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SYNAPTIC EFFECTS OF IDENTIFIED INTERNEURONS INNERVATING BOTH INTERNEURONS AND PYRAMIDAL CELLS IN THE RAT HIPPOCAMPUS

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Abstract—GABAergic interneurons sculpt the activity of principal cells and are themselves governed by GABAergic inputs. To determine directly some of the sources and mechanisms of this GABAergic innervation, we have used dual intracellular recordings with biocytin-filled microelectrodes and investigated synaptic interactions between pairs of interneurons in area CA1 of the adult rat hippocampus.

Of four synaptically-coupled interneuron-to-interneuron cell pairs, three presynaptic cells were identified as basket cells, preferentially innervating somata and proximal dendrites of pyramidal cells, but one differing from the other two in the laminar distribution of its dendritic and axonal fields. The fourth presynaptic interneuron was located at the border between strata lacunosum moleculare and radiatum, with axon ramifying within stratum radiatum. Action potentials evoked in all four presynaptic interneurons were found to elicit fast hyperpolarizing inhibitory postsynaptic potentials (mean amplitude 0.35 ± 0.10 mV at a membrane potential of -59 ± 2.8 mV) in other simultaneously recorded interneurons (n=4). In addition, three of the presynaptic interneurons were also shown to produce similar postsynaptic responses in subsequently recorded pyramidal cells (n=4). Electron microscopic evaluation revealed one of the presynaptic basket cells to form 12 synaptic junctions with the perisomatic domain (seven somatic synapses and five synapses onto proximal dendritic shafts) of the postsynaptic interneuron in addition to innervating the same compartments of randomly-selected local pyramidal cells (50% somatic and 50% proximal dendritic synapses, n=12). In addition, light microscopic analysis also indicated autaptic self-innervation in basket (12 of 12) and bistratified cells (six of six). Electron microscopic investigation of one basket cell confirmed six autaptic junctions made by five of its boutons.

Together, these data demonstrate that several distinct types of interneuron have divergent output to both principal cells and local interneurons of the same (basket cells) or different type. The fast synaptic effects, probably mediated by GABA in both postsynaptic interneurons and principal cells are similar. These additional sources of GABA identified here in the input to GABAergic cells could contribute to the differential temporal patterning of distinct GABAergic synaptic networks. © 1997 IBRO. Published by Elsevier Science Ltd.

Key words: basket cell, IPSP, microcircuits, inhibition, disinhibition, GABA.

The activity of the large network of principal excitatory neurons in the hippocampus is governed by local-circuit neurons releasing the neurotransmitter GABA. Such GABAergic interneurons occupy distinct positions within hippocampal microcircuits where they form mainly local connections and are thought to perform a range of distinct functions. At the cellular level, these range from governing action potential generation,[6,40,51,66] firing pattern,[7,8,65] membrane potential oscillations[17,76] and dendritic calcium spikes.[64] Moreover, at the network level, GABAergic interneurons are considered to be important in controlling synaptic strength[19] and synchronizing neuronal population activity.[17,92]

Unlike the relatively stereotyped principal cells, hippocampal GABAergic interneurons show great morphological and neurochemical diversity.[24] They may differ with respect to their content of various neuropeptides,[1,20,46,81,82] calcium-binding proteins[32,45,62,71] and in their expression of neurotransmitter receptors.[10,26,57,58] GABAergic interneurons also differ in their synaptic inputs[29,35,61] as well as their synaptic target selectivity.[2,13,31,34,36,37,83]

Although the division of function suggested by such heterogeneity remains to be fully established, some general rules regarding the functional significance of different types of interneuron are beginning

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Abbreviations: ACSF, artificial cerebrospinal fluid; LM-R, strata lacunosum-moleculare/radiatum border; IPSP, inhibitory postsynaptic potential.

to emerge. Indeed, it has been demonstrated that the axonal output of many types of GABAergic interneuron terminate in conjunction with the terminal field of specific excitatory input pathways,[13,31,37] whereas cells that specifically terminate onto the perisomatic domain of principal cells, an area which lacks significant excitatory input, are particularly effective at inhibiting and synchronizing the activity of principal cells.[17,64]

GABAergic interneurons are themselves under GABAergic control. They show both fast (putative $GABA_A$) and slow (putative $GABA_B$) inhibitory postsynaptic potentials (IPSPs)[14,15,48,49,68,80] which are thought to originate from three main sources. These include a selective extrinsic input from GABAergic cells in the medial septum;[3,23,29,61] intrinsic inputs from hippocampal GABAergic interneurons, which also innervate principal cells;[25,38,72,80] and recently described GABAergic interneurons, which selectively target other interneurons.[2,30]

Although some physiological evidence suggests the existence of an interconnected network(s) of inhibitory interneurons,[51,60,78,89,92] electrophysiological studies of GABAergic interneurons, to date, have focused almost exclusively on their influence over the local population of principal cells,[12,13,44,49,51,63,65,66,78] and the mechanism and site of their action on GABAergic cells remain to be established. In this study, we have used dual intracellular recording techniques in conjunction with biocytin labelling to identify directly those types of interneurons in area CA1 of the rat hippocampus that innervate other interneurons in addition to pyramidal cells. Moreover, we sought to establish whether such synaptic interactions differ from those with pyramidal cells. A preliminary report of these findings has been published in abstract form.[18]

EXPERIMENTAL PROCEDURES

Slice preparation

Young adult female Wistar rats (150 g, Charles River, U.K.) were deeply anaesthetized by intramuscular injection of ketamine (100 mg/kg) and xylazine (10 mg/kg). After cessation of pain reflexes the animals were intracardially perfused with approximately 30 ml of chilled artificial cerebrospinal fluid (ACSF), their brains were quickly removed and immersed in a beaker with chilled ACSF. With the aid of a Vibroslice (Campden Instruments, U.K.) 400-μm-thick slices were cut in the horizontal plane. The hippocampi were dissected free and transferred to a recording chamber where they were maintained at 34–35°C on a nylon mesh at the interface between oxygenated ACSF and a humidified atmosphere saturated with 95% O_2 and 5% CO_2. The flow rate was adjusted to 1.5 ml/min and the slices were allowed to equilibrate for 1 h. The ACSF for electrophysiological recordings was composed of (in mM) 126 NaCl, 3.0 KCl, 1.25 NaH_2PO_4, 24 $NaHCO_3$, 2.0 $MgSO_4$, 2.0 $CaCl_2$ and 10 glucose. During the initial stages of the experiments (perfusion, cutting, incubation) all NaCl (126 mM) was replaced by equiosmolar sucrose (256 mM), thus preventing passive chloride entry which has been suggested to be acutely responsible for neurotoxicity during slice preparation.[4] Usually the slices remained 30 min in the sucrose solution before the perfusion medium was changed to normal ACSF.

Intracellular recordings and data analysis

Recording electrodes were pulled from standard wall borosilicate tubing, filled with 2% biocytin[39] in 1.5 M KCH_3SO_4 and bevelled to a d.c. resistance of 80–150 MΩ. All recordings were obtained in the CA1 subfield. Putative interneurons were identified on the basis of their physiological characteristics, such as short-duration action potentials followed by large amplitude fast after-hyperpolarizing potentials. Once a stable recording had been obtained, a search was made for an adjacent neuron. When a stable recording had been established in the second neuron, on-line spike-triggered averaging was used to test for synaptic coupling between the cells in both directions. One of the cells was depolarized by either depolarizing current pulses or tonic current injection to elicit firing at rates of approximately 0.1–1 Hz and responses to up to 64 action potentials were averaged. If a synaptic response was not detected visually, the electrode was advanced, usually destroying the cell, or withdrawn and a new search was made for another cell. Current-clamp recordings were obtained with an Axoprobe amplifier (Axon Instruments, Foster City, U.S.A.) and capacitive coupling was eliminated on-line.[56] Experimental data was acquired using a PCM instrumentation recorder and stored on videotapes. Data analysis was continued off-line by (re)digitizing the data at 5–20 kHz, using a commercially available 12 Bit A/D board (RC Electronics Computerscope, Santa Barbara, U.S.A.). Standard data analysis was carried out using Axograph (Axon Instruments) software.

Resting membrane potentials were determined following electrode withdrawal and are given as the difference between surface d.c. potential and the steady-state membrane potential without bias current injection. Membrane time constants were obtained from small hyperpolarizing current pulses as the time necessary to reach e^{-1} (63%) of the maximum voltage deflection. Likewise, input resistance was determined from measuring the maximal deflection of small amplitude hyperpolarizing current pulses. IPSP measurements include duration (at half-amplitude), rise-time (10–90%) and averaged peak amplitude. Unless indicated otherwise, data is expressed as mean ± S.D.

Histological processing and anatomical evaluation

Following withdrawal of the recording electrodes, slices were sandwiched between two Millipore filters and fixed overnight in 2.5% paraformaldehyde, 1.25% glutaraldehyde and 15% (v/v) picric acid in 0.1 M phosphate buffer (pH 7.4). Following gelatine embedding, slices were resectioned on a Vibratome at 50–60 μm thickness and processed for light and electron microscopy using the avidin–biotinylated–horseradish peroxidase complex (Vector Laboratories), closely following previously described procedures.[37] Following their embedding into Durcupan resin (Fluka, U.K.) on glass microscope slides, all recovered interneurons were scrutinized in the light microscope to establish preliminary classification according to their salient morphological features. When possible, portions of the axonal arbor of all recovered interneurons were re-embedded for electron microscopy.[84] Labelled terminal branches of the axon were traced in serial ultrathin sections to determine their postsynaptic targets. In all cases tested, a minimum of 10 synaptic contacts were rigorously identified. In the case of the synaptically-coupled basket cell pair (Fig. 1), where the axons partially overlapped, care was taken to sample only non-overlapping areas from the two most lateral sectors of the axonal fields. The branches in these areas were traced to the two respective main axons (Fig. 1). In one instance, all putative sites of synaptic interaction from a basket cell to a putative bistratified cell

were mapped in the light microscope and subsequently scrutinized in serial electron microscopic sections (Fig. 2 and Fig. 3).

RESULTS

In the course of dual intracellular recordings between interneurons and pyramidal cells in area CA1 of the rat hippocampus, on several occasions, due to the fortuitous penetration of a second interneuron, the presynaptic interneuron could also be tested for synaptic coupling with other CA1 interneurons. This paper presents the synaptic interactions of these four interneurons with other hippocampal interneurons as well as pyramidal cells from a combined electrophysiological and anatomical perspective. In addition to showing interneurons to innervate other interneurons, we also demonstrate a high incidence of autaptic self-innervation amongst hippocampal interneurons.

Basket cell-to-basket cell pair

Anatomical analysis. Following simultaneous recording of two fast-spiking putative interneurons, visualization of the biocytin labelling confirmed that both cells shared morphological features common to many GABAergic interneurons located within the proximal part of the CA1 area (Fig. 1A). The axonal and dendritic arbors of both cells were extensively labelled without any weakening of the intracellular marker towards distal dendritic and axonal processes. Both cells had their cell bodies located within the pyramidal layer. The presynaptic cell (Fig. 1A; dendrites displayed in red, axon in black) was located within the proximal part of the CA1 subfield, adjacent to the CA3/CA1 border. Both somata gave rise to several basal and apical dendrites which were beaded in their appearance and extended throughout all hippocampal layers, including the lower half of stratum lacunosum moleculare, a characteristic of basket cells.[12,34] The cells also resembled the presynaptic basket cell illustrated in Fig. 2. Aided by the spatial separation of the two cells and the dense, homogeneous filling of their axons, it was feasible to trace accurately most collateral branches back to the parent cell body, thus enabling the untangling of the two axons in regions of spatial overlap. Several axon collaterals of the presynaptic cell overlapped with the somatodendritic domain of the postsynaptic cell. Both axons were very similar in their appearance, forming a dense meshwork of terminal branches criss-crossing the cell body layer and, to a lesser extent, ramifying in adjacent regions of strata radiatum and oriens. For the sake of clarity, only those axonal branches of both cells are presented (Fig. 1A) which were in continuity with the respective main axons in two consecutive 60-μm-thick sections.

The efferent target profile of both neurons was determined by means of random electron microscopic sampling of synaptic boutons (n=29). One sample from each cell was taken from representative non-overlapping parts of the respective axons. The presynaptic cell made synapses with somata (n=5, 50%) and dendritic shafts (n=5, 50%), one of which was a dendrite of an unidentified interneuron as it received several type 1 synaptic junctions. The other dendrites and the somata had the characteristics of pyramidal cells. The synaptic target profile of the postsynaptic neuron was similar, as it also gave synapses to somata (n=9, 47%) and proximal dendrites (n=8, 42%) in roughly equal proportions, as well as to a pyramidal axon initial segment (5%) and a spine (5%). One of the dendritic shafts was identified as originating from an unidentified interneuron on the basis of type 1 synaptic junctions that it received. Thus not only the distinct light microscopic appearance but also the characteristic efferent synaptic connectivity helps to identify both neurons as basket cells.[12,34] The synaptic terminals of similar basket cells have been demonstrated extensively,[34] therefore electron micrographs are not presented for this pair. Since there was a very dense axonal plexus around the postsynaptic cell body, and basket cells can innervate themselves (see below), no attempt was made to identify electron microscopically the specific boutons that mediated the synaptic interaction.

Synaptic coupling. When elicited at a frequency of 1 Hz, single action potentials in the presynaptic basket cell (Fig. 1B) evoked small amplitude short-latency IPSPs (Fig. 1C, 0.25 mV mean amplitude at a membrane potential of −59 mV). The averaged IPSP was characterized by fast rise and decay kinetics (Table 1, 1.3 ms rise time and 27.0 ms duration at half-amplitude). Following the sudden loss of the presynaptic interneuron it was not possible to test either for reciprocal coupling or demonstrate the divergent innervation of pyramidal cells, although the latter scenario is fairly plausible in view of the anatomical data indicating the predominance of pyramidal neurons as the synaptic target of both pre- and postsynaptic basket cells. It was possible, however, to study membrane and firing properties of the postsynaptic basket cell which was characterized by short-duration action potentials, large-amplitude fast afterhyperpolarizations and a modest degree of spike frequency adaptation (Fig. 1D). Subsequently, 0.1 nA hyperpolarizing pulses (not shown) were injected at resting membrane potential (−64 mV) to measure the membrane time constant (12.8 ms) and input resistance (22 MΩ). Higher current intensities revealed some time-dependent inward rectification (Fig. 1E).

Basket cell-to-putative bistratified cell pair

Anatomical analysis. Following simultaneous recording of two fast-spiking putative interneurons, visualization of the biocytin labelling confirmed that both cells share morphological features common to

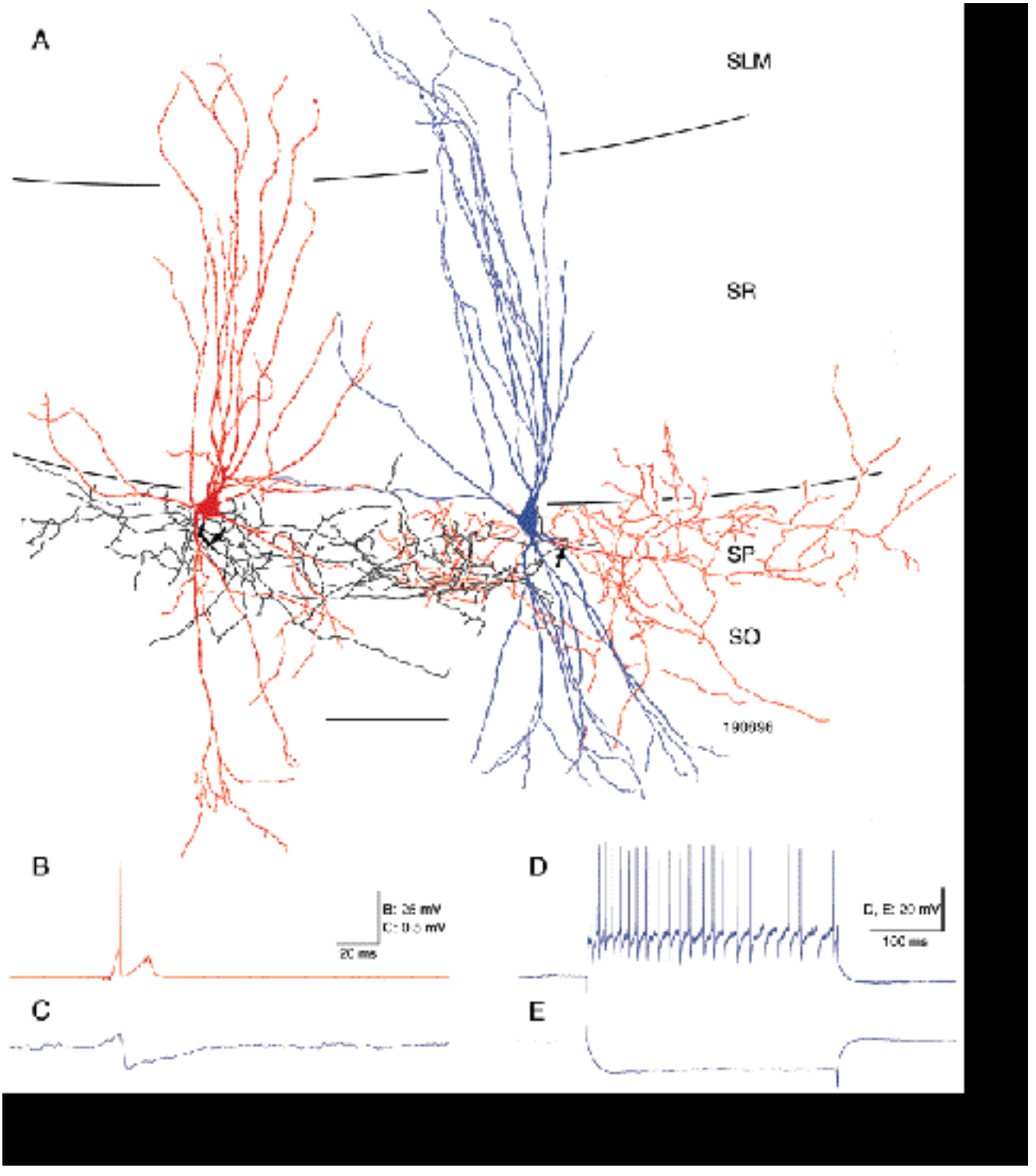


Fig. 1. Light microscopic reconstruction and physiological characterization of basket-to-basket cell interaction. (A) The presynaptic cell (soma and dendrites in red, axon in black) and the postsynaptic cell (soma and dendrites in blue, axon in red) are similar, showing a concentration of axon in the pyramidal cell layer (SP), which is characteristic of basket cells. Electron microscopic analysis of the postsynaptic targets identified both cells as basket cells. Both dendritic arbors were reconstructed from the entire slice, but, for clarity, from the axonal arbors only those collaterals are illustrated which were in continuity with the respective initial segments in two adjacent 60-μm-thick sections. Note that in these sections, the presynaptic axon overlaps with the dendrites of the postsynaptic cell. (B, C) Synaptic coupling was revealed by evoking single action potentials in the presynaptic basket neuron (red) and concomitantly monitoring the evoked response in the postsynaptic neuron (blue). In the average (n=828 sweeps) it is apparent that presynaptic firing elicited short-latency hyperpolarizing IPSPs with fast kinetics. (D) In response to depolarizing current pulses (350 ms; 1.1 nA) the postsynaptic basket cell fired a weakly accommodating train of short-duration action potentials. (E) Upon injection of hyperpolarizing pulses a small degree of time-dependent inward rectification became apparent. SR, stratum radiatum; SO, stratum oriens; SLM, stratum lacunosum moleculare. Scale bar=100 μm.

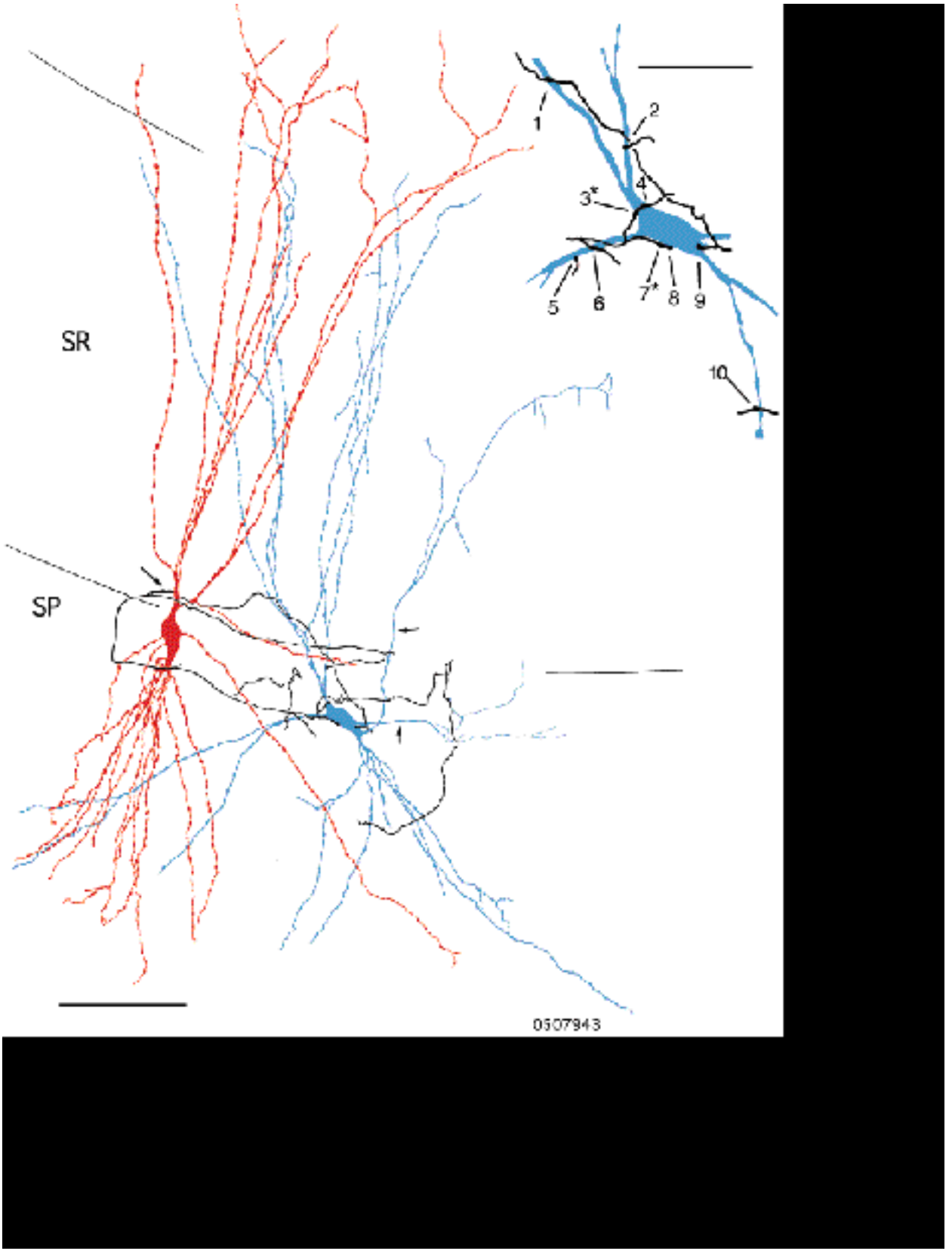


Fig. 2. Intracellularly labelled basket cell-to-bistratified cell pair. The light microscopic reconstruction shows the presynaptic basket cell (soma and dendrites in red, axon in black) and the nearby postsynaptic interneuron (blue) which has the distinctive dendritic characteristics of a bistratified cell.[31] Arrows indicate the main axons originating from each cell. The axon of the basket cell was similar to those published earlier,[31] therefore only the branches innervating the putative bistratified cell are indicated for clarity. The postsynaptic cell gives rise to two main axons, which emerge from the basal portion of the soma. The inset indicates all electron microscopically identified sites of synaptic contact (n=12; see Fig. 3) made by 10 boutons of the presynaptic basket cell axon onto the postsynaptic bistratified cell. In two instances (marked with an asterisk), a single bouton formed two active zones, so that in total, seven synaptic junctions were made onto the soma and the remaining five onto proximal dendritic shafts. Electron microscopic evaluation of randomly selected synaptic terminals (n=12) showed that the basket cell innervated somata (50% of targets tested) and proximal dendritic shafts (50%) of unlabelled pyramidal cells. SP, stratum pyramidale; SR, stratum radiatum. Scale bar=100 μm; Inset scale bar=50 μm.

Table 1. Summary of unitary postsynaptic response amplitude and kinetics

Presynaptic cell (code)	Postsynaptic cell	IPSP amplitude (mV)	Holding potential (mV)	IPSP 10–90% rise time (ms)	IPSP duration at 1/2 amplitude (ms)
basket cell (190896)	basket cell	0.25	− 59	1.3	27
basket cell (I1) (050794/3)	bistratified cell (I2)	0.37	− 55	1.0	5.6
	pyramidal cell (P1)	0.31	− 57	2.6	21.0
	pyramidal cell (P2)	0.28	− 60	3.2	16.4
basket cell (I1) (200694/3)	fast-spiking interneuron (I2)	0.30	− 61	15.2	49.6
	pyramidal cell (P1)	0.60	− 57	11.8	38.0
LM-R† interneuron (I1) (070695/1)	bistratified cell (I2)	0.48*	− 61	n/a	n/a
	pyramidal cell (P1)	0.46*	− 60	n/a	n/a

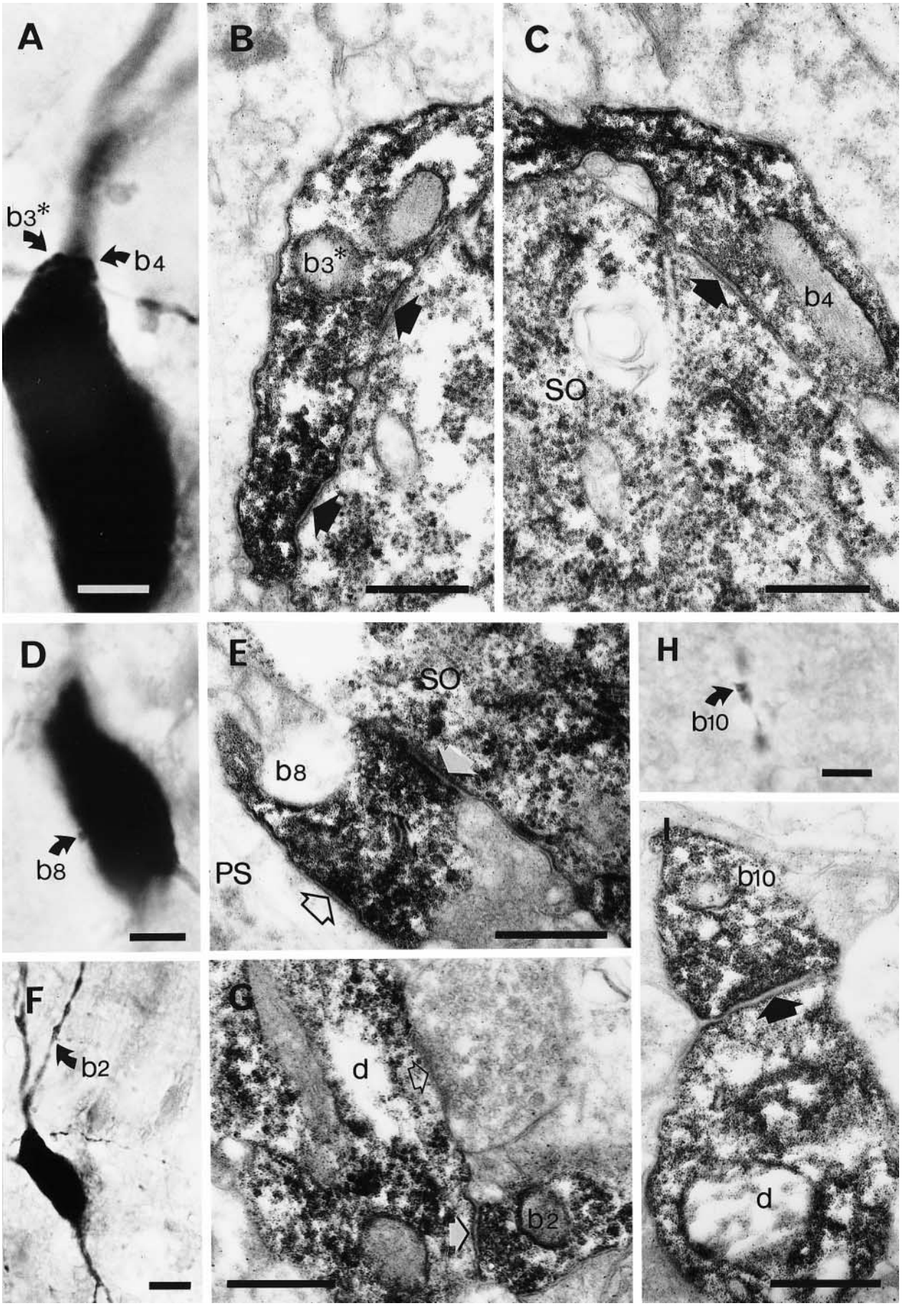
*Indicates summated response.
n/a, Not applicable.
Rise times were measured as the time difference of 10 and 90% of the respective peak-amplitudes.
†LM-R, strata lacunosum-moleculare/radiatum border.

many GABAergic interneurons. Both cells had fusiform somata located in stratum pyramidale, approximately 150 μm apart (Fig. 2). Their somata gave rise to several basal and apical dendrites which were smooth and beaded in appearance. However, the two cells differed with respect to the extent that their apical dendrites ramified within the molecular layer. Whilst the presynaptic cell had extensive dendrites which fanned out, traversing stratum radiatum before branching in a dichotomous manner within stratum lacunosum moleculare, the apical dendrites of the postsynaptic neuron ramified extensively within stratum radiatum, but without extending into the molecular layer. These latter dendritic features are highly characteristic of bistratified cells.[13,34]

The axons of both cells branched extensively, giving rise to multiple local collaterals. A partial reconstruction of their axons is shown on Fig. 2. The axon of the presynaptic cell was confined mainly to an area in and adjacent to stratum pyramidale (only the connecting branches are shown for clarity of display, see previous reconstructions for similar cells in Fig. 1 and Ref. 34). A representative portion of this well-filled axon was examined at the electron microscopic level, allowing evaluation of a randomly-selected sample of boutons and their postsynaptic targets (n=12). This cell made synapses with somata (50% of synaptic targets examined) of pyramidal cells and proximal dendritic shafts. Such selective innervation of the perisomatic domain of its target neurons confirms this cell to be a basket cell.[12]

By continuously following the axon from the presynaptic basket cell, several axonal branches

Fig. 3. Light and electron micrographs of selected basket cell synaptic boutons established with a postsynaptic putative bistratified cell shown in Fig. 2. Bouton numbers correspond to those in Fig. 2, inset. (A) Light micrograph showing basket cell axon crossing the apical portion of the bistratified cell soma and forming two boutons, b3* and b4. (B, C) Electron micrographs of boutons b3* and b4 respectively, making type 2 synaptic junctions (arrows) with the bistratified cell soma (SO). Bouton 3* forms two separate synaptic junctions which were not joined as proved in serial sections. (D) Light micrograph of bouton 8 (arrow) in close apposition to the bistratified cell soma. (E) The corresponding electron micrograph confirms the presence of a type 2 synaptic junction with the postsynaptic neuron (white arrow). The opposite side of the same bouton makes a second synapse (open arrow) with an adjacent pyramidal cell soma (PS). The identified bouton (b8) is adjacent to another vesicle filled bouton (lower right) that is free of reaction product. (F) Light micrograph showing the basket cell axon forming a bouton (arrow, b2) close to a proximal dendrite of the postsynaptic bistratified cell. (G) Corresponding electron micrograph of the bouton (b2) showing the synaptic junction (arrow) with the dendrite (d) which also receives synaptic input (open arrow) from an adjacent unlabelled synaptic terminal. (H) Light micrograph of a basket cell bouton (arrow; b10) in close apposition to a basal dendrite of the bistratified cell. (I) The corresponding electron micrograph shows b10 forming a type 2 synaptic junction (arrow) with the dendrite (d). Scale bars: A, D, F, H=2 μm; B, C, E, G, I,=0.5 μm.


A
b3*
b4
B
b3*
C
b4
SO
D
b8
E
SO
b8
PS
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I
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d
F
b2
G
d
b2

were found to project towards the labelled postsynaptic interneuron where several boutons appeared to be apposed to its somatic and proximal dendritic membrane. A total of 11 suspected sites of synaptic contact were predicted at the light microscopic level. A block containing all presumed sites of contact was re-embedded and serial ultrathin sections were cut for further evaluation at the electron microscopic level (Fig. 3). Of the 11 boutons tested, 10 were confirmed to form type 2 (symmetrical) synaptic junctions,[11,28] whereas one bouton did not form a synapse with the postsynaptic neuron. In two instances, a single bouton made two synaptic junctions (Fig. 2 inset, Fig. 3B), which were completely separated as proved in serial sections. In total, the presynaptic basket cell established a total of 12 synapses with the soma (n=7) and proximal apical (n=2) as well as basal (n=3) dendritic shafts of the postsynaptic interneuron.

The axon of the postsynaptic cell was weakly filled and could be only partially visualized. Due to the difference in biocytin filling, the axonal branches of the two cells could be identified and differentiated clearly. Unusually, two separate axons emerged from the soma, one projecting into stratum radiatum where it branched extensively, whilst the other one ramified close to the border between strata pyramidale and radiatum. Primarily from its characteristic dendritic arbor, and also from the apparent innervation of the dendritic layers, this cell was identified as a putative bistratified cell.[13,34]

Synaptic coupling. Action potentials evoked in the basket cell (Fig. 4A) were found to elicit hyperpolarizing IPSPs in the postsynaptic putative bistratified cell (Fig. 4B, IPSP amplitude 0.37 mV at −55 mV membrane potential). Evoked IPSPs had fast kinetics (rise time ~1 ms, Table 1) and were of short-duration (duration 5.6 ms at half-amplitude). In contrast to the basket-to-bistratified cell interaction, action potentials evoked in the putative bistratified cell failed to elicit detectable reciprocal synaptic responses in the basket cell (data not shown).

On withdrawal of the microelectrode from the putative bistratified cell, two pyramidal cells (P1 and P2) were subsequently impaled whilst maintaining the same presynaptic basket cell on the other microelectrode. Prior to morphological verification following biocytin visualization, pyramidal cells were tentatively distinguished from interneurons by their distinctive firing response to a depolarizing current injection, which occurred in the form of a strongly accommodating train of action potentials. Moreover, pyramidal cell action potentials were generally overshooting, broader and lacked the fast, deep afterhyperpolarization seen in interneurons (data not shown). For both pyramidal cells, activation of the common presynaptic basket cell elicited hyperpolarizing IPSPs (Fig. 4C, D; averaged IPSP amplitudes

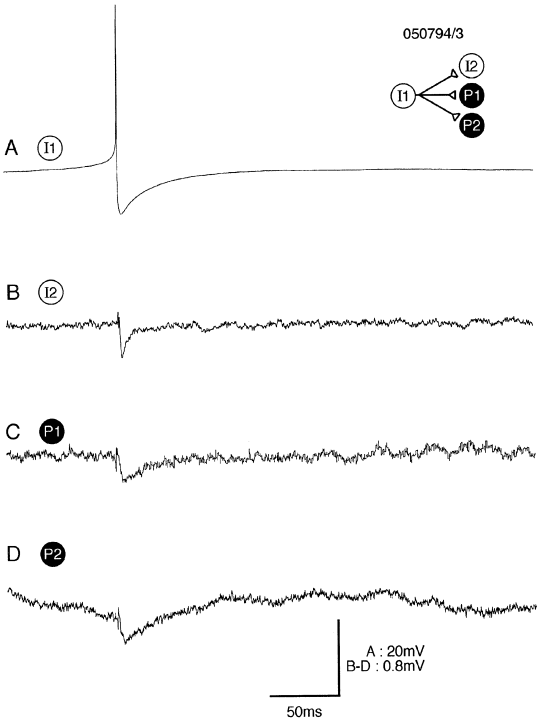

Fig. 4. Synaptic responses in a putative bistratified cell (I2) and two pyramidal cells (P1, P2), following activation of a common presynaptic basket cell (I1). Action potentials (A, single sweep) evoked in the presynaptic basket cell shown in Fig. 2 elicit fast hyperpolarizing IPSPs in the simultaneously recorded bistratified cell (B; average of 82 sweeps). Following subsequent sequential impalement of nearby pyramidal cells, activation of the same presynaptic basket cell elicited fast hyperpolarizing responses (C, average of 34 sweeps; D, average of 24 sweeps).

0.31 mV and 0.28 mV at −57 mV and −60 mV membrane potential for P1 and P2, respectively), which also had fast kinetics (rise time 2.6 ms and 3.2 ms; duration 21 ms and 16.4 ms for P1 and P2, respectively).

Basket cell-to-fast-spiking interneuron pair

Anatomical analysis. Following simultaneous recording from two fast-spiking putative interneurons, visualization of the biocytin labelling revealed only a single filled cell in a position corresponding to the first (presynaptic) recording microelectrode. One interneuron was recorded for 1.3 h which usually provides strong and continuous labelling of cells with our method, therefore we assume that the presynaptic cell was recovered. Failure to recover the second biocytin-injected cell may have resulted from inadequate amounts of biocytin entering the cell, owing to the relatively short (~20 min) impalement period, or to the disintegration of the cell following its loss from the electrode. However, the characteristic response to a depolarizing current pulse in the form of a rapid train of very brief, non-accommodating

action potentials with fast, deep afterhyperpolarizations (Fig. 7F), strongly indicates that the postsynaptic cell was an interneuron. Unfortunately, in the absence of morphological verification, the exact type of interneuron cannot be determined from physiological characteristics alone.[15]

Evaluation of the morphologically recovered presynaptic cell and subsequent reconstruction (Fig. 5) showed the multipolar soma (red) located in stratum pyramidale giving rise to an extensive, stellate-like dendritic arbor (red). Dendrites extended radially into strata oriens and radiatum adjacent to the pyramidal cell layer, but no dendrite entered stratum lacunosum moleculare. The dendrites were smooth and beaded and did not branch except in stratum oriens, close to the alveus (Fig. 5 and Fig. 6A).

Reconstruction of the axon (black) showed this cell to possess an unusually dense axonal arbor, which was most dense in stratum pyramidale, but which also extensively ramified in both strata oriens and lower radiatum (Fig. 5 and Fig. 6A). In addition, the lateral extent of the axonal arbor was considerable, covering almost 900 μm of the CA1 subfield and implying a highly divergent output. Within stratum pyramidale, the axon appeared to target somata, occasionally forming "pericellular baskets" (Fig. 6B) which, at the light microscopic level, indicated that the cell was a basket cell. However, an unusual feature of this cell was that the axon frequently formed vertically elongated axonal terminal branches which extended from stratum pyramidale into stratum radiatum. These branches appeared to run alongside apical dendritic trunks, forming multiple boutons along their length (Fig. 6C), but we have not tested the number of synapses provided to individual apical dendrites. Electron microscopic evaluation revealed the overall postsynaptic targets (n=25, Fig. 6D, E) to be somata of pyramidal cells (40%), proximal dendritic shafts (56%) and spines (4%). Such perisomatic innervation of postsynaptic target neurons confirms this cell to be a basket cell, although several of its features differ from all basket cells previously reported in the CA1 area.

Synaptic coupling. Action potentials (duration at half amplitude 0.40 ms) evoked in the presynaptic basket cell (Fig. 7A) were found to elicit hyperpolarizing IPSPs (Table 1, amplitude 0.3 mV at −61 mV membrane potential) with a rise time of 15.2 ms and duration at half-amplitude of 49.6 ms in the postsynaptic interneuron (Fig. 7B). As with the previous interneuron pair, activation of the second interneuron failed to elicit any detectable reciprocal synaptic response (data not shown). A short train of fast (80 Hz), non-accommodating action potentials (amplitude 60 mV) evoked by a depolarizing current pulse in the presynaptic basket cell, resulted in a summated IPSP in the postsynaptic interneuron (Fig. 7E). After reaching a plateau (summated IPSP amplitude 0.85 mV at −65 mV), the synaptic response diminished in spite of the continued activation of the presynaptic basket cell. However, like the effect seen in pyramidal cells during repetitive high frequency activation of presynaptic basket cells,[12] the synaptic response never completely faded.

Upon withdrawal of the microelectrode from the postsynaptic interneuron an adjacent pyramidal cell (identified as such by its distinctive electrophysiological characteristics, see above) was subsequently impaled. Activation of the common presynaptic basket cell elicited hyperpolarizing IPSPs (Fig. 7C, IPSP amplitude 0.6 mV at −57 mV membrane potential) with a rise time of 11.8 ms and a duration of 38 ms, similar to those produced in the postsynaptic interneuron.

Stratum lacunosum moleculare/radiatum border interneuron-to-bistratified cell pair

Anatomical analysis. After simultaneous recording of a fast-spiking putative interneuron and a second fast-spiking interneuron, followed by a putative pyramidal cell, subsequent visualization of biocytin labelling revealed three cells, all in good correspondence with the respective recording sites (Fig. 8). Consistent with the electrophysiological data, two of the cells (red, blue) revealed morphological characteristics of interneurons whilst, the third (black) was a CA1 pyramidal cell.

The soma of the first impaled cell (red) was located in stratum radiatum, close to the border with the stratum lacunosum moleculare (LM-R interneuron). This soma gave rise to several main dendrites which ramified both in strata radiatum and lacunosum moleculare where they branched, though not extensively. The dendrites could not be completely reconstructed due to the loss of part of a section containing one of the dendrites. The dendrites were mostly smooth, but some proximal main dendritic trunks protruded large thorn-like processes similar to a type of interneuron described previously.[50,51] The LM-R interneuron axon was weakly filled, but nevertheless could be seen to ramify in stratum radiatum. The postsynaptic target profile of the LM-R interneuron was not determined. However, since most of the axon was contained in the stratum radiatum, it seems likely that it innervated mainly dendritic targets.

The second (postsynaptic, see below) interneuron (blue) soma was located in stratum pyramidale and gave rise to smooth, slightly beaded dendrites ramifying in stratum oriens and radiatum, but not projecting into stratum lacunosum moleculare. These characteristics are consistent with the cell being a bistratified type interneuron.[13,34] Labelling of the axon was weak and could not be traced far. Evaluation of randomly selected boutons and their postsynaptic profiles at the electron microscopic level (n=11) showed this cell to innervate dendritic shafts (82%) and dendritic spines (18%), thus confirming the notion from the dendritic branching pattern (Fig. 8)

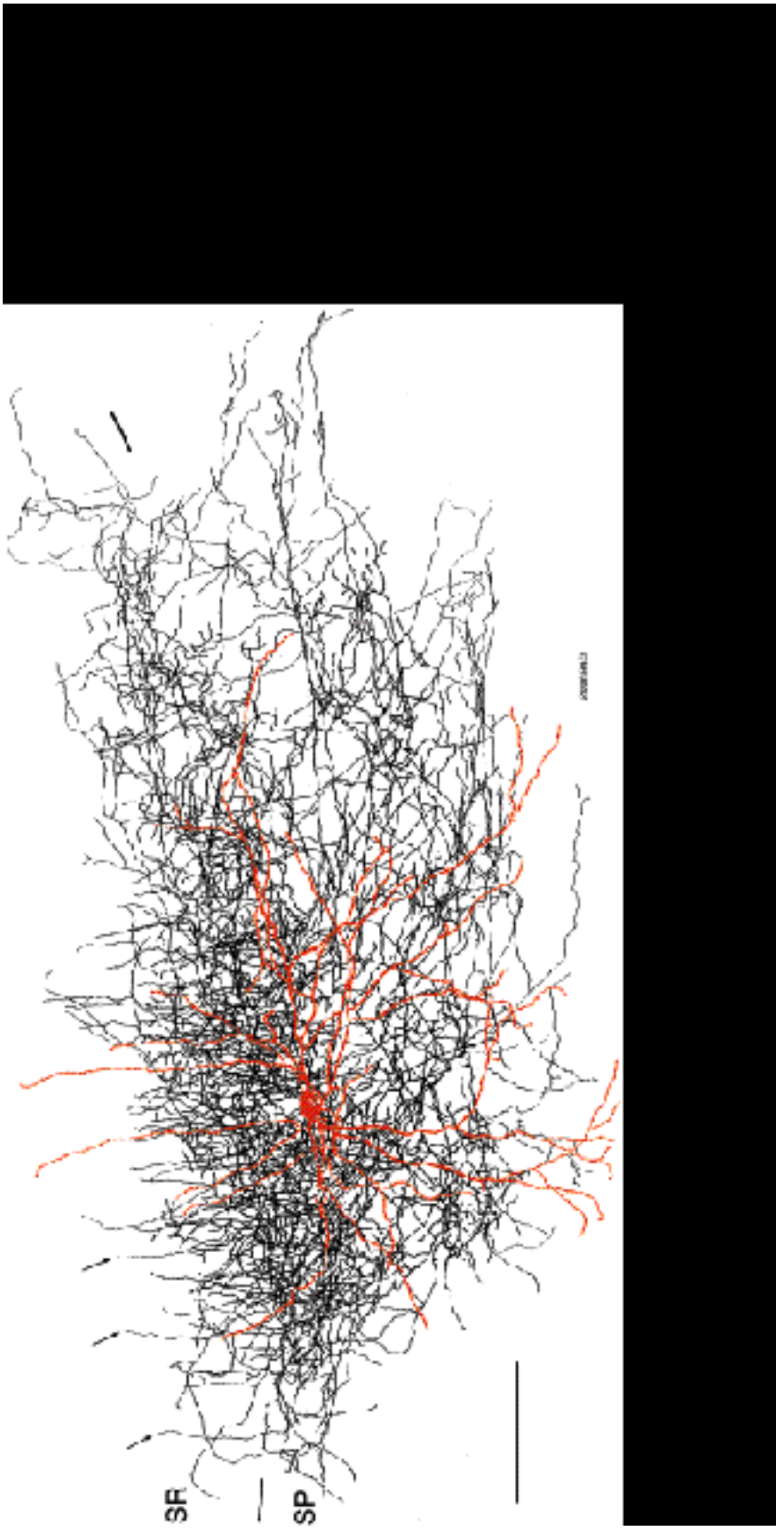

Fig. 5. Reconstruction of a novel type of basket cell, showing the soma, dendrites (red) and the axon (black). The soma is located in stratum pyramidale, giving rise to smooth, aspinous dendrites which only extend radially into strata oriens and radiatum (SR) adjacent to pyramidale (SP). Note the unusually dense axonal arbor which extends laterally ~900 μm in the CA1 subfield, but is restricted mainly to stratum pyramidale and adjoining regions of strata oriens and radiatum. Arrows indicate characteristic vertically orientated axon collaterals. Electron microscopic evaluation of randomly selected postsynaptic profiles (n=25) showed this cell to innervate somata (40%), proximal dendrites (56%) and dendritic spines (4%), confirming its basket cell status. Scale bar=100 μm.

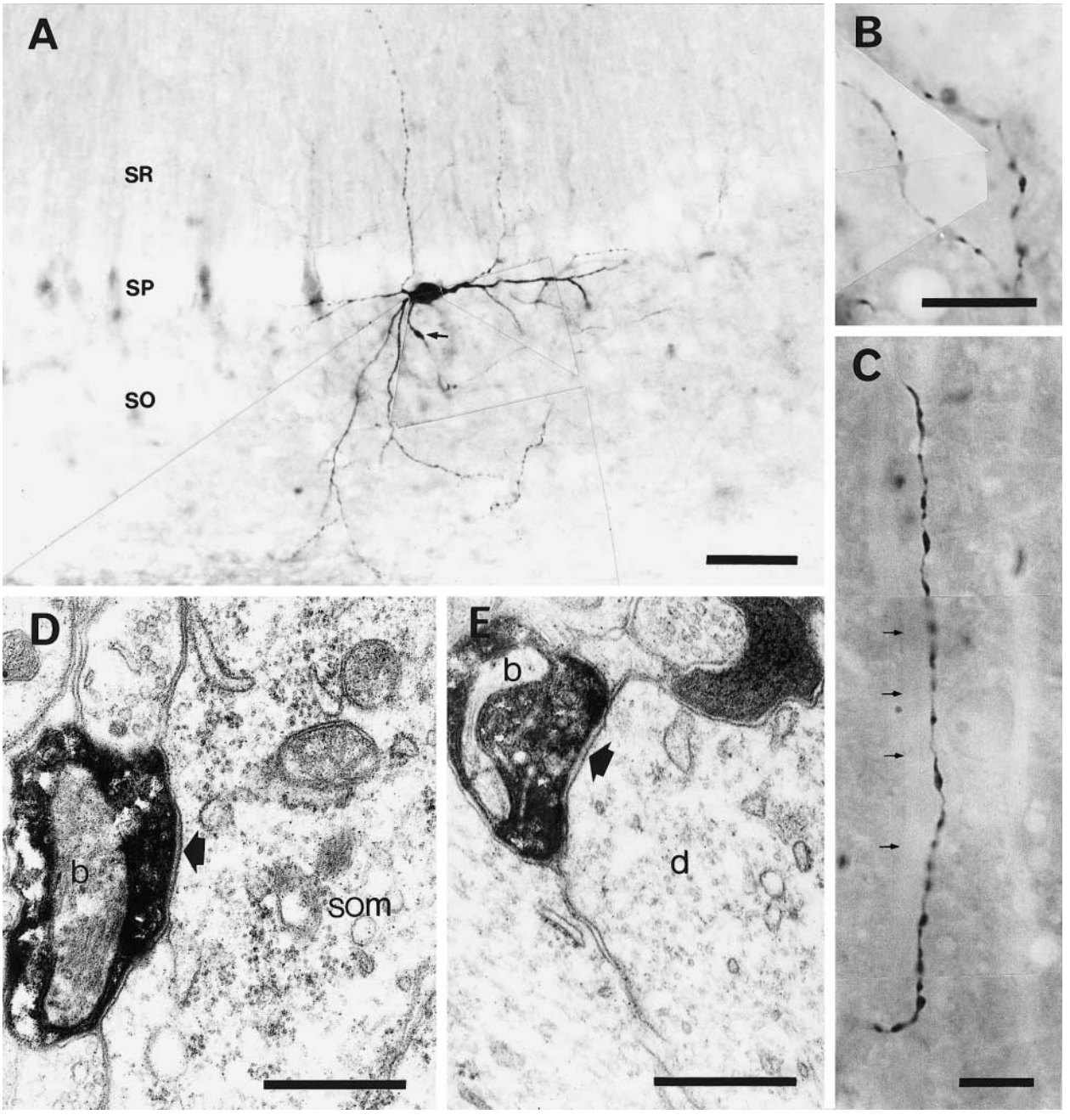

Fig. 6. Light and electron micrographs of the basket cell shown in Fig. 5 and its postsynaptic targets. (A) Light microscopic montage of the basket cell. At higher magnification, axons were seen to form characteristic "perisomatic baskets" (B) and vertically elongated collaterals (C), forming multiple boutons, which appeared to follow unlabelled apical dendrites (arrows). (D, E) Electron micrographs of labelled basket cell boutons (b), forming synaptic junctions (arrows) with an unlabelled pyramidal cell soma (som) and a proximal apical dendrite (d). SO, stratum oriens; SP, stratum pyramidale; SR, stratum radiatum. Scale bars: A=50 μm; B=20 μm; C=10 μm; D and E=0.5 μm.

that this interneuron was a bistratified cell, presumably innervating the dendritic domain.

The third cell (Fig. 8, black) was identified as a CA1 pyramidal cell whose soma was located in stratum pyramidale and whose apical and basal tuft of highly spinous dendrites extended and branched extensively in all layers of the CA1 subfield. The axon of the pyramidal cell (arrowhead indicates main axon) coursed towards the alveus where it branched, sending collaterals towards both the fimbria and the subiculum.

Synaptic coupling. Short trains of 8–10 action potentials were evoked in the LM-R interneuron

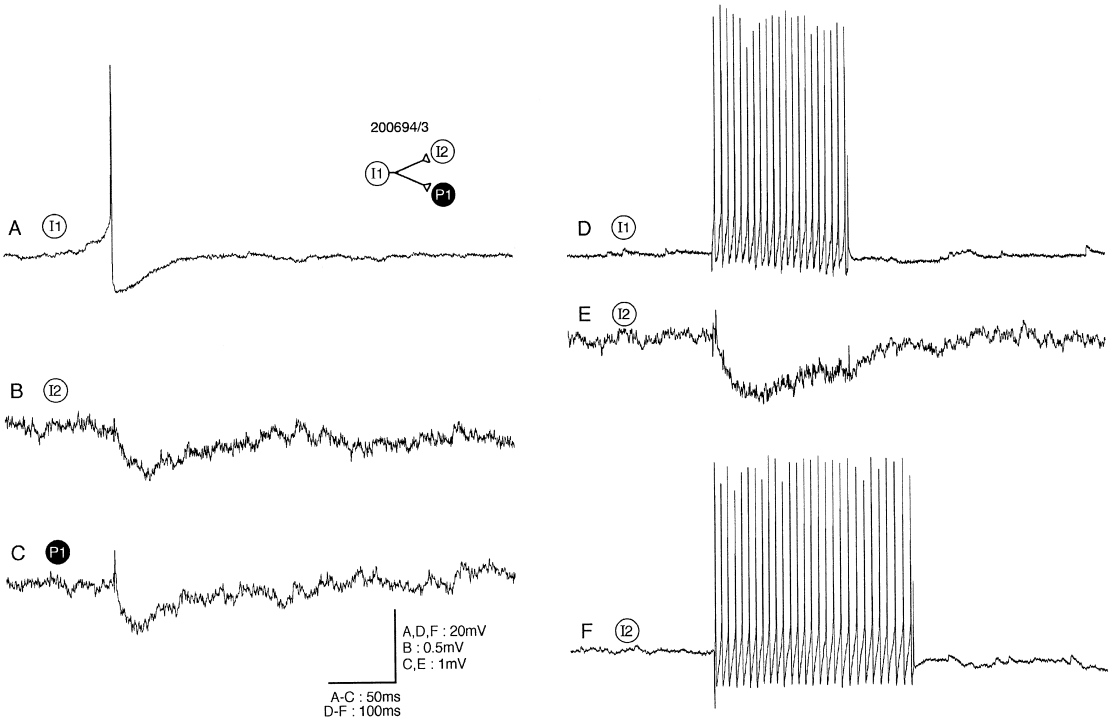

Fig. 7. Basket cell-evoked unitary synaptic responses in consecutively recorded interneuron and pyramidal cell. (A) Action potential (duration at half-amplitude 0.4 ms) evoked in the basket cell shown in Fig. 5 and Fig. 6 elicits short latency hyperpolarizing IPSPs in a simultaneously recorded interneuron (B; average of 85 sweeps) and in a subsequently recorded pyramidal cell (C, average of 34 sweeps). (D) A train of action potentials (80 Hz) evoked in the presynaptic basket cell (200 ms, 0.5 nA depolarizing current pulse) elicit a summated fast hyperpolarizing IPSP in the postsynaptic interneuron (E; average of eight sweeps). Note that the initial 5–6 IPSPs summate before the synaptic response fatigues (F). A similar intracellular depolarizing current injection (300 ms, 0.2 nA) produces the same characteristic fast-spiking response (102 Hz, action potential duration at half-amplitude 0.4 ms) in the postsynaptic interneuron. Inset: Schematic diagram of synaptic interactions. Reciprocal synaptic coupling was not observed at either connection (data not shown).

when testing for synaptic responses in the simultaneously recorded bistratified and pyramidal cells. This protocol was adopted as previous data have indicated that single action potentials are often insufficient to elicit any detectable response mediated by single interneurons in stratum lacunosum moleculare.[51] Therefore this pair was not tested by eliciting single action potentials. The LM-R interneuron was found to be synaptically coupled to both the bistratified cell and the subsequently recorded pyramidal cell (Fig. 9). Synaptic responses were similar in each case and were in the form of summated, short latency, hyperpolarizing IPSPs (Table 1, peak amplitude 0.48 at -61 mV and 0.46 mV at -60 mV for the bistratified and pyramidal cells, respectively). Since the presynaptic cell was an anatomically identified interneuron that also made an IPSP in a pyramidal cell, it is very unlikely that the effect was produced through multisynaptic connections. No reciprocal synaptic coupling was observed in either cell pair (not shown). Because of the summated nature of synaptic responses, kinetic measurements of such responses were not obtained.

Autaptic connectivity of GABAergic interneurons

In addition to targeting interneurons and pyramidal cells, individual basket and bistratified cells also appeared to form multiple sites of autaptic[90] self-innervation with their own membrane surface. For each of 18 interneurons, one 60–70 μm section containing the cell body and the majority of the dendrites was analysed. For 12 of 12 basket cells and six of six bistratified cells tested, light microscopic evaluation revealed bouton-laden axon collaterals of the same cell closely apposed to its somata and /or dendrites. In a single section, at least two and up to seven boutons appeared to be apposed to the somatodendritic domain of each cell. Conversely, axo-axonic cells which exclusively innervate the axon initial segment domain of pyramidal cells[13,14,52,83] showed no such autaptic innervation in any of the 12 biocytin-filled cells examined (not shown). In order to confirm the synaptic nature of such sites seen at the light microscopic level in basket and bistratified cells, a single biocytin-filled basket cell (Fig. 10; full reconstruction shown in Fig. 2 of Ref. 12) was

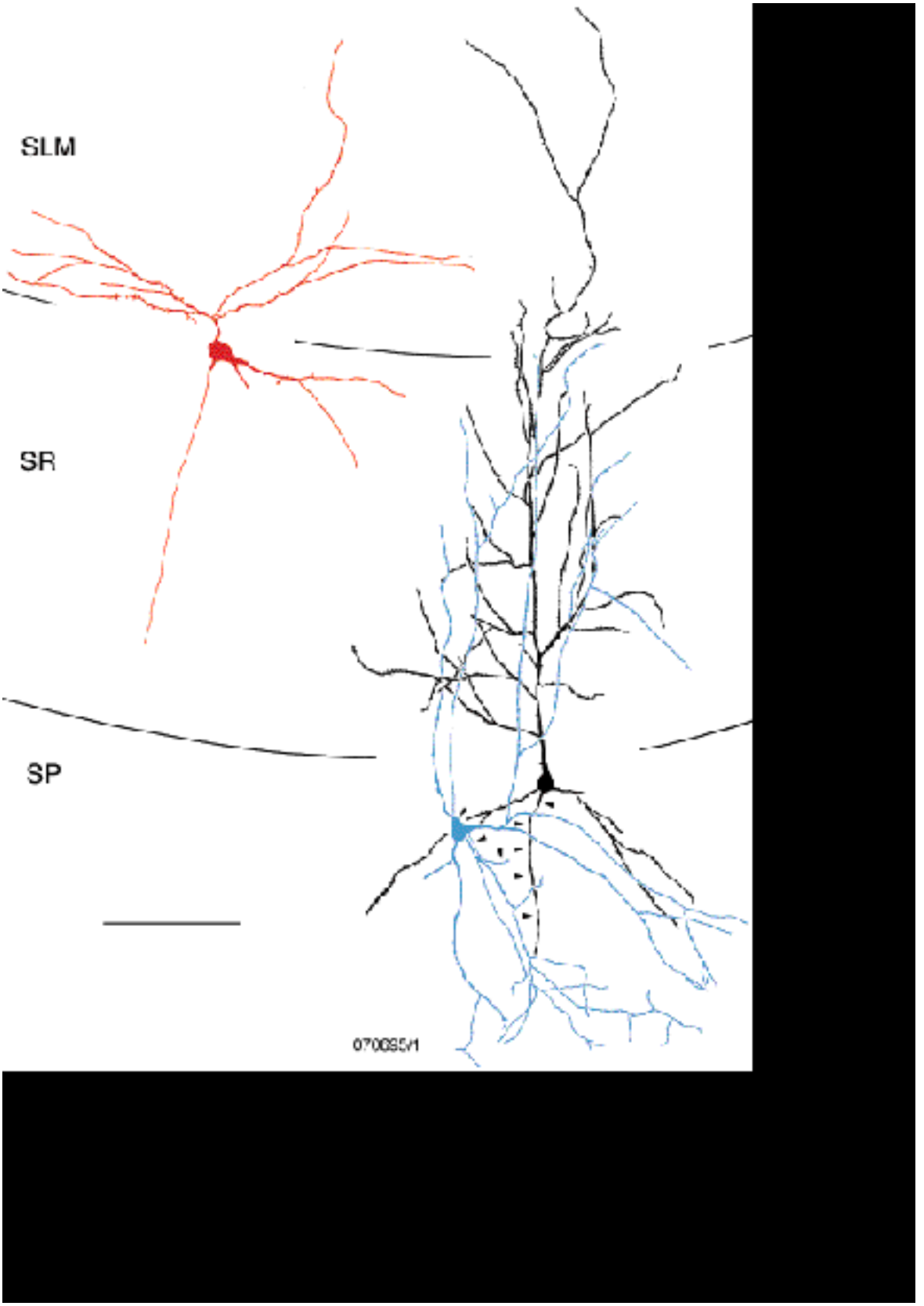

Fig. 8. Reconstruction of an interneuron at the border of stratum lacunosum/radiatum and postsynaptic bistratified and pyramidal cells. The soma of the presynaptic cell (red) is situated in stratum radiatum (SR) close to the border with stratum lacunosum moleculare (SLM). Dendrites (incomplete) project into both strata radiatum and lacunosum moleculare. The postsynaptic bistratified cell (blue) and pyramidal cell (black) somata are situated in stratum pyramidale (SP). Apical dendrites of the bistratified cell are beaded and confined to stratum radiatum, whereas the spiny pyramidal cell dendrites extensively ramify in all layers. Only the main axons (arrowheads) of the bistratified and pyramidal cells and two of the pyramidal basal dendrites are shown for clarity. The axon of the bistratified cell originated from the soma towards stratum radiatum and turned immediately towards stratum oriens. Scale bar=100 μm.

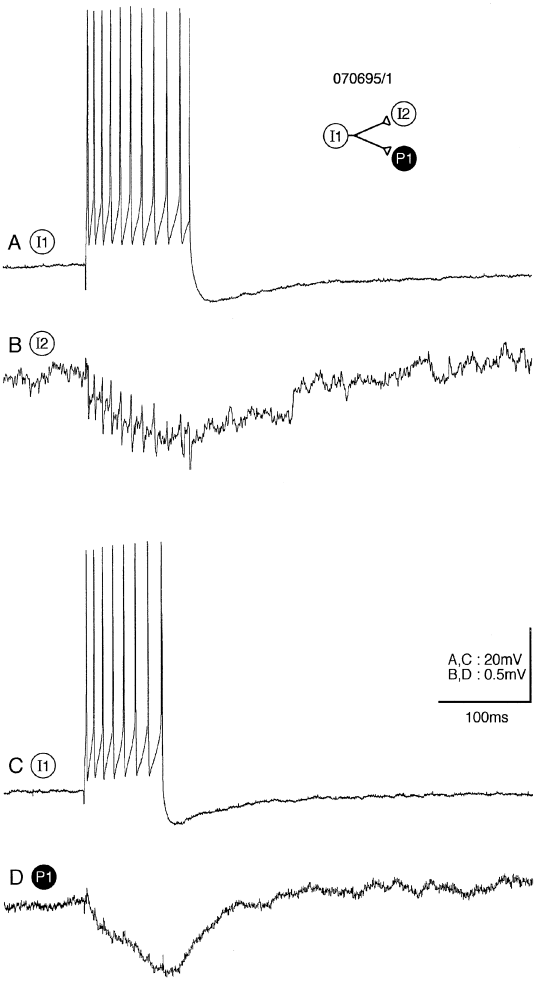

Fig. 9. Synaptic responses recorded in a bistratified and pyramidal cell, following activation of a common presynaptic LM-R interneuron. (A) A short train of action potentials evoked in the presynaptic LM-R interneuron (shown in Fig. 8) elicits a fast hyperpolarizing synaptic response in the simultaneously recorded bistratified cell (B; average of 49 sweeps). Repeated activation of the same presynaptic LM-R interneuron (C) elicits similar fast hyperpolarizing responses in a subsequently recorded pyramidal cell (D; average of 106 single sweeps). Inset: summary of synaptic interactions where, I1, I2 and P1 correspond to presynaptic LM-R interneuron, postsynaptic bistratified cell and pyramidal cell, respectively. No reciprocal synaptic coupling was observed (data not shown).

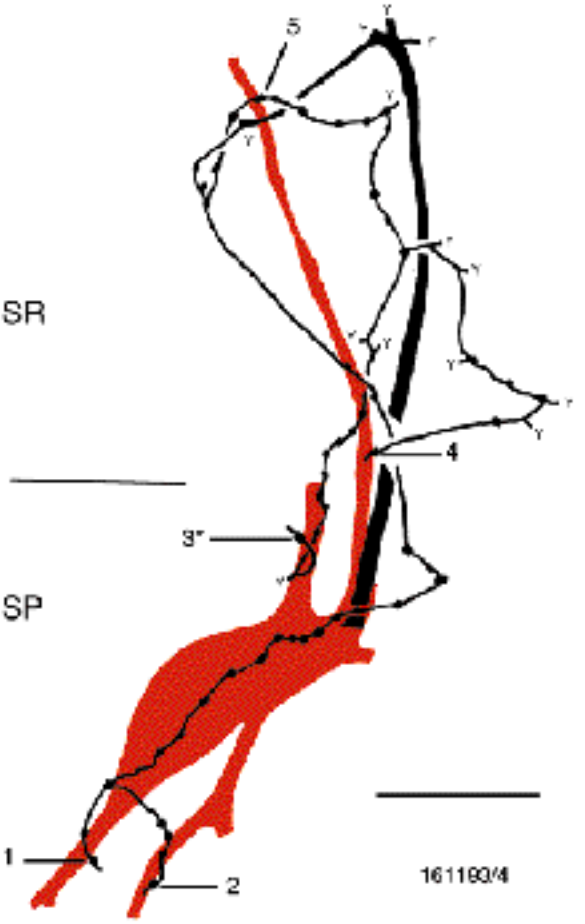

Fig. 10. Light microscopic reconstruction of a basket cell soma and proximal dendrites, showing multiple sites of autaptic innervation. The basket cell axon (black) emerges from the soma (red), branches extensively (Ys indicate branch points), forming multiple (5) sites of autaptic self-innervation. Electron microscopic analysis confirmed all the numbered sites to represent synaptic junctions (see Fig. 11) and showed one particular bouton (3*) to form two separate synaptic membrane specializations. SR, stratum radiatum; SP, stratum pyramidale. Scale bar=20 μm.

re-embedded in resin and the entire perisomatic region was re-sectioned for electron microscopy. Light microscopic analysis predicted that seven boutons, originating from five axon collaterals which could be continuously traced to the main axon, were closely apposed to the somatodendritic domain of the basket cell. Evaluation of serial ultrathin sections at the electron microscopic level confirmed that five boutons made autaptic junctions (Fig. 11). In one instance (site 3* in Fig. 10), a single bouton formed two active zones on a principal apical dendrite, close to where it originated from the parent basket cell soma (Fig. 11). All autaptic junctions (n=6) were on very proximal apical and basal dendrites. The perisomatic placement of autapses is similar to the synaptic target profile of the same cell to local pyramidal cell targets (48% somata, 49% proximal dendrites and 2% spines; n=61; reported in Ref. 12).

DISCUSSION

This study demonstrates directly that local interneurons located in the CA1 area of the rat hippocampus receive a GABAergic input from several discrete intrinsic sources. Moreover, individual interneurons may provide a common divergent output to both principal cells and other interneurons of the same (basket cells) or different type, whilst retaining target selectivity towards a particular membrane domain. Indeed, both the number and placement of

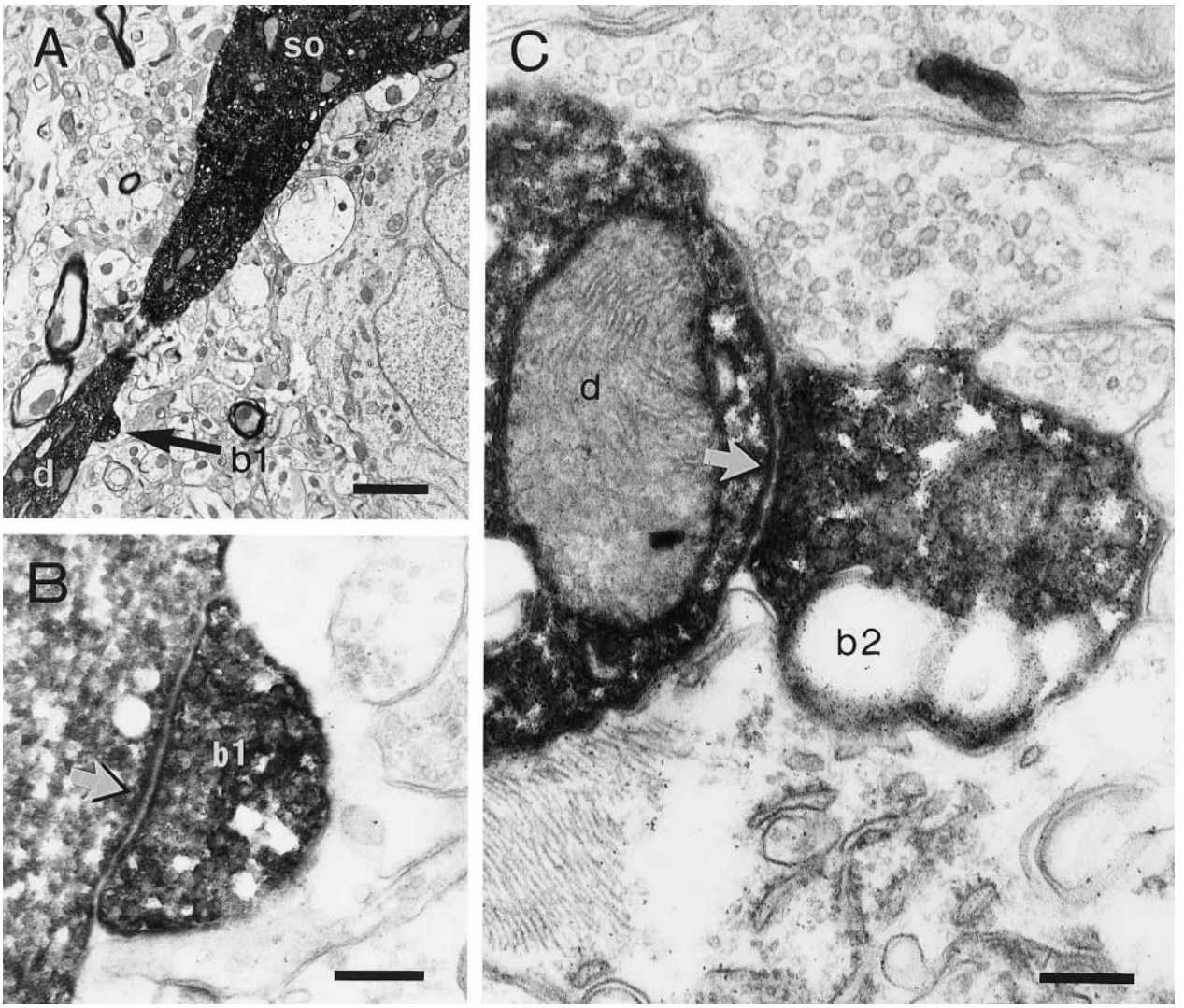

Fig. 11. Electron microscopic demonstration of autaptic junctions (also illustrated in Fig. 10). (A) Low power electron micrograph of the basket cell soma (so) and a proximal basal dendrite (d). Arrow indicates a bouton of the same basket cell in close apposition to the basal dendrite. At higher power (B) the same bouton (b1; corresponds to site 1 in Fig. 10) is seen to form an autaptic junction with the dendrite (arrow). (C) Electron micrograph of a second bouton (b2, site 2 in Fig. 10), forming an autaptic junction (arrow) onto a basal dendrite (d) of the basket cell. Scale bars: A=2 μm; B, C=0.2 μm.

synapses provided by a basket cell to a putative bistratified cell were similar to those provided to pyramidal cells by other basket cells in the CA1 area of the rat.[12,13] In agreement, the kinetics and amplitudes of the postsynaptic responses were similar in both pyramidal cell and interneuron targets and were consistent with $GABA_A$ receptor-mediated synaptic responses. In addition, a high incidence of autaptic self-innervation was also detected amongst certain types of interneuron and the strength (i.e. number) and placement of autapses are similar to the synaptic output onto other target neurons.

Identification of pre- and postsynaptic neurons

All recovered fast-spiking cells shared common morphological features, including smooth aspinous dendrites and dense local axon, which formed symmetrical type synapses. These results are consistent with the consensus that many interneurons can be distinguished from principal cells by their distinctive firing and other salient membrane properties alone.[9,12–15,42,43,50,68,78,79] However, specific morphological subtypes of interneuron cannot be reliably differentiated from each other solely on the basis of their intrinsic membrane properties[12,13,15] and therefore identification requires assessment at the light and electron microscopic levels.[31,41] One of the basket cells identified in this study (Fig. 5) had dendritic and axonal morphology different from the other three cells and those described in previous studies.[12,13,34] It appears to resemble a "trilaminar cell" described previously by Sik *et al.*[80] or the "wide axonal basket cell" described in the CA3 area by Gulyas *et al.*[31]

Such differences in axonal and dendritic patterns may reflect different subtypes of basket cell that are known to exist, based on differential immunoreactivity to neurochemical markers.[38,72,80] Likewise, the LM-R interneuron described in Fig. 8 represents only one example of a number of different interneurons which are known to occur at the stratum radiatum/lacunosum moleculare border.[91]

Properties of postsynaptic responses

Hippocampal interneurons are known to express a range of $GABA_A$ receptor subunits[26,73,93] and exhibit both fast (putative $GABA_A$) and slow (putative $GABA_B$) IPSPs,[13,15,48,49,68,78,80] indicating that GABAergic neurons are themselves under GABAergic control. This study indicates that synaptic responses mediated by basket cells are similar in both pyramidal cells and other interneurons and their properties are consistent with unitary IPSPs described previously.[12,13,17,44,49,51,63,65,66,78]

In the absence of pharmacological verification, the finding that all unitary synaptic responses were hyperpolarizing in the range of membrane potentials tested (-55 to -65 mV) and had fast kinetics (rise times 1–15.2 ms) is highly suggestive of a $GABA_A$ receptor-mediated effect,[12,13,63] as compared to a $GABA_B$ receptor-mediated response which has a slow onset and a time to peak of $\sim$200 ms.[67,70] It therefore seems likely that responses elicited by individual interneurons in both pyramidal cells and other interneurons are mediated by similar receptor mechanisms. However, $GABA_A$ receptor subunits may not be uniformly expressed across different subtypes of interneuron.[22,26,73] In the future, detailed pharmacology of interneuron-to-interneuron connections may therefore reveal functional differences corresponding to different $GABA_A$ receptors with varying subunit composition.

The hyperpolarizing nature of the unitary synaptic responses seen in this study is at variance with earlier studies addressing interneuron-to-interneuron network interactions in which the authors described a $GABA_A$ receptor-mediated excitatory effect over other interneurons.[59,60] However, the hyperpolarizing nature of the responses seen here is consistent with other studies which examined unitary interactions between unidentified pairs of putative interneurons.[51,78] The previously reported depolarizing responses evoked by population activity[59,60] could be due to changes in the intracellular concentration of chloride and bicarbonate ions in the cells after synchronous activation of very large numbers of synapses.[85]

The synaptic response mediated by the LM-R interneuron needs special consideration. Previous studies have shown that single action potentials elicited in presynaptic LM-R interneurons may fail to evoke any postsynaptic response, whereas a short train of action potentials produces summated hyperpolarizing synaptic responses with slow rise and decay kinetics,[50,51] which may indicate different receptor mechanisms. However, summated responses evoked by trains of action potentials make accurate kinetic measurements difficult to obtain and interpret. Moreover, recent evidence shows that interneurons in stratum lacunosum moleculare are morphologically more heterogeneous than previously supposed and their hyperpolarizing postsynaptic responses can be abolished by $GABA_A$ receptor antagonists.[91]

Sources of GABAergic input

GABAergic interneurons receive GABAergic input from three main sources. The primary extrinsic source originates from GABAergic cells in the medial septum which selectively target several classes of hippocampal interneuron.[3,23,25,29,61] The second source is from several recently described populations of hippocampal interneurons, identified by the presence of distinct neurochemical markers such as calretinin and vasoactive intestinal peptide, which selectively target some types of hippocampal interneuron, whilst avoiding others.[2,30] In contrast, other types of interneuron, including those reported here, mainly target principal cells as well as provide an intrinsic source of input onto other interneurons. These cell classes represent the third main source of GABAergic input onto hippocampal interneurons and those reported previously include cells immunoreactive for cholecystokinin[38,72] and parvalbumin.[80] By innervating both other interneurons as well as principal cells, all four presynaptic cells described in this study belong to this third category. The results indicate that the strength of synaptic connections given to postsynaptic pyramidal cells is approximately equivalent to that given to interneurons, since both the amplitude of evoked responses and the number of synapses provided by a single basket cell to a postsynaptic putative bistratified cell are similar to those received by individual pyramidal cells in the rat hippocampus.[12,13] Although the selectivity of a single interneuron towards one type of postsynaptic cell over another cannot be established from these data, a previous study suggested an individual interneuron to innervate both other interneurons and pyramidal cells at a ratio[80] equivalent to which the two cell types occur in the hippocampus,[5] thus implying no preference for one postsynaptic target cell over the other. However, considerable uncertainties remain. For example, although we have found evidence that basket cells of the same type are interconnected, the extent and strength of such synaptic links remains to be determined. The interconnection between basket cells is supported by previous indirect evidence showing the innervation of parvalbumin-immunopositive cells by a basket cell[80] and the abundance of GABAergic, parvalbumin-immunopositive innervation of parvalbumin-positive

cells.[25] It remains to be determined whether the innervated parvalbumin-positive cells are basket or axoaxonic cells, as both interneuron types express parvalbumin.

The high incidence of autaptic self-innervation seen in this study is surprising and represents a fourth source of GABAergic input onto hippocampal interneurons. The effect of autapses is expected to be strongly correlated with the action potential. Autapses are not uncommon within the central nervous system,[27,41,54,74,75,77,86,87,90] yet their physiological significance remains to be established. It is not yet clear whether autaptic innervation simply reflects the fact that these neurons have not evolved a mechanism to eliminate inappropriately placed synaptic junctions on their own membrane. However, given that specific interneurons can selectively target specific subtypes of interneuron whilst avoiding others,[2,30] this seems unlikely. In support of this, axoaxonic cells which exclusively innervate the axon initial segment domain of principal cells show no autaptic innervation. Whether self-innervation described here mediates any functional role remains unknown, but given the emerging importance of GABAergic interneurons in timing and synchronizing neuronal activity during rhythmic neuronal states, it is reasonable to speculate that self-innervation may help to temporally sculpt a cell's own firing during periods of rhythmic activity.

Functional implications

The existence of extensive synaptic cross-talk between GABAergic interneurons adds further complexity to the way we view hippocampal circuitry. Indeed, interconnectivity amongst interneurons may serve a role in controlling both spatial and temporal aspects of inhibition. The traditional view of an inhibitory neuron innervating a second such cell is that the consequent downstream effect would be disinhibition. Recent evidence suggests that the GABAergic septohippocampal input onto hippocampal interneurons does suppress spontaneous IPSP activity in pyramidal cells.[88] However, as previous anatomical and the present results suggest, in the case of one class of interneuron evoking fast inhibition in another, it is possible to change the balance of inhibition from one membrane domain of a principal neuron to another.[2,33] For example, the result of basket cell synaptic effects on bistratified cells may be a shift from dendritic to perisomatic inhibition. This would be achieved by direct input to the perisomatic domain of pyramidal cells, concurrent with a reduction of inhibition at the dendritic membrane caused by the same basket cell inhibiting the activity of dendrite-targeting interneurons. Switches in spatial aspects of inhibition have been reported to occur *in vivo* during exploratory behaviour in the dentate gyrus, where perisomatic inhibition is decreased when dendritic inhibition is increased.[69]

A second role for interconnectivity amongst interneurons is predictable, given the observations that many types of interneuron fire within characteristic frequency bands during specific behavioural states (see Ref.[24]). Rhythmic firing, coupled with the complex postsynaptic responses that assist coherence in the population,[17] greatly extends the role of GABAergic neurons. Neuronal integration and coding is based both on the magnitude and temporal structure of responses.[21,47,55] The latter is implemented through the interaction of numerous voltage-gated intrinsic conductances within neurons, which profoundly shape neuronal responses and may endow individual neurons with intrinsic oscillatory properties, which may, in turn, facilitate coherent population activities.[53] Interconnected interneuron networks may therefore form a system of coupled oscillators, leading to emergent network properties[16,89,92] which may provide a co-ordinated temporal structure across areas of the hippocampus, relative to which specific information is coded. The synaptic interconnection between the same type of basket cells demonstrated here could be one component contributing to coherent basket cell population activity. As is evident from the other demonstrated interactions, more complex spatiotemporal patterns of GABAergic influence may emerge from the interconnections of several distinct types of interneurons.

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REFERENCES

1. Acsády L., Arabadzisz D. and Freund T. F. (1996) Correlated morphological and neurochemical features identify different subsets of vasoactive intestinal polypeptide-immunoreactive interneurons in rat hippocampus. *Neuroscience* **73,** 299–315.
2. Acsády L., Görcs T. J. and Freund T. F. (1996) Different populations of vasoactive intestinal polypeptide-immunoreactive interneurons are specialized to control pyramidal cells or interneurons in the hippocampus. *Neuroscience* **73,** 317–334.
3. Acsády L., Halasy K. and Freund T. F. (1993) Calretinin is present in non-pyramidal cells of the rat hippocampus — III. Their inputs from the median raphe and medial septal nuclei. *Neuroscience* **52,** 829–841.
4. Aghajanian G. K. and Rasmussen K. (1989) Intracellular studies in the facial nucleus illustrating a simple new method for obtaining viable motoneurons in adult rat brain slices. *Synapse* **3,** 331–338.

5. Aika Y., Ren J. Q., Kosaka K. and Kosaka T. (1994) Quantitative analysis of GABA-like-immunoreactive and parvalbumin-containing neurons in the CA1 region of the rat hippocampus using a stereological method, the disector. *Expl Brain. Res.* **99,** 267–276.
6. Andersen P., Eccles J. C. and Loyning Y. (1963) Recurrent inhibition in the hippocampus with identification of the inhibitory cell and its synapses. *Nature* **198,** 540–542.
7. Andersen P., Eccles J. C. and Loyning Y. (1964) Location of postsynaptic inhibitory synapses on hippocampal pyramids. *J. Neurophysiol.* **27,** 592–607.
8. Andersen P., Eccles J. C. and Loyning Y. (1964) Pathway of postsynaptic inhibition in the hippocampus. *J. Neurophysiol.* **27,** 608–619.
9. Ashwood T. J., Lancaster B. and Wheal H. V. (1984) *In vivo* and *in vitro* studies on putative interneurones in the rat hippocampus: possible mediators of feed-forward inhibition. *Brain Res.* **293,** 279–291.
10. Baude A., Nusser Z., Roberts J. D. B., Mulvihill E., Mcilhinney R. A. J. and Somogyi P. (1993) The metabotropic glutamate receptor (mGluR1α) is concentrated at perisynaptic membrane of neuronal subpopulations as detected by immunogold reaction. *Neuron* **11,** 771–787.
11. Blackstad T. W. and Flood P. R. (1963) Ultrastructure of hippocampal axo-somatic synapses. *Nature* **198,** 542–543.
12. Buhl E. H., Cobb S. R., Halasy K. and Somogyi P. (1995) Properties of unitary IPSPs evoked by anatomically identified basket cells in the rat hippocampus. *Eur. J. Neurosci.* **7,** 1989–2004.
13. Buhl E. H., Halasy K. and Somogyi P. (1994) Diverse sources of hippocampal unitary inhibitory postsynaptic potentials and the number of synaptic release sites. *Nature* **368,** 823–828.
14. Buhl E. H., Han Z. S., Lörinczi Z., Stezhka V. V., Karnup S. V. and Somogyi P. (1994) Physiological properties of anatomically identified axo-axonic cells in the rat hippocampus. *J. Neurophysiol.* **71,** 1289–1307.
15. Buhl E. H., Szilágyi T., Halasy K. and Somogyi P. (1996) Physiological properties of anatomically identified basket and bistratified cells in the CA1 area of the rat hippocampus *in vitro*. *Hippocampus* **6,** 294–305.
16. Buzsáki G. and Chrobak J. J. (1995) Temporal structure in spatially organised neuronal ensembles: a role for interneuronal networks. *Curr. Opin. Neurobiol.* **5,** 504–510.
17. Cobb S. R., Buhl E. H., Halasy K., Paulsen P. and Somogyi P. (1995) Synchronisation of neuronal activity in hippocampus by individual GABAergic interneurones. *Nature* **378,** 75–78.
18. Cobb S. R., Halasy K., Vida I., Buhl E. H. and Somogyi P. (1996) Common sources and characteristics of GABAergic input to interneurones and pyramidal cells in the rat hippocampus. *J. Physiol., Lond.* **495,** 62P.
19. Davies C. H., Starkey S. J., Pozza M. F. and Collingridge G. L. (1991) $GABA_B$ autoreceptors regulate the induction of LTP. *Nature* **349,** 609–611.
20. Deller T. and Leránth C. (1990) Synaptic connections of neuropeptide Y (NPY) immunoreactive neurons in the hilar area of the rat hippocampus. *J. comp. Neurol.* **300,** 433–447.
21. Engel A. K., König P., Kreiter A. K., Schillen T. B. and Singer W. (1992) Temporal coding in the visual cortex: new vistas on integration in the nervous system. *Trends Neurosci.* **15,** 218–226.
22. Esclapez M., Chang D. K. and Houser C. R. (1996) Subpopulations of GABA neurons in the dentate gyrus express high levels of the α1 subunit of the $GABA_A$ receptor. *Hippocampus* **6,** 225–238.
23. Freund T. F. and Antal M. (1988) GABA-containing neurons in the septum control inhibitory interneurones in the hippocampus. *Nature* **336,** 170–173.
24. Freund T. F. and Buzsáki G. (1996) Interneurons of the hippocampus. *Hippocampus* **6,** 347–470.
25. Fukuda T., Aika Y., Heizmann C. W. and Kosaka T. (1996) Dense GABAergic input on somata of parvalbumin-immunoreactive GABAergic neurons in the hippocampus of the mouse. *Neurosci. Res.* **26,** 181–194.
26. Gao B. and Fritschy J. M. (1994) Selective allocation of $GABA_A$ receptors containing the α1 subunit to neurochemically distinct subpopulations of rat hippocampal interneurones. *Eur. J. Neurosci.* **6,** 837–853.
27. Guldner F. H. and Wolf J. R. (1978) Self-innervation of dendrites in the rat suprachiasmatic nucleus. *Expl Brain Res.* **32,** 77–82.
28. Gray E. G. (1959) Axo-somatic and axo-dendritic synapses of the cerebral cortex: an electron microscope study. *J. Anat.* **93,** 420–433.
29. Gulyás A. I., Görcs T. J. and Freund T. F. (1990) Innervation of different peptide-containing neurons in the hippocampus by GABAergic septal afferents. *Neuroscience* **37,** 31–44.
30. Gulyás A., Hájos N. and Freund T. (1996) Interneurones containing calretinin are specialized to control other interenurones in the rat hippocampus. *J. Neurosci.* **16,** 3397–3411.
31. Gulyás A. I., Miles R., Hájos N. and Freund T. F. (1993) Precision and variability in postsynaptic target selection of inhibitory cells in the hippocampal CA3 region. *Eur. J. Neurosci.* **5,** 1729–1751.
32. Gulyás A. I., Tóth K., Danos P. and Freund T. F. (1991) Subpopulations of GABAergic neurons containing parvalbumin, calbindin D28k, and cholecystokinin in the rat hippocampus. *J. comp. Neurol.* **312,** 371–378.
33. Hájos N., Acsády L. and Freund T. F. (1996) Target selectivity and neurochemical characteristics of VIP-immunoreactive interneurons in the rat dentate gyrus. *Eur. J. Neurosci.* **8,** 1415–1431.
34. Halasy K., Buhl E. H., Lörinczi Z., Tamás G. and Somogyi P. (1996) Synaptic target selectivity and input of GABAergic basket and bistratified interneurons in the CA1 area of the rat hippocampus. *Hippocampus* **6,** 306–329.
35. Halasy K., Miettinen R., Szabat E. and Freund T. F. (1992) GABAergic interneurons are the major postsynaptic targets of median raphe afferents in the rat dentate gyrus. *Eur. J. Neurosci.* **4,** 144–153.
36. Halasy K. and Somogyi P. (1993) Subdivisions in the multiple GABAergic innervation of granule cells in the dentate gyrus of the rat hippocampus. *Eur. J. Neurosci.* **5,** 411–429.
37. Han Z.-S., Buhl E. H., Lörinczi Z. and Somogyi P. (1993) A high degree of spatial selectivity in the axonal and dendritic domains of physiologically identified local-circuit neurons in the dentate gyrus of the rat hippocampus. *Eur. J. Neurosci.* **5,** 395–410.
38. Harris K. M., Marshall P. E. and Landis D. M. (1985) Ultrastructural study of cholecystokinin-immunoreactive cells and processes in area CA1 of the rat hippocampus. *J. comp. Neurol.* **233,** 147–159.
39. Horikawa K. and Armstrong W. E. (1988) A versatile means of intracellular labeling: injection of biocytin and its detection with avidin conjugates. *J. Neurosci. Meth.* **25,** 1–11.
40. Kandel E. R., Spencer W. A. and Brinley F. J. (1961) Electrophysiology of hippocampal neurons. 1. Sequential invasion and synaptic organisation. *J. Neurophysiol.* **24,** 225–242.

41. Karabelas A. B. and Purpura D. P. (1980) Evidence for autapses in the substantia nigra. *Brain Res.* **200,** 467–473.
42. Kawaguchi Y. and Hama K. (1987) Two subtypes of non-pyramidal cells in the rat hippocampal formation identified by intracellular recording and HRP injection. *Brain Res.* **411,** 190–195.
43. Kawaguchi Y. and Hama K. (1987) Fast-spiking non-pyramidal cells in the hippocampal CA3 region, dentate gyrus and subiculum of rats. *Brain Res.* **425,** 351–355.
44. Knowles W. D. and Schwartzkroin P. A. (1981) Local circuit synaptic interactions in hippocampal brain slices. *J. Neurosci.* **1,** 318–322.
45. Kosaka T., Katsumaru H., Hama K., Wu J.-Y. and Heizmann C. W. (1987) GABAergic neurons containing the Ca^{2+}-binding protein parvalbumin in the rat hippocampus and dentate gyrus. *Brain Res.* **419,** 119–130.
46. Kosaka T., Kosaka K., Tateishi K., Hamaoka Y., Yanaihara N., Wu J.-Y. and Hama K. (1985) GABAergic neurons containing CCK-8-like and/or VIP-like immunoreactivities in the rat hippocampus and dentate gyrus. *J. comp. Neurol.* **239,** 420–430.
47. Kruger J. and Becker J. D. (1991) Recognizing the visual stimulus from neuronal discharges. *Trends Neurosci.* **14,** 282–286.
48. Lacaille J.-C. (1991) Postsynaptic potentials mediated by excitatory and inhibitory amino acids in interneurons of stratum pyramidale of the CA1 region of rat hippocampal slices *in vitro*. *J. Neurophysiol.* **66,** 1441–1454.
49. Lacaille J.-C., Mueller A. L., Kunkel D. D. and Schwartzkroin P. A. (1987) Local circuit interactions between oriens/alveus interneurons and CA1 pyramidal cells in hippocampal slices: electrophysiology and morphology. *J. Neurosci.* **7,** 1979–1993.
50. Lacaille J.-C. and Schwartzkroin P. A. (1988) Stratum lacunosum-moleculare interneurons of hippocampal CA1 region. I. Intracellular response characteristics, synaptic responses, and morphology. *J. Neurosci.* **8,** 1400–1410.
51. Lacaille J.-C. and Schwartzkroin P. A. (1988) Stratum lacunosum-moleculare interneurons of hippocampal CA1 region. II. Intrasomatic and intradendritic recordings of local circuit synaptic interactions. *J. Neurosci.* **8,** 1411–1424.
52. Li X. G., Somogyi P., Tepper J. M. and Buzsáki G. (1992) Axonal and dendritic arborization of an intracellularly labeled chandelier cell in the CA1 region of rat hippocampus. *Expl Brain Res.* **90,** 519–525.
53. Llinás R. R. (1988) The intrinsic electrophysiological properties of mammalian neurons: insights into central nervous system function. *Science* **242,** 1654–1664.
54. Lübke J., Markram H., Frotscher M. and Sakmann B. (1996) Frequency and dendritic distribution of autapses established by layer 5 pyramidal neurones in the developing rat neocortex: comparison with synaptic innervation of adjacent neurons of the same class. *J. Neurosci.* **16,** 3209–3218.
55. Mainen Z. F. and Sejnowski T. J. (1995) Reliability of spike timing in neocortical neurons. *Science* **268,** 1503–1506.
56. Mason A., Nicoll A. and Stratford K. (1991) Synaptic transmission between individual pyramidal neurons of the rat visual cortex *in vitro*. *J. Neurosci.* **11,** 72–84.
57. McBain C. J., Dichiara T. J. and Kauer J. A. (1994) Activation of metabotropic glutamate receptors differentially affects two classes of hippocampal interneurons and potentiates excitatory synaptic transmission. *J. Neurosci.* **14,** 4433–4445.
58. McBain C. J. and Dingledine R. (1993) Heterogeneity of synaptic glutamate receptors on CA3 stratum radiatum interneurones of rat hippocampus. *J. Physiol., Lond.* **462,** 373–392.
59. Michelson H. B. and Wong R. K. S. (1994) Synchronization of inhibitory neurones in the guinea-pig hippocampus *in vitro*. *J. Physiol., Lond.* **477,** 35–45.
60. Michelson H. B. and Wong R. K. S. (1991) Excitatory synaptic responses mediated by $GABA_A$ receptors in the hippocampus. *Science* **253,** 1420–1423.
61. Miettinen R. and Freund T. F. (1992) Neuropeptide Y-containing interneurons in the hippocampus receive synaptic input from median raphe and GABAergic septal afferents. *Neuropeptides* **22,** 185–193.
62. Miettinen R., Gulyás A. I., Baimbridge K. G., Jacobowitz D. M. and Freund T. F. (1992) Calretinin is present in non-pyramidal cells of the rat hippocampus. II. Co-existence with other calcium binding proteins and GABA. *Neuroscience* **48,** 29–43.
63. Miles R. (1990) Variation in strength of inhibitory synapses in the CA3 region of guinea-pig hippocampus *in vitro*. *J. Physiol., Lond.* **431,** 659–676.
64. Miles R., Tóth K., Gulyás A. I., Hájos N. and Freund T. F. (1996) Differences between somatic and dendritic inhibition in the hippocampus. *Neuron* **16,** 815–823.
65. Miles R. and Wong R. K. S. (1984) Unitary inhibitory synaptic potentials in the guinea-pig hippocampus *in vitro*. *J. Physiol., Lond.* **356,** 97–113.
66. Miles R. and Wong R. K. S. (1987) Inhibitory control of local excitatory circuits in the guinea-pig hippocampus. *J. Physiol., Lond.* **388,** 611–629.
67. Misgeld U., Bijak M. and Jarolimek W. (1995) A physiological role for $GABA_B$ receptors and the effects of baclofen in the mammalian central nervous system. *Prog. Neurobiol.* **46,** 423–462.
68. Misgeld U. and Frotscher M. (1986) Postsynaptic-GABAergic inhibition of non-pyramidal neurons in the guinea-pig hippocampus. *Neuroscience* **19,** 193–206.
69. Moser E. I. (1996) Altered inhibition of granule cells during spatial learning in an exploration task. *J. Neurosci.* **16,** 1247–1259.
70. Newberry N. R. and Nicoll R. A. (1984) A bicuculline-resistant inhibitory post-synaptic potential in rat hippocampal pyramidal cells *in vitro*. *J. Physiol., Lond.* **348,** 239–254.
71. Nitsch R., Soriano E. and Frotscher M. (1990) The parvalbumin-containing nonpyramidal neurons in the rat hippocampus. *Anat. Embryol.* **181,** 413–425.
72. Nunzi N. G., Gorio A., Milan F., Freund T. F., Somogyi P. and Smith A. D. (1985) Cholecystokinin-immunoreactive cells form symmetrical synaptic contacts with pyramidal and non-pyramidal neurons in the hippocampus. *J. comp. Neurol.* **237,** 485–505.
73. Nusser Z., Roberts J. D. B., Baude A., Richards J. G., Sieghart W. and Somogyi P. (1995) Immunocytochemical localisation of the α1 and β2/3 subunits of the $GABA_A$ receptor in relation to specific GABAergic synapses in the hippocampal dentate gyrus. *Eur. J. Neurosci.* **7,** 630–646.

74. Park M. R., Lighthall J. W. and Kitai S. T. (1980) Recurrent inhibition in the rat neostriatum. *Brain Res.* **194,** 359–369.
75. Pasik P., Pasik T., Holstein G. R. and Hamori J. (1988) GABAergic elements in the neuronal circuits of the monkey neostriatum: a light and electron microscopic immunocytochemical study. *J. comp. Neurol.* **270,** 157–170.
76. Paulsen O. and Vida I. (1996) Sustained dendritic oscillations at theta frequencies elicited in CA1 pyramidal cells in the rat hippocampal slices. *J. Physiol., Lond.* **495,** 50P.
77. Peters A. and Proskauer C. C. (1980) Synaptic relationships between a multipolar stellate cell and a pyramidal neuron in the rat visual cortex. A combined Golgi-electron microscope study. *J. Neurocytol.* **9,** 163–183.
78. Scharfman H. E., Kunkel D. D. and Schwartzkroin P. A. (1990) Synaptic connections of dentate granule cells and hilar neurons: results of paired intracellular recordings and intracellular horseradish peroxidase injections. *Neuroscience* **37,** 693–707.
79. Schwartzkroin P. A. and Kunkel D. D. (1985) Morphology of identified interneurones in the CA1 regions of the guinea pig hippocampus. *J. comp. Neurol.* **232,** 205–218.
80. Sík A., Penttonen M., Ylinen A. and Buzsáki G. (1995) Hippocampal CA1 interneurons: an *in vivo* intracellular labeling study. *J. Neurosci.* **15,** 6651–6665.
81. Sloviter R. S. and Nilaver G. (1987) Immunocytochemical localization of GABA-cholecystokinin, vasoactive intestinal polypeptide-, and somatostatin-like immunoreactivity in the area dentata and hippocampus of the rat. *J. comp. Neurol.* **256,** 42–60.
82. Somogyi P., Hodgson A. J., Smith A. D., Nunzi M. G., Gorio A. and Wu J.-Y. (1984) Different populations of GABAergic neurons in the visual cortex and hippocampus of cat contain somatostatin- or cholecystokinin-immunoreactive material. *J. Neurosci.* **4,** 2590–2603.
83. Somogyi P., Nunzi M. G., Gorio A. and Smith A. D. (1983) A new type of specific interneuron in the monkey hippocampus forming synapses exclusively with the axon initial segments of pyramidal cells. *Brain Res.* **259,** 137–142.
84. Somogyi P. and Takagi H. (1982) A note on the use of picric acid-paraformaldehyde-glutaraldehyde fixative for correlated light and electron microscopic immunocytochemistry. *Neuroscience* **7,** 1779–1783.
85. Staley K. J., Soldo B. L. and Proctor W. R. (1995) Ionic mechanisms of neuronal excitation by inhibitory $GABA_A$ receptors. *Science* **269,** 977–981.
86. Takagi H., Somogyi P. and Smith A. D. (1984) Aspiny neurons and their local axons in the neostriatum of the rat: a correlated light and electron microscopic study of Golgi-impregnated material. *J. Neurocytol.* **13,** 239–265.
87. Tamas G., Buhl E. H. and Somogyi P. (1995) High degree of self-innervation by GABAergic basket cells in the visual cortex of the cat as revealed *in vitro*. *J. Physiol., Lond.* **487,** 63P.
88. Toth K., Freund T. F. and Miles R. (1995) Septal stimulation suppresses hippocampal synaptic inhibition in a combined slice preparation. *Soc. Neurosci. Abstr.* **21,** 1207.
89. Traub R. D., Whittington M. A., Stanford I. M. and Jefferys J. G. R. (1996) A mechanism for generation of long-range synchronous fast oscillations in the cortex. *Nature* **383,** 621–624.
90. Van der Loos H. and Glaser E. M. (1972) Autapses in neocortical cerebri: synapses between a pyramidal cell's axon and its own dendrites. *Brain Res.* **48,** 355–360.
91. Vida I., Halasy K., Szinyei C., Buhl E. H. and Somogyi P. (1997) Lacunosum-moleculare interneurones produce fast $GABA_A$ IPSPs in CA1 pyramidal cells of the rat hippocampus *in vitro*. (Submitted).
92. Whittington M. A., Traub R. D. and Jefferys J. G. R. (1995) Synchronized oscillations in interneurone networks driven by metabotropic glutamate receptor activation. *Nature* **373,** 612–615.
93. Zimprich F., Zezula J., Sieghart W. and Lassmann H. (1991) Immunohistochemical localisation of the α1, α2 and α3 subunit of the $GABA_A$ receptor in the rat brain. *Neurosci. Lett.* **127,** 125–128.

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