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Cholecystokinin-Immunoreactive Cells Form Symmetrical Synaptic Contacts With Pyramidal and Nonpyramidal Neurons in the Hippocampus

M.G. NUNZI, A. GORIO, F. MILAN, T.F. FREUND, P. SOMOGYI, AND A.D. SMITH
Department of Cytopharmacology, Fidia Research Laboratories, 35031 Abano Terme, Italy (M.G.N., A.G., F.M.), University Department of Pharmacology, Oxford OX1 3QT, Great Britain (A.D.S.), and 1st Dept. of Anatomy, Semmelweis University Medical School, Budapest 1450, Hungary (T.F.F., P.S.)

ABSTRACT

The ultrastructural features and synaptic relationships of cholecystokinin (CCK)-immunoreactive cells of rat and cat hippocampus were studied using the unlabeled antibody immunoperoxidase technique and correlated light and electron microscopy. CCK-positive perikarya of variable shape and size were distributed in all layers and were particularly concentrated in stratum pyramidale and radiatum: the CCK-immunoreactive neurons were nonpyramidal in shape and the three most common types had the morphological features of tufted, bipolar, and multipolar cells.

Electron microscopic examination revealed that all the CCK-positive boutons established symmetrical (Gray's type II) synaptic contacts with perikarya and dendrites of pyramidal and nonpyramidal neurons. The origin of some of the boutons was established by tracing fine collaterals that arose from the main axon of two CCK-immunostained cells and terminated in the stratum pyramidale; these collaterals were then examined in the electron microscope. The axon of one such neuron exhibited a course parallel to the pyramidal layer and formed pericellular nets of synaptic boutons upon the perikarya of pyramidal neurons. This pattern of axonal arborization is very similar to that of some of the basket cells, previously suggested to be the anatomical correlate for pyramidal cell inhibition. Typical dendrites of pyramidal cells also received symmetrical synaptic contacts from CCK-immunoreactive boutons, and some of these boutons could be shown to originate from a local neuron in stratum radiatum. Many CCK-immunoreactive cells received CCK-labeled boutons upon their soma and dendritic shafts. Synaptic relationship, established by multiple "en passant" boutons, was observed between CCK-positive interneurons of the stratum lacunosum-moleculare and radiatum. The soma and dendrites of the CCK-immunostained neurons also received symmetrical and asymmetrical synapses from nonimmunoreactive boutons.

These results indicate that the CCK-immunoreactive neurons participate in complex local synaptic interactions in the hippocampus.

Key words: immunocytochemistry, synaptic connections, interneurons

Cholecystokinin (CCK) was originally isolated from gastrointestinal tissue as a substance containing 33 amino acid residues (Mutt and Jorpes, '68) and later was also found in the brain along with its smaller fragments (Dockray et al., '78; see reviews by Beinfeld, '83; Dodd and Kelly, '81). Its

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P. Somogyi's present address is MRC Anatomical Neuropharmacology Unit, Department of Pharmacology, South Parks Road, Oxford OX1 3QT, U.K.

distribution has been described by both radioimmunoassay (Vanderhaeghen et al., '75; Rehfeld, '78; Robberecht et al., '78; Larsson and Rehfeld, '79; Dockray, '80; Beinfeld et al., '81; Emson et al., '82) and immunohistochemistry (Innis et al., '79; Loren et al., '79). In addition, in vitro biosynthetic studies have demonstrated that the peptide is synthesized in the brain (Rehfeld, '80; Golterman et al., '80). Many characteristics of CCK suggest a role in neurotransmission. Cholecystokinin occurs in synaptic vesicle fractions of brain homogenates (Pinget et al., '78; Emson et al., '80), can be released from synaptosomes and cortical slices with depolarizing stimuli in a Ca^{++}-dependent manner (Pinget et al., '79; Dodd et al., '80; Emson et al., '80), and a specific binding of radiolabeled CCK to high-affinity binding sites has been reported (Saito et al., '80; Snyder et al., '81; Hays et al., '81; Zarbin et al., '83). Moreover, studies on the electrophysiological action of CCK applied iontophoretically into the cortex and in hippocampal slices have shown an excitatory effect on pyramidal neurons (Phillis and Kirkpatrick, '80; Dodd and Kelly, '81).

In agreement with the above findings, immunohistochemical investigations, at the light microscopic level, in cortex and hippocampus have localized CCK-immunoreactive material in cell bodies and varicose fibers (Vanderhaeghen et al., '80; Emson and Hunt, '81; Greenwood et al., '81; Köhler and Chan-Palay, '82; Peters et al., '83; Hendry et al., '83a).

From these studies, it is apparent that CCK-containing cells form an heterogeneous population, with regard to size and distribution of perikarya and dendritic arborization pattern, and that the axons and terminals are primarily associated with pyramidal neurons. These results, together with the observations by Handelmann et al. ('81) showing that isolation of the hippocampal formation from its main afferent connections had no effect on the CCK content of this region, have led to the hypothesis that CCK-containing cells might act as interneurons, modulating pyramidal cell activity. At the same time other studies suggest that at least some of the CCK-immunoreactive neurons may project from the hippocampal formation (see Beinfeld, '83). Ultrastructural features of CCK-positive cells and terminals in cortical areas have not led to any firm conclusions about their role in cortical circuitry. CCK immunoreactivity has been reported to be present in neurons suggested to be either inhibitory or excitatory (Peters et al., '83; Hendry et al., '83a) on the basis of the axonal and dendritic morphology, and by analogy with cortical neurons displaying similar dendritic characteristics in Golgi-impregnated preparations.

Therefore, we decided to investigate further the cytological features and synaptic connections of CCK-containing cells and terminals in the hippocampus, a brain area where the role of inhibitory interneurons has been extensively studied (Andersen et al., '63, '64a,b; Knowles and Schwartzkroin, '81; Alger and Nicoll, '82; Somogyi et al., '83b,c). The main aim of the present study was to trace connections at the synaptic level of light microscopically identified CCK-immunoreactive neurons, in order to establish the existence of local interactions. Since pyramidal neurons in the hippocampus are known to receive significant input from local interneurons, the CCK input onto pyramidal cells was studied in greater detail. A preliminary report of some of the findings has been made (Nunzi et al., '84).

MATERIALS AND METHODS

Animals

Four albino Sprague-Dawley adult rats (Charles River, Breeding Labs., Wilmington, MA) and two adult cats were used in this study. Rats were anesthetized with an injection of either chloral hydrate (Merck, 0.04 g/100 g i.p.) or pentothal (Abbott, 0.04 g/100 g i.p.) and perfused at room temperature through the left ventricle with Tyrode's solution (gassed with a mixture of 95% O_2 and 5% CO_2). When the venous return was clear, the perfusion fluid was substituted with the fixative containing 4% paraformaldehyde (Serva), 0.05% glutaraldehyde (Merck), and 0.2% picric acid dissolved in 0.1 M sodium phosphate buffer pH 7.4 (Somogyi and Takagi, '82).

Six cats were sedated with ketamine hydroclorate (Ketanest, 0.4 mL/kg i.m.), anesthetized with chloral hydrate as above or with xylazine hydrochloride (Rompun, Bayer 1 ml/kg), and perfused with the same procedure and fixative described above for rats.

To increase the immunostaining of CCK-containing cell bodies and neuronal processes, one cat, anesthetized with ketamine and xylazine, received injections of colchicine 24 hours before perfusion as described earlier (Somogyi et al., '83b). The right hippocampal formation was injected through glass micropipettes having a tip diameter of about 50 μm, with colchicine (BDH Chemicals, 6 μg/ul) dissolved in artificial cerebrospinal fluid. Two vertical penetrations were made 3 mm apart in the anterior-posterior direction. While the capillary was withdrawn, colchicine (0.2 μl) was injected at each half-millimeter step over a total distance of 5 mm. Thus, 2 μl of solution, equivalent to 12 μg of colchicine, was injected following each vertical penetration.

Preparation of tissue sections and the immunocytochemical procedures were identical for both rats and cats.

Preparation of tissue sections

After perfusion, the brain was removed from the skull and blocks of 1–2 mm in thickness were cut from the hippocampus in a coronal plane. The blocks were postfixed for 1–2 hours at 4°C with the perfusion fixative; they were then washed several times with 0.1 M sodium phosphate buffer, pH 7.4, and subsequently, until the blocks sank, in 10% and then 20% sucrose dissolved in the same buffer. Blocks were then frozen in liquid nitrogen and thawed in 0.1 M phosphate buffer. Sections of 80-μm thickness were cut on a Vibratome (Oxford Instruments) and washed in 0.1 M phosphate buffer, pH 7.4.

Immunocytochemical procedure

The unlabeled antibody peroxidase-antiperoxidase (PAP) method (Sternberger et al., '70) was used to reveal CCK immunoreactivity. The procedure of section incubation was in the following order and performed at room temperature unless otherwise stated: 1 hour in 20% normal goat serum (Sigma); 2 × 15-minute washes; overnight at 4°C in primary antiserum diluted 1:200; 4 × 20-minute washes; 6–12 hours in goat antirabbit IgG (Miles Laboratories) diluted 1:40; 4 × 20-minute washes; overnight at 4°C in PAP complex (Bioproducts, Ltd.) diluted 1:80; 2 × 20-minute washes; 2 × 15-minute washes in Tris HCl buffer pH 7.4.

Phosphate-buffered saline (PBS) containing 1% of normal goat serum was used for dilutions of both primary anti-

serum, and goat antirabbit IgG and for each wash after each antiserum. PBS was used for PAP dilution. Sections were then pre-incubated at room temperature for 30–45 minutes in 0.5% 3,3′-diaminobenzidine tetrahydrochloride (Sigma) dissolved in 0.05 M Tris HCl buffer, pH 7.4, followed by a reaction for 4–6 minutes in the same solution but containing 0.01% hydrogen peroxide. After washing in Tris buffer, sections were postfixed for 1 hour with 1% OsO_4 in 0.1 M phosphate buffer, pH 7.4. Dehydration was performed with a graded series of ethyl alcohols 70%, 95%, and 100%. Block staining with 1% uranyl acetate for 40 minutes was done during the 70% ethyl alcohol step.

For correlated light and electron microscopic observations, sections were flat-embedded on slides in Durcupan ACM (Fluka) resin (Somogyi and Takagi, '82). Neurons selected for electron microscopy were drawn from the light microscope using a Leitz drawing tube attachment, photographed in the light microscope, and the areas containing the neurons and processes were re-embedded for correlated electron microscopy (Somogyi and Takagi, '82). The re-embedded neurons and processes were cut serially on an ultramicrotome and collected on Formvar-coated single-slot (2 × 1 mm) grids, stained with lead citrate, and examined with Philips 400T and JEOL 100 B electron microscopes.

Antiserum

Antiserum to CCK (code No. L112) was raised to the C-terminal tetrapeptide of CCK/gastrin, coupled to bovine thyroglobulin, by Dr. G. Dockray. The characteristics of this serum have been reported earlier (Dockray et al., '81).

Controls

To check for specificity of the immunostaining, some sections were incubated in normal rabbit serum, diluted 1:200, in place of the primary antiserum. Serum specificity was evaluated by pre-incubating the diluted CCK antiserum overnight with sulphated CCK-8 (Sigma, 10^{-5} M) before the immunohistochemical reaction.

No immunoreactivity was seen in the control sections, but peroxidase reaction endproduct was present over red blood cells and in small granules in the cell bodies of pyramidal neurons. These places represent endogenous peroxidase activity and are not immunoreactive sites.

RESULTS

Distribution and characterization of CCK-immunoreactive cells and fibers

Light microscopic analysis of 80-μm-thick immunostained sections revealed CCK-immunoreactive cells and fibers throughout all subfields and layers of the hippocampal formation, including the dentate fascia.

The number of CCK-positive neurons was greater and the intensity of the dendritic staining more pronounced in colchicine-injected hippocampus from the cat. For this reason, the distribution and morphological characteristics of immunostained neurons were examined in this material. However, no substantial differences were seen between the distribution of CCK-immunoreactive cells in nontreated hippocampi of cat and rat.

As previously reported by Greenwood et al. ('81) for rat hippocampus, most of the CCK-immunoreactive cells in the cat hippocampus lie within the stratum pyramidale and radiatum, while relatively fewer cells are scattered in the stratum oriens and lacunosum-moleculare (Fig. 1). In general, the CCK-positive neurons did not appear arranged in any particular pattern although a clustering of CCK-positive cells occurred frequently in the proximal region of subfield CA1 adjacent to the subiculum.

CCK-positive cells constitute a heterogeneous population that exhibits considerable variations in perikaryal shape and size and dendritic arborization. However, three basic morphological types of cells are commonly encountered and can be classified as tufted, bipolar, and multipolar. With the possible exception of the multipolar cells, which are mostly confined to the stratum pyramidale, the other neuronal types are spread out in all hippocampal layers. Tufted cells, by far the most frequently immunostained cell type, have ovoidal perikarya whose dimensions vary between 6 and 11 μm and 10–12 μm for minor and major axes, respectively (Fig. 2A). Tufts of dendrites originate either from one or, more frequently, from the two opposite poles of the cell body. The dendritic field has an elongated shape that is typically orientated across the borders of the adjacent layers.

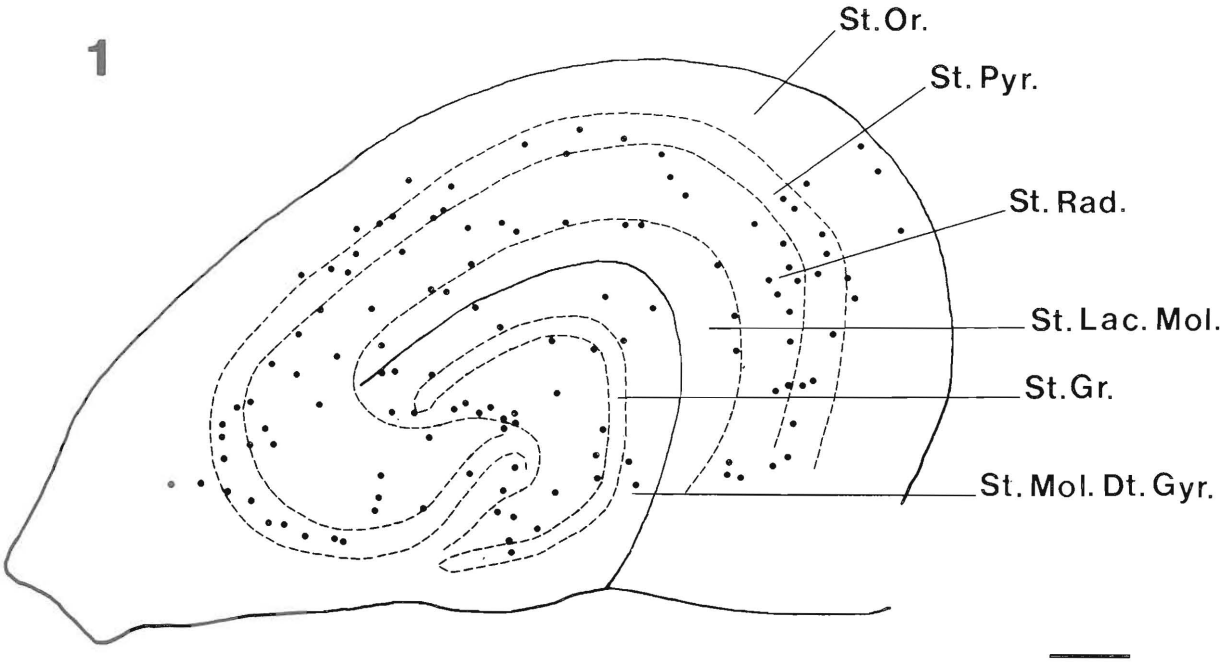
The second most common neuronal elements are bipolar cells, which are frequently found in the stratum pyramidale and at the lower border of the stratum radiatum (Fig. 2B). The cells display fusiform cell bodies, 26 × 13 μm in average size, oriented with their long axis perpendicular to the surface of the alveus. Two major dendritic trunks arise from the tapered ends of the perikaryon and, at a short distance, break up into a variable number of lateral branches arranged in a narrow vertical field. Each main dendrite may extend for 300–400 μm from the perikaryon. The remainder of the CCK-immunoreactive neurons have a multipolar appearance, since primary dendrites arise in random fashion from the perikarya (Fig. 2C). The perikarya are rounded, 14–18 μm in diameter, or markedly pyramidal in shape, with the major diameter of 25 μm or more (Fig. 2C). The primary dendrites give off secondary branches characterized by very thick and coarse beads.

CCK-immunoreactive neurons showed dendritic processes with smooth or irregular contours and spines were not evident. The axon initial segment of CCK-immunoreactive neurons originated from the proximal portion of a primary dendrite or directly from the perikaryon (Fig. 2A–C). In sections from hippocampi not treated with colchicine the main axons were seen pursuing a vertical or horizontal course giving off, at some distance from the cell body, a variable number of fine collaterals.

Immunoreactive fibers bearing numerous varicosities occurred in all hippocampal layers, with the highest density in stratum pyramidale and radiatum. CCK-immunostained varicosities were found mostly associated with profiles of unlabeled perikarya and apical dendritic shafts of pyramidal neurons, thus suggesting the presence of a prominent CCK projection onto these cells (Fig. 3A). Long strands of immunoreactive fibers could be traced next to both CCK-immunoreactive and nonimmunoreactive neurons of different morphology and size. Such neurons were very frequently contacted by CCK-positive fibers forming pericellular plexi upon cell bodies and dendritic shafts (Fig. 3B). This pattern of close apposition of CCK-immunoreactive varicosities with different structures is strongly suggestive of axosomatic and axodendritic synaptic terminals (see below).

Synaptic connections of CCK-immunoreactive cells and boutons

The main purpose of this part of the study is to describe the ultrastructure and synaptic connection with specific



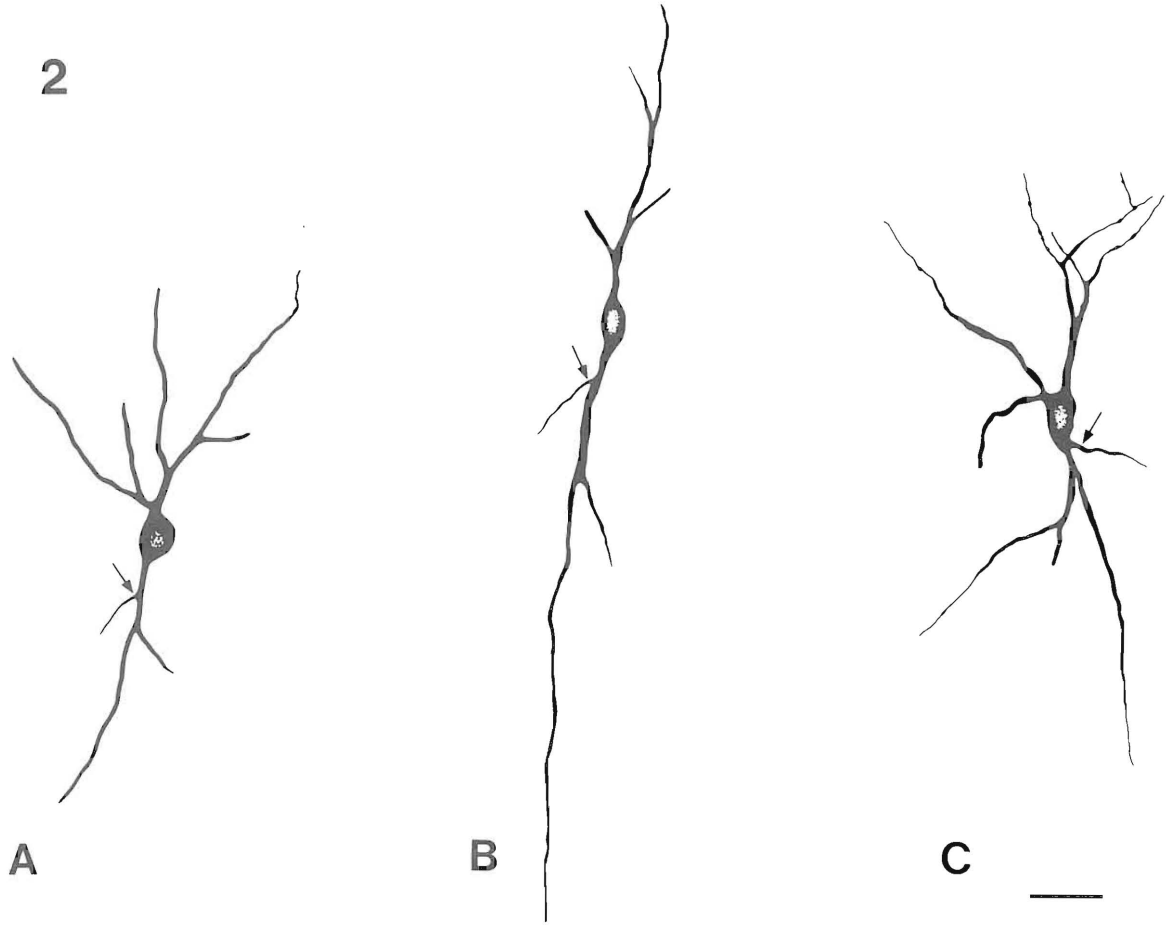


Fig. 1. Camera lucida drawing of a 80-μm-thick coronal section of colchicine-injected cat hippocampus, incubated in antiserum to CCK. The CCK-positive neurons are indicated by dots. The cells are present throughout the subfields and layers of the hippocampus. St. Gr., stratum granulosum; St. Lac. Mol., stratum lacunosum moleculare; St. Mol. Dt. Gy., stratum moleculare of the dentate gyrus; St. Or., stratum oriens; St. Pyr., stratum pyramidale; St. Rad., stratum radiatum. Scale = 100 μm.

Fig. 2. Camera lucida drawing of CCK-positive cells selected from the section illustrated in Figure 1. They represent the major types of CCK-positive neurons observed in the hippocampus. A Tufted cell in the stratum pyramidale of subfield CA1. B. Bipolar cell in the stratum radiatum of subfield CA2. C. Multipolar cell in the stratum pyramidale of subfield CA3. Arrows point to the axon initial segments identified in the light microscope. Scale = 30 μm.

target sites of identified CCK-immunoreactive neurons and nerve terminals. The following characteristics were examined: (1) CCK-immunoreactive axons originating from local neurons and forming contacts with pyramidal neurons or with CCK-positive nonpyramidal neurons; (2) CCK-immunoreactive boutons of unknown origin forming contacts on perikarya and dendrites.

We examined material from hippocampi not injected with colchicine. Although in these hippocampi the CCK-immunoreactive cells were less intensely stained, the immunoperoxidase reaction end-product was homogeneously distributed and revealed fine morphological details—in particular, extensive axonal processes. A total of 19 cells and a large number of axonal profiles were studied, all of which were in area CA1 in the cat and in areas CA1 and CA2 in the rat.

Synaptic contacts onto pyramidal neurons

Several CCK-immunoreactive cells in the stratum radiatum and oriens were found with an axon deep in the stratum pyramidale, where the beaded collaterals of the main axon ramified among the pyramidal neurons. Two CCK-positive cells, which from light microscopic examination could be seen to send their axon collaterals onto the perikarya of pyramidal neurons, were selected for further study in the electron microscope.

The CCK-labeled cell illustrated in Figure 4 was situated in the upper stratum radiatum of the cat hippocampus. It can be classified as a neuron of the bitufted variety with an ovoid-shaped perikaryon and with long and short axis of 18 and 14 μm, respectively. The axon initial segment originates from the lower pole of the cell body and, after taking a descending course, it branches into several beaded collaterals coursing in a tangential and vertical fashion through the stratum radiatum (Fig. 4B). Some collaterals reach the stratum pyramidale, then further collateralize into rows studded with varicosities, associated mostly with perikarya and proximal dendritic profiles of presumed pyramidal neurons (Fig. 4A,B).

A row of varicosities belonging to a terminal plexus of one of the axon collaterals and outlining the perikaryon of a pyramidal neuron (Fig. 4A–D) was examined in the electron microscope. Serial sections confirmed that the varicosities, four of which are shown in Figure 5A, were synaptic boutons establishing symmetrical (Gray's type II) contacts with a pyramidal neuron (Fig. 5B–D). The boutons were crowded with clear pleomorphic synaptic vesicles and had junctional contacts characterized by postsynaptic densities

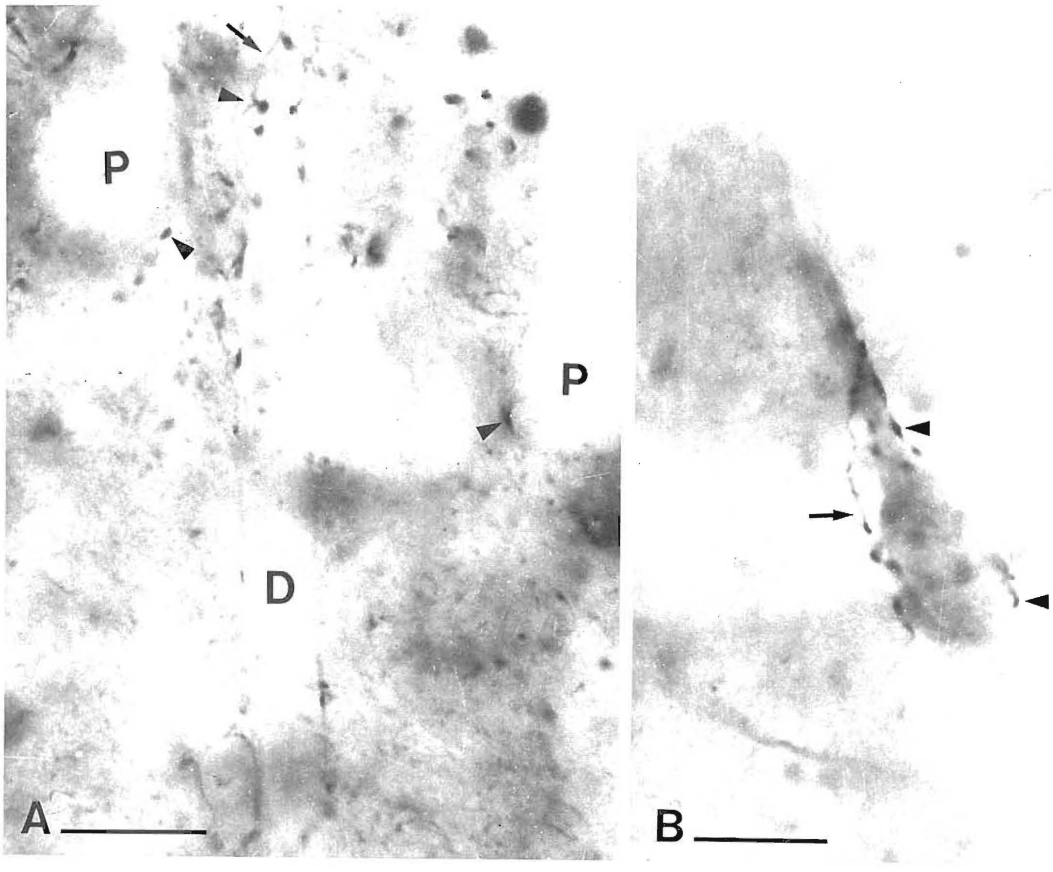


Fig. 3. Light micrographs of 80-μm-thick coronal sections of normal cat hippocampus, incubated to reveal CCK immunoreactivity. A. The micrograph shows the association of CCK-immunoreactive varicosities with different regions of pyramidal neurons. The varicosities (arrowheads) appear to be associated with the perikarya (P) and an apical dendrite (D). The varicosities are connected by a thin axon (arrow) climbing the shaft of the apical dendrite. The immunoreactive varicosities were found to make symmetrical synapses at the electron microscopic level (not illustrated). B. Micrograph showing a CCK-immunoreactive neuron of the stratum lacunosum-moleculare of cat hippocampus. The immunoreactive varicosities (arrowheads) are connected by axons (arrows) and surround the soma and dendrite of the immunoreactive neuron. Scales = 10 μm.

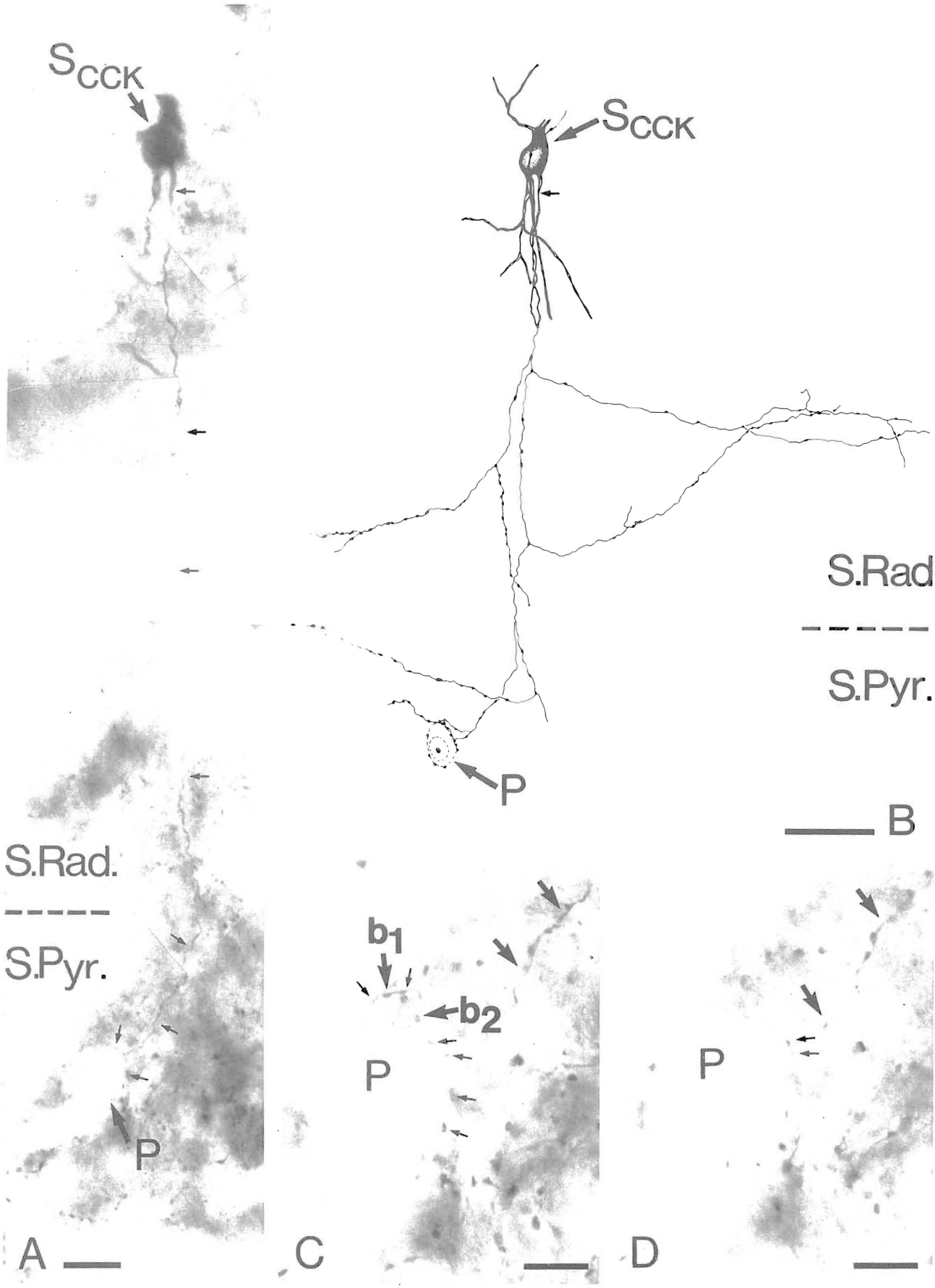


Figure 4

of almost negligible thickness. The immunoperoxidase reaction end-product within the axon terminals appears mostly concentrated upon the surfaces of synaptic vesicles and mitochondria.

In a similar case in the rat, pericellular CCK-immunoreactive nets of boutons in the stratum pyramidale of the hippocampus could be traced back to the parent axon and perikaryon of a neuron situated in the middle portion of the stratum oriens (Figs. 6, 7A). The immunoreactive neuron has a round cell body, 13 × 12 μm in size, and dendrites extending radially. The axon initial segment arises from a small hillock on the side of the perikaryon and ascends with a straight course toward the pyramidal layer. Upon entering the pyramidal layer, the axon bends into an horizontal course giving off beaded collaterals which remain confined into the pyramidal layer. Some of them further collateralize horizontally giving rise, almost at right angles, to beaded terminal segments which arborize around a population of pyramidal neurons (Figs. 6, 7A,B). The framed area in Figure 7A, containing the perisomatic branches of the axon (Figs. 6, 7B), was studied at the electron microscopic level, and 48 synaptic boutons immunoreactive for CCK were identified. The great majority of the synapses (75%) were upon the soma of pyramidal neurons and the remaining ones were upon the shaft of the proximal portions of basal dendrites (Fig. 7C). Synaptic junctions were symmetric with minute synaptic specializations (Fig. 7D). The number of immunoreactive boutons forming synapses correlates well with the number of varicosities seen at the light microscopic level. In many sections, a rather large proportion of the terminals making synapses with the somata of pyramidal neurons were found to be CCK-positive. However, in some sections it was possible to identify unlabeled synaptic terminals.

Thus, CCK-immunoreactive neurons from the rat and from the cat and which are located in different layers of the hippocampus have a similar pattern of ramification of their terminal axonal branches and similar postsynaptic targets (Figs. 4B, 6).

CCK-immunoreactive boutons of an identified cell provide synapses to a pyramidal cell apical dendrite

Small-caliber CCK-labeled processes, encrusted by irregular shaped protrusions, have been observed several times crossing, in an almost vertical direction, the width of the stratum radiatum and lacunosum-moleculare. Two of them could be traced back, in one case, to a CCK-immunoreactive multipolar neuron of the stratum lacunosum-moleculare and, in the other case, to an immunoreactive neuron located at the bottom of the stratum radiatum. The latter neuron displays an unusual tadpolelike perikaryon, being characterized by a stout apical dendrite originating at the upper surface of the cell body and spanning more than 100 μm of the width of the stratum radiatum (Fig. 8A,B). The small-caliber process, studded by coarse swellings, bulging at rather regular intervals from the main shaft, arises directly from the perikaryon, at the opposite point of the site of origin of another process (Fig. 8B). This latter process ramifies into thin beaded collaterals, one of which projects into the pyramidal layer. The ascending process extends through the stratum radiatum with an almost straight course similar to the trajectory of the main apical dendrite, and has diameters varying between 0.25 and 0.4 μm. At the ultrastructural level, the shaft of the process swells into ovoidal or elliptical enlargements (Fig. 9A), which correspond to the protrusions observed in the light microscope. The protrusions are characterized by large aggregates of clear vesicles, of 32–50 nm in diameter, mostly accumulated at membrane thickenings that were found to be junctional sites of the symmetric type (Fig. 9A–C). The synaptic vesicles are often found in association with both immunoreactive and nonreactive large granulated vesicles ranging from 80 to 120 nm in size. The main shaft of the process contains arrays of tightly packed microtubules thickened by the precipitate of the immunoperoxidase reaction end-product and, among them, fairly dispersed clear vesicles as well as immunoreactive and nonimmunoreactive granulated vesicles.

The postsynaptic profiles were unstained structures, mostly viewed in cross and oblique orientation, ranging from 0.6 to 1.8 μm in diameter and containing neurofilaments and occasional cisternae of smooth endoplasmic reticulum (Fig. 9A–C). Although we could not relate the majority of them to any identifiable element, one postsynaptic profile could be cytologically classified as a pyramidal cell apical dendrite measuring 1.4 μm in diameter (Fig. 9B). Serial sections showed that the dilatations of the immunostained shaft, apposed to the dendrite, contained a few large granulated vesicles interspersed among tightly packed clear pleomorphic vesicles (Fig. 9B,D). The immunostained structure was considered presynaptic because of the large aggregate of synaptic vesicles and the presence of electron-dense material at the inner surface of the membrane apposed to the dendritic shaft. Since the swellings are quite separate from each other, and the postsynaptic profiles display different diameters, it is likely that the immunoreactive process made synapses with different dendrites, or with collateral branches of the same dendrite. Because the presynaptic process was not found to be postsynaptic to any structure along its entire length, and its varicosities contained synaptic vesicles, it was concluded that it represented the proximal axon of the neuron.

Synaptic connections between CCK-immunoreactive cells

A synaptic relationship between CCK-immunoreactive cells was observed in the stratum laconosum-moleculare and upper stratum radiatum of both cat and rat hippocampus.

As shown in Figure 10, the axon of a multipolar, pyramid-shaped, CCK-positive neuron, 16 × 10 μm in size, arises from the proximal portion of a basal dendrite, ascends in a oblique direction, and then descends toward the soma of a lightly labeled CCK-positive cell exhibiting a rounded cell body of 10-μm size (Fig. 10). The axon (0.4 μm in caliber)

Fig. 4. A. Low-power micrograph of an immunoreactive cell (S_{CCK}) in the cat hippocampus (CA1) at the top of the stratum radiatum, with its main axon (small arrows) descending into the stratum pyramidale. The collaterals of the axon surround the perikaryon of an unstained pyramidal cell (P). The camera lucida drawing of the same cell (S_{CCK}) is shown in B. C,D. High-power light micrographs at different focal planes of the pyramidal neuron perikaryon (P) that is also shown in A and B. The perikaryon is surrounded by varicosities of the axon originating from S_{CCK}. Large arrows indicate the axon collaterals approaching the perikaryon (P), and small arrows label the varicosities surrounding the soma (P). The boutons on C, labeled by b_1 and b_2, are also shown at the electron microscopic level in Figure 5A–C. The two varicosities labeled by small arrows on D, are also shown in 5D. Scales: A = 20 μm; B = 50 μm; C,D = 10 μm.

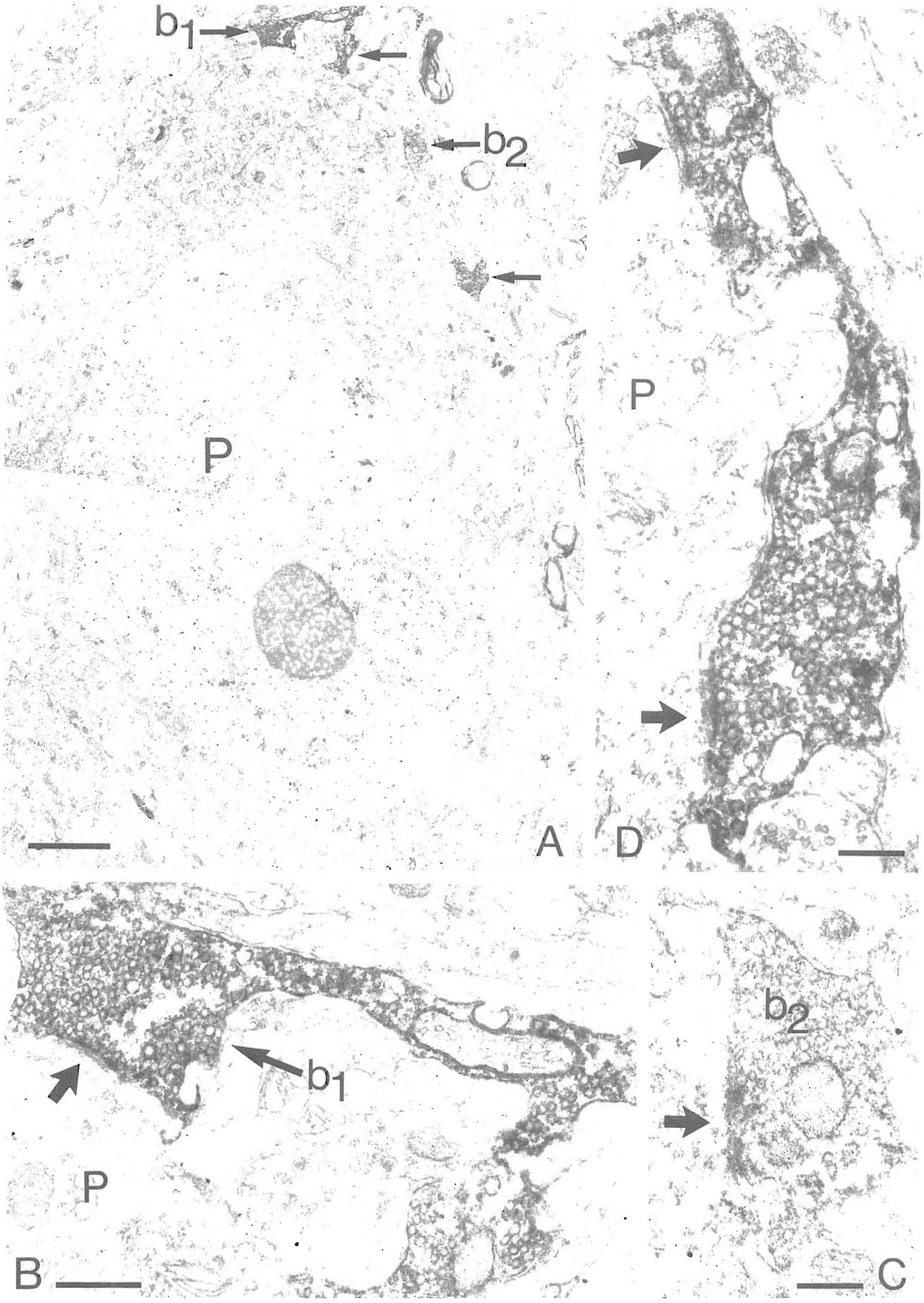


Figure 5

enlarges into small boutons "en passant" while passing along the surface of the CCK-positive cell body and then continues its descending course toward the lower portion of the stratum lacunosum-moleculare. The immunostaining of the axon ceased abruptly in the middle of the section, perhaps due to myelination.

Correlated ultrastructural analysis carried out through the cell body of the CCK-immunoreactive target cell shows that all the immunostained "en passant" boutons identified in the light microscope (Fig. 11A) are vesicle-filled axon terminals forming symmetric-type synapses with the soma of the CCK-positive cell (Fig. 11C,D). The postsynaptic neuron displays an irregular contour and is characterized by an ovoidal, indented nucleus, almost as large as the cell body, and by a thin layer of cytoplasm (Fig. 11B). The neuron was examined for axosomatic synapses using serial sections taken through the region of the cell body. Synaptic boutons were rare and nonimmunoreactive and exhibited either symmetrical or asymmetrical membrane thickenings at the junctional sites (Fig. 11E). The immunoreactive "efferent" neuron received several CCK-immunoreactive boutons on the shaft of its apical and basal dendrites and on the soma. The axon gave off thin collaterals ascending toward the upper stratum moleculare and several short side branches extended almost at right angles into the surrounding tissue (Fig. 11A). The branch we examined formed a symmetrical synaptic contact with a dendrite (Fig. 11F), possibly derived from a nonpyramidal cell, since the observed large mitochondria and multivesicular bodies are very often associated with dendrites and perikarya of nonpyramidal neurons.

The axoplasm of the main axon contained both clear vesicles, of different size and shape, and large immunoreactive, granulated vesicles, measuring 110 $\pm$ 25 nm in diameter, interspersed among aggregates of the fine granular material of the immunoperoxidase reaction end-product.

The same pattern of axonal projection has been observed at light microscopic level between two CCK-immunoreactive neurons located in the hilar region of cat hippocampus. The afferent cell was of the bitufted variety and its main axon, after a short loop, appeared to contact the cell body of the target immunoreactive neuron by means of two boutons"en passant."

CCK-immunoreactive cells postsynaptic to CCK-immunoreactive boutons

As pointed out in the first section of the Results, a large number of CCK-positive cells were found to be associated with CCK-immunoreactive boutons impinging onto perikarya and dendritic shafts. Three light microscopically identified CCK-positive neurons receiving CCK-immunoreactive boutons were examined at the electron microscopic level in order to study their ultrastructural features and synaptic connections (Figs. 12–14).

The cell illustrated in Figure 12 was located in the upper stratum radiatum of the cat hippocampus and could be classified as a bitufted neuron (Fig. 12A). The perikaryon of the cell was contacted by several CCK-immunoreactive boutons interconnected by a thin fiber. The neuron contained a large ovoid nucleus and an extensive parallel array of cisternae of rough endoplasmic reticulum, mostly accumulated at the two opposite cytoplasmic poles of the cell body (Fig. 12B). The nucleus, whose envelope is deeply infolded, displays homogeneously dispersed chromatin and a long filamentous rod approaching at one extremity the prominent nucleolus. The intranuclear rods have been found only in a small population of CCK-immunoreactive cells. The immunoperoxidase reaction end-product appears as a uniform, coarse precipitate onto the surface of cytoplasmic organelles. The CCK-immunoreactive boutons observed at the light microscope were found to make symmetrical synaptic contacts with the perikaryon. One of these boutons is shown at both light and ultrastructural level (Fig. 12A,E) and has the synaptic junction characterized by small postsynaptic densities and prominent presynaptic dense projections (Fig. 12H). The axoplasm inside the bouton is filled with clear synaptic vesicles encircled by a heavy deposit of the immunoperoxidase precipitate and contains both immunoreactive and nonreactive large granulated vesicles (Fig. 12F,G). Large granulated vesicles of both types were also found scattered in the perikaryon and proximal dendrites of the neuron.

Numerous nonreactive axon terminals, displaying symmetrical and asymmetrical synapses, were identified upon the surface of the soma (Fig. 12C,D). The terminals that exhibited asymmetrical membrane thickening at the junctional site were usually large in size and contained many clear rounded vesicles (Fig. 12D).

The cytoplasmic features reported for this neuron were also shared by the other CCK-immunoreactive cells examined at the ultrastructural level. However, the presence of numerous synaptic boutons, most of them forming asymmetric synaptic contacts (Fig. 12C,D) on the perikaryon, is a rare feature observed in a few CCK-positive cells only.

CCK-immunoreactive fibers were also observed making symmetrical synapses with the proximal portion of dendrites belonging to bitufted CCK-immunoreactive cells of the stratum lacunosum-moleculare (Figs. 13, 14). In addition to making synapses with immunoreactive dendrites, one fiber established symmetrical synaptic contacts with two nonreactive profiles (Fig. 13A,B). The latter recipient structures, on the basis of their dimension (1.7–1.6 μm in diameter) and cytological features (i.e., microtubules and small mitochondria), are very likely branches of apical dendrites of pyramidal neurons (Fig. 13C,D). Unlike the perikarya, the dendritic region of CCK-immunoreactive cells receives a substantial number of synaptic contacts from unreactive axon terminals, forming mostly asymmetrical synapses.

The CCK-positive synaptic boutons on the basal dendrite of the CCK-positive cell shown in Figure 14 were linked by a thin axonal profile to a plexus of interconnected, strongly immunoreactive varicosities flattened against the cell body and the main dendrite of the immunoreactive cell (Fig. 14A,B). Unfortunately, the varicosities were sectioned in a plane grazing the synaptic sites, thus making the analysis of the junction difficult, but at least one bouton formed a

Fig. 5. A correlated electron micrographs of the pyramidal cell (P) and CCK-positive boutons (arrows) shown at light microscopic level in Figure 4A. Low-power electron micrograph which serves as a guide for correlation to Figure 5C. Two boutons b_1 and b_2 (also labeled in Fig. 4C) are shown at higher magnification in B and C. The two immunoreactive boutons (b_1 and b_2) belonging to an identified CCK-positive cell of the stratum radiatum establish symmetrical synaptic contacts (large arrows) with the soma of the pyramidal cell (P). The immunoperoxidase reaction end-product fills the axoplasm of the stained terminals and is also present around synaptic vesicles and mictochondria. D. Two other boutons derived from the CCK-positive cell (labeled by small arrows in Fig. 4D) make symmetrical synaptic contacts (arrows) on the same pyramidal perikaryon (P). Scales: A = 2 μm; B = 0.5 μm; C,D = 0.25 μm.

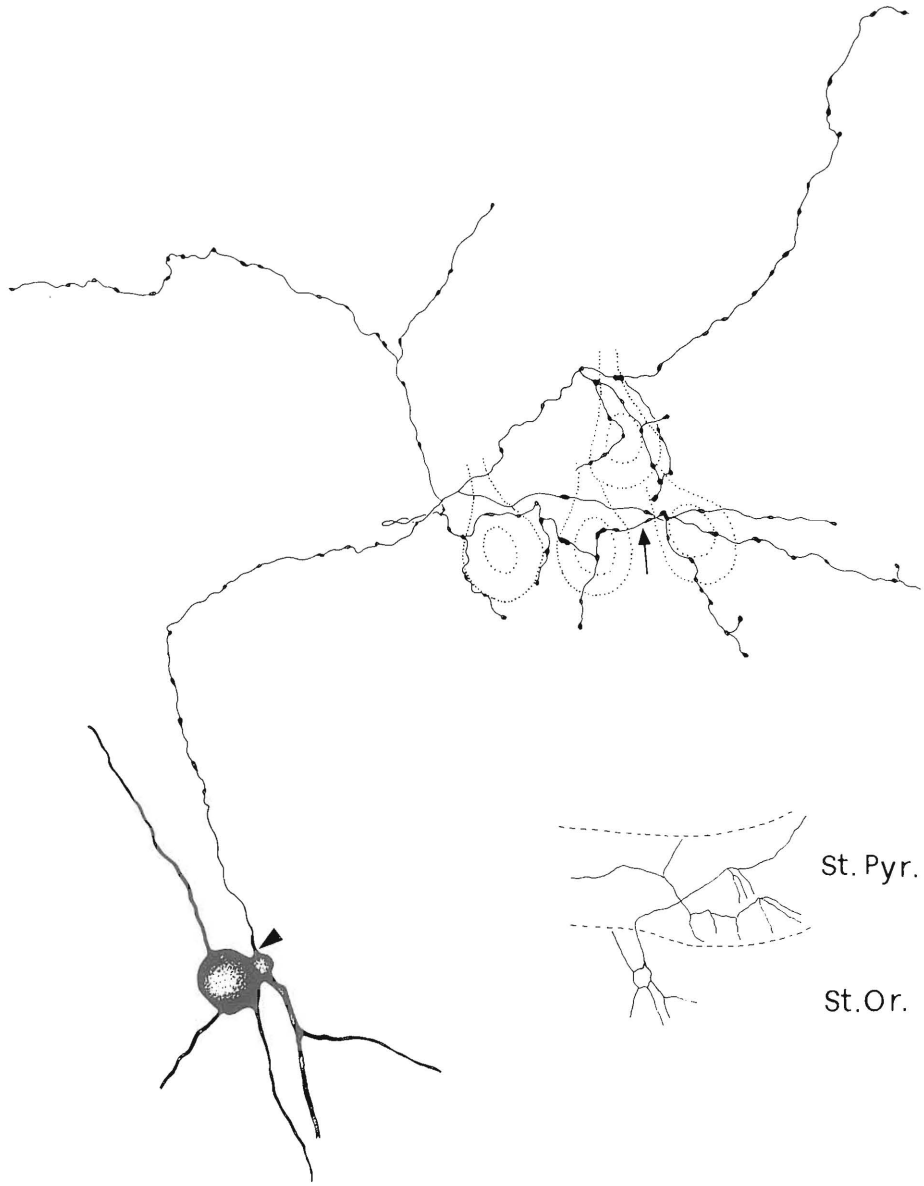


Fig. 6. Camera lucida drawing of a CCK-immunoreactive neuron located in the middle portion of the stratum oriens (St. Or.) of area CA1 of the rat hippocampus. The axon initial segment originates from a small hillock of the multipolar cell body (arrowhead) and the axon ascends through the stratum oriens. Upon entering the pyramidal layer, the axon takes an horizontal course giving off collaterals which then give rise, at almost right angles, to terminal branches. The arborization pattern of the axon through the stratum pyramidale (St. Pyr.) is indicated in the schematic drawing. The terminal branches form pericellular nets of boutons, around the perikarya of pyramidal neurons (dotted profiles). The arrow indicates the immunoreactive process shown in Figure 7B.

symmetrical synaptic contact (Fig. 14C). These findings further demonstrate the convergence of multiple synaptic interaction among CCK-immunostained neurons. Finally, a constant finding was a close association of one or two glial cells with perikarya and proximal dendrites of CCK-positive neurons (Fig. 14A).

DISCUSSION

We have provided direct evidence that CCK-immunoreactive cells are involved in intrahippocampal connections with different types of neurons and that they have various patterns of axonal and synaptic morphology. Our main findings can be summarized as follows: (1) CCK-immunoreactive neurons are morphologically heterogeneous, with a predominance of three major kinds of neurons: tufted, bipolar, and multipolar. (2) All CCK-immunoreactive boutons form symmetrical synaptic contacts, very similar to those formed by GAD-immunoreactive boutons. (3) Some CCK-immunoreactive boutons of the above type were shown to originate from local neurons.

Previous accounts on CCK immunoreactivity in cortical areas have pointed out that all CCK-positive cells are non pyramidal, raising the possibility that differential distribution and varying morphology among CCK-containing

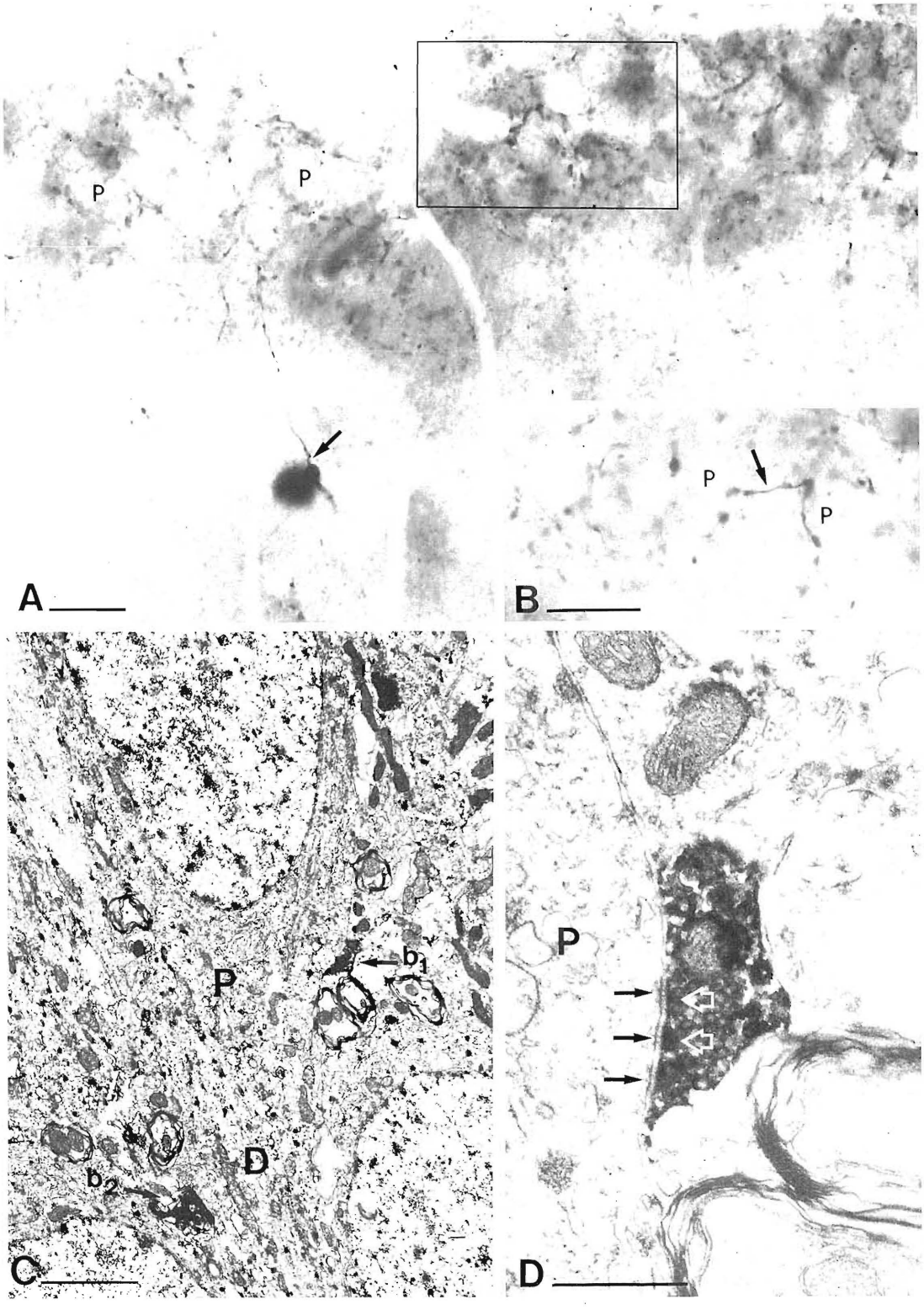


Fig. 7. A. Light micrograph of a 80-μm-thick section of rat hippocampus showing the CCK-immunoreactive cell body, axon initial segment (arrow), and proximal portion of the axon of the neuron illustrated in figure 6. The dark punctate structures around the cell bodies of pyramidal neurons (P) represent CCK-immunoreactive varicosities. The framed area encloses the pyramidal neurons contacted by the terminal branches of the identified axon. B. High-magnification of the area framed in A, showing, at a different focal depth, the profiles of two pyramidal neurons (P) associated with the axonal plexus of the CCK-immunoreactive neuron (arrow). C. Low-power electron micrograph of one of the two latter pyramidal neurons showing the perikaryon (P) and basal dendrite (D) contacted by two CCK-immunoreactive boutons (b_1, b_2). D. High-power electron micrograph of the bouton b_1, shown in C, in a serial section. The bouton forms symmetrical synaptic contacts (arrows) with the perikaryon (P). Note the prominent presynaptic dense projections (open arrows). Scales: A = 20 μm; B = 10 μm; C = 1.5 μm; D = 0.5 μm.

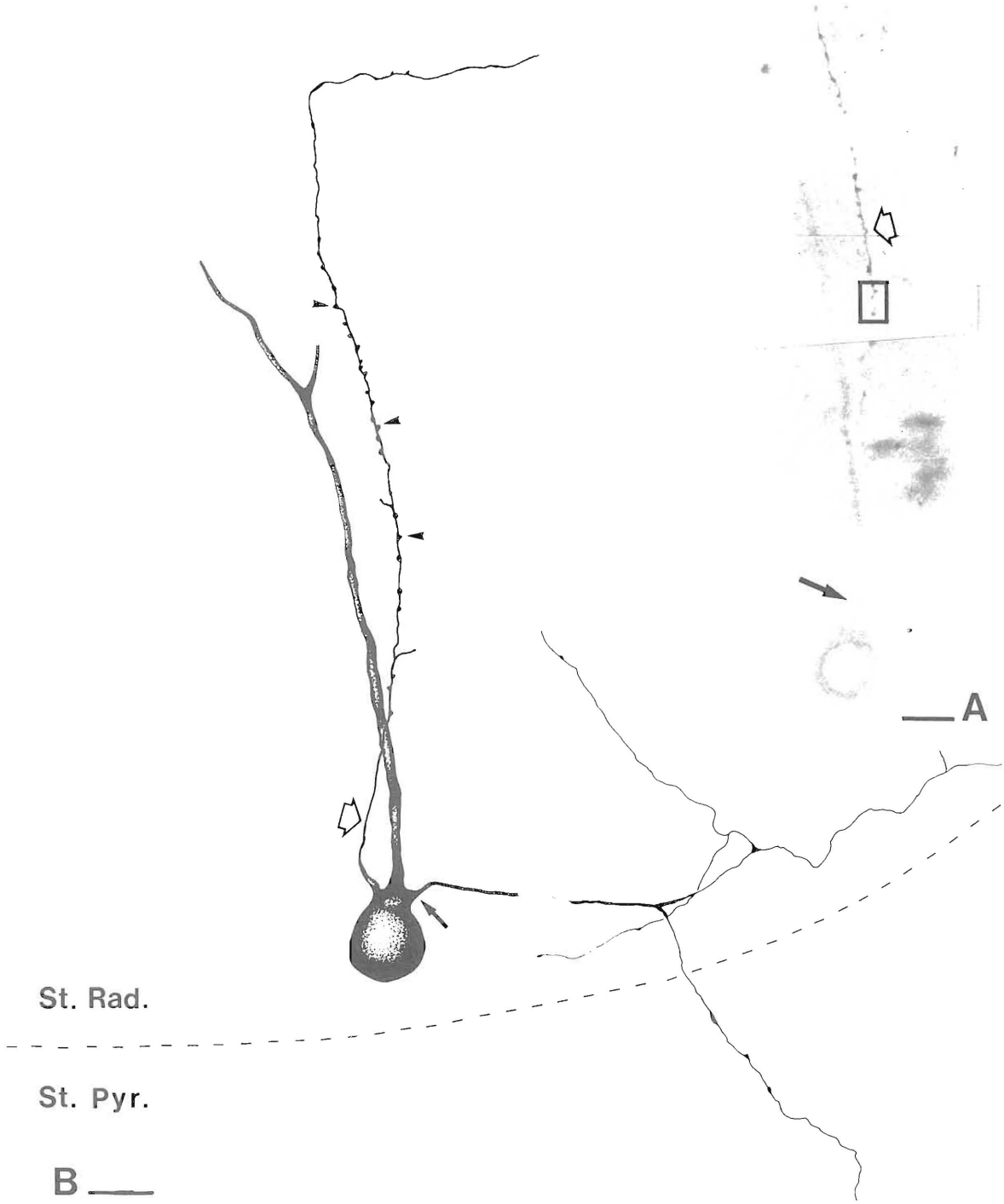


Fig. 8. A. Photomontage of a CCK-immunoreactive neuron situated in the lower portion of the stratum radiatum of the cat hippocampus in area CA2. A thick dendrite (arrow) arises from the upper surface of the ovoidal shaped cell body. A thinner process (open arrow) endowed with strongly immunoreactive protrusions and shown in Figure 9 to be an axon is also evident. The framed area is correlated at the electron microscope level in Figure 9A. B. Camera lucida drawing of the neuron in Figure 8A. The axon initial segment is indicated (open arrow); arrowheads mark axonal varicosities in stratum radiatum (St. Rad.). Another thin branching process (arrow) penetrates the stratum pyramidale (St. Pyr.). Scales = 10 μm.

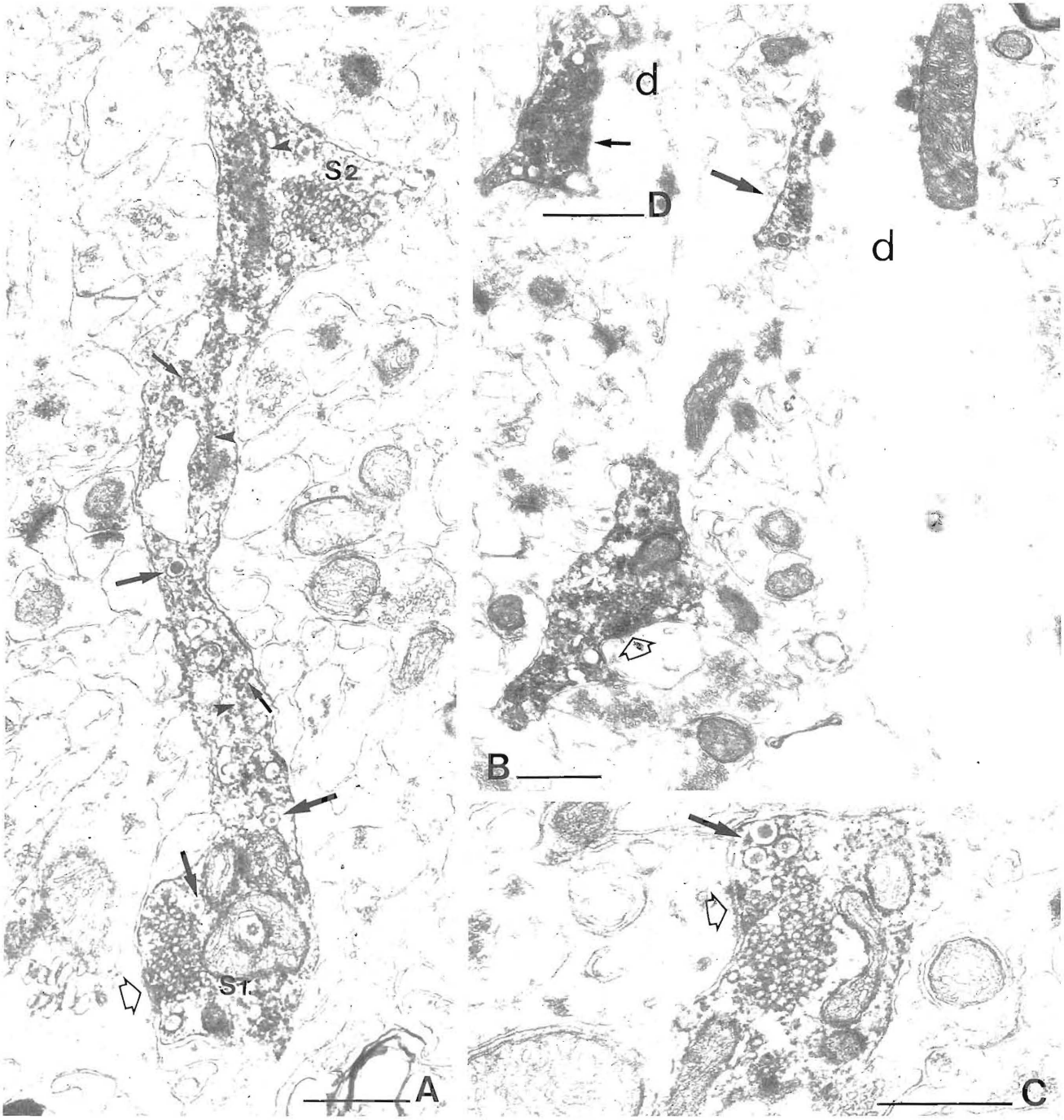


Fig. 9. Electron micrographs illustrating the synaptic connections established by the process indicated by the open arrow in Figure 8A, B. A. Micrograph illustrating the framed area in Figure 8A. A portion of the main shaft of the CCK-positive process enlarges in two swellings (s_1 and s_2) containing a large number of small pleomorphic clear vesicles. One swelling (s_1) is in symmetrical synaptic contact (open arrow) with an unreactive irregular profile. The main shaft of the process contains clear vesicles (small arrows) and large granulated vesicles (large arrows). Arrowheads indicate immunoperoxidase reaction end-product associated with fascicles of neurotubules. B. Swelling (asterisk) belonging to the same axon is seen making a synaptic contact of symmetric type (open arrow) with a small profile. The vesicle containing immunostained structure, indicated by the arrow and apposed to the apical dendrite (d) of a pyramidal cell, was found to be presynaptic to the dendrite (d) in serial sections (D). C. High-power electron micrograph showing an immunoreactive enlargement which forms a symmetric synaptic contact (open arrow). The varicosity contains clear synaptic vesicles and a group of large granulated vesicles (arrow). Arrowheads indicate the presynaptic dense projections. Scales = 0.5 μm.

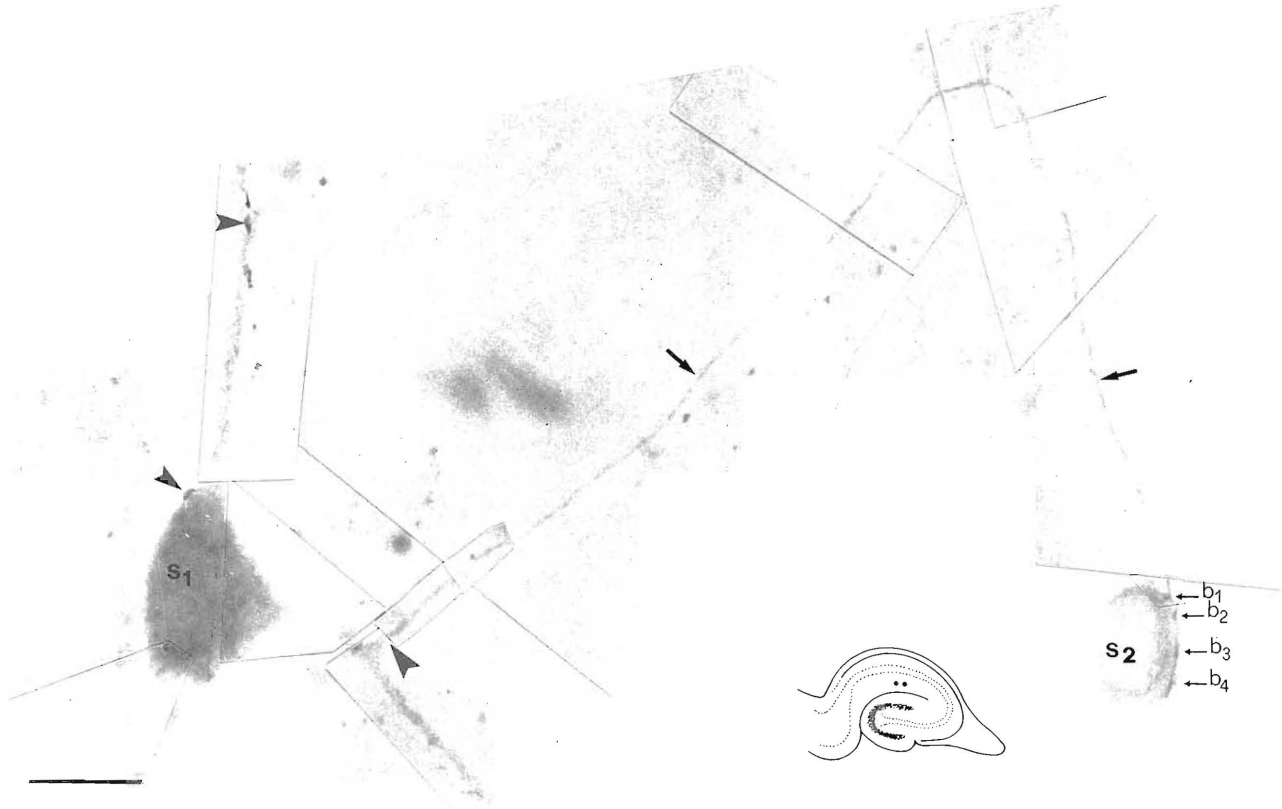


Fig. 10. Light micrograph of a 80-μm-thick section showing CCK-immunoreactive neurons (s_1 and s_2) connected by the axon (arrows) of a multipolar CCK-immunoreactive cell (s_1) in area CA2 of the rat hippocampus. The origin of the axon is indicated by the large arrowhead. Four boutons "en passant" of the multipolar cell axon are apposed to the soma of the target cell (b_1, b_2, b_3, b_4). CCK-immunoreactive varicosities appear also in contact with the soma and dendritic shaft of the afferent neuron (small arrowheads). The diagram indicates the positions of the two CCK-positive neurons (dots) in the upper stratum lacunosum-moleculare of the rat hippocampus. Scale = 10 μm.

cells may reflect differences in postsynaptic targets as well as in functions (Köhler and Chan-Palay, '83; Peters et al., '83). In particular, it has been suggested that in the neocortex the multipolar and bitufted CCK-immunoreactive cells have an inhibitory function (Peters et al., '83), by analogy with the same type of neurons studied in Golgi-electron microscopic studies in the rat visual cortex (Peters and Fairén, '78; Peters and Proskauer, '80). Some cortical multipolar and bitufted neurons have been shown to give rise to axon terminals with symmetrical membrane specializations, some of which, as shown by Ribak ('78) in the visual cortex, are similar to axon terminals immunoreactive for glutamate decarboxylase (GAD).

Our findings confirm previous reports on the structure of CCK neurons (Greenwood et al., '81; Peters et al., '83). Thus, ultrastructural analysis has shown that axon terminals either arising from multipolar and tufted CCK-positive cells or from unknown origin contain pleomorphic vesicles and make symmetrical synaptic contacts irrespective of the postsynaptic target. The synaptic contacts are associated with perikarya and dendrites of pyramidal and nonpyramidal neurons, as already reported for monkey and rat neocortex (Hendry et al., '83a). Unfortunately, we could not trace axons from bipolar CCK-immunoreactive neurons in spite of their abundance in all hippocampal layers. Some of the bipolar neurons found in the visual cortex were shown to form asymmetrical, possibly excitatory, synapses with somata and dendrites of nonpyramidal cells (Peters and Kimerer, '81). The most likely explanation, as already suggested by Hendry et al. ('83a), is that two or more populations of neurons with bipolar morphology may exist, since our findings indicate that the axonal boutons contain-

Fig. 11. A–D. Micrographs illustrating the synaptic connections of the target CCK-positive neuron shown in Figure 10. A. High-power light micrograph showing the CCK-immunoreactive perikaryon (S_2), the descending portion of the axon (arrow), and the associated boutons (small arrows). The framed area encloses a portion of the main axon, which gives rise to a short side branch (arrowhead). The axon, perikaryon, and boutons b_1 and b_4 are seen at the ultrastructural level in B–C and D. F. The low-power electron micrograph illustrates the perikaryon (S_2) of the CCK-immunoreactive cell, almost filled by the ovoidal nucleus. The boundary of the perikaryon is underlined by dots. The cell is approached by the immunoreactive axon (arrow) of the multipolar neuron. Upon reaching the cell body it swells into a bouton (b_1). C,D. High-power electron micrographs of boutons b_1 and b_4. The boutons contain clear pleomorphic synaptic vesicles and make symmetrical-type synapses with the membrane of the CCK-immunoreactive cell body. E. Nonimmunoreactive axon terminals (arrows) containing pleomorphic vesicles are in synaptic contact with the same cell. One of the axon terminals has an asymmetrical thickening at the junctional site (arrowhead). F. The framed area shown in A is shown at the electron microscopic level. The side branch of the main axon (large arrowhead) makes a symmetrical synapse with a small nonimmunoreactive profile (small arrowhead) containing a large mitochondria and a multivesicular body. The axoplasm contains clear nonreactive vesicles of different size and shape (open arrows) and occasional granulated immunoreactive vesicles (arrow). Scales: A = 5 μm; B = 2 μm; C–F = 0.5 μm.

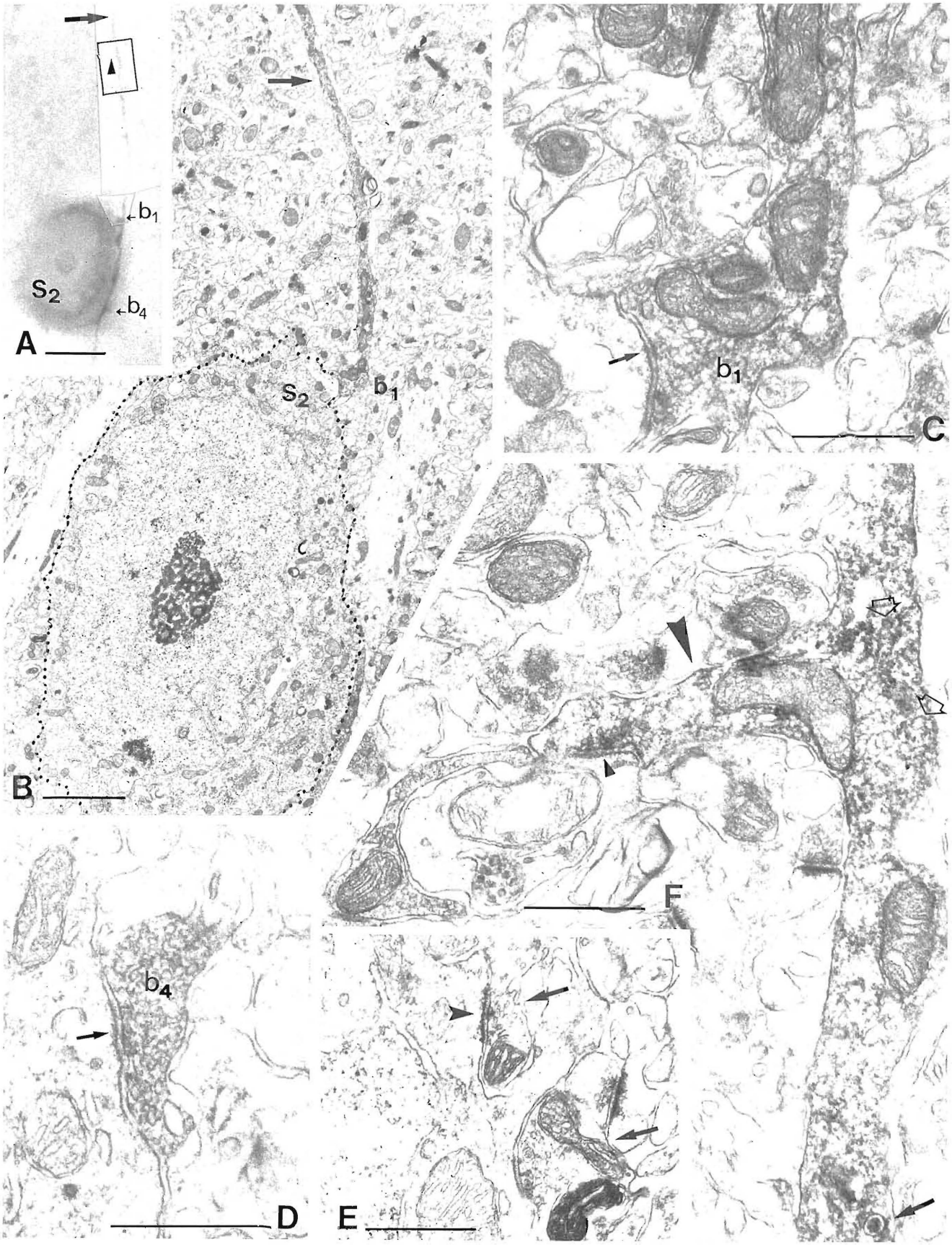


Figure 11

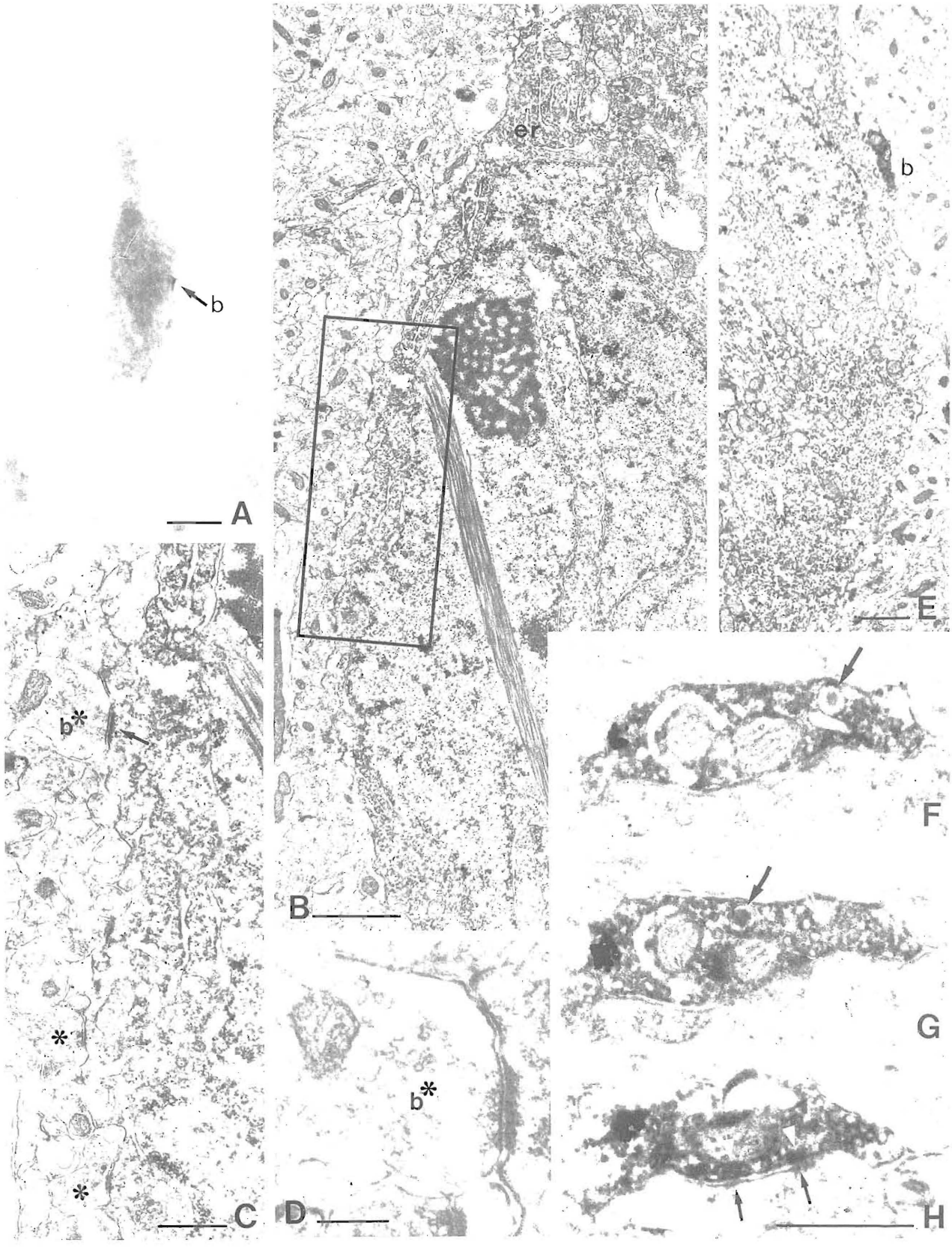


Figure 12

ing CCK immunoreactivity always formed symmetrical snyapses in the hippocampus.

CCK-immunoreactive boutons in synaptic contact with pyramidal neurons

By comparing the somatic and dendritic features of CCK-positive cells with those of neurons described in Golgi studies (Ramón y Cajal, '68; Lorente de Nó, '34; Amaral, '78), Greenwood et al. ('81) suggested that CCK-positive cells could form part of the population of local-circuit neurons in the hippocampus. In particular, they speculated that CCK-immunoreactive cells of the stratum oriens might provide the dense CCK-immunoreactive innervation around the perikarya of pyramidal neurons, thus resembling basket cells, whose terminal axonal arborization has been considered as the source of the powerful inhibitory postsynaptic potentials recorded in pyramidal neurons (Andersen et al., '64a,b).

Our results provide direct evidence that at least some of the CCK-positive axon terminals on the perikarya of pyramidal neurons arise from neurons situated within the layers bordering the stratum pyramidale. The bitufted, ovoid, CCK-positive neuron of the stratum radiatum, and the rounded, multipolar, CCK-positive neuron of the stratum oriens had axonal branches forming pericellular nets of densely packed synaptic boutons surrounding the somata of pyramidal neurons. In particular, the multipolar cell strikingly resembles the classical basket cell defined in Golgi studies for its topographical location and horizontally expanding axonal arborization (Ramón y Cajal, '68; Lorente de Nó, '34).

The origin of numerous synaptic contacts on pyramidal cell bodies from nonpyramidal neurons of the stratum oriens has been recently substantiated by intracellular studies. HRP-filled aspiny neurons in the stratum oriens of the guinea pig hippocampus have electrophysiological characteristics of inhibitory interneurons (Schwartzkroin and Mathers, '78) and their axonal terminals form symmetrical synaptic contacts mostly with perikarya and also with apical and basal dendrites of pyramidal neurons (Schwartzkroin and Kunkel, personal communication).

The somatic region of pyramidal neurons is known to receive exclusively symmetric synapses (Jones and Powell, '70; Sloper, '73), and in the hippocampus it has been considered to be the target site for some of the inhibitory input to pyramidal cells from interneurons (Kandel et al., '61; Spencer and Kandel, '61). Physiological and pharmacological studies indicate that inhibitory interneurons in the hippocampus use GABA as neurotransmitter (Biscoe and Straughan, '66; Curtis et al., '70). This observation is further supported by the immunocytochemical localization of GAD-positive neurons and boutons in all hippocampal layers (Barber and Saito, '76; Ribak et al., '78; Somogyi et al., '83b). In particular, most, if not all, of the axon terminals forming pericellular plexuses around the perikarya of pyramidal neurons have been shown to contain GAD (Somogyi et al., '83b). Thus, either there are two classes of pyramidal neuron, one receiving somatic input exclusively from GAD-immunoreactive boutons and the other from CCK-immunoreactive boutons, or some of the GAD-immunoreactive boutons might also contain CCK-immunoreactive material. The latter view is supported by a postembedding immunocytochemical study which indicates that in the hippocampus a small population of GABAergic neurons contain neuroactive peptides such as CCK or somatostatin (Somogyi et al., '84). In particular, CCK-immunoreactive material could be demonstrated in some small- to medium-size GABAergic cells located in all layers of the hippocampal formation (Somogyi et al., '84).

Many unlabeled synaptic terminals were also found among CCK-positive boutons making synaptic contacts on pyramidal cell perikarya. This may indicate that only some of the GABAergic interneurons contain CCK; conceivably the others might contain peptides such as VIP and somatostatin (Siggins et al., '82; Petrusz et al., '77). Therefore, the presence of CCK may define a subgroup of interneurons within a wider population of hippocampal GABAerigc interneurons responsible for the somatic inhibition. In the neocortex (Peters et al., '82; Houser et al., '83; Freund et al., '83; Hendry et al., '83b) and in the hippocampus (Somogyi et al., '83b) pyramidal neurons receive axon terminals from several classes of GABAergic interneurons. Thus, recently, it has been demonstrated with Golgi impregnation and GAD immunocytochemistry that the axon initial segment of pyramidal neurons in the monkey and cat hippocampus receives a prominent GAD-positive input from axo-axonic (chandelier) cells (Somogyi et al., '83a,b). However, we have never observed, in the present study, CCK-immunoreactive synaptic boutons forming structures similar to the tubelike GAD-positive structures that surround the axon initial segments of pyramidal neurons and which are characteristic of the axon terminal segments of axo-axonic cells (Freund et al., '83; Somogyi et al., '83b). In conclusion, it is apparent that different regions, i.e., soma and axon initial segments of pyramidal neurons, receive GABAergic inputs from at least two distinct cell types that differ from each other not only in their efferent connections but also in their peptide content.

CCK-immunoreactive synapses on non-pyramidal neurons

We have found direct evidence of the existence of local synaptic interactions established by the axons of a CCK-immunoreactive neuron with the perikarya of nonpyramidal cells, which may themselves either be immunoreactive or not for CCK (Figs. 10, 11). These connections are indicative of the target heterogeneity of CCK-immunoreactive neurons. Such a heterogenity has also been described for GAD-immunoreactive neurons in the neocortex (Houser et al., '83); Somogyi et al., '83a) and in the hippocampus (our unpublished observations).

In addition to a pattern of termination consisting of occasional CCK-positive boutons in synaptic contact with indi-

Fig. 12. A. High-power light micrograph of the cell body of a bitufted CCK-positive cell rich in endoplasmic reticulum (er) and situated in the stratum radiatum of the cat hippocampus (CA1). One of the CCK-immunoreactive boutons contacting the soma is visible (b). The cell body of the neuron is illustrated at the electron microscope level in B. The large indented nucleus is crossed by a filamentous rod which approaches at one extremity the prominent nucleolus. The framed area in B is shown at a higher magnification in C. Several axon terminals (asterisks) are closely apposed to the surface of the CCK-positive soma. One bouton (b*) establishing an asymmetrical synaptic contact (arrow) is shown at high power in a serial section in D. The bouton contains clear synaptic vesicles and both immunoreactive and nonreactive large granulated vesicles (F, G, arrows). H. The bouton makes focal symmetrical synaptic contacts (arrows) and also has prominent presynaptic projections (arrowheads). Scales: A = 5 μm; B = 2 μm; C = 0.5 μm; D = 0.25 μm; E = 1 μm; F–H = 0.5 μm.

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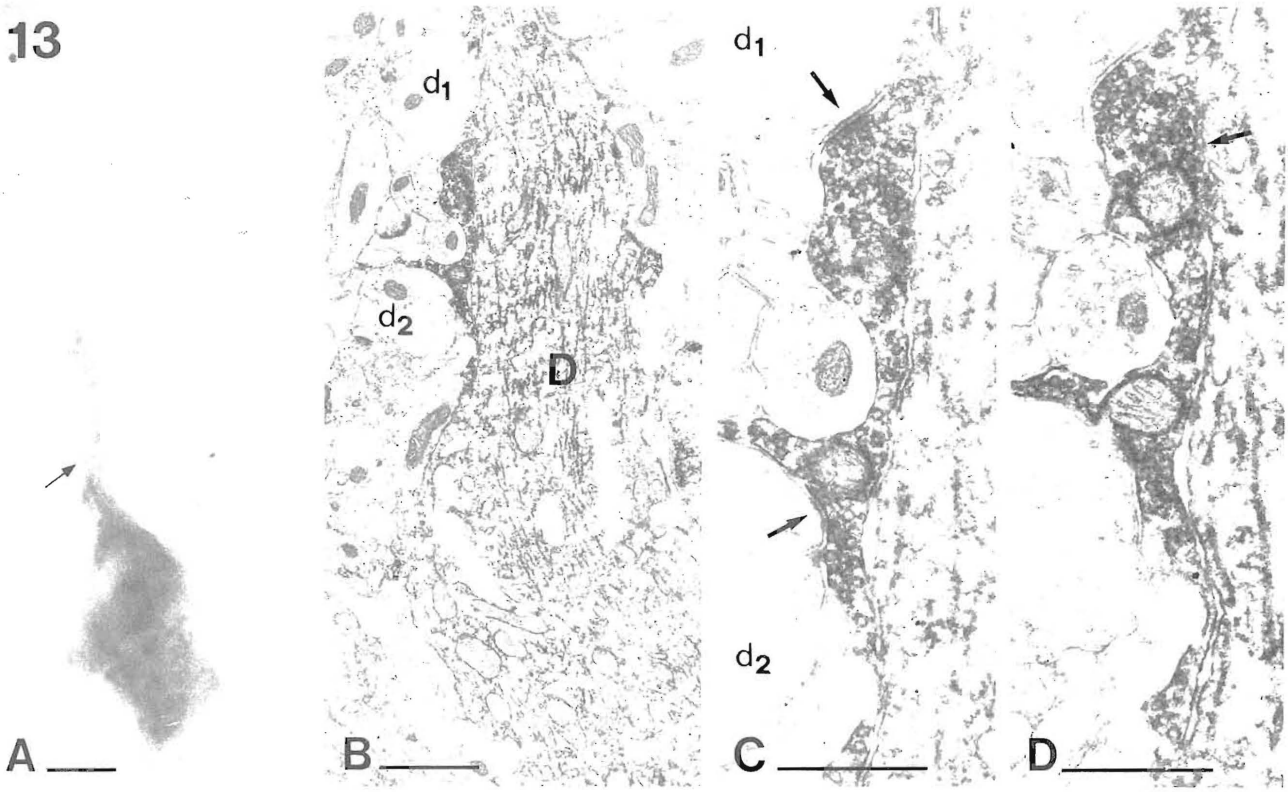

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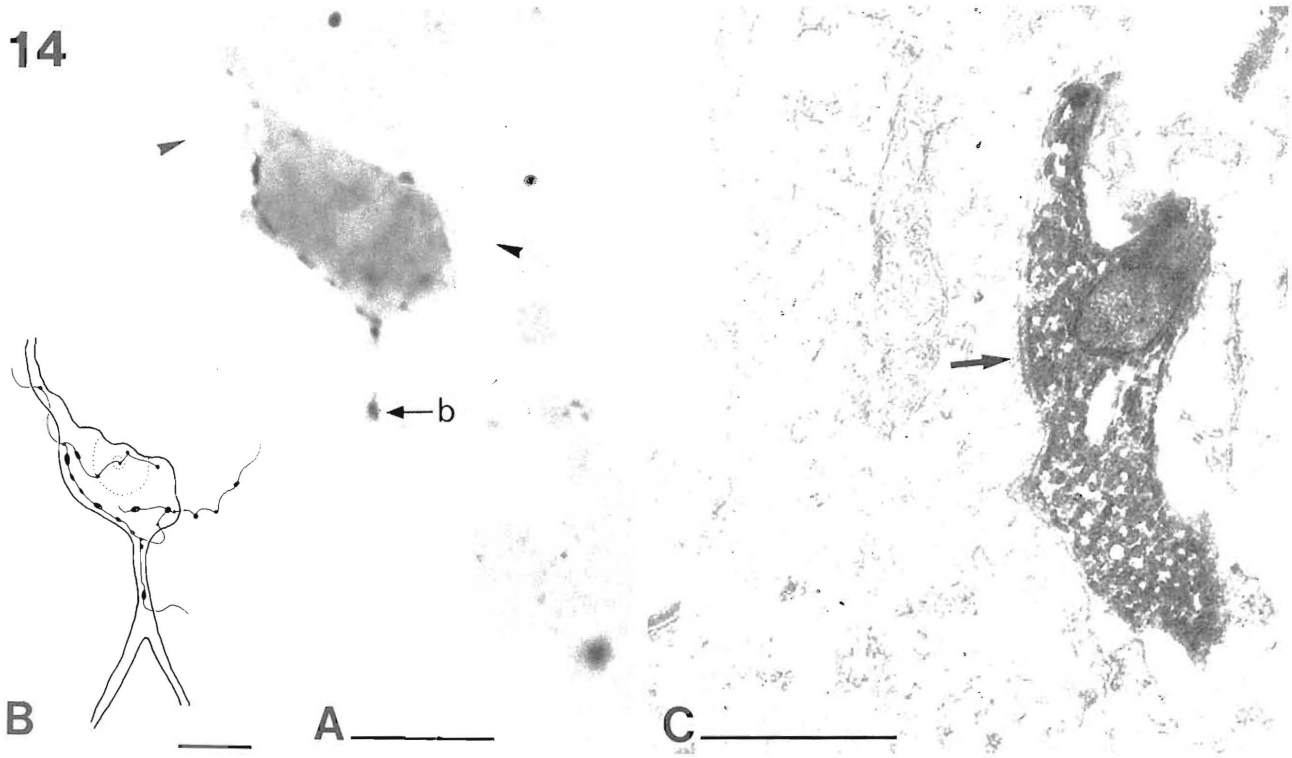

vidual CCK-positive or CCK-negative cells, large plexuses of CCK-immunoreactive boutons, mostly surrounding the perikarya of CCK-positive cells, were frequently found in the stratum moleculare, but unfortunately the origin of the axon forming such plexuses could not be traced. Furthermore, it was not always possible to determine whether a single or several afferent CCK-positive fibers contributed to the plexuses. In view of the finding that most CCK-positive cells contain GABA (Somogyi et al., '84), the presence of a large number of CCK-positive boutons in synaptic contact with CCK-containing cells may provide the anatomical substrate for disinhibition in the hippocampus. In this respect, it is noteworthy that the excitation of pyramidal neurons by enkephalin may be due to the depressant action of enkephalins on GABAergic interneurons that mediate the inhibition of pyramidal cells (Lee et al., '80; Nicoll et al., '80). Enkephalin-immunoreactive cell bodies and terminals have been found in all hippocampal layers (Gall, '81) but whether they terminate on inhibitory interneurons has not been determined.

Ultrastructural features of CCK-immunoreactive cells

In both the cat and the rat hippocampus, despite the differences in size, shape, and dendritic arborization the different classes of CCK-positive cells exhibit ultrastructural features which overlap in many respects. Furthermore, neither their cytology nor their synaptology provide criterias for distinguishing them from other hippocampal nonpyramidal neurons.

The general somatic features of CCK-immunoreactive cells resemble those reported for inhibitory interneurons in the hippocampus (Schwartzkroin and Kunkel, personal communication) and GABAergic neurons in the neocortex (Ribak et al., '78). A peculiar feature, which was often found in CCK-positive perikarya of both cat and rat hippocampus, was the presence of intranuclear rods; these were also observed in the nuclei of GAD-positive neurons (Ribak, '78) and axo-axonic cells (Somogyi et al., '82) of the rat visual cortex and in pyramidal basket cells of the rat hippocampus (Ribak and Anderson, '80). The presence of intranuclear rods has been related to a high degree of physiological activity (Feldman and Peters, '72). In this connection it is noteworthy that hippocampal neurons with inhibitory function have a high tonic discharge rate (Schwartzkroin and Mathers, '78). Another characteristic shared by CCK-immunoreactive cells and other hippocampal intrinsic neurons is the presence of both symmetrical and asymmetrical synapses on their perikarya.

Possible functional implications

Studies on the electrophysiological action of cholecystokinin indicate that both CCK-8 and CCK-4 cause depolarization accompanied by a marked increase of excitability of pyramidal neurons (Phillis and Kirkpatrick, '80; Dodd and Kelly, '81). Thus, CCK might act as an excitatory neuroactive substance at certain synapses. However, the precise role of CCK remains to be established; this peptide might have a more subtle role than simple excitation or inhibition since it coexists with GABA in many hippocampal neurons (Somogyi et al., '84).

Due to the large number of CCK-positive cells and terminals associated with pyramidal and nonpyramidal neurons in the hippocampal formation, it seems reasonable to suppose that CCK/GABA-containing cells would exert a significant influence in the hippocampal circuitry. Recent observations indicate that several peptides may coexist in central neurons with small neurotransmitter molecules such as acetylcholine, serotonin, and noradrenaline (see Hökfelt et al., '80a; Lundberg and Hökfelt, '83). CCK has also been found together with dopamine in a subpopulation of mesencephalic neurons in rat and man (Hökfelt et al., '80a–c). It has been shown that CCK modulates the release of dopamine in the striatum (Starr, '82; Meyer and Krauss, '83) and cerebral cortex (Klaff et al., '82) and, in addition, that interactions exist between dopamine and CCK at the level of the dopamine receptors (Fuxe et al., '81). CCK might modulate GABA transmission in the neocortex, since Sheehan and de Belleroche ('83) found that CCK-8 facilitates the release of GABA in vitro. Thus, in the hippocampus, CCK and GABA might interact at either the presynaptic terminal and/or at the postsynaptic target. Furthermore, the codistribution of binding sites for CCK (Zarbin et al., '83) and GABA (Chan-Palay, '78) in the hippocampus is also indicative of their possible interaction.

Fig. 13. A. Light micrograph of a CCK-positive neuron in the upper stratum lacunosum-moleculare of the cat hippocampus (CA1). The arrow indicates the profile of a CCK-immunoreactive fiber apposed to the proximal portion of the dendrite. B. Electron micrograph illustrating the relation of the CCK-positive fiber shown in A with immunostained dendrite (D) of the neuron and two nonreactive profiles (d_1 and d_2) probably belonging to pyramidal neuron apical dendrites. C,D. Electron micrographs of two serial sections through the CCK-positive fiber. In C symmetric synaptic contacts (arrows) made by the fiber with the pyramidal cell dendrites (d_1 and d_2) are seen. D. The CCK-positive fiber also makes a symmetrical synaptic contact with the dendrite of the CCK-positive neuron (arrow). Scales: A = 5 μm; B = 1 μm; C,D = 0.5 μm.

Fig. 14. A. Light micrograph of an 80-μm-thick immunostained section, illustrating a CCK-positive neuron in the stratum lacunosum-moleculare of the rat hippocampus (CA1) whose profile is outlined by strongly immunostained boutons. The bouton (b) indicated by the arrow is contacting the proximal portion of the basal dendrite. Arrowheads indicate glia cell profiles associated with the perikaryon and proximal dendrite. B. Camera lucida drawing of the neuron shown in A. The varicosities, which represent the CCK-immunoreactive boutons, are interconnected by thin fibers forming a plexus around the perikaryon and dendrites. C. The high-power electron micrograph shows the bouton (b), indicated by the arrow in A, in symmetrical synaptic contact (arrow) with the faintly immunostained basal dendrite of the neuron. Scales: A,B = 10 μm; C = 0.5 μm.

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