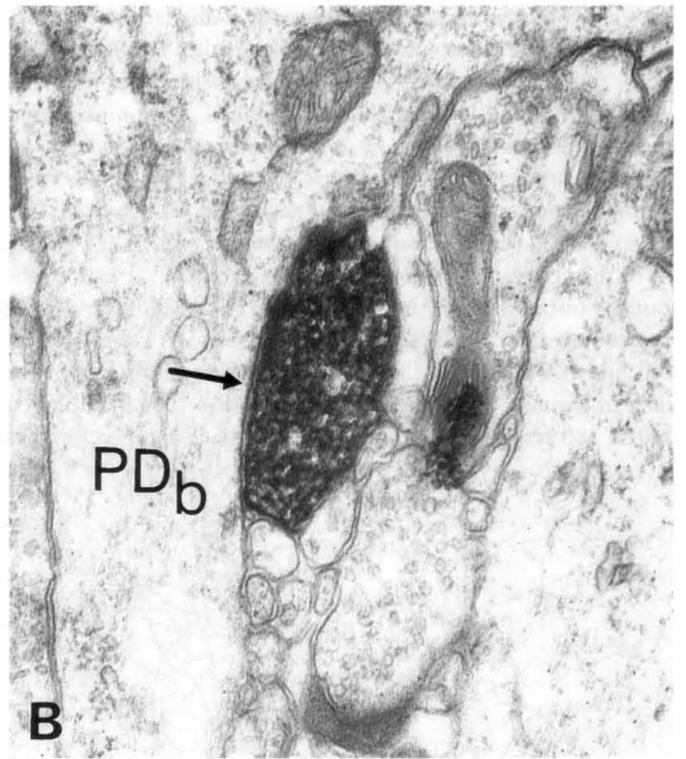
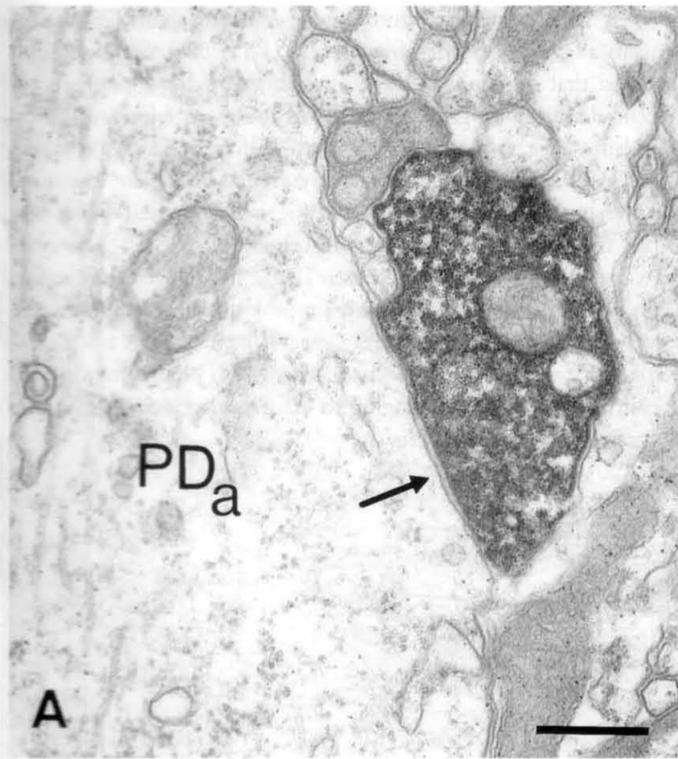


FIGURE 7. Electron micrographs of biocytin-filled basket cell boutons establishing type 2 synaptic junctions (arrows) with the soma (PS), axon initial segments (PAIS), and somatic spine (sp) of pyramidal cells. The presynaptic boutons of basket cells are readily identified by the presence of peroxidase reaction product. Asterisks denote unlabeled boutons in synaptic contact (solid triangles) with

the same postsynaptic element. Open triangles point to the undercoating of the plasma membrane; open circles label microtubule bundles, both being characteristic ultrastructural features of axon initial segments. A and B are from cell No. 3 in Table 1. C and D are serial sections from cell No. 12 in Table 1. Scale bars = 0.4 μm in A and B, 0.3 μm in C and D.

FIGURE 8. Electron micrographs of biocytin-filled basket cell boutons (cell No. 12 in Table 1) in synaptic contact (arrows) with the apical (PD_a , A) or basal (PD_b , B) dendrites and somata of pyramidal cells. The same bouton in C and D (serial sections) makes

synapses with both pyramidal cells (PS_1 and PS_2). Asterisk denotes an unlabeled bouton in synaptic contact (triangle) with PS_2 . Scale bar = 0.3 μm in A–D (same magnification).



The area of synaptic boutons, measured at the largest extent of the synaptic junction, was significantly smaller ($0.186 \pm 0.011 \mu\text{m}^2$, $n = 43$ boutons, $n = 2$ cells, Mann-Whitney U-test, $Z = -6.85$, $P < 0.0001$) than that of basket cells. In all nine bistratified cells, the low proportion ($n = 3$) or absence ($n = 6$) of somatic synaptic targets (Table 1) was reflected (correlation -0.84) by the high proportion of dendritic shafts (Fig. 2). One cell (No. 20 in Table 1) made more somatic synapses ($n = 6$, 24%, Fig. 14B) than the remainder of the bistratified cells ($2 \pm 4\%$). In the case of bistratified cell No. 21 (Table 1), in addition to one synapse on a pyramidal soma, three synapses were made on the cell body of a non-principal neuron, but for the display of the data in Figure 11 they were lumped together. The main targets of the 186 synapses ($n = 151$ this study) of bistratified boutons

($n = 176$, 1.06 synapse/bouton) were dendritic shafts ($79 \pm 8\%$) and to a lesser extent dendritic spines ($17 \pm 8\%$). Serial sections showed that one bouton sometimes innervated two to three adjacent dendrites (Figs. 13D,E). Both the main trunks of apical dendrites (Fig. 13A) and their side branches received synapses (Figs. 13D,E). Basal dendrites of pyramidal cells were identified on the basis of sparse type 2 synaptic input to their shafts and from the appearance of emerging spines (Fig. 13C). The symmetrical synapses given to spines (Figs. 14C,D) by bistratified cells were always associated with a type 1 (asymmetrical) synapse received by the same spine (Fig. 14D), but this was not necessarily apparent in a single section (Fig. 14C). Non-spiny, beaded dendrites (Fig. 14A), belonging to other non-principal cells, also occurred among the targets ($n = 3$).

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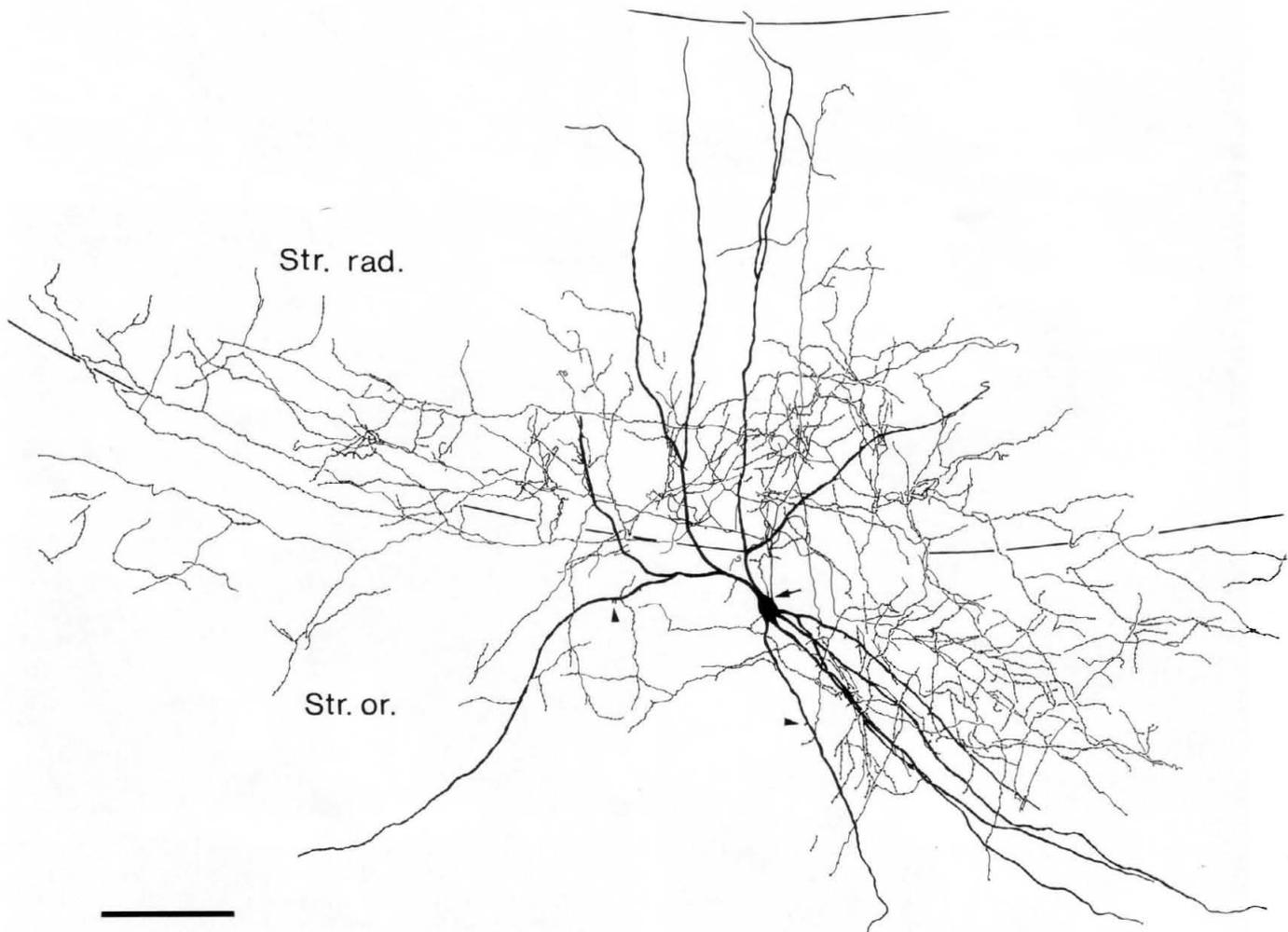


FIGURE 9. Axonal and dendritic arborization of an intracellularly recorded bistratified cell (No. 24 in Table 1). Dendrites were reconstructed from the entire slice, but the axon was only reconstructed from two 60- μm -thick sections. The main axon (arrow) emerges from the pyramidal-shaped soma which is situated in the pyramidal cell layer. The majority of axonal branches are within the inner one-third of stratum radiatum and throughout stratum oriens,

whereas the pyramidal cell layer contains relatively little axon. The dendrites rarely emit spines (arrowheads), and they occupy stratum radiatum and oriens, penetrating into the alveus, but avoiding stratum lacunosum-moleculare. A random electron microscopic sample of labeled synaptic boutons (see also Figs. 13C, 14A) revealed that this cell targeted pyramidal dendritic shafts (89%) and spines (11%). Scale bar = 100 μm .

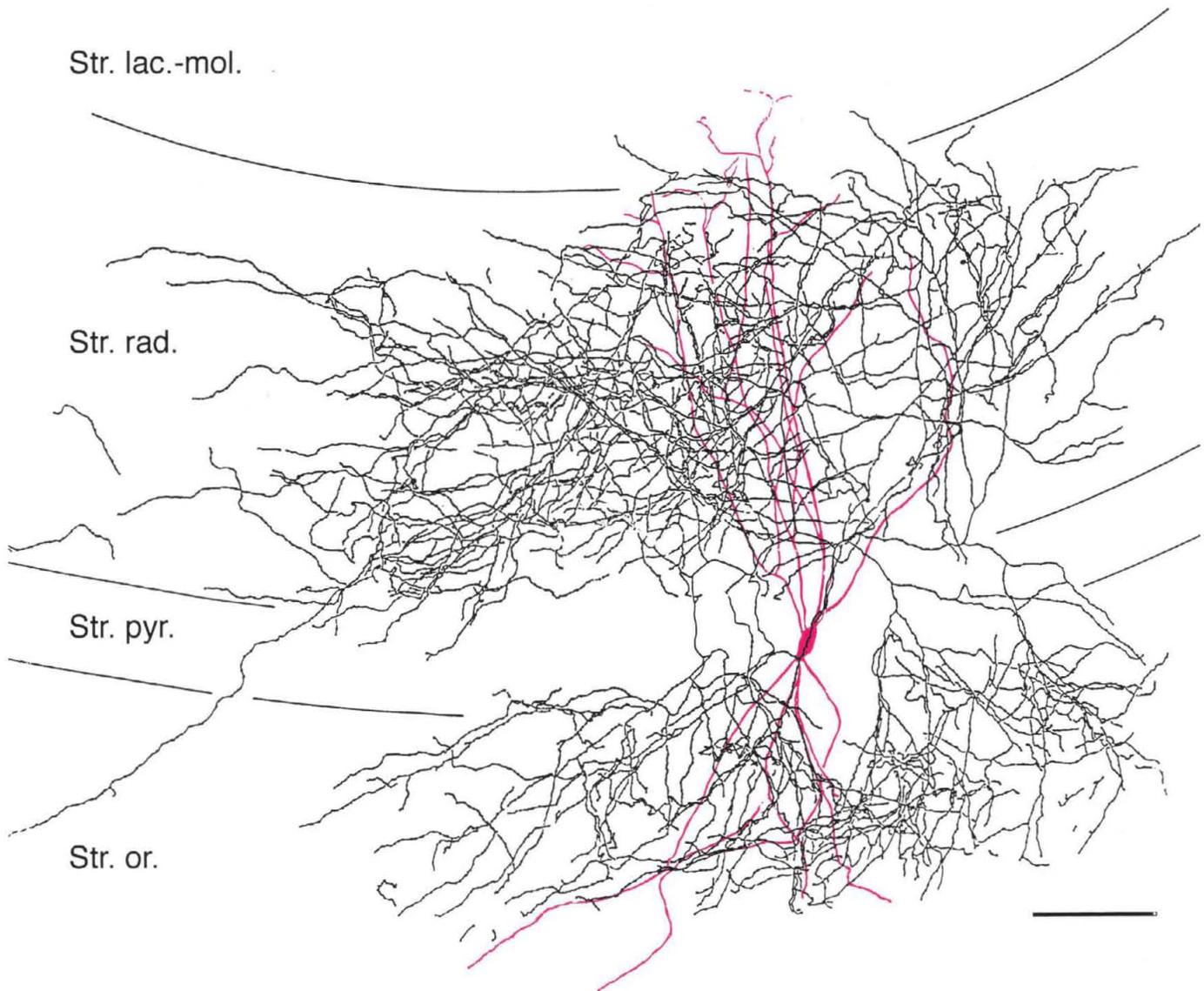


FIGURE 10. A-C. Axonal and dendritic arborization of a bistratified cell (No. 25 in Table 1), which was reconstructed from the entire 400- μm -thick slice. The elongated soma and dendrites (red) are similar to the bistratified cell shown in Figure 9, but the axonal

arbor (black) covers the full radial depth of both strata oriens and radiatum with only few branches traversing stratum pyramidale. The synaptic targets of this cell were confined to pyramidal cell dendritic shafts (85%) and spines (15%). Scale bar = 100 μm .

GABA Immunoreactivity of Basket and Bistratified Cells

Antibodies against GABA and a highly sensitive postembedding immunogold method (Halasy and Somogyi, 1993) were applied to test the transmitter content of 19 terminals of two bistratified cells and 12 terminals of two basket cells. As shown by the accumulation of immunoparticles over the biocytin-filled boutons of both basket (Fig. 15A,B) and bistratified (Fig. 15C,D) cells, the terminals of the tested neurons are enriched in GABA. In the examined cases, the postsynaptic targets of the identified basket and bistratified boutons were GABA immunonegative. The postsynaptic somata that received synapses from the identified

GABA immunopositive basket cell terminals also received synapses from GABA-immunopositive unidentified terminals.

DISCUSSION

The non-pyramidal cells analyzed quantitatively in this study clearly fall into two distinct categories in terms of their synaptic connections. The spatial distribution of their axons, reflecting postsynaptic target preference, and their dendrites, indicating input preference, are significantly different. In addition, their synaptic boutons are of different size, presumably reflecting differences

in the frequency of transmitter release, as in other axons (Ryugo et al., 1996).

How Distinct Are the Synaptic Targets of Basket and Bistratified Cells?

On the basis of their postsynaptic elements, three main types of GABAergic interneuron are known so far to have their somata in or near the pyramidal cell layer of the CA1 hippocampal field: axo-axonic cells (Somogyi et al., 1985; Li et al., 1992; Buhl et al., 1994b), basket cells (Lorente de Nó, 1934; Buhl et al., 1994a; Sík et al., 1995), and bistratified cells (Buhl et al., 1994a; Sík et al., 1995). Some other intracellularly marked cells reported earlier (Schwartzkroin and Mathers, 1978; Schwartzkroin and Kunkel, 1985; Lacaille et al., 1987; Lacaille and Williams, 1990) may also fall into these categories, but without a quantitative assessment of their synaptic targets, the identity of the cells remains uncertain. The distinction between the above-mentioned three classes of interneuron is based most safely on their postsynaptic target area on pyramidal cells. Although some degree of overlap may be found in the targets of individual cells from two categories, most cells terminate in completely segregated postsynaptic surfaces. There is no overlap between axo-axonic and bistratified cells, as none of the 186 postsynaptic elements were axon initial segments. Similarly, there is little overlap on the post-synaptic somatic surface of bistratified and basket cells, since only three out of nine bistratified cells made a few synapses on somata.

An overlap in the placement of the basket and bistratified cell synapses appears to be present in the dendritic domain of pyram-

idal cells, as basket cells target about half of their terminals to proximal dendrites, which may also be innervated by some bistratified cells; the latter innervate more distal dendrites as well. The degree of overlap is not yet known, since the synapse distribution of only one bistratified cell has been partly established on an identified pyramidal cell (Buhl et al., 1994a), but this particular cell concentrated its terminals close to the pyramidal layer. Other bistratified cells, such as the one shown in Figure 10, would probably have little overlap with basket cells. The surface of the pyramidal soma is only sparsely covered by GABAergic synapses; the total number of synapses on one cell was 125 (Buhl et al., 1994a). Therefore, the placement of basket cell synapses also on the dendrites probably reflects a statistical optimization of a collective effect of the ten to 12 synaptic junctions provided by a single basket cell and not the lack of available postsynaptic somatic membrane. Similarly, the occasional synapse of a bistratified cell to the soma may represent an element in the collective and statistical optimization of providing a postsynaptic effect with particular kinetics.

How Homogeneous Are the Basket and Bistratified Cell Categories?

The fast transmitter of both cell types is GABA, as evident from the postsynaptic responses to their action (Buhl et al., 1994a) and the presence of GABA in their terminals. However, other neurochemical characteristics influencing their synaptic action, such as the possible co-release of neuroactive peptides or the presence of calcium-binding proteins in the terminals and in the somato-dendritic domain, are likely to be different.

Basket cells

Synaptic terminals on pyramidal cell bodies are immunoreactive either for parvalbumin (Kosaka et al., 1987; Katsumaru et al., 1988; Sloviter, 1989; Celio, 1990; Sík et al., 1995) or cholecystokinin (CCK) (Harris et al., 1985; Hendry and Jones, 1985; Nunzi et al., 1985; Totterdell and Smith, 1986), which is probably co-localized with vasoactive intestinal polypeptide (VIP) (Acsády et al., 1996). Therefore, basket cells, which provide most of these terminals, are neurochemically heterogeneous. Since parvalbumin-immunopositive somata are mostly restricted to the pyramidal cell layer and stratum oriens (Kosaka et al., 1987; Sloviter, 1989; Gulyás et al., 1991), but CCK-containing cell bodies are also present in strata radiatum and lacunosum (Greenwood et al., 1981; Gall, 1984; Somogyi et al., 1984; Nunzi et al., 1985; Gulyás et al., 1991), these two neurochemical markers may also delineate functionally distinct classes of basket cells. The cells presented in this report may include both classes of basket cells. The dendritic and axonal arbors of a parvalbumin-immunoreactive basket cell have been documented (Sík et al., 1995) and are similar to the majority of our basket cells, as exemplified in Figures 3 and 4. One feature of these cells is the frequent branching of dendrites in stratum radiatum and particularly in lacunosum-moleculare. Other, more rarely encountered basket cells, such as the one shown in Figure 5, showed little branching of the dendrites in strata radiatum and lacunosum-moleculare. The somatic characteristics of the latter group is remarkably similar to synaptically

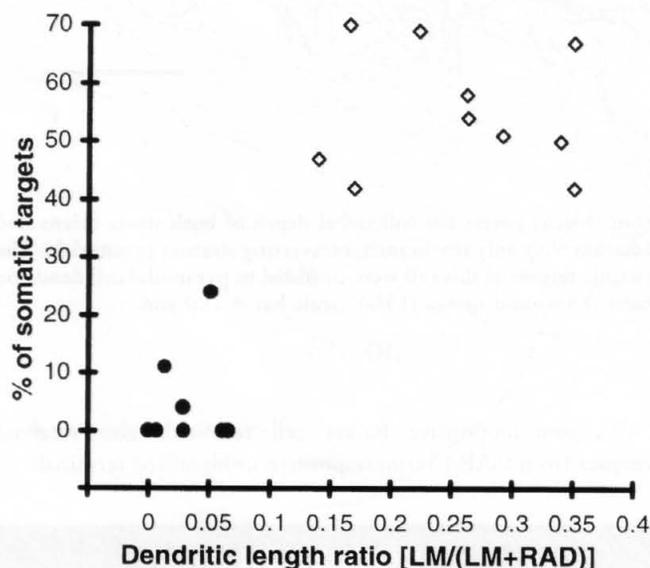


FIGURE 11. Comparison of 19 non-principal cells having well-filled dendrites (marked in Table 1), with respect to the relative extent of their dendrites in stratum lacunosum-moleculare and proportion of their synapses on pyramidal cell bodies. The cells fall in two non-overlapping populations, basket cells (diamonds) having a significantly larger proportion of their dendrites in lacunosum-moleculare ($P < 0.0002$) than bistratified cells (solid circles; lower right double data point = 3 cells).

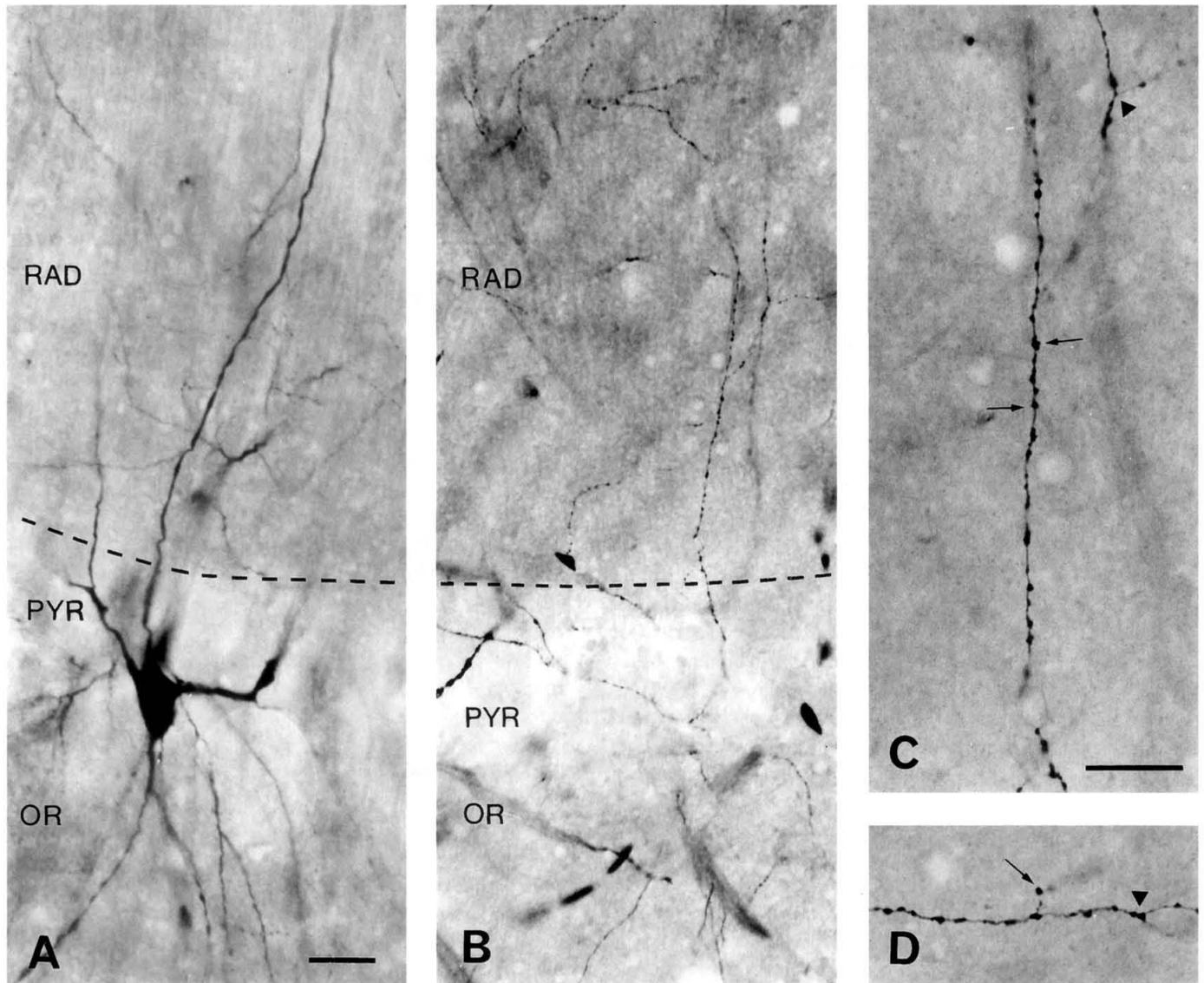


FIGURE 12. Light micrographs of biocytin-injected bistratified cells. **A:** Cell body, dendrites, and axon branches of a bistratified cell (No. 23 in Table 1). **B:** Detail from the axonal arbor of the bistratified cell shown in Figure 10. The relative paucity of branches is due to the narrow depth of focus. **C,D:** Higher-magnification view of bistratified cell axon collaterals in stratum radiatum. Note the high

density of "en passant" axonal varicosities (e.g., arrows), shown by electron microscopy to represent synaptic boutons. **D:** Boutons "en terminaux" (arrow) is an additional feature of bistratified cell axons. Arrowheads show bifurcations of the axon. Broken lines mark the borders of hippocampal layers. Scale bars = 25 μm in A and B (same magnification), 15 μm in C and D (same magnification).

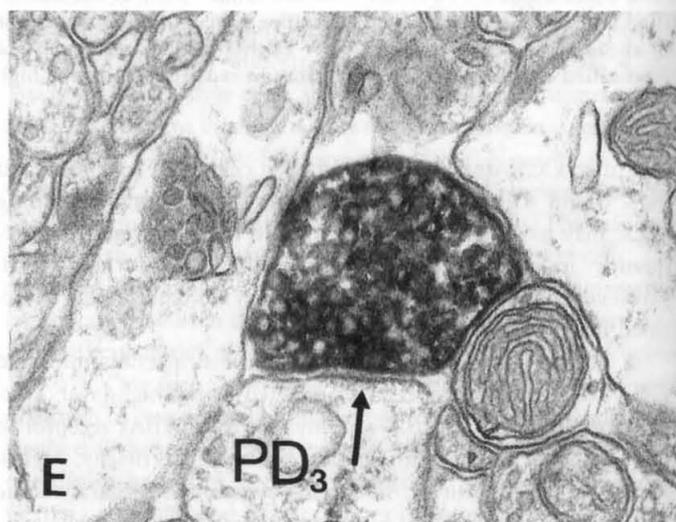
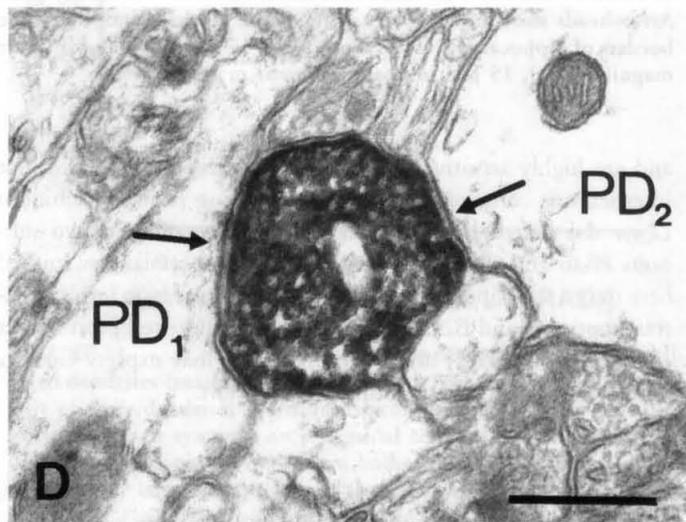
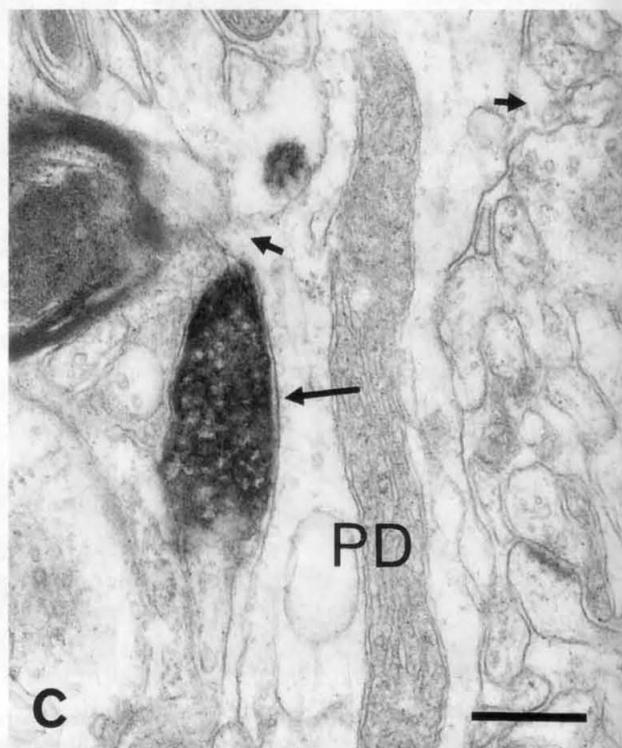
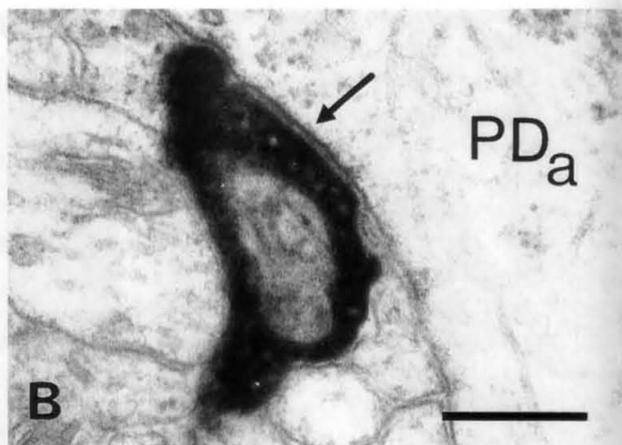
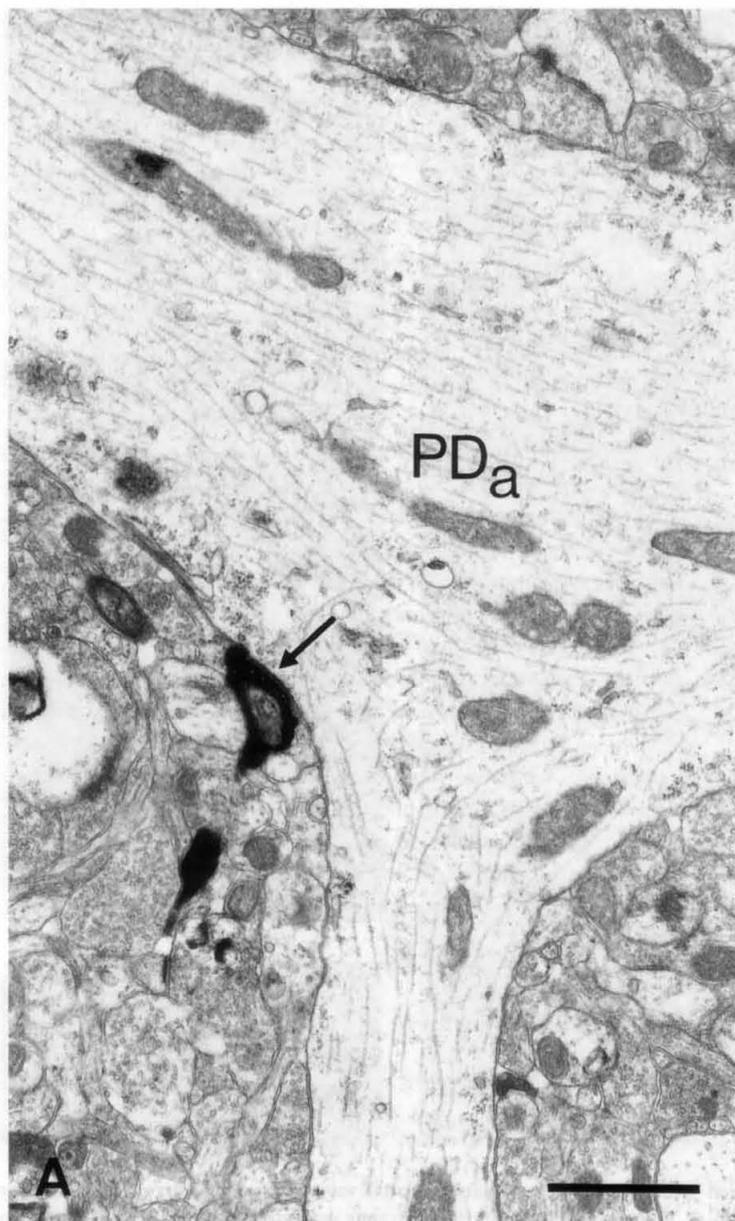
identified CCK-immunopositive basket cells (cf. Fig. 5 here, and Figs. 6 and 8 in Nunzi et al., 1985). Interneurons containing CCK may have their somata in stratum radiatum, and they frequently innervate each other as well (Nunzi et al., 1985). However, not all CCK-positive cells may be basket cells, as defined by their synaptic output.

A further functional difference between parvalbumin and CCK-containing basket cells is predicted by the high level of expression of the $\alpha 1$ and $\beta 2/3$ subunits of the GABA_A receptor in the former in contrast to the latter (Gao and Fritschy, 1994). Most of this receptor in the plasma membrane is extrasynaptic (Nusser et al., 1995). The high density of GABA_A receptors predicts that these basket cells are under strong GABAergic control

and are highly sensitive to type I benzodiazepine ligands. Since interneurons containing the calcium-binding protein, calbindin D_{28k}, also lacked high levels of the above-mentioned two subunits (Gao and Fritschy, 1994), in the cell populations studied here only a subpopulation of basket cells express high levels of extrasynaptic $\alpha 1$ and $\beta 2/3$ subunits of the GABA_A receptor. Basket cells containing CCK and bistratified cells may express GABA_A receptors with different subunit composition.

Bistratified cells

The somata of all bistratified cells reported here were in stratum pyramidale, but this may be due to our method of sampling.



A bistratified cell having a cell body in stratum oriens was shown to be immunoreactive for calbindin (Sík et al., 1995). Calbindin-immunopositive cells are present throughout strata oriens, pyramidale, and radiatum, and their dendrites, like those of bistratified cells, do not seem to enter stratum lacunosum-moleculare (Sloviter, 1989; Gulyás et al., 1991). It is therefore likely that interneurons with bistratified axonal arbors paralleling the Schaffer collateral/commissural terminal fields are present in all layers but lacunosum-moleculare (A. Gulyás and T.F. Freund, personal communication).

Calbindin-immunoreactive boutons have not been reported to make synaptic contacts with cell bodies of pyramidal cells (Seress et al., 1993). However, about 10% of calbindin-immunoreactive interneurons also contained immunoreactive parvalbumin (Gulyás et al., 1991). The synaptic connections of cells containing both calcium-binding proteins are unknown. Since calbindin is present in at least some bistratified cells (Sík et al., 1995), it could be argued that some interneurons which contain both calcium-binding proteins are transitional cells between the two classes. This is unlikely, because parvalbumin-immunopositive terminals are largely restricted to the perisomatic region and do not occur in distal stratum radiatum which contains bistratified cell terminals.

As discussed above, some basket cells contain CCK, and it remains to be established if bistratified cells contain any neuroactive peptide. In stratum pyramidale, where bistratified cell bodies are located, some calbindin-immunopositive interneuron cell bodies have been shown to contain CCK (Gulyás et al., 1991), but whether any of these cells make synaptic connections similar to bistratified cells is not yet known. There appear to be few, if any, CCK-immunopositive boutons on distal stratum radiatum dendrites of pyramidal cells in the CA1 area; therefore, bistratified cells are distinct from CCK-containing basket cells.

Bistratified cell axons show cell-to-cell variability in their radial extent in strata radiatum and oriens. Some axons are more biased toward stratum oriens and the proximal radiatum; others innervate stratum radiatum more extensively (cf. Fig. 10 here and Fig. 2 in Buhl et al., 1994a). This is remarkably similar to the systematic variation in the extent of the CA3 axons innervating the CA1 area (Ishizuka et al., 1990; Li et al., 1994). As pyramidal cells are topographically arranged from the hilus toward the border with the CA1 area, they innervate progressively more of stratum oriens and less of radiatum. It remains to be tested whether bistratified cells with an axonal bias for certain laminae

are preferentially innervated by Schaffer collateral/commissural axons with similar or opposite laminar preference and whether the GABAergic terminals selectively associate on the postsynaptic dendritic surface with subsets of the glutamatergic Schaffer collateral/commissural terminals. Axonal selectivity of dendrite targeting interneurons for different laminae of the CA3 area has also been demonstrated in the guinea pig (Gulyás et al., 1993a).

Divergence and Convergence of GABAergic Connections

The divergence of basket cells labeled *in vivo* has been calculated by Sík et al. (1995), using two-dimensional axon length measurements and bouton density estimates along the axon, in addition to an average value of six synapses from a basket to a pyramidal cell. Our estimated total bouton number for a basket cell (10,800 boutons) is in the range (9,200–12,100) of that calculated by Sík et al. (1995). However, previous measurements show that in two directly determined cases, the total number of synapses from a basket to a pyramidal cell were 10 and 12, respectively (Buhl et al., 1994a, 1995). If these values are representative for the population, then the divergence calculated by Sík et al. (1995) will require downward revision. Our estimate of 1,140 pyramidal cells innervated by a basket cell is remarkably similar to the approximately 1,200 postsynaptic pyramidal cells estimated for an axo-axonic cell (Li et al., 1992), suggesting a modular organization of GABAergic innervation.

A single basket cell provides only a small proportion of the GABAergic synapses to the perisomatic region of a pyramidal cell. The pyramidal cell soma shown in Figure 6 appears to be contacted by six boutons. Two basket cells were established to provide five synapses each to the somata of two identified postsynaptic pyramidal cells (Buhl et al., 1994a, 1995). One of these pyramidal cells received 125 synapses on the soma, leading to an estimated convergence of 25 basket cells (Buhl et al., 1994a). Such a calculation assumes small variation in the contribution of individual cells, which remains to be fully examined, particularly with respect to the somatic innervation provided by cells located outside the pyramidal cell layer. Taking into account only the parvalbumin-containing interneurons, Sík et al. (1995) arrived at a higher estimate of 30–40 converging interneurons that included axo-axonic cells, but not the CCK/VIP-immunopositive basket cells (Acsády et al., 1996). Their calculation included the estimated value of six basket-to-pyramidal cell synapses, which is lower than our direct measurement.

Similar calculations are not yet possible for bistratified cells, since, due to their dispersed distribution on the dendritic surface, it is much more difficult to determine accurately the number of synapses provided by a bistratified cell to a pyramidal cell. In one case, six contacts were observed between the axon of a bistratified cell and the dendrites of a pyramidal cell, and out of the six sites three synaptic junctions could be positively identified (Buhl et al., 1994a); the other three sites were not tested. Using a value of six synapses and two-dimensional axon length measurements, Sík et al. (1995) estimated a divergence of 2,500. For obtaining an estimate of convergence, unfortunately it is not yet possible to

FIGURE 13. Electron micrographs of synaptic junctions (long arrows) established by bistratified cell axon terminals with dendrites. A: A bistratified cell (No. 20 in Table 1) bouton makes a synapse with an apical dendrite (PD_a), where it gives rise to a secondary dendritic branch. B: Higher magnification of the synapse shown in A. C: A pyramidal cell basal dendrite (PD) in stratum oriens receives a synapse (arrow) from a bistratified cell (No. 24 in Table 1) bouton. Small arrows mark origin of spines. D,E: Serial sections of a bouton from cell No. 23 in Table 1 establishing synapses with three small-diameter dendrites (PD₁, PD₂, PD₃) in stratum radiatum. Scale bars = 1 μm in A; 0.4 μm in C; 0.3 μm in B, D, and E (same magnification).

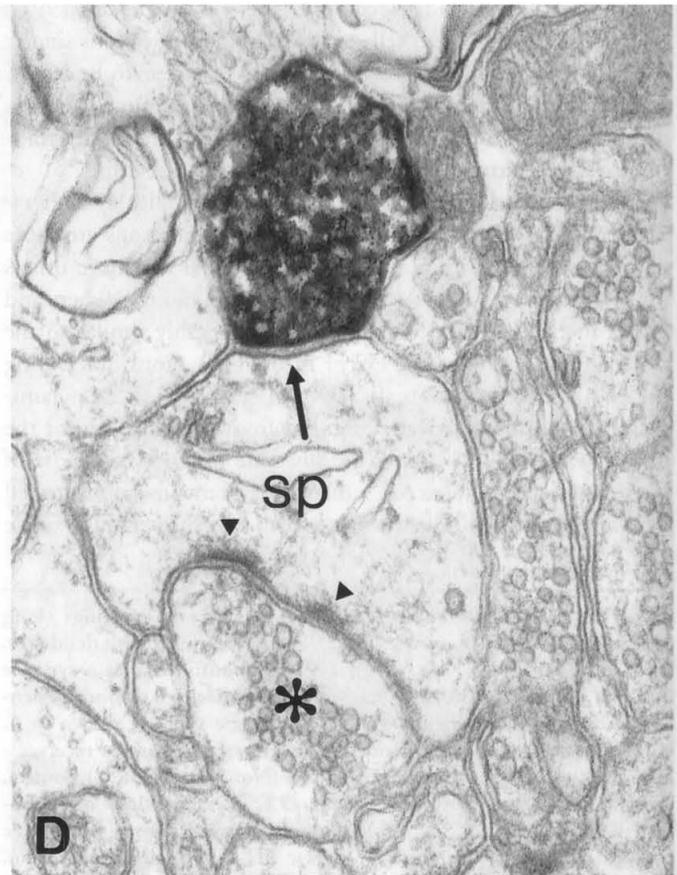
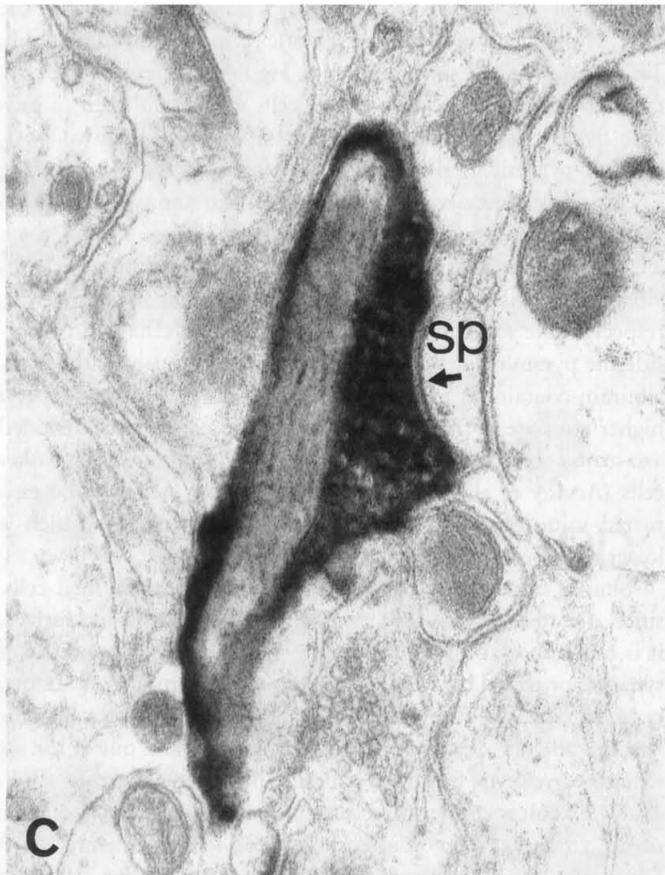
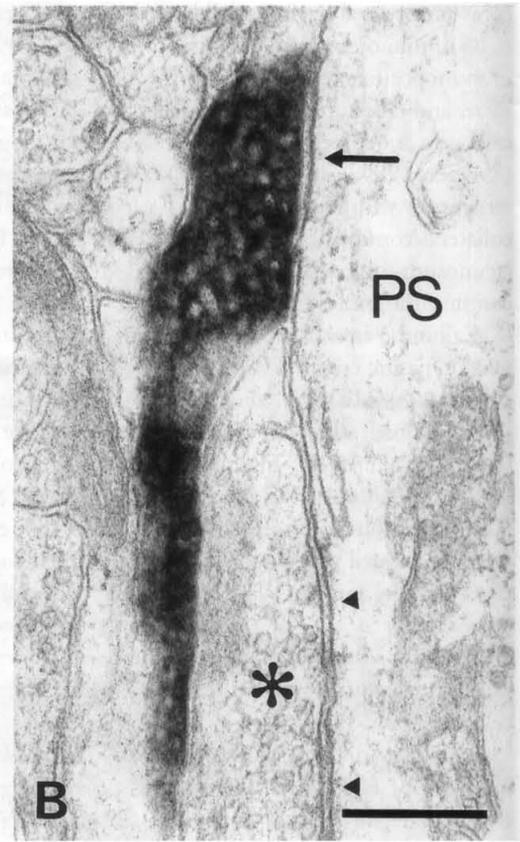
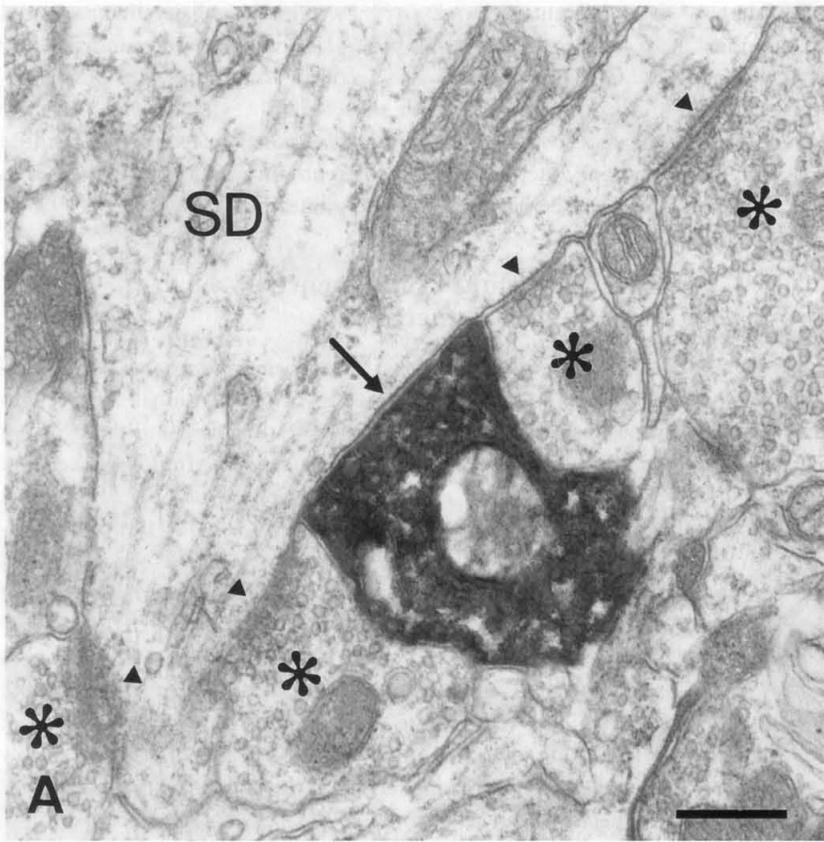


FIGURE 14. Electron micrographs of biocytin-filled bistratified cell boutons and their postsynaptic targets. **A:** A smooth dendrite (SD), with characteristics of non-pyramidal cells, is in synaptic contact with a labeled bouton (arrow, from cell No. 24 in Table 1) and four non-identified boutons (asterisks and arrowheads). **B:** Pyramidal cell somata (PS) rarely receive synapses from bistratified cells (arrow, cell No. 20 in Table 1). Asterisk denotes an unlabeled bouton, and arrowheads mark synaptic junctions established by an unidentified bouton. **C:** A small dendritic spine (sp) in synaptic contact with a bistratified cell bouton (from cell No. 20 in Table 1). **D:** A large spine (sp) is postsynaptic to a bistratified cell bouton (arrow, cell No. 23 in Table 1) as well as to an unidentified bouton (asterisk) making an asymmetrical synaptic junction (arrowhead). Scale bars = 0.4 μm in A; 0.3 μm in B–D (same magnification).

estimate the total number of bistratified cell boutons received by a pyramidal cell. If all bistratified cells contain calbindin, and calbindin is present in detectable concentration in their terminals, quantitative immunocytochemical determination of the volume density of terminals that contain both calbindin and GABA could be used to calculate the total number of bistratified cell synapses per pyramidal cell. To our knowledge, synapses made by calbindin-containing hippocampal interneurons have not been reported, but work in progress indicates that they are mainly on the dendritic trees of pyramidal cells (A. Gulyás and T.F. Freund, personal communication).

Inputs to Basket and Bistratified Cells

The two interneuron classes studied here exhibited similarities and differences in the spatial distribution of their dendritic trees. Both types had extensive dendritic arbors in strata radiatum and oriens, where they are in a position to receive excitatory input from the Schaffer-collateral/commissural pathway (Buhl et al., 1996). Both cell types send dendrites equally deep into stratum oriens and the alveus, where they overlap with the local axon collateral plexus of pyramidal cells. An identified basket cell was shown to receive a single synaptic contact from the axon collateral of a pyramidal cell in the alveus of the CA1 area (Buhl et al., 1994a). Basket cells in the CA3 area of the guinea pig are also innervated by pyramidal cells through single or few boutons (Gulyás et al., 1993b; Arancio et al., 1994). Although each pyramidal cell may contribute few synapses to a given basket cell, their local recurrent input could still be significant, since pyramidal cells outnumber basket cells by at least two orders of magnitude. Bistratified cells have not been shown to receive recurrent pyramidal cell input. None of the bistratified cells in our material were closely approached by the axons of simultaneously recorded and filled pyramidal cells in the CA1 area. Although it remains to be tested directly, it is possible that for bistratified cells the dominant excitatory input comes only from pyramidal cells of the CA3 area.

The small extent, or in some cases, the complete lack of bistratified cell dendrites in stratum lacunosum indicates that they receive little, if any, direct input from the entorhinal cortex or the thalamic nucleus reuniens. In contrast, basket cells always ex-

tend dendritic branches into stratum lacunosum-moleculare, where they are likely to receive direct entorhinal input. Electrical stimulation of entorhinal afferents evokes prominent inhibition in CA1 pyramidal cells in the rat (Colbert and Levy 1992; Soltész and Deschênes, 1993; Buzsáki et al., 1995; Soltész, 1995), but it is not known which interneurons mediate this action. In addition to basket cells, axo-axonic cells and different GABAergic neurons having cell bodies in stratum radiatum and lacunosum-moleculare also send dendrites to the entorhinal termination zone (Lorente de Nó, 1934). The entorhinal input promotes gamma frequency (30–80 Hz) oscillatory activity observed in the CA1 area (Chapack et al., 1995; see also Bragin et al., 1995), which is modulated at theta (4–12 Hz) frequency (Soltész and Deschênes, 1993; Bragin et al., 1995). Basket cells fire action potentials on the positive phase of the hippocampal field theta activity, as recorded in the pyramidal cell layer in anesthetized animals (Sik et al., 1995). *In vitro*, even a single basket cell can phase-lock and synchronize pyramidal cells in the theta frequency range (Cobb et al., 1995). Thus, it is likely that their entorhinal input, which is also modulated at theta frequency during theta activity (Boeijinga and Lopes da Silva, 1988; Chrobak and Buzsáki, 1994), assists in the phasing of basket cell activity and maintains coherence in the postsynaptic pyramidal cell population.

Hippocampal interneurons are targets of subcortical afferents, such as the GABAergic and cholinergic septo-hippocampal pathways. Several types of GABAergic neuron, including those containing parvalbumin, calbindin, and CCK, are directly innervated by the GABAergic axons (Freund and Antal, 1988; Gulyás et al., 1990), whereas serotonergic median raphe afferents innervate calbindin- (Freund et al., 1990) and calretinin-containing (Acsády et al., 1993) interneurons. One bistratified cell has been shown to contain calbindin (Sik et al., 1995); thus, these cells might receive inputs from one or both subcortical pathways, whereas basket cells, some of which contain parvalbumin or CCK, may be innervated only by the septal afferents. Our physiological results show that both basket and bistratified cells receive GABAergic input, probably acting through both GABA_A and GABA_B receptors (Buhl et al., 1996), but the source of these inputs remains largely unknown. A parvalbumin-immunopositive basket cell has been shown to innervate other parvalbumin-immunoreactive cells in the CA1 area, and some of these cells could have been other basket cells (Sik et al., 1995).

Other Types of Interneuron

Basket and bistratified cells are only two of the numerous GABAergic interneuron types present in the CA1 area of the hippocampus. Below, we briefly summarize the most distinct cell types described so far, which are unlikely to be part of the cell populations presented in this article. In the pyramidal cell layer, in addition to axo-axonic cells (Somogyi et al., 1985; Li et al., 1992), there are numerous calretinin-immunoreactive neurons (Miettinen et al., 1992) which do not belong to either of the above three types of non-principal cells (Acsády et al., 1996). Some calretinin-immunopositive cells are immunoreactive for va-

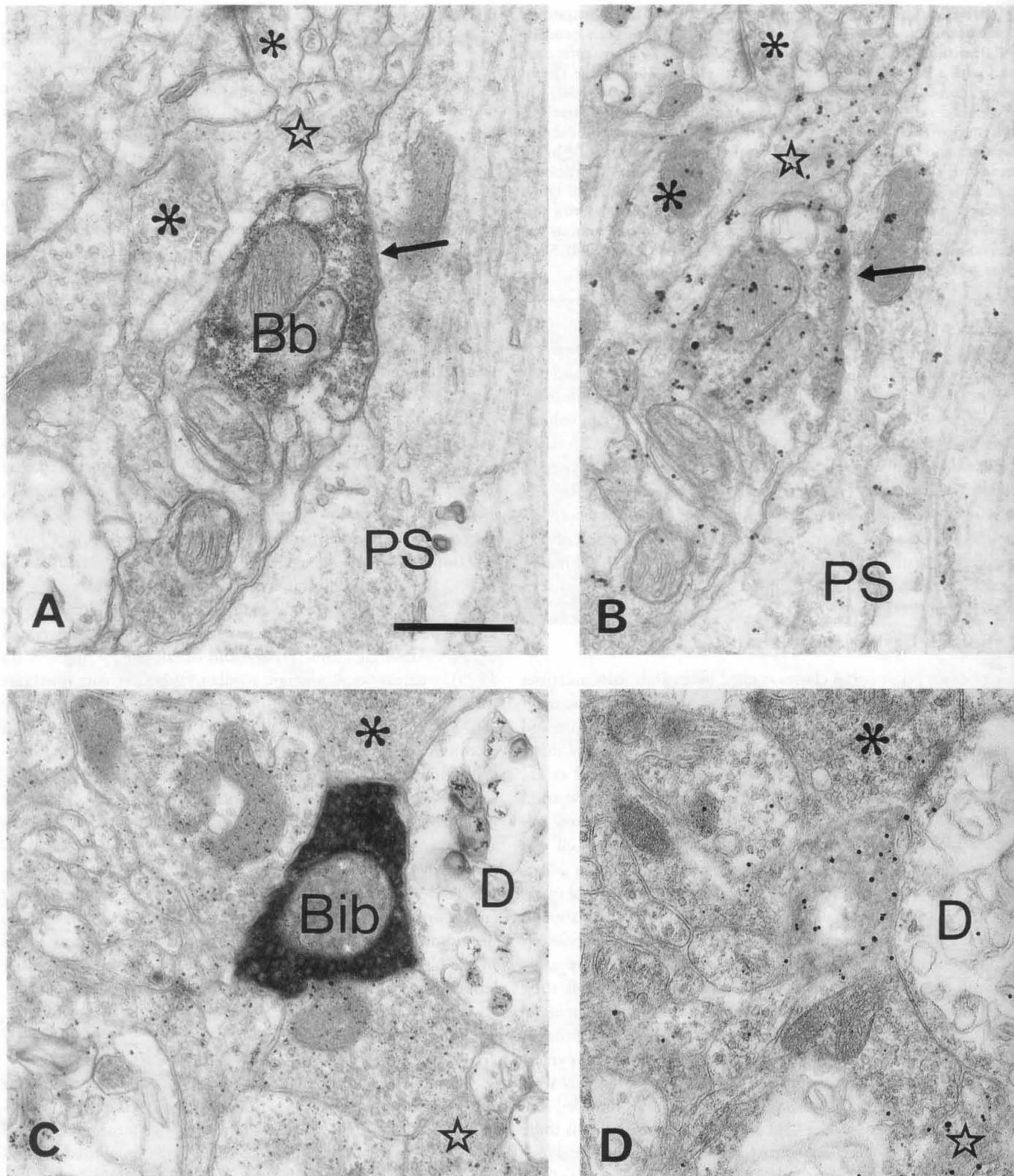


FIGURE 15. Electron micrographs showing immunoreactivity for GABA in terminals of identified basket (No. 9 in Table 1) and bistratified (No. 25 in Table 1) cells. A,B: Serial sections of basket cell bouton (Bb) contacting (arrow) a pyramidal cell soma (PS). Following removal of osmium tetroxide, the section in B was immunoreacted with antibodies to GABA. The enrichment of silver-intensified gold particles over the basket cell bouton demonstrates that it is immunopositive. Note the paucity of labeling over the post-

synaptic soma. C,D: Serial sections of a bistratified cell bouton (Bib) next to a dendrite (D). Following immunogold reaction for GABA, electron-dense metal particles are enriched over the bistratified cell bouton. Open stars mark unidentified GABA-immunopositive boutons, asterisks label immunonegative boutons. The small granular electron-dense precipitate in C is due to the slice-processing procedure. Scale bar = 0.5 μ m in A–D (same magnification).

soactive intestinal polypeptide (VIP), which is also present in at least two other, different interneuron populations (Acsády et al., 1996), as well as in the CCK-immunoreactive basket cells discussed above.

There are also occasional cells in the pyramidal layer which are immunoreactive for both somatostatin (Kosaka et al., 1988) and calbindin (Tóth and Freund, 1992); however, they are probably displaced members from populations in stratum oriens/alveus. The somatostatin-immunopositive GABAergic cells (Somogyi et al., 1984; Kosaka et al., 1988; Kunkel and Schwartzkroin, 1988) are a distinct cell type innervating mainly stratum lacunosum-moleculare (McBain et al., 1994; Sík et al., 1995).

Stratum oriens/alveus also contains cells whose dendrites remain in the same layers, but their axon innervates the entire Ammon's horn and the dentate gyrus (Sík et al., 1994, 1995). These cells were suggested to correspond to the few cells that stain very intensely with NADPH diaphorase reaction and are distinct from basket and bistratified cells. A different cell with dendrites also confined to stratum oriens was reported by Sík et al. (1995), who called it the *trilaminar cell*, referring to the axon being distributed from oriens to radiatum, but being most extensive in radiatum. Although the axon of this cell resembled those of bistratified cells, its dendritic arbor and physiological properties appeared to be very different. A detailed quantitative comparison of the postsynaptic targets of trilaminar and bistratified cells may help to investigate differences in their function.

Stratum lacunosum-moleculare contains many interneurons, particularly at the border with radiatum (Ramón y Cajal, 1893; Lorente de Nó, 1934; Kawaguchi and Hama, 1987, 1988; Lacaille and Schwartzkroin, 1988a,b; Kunkel et al., 1988). The interneuron population here probably contains cells specific to this layer and associated with the entorhinal innervation, in addition to cell types also present in stratum radiatum and the other layers, but their synaptic targets remain to be evaluated quantitatively. It is likely that the somata of GABAergic cells innervating the Schaffer collateral/commissural termination zone, i.e., bistratified cells, are also present at the border of strata radiatum and lacunosum-moleculare.

Functional Implications

The segregated, GABAergic innervation of the perisomatic and dendritic regions of pyramidal cells by basket and bistratified cells, respectively, allows independent control of the input and output of the principal cells. Although both types of GABAergic interneuron appear to act exclusively through GABA_A receptors (Buhl et al., 1994a, 1995), the onset and time course of their synaptic action in combination with the precise placement of their synapses may lead to very distinct physiological roles. Basket cells influence the firing pattern of pyramidal cells (Cobb et al., 1995; for review, see Buzsáki and Chrobak, 1995) and are responsible for the control of the final output. Their relatively large boutons contain numerous mitochondria and suggest tonic release of GABA. Physiological evidence suggests that tonic inhibitory input arrives in the perisomatic region (Soltész et al., 1995).

In contrast, bistratified cells are in a key position to influence

selectively the effect of the glutamatergic Schaffer collateral/commissural input from the CA3 area. In this respect they represent a well-developed example of the recently discovered co-stratification of glutamatergic and GABAergic afferents in the dendritic domain of hippocampal principal cells (Han et al., 1993; Halasy and Somogyi, 1993; Gulyás et al., 1993a). Bistratified cells elicit IPSPs with significantly longer rise and decay parameters than those of basket cells (Buhl et al., 1994a) and could primarily modulate the *N*-methyl-D-aspartate receptor-mediated component of the CA3 input (Staley and Mody, 1992) and/or the activation of voltage-dependent conductances in the dendrites. Indeed, the activation of dendritic inhibition prevented calcium-dependent burst firing in pyramidal cells of the CA3 area (Miles et al., 1994). Pathway-specific GABAergic reduction of excitatory postsynaptic potentials (EPSPs) could also extend the dynamic range for temporal summation (Halasy and Somogyi, 1993). In a detailed model of the effect of conjoint glutamatergic and GABAergic inputs to the dendrites of cerebellar granule cells, Gabbiani et al. (1994) calculated that a small dendritic inhibitory input could greatly increase the sensitivity of the postsynaptic cell to the temporal summation of repetitive EPSPs, with a maximal response evoked by coinciding inputs. The GABAergic bistratified cell input to the dendritic area of pyramidal cells innervated by the Schaffer collateral/commissural input may act in a similar manner. By reducing the amplitude of EPSPs, it could enhance subthreshold integration from multiple input fibers and, by narrowing the temporal window for integration, it would predominantly favor coincident inputs.

CONCLUSIONS

The results demonstrate that a quantitative evaluation of synaptic relationships in a heterogeneous neuronal network helps to define distinct cell types and also provides predictions for their roles in the system. The spatial segregation of basket and bistratified cell GABAergic inputs to pyramidal cells is probably related to the role of basket cells in synchronizing both subthreshold and suprathreshold pyramidal cell activity (Cobb et al., 1995) and to the role of bistratified cells in governing the kinetics of EPSPs evoked by the input from the CA3 area. Individual members of both cell types provide synaptic input, in a stereotypical manner, only to a subpopulation of pyramidal cells within their axonal field. This specific termination pattern predicts that they act as selectors, probably in conjunction with selective inputs from CA3 pyramidal cells, for delineating functionally related pyramidal cell populations.

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