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Five types of basket cell in the hippocampal dentate gyrus: a combined Golgi and electron microscopic study

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Summary

Five types of basket cell in the hippocampal dentate gyrus of rats were analysed with a combined Golgi and electron microscopic method. Light microscopic observations show that the large somata of these different cell types are located either in the granule cell layer or within 30–50 μm of this layer. The somata of basket cells are pyramidal, horizontal, fusiform or multipolar. Dendrites of basket cells are aspiny or sparsely spiny and are found in all layers of the dentate gyrus. Their axons form an extensive plexus in the granule cell and lower molecular layers.

Electron microscopic preparations of Golgi-impregnated, gold-toned basket cells revealed gold-labelled neurons with distinct ultrastructural features. All somata of basket cells displayed an extensive perikaryal cytoplasm with large Nissl bodies and nuclei with infoldings, euchromatin, intranuclear rods and sheets, and large nucleoli. The aspiny dendrites as well as the somata had a mixture of asymmetric and symmetric synapses on their surfaces. Basket cell dendrites located in the hilus were contacted by numerous terminals with characteristics of mossy fibres derived from granule cells. Some of these terminals were identified positively in preparations that also contained impregnated granule cells. The axons of basket cells formed exclusively symmetric synapses. The most common postsynaptic structures to these terminals were the somata and dendrites of granule cells. Dendritic spines were rarely contacted by basket cell axons while the axon hillocks and initial segments of granule cells were never contacted. These findings are consistent with previous immunocytochemical, and physiological data that indicate feedback inhibitory mechanisms in the dentate gyrus are mediated via mossy fibre collaterals which synapse with GABAergic basket cells. In addition, the electron microscopic data for basket cells are similar to those for aspiny stellate cells in the neocortex, another type of cortical, GABAergic local circuit neuron. Thus, the basket cells in the dentate gyrus may have a function similar to other inhibitory, cortical local circuit neurons.

Introduction

Two types of basket cell in the dentate gyrus of the hippocampus have been described in Golgi preparations by Cajal (1911) and Lorente de Nó (1934). Both send their axons

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into the granule cell layer (GL) to form a basket plexus with the somata of granule cells (10–15 μm diameter). The larger somata of pyramidal basket cells (25–35 μm) lie either embedded in the hilar margin of the GL or directly beneath it. These cells have an apical dendrite which ascends through the GL and basal dendrites that run along the hilar border or penetrate the hilus. In contrast, the horizontal basket cell generally has only one main basal dendrite which arises from its soma and courses beneath the GL.

More recent Golgi studies at the light microscopic level have confirmed these results (Amaral, 1978; Gayoso *et al.*, 1979; Seress & Pokorny, 1981). Further, the results of these latter studies have revealed many types of local circuit neuron in the dentate gyrus as well as three additional types of basket cell. Two of these types have somata within the GL whereas the third type has its soma in the molecular layer. This latter type of basket cell is found in the part of the molecular layer adjacent to the GL and has a multipolar dendritic field and a large soma (Hazlett & Farkas, 1978; Seress & Pokorny, 1981; Seress & Ribak, 1983). One of the other basket cell types in the GL has its soma in the lower half of this layer and an apical dendrite similar to the pyramidal basket cell. However, this cell type, the fusiform basket cell, usually has one main basal dendrite which is oriented directly opposite its apical dendrite (Seress & Ribak, 1983). The remaining cell type is the inverted fusiform cell which is characterized by a soma located in the outer half of the GL and apical dendrites directed toward the hilus instead of the molecular layer (Seress & Pokorny, 1981; Seress & Ribak, 1983). Results from immunocytochemical studies have indicated that the basket axonal plexus (Barber & Saito, 1976; Ribak *et al.*, 1978) and the somata of all five types of basket cell (Seress & Ribak, 1983) contain glutamate decarboxylase, the synthesizing enzyme for the inhibitory neurotransmitter GABA. Thus, the somata and dendrites of basket cells of the dentate gyrus are morphologically heterogenous but the axons of these cells may all perform the same function of mediating GABAergic inhibition of granule cell somata.

Electron microscopic studies of the dentate gyrus have only recently concentrated on the description of local circuit neurons (Gayoso *et al.*, 1979), and, in particular, basket cells (Ribak & Anderson, 1980). The results from this latter study have shown that the morphology of pyramidal basket cells differs in many ways from that of the granule cell, aside from the obvious difference of their greater size (e.g. pyramidal basket cells display infolded nuclei, intranuclear rods and sheets, abundance of cisternae of granular endoplasmic reticulum and other perikaryal organelles). These characteristics are shared by other GABAergic neurons in the cerebral cortex (Ribak, 1978; Ribak *et al.*, 1978).

Since the ultrastructural analysis of basket cells has been limited to only the somata and the main proximal dendrites of one type of basket cell (Ribak & Anderson, 1980), much remains to be learnt about the basket cells to understand their role in hippocampal neuronal circuitry. For example, an analysis of the distal dendrites can provide information about the types of terminals that synapse with these cells. Furthermore, an examination of the distribution of synapses made by a basket cell's axon will allow for an understanding of the neurons that receive inhibitory terminals. The use of a combined Golgi–electron microscopic method (Fairén *et al.*, 1977) can provide this information

because an individual neuron is labelled with gold particles that facilitate its identification in both light and electron microscopic preparations. Therefore, this method was utilized in the present study to assess the ultrastructural characteristics of the five types of basket cell in the hippocampal dentate gyrus. A preliminary report of these results was presented recently (Ribak, 1982).

Methods

Fifty young albino rats (1–2 months of age) were utilized for these studies because impregnation of neurons is better in these preparations than in older adults. The brains of these rats were fixed by intracardiac perfusions with a single solution containing 4.0% paraformaldehyde, 1.25% glutaraldehyde and 0.002% calcium chloride in a 0.12 M phosphate buffer at pH 7.2. The perfused animals were stored overnight in the refrigerator before dissecting out the hippocampus the following day.

The hippocampus was processed for the Golgi–electron microscopic method according to Fairén *et al.* (1977). Briefly, the entire hippocampus was rinsed and placed into an osmium dichromate solution (1 g of osmium tetroxide and 12 g of potassium dichromate in 500 ml of distilled water). Each specimen was immersed in 50 ml of this solution and kept in the dark for four days. Then, the tissue was washed briefly in 0.75% silver nitrate and stored in this solution for three days. Following impregnation, the blocks were passed through 20, 40, 60, 80 and 100% solutions of glycerol before being cut.

These impregnated blocks were embedded in agar and were sectioned with the Sorvall tissue chopper. Sections were cut at 75 to 100 μm , collected on slides, coverslipped and examined in the light microscope. Basket cells in the dentate gyrus were identified, drawn with a Zeiss microscope equipped with a drawing tube and photographed.

The pieces of tissue that contained basket cells were hydrated to distilled water through a series of glycerol solutions. Then, the pieces 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, the tissue pieces were placed into cold 0.05% oxalic acid for 2 min. The tissue was then brought to room temperature and placed into a 1% solution of thiosulphate at 20° C for 1–1.5 h. Afterwards, the sections were rinsed in distilled water and examined in the light microscope to confirm the presence of the de-impregnated somata, dendrites and axons.

At this point, cells were processed for electron microscopy using a routine schedule that included post-staining with osmium tetroxide, rapid dehydration with acetone, and embedding in Epon. The use of 75–100 μm thick sections allowed us to visualize the de-impregnated cells in the cured block of resin. Serial thin sections were taken of critical structures and all sections were stained with uranyl acetate and lead citrate before examination in the electron microscope.

Description

LIGHT MICROSCOPIC OBSERVATIONS

All five types of basket cell were observed in Golgi preparations of the dentate gyrus. These neurons were identified by their characteristic features: large somal size, location within or close to the GL, smooth dendrites, and an axonal plexus above and within the GL. The observations were made on a total of 12 basket cells which included five pyramidal, two fusiform, two horizontal, one inverted fusiform and two molecular layer

types. This distribution of neurons obtained from Golgi preparations compared well with the distribution of basket cells observed in Nissl preparations (Seress & Pokorny, 1981).

Pyramidal basket cells

This cell type has a triangular-shaped soma located on the hilar border of the GL (Figs. 1A,C,4). The base of the soma is either aligned with the lower margin of the GL or protrudes beneath that margin for 10–30 μm into the hilus. A main apical dendrite ascends through the GL and into the molecular layer where it branches among the granule cell dendrites (Fig. 4). The axons of pyramidal basket cells originate from either this apical dendrite or the apical portion of the soma (arrows, Figs. 1A,C,4). Fine branches of the axon terminate in the GL where they appear to outline the somata and dendrites of granule cells. Basal dendrites of pyramidal basket cells arise from both sides of the somal base and extend into the hilar region subjacent to the GL. Both basal and apical dendrites lack spines but occasionally display filiform processes which are larger than typical spines.

Fusiform basket cells

Although the upper half of this cell type resembles that of the pyramidal basket cell (i.e. a major apical dendrite in the GL, numerous branches in the molecular layer, and a typical basket cell axon which originates from the apical dendritic tree), its soma and basal dendrites are different. The soma of the fusiform basket cell is widest in the middle and tapers down in width at each end (Fig. 1D). The apical and basal dendrites are usually oriented directly opposite to each other. Occasionally, smaller calibre dendrites

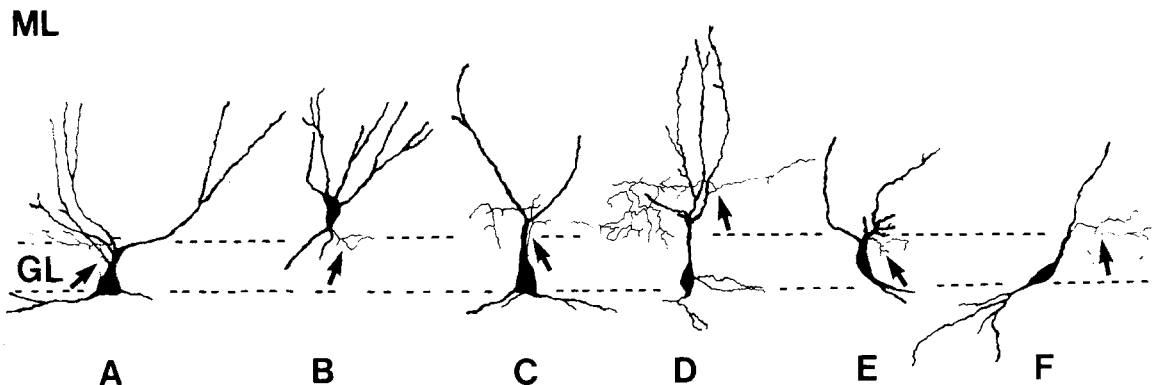


Fig. 1. Drawings of Golgi-impregnated and gold-toned basket cells examined by electron microscopy. (A) and (C) are pyramidal basket cells, (B) is a molecular layer basket cell, (D) is a fusiform type, (E) is an inverted fusiform type and (F) is a horizontal type of basket cell. The axons (arrows) of these cells arise from either somata or apical dendrites and send branches into both the molecular layer (ML) and the granule cell layer (GL), the boundaries of which are indicated roughly by the broken lines. $\times 110$.

emanate from the centre of the soma and extend into the GL, but their size is minor in comparison to the dendritic branches which arise from the apical and basal dendrites. The dendrites of fusiform basket cells lack spines. The axons of these cells terminate in the manner described for those of pyramidal basket cells (Fig. 18).

Horizontal basket cell

This cell type is an intermediate form between the pyramidal and fusiform basket cells (Figs. 1F,6,7). The soma has a wider central region than the fusiform cell type but is clearly not pyramid-shaped. The long axis of the soma is oriented obliquely to the GL (Figs. 1F,6,7). The single basal dendrite of this cell type is oriented parallel to the GL and located entirely within the hilus (Fig. 6). A major portion of the soma is also located subjacent to the GL. A prominent apical dendrite emerges from the soma and courses through the GL to enter the overlying molecular layer (Figs. 6,7). The axon which arises from this dendrite has a typical basket plexus. Both basal and apical dendrites lack spines.

Inverted fusiform basket cell

Unlike the previous cell types which have their somata located in the lower half of the GL or subjacent to the GL, this cell type has its soma located in the outer half of the GL, the part adjacent to the molecular layer (Fig. 1E). Apical dendrites of inverted basket cells descend into the hilus whereas basal dendrites branch into the molecular layer. Branches derived from the basal dendrites run obliquely to the long axis of the soma and thus define the basal dendritic tree. Both sets of dendrites are sparsely spinous. The axon arises from the dendrites in the molecular layer.

Molecular layer basket cell

This cell type has a multipolar soma located in the part of the molecular layer adjacent to the GL (Figs. 1B,9). The sizes of these somata vary more than any of the other types of basket cell. Dendrites are oriented in many different directions with at least one or two that penetrate through the GL (Fig. 9). All dendrites of this cell type are aspiny (Fig. 9); their axons branch in the GL.

ELECTRON MICROSCOPIC OBSERVATIONS

The ultrastructural features of all five basket cell types were very similar. For this reason they are considered together in the following description. The minor differences observed between the cell types are described under the following subheadings: somata, dendrites and axons.

Somata

The somata of gold-toned, Golgi-impregnated basket cells are readily identified in electron micrographs by the numerous, gold particles concentrated beneath the plasma membrane (Figs. 2,3,5,8,10). Occasionally, gold label is found in other parts of the soma.

These deposits of label clearly distinguish the gold-toned basket cell somata from neighbouring unlabelled granule cells.

Basket cell somata are found either in the GL or within 30–50 μm of this layer as predicted by the light microscopic data. The shapes and sizes of these somata vary between the different basket cell types. For example, the somata of pyramidal basket cells are triangular-shaped in thin sections (Fig. 2) and those of fusiform basket cells are ovoid with their long axes perpendicular to the GL (Fig. 5). In general, the labelled somata of basket cells are twice the size of the somata of granule cells (Figs. 2,5,8). This size difference is due partly to the large nuclei in basket cells but is mainly a result of a much thicker shell of perikaryal cytoplasm in these cells.

The nuclei of all types of basket cells display single large nucleoli, euchromatin, extensive nuclear infoldings, and intranuclear rods and sheets. Although all of these

Fig. 2. Electron micrograph of the pyramidal basket cell soma shown in Fig. 1C. This section contains only a small part of the nucleus (N) but illustrates two of the three basal dendrites (arrows) that arise from the soma. The perikaryal cytoplasm is packed with organelles and is much larger than that of neighbouring granule cells (G). An oligodendroglia (O) separates the granule cell layer from the subjacent hilus (H). $\times 7200$.

Fig. 3. Electron micrograph of another section through the soma of the pyramidal basket cell shown in Fig. 2. The nucleus displays an intranuclear rod (arrows) and, just below it, a nucleolus. The somata of granule cells (G) are the same as the ones in Fig. 2. $\times 7500$.

Fig. 4. Light micrograph of the pyramidal basket cell shown in Figs. 2 and 3. The soma and proximal apical dendrite are located in the granule cell layer along with an impregnated granule cell (G) that is out of the focal plane. The axon (arrow) arises from the apical dendrite. Two basal dendrites (white arrows) are the same ones shown in Fig. 2. $\times 700$.

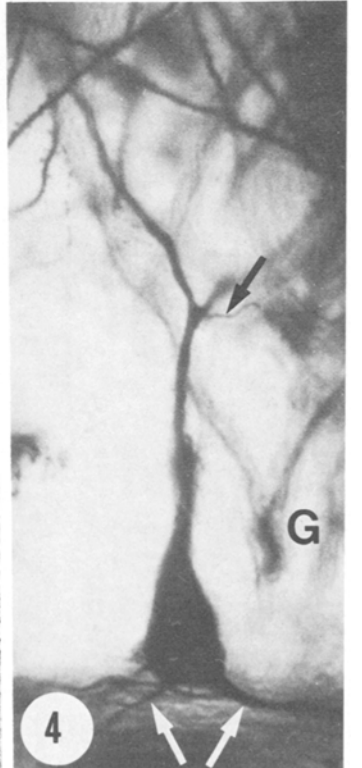
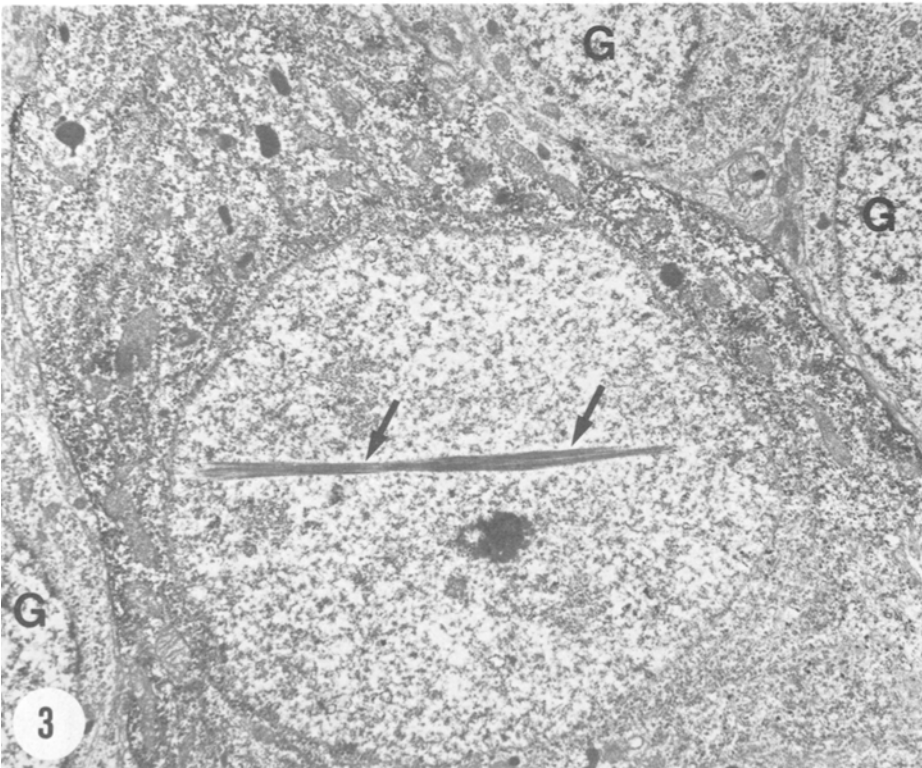
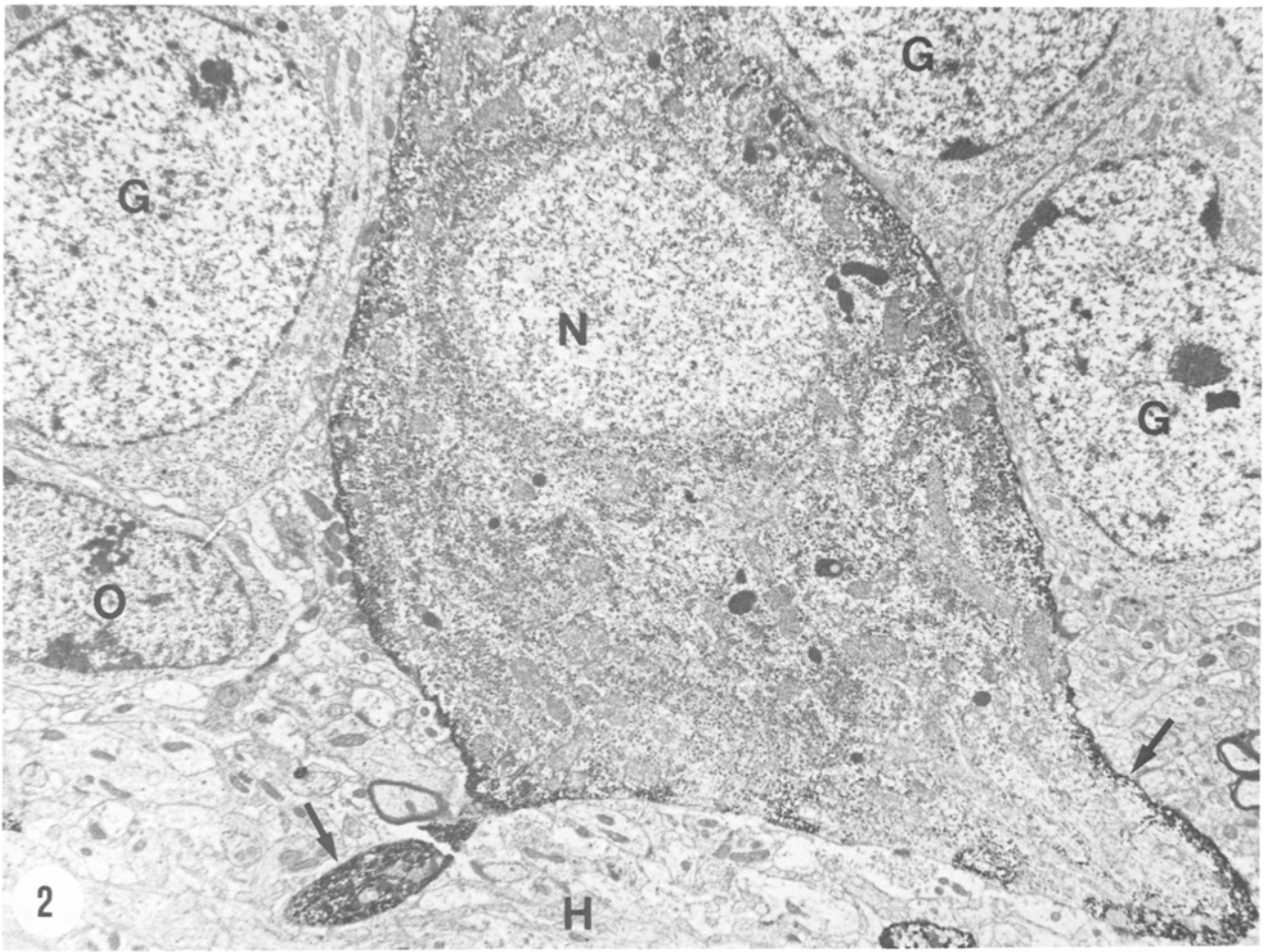
Fig. 5. Electron micrograph of the oval soma of the fusiform basket cell shown in Fig. 1D. The nucleus (N) appears bilobed in this section because deep infoldings (arrows) have occurred near the nucleolus which is located in the right part of the nucleus. The gold label is spread more homogeneously in this soma than in the others and has covered many of the cytoplasmic structures. This soma is located at the same level as the somata in the lowest row of granule cells (G) but also extends into the hilus (H). $\times 14\,000$.

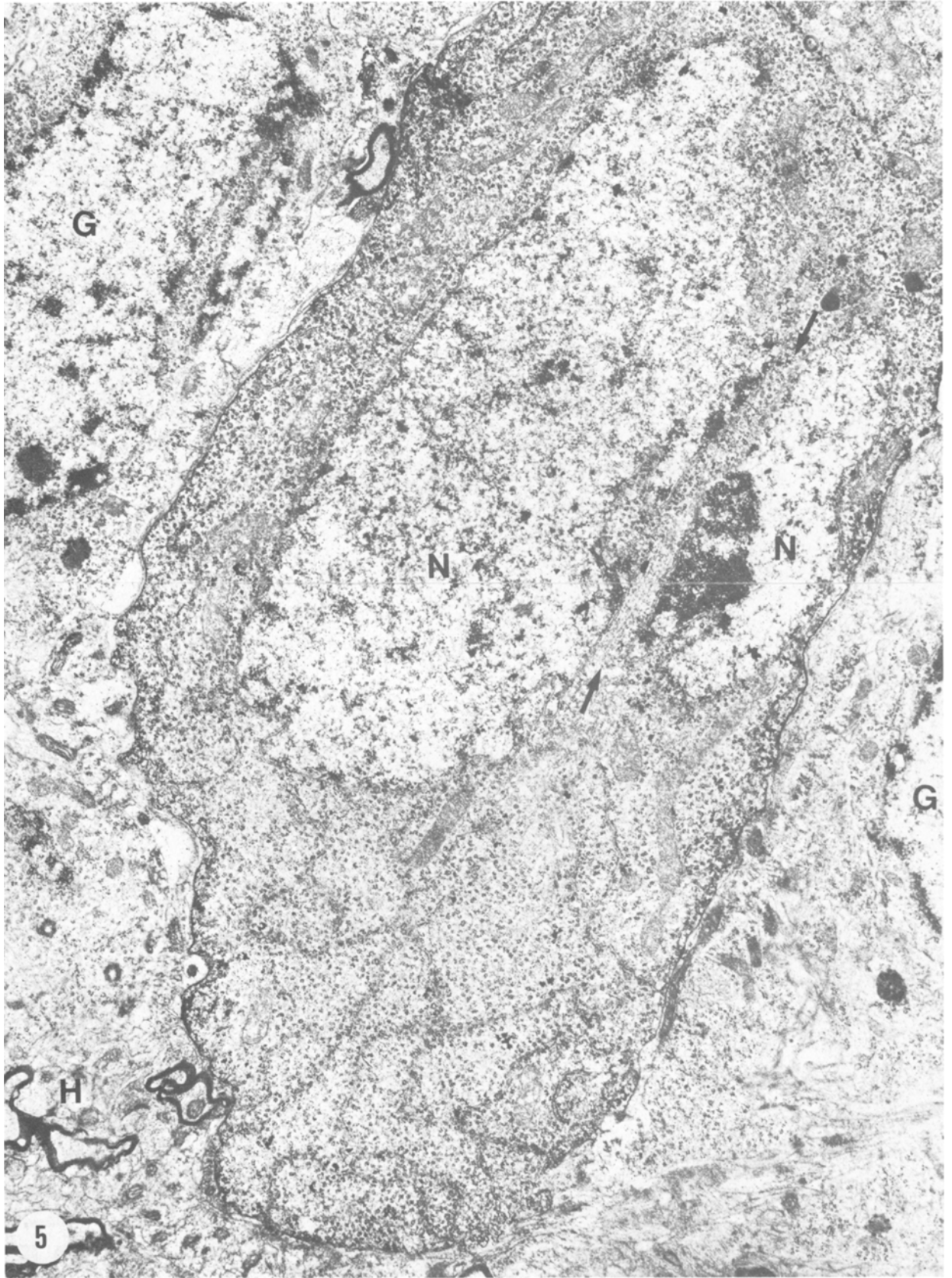
Figs. 6–8. Photomicrographs of the horizontal basket cell illustrated in Fig. 1F.

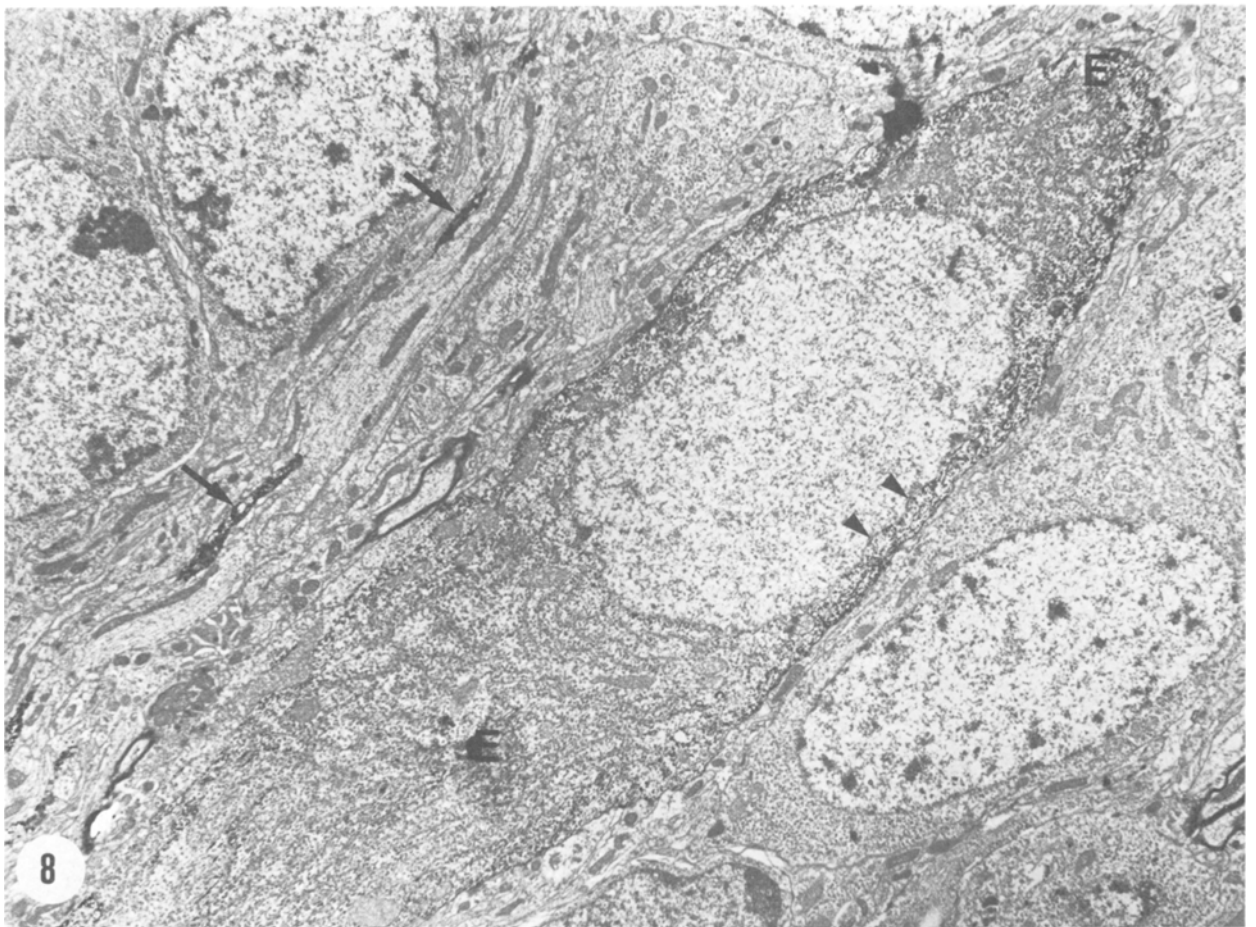
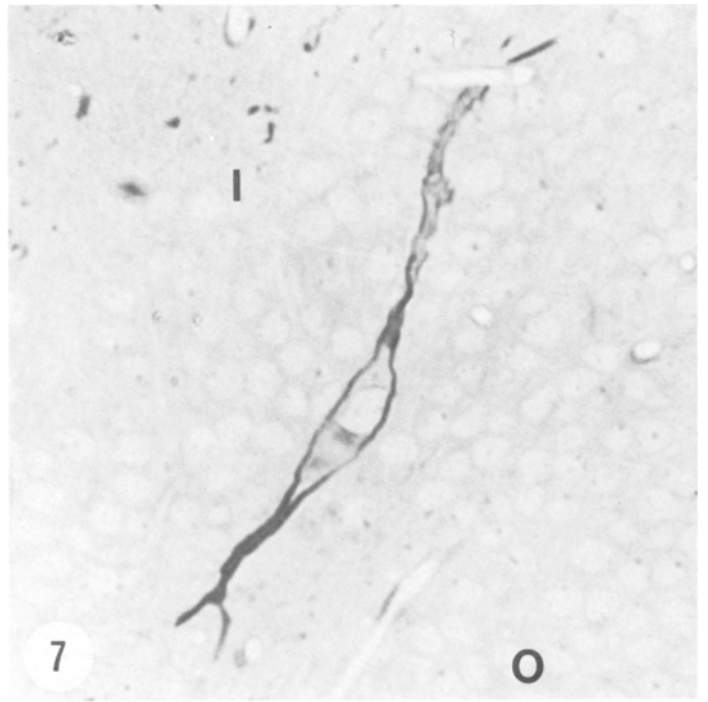
Fig. 6. Light micrograph of the silver-impregnated cell in a 100 μm thick section. A single large apical dendrite is directed toward numerous silver deposits in the molecular layer (ML). The soma gives rise to a single basal dendrite which has several branches in the hilus (H). An impregnated axon (arrow) of a probable granule cell lies just to the left of the soma of this horizontal basket cell. $\times 470$.

Fig. 7. A 1 μm section taken through the cell in Fig. 6 after it was gold-toned and embedded in plastic. This soma is located in the peak of the dentate gyrus where the inner (I) and outer (O) blades of granule cells meet. $\times 770$.

Fig. 8. An electron micrograph obtained from an adjacent thin section to that in Fig. 7. This soma is oval-shaped, has a slight ruffling to its nucleus (arrowheads) and large Nissl bodies (E) at the base of the basal and apical dendrites. The impregnated granule cell axon (arrows) shown in Fig. 6 runs parallel with the gold-labelled soma. $\times 6700$.







features are not present in every section, the use of serial sections has confirmed that every basket cell examined displays these characteristics. The nucleolus of basket cells is usually centrally located in the nucleus (Figs. 3,10). However, deep nuclear infoldings occasionally bring the nuclear envelope into apposition with the nucleolus (Fig. 5). The extent of these infoldings varies between simple ruffling of the nuclear envelope (Figs. 3,8) to instances where the nucleus appears bilobed (Fig. 5) and is not related to any particular basket cell type. The euchromatin nature of basket cell nuclei is based on the relatively greater amounts of heterochromatin observed along the inner surfaces of the nuclear envelopes of granule cells (Figs. 2,5,8). Most of the basket cells lack heterochromatin. Intranuclear rods (Figs. 3,10) or sheets are also observed in the nuclei of basket cells. The rods are composed of a loosely aggregated, parallel-oriented group of filaments whereas the sheets are formed by a regular lattice of filaments which in some cases form a cylindrical structure. These nuclear characteristics were observed clearly in all of the gold-labelled basket cells examined in this study and were not obscured by gold particles.

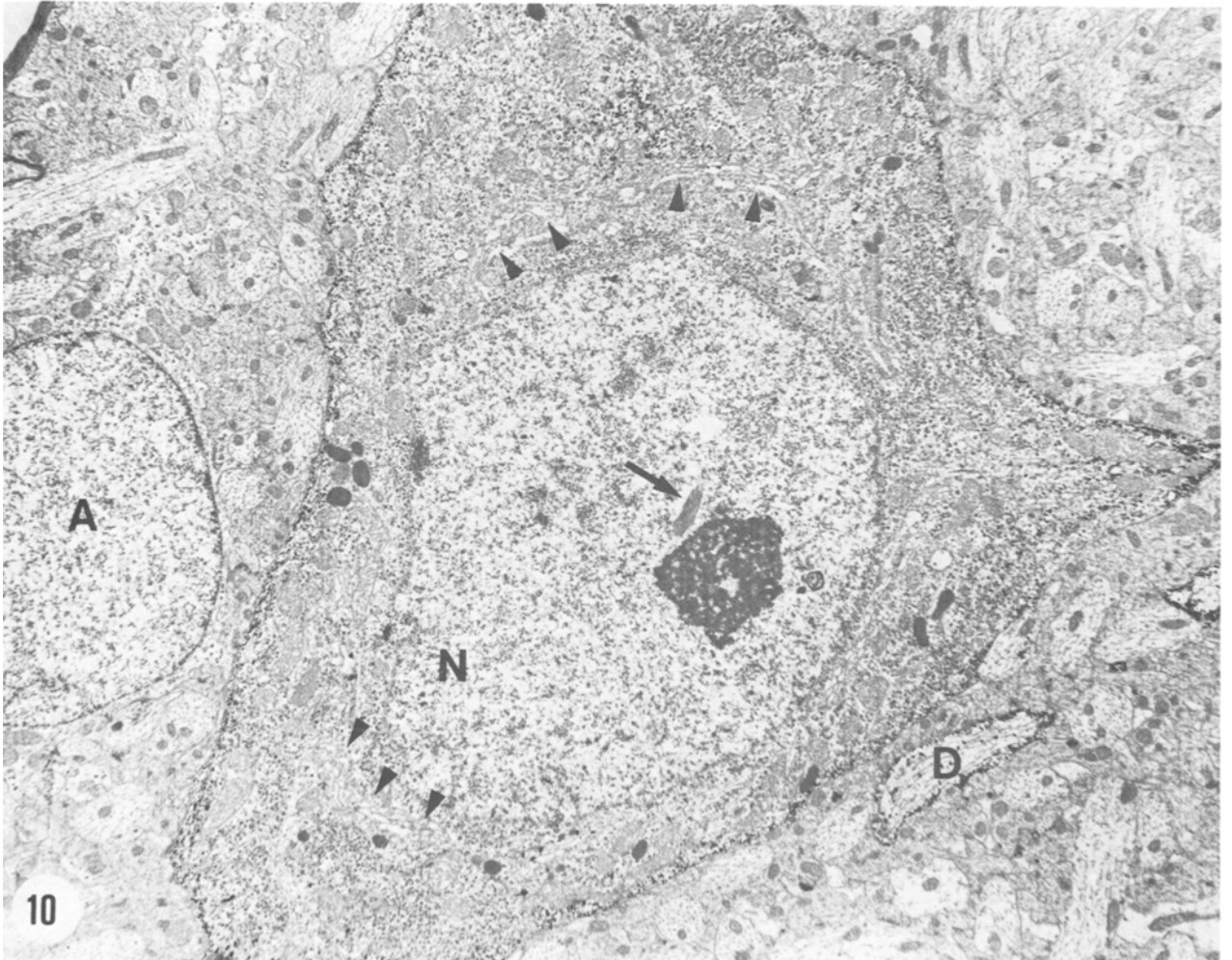
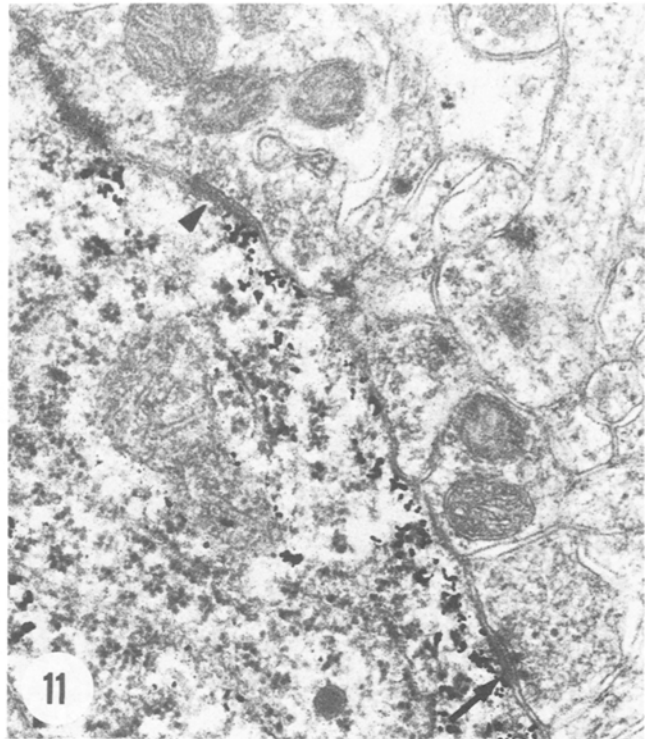
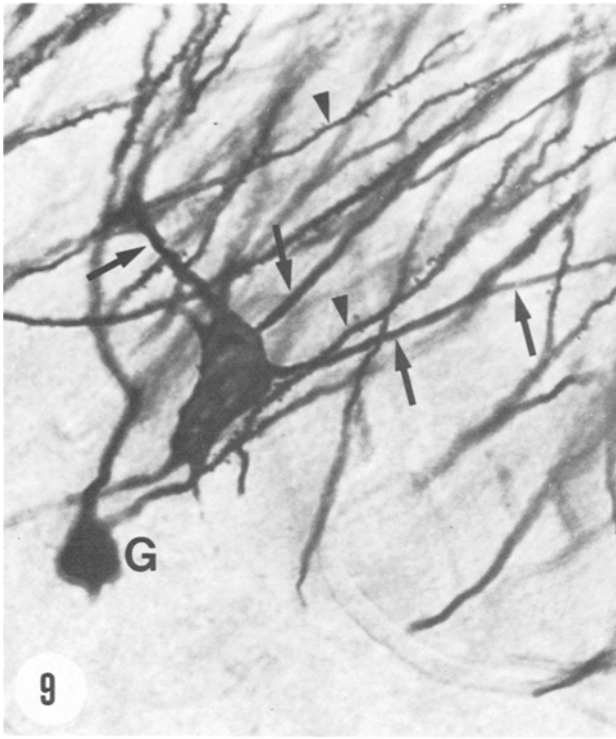
The thickness of the rim of cytoplasm that surrounds the nuclei of basket cells varies somewhat between cell types. However, the number and types of organelles observed in this cytoplasm are consistent for all of the varieties. The most striking feature is the large quantity of cisternae of granular endoplasmic reticulum which aggregate to form Nissl bodies between the nucleus and each of the proximal dendrites (Figs. 5,8). The cisternae and vesicles of the Golgi complex are also well-developed in the perikaryal cytoplasm (Fig. 10). In addition, numerous mitochondria, free ribosomes and lysosomes are present.

The somal surface is contacted by axon terminals that form both asymmetric and symmetric synapses (Fig. 11). The number of these synapses varies greatly for individual basket cells but most somata display at least two or three synapses per thin section. Most of the basket cell somata are embedded in the GL where they are mainly apposed by the somata of granule cells or thin glial sheaths that separate the somata of these two cell types.

Fig. 9. Light micrograph of the gold-toned molecular layer basket cell illustrated in Fig. 1B. The long dendrites (arrows) of this cell lack spines. In contrast, the impregnated dendrites (arrowheads) of granule cells display spines. A soma of a granule cell (G) is shown to the left of the molecular layer basket cell. $\times 900$.

Fig. 10. Electron micrograph of the same basket cell soma as in Fig. 9. The nucleus (N) contains a nucleolus and an intranuclear rod (arrow). Numerous cisternae and vesicles of the Golgi complex (arrowheads) are adjacent to the nucleus. Also shown are an astrocyte (A) and a gold-labelled spinous dendrite of a granule cell (D). $\times 7000$.

Fig. 11. Electron micrograph of asymmetric (arrow) and symmetric (arrowhead) synapses that contact the soma of the molecular layer basket cell in Fig. 10. $\times 41\ 000$.



Dendrites

The dendrites of basket cells are present in the GL (Fig. 12), molecular layer (Fig. 14) and the hilus (Figs. 15–17). In general, the proximal portions of basket cell dendrites are larger in diameter than the distal parts. Both proximal and distal dendrites lack spines and are contacted by axon terminals that make both asymmetric and symmetric synapses (Figs. 13–17). Even dendrites that course in the GL have numerous synapses upon their surfaces. However, the basket cell dendrites in the hilus appear to have the greatest concentration of terminals apposed to their surfaces. In some instances 10 μm lengths of these dendrites are contacted by contiguous axon terminals (Figs. 15,17). Many of these terminals are large (1–2 μm), contain round synaptic vesicles spread throughout the terminal, and have occasional dense-core vesicles and mitochondria that are located away from the active synaptic sites (Fig. 16). Such terminals synapse with hilar dendrites from all basket cells except for those of the molecular layer type. Some of these terminals arise from identified granule cell axons (Fig. 17). In contrast, the terminals that synapse with basket cell dendrites in the molecular layer have not been characterized as well. However, some of these latter terminals also make synaptic contact with neighbouring dendritic spines that are probably derived from granule cells (Fig. 14). Thus, the basket cells may receive the same afferents as the granule cells. These findings are based on basket cell dendrites found in the inner half of the molecular layer because other impregnated structures made it difficult to identify the basket cell dendrites in the outer half of this layer.

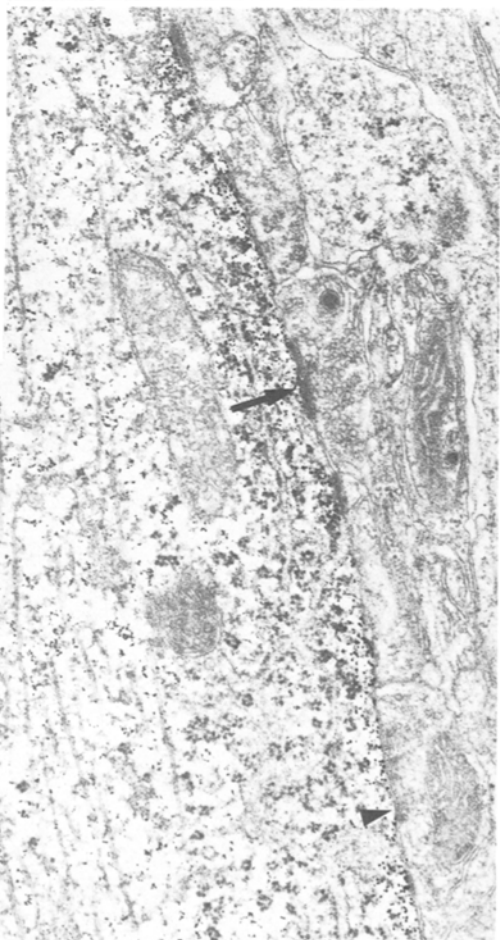
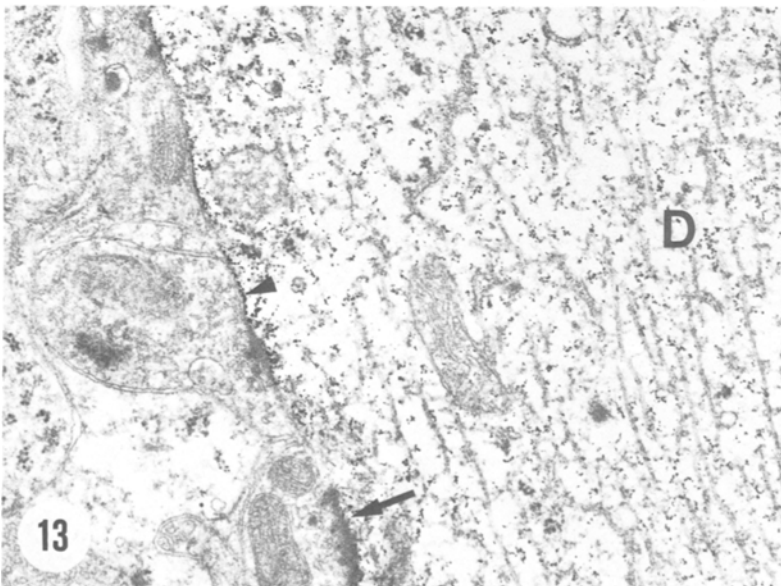
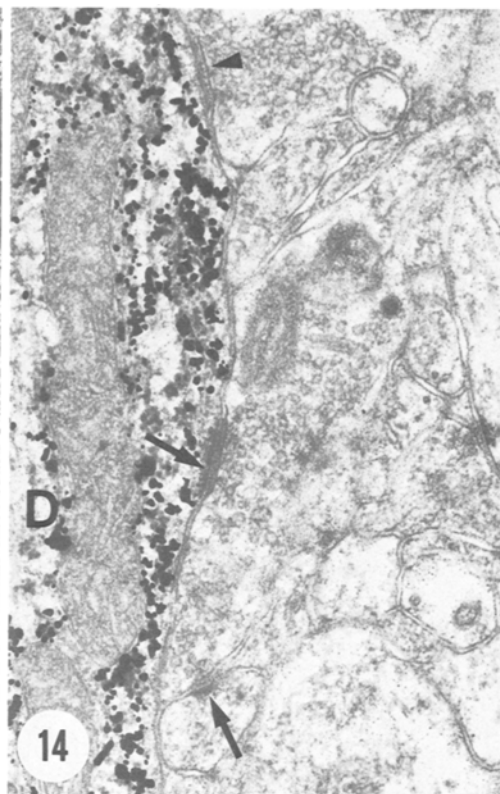
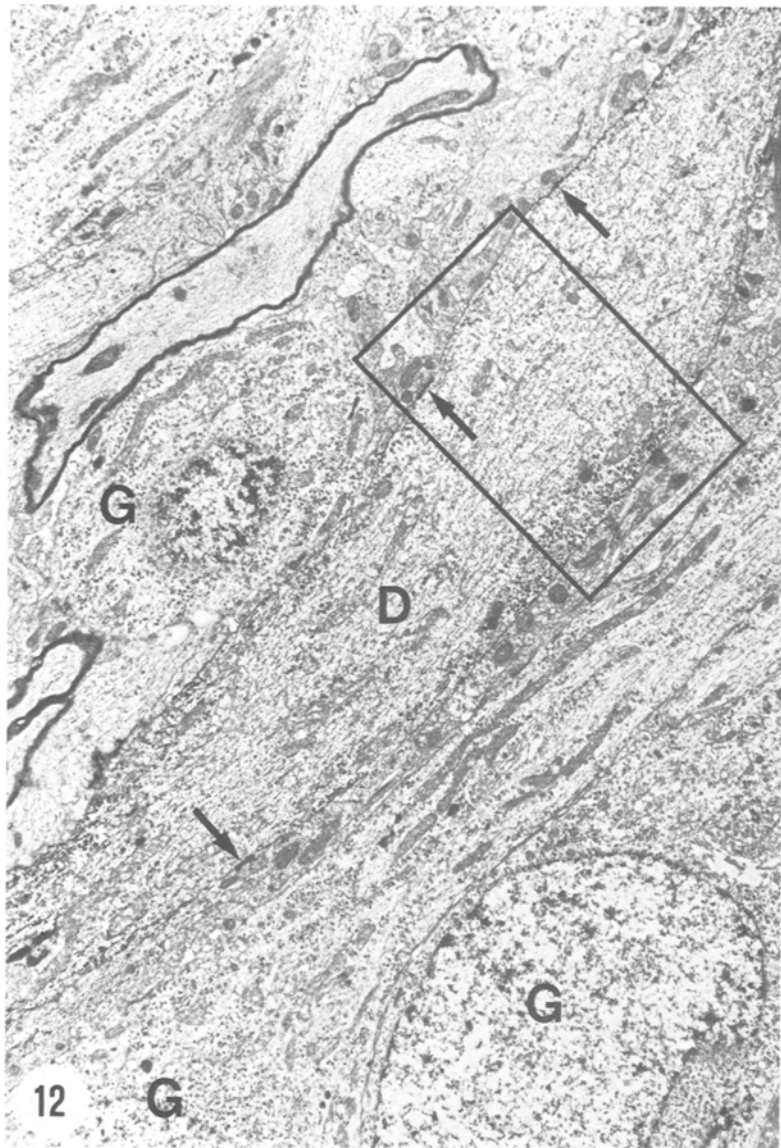
Axons

The axons of basket cells arborize in the GL and adjacent part of the molecular layer (Figs. 18–20). Terminals from these axons form exclusively symmetric synapses (Figs. 21–23). The gold-labelled terminals are 1 μm in diameter or less. These terminals are characterized by numerous mitochondria located near the active site which has a sparse number of pleomorphic synaptic vesicles associated with it (Fig. 22). In most instances

Fig. 12. Electron micrograph of the apical dendrite (D) of the pyramidal basket cell shown in Fig. 1A as it leaves the layer of granule cells (G) to enter the molecular layer. Several synapses (arrows) occur along the length of the dendrite and some are shown at higher magnification in Fig. 13. $\times 7000$.

Fig. 13. Enlargement of a portion of the apical dendrite (D) of the pyramidal basket cell as indicated by the frame in Fig. 12. The gold label is distributed throughout the cytoplasm and numerous asymmetric (arrows) and symmetric (arrowheads) synapses are found along the dendritic surface. $\times 26\ 000$.

Fig. 14. Enlargement of a part of the dendrite from the molecular layer basket cell shown in Figs. 9–11. A large terminal in the centre forms asymmetric synapses (arrows) with the labelled dendrite (D) and with an unlabelled spine. Another terminal makes a symmetric synapse (arrowhead) with the labelled dendrite (D). $\times 45\ 000$.



the gold particles are contained within the terminal so the presynaptic and postsynaptic membranes are clearly visible as well as the synaptic cleft material.

The most common postsynaptic structures are the somata (Fig. 21) and dendrites (Figs. 22–24) of granule cells. Dendritic spines from these cells are contacted rarely by basket cell axons. More than one gold-labelled axon terminal from a single basket cell may form synapses with an individual cell soma. However, in these cases, the granule cell soma is often contacted by unlabelled terminals which make either symmetric or asymmetric synapses. From a series of serial sections, one fusiform basket cell axon which was partially studied was shown to form more than 30 synapses with granule cell somata and dendrites. About half of these synapses were axosomatic and the remainder were axodendritic. The total number of synapses made by that basket axon is probably an order of magnitude greater when one considers the small size of the part which was

Fig. 15. Electron micrograph of a portion of a basal dendrite (D) from the same pyramidal basket cell shown in Fig. 1C. This gold-labelled dendrite is about 20 μm below the granule cell layer (not shown). Numerous, large axon terminals (T) establish synaptic contact with this dendrite (see Fig. 16). $\times 19\ 000$.

Fig. 16. Enlargement of the dendrite (D) in Fig. 15 and some of the mossy fibre-like terminals that form asymmetric axodendritic synapses (arrows). $\times 37\ 000$.

Fig. 17. Electron micrograph of a gold-labelled basal dendrite (D) from the pyramidal basket cell and gold-labelled terminals (T) and unmyelinated axon (A) from the granule cell in Fig. 4. One of these terminals forms an asymmetric synapse (arrowhead) with the basal dendrite. The other labelled terminal also synapses with this same dendrite in another section. Other terminals form asymmetric synapses (arrows) with the dendrite. $\times 16\ 000$.

Fig. 18. Light micrograph of a gold-labelled axonal plexus that arises from the apical dendrite (D) of the fusiform basket cell in Fig. 1D. Numerous varicose swellings (arrows) of this axon appear to encircle a granule cell soma (G). $\times 1400$.

Fig. 19. Electron micrograph of a granule cell soma (G) and a gold-labelled axon of a pyramidal basket cell. Boutons (arrowheads) from this axon contact the soma and base of the main dendrite (D) while other boutons (arrows) appear in the adjacent neuropil. $\times 10\ 000$.

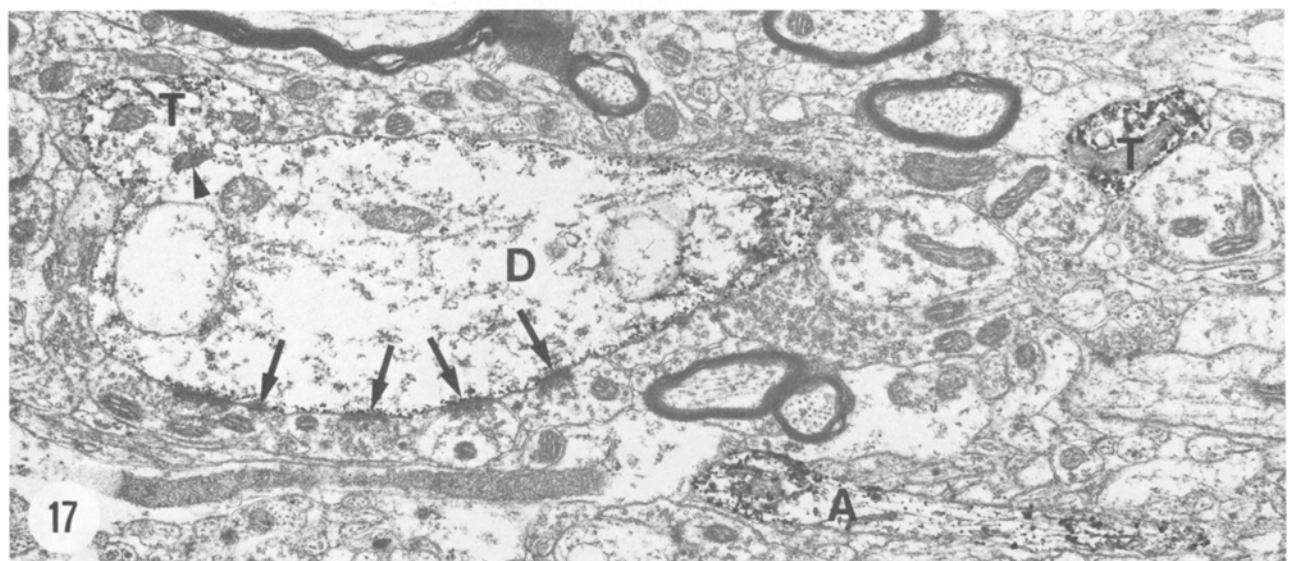
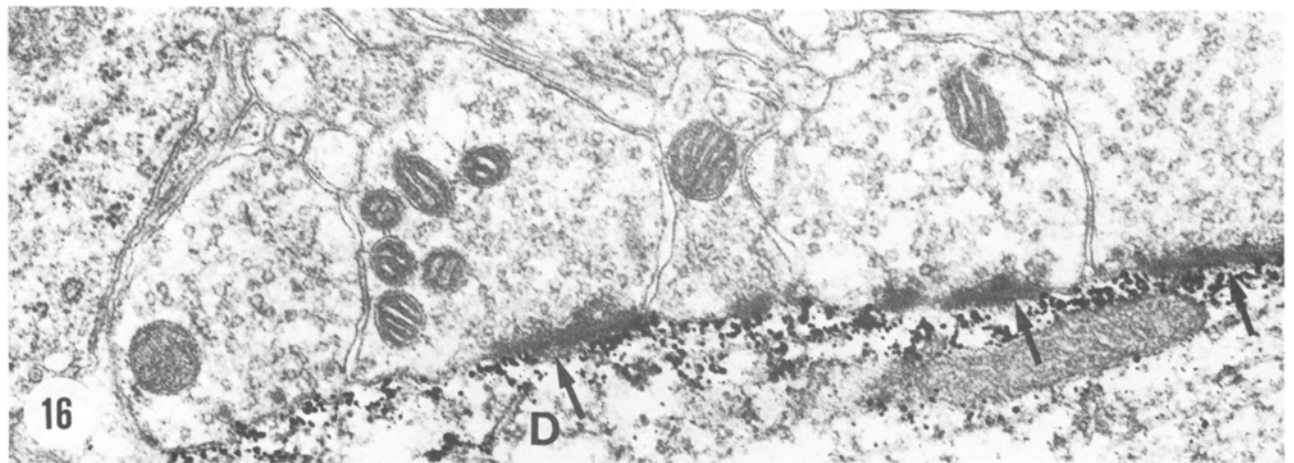
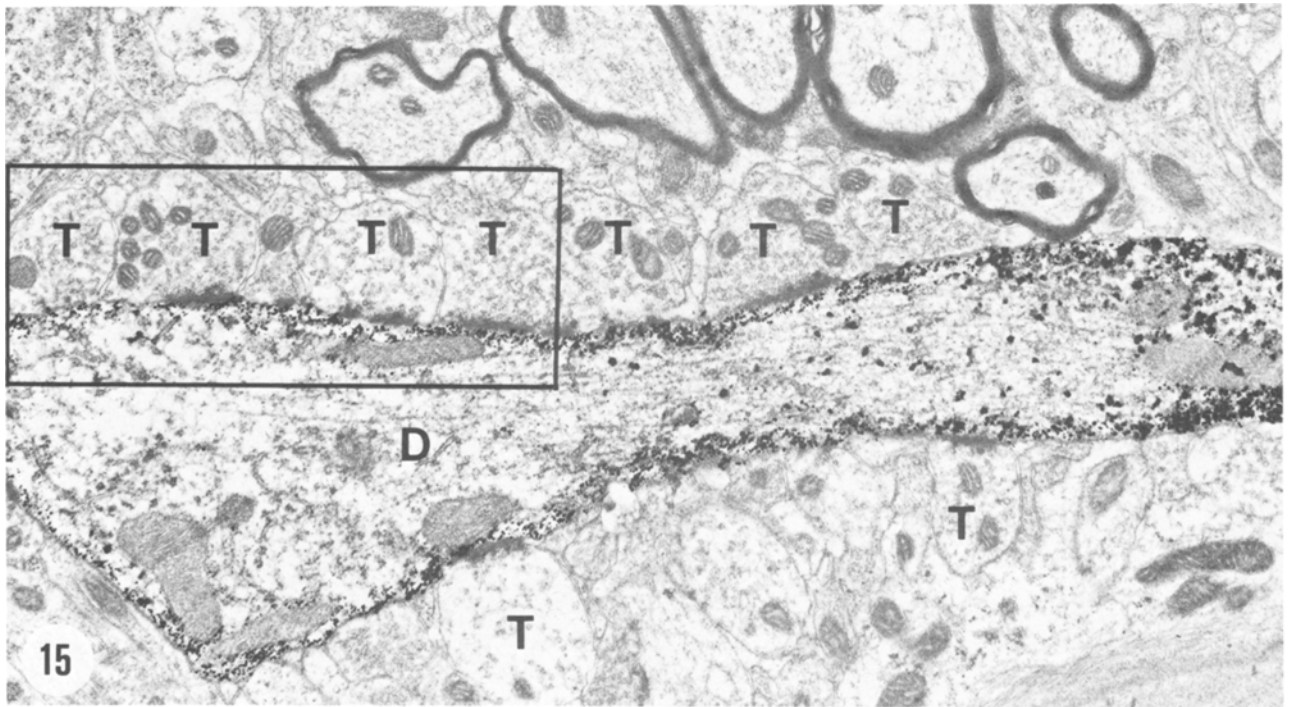
Fig. 20. Electron micrograph of a section adjacent to Fig. 19 showing the labelled bouton (arrows) that encircles the base of the main dendrite (D) of the granule cell. An example of a synapse at this location is shown in Fig. 22. $\times 22\ 800$.

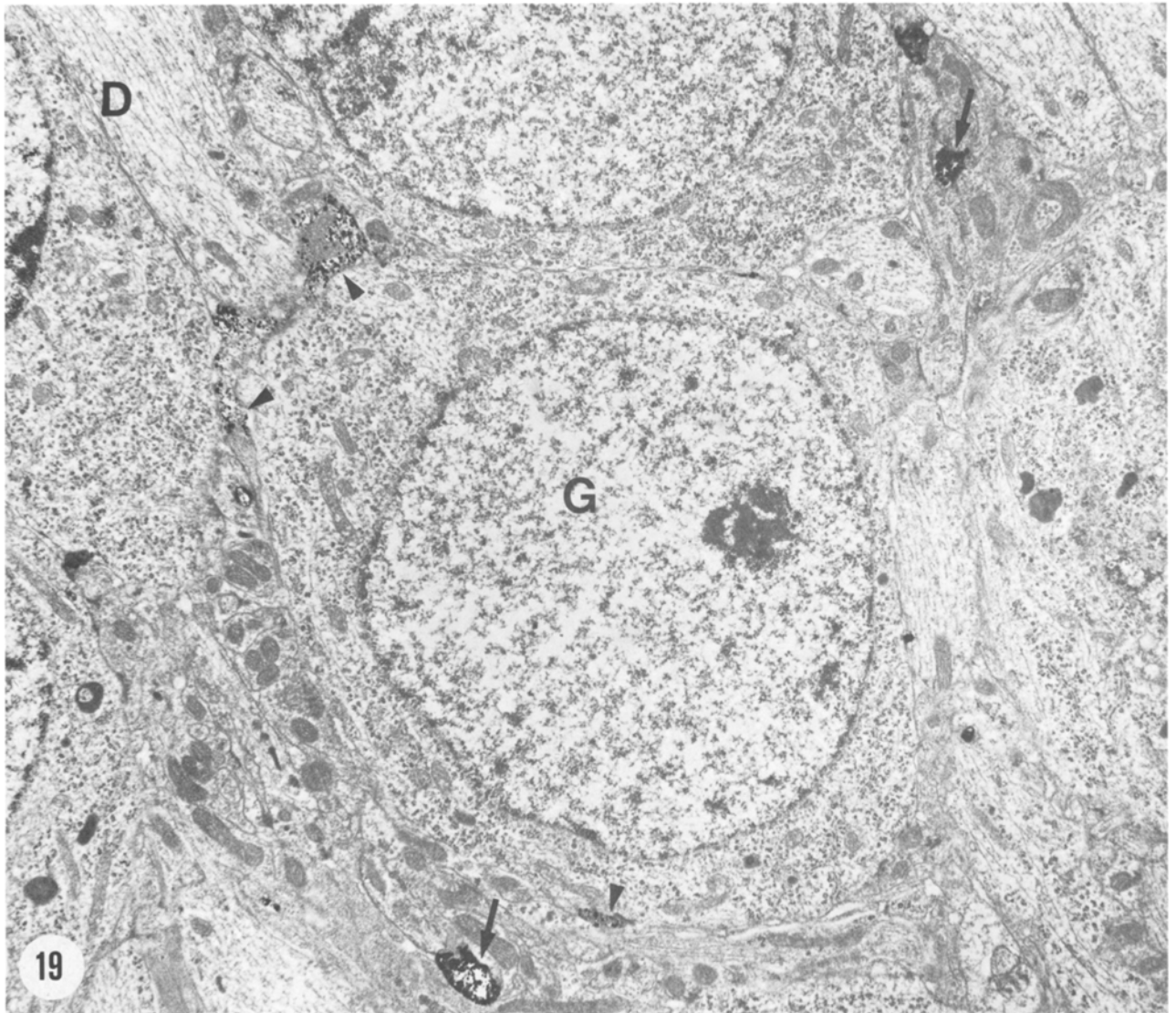
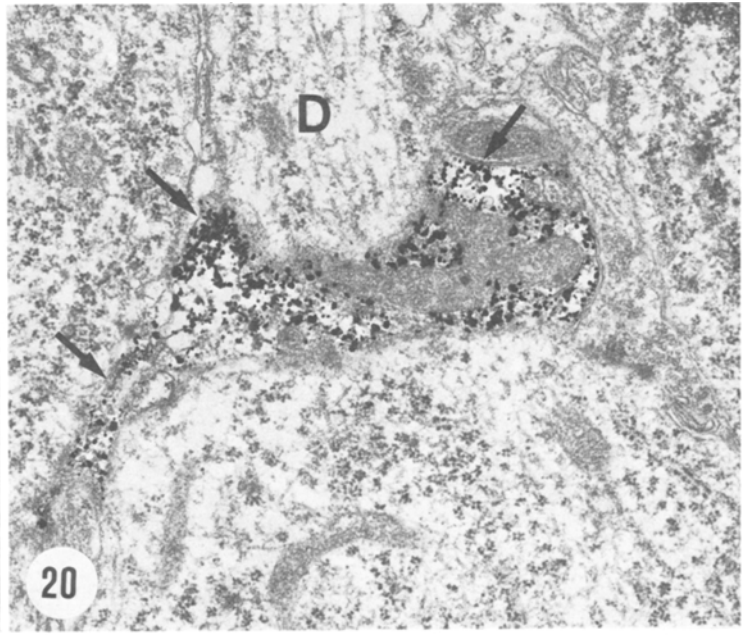
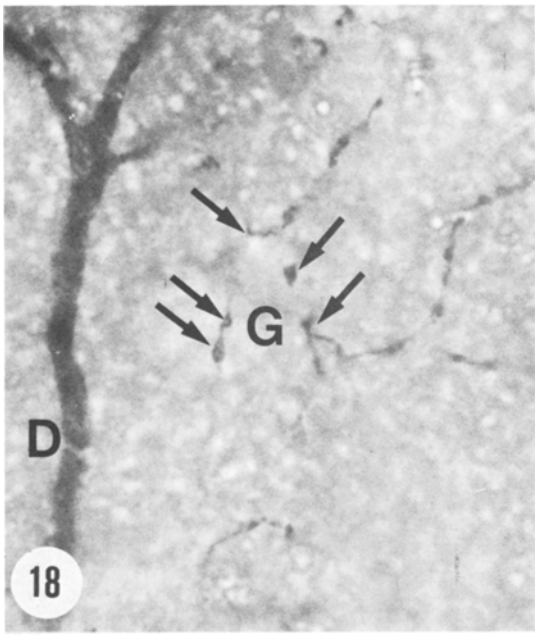
Figs. 21–24. Electron micrographs of gold-labelled axon terminals from basket cells.

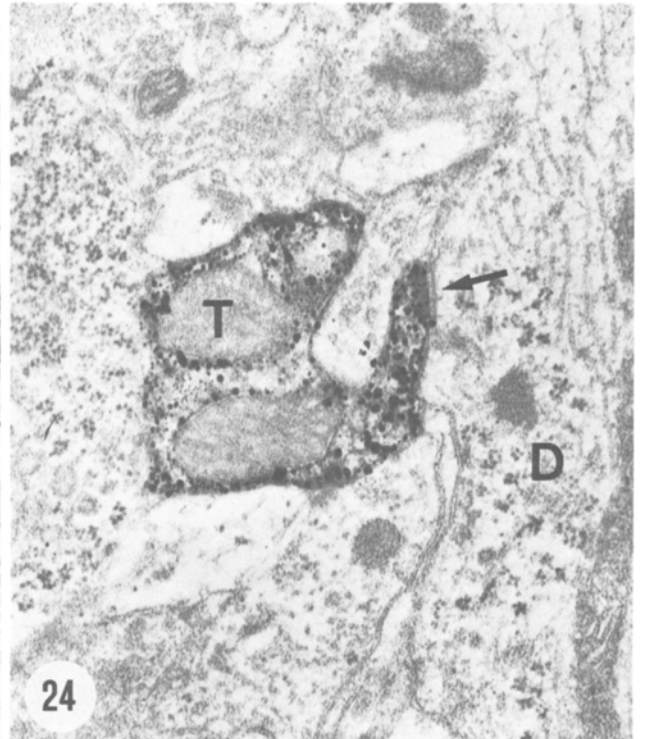
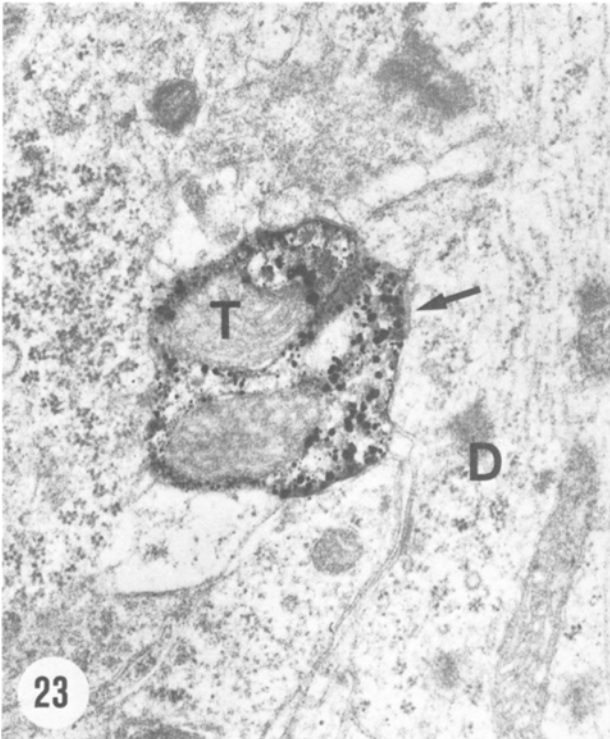
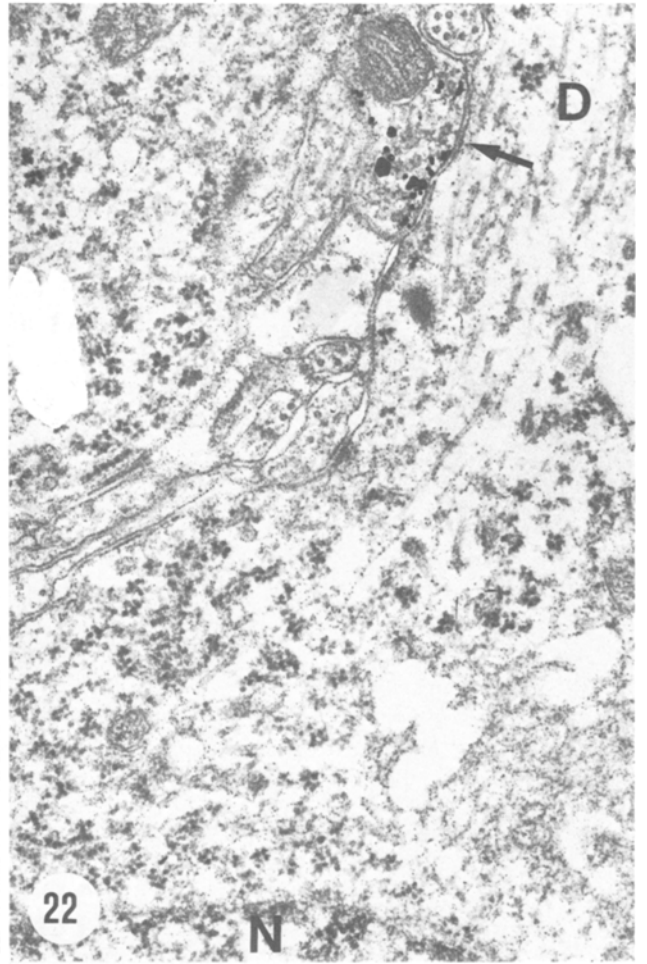
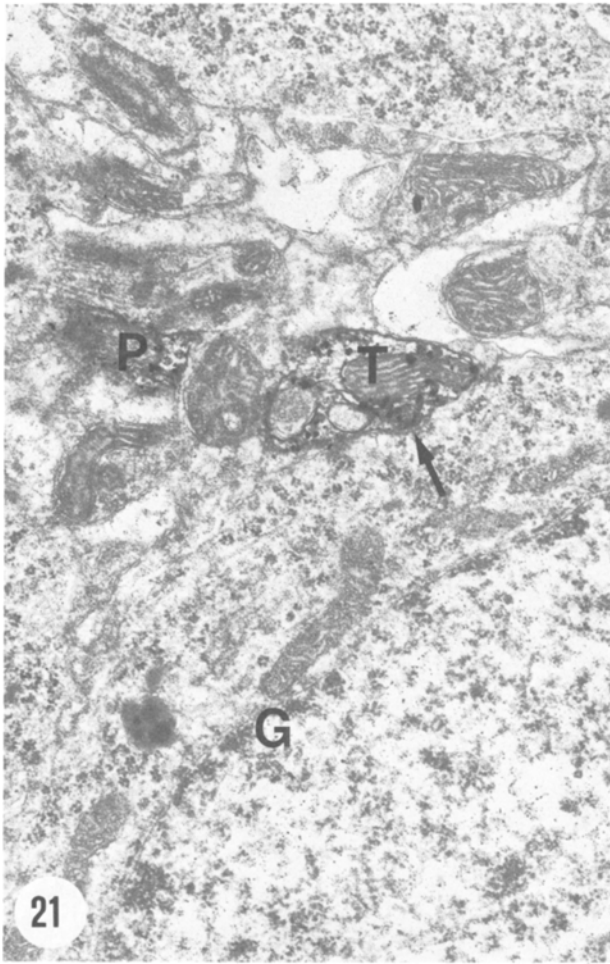
Fig. 21. A terminal (T) from a fusiform basket cell that forms a symmetric synapse (arrow) with the soma of a granule cell (G) is shown. The preterminal (P) of this axon is also shown. $\times 22\ 300$.

Fig. 22. A terminal from a pyramidal basket cell forming a symmetric synapse (arrow) at the base of the main dendrite (D) of a granule cell. Note the proximity of this synapse to the nucleus (N). $\times 37\ 000$.

Figs. 23 and 24. Adjacent serial sections which show another gold-labelled terminal (T) from the fusiform basket cell. A spine from a granule cell dendrite (D) protrudes into the terminal which forms a symmetric synapse (arrows) with the dendritic shaft. Possible sites of synaptic contact with the spine are obscured by the gold particles. $\times 31\ 000$.







analysed. Gold-labelled basket cell terminals synapse with the proximal dendrites of granule cells both close to the soma (Fig. 22) and as far away as 50 μm into the molecular layer. These axodendritic synapses may occur in the granule cell layer as well. When terminals of basket cells are apposed to granule cell dendritic spines, the terminal either synapses with the spine or ensheathes the spine and synapses with the dendritic shaft (Figs. 23,24). Basket terminals were never found contacting the axon initial segments of granule cells even though numerous terminals that form symmetric synapses are present at this location.

Discussion

The results of this study indicate that the five types of basket cell observed in light microscopic preparations share similar ultrastructural features. The nuclei of all basket cell types display euchromatin, infolded nuclei, intranuclear rods and sheets, and single, large nucleoli. The dendrites of basket cells all have smooth surfaces due to a lack of spines. Basket cell axons all form symmetric synapses and contain flat vesicles. It is remarkable that these characteristics are consistent for all basket cell types since these cells show a diverse morphology in the light microscope with regard to the shapes and locations of their somata and dendrites.

Somata and dendrites of basket cells

The five types of basket cell share similar ultrastructural features of their somata and smooth dendrites. The same somal ultrastructure was previously described for pyramidal basket cells (Ribak & Anderson, 1980) and it was suggested that these cells probably have high metabolic rates that may reflect a high level of physiological activity. Since the other types of basket cell display these same somal features, all basket cells in the dentate gyrus might be expected to have higher spontaneous firing rates than the projection neurons, the granule cells.

The different shapes and locations of the basket cell dendrites and somata may indicate that different afferents synapse with specific types of basket cells. For example, molecular layer basket cells receive synaptic input from axons in the GL and the molecular layer, whereas the other four types of basket cell have dendrites in these layers as well as in the hilus, and may receive different afferents. For example, a common type of terminal that contacts the hilar dendrites of basket cells has similar ultrastructural features to the mossy fibre terminal derived from granule cells (Blackstad, 1963; Laatsch & Cowan, 1966). Indeed, Amaral (1978) has shown small mossy fibre terminals apposed to these dendrites in light microscopic preparations, and this observation suggested a synaptic contact at the site of apposition. Such a contact has been demonstrated in the present study between identified hilar dendrites of basket cells and terminals that arise from identified granule cells. Since mossy fibre collaterals do not enter the GL or molecular layer, the molecular layer basket cells lack this input. If we assume that the lengths of dendrites in the hilus are related to the number of mossy

fibre synapses, then pyramidal basket cells would appear to receive the largest granule cell input. In comparison, the horizontal, fusiform and inverted fusiform basket cells have fewer dendrites that enter the hilus, and they probably have a smaller number of synapses from granule cell axon collaterals. Therefore, a hierarchy might exist for basket cells which receive varying amounts of synaptic input from granule cell axon collaterals: pyramidal > fusiform, horizontal, inverted fusiform > molecular basket cells. It is possible that other sources of synaptic input to basket cells also display a hierarchical preference. Further studies will attempt to determine whether this is so.

Axons of basket cells

A unifying characteristic of the five types of basket cell is the distribution of their axons into a basket plexus in the GL and adjacent molecular layer. For almost a century, neuroanatomists have speculated that these axons made only axosomatic synapses with granule cells (Cajal, 1911; Lorente de Nó, 1934; Amaral, 1978). More recently, Seress & Pokorny (1981) have predicted that the basket cell axons also form synapses with the proximal dendrites of granule cells. The present study shows conclusively that these predictions are both true, i.e. the axons of basket cells form synapses with both the somata and dendrites of granule cells.

In any type of Golgi study, it is difficult to assess the negative findings because complete impregnation of a neuron is not always assured. The fact that individual granule cell somata were contacted by both labelled and unlabelled terminals indicates that more than one basket cell may give rise to a single pericellular plexus. Such a finding suggests that basket cells have overlapping influences on the same granule cell. Another negative finding was the lack of labelled terminals apposed to axon initial segments of granule cells. Terminals found at this site on cerebellar Purkinje cells are derived from basket cells (Palay & Chan-Palay, 1974). However, data from the cerebral neocortex indicate that the cortical basket cell forms only axosomatic and axodendritic synapses (Marin-Padilla, 1969; Jones, 1975; Gatter *et al.*, 1978; De Filipe & Fairén, 1982), whereas another cell type, the chandelier cell, is specialized to contact exclusively axon initial segments (Somogyi, 1977; Fairén & Valverde, 1980; Peters *et al.*, 1982). A neuron analogous to the cortical chandelier cell has been reported recently in Golgi studies of the Ammon's horn region of the hippocampus (Nunzi *et al.*, 1982). It is likely that a similar neuron exists in the dentate gyrus because numerous terminals with the same morphology as chandelier cell terminals occur alongside the axon initial segments of granule cells. At the present time, however, such a cell has yet to be described. For these reasons, the basket cells of the dentate gyrus are more similar to the basket cells in the neocortex than to those in the cerebellar cortex.

Basket cell function

The localization of glutamate decarboxylase with immunocytochemical methods to axon terminals (Barber & Saito, 1976; Ribak *et al.*, 1978) and somata (Ribak *et al.*, 1978; Seress & Ribak, 1983) of all basket cell types in the dentate gyrus indicates that the basket cells

probably mediate GABAergic inhibition. Since four out of five basket cell types are contacted by presumed mossy fibre collateral terminals, these morphological data are consistent with the notion obtained from physiological studies that recurrent feedback inhibition of granule cell activity is mediated by basket cells (Andersen, 1975). The synaptic sites of basket cell terminals on the somata and proximal dendrites of granule cells are important because this input is much closer to the granule cell axon hillock than that which terminates in the outer molecular layer (e.g. perforant path fibres). This fact taken together with the postulated higher firing rates of basket cells indicates that basket cells probably provide a strong, tonic inhibition of granule cells.

Recent quantitative data indicate that the granule:basket cell ratio in the dentate gyrus is 150–200:1. (Seress & Pokorny, 1981). Since basket cells have axons that extend for up to 1 mm, they have the potential to contact a large number of granule cells (Struble *et al.*, 1978). This number of granule cell somata may be as high as 500 when considering the average 10 μ m somal diameter of granule cells and their packing thickness of four or five somata in the GL. Although basket cells are greatly outnumbered in the dentate gyrus by granule cells, the wide distribution of basket cell axons compensates in the balance of inhibitory and excitatory functions in this brain region. Thus, the relatively smaller numbers of basket cells would be able to provide a vast inhibitory influence over the granule cells.

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