

# UC Irvine

## UC Irvine Previously Published Works

### Title

A combined Golgi-electron microscopic study of non-pyramidal neurons in the CA 1 area of the hippocampus

### Permalink

<https://escholarship.org/uc/item/3td6330v>

### Journal

Brain Cell Biology, 14(5)

### ISSN

1559-7105

### Authors

Seress, László  
Ribak, Charles E

### Publication Date

1985-10-01

### DOI

10.1007/bf01170824

### Copyright Information

This work is made available under the terms of a Creative Commons Attribution License, available at <https://creativecommons.org/licenses/by/4.0/>

Peer reviewed

# A combined Golgi–electron microscopic study of non-pyramidal neurons in the CA 1 area of the hippocampus

LÁSZLÓ SERESS<sup>1</sup> and CHARLES E. RIBAK<sup>2\*</sup>

<sup>1</sup>*Department of Physiology, University Medical School, Pécs, Hungary*

<sup>2</sup>*Department of Anatomy, University of California, Irvine, California 92717, USA*

Received 15 January 1985; revised 16 April 1985; accepted 1 May 1985

---

## Summary

Non-pyramidal neurons of the CA 1 area of the rat hippocampus were identified with a combined Golgi–electron microscopic method. They were observed to have distinctive light and electron microscopic characteristics that are different from those of pyramidal cells. These features included smooth dendrites, locally arborizing axons, infolded cell nuclei with intranuclear rods or sheets, and a well-developed perikaryal cytoplasm with many organelles. In addition, the axon terminals that contact the somata and dendrites of local circuit neurons may form asymmetric as well as symmetric synapses. The axons of these cells form symmetric synapses with dendrites and somata of pyramidal cells. Some of these features were utilized to identify non-pyramidal neurons of the CA 1 area for studies of connectivity. Degenerating commissural terminals were found to form synapses with the dendrites and somata of non-pyramidal neurons. These results indicate that these neurons are a significant population of hippocampal neurons that may provide feed-forward inhibition of pyramidal neurons.

## Introduction

The morphological features of the non-pyramidal neurons of CA 1 have been described in separate light and electron microscopic preparations (Ramón y Cajal, 1911; Lorente de Nó, 1934; Gayoso *et al.*, 1979; Tömböl *et al.*, 1979). These neurons have multipolar, fusiform or stellate shaped somata that are located in all layers. Smooth, beaded dendrites arise from these somata and spread out in all directions. Their axons arborize locally in the different layers of CA 1.

At the electron microscopic level, the basic criterion used for the identification of a non-pyramidal neuron has been the location of a soma outside the stratum pyramidale

\*To whom all correspondence should be addressed.

(Gayoso *et al.*, 1979; Tömböl *et al.*, 1979; Frotscher & Zimmer, 1983). These studies pointed out that such neurons have deeply infolded nuclear envelopes and intranuclear rods, two features that are not found in pyramidal neurons. However, many non-pyramidal neurons are located within stratum pyramidale, and these cells have not been analysed. In addition, the identification of a local circuit neuron based on its somal location is probably not accurate because recent studies have shown that some non-pyramidal cells of CA 1 project to the septum (Chronister & DeFrance, 1979; Alonso & Köhler, 1982). Furthermore, the ultrastructural characteristic of the nuclear infoldings is not diagnostic for non-pyramidal neurons because some pyramidal neurons of CA 1 in the cat display this feature to a certain degree (Somogyi *et al.*, 1983). Therefore, it is difficult to distinguish between pyramidal and non-pyramidal neurons in normal electron microscopic preparations of CA 1.

For this reason, we have approached the identification of non-pyramidal neurons using a combined Golgi–electron microscopic method (Fairén *et al.*, 1977). This method facilitates the examination of light and electron microscopic features of the non-pyramidal neurons in the different layers of CA 1. Our previous studies with this method in the dentate gyrus of the hippocampal formation have shown the ultrastructural features of basket cells (Ribak & Seress, 1983; Seress & Ribak, 1984), and facilitated their identification in ordinary electron microscopic preparations (Chang & Dyer, 1984). The identification of non-pyramidal neurons in CA 1 in Golgi–electron microscopic preparations would benefit their future analysis.

## Materials and methods

Twenty albino rats of the Wistar strain and six Long-Evans rats (1–2 months of age) were utilized for these studies. Animals were anaesthetized with sodium pentobarbital and the brains were fixed by transcardiac perfusion with a solution containing 2.0% paraformaldehyde, 1.25% glutaraldehyde and 0.002% calcium chloride in a 0.12 M phosphate buffer at pH 7.2. The perfused rats were stored overnight in the refrigerator before dissecting out the hippocampus the following day. Five rats had their ventral hippocampal commissure surgically cut on the right side using an L-shaped stainless steel knife. These rats were perfused 4 days after the surgery and the commissural lesion was assessed in 100 µm thick Vibratome sections.

All brains were processed for the Golgi–electron microscopic method described by Fairén *et al.* (1977). Briefly, the entire hippocampus was rinsed in buffer and placed into an osmium dichromate solution (1 g osmium tetroxide and 12 g potassium dichromate in 500 ml distilled water). Each specimen was immersed in 50 ml of this solution and kept in the dark at room temperature for 4 days. Then the tissue was washed briefly in 0.75% silver nitrate and stored in this solution for 3 days. Following impregnation, the blocks were processed through 20, 40, 60, 80 and 100% solutions of glycerol before being cut. Then, the blocks were embedded in agar and were sectioned on a Sorvall tissue chopper at a thickness of 100 µm, mounted on slides and examined with a light microscope. Non-pyramidal neurons with visible axons in the hippocampus were identified, drawn with a Zeiss microscope equipped with a drawing tube and photographed. Sections that contained such impregnated neurons were hydrated to distilled water through a series of glycerol solutions. Then, they were placed into a chilled 0.05% gold chloride solution for about 60 min with agitation in the refrigerator. After three rinses in cold distilled water to remove

excess gold chloride, sections were placed into cold 0.05% oxalic acid for 2 min, brought to room temperature and placed into a 1% solution of thiosulphate at 20° C for 1–1.5 h.

Sections were processed for electron microscopy using a routine schedule including poststaining with 2% osmium tetroxide, rapid dehydration and embedding in Epon. The use of 80–100 µm thick sections allowed us to visualize the de-impregnated cells in the block of resin. Serial thin sections were taken of critical structures and all sections were stained with uranyl acetate and lead citrate before examination with a Philips 300 electron microscope.

## Results

### *Golgi–electron microscopic description of non-pyramidal neurons*

Seven non-pyramidal and four pyramidal neurons of the CA 1 area were included in this description from which three non-pyramidal neurons are illustrated (Figs 1–14). The non-pyramidal neurons included in this study were different from pyramidal neurons in regard to their light and electron microscopic characteristics. Somata of these neurons were located in any of the strata of the CA 1 area. The somata illustrated in this study were obtained from strata pyramidale and oriens, but neurons from the other layers displayed the same features.

The somata of non-pyramidal neurons had ovoid or elliptical shapes and were usually multipolar, giving rise to three or more dendrites (Figs 1–3). These dendrites lacked spines and were often beaded with numerous swellings or varicosities along their entire length (Fig. 2). The non-pyramidal neurons of stratum pyramidale had both basal and apical dendritic trees, similar to those of pyramidal cells. However, the non-pyramidal neurons had very few dendritic branches. In addition, the basal dendrites that extended into stratum oriens were shorter than the apical dendrites that reached into stratum moleculare (Figs 1, 2). In contrast, the non-pyramidal neurons of stratum oriens usually had dendrites restricted to this layer with an occasional branch that penetrated into strata pyramidale and radiatum (Fig. 3).

The axon of non-pyramidal neurons originated from either the cell body or one of the proximal dendrites and arborized adjacent to the cell body (Figs 1, 2). Some of the axons arborized mainly in the stratum radiatum, while others arborized in the strata pyramidale and oriens. Basket cells (Ramón y Cajal, 1911; Lorente de Nó, 1934) and chandelier cells (Somogyi *et al.*, 1983) that give rise to an extensive axonal plexus among pyramidal cell bodies and axon initial segments, respectively, were not observed in this analysis.

In electron microscopic preparations, one of the most obvious features of these non-pyramidal neurons was the multiple, deep and complex nuclear infoldings that could always be found for each neuron in serial sections (Figs 4, 6, 7). In addition, the nuclei of non-pyramidal neurons contained intranuclear rods or sheets (Fig. 6). The perikaryal cytoplasm displayed numerous mitochondria and cisternae of both granular endoplasmic reticulum and Golgi complex. Asymmetric axosomatic synapses were frequently observed between terminals and non-pyramidal cell bodies, and formed about 20% of the total number of axosomatic synapses (Figs 5, 8). The dendrites of these

neurons lacked spines. Instead, they displayed swellings or varicosities that contained microtubules and numerous mitochondria (Figs 9, 10). Both symmetric and asymmetric synapses appeared on all portions of these dendrites (Fig. 10).

The axons of the analysed Golgi-impregnated non-pyramidal neurons formed symmetric synapses mainly with dendritic shafts that were found in both strata radiatum and oriens (Figs 11, 12). Only a few axons formed axosomatic synapses with pyramidal cells in our preparations. This latter finding was probably due to our inability to impregnate basket cells.

Pyramidal neurons had their somata within stratum pyramidale and displayed spiny dendrites (Fig. 2). Their somata contained a nucleus with either small, finger-like protrusions or, rarely, a deeper infolding that appeared to split the nucleus. However, they never displayed either intranuclear rods and sheets or multiple complex nuclear infoldings, two features found for non-pyramidal neurons. About 10% of the pyramidal neurons displayed two nucleoli within their nuclei. The axosomatic synapses formed

**Fig. 1.** Camera lucida drawing of two non-pyramidal neurons (A, B) from an 80  $\mu\text{m}$  thick section. Both neurons are located in the stratum pyramidale close to the border with stratum oriens and have dendrites that enter into both stratum oriens and radiatum. Neuron A has an axonal plexus (small arrows) in stratum oriens in the vicinity of its cell body whereas neuron B has an axon arborization in the outer part of stratum oriens (large arrows). The two axons appear to cross each other in this diagram but in actuality they were located at different depths of focus. Scale bar: 50  $\mu\text{m}$ .

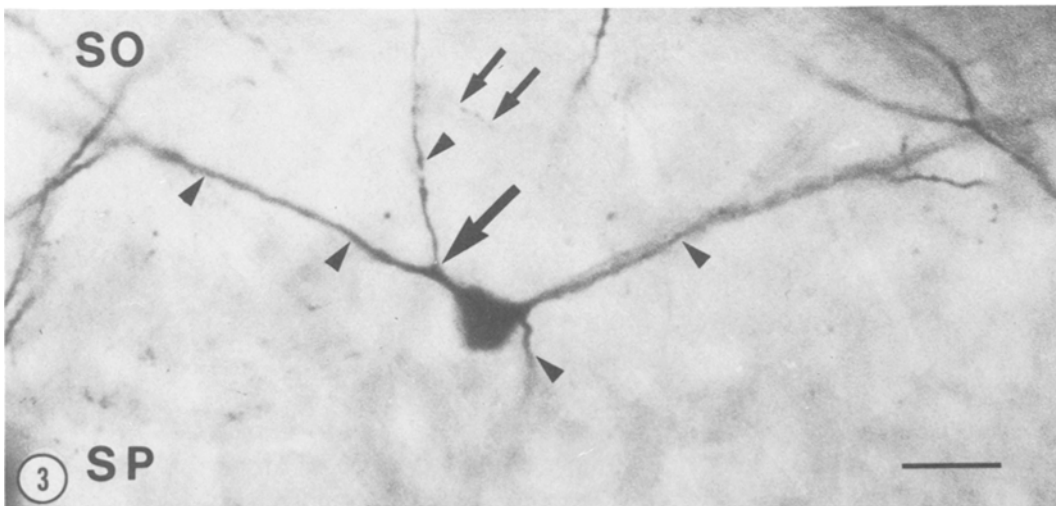
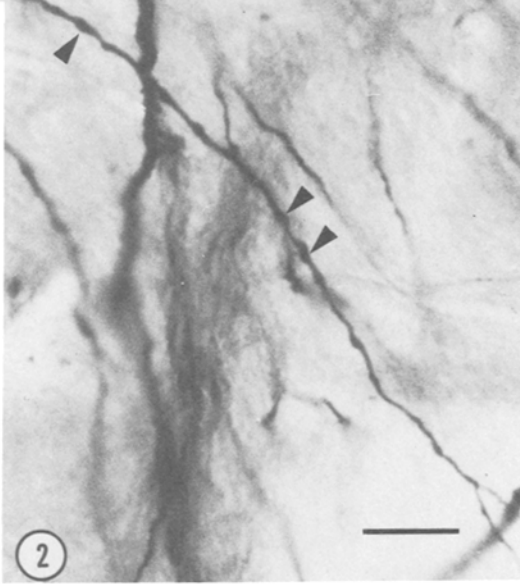
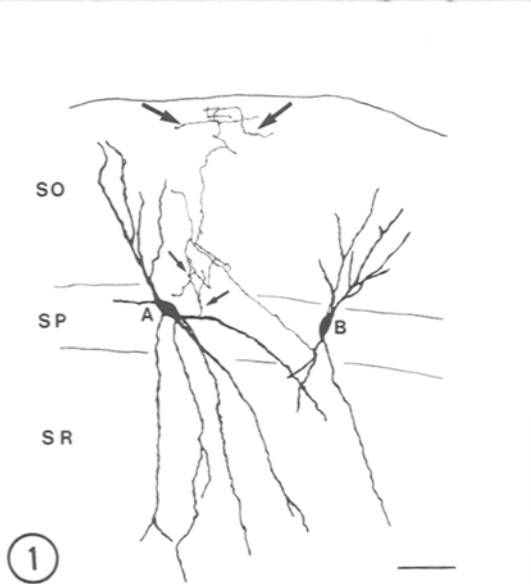
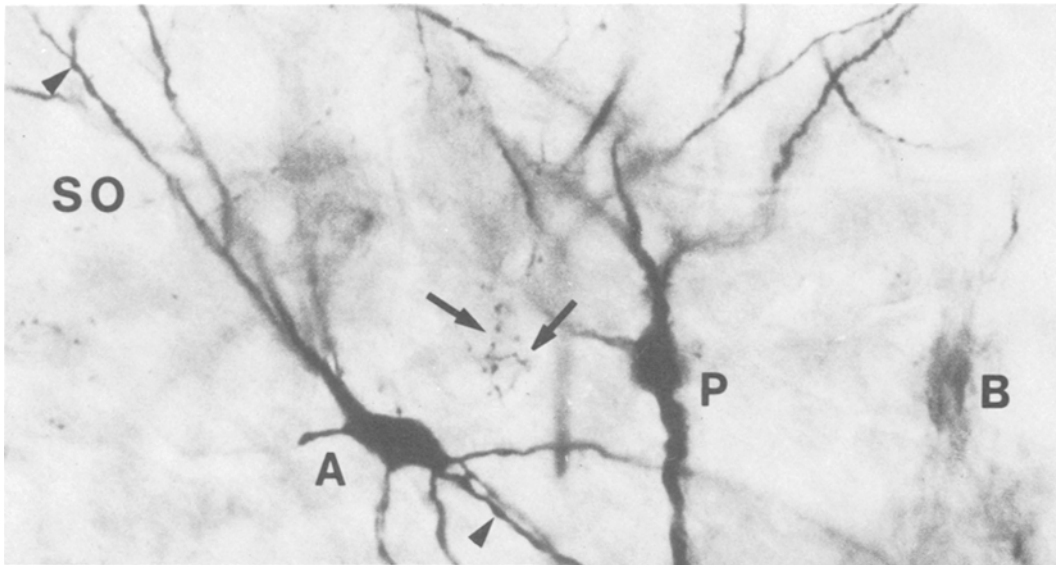
**Fig. 2.** Photomicrograph of a non-pyramidal neuron A (from Fig. 1) and an adjacent pyramidal neuron (P) with a prominent apical dendrite that extends into stratum radiatum. The soma of the other non-pyramidal neuron (B) illustrated in Fig. 1 is out of the plane of focus. Neuron A displays beaded dendrites (arrowheads) in both stratum radiatum and stratum oriens. The axon of this neuron forms an axonal plexus (arrows) close to its soma. Scale bar: 25  $\mu\text{m}$ .

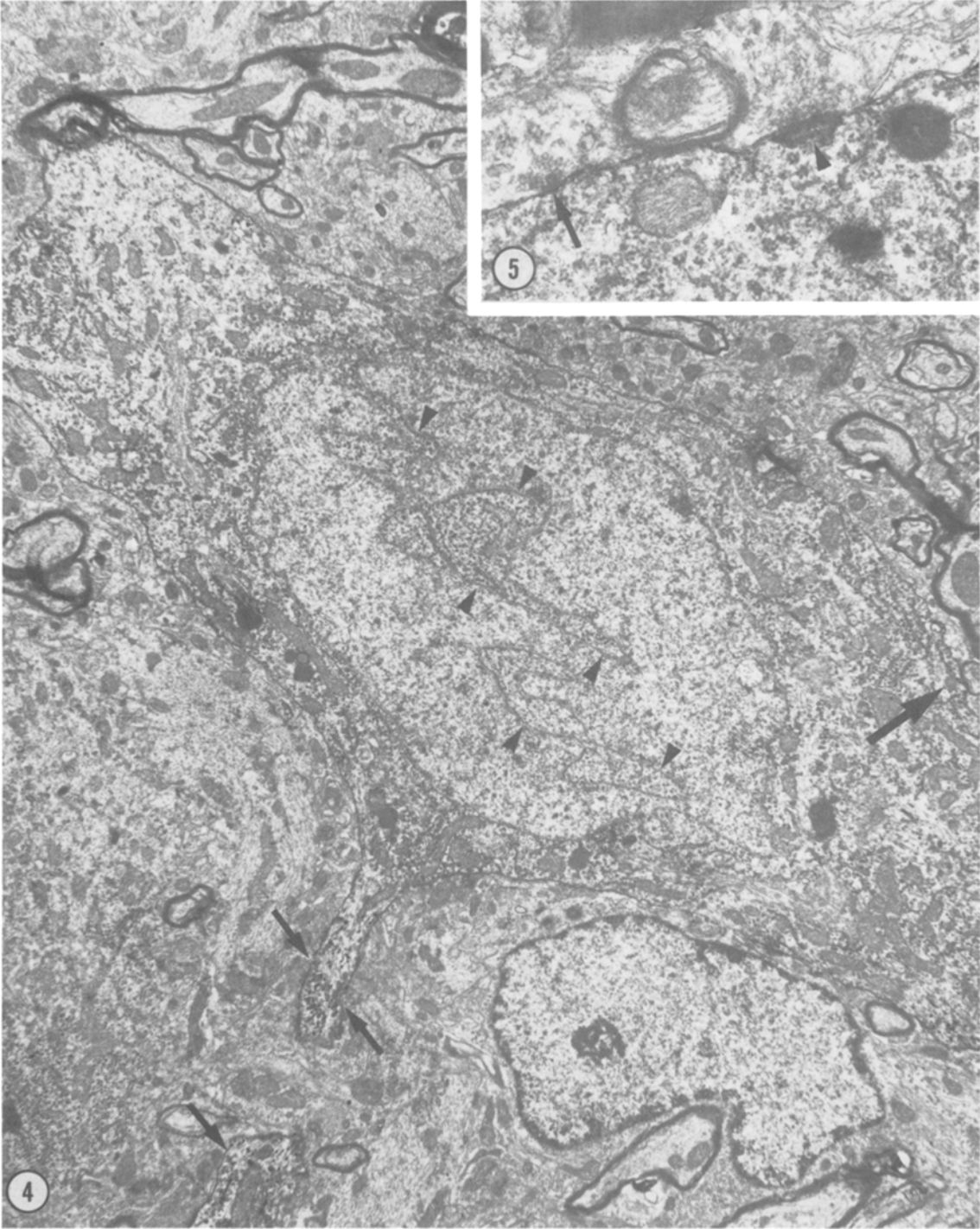
**Fig. 3.** Photomicrograph of a non-pyramidal neuron in stratum oriens near the border with stratum pyramidale. This neuron has smooth dendrites (arrowheads) that remain mainly in stratum oriens. The axon of this neuron is partly impregnated (small arrows). The fine structure of the dendritic bifurcation (large arrow) is shown in Fig. 9. Scale bar: 25  $\mu\text{m}$ .

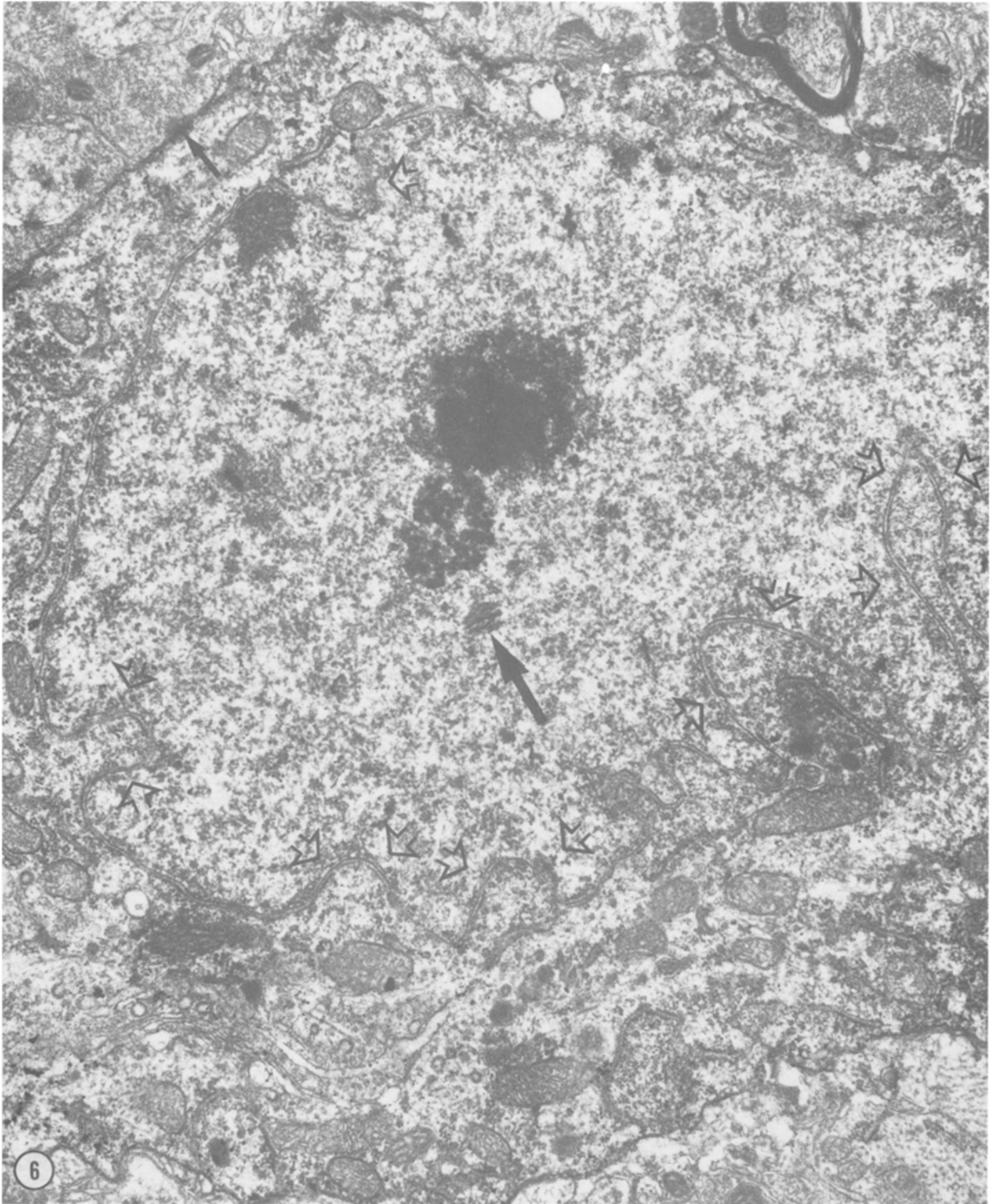
**Fig. 4.** Electron micrograph of the soma of non-pyramidal neuron A from Figs 1 and 2. Gold particles are shown in the cell body and within one of the proximal dendrites (small arrows). The cell nucleus is divided in half by deep and multiple infoldings (arrowheads). This soma is contacted by axon terminals, two of which are shown in Fig. 5 (large arrow).  $\times 10\ 000$ .

**Fig. 5.** Electron micrograph of two terminals that form axosomatic synapses with the non-pyramidal neuron in Fig. 4. One terminal forms an asymmetric synapse with a subjunctional dense body (arrowhead) whereas the other terminal forms a symmetric synapse (arrow).  $\times 22\ 000$ .

**Fig. 6.** Electron micrograph of the soma of non-pyramidal neuron B from Figs 1 and 2. Two typical features of non-pyramidal neurons are present in this single section: an intranuclear rod (large arrow) and an asymmetric axosomatic synapse (small arrow). Although some nuclear infoldings (open arrows) are shown in this section, other sections of this soma displayed more severe infoldings.  $\times 15\ 000$ .









with pyramidal cell bodies appeared to be symmetric or an intermediate type with a wide synaptic cleft.

*Use of electron microscopic features for the identification of afferents*

Degenerating terminals from the commissural pathway formed asymmetric synapses with dendrites of the Golgi-impregnated non-pyramidal neurons that were found in both strata radiatum and oriens (Figs 13, 14). Degenerating terminals that formed axosomatic synapses with these non-pyramidal neurons were less frequent. In general, the degenerating terminals were mainly associated with pyramidal cell dendrites in stratum oriens. However, many synapses made by degenerating terminals were difficult to classify because the presynaptic and postsynaptic elements were obscured by degenerating electron opaque structures and/or gold particles. Therefore, a number of non-impregnated, non-pyramidal neurons were analysed. These somata were identified

---

**Fig. 7.** Electron micrograph of the stratum oriens neuron from Fig. 3. Two features of non-pyramidal neurons are present in this section: the deep infoldings (arrowheads) in the cell nucleus (N) and an asymmetric axosomatic synapse (arrow).  $\times 6500$ .

**Fig. 8.** Enlargement of the asymmetric axosomatic synapse with an associated subjunctional dense body (arrow) shown in Fig. 7.  $\times 32\ 000$ .

**Fig. 9.** Electron micrograph of the site of dendritic bifurcation indicated by the large arrow in Fig. 3. Terminals form synapses with these dendrites (D) at sites of varicosities (arrow) and at sites of constrictions (arrowheads).  $\times 14\ 300$ .

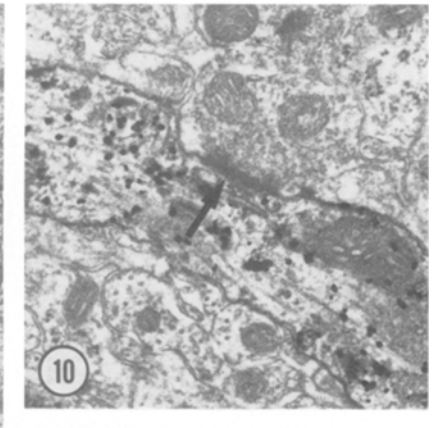
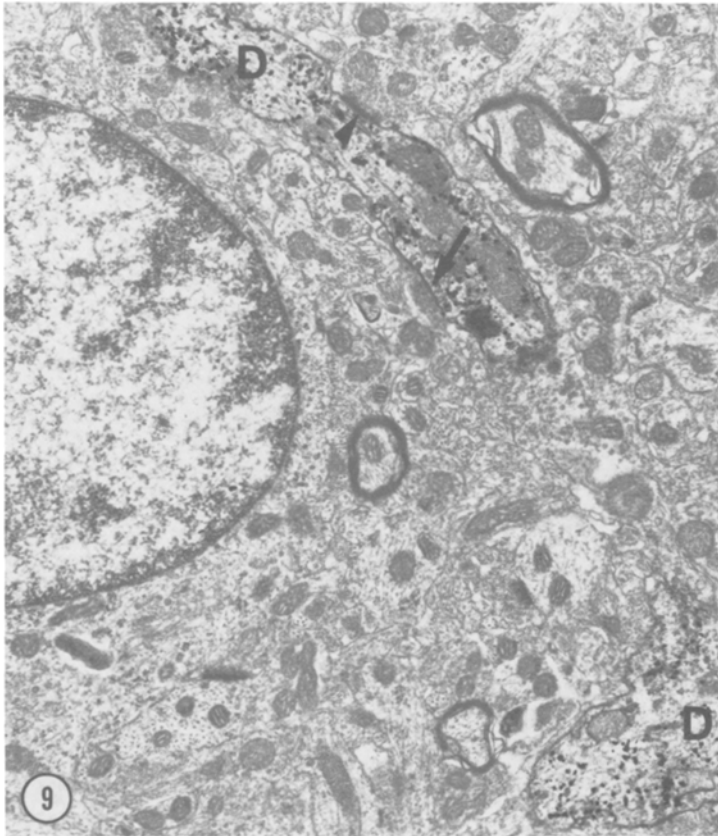
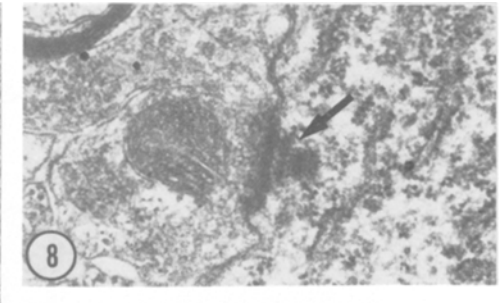
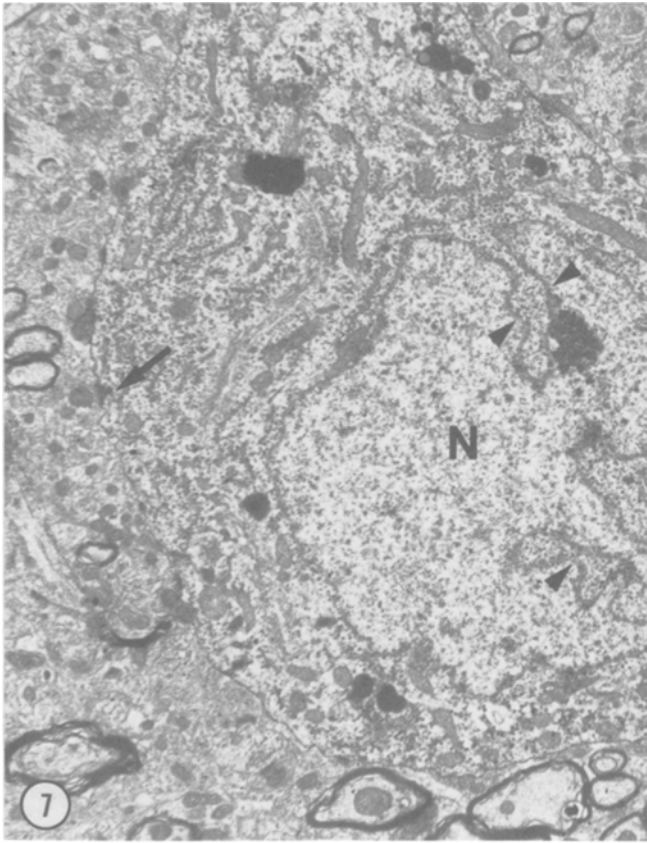
**Fig. 10.** Enlargement of a portion of the dendrite in Fig. 9 showing an asymmetric axodendritic synapse (arrow).  $\times 25\ 000$ .

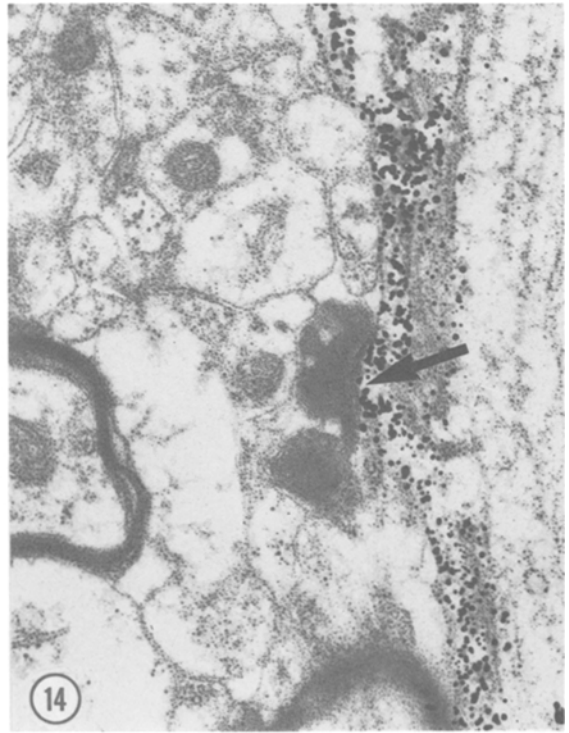
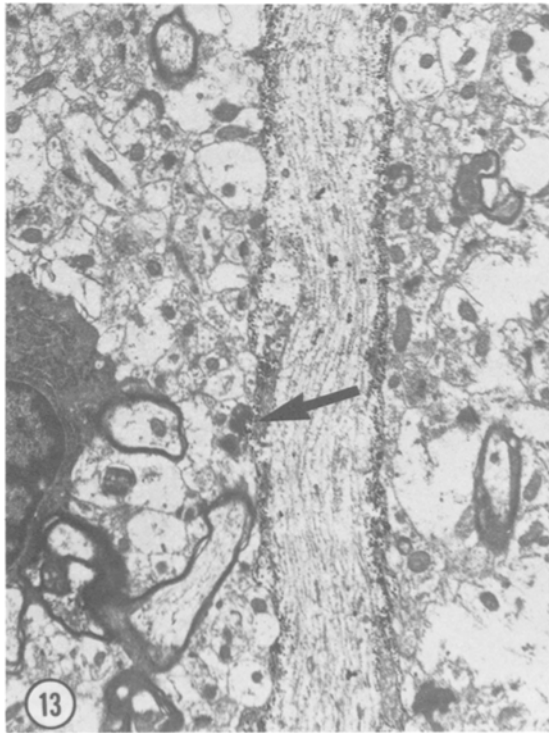
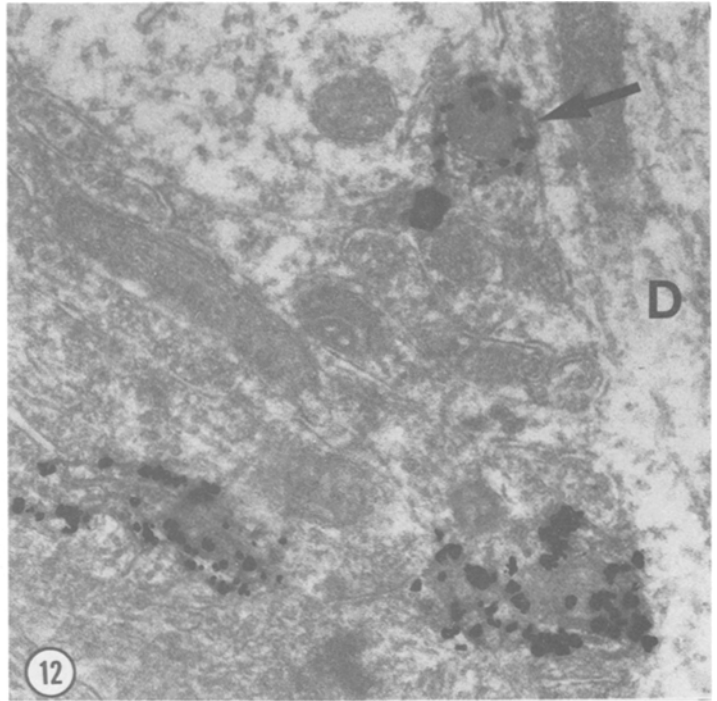
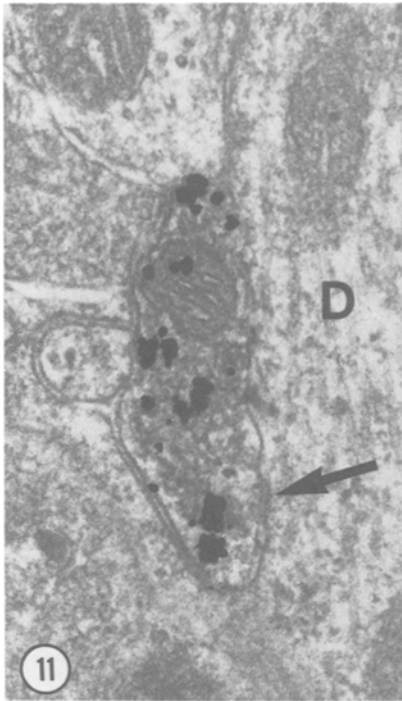
**Figs 11, 12.** Electron micrographs of Golgi-impregnated axons from non-pyramidal neurons. Fig. 11 shows a terminal from the stratum oriens non-pyramidal neuron from Fig. 3. It forms a symmetric synapse (arrow) with a dendrite (D).  $\times 60\ 000$ . Fig. 12 shows a branched axon from non-pyramidal neuron A and one axonal branch forms a symmetric synapse (arrow) with a dendrite (D).  $\times 60\ 000$ .

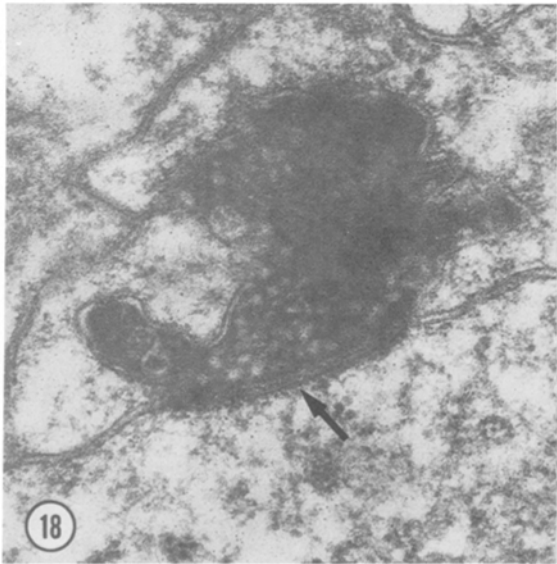
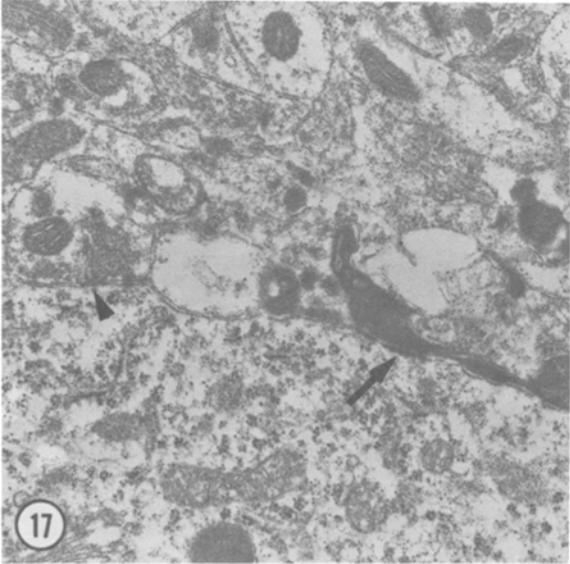
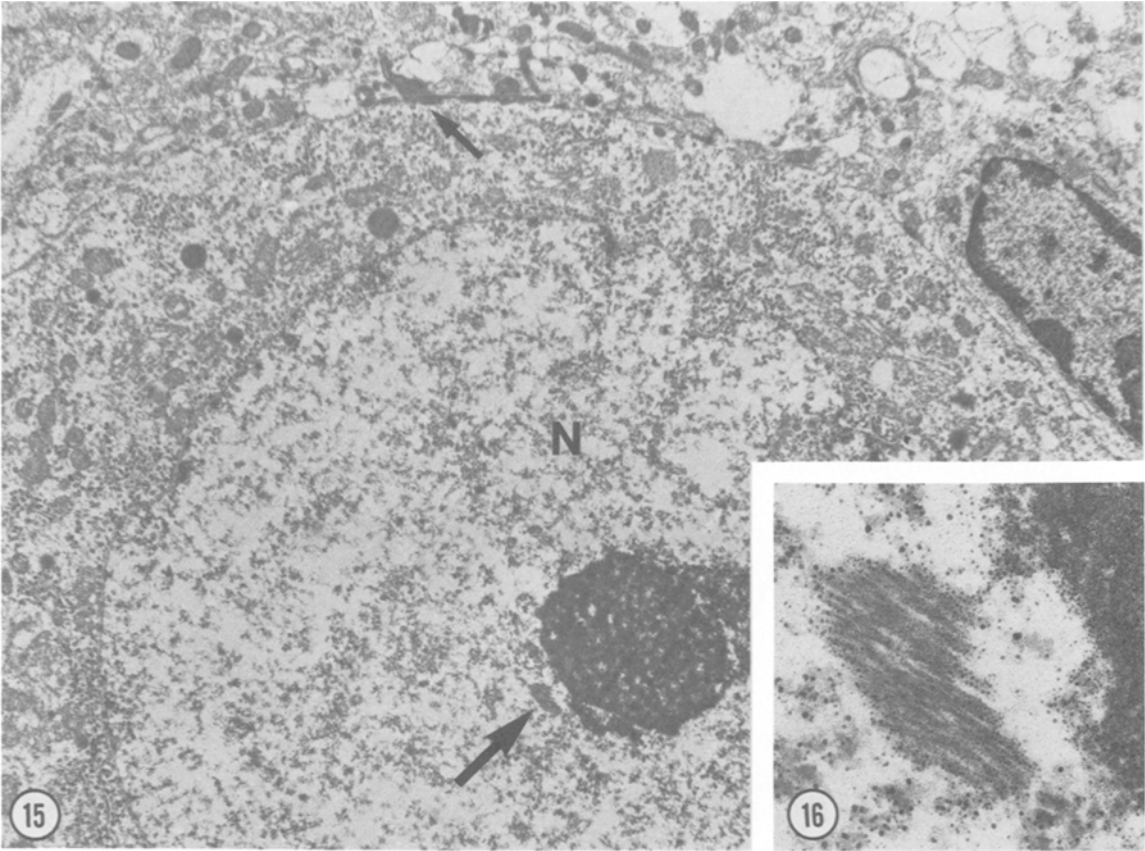
**Figs 13, 14.** Electron micrographs of a degenerating commissural terminal that forms an asymmetric synapse (arrows) with a smooth dendrite of the non-pyramidal neuron in stratum oriens.  $\times 10\ 000$  and  $\times 35\ 000$ , respectively.

**Figs 15–17.** Electron micrographs of a soma of a non-impregnated, non-pyramidal neuron from the stratum oriens. Fig. 15 shows a portion of this soma including the nucleus (N), intranuclear rod (large arrow) and a degenerating commissural terminal (small arrow). In other sections through this soma, we observed deep nuclear infoldings and asymmetric axosomatic synapses formed by non-degenerating terminals.  $\times 12\ 000$ . Fig. 16 shows an enlargement of the intranuclear rod.  $\times 60\ 000$ . Fig. 17 is an enlargement of the degenerating commissural terminal that forms an asymmetric axosomatic synapse (arrow). A nearby, normal terminal forms a symmetric synapse (arrowhead) with this same soma.  $\times 19\ 000$ .

**Fig. 18.** Electron micrograph of another degenerating commissural terminal that forms an asymmetric synapse (arrow) with a soma of a non-pyramidal neuron that was identified with the criteria established in this report.  $\times 55\ 000$ .







using the same criteria that were utilized for quantitative analysis (unpublished observations). Degenerating commissural terminals were found to form asymmetric synapses with the somata of non-pyramidal neurons (Figs 15–18). This observation was consistent with the findings obtained from the Golgi preparations.

## Discussion

The present study has utilized a combined Golgi–electron microscopic method to analyse non-pyramidal neurons in the rat hippocampus. This method facilitated the ultrastructural analysis of these cells because neurons with established light microscopic features can readily be identified in electron microscopic preparations by the deposition of gold particles within their cytoplasm. Since the label extends into the dendrites and axons, the synaptic connections of these labelled neurons may also be analysed.

Non-pyramidal neurons have certain ultrastructural characteristics that appear to be specific for this cell type in CA 1. These features include deeply infolded nuclear envelopes, the presence of intranuclear rods or sheets, and asymmetric axosomatic synapses. All three criteria are infrequently observed together in a single thin section because the intranuclear rods can vary in size, but most are small. In contrast, intranuclear rods or sheets were never observed in pyramidal neurons. However, pyramidal neurons may often contain double nucleoli and these have not been found in non-pyramidal neurons. These differences between the two neuronal types may help to identify their somata in preparations which lack Golgi impregnation.

In addition to the somal differences between pyramidal and non-pyramidal neurons, differences were also observed for their dendrites and axons. For example, the dendrites of non-pyramidal neurons are smooth and varicose, whereas pyramidal cells have spiny dendrites. Also, the axons of non-pyramidal neurons form symmetric synapses with postsynaptic structures, such as dendrites and somata. The axons of pyramidal neurons form asymmetric synapses. Together, these features distinguish non-pyramidal neurons from pyramidal neurons.

Our findings that demonstrate an anatomical circuit for commissural axons terminating upon the dendrites and somata of non-pyramidal neurons may provide a basis for feed-forward inhibition in the commissural pathway of the rat because neuronal somata with features described here for non-pyramidal neurons contain glutamate decarboxylase (GAD), the synthesizing enzyme for the inhibitory neurotransmitter GABA (Ribak *et al.*, 1978). This conclusion is consistent with results from previous anatomical studies in the guinea-pig that have demonstrated commissural afferents forming asymmetric synapses with non-pyramidal neurons, some of which contain immunoreactivity for GAD (Frotscher & Zimmer, 1983; Frotscher *et al.*, 1984). Such connections of non-pyramidal neurons are consistent with electrophysiological data that suggest commissural fibres form direct synapses with inhibitory local circuit neurons (Buzsáki & Czéh, 1981; Buzsáki & Eidelberg, 1982).

In the present study, degenerating commissural terminals were not observed to form

axosomatic contacts with pyramidal neurons, although they did contact their dendrites. These findings are different from those in the dentate gyrus where both basket and granule cells receive direct commissural connections onto both their dendrites and somata (Seress & Ribak, 1984). This difference in the locations of commissural terminals might be the basis for some of the differences that exist between the electrophysiological findings in the hippocampus and dentate gyrus.

### Acknowledgements

The authors gratefully acknowledge Margot Brundage, Yashoda Jhurani and Mária Tárnok for technical assistance. This work was supported by Grant NS-20228 from the National Institutes of Health.

### References

- ALONSO, A. & KÖHLER, C. (1982) Evidence for separate projections of hippocampal pyramidal and non-pyramidal neurons to different parts of the septum in the rat brain. *Neuroscience Letters* **31**, 209–14.
- BUZSÁKI, G. & CZÉH, G. (1981) Commissural and perforant path interaction in the rat hippocampus. *Experimental Brain Research* **43**, 429–38.
- BUZSÁKI, G. & EIDELBERG, E. (1982) Direct afferent excitation and long-term potentiation of hippocampal interneurons. *Journal of Neurophysiology* **48**, 597–607.
- CHANG, L. W. & DYER, R. S. (1984) Early pathological effects of trimethyltin (TMT) on the basket cells in the fascia dentata of the hippocampal formation. *Society for Neuroscience Abstracts* **10**, 816.
- CHRONISTER, R. B. & DeFRANCE, J. F. (1979) Organization of projection neurons of the hippocampus. *Experimental Neurology* **66**, 509–23.
- FAIRÉN, A., PETERS, A. & SALDANHA, S. (1977) A new procedure for examining Golgi impregnated neurons by light and electron microscopy. *Journal of Neurocytology* **6**, 311–37.
- FROTSCHER, M. & ZIMMER, J. (1983) Commissural fibers terminate on non-pyramidal neurons in the guinea pig hippocampus – a combined Golgi/EM degeneration study. *Brain Research* **265**, 289–93.
- FROTSCHER, M., LÉRÁNT, CS., LÜBBERS, K. & OERTEL, W. H. (1984) Commissural afferents innervate glutamate decarboxylase immunoreactive non-pyramidal neurons in the guinea pig hippocampus. *Neuroscience Letters* **46**, 137–43.
- GAYOSO, M. S., DIAZ-FLORES, L., GARRIDO, M., SANCHEZ, G. & VELASCO, E. (1979) Hippocampal formation. IV. Interneurons. *Morphología Normal y Patológica* **3**, 247–77.
- LORENTE DE NÓ, R. (1934) Studies on the structure of the cerebral cortex. II. Continuation of the study of the Ammonic system. *Journal für Psychologie und Neurologie* **46**, 113–77.
- RAMÓN Y CAJAL, S. (1911) *Histologie du Système Nerveux de l'Homme et des Vertébrés*. Vol. II. Paris: S. A. Maloine.
- RIBAK, C. E. & SERESS, L. (1983) Five types of basket cell in the hippocampal dentate gyrus: a combined Golgi and electron microscopic study. *Journal of Neurocytology* **12**, 577–97.
- RIBAK, C. E., VAUGHN, J. E. & SAITO, K. (1978) Immunocytochemical localization of glutamic acid decarboxylase in neuronal somata following colchicine inhibition of axonal transport. *Brain Research* **140**, 315–32.

- SERESS, L. & RIBAK, C. E. (1984) Commissural axons synapse with identified basket cells in the rat dentate gyrus: a combined degeneration-Golgi-electron microscopic study. *Journal of Neurocytology* **13**, 215-25.
- SOMOGYI, P., SMITH, A. D., NUNZI, M. G., GORIO, A., TAKAGI, H. & WU, J. Y. (1983) Glutamate decarboxylase immunoreactivity in the hippocampus of the cat. Distribution of immunoreactive terminals with special reference to the axon initial segment of pyramidal neurons. *Journal of Neuroscience* **3**, 1450-68.
- TÖMBÖL, T., BABOSA, M., HAJDU, F. & SOMOGYI, G. (1979) Interneurons: an electron microscopic study of the cat's hippocampal formation, II. *Acta morphologica Academiae scientiarum hungaricae* **27**, 297-313.