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Ultrastructural Study of Cholecystokinin-Immunoreactive Cells and Processes in Area CA1 of the Rat Hippocampus

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ABSTRACT

We used light and electron microscopic immunocytochemical methods to examine the structure of neuronal perikarya and processes containing cholecystokinin-like immunoreactivity (CCK-IR) in area CA1 of the rat hippocampus. The morphology of stained perikarya, their positions within all laminae, and the orientation of their dendrites indicate that CCK-IR is located in interneurons. These cells were seen in the electron microscope to have deeply folded nuclei and to receive both symmetric and asymmetric synaptic junctions on their cell somata and dendritic shafts. Their dendrites are essentially spine-free, but form bulges at the site of some asymmetric synaptic junctions. Axonal varicosities containing CCK-IR make symmetric synaptic junctions with cell somata and dendritic shafts of both pyramidal and non-pyramidal neurons. In addition, CCK-IR varicosities form symmetric junctions with unstained non-pyramidal neurons and with CCK-IR cells, suggesting either recurrent innervation of one cell on itself or interaction between interneurons. The presence of CCK-IR varicosities and synaptic junctions on pyramidal cells is in agreement with physiological data which indicate that CCK has a direct postsynaptic action. The observation of CCK-IR varicosities forming synaptic junctions on non-pyramidal cells suggests that CCK might also modify the response of interneurons.

Key words: interneurons, electron microscopy, synapses, GABA, nucleus

Cholecystokinin (CCK) is a neuroactive peptide that has been isolated from the gut and central nervous system (for review see Rehfeld, '80; Morley, '82; Dockray, '82). Recently, immunocytochemical methods have localized CCK-like immunoreactivity (CCK-IR) in neurons and their processes throughout the central nervous system (e.g., Hökfelt et al., '80; Vanderhaegen et al., '80; Köhler and Chan-Palay, '82; Maderut et al., '82; Roberts et al., '83). In all layers and subfields of the rat hippocampus, CCK-IR occurs in cells that resemble interneurons, as shown with Golgi preparations (Ramón y Cajal, 1893a,b; Lorente de Nó, '34; Gayoso, et al. '79; and Tömböl et al., '79; Greenwood et al., '81; Handelmann et al., '81; Roberts et al., '84). Cells with similar shapes and in similar positions of the hippocampus contain GABA (Ribak et al., '81; Somogyi et al., '83), as do some of the CCK-containing cells (Vickrey et al., '83). Pressure application of CCK to pyramidal cells in area CA1 increases their spontaneous and evoked activity (Dodd and Kelly, '81; Kelly and Dodd, '81); however, anatomical evidence for synaptic interaction has not yet been presented. We describe here the morphological characteristics of neurons and neuronal processes containing CCK-IR in area CA1 of the rat hippocampus, with particular attention to the location and structure of their synaptic junctions. Preliminary results from this study have been reported elsewhere (Marshall et al., '83).

METHODS

Animals

Observations were made in area CA1 of the hippocampus from seven male rats weighing between 275 and 375 gm. One animal was pretreated with colchicine (60 μg in 15 μl of distilled water injected over 15 minutes, into the right lateral ventricle 24 hours before perfusion). This animal

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and four others were perfused with 4% paraformaldehyde and 0.1% glutaraldehyde in 0.1 M sodium phosphate buffer pH 7.4 (PB), at 37°C for 20–30 minutes. Two animals were perfused with a mixture of 2% paraformaldehyde, in 0.075 M lysine and 0.01 M periodate (PLP) in PB under the same perfusion conditions. The brains were dissected free from the cranium after 1 hour and stored in the same fixative for 4–18 hours at 4°C.

Immunocytochemistry

Coronal vibratome sections (25–30 μm) were obtained through the hippocampus of each animal. For light microscopy, 0.2% Triton X-100 was added to a 10% normal goat serum (NGS) in PB wash and to the first antibody solutions. For electron microscopy, some sections were washed in 0.1% Triton X-100 in 10% NGS in PB for 10 minutes and then rewashed with 10% NGS without Triton. Other sections received only a 10% NGS wash with no Triton in this prewash or subsequent solutions. Sections were then incubated in antiserum to CCK-8 (Immunonuclear) at dilutions of 1:2,000, 1:2,500, or 1:3,000 for 24 or 36 hours at 4°C. Control sections were exposed to antiserum preadsorbed with CCK-8 (Boehringher-Mannheim). Sites of antibody binding were visualized by means of the avidin-biotin peroxidase technique (Hsu et al., '81) (Vectastain kit, Vector Labs). Diaminobenzidine was the substrate for the peroxidase reaction.

For electron microscopy, tissue from glutaraldehyde-fixed animals was processed through 2% OsO_4 for 1 hour, dehydrated through ascending ethanol concentrations, and propylene oxide, and embedded in Epon between dimethyldichlorosilane-treated glass slides and coverslips. CCK-IR cells were identified and photographed in the light microscope, dissected from the Epon, and mounted on Epon blanks. Serial thin sections were positioned on Formvar-coated slot grids and viewed in a JEOL 100CX electron microscope. Two of these series were grid stained with methanolic uranyl acetate (Figs. 4–6). Other series were studied without grid stain.

RESULTS

Light microscopy

Densely stained neuronal perikarya giving rise to at least one well-stained dendritic process were studied throughout all layers of area CA1 across the septo-temporal axis (Fig. 1). Of 207 cells measured in seven sections from the hippocampus of two animals, 25% had cell bodies in s. pyramidale, 11% were in s. oriens or the alveus (basilar field); and 64% were in s. radiatum or lacunosum-moleculare (apical field). Cell soma diameters ranged from 12 to 28 μm in s. pyramidale, 15 to 30 μm in s. radiatum, 9 to 27 μm in s. lacunosum-moleculare, and 9 to 27 μm in the fissure. Both the apical and basilar fields contained multipolar and bipolar cells with spine-free processes. Cells in s. oriens were often larger and more fusiform than cells found in other regions. Many CCK-IR varicose processes were observed in s. pyramidale and the proximal apical field. At the light microscopic level, diffuse immunoreactive product was seen in the alveus and near the hippocampal fissure; and occasionally CCK-IR varicosities were discerned in these regions. Sections treated with pre-adsorbed antisera had no CCK immunoreactive cells or terminals. All of these observations were further confirmed in hippocampal sections prepared for electron microscopy. Colchicine and Triton X-100 pretreatment increased the frequency of cell staining, but staining of comparable intensity was present in tissue that was not pretreated.

Electron miscroscopy

We studied the ultrastructure of individual CCK-IR cells in each layer of area CA1 in 25–45 serial sections. The reaction product accumulated throughout the cytoplasm around vesicular organelles, around the outer membrane of mitochondria, and on microtubules. CCK-IR axonal varicosities were densely stained, cell bodies were moderately stained, and the concentration of reaction product diminished in the dendrites with increasing distance from the cell soma. Intensity of the stain also decreased with depth from the surface of the vibratome-cut section.

Stratum radiatum. Three CCK-IR cells in s. radiatum were examined. The nucleus was deeply indented in these and in the CCK-IR cells from the other layers of area CA1 (Fig. 2a). Synaptic junctions were found on the cell somata and proximal dendrites. The junctions were identified as asymmetric if the postsynaptic thickening was as great as that seen on unstained dendritic spines in the same preparation. All junctions with less electron-dense thickening on the postsynaptic side were referred to as symmetric, even where the CCK reaction product enhanced the thickening so that it was intermediate in thickness.

Three morphologically distinct classes of axonal varicosity formed synaptic junctions with these cells. First, axonal varicosities formed synaptic junctions on cell somata with symmetric pre- and postsynaptic electron-dense specializations. The presynaptic varicosity contained pleomorphic synaptic vesicles whose shapes ranged from round to ellipsoid (Fig. 2b). In single sections, these axons seemed to form several discrete junctional specializations. In serial sections, however, the electron-dense specializations proved to be part of a single vermiform junction.

In addition to this first class of vermiform synapse, two other classes of synapses were observed on dendrites of this CCK-IR cell (Fig. 2c–e). A second class of varicosities contained pleomorphic vesicles and formed macular synaptic junctions with symmetric electron-dense specializations on dendritic shafts of secondary branches (Fig. 2c). Finally, smaller varicosities containing round vesicles formed macular junctions with asymmetric specializations on the dendritic shaft (Fig. 2d,e). The dendritic surface was irregular, but no dendritic spines were seen within 40 μm of the cell soma.

Stratum lacunosum-moleculare. The CCK-IR cell shown in Figure 3a was located in s. lacunosum-moleculare about 75 μm from the hippocampal fissure and gave rise to a major dendrite that followed a course parallel to the fissure. Two varicosities making synaptic junctions on this soma were distinctly different from those forming somatic junctions in s. radiatum (Fig. 3b–d). They might be of the same synaptic class as those found making asymmetric junctions with the dendritic shafts of the s. radiatum cell. These varicosities contained more uniformly round vesicles and formed asymmetric electron-dense thickenings at the postsynaptic site. They were usually smaller than those forming symmetric vermiform junctions with the s. radiatum somata, and the junction sites occurred on bulges in the somatic plasmalemma—especially evident in Figure 3c.

The CCK-IR dendrite was traced about 50 μm from the soma in serial sections. Macular asymmetric and symmetric synaptic specializations were found along the dendritic surface. Some of the asymmetric junctions occurred on dendritic bulges similar to those on the somal surface. An

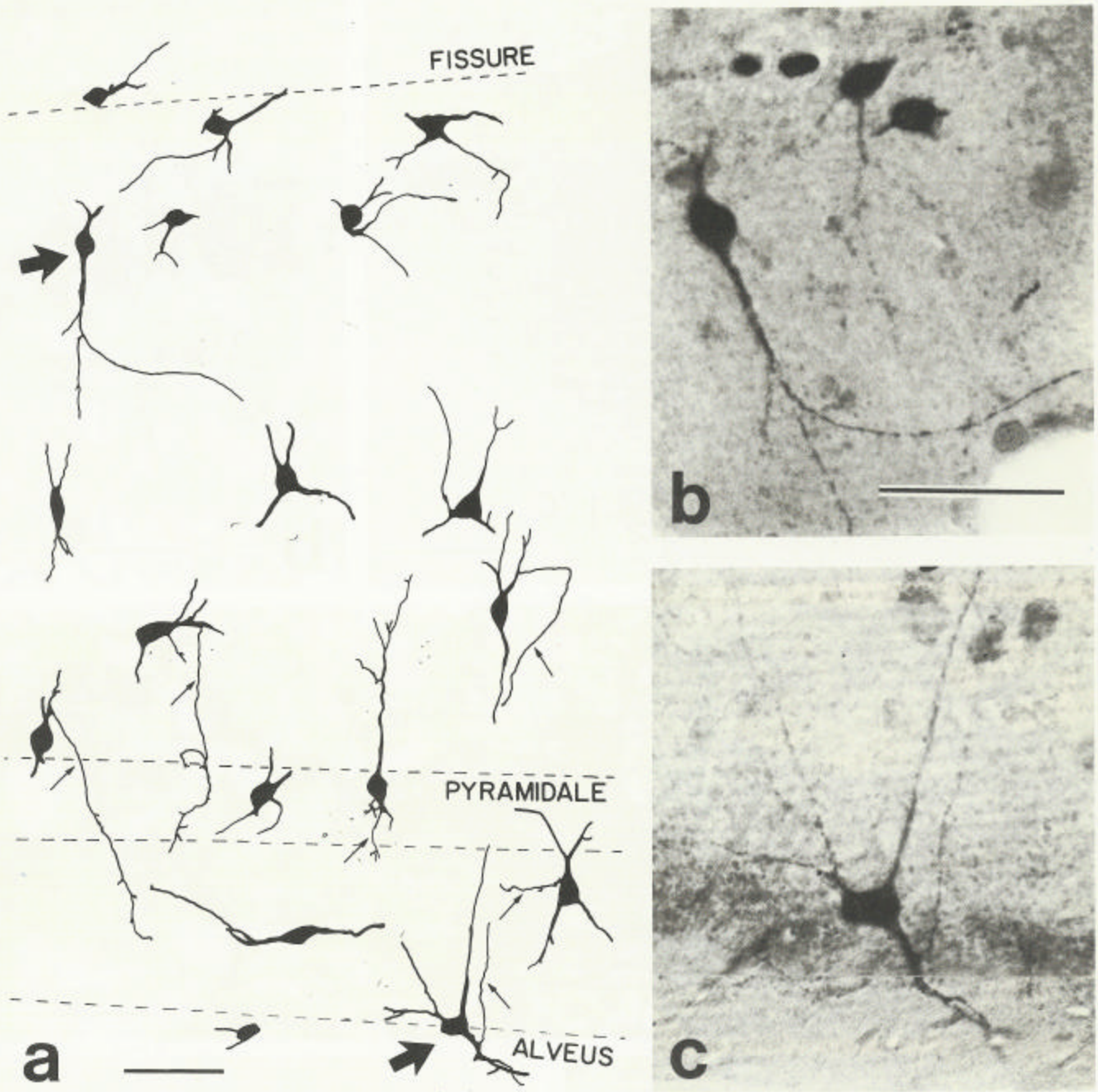

Fig. 1. Distribution of CCK-IR cells in area CA1 of the rat hippocampus. a. Camera lucida tracings of CCK-IR cells in area CA1. All of the processes appeared spine-free and for some cells, one process of finer caliber with a different branching pattern was likely to be an axon (small arrows). Large arrows indicate two representative cells shown in b and c. S. radiatum and s. lacunosum-moleculare occupy the inner two thirds and outer one third, respectively, of the apical field between s. pyramidale and the fissure, and s. oriens is the basilar dendritic field between s. pyramidale and the alveus. b. CCK-IR cell in s. radiatum. c. CCK-IR cell at the s. oriens-alveus border. Calibration bars in a, and b (for b and c) = 50 μm.

elongated varicosity of an unstained axon formed an asymmetric junction with the CCK-IR dendrite, and also two asymmetric junctions with unstained dendritic spines (Fig. 3e). A CCK-IR varicosity made a symmetric junction nearby on the same CCK-IR dendrite (Fig. 3f). This CCK-IR varicosity was similar to unstained varicosities seen elsewhere which formed symmetric synaptic junctions on dendritic shafts and soma (see below) that appeared macular, not vermiform, in serial sections.

Stratum pyramidale. Other CCK-IR cells were found in and around stratum pyramidale. Their nuclei were also indented, in contrast to the smooth and approximately spherical nuclei of adjacent pyramidal cells. The perikaryon of one CCK-IR cell was viewed intermittently in 27 of 45 serial sections (Fig. 4a, left). Only two varicosities were found making junctions with the soma of this cell; both had pleomorphic vesicles and formed symmetric junctions on the flat surface of the soma. One of these varicosities con-

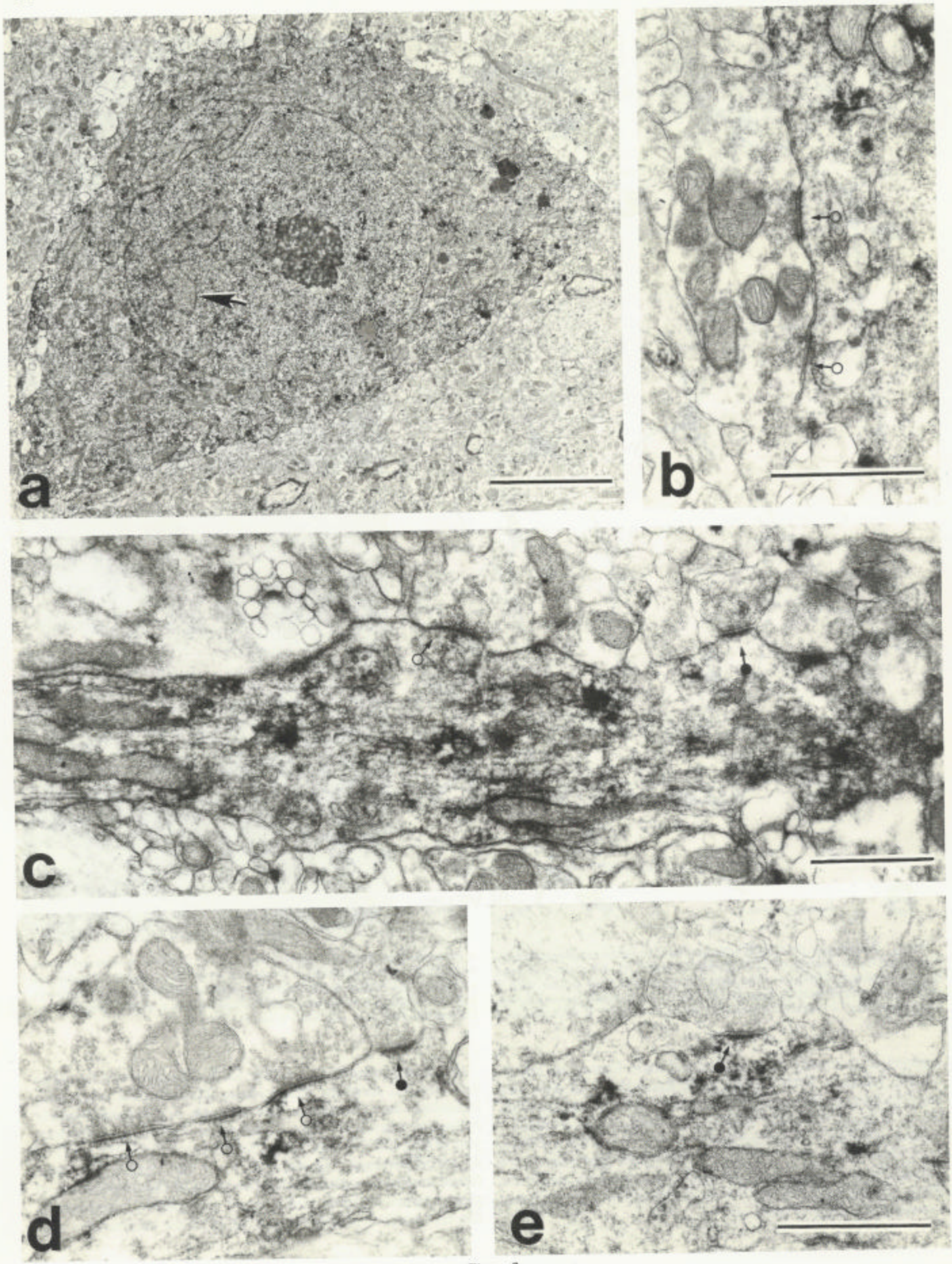

Figure 2

tained CCK-IR (Fig. 4b). The dendrite of this CCK-IR cell was traced about 50 μm into the apical field. The reaction product diminished with distance from the soma and was discontinuous where it appeared faint in the light microscope. This dendrite had both symmetric and asymmetric synaptic junctions on the dendritic shaft (Fig. 4c). The surface of the dendritic shaft was irregular and the asymmetric junction sites often appeared on bulges (Fig. 4d). These bulges contrasted with typical dendritic spines that emerged from unstained dendrites in the adjacent field (Fig. 4d, arrow).

Stratum oriens. Several classes of somatic synapses were found on a CCK-IR cell in s. oriens. We traced ten varicosities to junctional sites on this soma, and photographed them in at least two sections surrounding the junction site. The varicosities illustrated in Figure 5b and c represent the range of features observed at these junction sites. One varicosity made an asymmetric junction on a bulge in the soma membrane and contained large round vesicles (Fig. 5b). Nine varicosities formed symmetric synaptic junctions and contained pleomorphic vesicles clustering at the synaptic junctions. Two of the varicosities also had attachment plaques with dense membrane thickenings both pre- and postjunctionally, but no clustering of vesicles in the immediate vicinity (Fig. 5c). Three of these varicosities were adjacent to one another on the cell soma with no intervening glial processes.

The secondary and tertiary branches of a dendrite traced from this cell (Fig. 6a) had shaft synapses with either macular symmetric or asymmetric specializations. Varicosities containing pleomorphic vesicles formed symmetric junctions on flattened dendritic surfaces, while varicosities containing round vesicles formed asymmetric junctions on bulges (Fig. 6b). We observed a CCK-IR varicosity establishing a symmetric synaptic junction with a tertiary CCK-IR dendritic branch (Fig. 6c). Another symmetric junction was made by the same CCK-IR varicosity on an adjacent unstained soma (Fig. 6d). This unstained cell had deep folds in its nucleus and spine-free dendrites which formed synapses with symmetric and asymmetric specializations.

CCK-IR varicosities making synaptic contacts elsewhere in area CA1. In addition to the symmetric synaptic junctions made by CCK-IR varicosities on CCK-IR somas and processes, CCK-IR varicosities formed symmetric junctions on both proximal (Fig. 7a) and distal (Fig. 7b) portions of the apical dendritic shafts of pyramidal cells. A CCK-IR varicosity made a symmetric junction with an unstained non-spiny dendrite in s. lacunosum-moleculare that also had asymmetric shaft contacts, suggesting it was of non-pyramidal origin. CCK-IR varicosities formed symmetric junctions with pyramidal cell somas that were distant from CCK-IR cells (Fig. 7c). In s. oriens CCK-IR varicosities made symmetric synaptic junctions with dendritic shafts (Fig. 7d).

Including the CCK-IR varicosities found on CCK-IR dendrites and somas, 40 CCK-IR varicosities have been photographed and measured in serial sections. They were seen only to form junctions with dendritic shafts or cell somas, and not with dendritic spines. While the reaction product obscures many of the vesicles, at least a few flattened and round vesicles could be discerned in most of these varicosities. Thirty-five of these varicosities formed junctions with symmetric electron-dense pre- and postsynaptic specializations. Five other varicosities, all at the alveus-oriens border, made synaptic junctions that had postsynaptic specializations intermediate in density thickness between the junctions we have described as symmetric or asymmetric junctions. CCK-IR varicosities were 1.3 ± 0.07 μm long by 0.6 ± 0.05 μm wide at synaptic contacts 0.4 ± 0.02 μm long. All of these measurements were made in the section where the junction site could be most easily discerned.

We have not seen a CCK-IR varicosity forming a synaptic junction with a dendritic spine, although 96 dendritic spines less than 5 μm from a CCK-IR varicosity have been traced in serial sections (e.g., Fig. 4d). Some of these spines were adjacent to CCK-IR varicosities but no synaptic junctions were observed. Some unstained axons forming asymmetric junctions with CCK-IR dendritic shafts also formed asymmetric junctions on unstained dendritic spines (cf. Fig. 3e).

CCK-IR processes in and near the alveus were also studied. We were concerned that the immunoreactivity seen there in the light microscope might be found in the myelinated axons, suggesting that non-interneuronal cells might be contributing to the population of CCK-IR terminals, or that the CCK-IR cells in area CA1 were projection neurons rather than interneuronal. We photographed and counted 1,768 myelinated axons on single sections from the alveus and only two were seen to have evidence of even light CCK staining. Of these axons, 1,064 had mitochondrial profiles and no evidence of CCK reaction product was seen around their outer membranes; therefore we are reasonably confident that most if not all the myelinated axons were devoid of CCK reaction product. In these same fields, 483 CCK-IR processes were observed but none of them were myelinated. In these single section views it was usually impossible to identify unambiguously the source(s) of these CCK-IR processes.

DISCUSSION

Neuronal perikarya in area CA1 of the rat hippocampus containing CCK-like immunoreactivity (CCK-IR) are different in morphology and distribution from the spiny pyramidal neurons. Their nuclei are deeply folded and they have both symmetric and asymmetric synaptic junctions on their somata and dendritic shafts. Their dendrites are spine-free but frequently have bulges at sites of asymmetric synaptic junctions. CCK-IR axons form symmetric synaptic junctions on both pyramidal and non-pyramidal cell somata and dendritic shafts. There was no evidence of CCK-IR varicosities forming synaptic junctions with dendritic spines of the pyramidal cells. Finally, most myelinated axons of the alveus do not contain CCK immunoreactivity, suggesting that CCK-IR axons are not likely to project out of the hippocampus.

Three classes of synaptic junction were identified on CCK-IR cells. First, large varicosities form symmetric junctions

Fig. 2. CCK-IR cell in s. radiatum. a. The nuclear membrane is deeply invaginated (arrow). b. Large unstained varicosity with two discrete sites of junctional specialization on the soma (open circle). The varicosity contains pleomorphic synaptic vesicles and the electron-dense thickenings are symmetric. Viewed in serial sections these specializations merge to form a vermiform junction. c. Secondary branch of a dendrite traced from this CCK-IR cell. Varicosities with pleomorphic vesicles form symmetric synaptic junctions (open circle). A varicosity containing primarily round vesicles forms an asymmetric synaptic junction on a bulge in the dendritic surface (closed circle). d. Varicosity with a vermiform junction and pleomorphic vesicles in the proximal region of this dendrite (open circles). e. Smaller varicosities forming asymmetric junctions with round vesicles occur more distally on the same dendrite (closed circles d, e). Calibration bars: a = 5 μm, b and c = 1 μm, e (for d, e) = 1 μm.

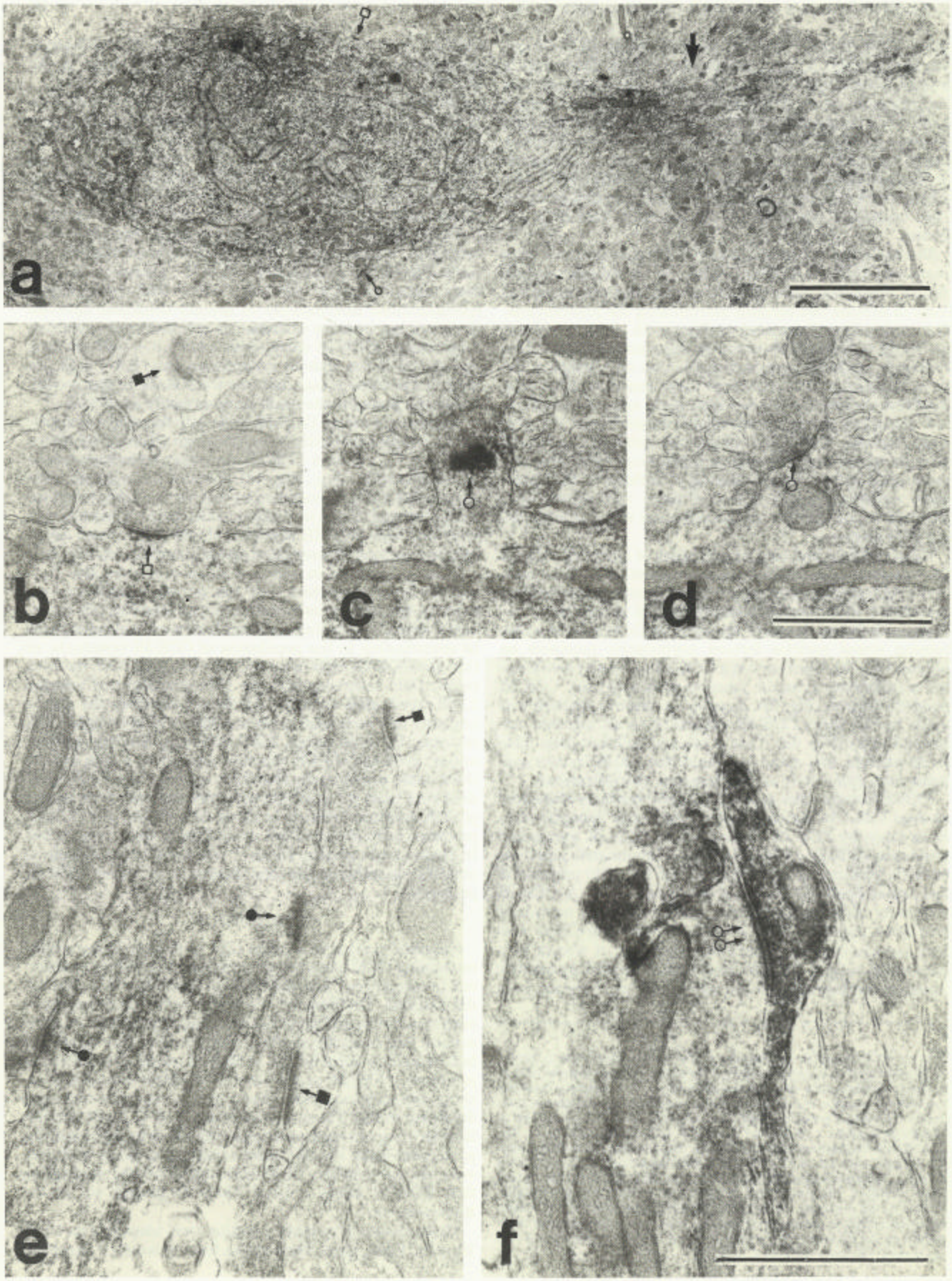

Figure 3

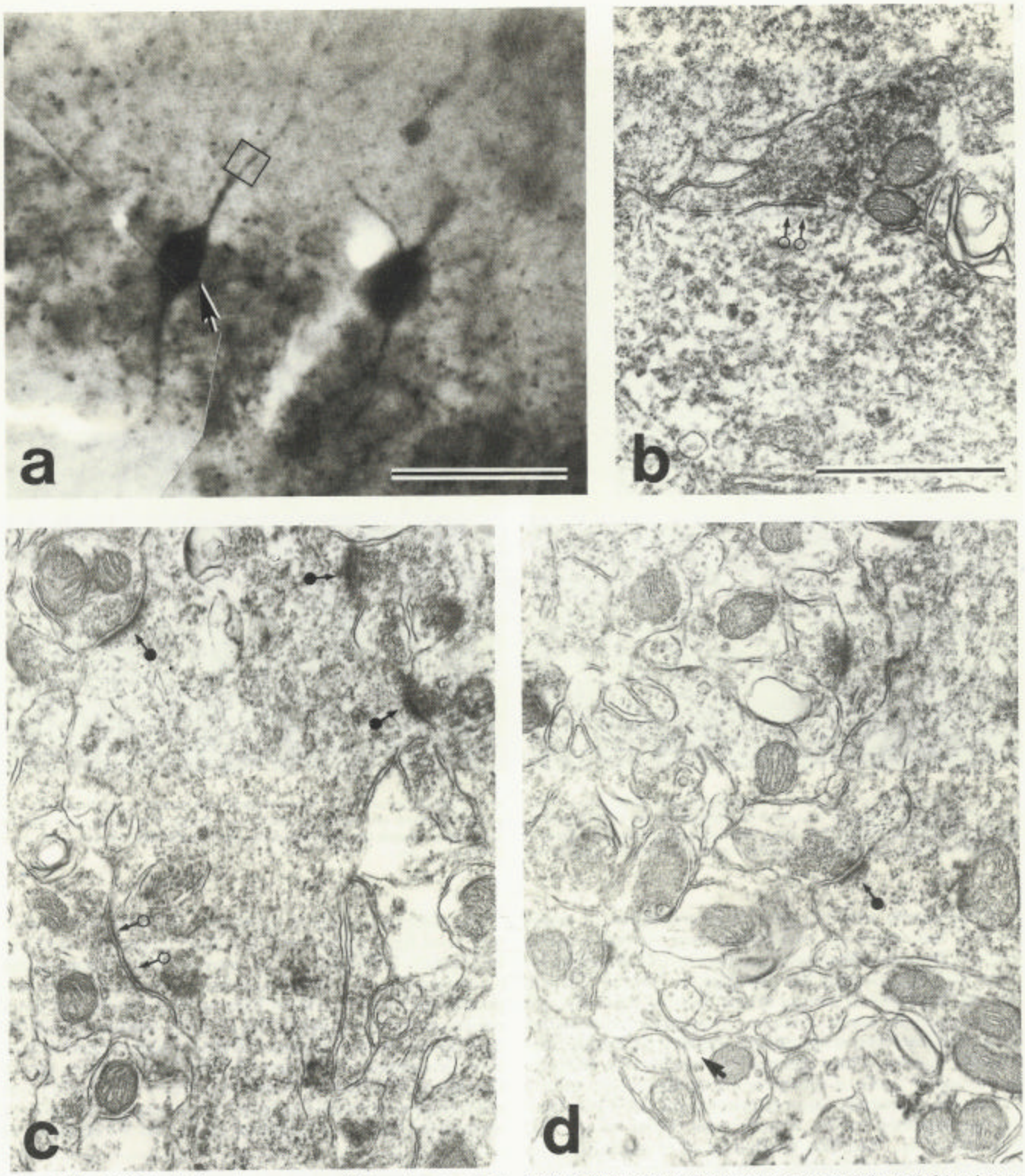

Fig. 4. Two CCK-IR cells in and near s. pyramidale as they appeared prior to serial thin sectioning. a. The large arrow indicates the location of the CCK-IR varicosity illustrated in b. The square encloses the less densely stained region of the CCK-IR dendrite which is shown at higher magnification in c and d. b. A CCK-IR varicosity forms a symmetric synaptic junction with the left CCK-IR soma (double open circles). The reaction product in the varicosity surrounds vesicles and the outer membrane of the mitochondria. c. The CCK-IR apical dendrite receives symmetric (open circle) and asymmetric synaptic junctions (closed circles) on the shaft. d. Synapse with an asymmetric junction on a bulge in the surface of the CCK-IR dendrite (closed circle). The bulge is clearly different from the dendritic spine of an unstained dendrite in the adjacent field (large arrow). Calibration bars: a = 50 μm, b (for b–d) = 1 μm.

Fig. 3. CCK-IR cell in s. lacunosum-moleculare. a. The nucleus is deeply invaginated. The locations of the two synaptic junctions (illustrated in b, c, and d) on the soma are indicated by an open square and open circle. The large arrow indicates a primary dendrite that is illustrated in e and f. b. Somatic synapse (open square) that was followed through serial sections and found to have a macular asymmetric specialization. Compare this junction with that found on a dendritic spine (closed square). c, d. Somatic synapse viewed in two sections, separated by one section not shown here. The macular, electron-dense postsynaptic specialization is present in oblique section in c. This junction site is on bulge in the soma. e. An unstained thin axon with round vesicles forms asymmetric synaptic junctions with two unstained dendritic spines (closed squares) and with the CCK-IR dendritic shaft (closed circle). There is a second asymmetric junction at the lower left on the same dendrite (closed circle). F. This CCK-IR dendrite also makes a synaptic junction having a macular, symmetric specialization, with a CCK-IR varicosity (double open circles). Calibration bars: a = 5 μm, d (for b–d) = 1 μm, f (for e, f) = 1 μm.

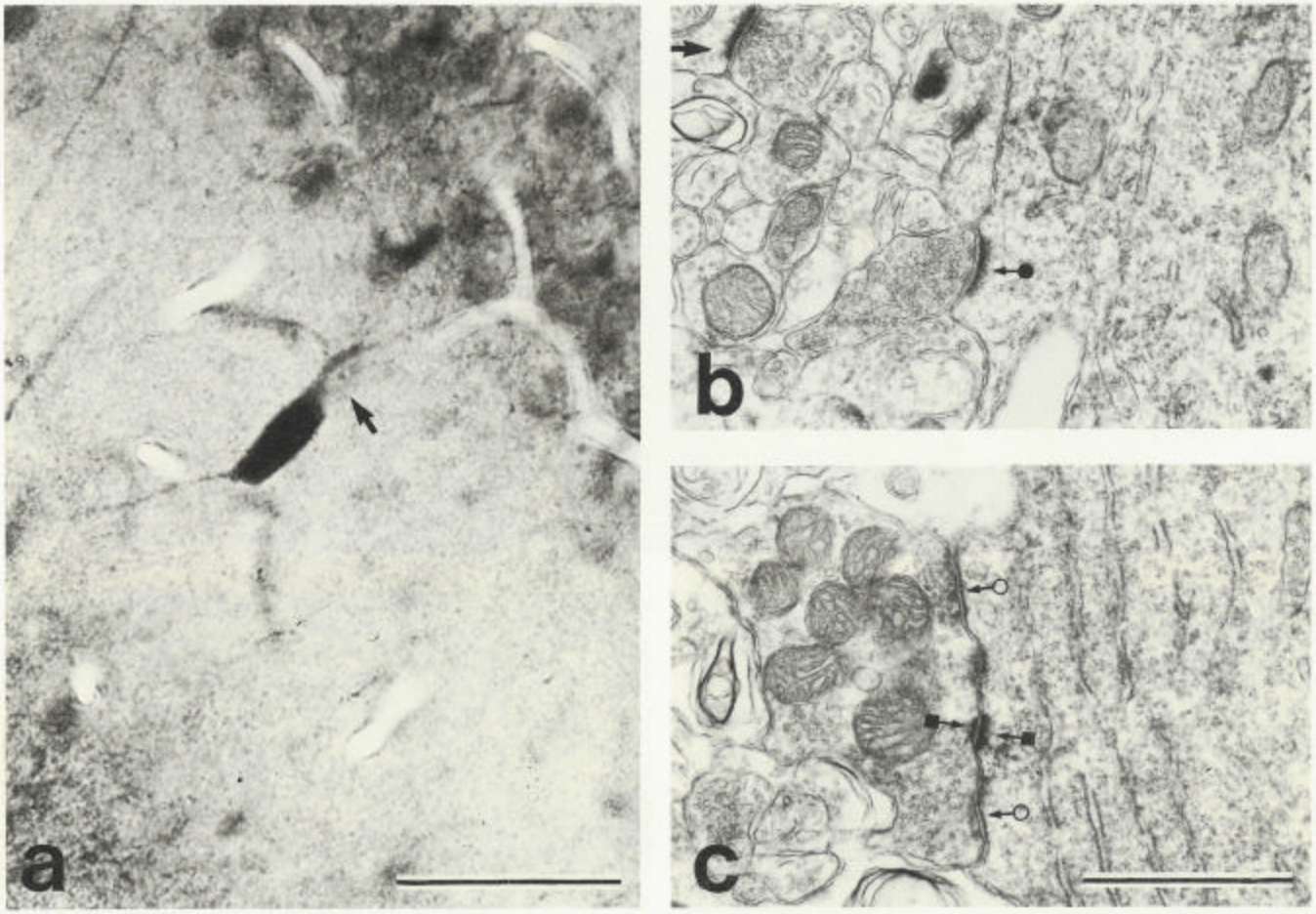

Fig. 5. CCK-IR cell in s. oriens as it appeared prior to serial thin sectioning. a. The arrow indicates an unstained cell adjacent to the CCK-IR cell. b. Synaptic junction on the CCK-IR soma with an asymmetric specialization (closed black circle); compare to synaptic junction on a dendritic spine in the same field (large arrow). c. Varicosities forming several junctions with the soma, including symmetric synaptic specializations (open circles), and an attachment plaque (opposed closed squares). Calibration bars: a = 50 μm, c (for b, c) = 1 μm.

with apparently multiple sites of specialization in single sections, but which are vermiform when viewed in serial sections. In some instances these varicosities also form attachment plaques with the postsynaptic neuron. Second, symmetric junctions having single sites of specialization are macular in serial sections. Presynaptic varicosities forming either type of symmetric junction contained pleomorphic vesicles. These symmetric junctions are likely to be formed by axons of interneurons, as their features and distribution resemble those of axons from GABA-containing interneurons in the hippocampus (Ribak et al., '81; Somogyi et al., '83). The third class of synaptic junction involved axonal varicosities with large, clear, round vesicles, forming asymmetric specializations, frequently on bulges in the dendritic or somatic membrane of CCK-IR cells. In our preparations, asymmetric junctions on flat regions of the dendritic shaft were similar to those occurring on bulges, and both had presynaptic varicosities which contained round vesicles; therefore these are treated together as one class of synaptic junction. These axons are likely to be from area CA3 cells, because ablation of CA3 axons reveals degenerating varicosities at asymmetric junction sites on the dendrites of CA1 interneurons (Frotscher and Zimmer, '83).

Our observations are in agreement with previous studies that used light microscopic immunocytochemical methods to identify CCK-IR cells in area CA1 as interneurons (Greenwood et al., '81; Handelmann et al., '81). Retrogradely transported tracers injected into septum and entorhinal cortex do not label CCK-IR cells; therefore, CCK-IR cells do not seem to project out of the hippocampal formation (Greenwood and Winstead, '83). These authors report that some CCK-IR cells in rostral portions of area CA1 and subiculum send axons caudally to subiculum. These might account for the two myelinated axons that we observed to

Fig. 6. The same CCK-IR cell in s. oriens that is shown in Figure 5. a. CCK-IR cell and an unstained non-pyramidal neuron in s. oriens (arrow is at the same position as that shown in 5a, but the image is reversed, right for left). A CCK-IR varicosity forms symmetric synaptic junctions with a tertiary dendrite of the CCK-IR cell and with the soma of the unstained cell (double open circles, upper left). The curved arrow is at the dendrite that was traced about 40 μm from the soma to synapses illustrated in b. b. Symmetric synaptic junction (open circle) on the CCK-IR dendrite and asymmetric junction (closed circle) on bulge in the dendritic surface. c. A higher magnification view of the symmetric synaptic junction between the CCK-IR varicosity and the CCK-IR dendrite illustrated in a (double open arrows). d. The same CCK-IR varicosity illustrated in c, here viewed several sections deeper, forming a synapse with symmetric specialization on the unstained soma (open circle). Calibration bars: a = 5 μm, d (for b–d) = 1 μm.

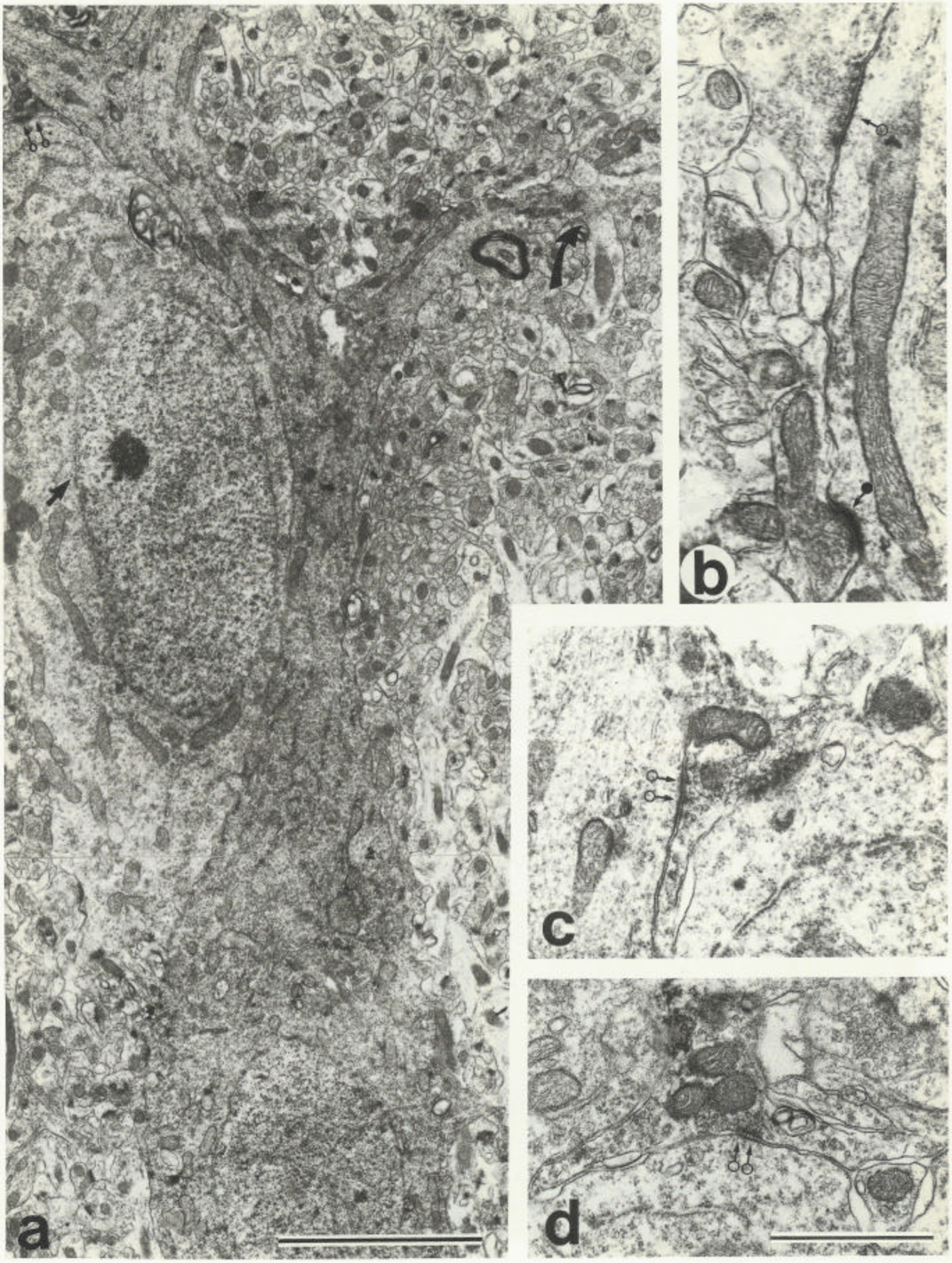

Figure 6

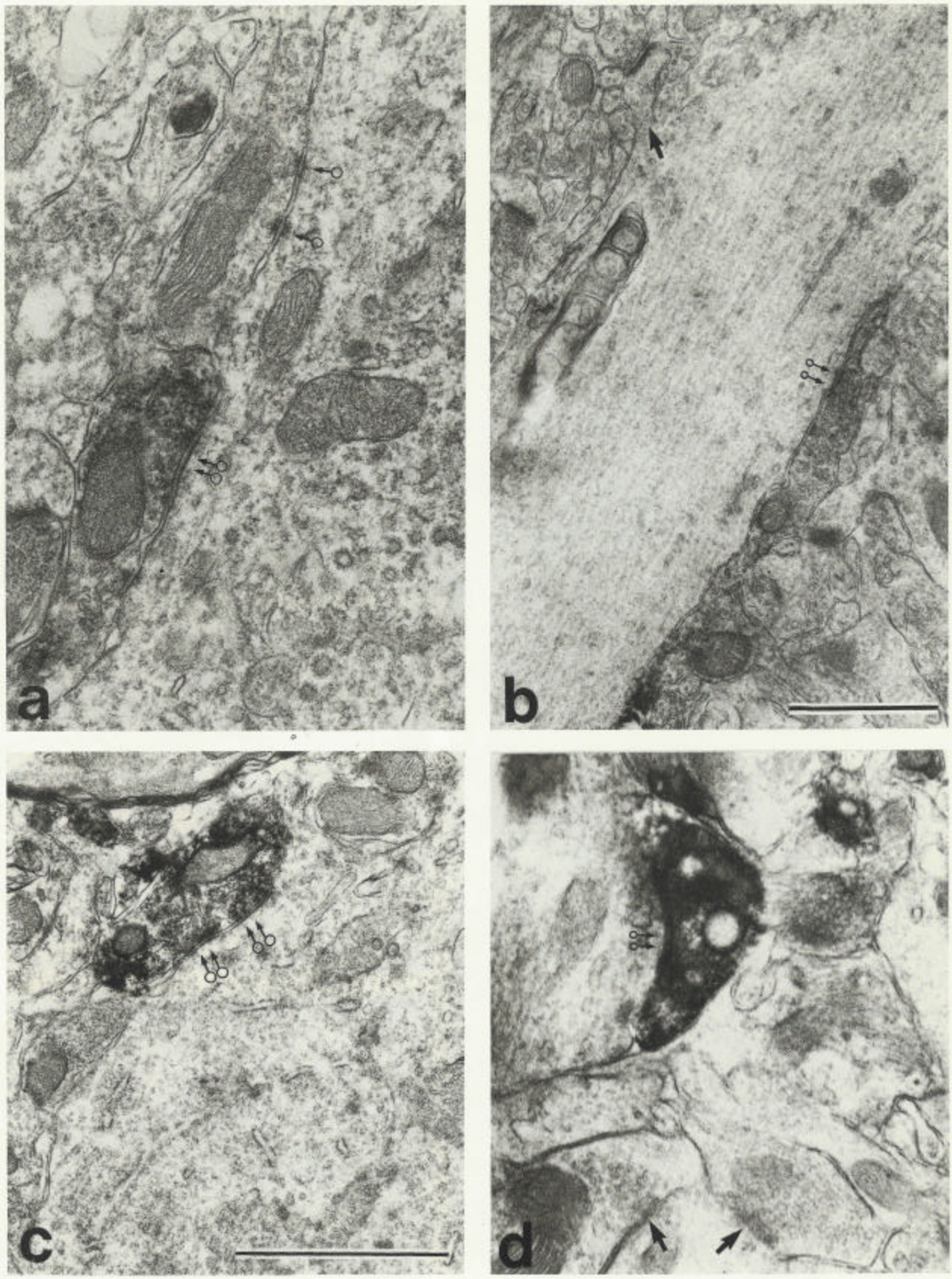

Figure 7

be lightly stained. Ablation of afferent projections to the hippocampus has no effect on the CCK content in area CA1, suggesting that these local CCK-IR neurons supply most of the CCK-IR axons found here (Nelson et al., '83). Finally, the morphology of CCK-IR varicosities and characteristics of their synaptic junctions resemble those of CCK-IR varicosities in cerebral cortex of rats and monkeys, where CCK-IR cells are also likely to be interneurons (Peters et al., '83, Hendry et al., '83).

We have shown that CCK-IR varicosities make symmetric synaptic junctions with CA1 pyramidal neurons. Application of CCK in the vicinity of CA1 pyramidal somata increases spontaneous activity and occurrence of action potentials following depolarization of the cells. These effects occur in low-Ca^{++} medium and hence may occur independently of synaptic transmitter release (Dodd and Kelly, '81). In homogenized rat cerebral cortex, CCK-IR has been localized in a vesicle-rich fraction and is released in a K^+-mediated, Ca^{++}-dependent fashion (Rehfeld et al., '79; Emson et al., '80, '82). It is reasonable, therefore, to postulate that CCK is released upon depolarization of CCK-containing cells and that it has a direct postsynaptic action on the CA1 pyramidal cells.

Recently, Vickrey and colleagues ('83) have shown that 75% of the CCK-IR cells in the hippocampus also contain glutamic acid decarboxylase immunoreactivity (GAD-IR), the synthesizing enzyme for GABA, a neurotransmitter known to be present in the hippocampus (Storm-Mathisen and Fonnum, '72). The CCK-IR varicosities we observed are similar to GAD-IR varicosities found synapsing on cat CA1 pyramidal cells (Somogyi et al., '83). In that report, GAD-IR terminals were described making symmetric junctions with somas and dendritic shafts in addition to many symmetric junctions with axon initial segments. These GAD-IR terminals also did not form synaptic junctions with dendritic spines. Nearly all the varicosities making symmetric junctions in area CA1 contained GAD-IR, while in the present study we found only a subpopulation of these varicosities to contain CCK immunoreactivity (e.g., Fig. 7a,d). Therefore, it is unlikely that all GAD-IR terminals in area CA1 also contain CCK.

The presence of CCK and GABA in the same cells raises the question of whether both are released upon depolarization of axonal terminals. There is evidence from other systems that repetitive axonal firing may result in release of a biologically active peptide while slower rates of firing do not. For example, in frog sympathetic ganglia, repetitive stimulation of preganglionic axons which contain both acetylcholine and a peptide similar to leutinizing hormone-releasing hormone produces a peptide-mediated excitatory response that is not observed following single stimuli (Jan and Jan, '82; Kuffler and Sjenowski, '83). In the hippocampus, too, there is an interesting contrast between the effects of single and repetitive stimulation. A single orthodromic stimulus of afferents to CA1 pyramidal cells results in a long-latency hyperpolarization at the soma. This hyperpolarization is believed to result from activation of recurrent collaterals from the pyramidal cell axons which synapse on GABA-containing interneurons, in turn causing release of GABA at inhibitory synapses on the pyramidal cell somas and proximal dendrites (Andersen et al., and Loyning, '64a,b, '80; Alger and Nicoll, '82; Wong and Watkins, '82). In contrast, repetitive orthodromic stimulation (e.g., 10 Hz for 10 seconds) reverses this hyperpolarization, and depolarization of the pyramidal cells occurs at the latency where GABA previously caused hyperpolarization (Wong and Watkins, '82). Although not yet tested, frequency-mediated differential release of GABA and CCK might account for part of this effect.

The mechanism of CCK-mediated postsynaptic effects on the pyramidal cells is unknown. In addition to a direct postsynaptic action at the CCK-IR synapses, CCK might also modulate the postsynaptic effect of GABA at these same, or at adjacent synaptic junctions on the pyramidal cells (e.g., Fig. 7a). Evidence is accumulating in other systems that neuroactive peptides can modify the action of neurotransmitters. For example, substance P blocks channels opened by acetylcholine applied to chromaffin cells (Clapham and Neher, '84).

CCK might also influence the excitability of pyramidal neurons indirectly via interneurons. We have observed that CCK-IR varicosities form symmetric synaptic junctions with CCK-IR somata and dendrites. These might be recurrent collaterals of one CCK-IR cell on itself, or reflect synaptic interactions between different CCK-IR cells. CCK-IR varicosities were also seen forming symmetric synapses with unstained non-pyramidal neurons. Subpopulations of interneurons in area CA1 also manifest immunoreactivity to other neuroactive peptides, including enkephalin, somatostatin, and vasoactive intestinal polypeptide (VIP) (Sims et al., '80; Gall et al., '81; Kunkel et al., '83; Roberts et al., '84). Like CCK, these peptides also excite CA1 pyramidal cells (Dodd et al., '79; Dodd and Kelly, '78; Gahwiler, '80; Lee et al., '80; Nicoll et al., '80; Siggins et al., '82; Mueller and Schwartzkroin, '83). The excitatory action of enkephalin is known to occur via presynaptic modulation of GABA release from interneurons (Gahwiler, '80; Lee et al., '80; Nicoll et al., '80). If CCK has a similar action on the GABA-containing interneurons, part of its excitatory action may occur via disinhibition of pyramidal cells by preventing GABA release.

Fig. 7. CCK-IR varicosities synapsing with unstained pyramidal neurons in area CA1. a. CCK-IR varicosity forming a symmetric synaptic junction with a proximal region of an apical dendritic shaft from a pyramidal cell (double open circles). A similar, unstained varicosity forming attachment plaques (single open circle) is located adjacent to the CCK-IR varicosity. Both classes of axonal varicosity contain pleomorphic vesicles. b. A CCK-IR varicosity forms a symmetric synaptic junction (double open circles) on the dendritic shaft of a spiny pyramidal cell (large arrow indicates a spine). c. In s. pyramidale, a CCK-IR varicosity makes a symmetric synaptic junction with a pyramidal cell soma (double open circles). d. CCK-IR varicosity in s. oriens forming a symmetric synaptic junction on a large dendritic shaft. This varicosity was located near the surface of the vibratome section and the dense reaction product obscures most of the presynaptic vesicles. Two unstained varicosities (large arrows) also make symmetric synaptic junctions on an unstained dendrite in the same field. Calibration bars = 1 μm, (bar in c is for a, c, d).

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