UC Irvine
UC Irvine Previously Published Works

Title
The development, ultrastructure and synaptic connections of the mossy cells of the dentate gyrus.

Permalink
https://escholarship.org/uc/item/0wz3x038

Journal
Journal of neurocytology, 14(5)

ISSN
0300-4864

Authors
Ribak, CE
Seress, L
Amaral, DG

Publication Date
1985-10-01

DOI
10.1007/bf01170832

Copyright Information

Peer reviewed

The development, ultrastructure and synaptic connections of the mossy cells of the dentate gyrus

CHARLES E. RIBAK[1], LÁSZLÓ SERESS[1*] and DAVID G. AMARAL[2]

[1]*Department of Anatomy, University of California, (Irvine), Irvine, CA 92717, USA*
[2]*The Salk Institute and The Clayton Foundation for Research – California Division, P.O. Box 85800, San Diego, CA 92138, USA*

Summary

One of the most distinctive and common cell types in Golgi preparations of the hilus of the rat dentate gyrus is the mossy cell. We have used a variety of techniques including the Golgi method, the combined Golgi and electron microscopic (EM) method and the retrograde transport of horseradish peroxidase (HRP) to study the development, ultrastructure and synaptic connections of this cell type. The mossy cells identified in our light microscopic preparations are characterized by: (1) triangular or multipolar shaped somata; (2) three to four primary dendrites that arise from the soma and bifurcate once or more to produce an extensive dendritic arborization restricted, for the most part, to the hilus; (3) numerous thorny excrescences on their somata and proximal dendrites with typical spines on distal dendrites; and (4) axons that bifurcate and are directed toward the fimbria and the molecular layer of the dentate gyrus.

The mossy cells have an immature appearance at birth and on subsequent days their maturation appears to lag somewhat behind that of the hippocampal pyramidal cells. On postnatal day 1, many of the dendrites bear growth cones primarily at their termini and have long, thin filipodia emanating from various points along their lengths. Many of the dendrites enter the molecular layer of the dentate gyrus, though this is rarely seen in the mature brain. Typical pedunculate spines are first commonly seen on the distal dendrites around postnatal day 7 while thorny excrescences are first commonly seen between postnatal days 11 and 14. By postnatal day 21, the dendrites have attained a mature appearance although the density of both typical spines and thorny excrescences is less than that found in adults.

Two different retrograde transport methods were used to confirm that mossy cells give rise to the commissural projection to the contralateral dentate gyrus. The first method combined HRP histochemistry with a silver intensification procedure and the second method combined HRP histochemistry with Golgi staining. While the majority of commissurally projecting hilar neurons had the appearance of mossy cells, there were others that were smaller and either ovoid or fusiform.

*Present address: Department of Physiology, University Medical School, Pécs, Hungary.

In EM preparations, the somata of mature mossy cells display round nuclei that lack infoldings and intranuclear rods. The perikaryal cytoplasm contains the organelles typically found in pyramidal cells of the hippocampus. Somal spines with complex shapes and branching patterns are commonly observed. The thorny excrescences on the proximal dendrites correspond to spines with long thin stalks and complex end bulbs that may appear mushroom shaped. Large, round vesicle-filled axon terminals similar to the expansions of mossy fibres of the dentate granule cells contact both somal and dendritic thorny excrescences. The initial segment of the mossy cell axon arises from a hillock and is contacted by a few terminals that form symmetric synapses. Axon terminals of mossy cell axon collaterals identified in Golgi–EM preparations form asymmetric axodendritic synapses in the hilus. Since the mossy cells are dominated by synaptic input from the granule cells, they are probably more easily influenced by this input than are pyramidal cells. Based on this input and the findings that mossy cells have commissural projections, we conclude that this cell participates in an important recurrent excitatory circuit for the granule cells of the dentate gyrus.

Introduction

One of the most distinctive and common cell types in the hilus of the hippocampal dentate gyrus is the mossy cell (Amaral, 1978). This cell is distinguished from other neurons of the hilar region primarily by the clusters of complex spines or 'thorny excrescences' located at several locations on the soma and proximal dendrites. In contrast, the distal dendrites have only more ordinary pedunculate spines. Lorente de Nó (1934) suggested that the large, complex spines were characteristic for structures postsynaptic to the mossy fibres of the dentate gyrus granule cells. Subsequently, a number of studies have shown this synaptic relationship for mossy fibres and complex spines on pyramidal cell dendrites in CA3 of the hippocampus and 'modified pyramidal cells' in the hilus (Blackstad & Kjaerheim, 1961; Hamlyn, 1962; Laatsch & Cowan, 1966a).

The mossy cell typically has an extensive arbor of branching dendrites that extends throughout much of the hilus. The mature dendrites do not generally leave the confines of the hilus, but occasionally a dendrite may enter the granule cell and molecular layers of the dentate gyrus. Normally, the dendrites come close to either the granule cell layer or the CA3 region of the hippocampus, and most appear to bend back into the hilus.

Lorente de Nó (1934) has suggested that mossy cell ('modified pyramidal cell') axons give rise to Schaffer collaterals similar to those of the pyramidal cells of the CA3 region of the hippocampus. However, recent data from retrograde transport studies show that large hilar cells, similar in size to the mossy cells, give rise to ipsilateral associational and commissural projections to only the dentate gyrus (West et al., 1979; Berger et al., 1981; Swanson et al., 1981; Voneida et al., 1981; Seroogy et al., 1983; Zimmer et al., 1983). However, the mossy cells have not been identified with certainty in such preparations.

Previous EM studies of the dentate gyrus have not described the ultrastructure or the synaptic connections of the mossy cells. Therefore, to reveal such features a combined Golgi–EM method was used in the present study. We have also analysed Golgi preparations from rats at several postnatal ages to describe the maturation of the mossy cell and, in particular, to determine the age at which the thorny excrescences appear.

Finally, we have employed two different procedures that utilize the retrograde transport of HRP to confirm that mossy cells contribute axons to the commissural projection of the dentate gyrus.

Methods

Golgi impregnation of developing brains
Among several Golgi procedures tested, the following was found to yield the most uniform and consistent staining of the immature hippocampal formation. Young rats (ages 1, 3, 5, 11, 14, 21 days postnatal with day 0 being the day of birth) were first perfused with a solution containing 3.3% $K_2Cr_2O_7$, 4.1% glutaraldehyde and 6.6% formaldehyde. Blocks of the brains containing the hippocampal formation were either postfixed in this same solution for 1 or 2 days (with the solution changed daily), or placed in a solution containing 3.3% $K_2Cr_2O_7$, and 0.33% OsO_4 for 1 to 3 days. The brains were then blotted and placed in a solution of 0.75% $AgNO_3$ for 1 or 2 days. Subsequently, the brains were embedded in low viscosity nitrocellulose and sectioned at 100 μm on a sliding microtome. The sections were cleared, dehydrated and mounted with DPX and coverslipped.

Retrograde transport
Ten adult rats (250–350 g) were used in these studies. After injections of 35% chloral hydrate (0.1 mg per 100 g body weight, i.p.) the dentate gyrus was stereotaxically injected using a Hamilton syringe with either 0.10–0.20 μl of 30% HRP or 5% wheat-germ agglutinin-conjugated HRP in distilled water. The injections were made in 0.025 μl increments and were completed in 10–20 min. The tip of the syringe remained in place for an additional 15 min before withdrawal. After a 48 h survival period, the rats were deeply anaesthetized and perfused with 0.9% saline followed by either a solution of 4% paraformaldehyde and 1% glutaraldehyde in 0.12 M phosphate buffer (pH 7.2), or two 4% paraformaldehyde solutions in 0.1 M buffer at pH 6.5 and 11, respectively (Berod *et al.*, 1981). When the latter fixative was used, it was followed by a phosphate-buffered saline solution containing 30% sucrose. The rat brains were removed from the cranium and sections 80 μm thick were cut on a freezing microtome. The glutaraldehyde-perfused brains were refrigerated overnight and sectioned at the same thickness with a Vibratome. The sections were processed by one of two methods for the visualization of HRP using diaminobenzidine (DAB) as the chromogen. The first method involved a modified silver staining technique for HRP intensification (Gallyas, 1971; Liposits *et al.*, 1982). Briefly, the 80 μm frozen sections were washed in 3.0% sodium acetate after DAB processing and refrigerated overnight in a solution of 8.0% thioglycolic acid. The following day, the sections were washed in 2% sodium acetate and placed in a physical developer (2.5% sodium carbonate, 0.1% silver nitrate, 0.095% ammonium nitrate, 0.5% tungstosilicic acid and 0.013–0.125% formalin). The intensity of staining was controlled by varying the time of development from 10 to 40 min. The reaction was stopped by immersion of the sections in 1% acetic acid followed by washes in 2.0% sodium acetate. The sections were then placed in a 0.2% gold chloride solution for 10 s, briefly washed, placed in 3.0% sodium thiosulphate and again washed with 2.0% sodium acetate. Finally, the sections were dehydrated in ethanol, cleared in xylene, mounted onto glass slides and coverslipped.

The second method combined the HRP retrograde and Golgi methods (Freund & Somogyi, 1983). Sections that were processed with DAB but not intensified as above, were washed in 0.1 M phosphate buffer and postfixed with 1% OsO_4 for 1 h. Coronal sections through the contralateral dentate gyrus were stacked on a silicone rubber plate and 5% agar-agar was poured onto the pile of sections. Excess agar was trimmed away and the block was placed into 3.5% potassium

dichromate solution for 3 days. Following brief rinses in H_2O, the blocks were placed into 1.0% silver nitrate for 1 day. After this impregnation, sections were separated from the blocks and examined for Golgi-stained mossy cells. Sections containing mossy cells were gold-toned (Fairén et al., 1977) and examined for the presence of HRP reaction product in perikaryal cytoplasm.

Golgi–electron microscopy

Sprague-Dawley rats, 1–2 months of age, 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 at 4° C, and the hippocampus dissected out and processed for the Golgi–EM method according to Fairén et al. (1977). Briefly, the entire hippocampus was rinsed and placed into a solution containing 2.0% OsO_4 and 2.4% $K_2Cr_2O_7$. Each specimen was immersed in 50 ml of this solution and kept in the dark for 4 days. The tissue was then washed briefly in 0.75% $AgNO_3$ and stored in this solution for 3 days. Following impregnation, the blocks were passed through 20, 40, 60, 80 and 100% solutions of glycerol, embedded in agar and sectioned with the Sorvall tissue chopper. Sections were cut at 75–100 μm, collected on slides, coverslipped with 100% glycerol and examined with the light microscope. Identified mossy cells were drawn with a Zeiss microscope equipped with a drawing tube, and photographed.

The sections with mossy cells were hydrated through a series of glycerol solutions and placed into a chilled (4° C) 0.05% gold chloride solution for about 60 min with agitation. After three rinses in cold distilled water they were placed into cold 0.05% oxalic acid for 2 min, brought to room temperature and placed into a 1% solution of sodium thiosulphate for 1–1.5 h. The sections were then rinsed in distilled water and examined with the 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 poststaining with OsO_4, rapid dehydration with acetone, and embedding in Epon. The use of sections 75–100 μm thick allowed us to visualize the de-impregnated cells in the polymerized blocks of resin. Serial thin sections were taken of critical structures. All sections were stained with uranyl acetate and lead citrate before examination with the electron microscope.

Results

*Light microscopy of Golgi preparations*

Twelve mature mossy cells selected for electron microscopy were initially analysed with the light microscope. The mossy cells were identified by: (1) a triangular or multipolar

Fig. 1. Drawing of a mossy cell with the aid of a drawing tube. The soma of this cell (open arrow) is located close to the granule cell layer (GL). The dendrites of this cell radiate in various directions and remain within the hilus. An axon (a) arises from the cell body and loops underneath a dendrite in the direction of the pyramidal cell layer. Two dendrites (arrows) of this cell are also shown in Fig. 2. × 150.

Fig. 2. Light photomicrograph of the plastic block that contains the mossy cell shown in Fig. 1. Part of this cell has been sectioned and, thus, allows for the visualization of the axon hillock (h) and initial segment (a) as it passes beneath a dendrite. The dendrite indicated on the right is found within 20 μm of the granule cell layer (partially shown on the bottom). Note that this dendrite lacks thorny excrescences and is thinner than the dendrite indicated on the left. The identification of these profiles was confirmed in electron microscopic preparations. × 1000.

H

GL

a

①

a

h

②
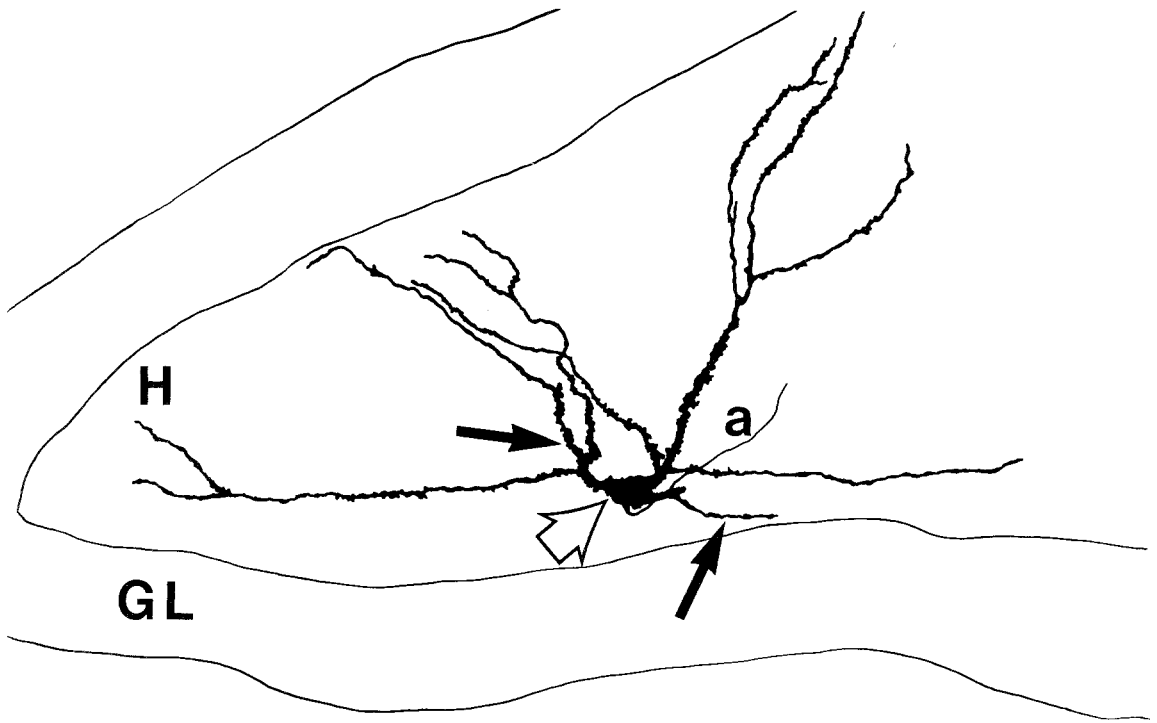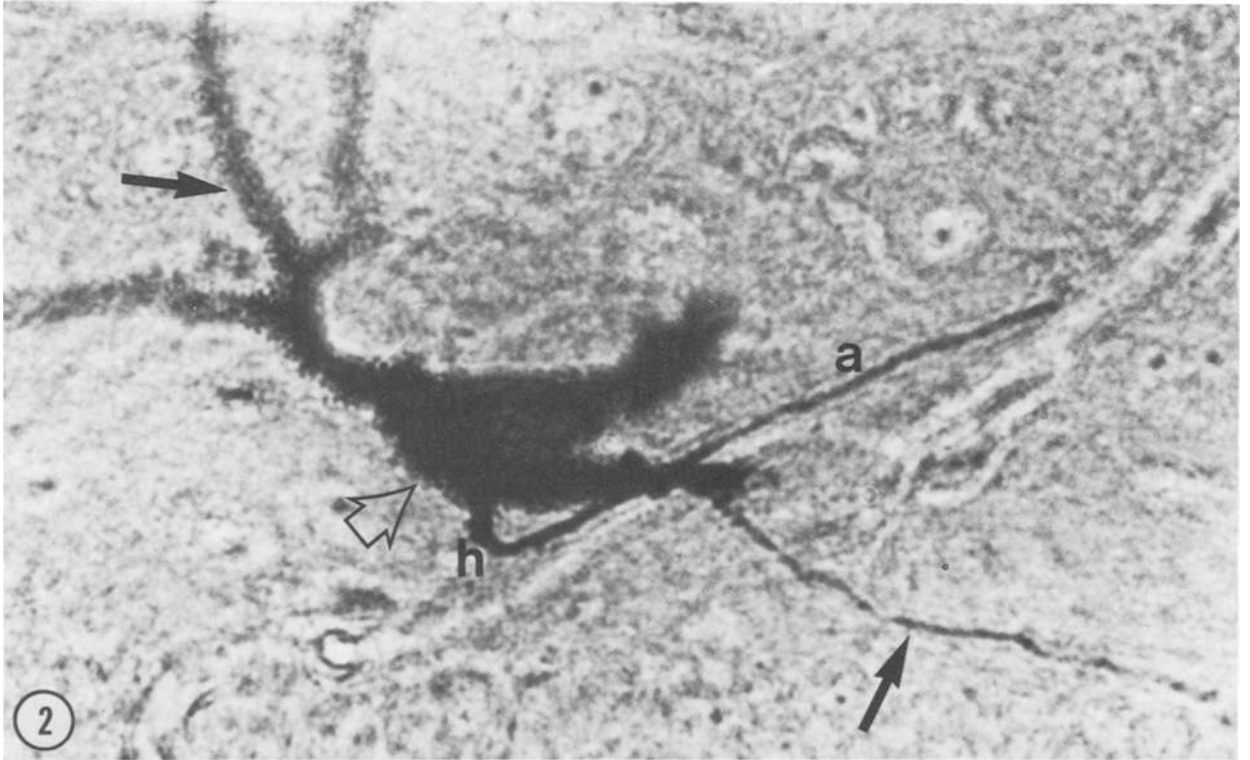
shaped soma, 20–25 µm in diameter; (2) an extensive dendritic arborization restricted primarily to the hilus; and (3) numerous thorny excrescences on the somata and proximal dendrites (Figs 1, 2). The shape of the soma depended somewhat on its location because those in the centre of the hilus appeared multipolar whereas the ones adjacent to the granule cell layer tended to be fusiform or triangular. The length of each proximal dendrite that is covered with the large, complex spines can range from 100 to 500 µm. The distal branches of the dendrites are much thinner than the proximal portions. Although the distal dendrites lack the large, complex spines, they are studded with smaller spines like those typical of pyramidal cells. The axons of the mossy cells usually arise from the somata (Figs 1, 2). Most of them are impregnated for only a short distance but some local collaterals are apparent in the hilus. Further features of mossy cell morphology are provided in the Electron Microscopy section of the Results.

Development of the mossy cells

Although the most distinctive characteristic of the mossy cell, namely the dense investiture of thorny excrescences, is not present in the early postnatal period, other

---

Fig. 3. Camera lucida drawing of a Golgi-stained mossy cell from a 1-day-old rat. There are numerous growth cones (gc) both at the ends of the major dendrites and at points along their course. The growth profiles marked with a star are shown in Fig. 4. There are also several filipodial spines (fs) along the course of the dendrites. The mossy cell axon (a) gives rise to a number of varicose, local collaterals (ac). Bulbous axon terminals (ax) are in close apposition to the soma and proximal dendrite of this cell. The insert (top left) shows the position of this cell in the anlage of the dentate gyrus. Note that one of the main dendrites enters the region of the developing granule cell and molecular layers. Scale bar: 20 µm.

Fig. 4. Photomicrograph showing two growth cones that emanate from a major dendrite of a 1-day-old mossy cell (Fig. 3). There is an expanded portion (open arrow) at the end of a thin stalk and numerous filipodia (arrows) all along. Scale bar: 1 µm.

Fig. 5. Photomicrograph showing the long, filipodial spines (arrows) on a 1-day-old mossy cell. Scale bar: 1 µm.

Fig. 6. The terminal portion of a mossy cell major dendrite with two growth cones (open arrows). One extends from the dendrite with a fine collateral (arrows) and has a bulbous expansion near its end. Scale bar: 5 µm.

Fig. 7. Golgi impregnated 3-day-old mossy cell. Two growth cones (open arrows) extend from a proximal dendrite. One of the main dendritic processes (arrow) enters the granule cell layer (GL). Scale bar: 10 µm.

Fig. 8. Mossy cell from a 5-day-old preparation. While there are occasional filipodia (arrow) or growth cones, the soma and proximal dendrites are generally smooth at this age. Scale bar: 10 µm.

Fig. 9. Golgi-impregnated mossy cell from a 14-day-old preparation. There are many patches (arrows) of typical thorny excrescences at this age. The axon (a) also has a mature appearance. Scale bar: 10 µm.

Fig. 10. A distal dendrite from the mossy cell shown in Fig. 9. There are numerous pedunculate spines (arrows) along the course of these branches. There are also longer filipodia (open arrows) which are reminiscent of the filipodial spines seen at earlier ages. Scale bar: 10 µm.

3

GL
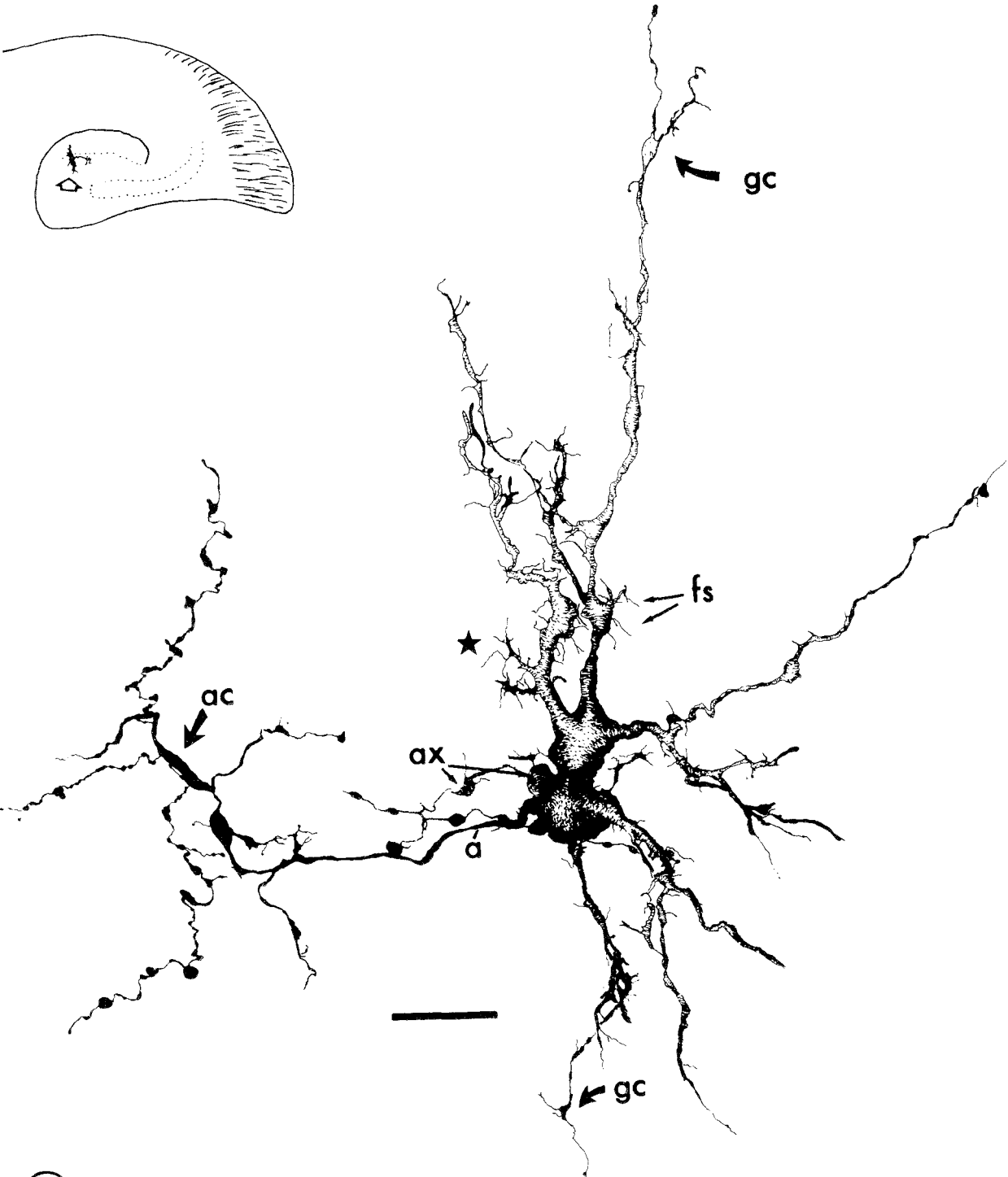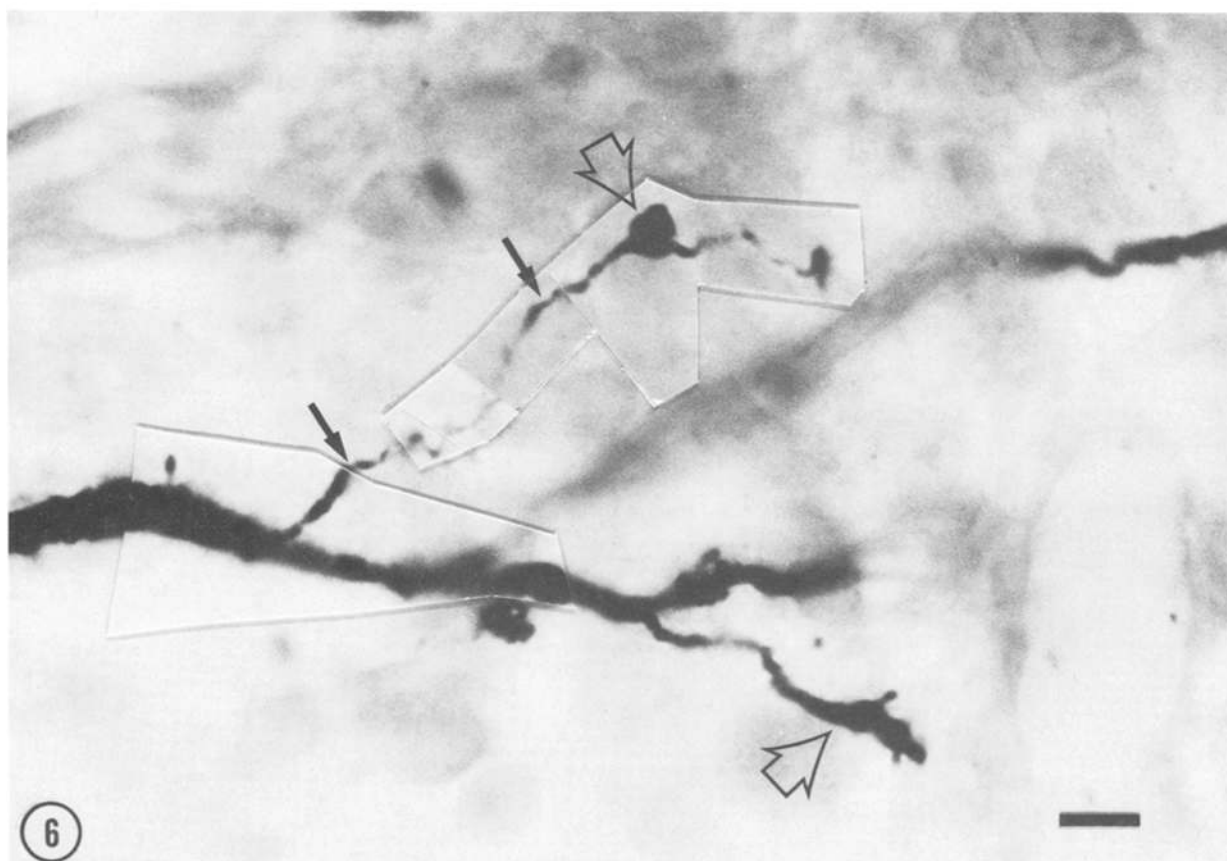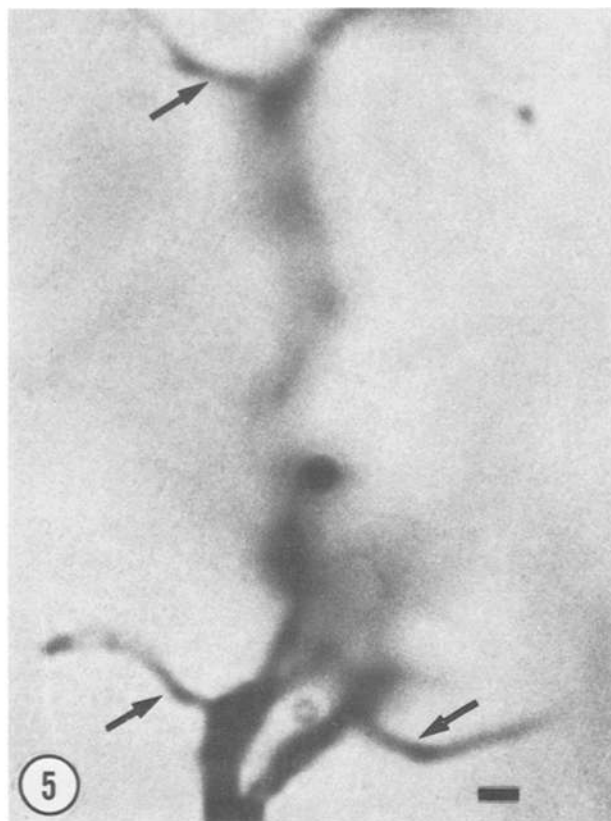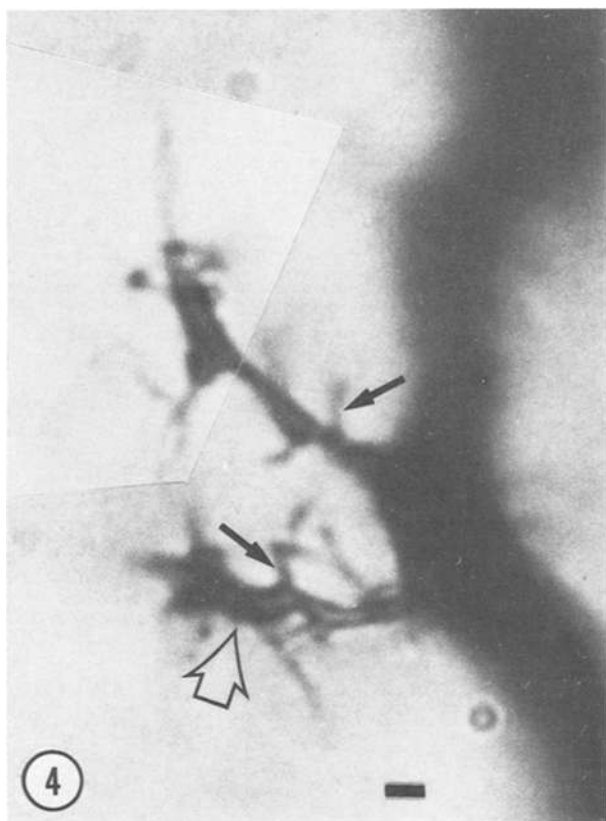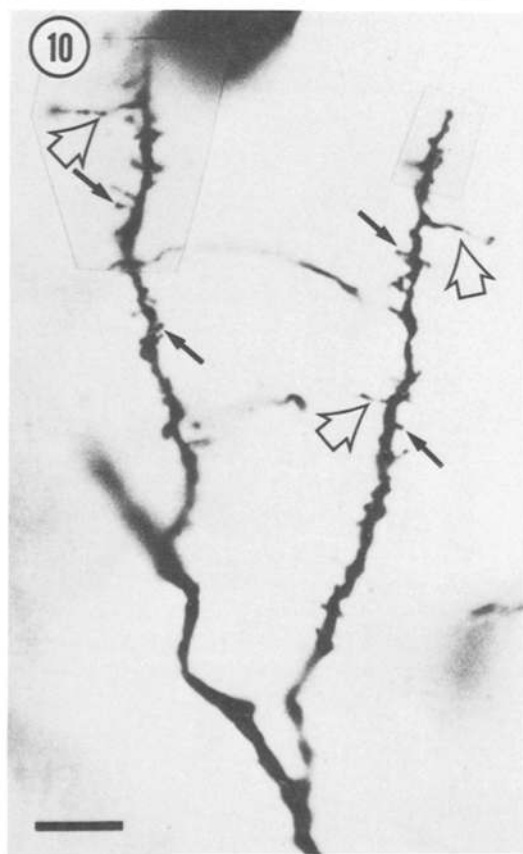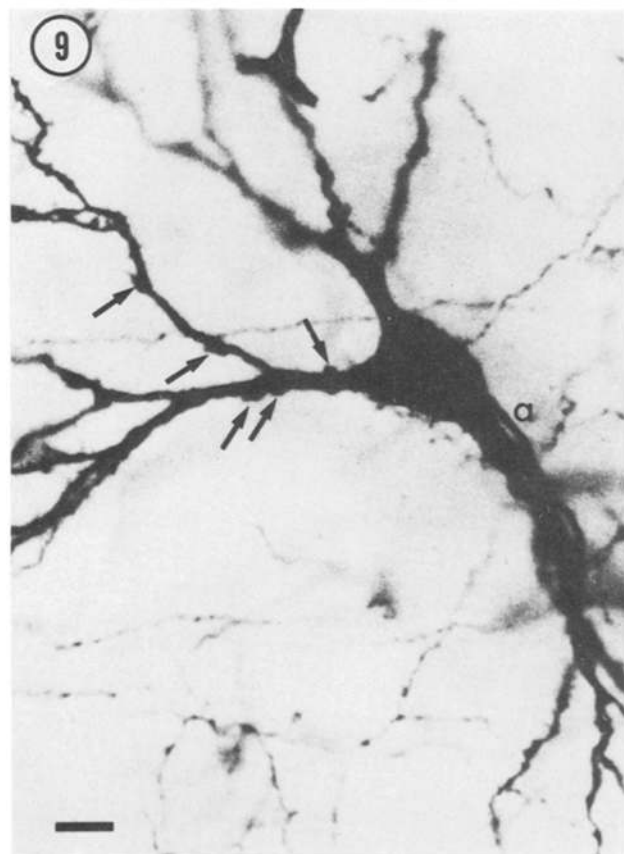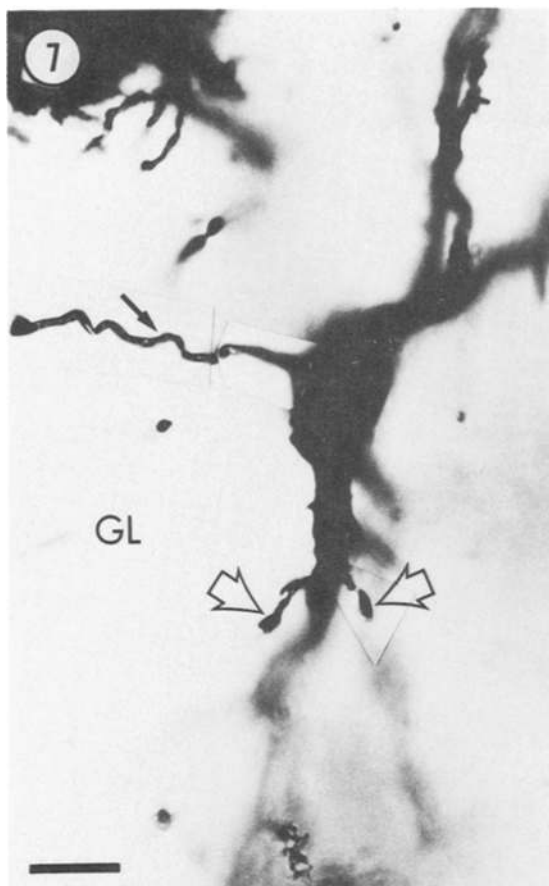
distinguishing features were used to identify them at early stages of development. These features included a relatively large soma, the number, the orientation and branching pattern of the primary dendrites, a location within the hilus and a thick axon hillock originating from the soma.

On postnatal day 1 the dendrites of the mossy cells have a very immature appearance, even less mature than those of the nearby CA3 pyramidal cells of the hippocampus (Fig. 3). Their tips generally bear typical dendritic growth cones which have one or more filipodia (Figs 3, 4). Similar growth profiles are seen at several points along the course of the major dendrites which also display several thin filipodial spines which can be as long as 10 μm. There is no indication of either typical spines on the distal dendrites or thorny excrescences on the proximal dendrites. However, several bulbous axon terminals are found in close apposition to the soma and proximal dendrites and some of these have the same size and shape as immature mossy fibres in stratum lucidum of the hippocampus (Amaral & Dent, 1981).

While dendrites of the mature mossy cell only occasionally enter the granule cell layer, several of the mossy cell dendrites extend through the incipient granule cell layer into the molecular layer during the early postnatal period (Figs 3, 7). At later stages, many of these dendrites appear to thin out progressively and it is possible that some of them are in the process of regression. However, it is also conceivable that these processes are present in the mature brain but are not visualized due to some artifact in the Golgi staining process.

On postnatal day 3, many dendritic growth cones and filipodial spines are still observed, but at later ages there appears to be a progressive decrease in these profiles (cf. Figs 3–10). The filipodial spines, in particular, are far less common on postnatal day 5, especially on the soma and proximal dendrites (Fig. 8). There is no indication that these long spines may be the predecessors of the thorny excrescences. On day 5 a few typical spines are seen on the distal dendrites and these often occur in patches. At postnatal day 7, several of the mossy cell dendrites still bear growth cones either at their tips or at points along their course. However, their number has continued to decline and by day 11 few if any remain. On the distal dendrites, typical pedunculate spines are commonly seen on day 7, but their density is low. While many of them have the typical stalk and bulb appearance, others appear to be long filipodia similar to the filipodial

Figs 11 & 12. Light micrographs of three labelled hilar neurons following an HRP injection into the contralateral dentate gyrus. One of the neurons (arrow) displays the features that are characteristic for mossy cells (cf. Fig. 1). Silver intensification method. × 165 and × 500, respectively.

Fig. 13. Electron micrograph of the soma of a Golgi-impregnated mossy cell. Most of the cell body is occupied by a large round nucleus (N). A large somal spine (arrow) is found to the right of the cell body. On the opposite side, the cell body is apposed by a satellite cell. Two dendrites (D) arise from the cell body. × 10 000.
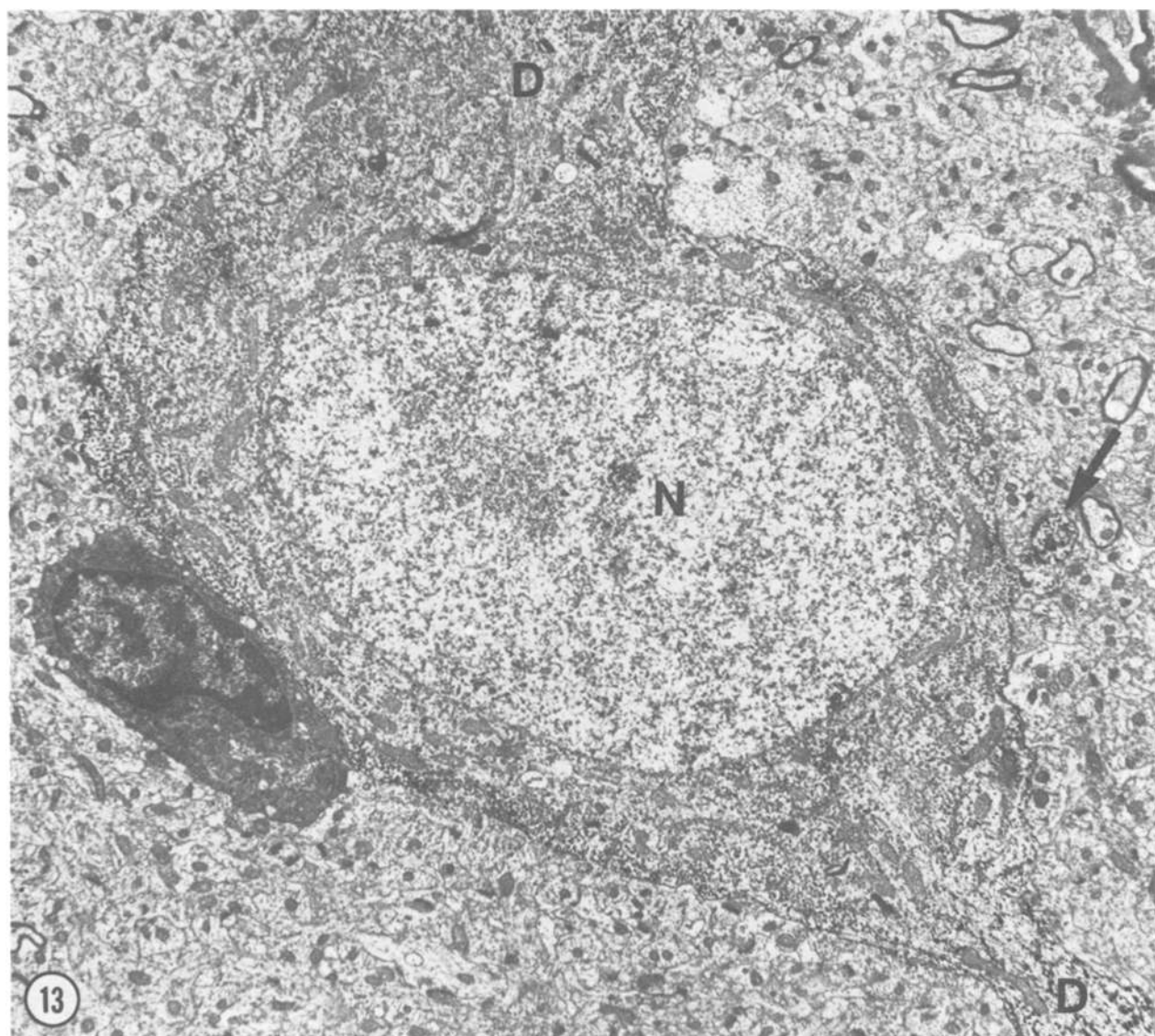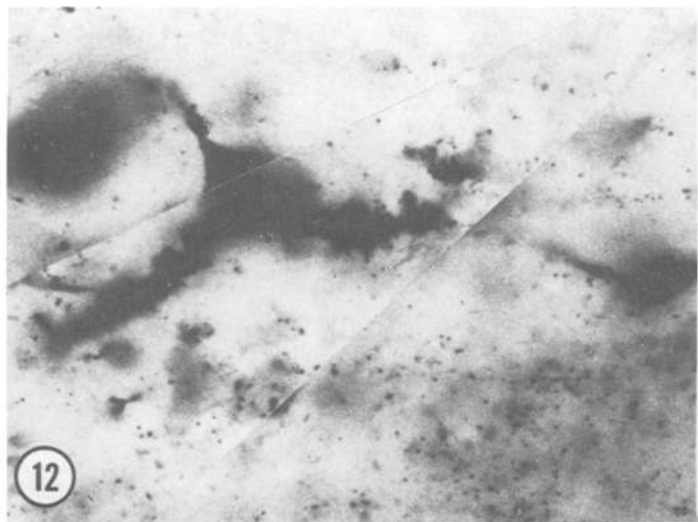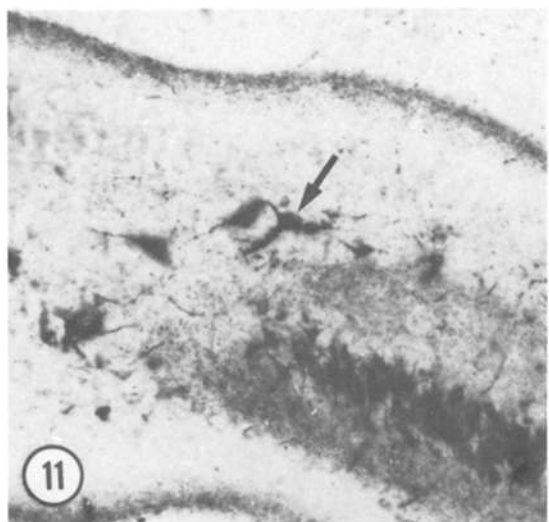
spines seen at earlier ages. The latter are seen on distal dendrites at least until postnatal day 14 (Fig. 10).

The thorny excrescences appear much later than the penduculate spines. While isolated, immature thorny excrescences may be seen as early as day 7, they are not common before day 14 (Fig. 9). A rather rapid proliferation of these special spines then occurs, so that by day 21 the proximal dendrites of the mossy cells have an adult appearance. Thereafter, the thorny excrescences appear to continue to increase in complexity and density into adulthood.

Portions of the mossy cell axon are also immature on postnatal day 1. The axon is varicose and there are several local collaterals which end with growth cones. At postnatal day 7 the axon is far less varicose and by day 14 it has a mature appearance (Fig. 9). In at least a few cases, the axon could be seen to bifurcate and give rise to two relatively thick collaterals and several finer ones. In one case, one of the thick collaterals could be followed through the granule cell layer into the molecular layer.

Retrograde studies

The HRP injection sites included the hilus and granule cell and molecular layers of the dentate gyrus. Cells labelled with HRP are found in the contralateral hilus as described earlier (West *et al.*, 1979; Berger *et al.*, 1981; Voneida *et al.*, 1981; Seroogy *et al.*, 1983). In the silver-intensified preparations, the HRP-labelled neurons appeared similar to those observed in Golgi preparations (Fig. 11). Although they formed a morphologically heterogeneous group, numerous examples of mossy cells were observed among them (Fig. 12). They had thick proximal dendrites covered with thorny excrescences. Most of the labelled mossy cells are located deep in the hilus while others are found just below the granule cell layer of the suprapyramidal limb.

To confirm that retrogradely-labelled commissural cells are mossy cells, we used the


Figs 14 & 15. Electron micrographs of the soma of the mossy cell shown in Figs 1 and 2. Fig. 14 shows a part of the cell body that contains the nucleus (N) and a Golgi complex (G). Numerous somal spines are shown and some of these spines (arrows) appear in Fig. 15 at a higher magnification. Fig. 15 shows complex somal spines that often contain mitochondria (m). Axon terminals (t) that contain numerous round synaptic vesicles contact these spines. One of these contacts (arrow) is shown to be an asymmetric synapse. × 8640 and × 19200, respectively.

Fig. 16. Low-power electron micrograph of a mossy cell dendrite (D). Numerous spines from this dendrite are labelled with gold particles and are contacted by large axon terminals (M) that resemble mossy fibre tufts. × 17000.

Fig. 17. Electron micrograph of an outlined mossy tuft (M) that contacts (arrow) a mossy cell dendrite and one of its spines (S). × 32000.

Fig. 18. Numerous dendritic spines (S) from a mossy cell dendrite (D) are contacted (arrows) by mossy fibre tufts (M). × 34000.

Fig. 19. Three dendritic spines (S) are contacted by the same mossy fibre tuft (M). Note that the mossy fibre tuft also forms a punctum adherens (arrow) with the labelled dendrite. × 41000.

Figure 14

Figure 15
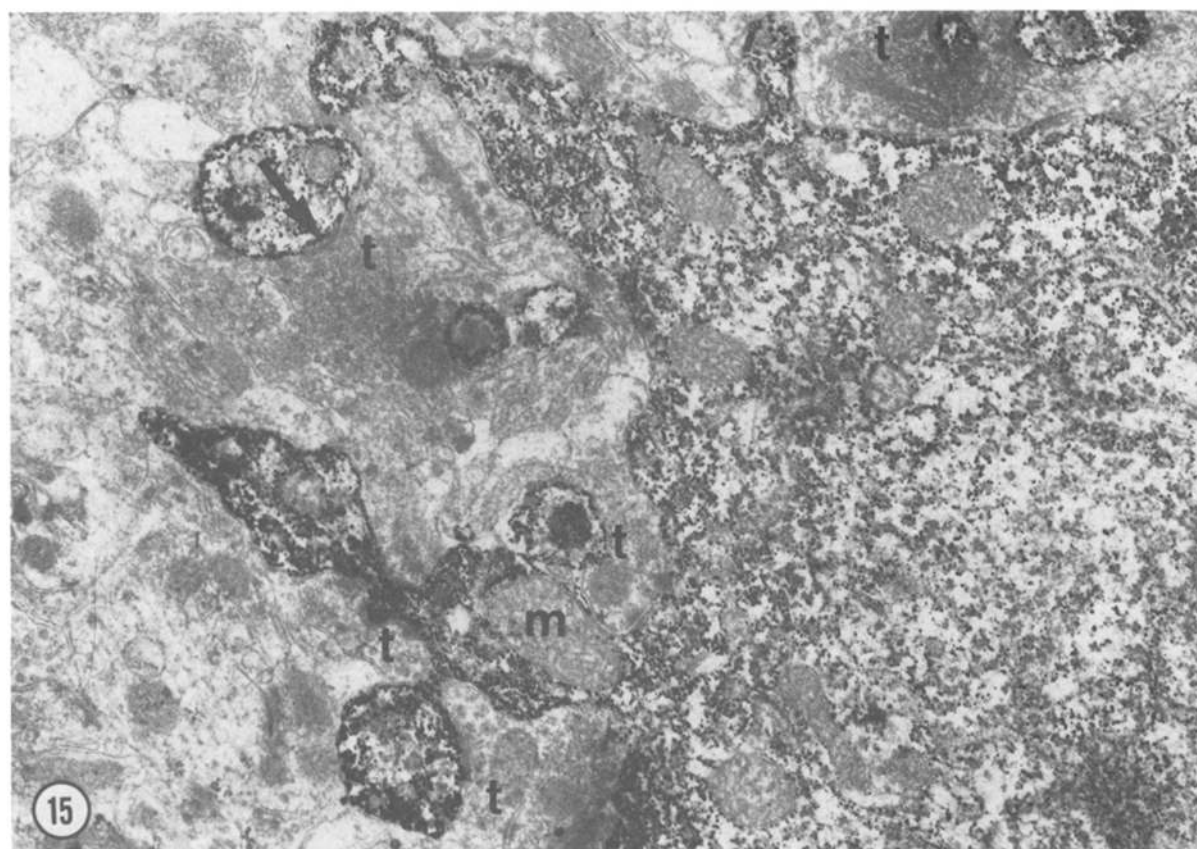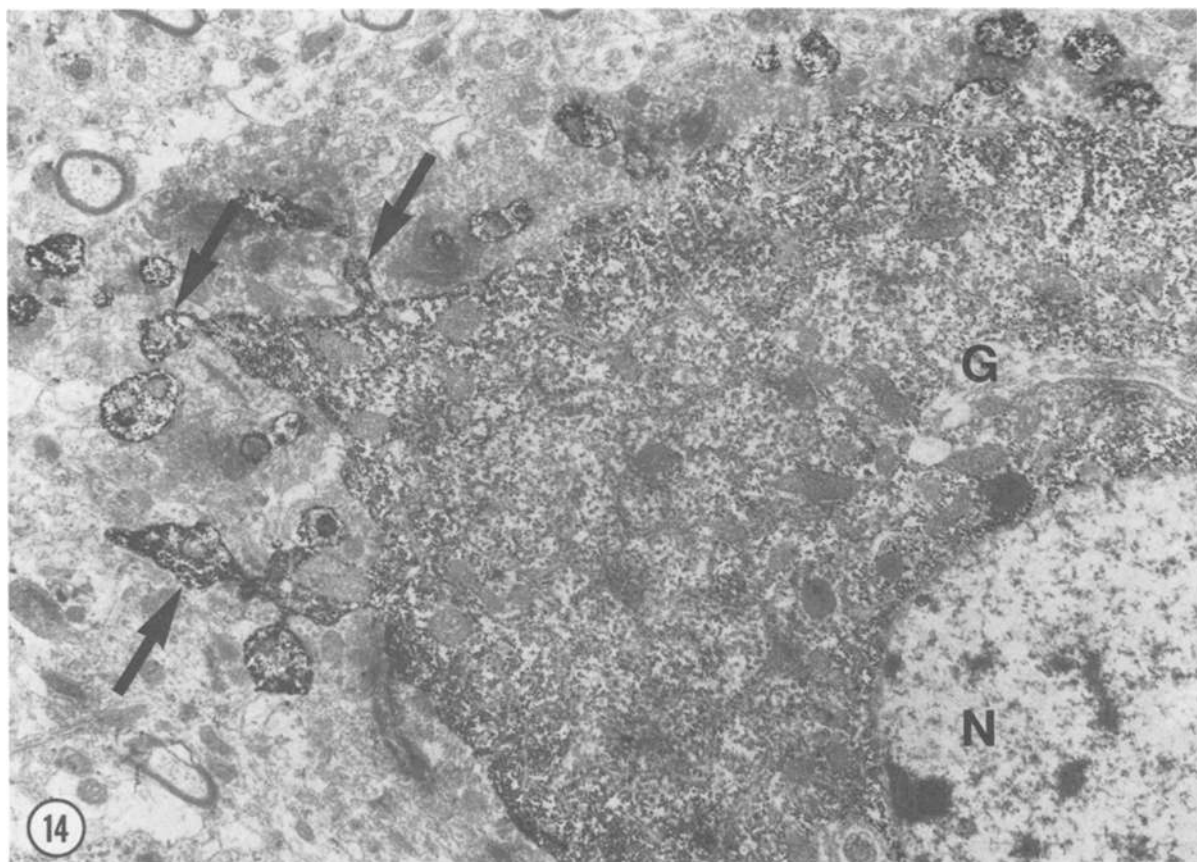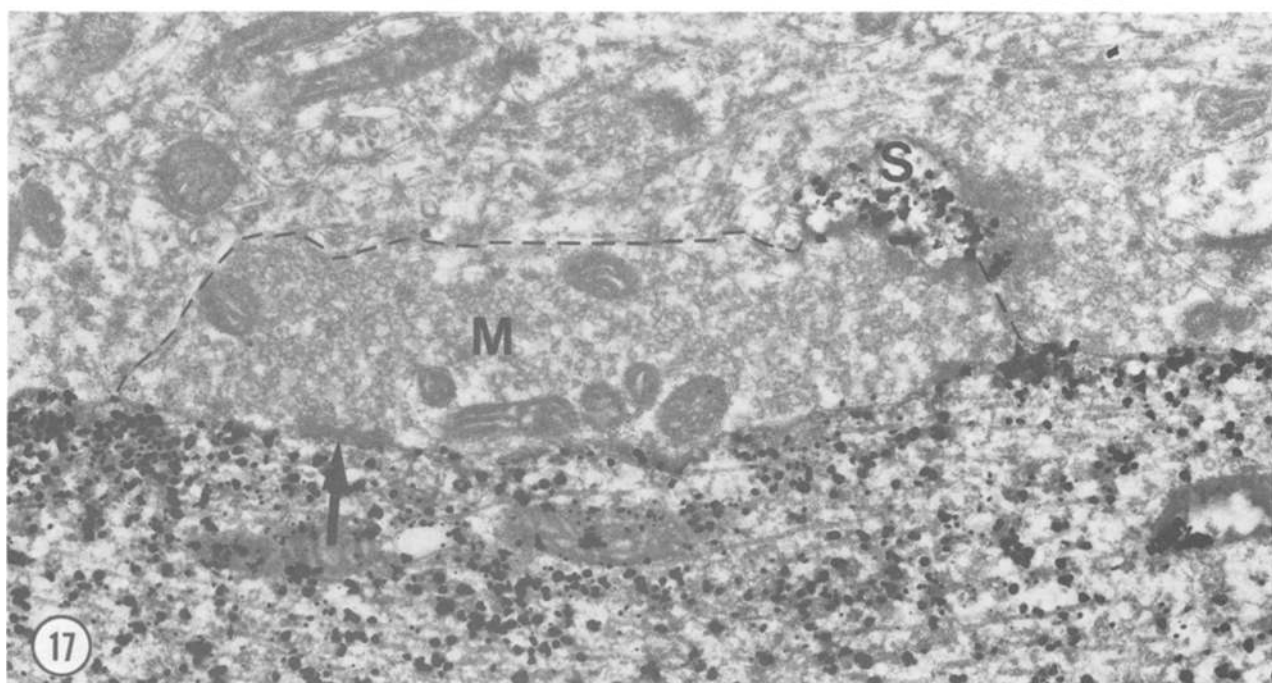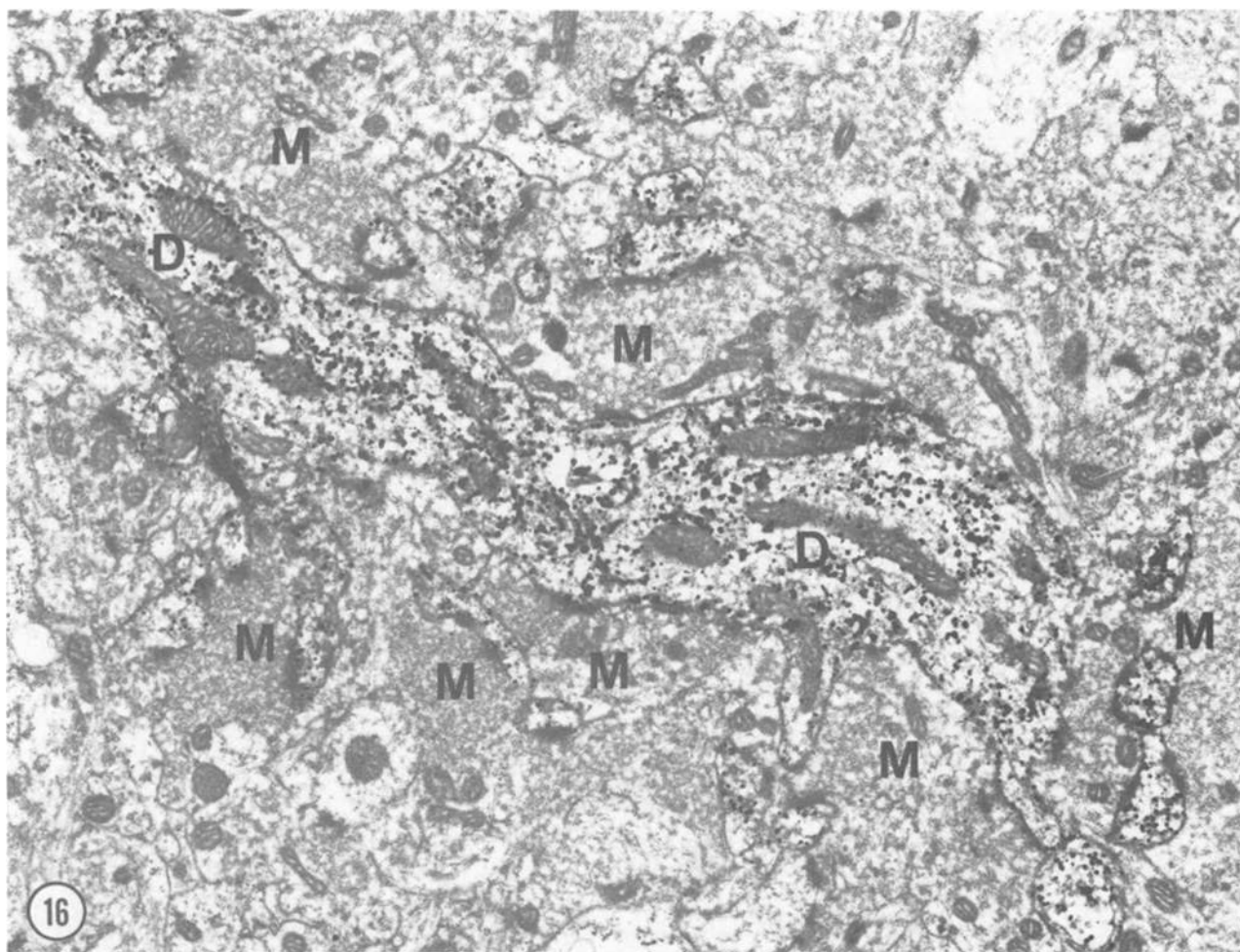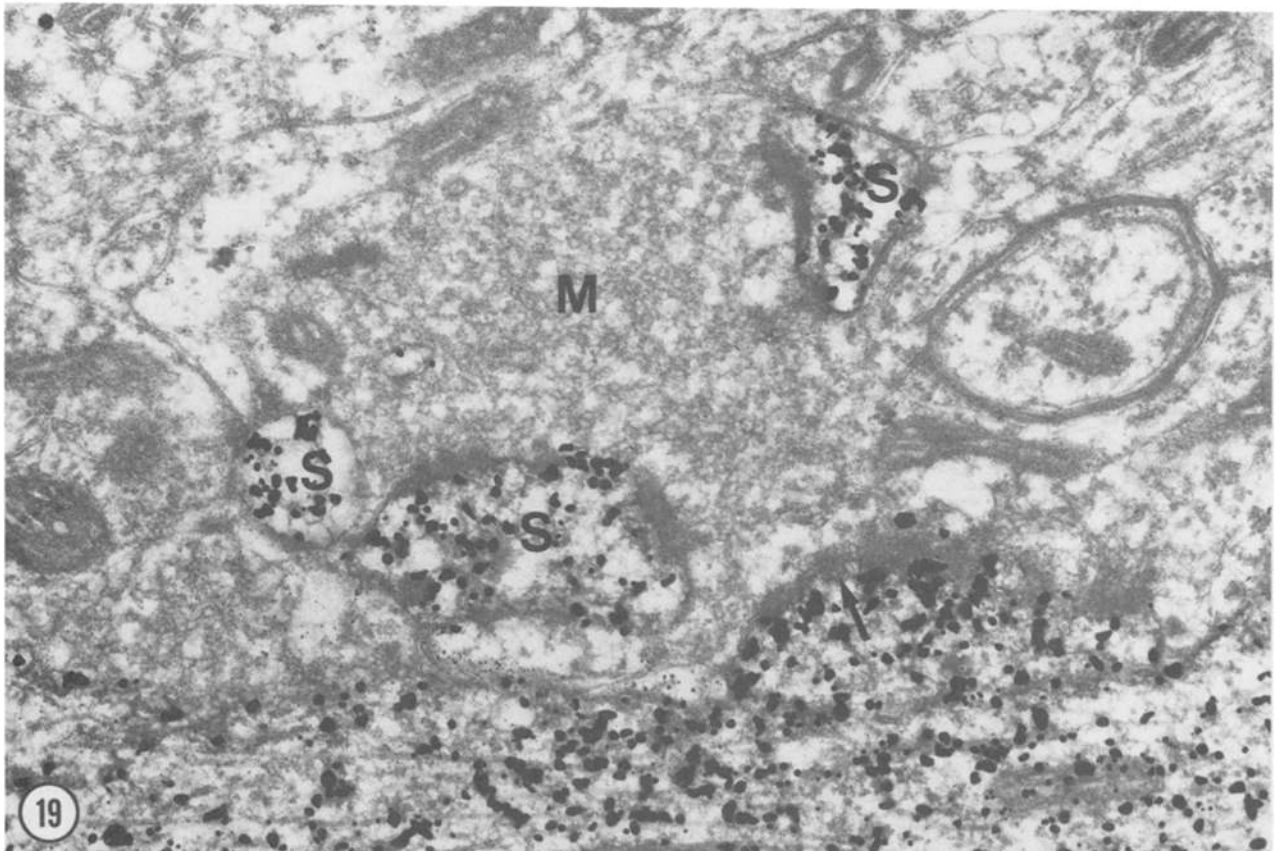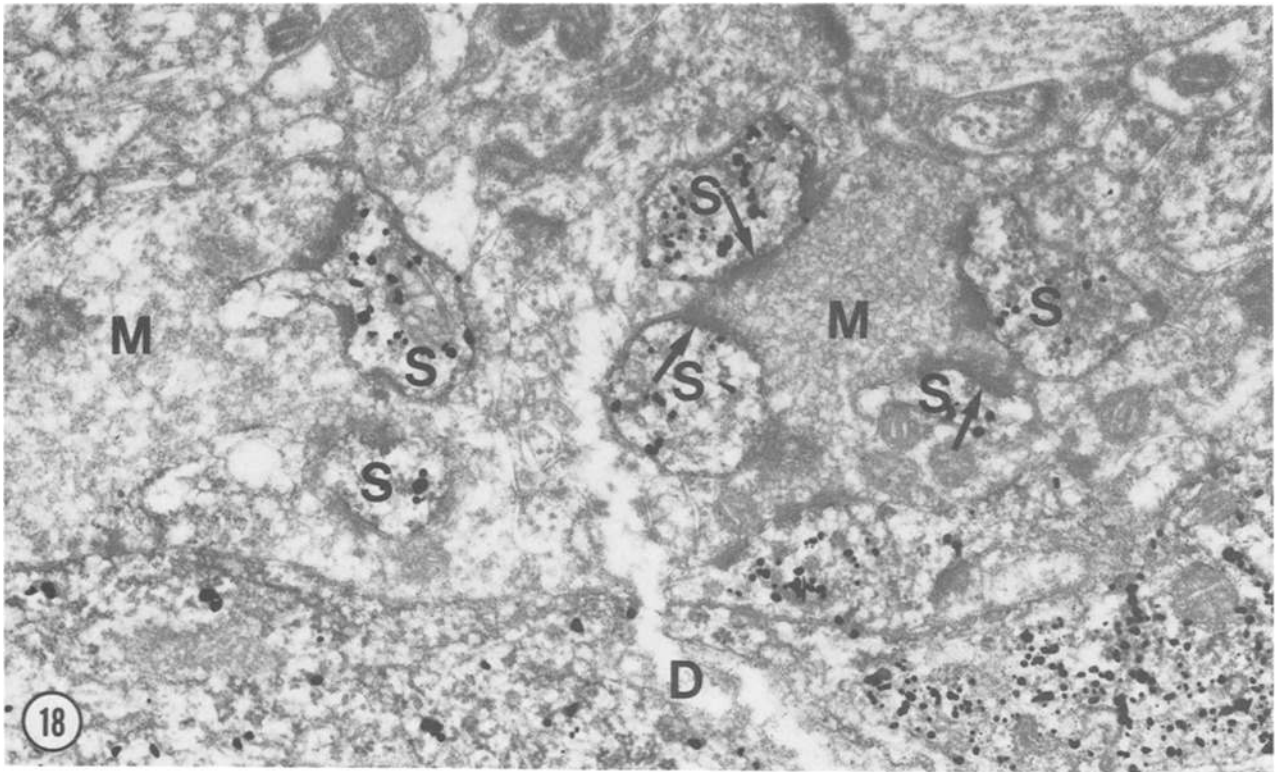
combined Golgi and HRP technique. The results were consistent with the findings reported above. In these preparations, five examples of Golgi-impregnated mossy cells were found among the numerous hilar cell types labelled with HRP. They all had thick dendrites with thorny excrescences and displayed typical arborizations throughout the hilus.

Electron microscopy of Golgi preparations

The mossy cells contain round or oval nuclei that lack infoldings and intranuclear rods (Figs 13, 14). The nuclei contain euchromatin with only rare condensations of chromatin. The perikaryal cytoplasm contains organelles typical of comparably sized pyramidal cell bodies (Figs 13, 14), namely cisternae of granular endoplasmic reticulum, Golgi complex, mitochondria, microtubules and neurofilaments. Nissl bodies are not prominent. The mossy cell bodies also display numerous somal spines (Figs 13–15). Most of these are complex in shape and may branch into three or four smaller spines (Fig. 15). They arise from a thin stalk (0.05–0.2 μm) which generally swells into a varicosity that may contain a mitochondrion (Fig. 15). All of these complex spine structures are contacted by large axon terminals that contain numerous round synaptic vesicles and form asymmetric synapses. These terminals which are similar in size and organelle content to the mossy fibres are often indented by the somal spines but they are rarely in contact with the somata proper. Another group of smaller terminals form symmetric synapses with the smooth surfaces of the mossy cell somata.

The dendrites arise from the cell bodies of mossy cells as large thick tapering structures (Fig. 13). The number of complex spines on the proximal dendrites is extremely large (Fig. 16). These spines generally do not display mitochondria as found for somal spines. In contrast, they are characterized by long thin stalks that either expand to form complex end bulbs that may appear mushroom-shaped or be long and slender with no distinct end bulbs. In almost all micrographs, they are seen to be contacted by large, vesicle-filled axon terminals which form asymmetric synapses that are often perforated (Figs 17–19). The same terminal will often form puncta adherentia (Fig. 19) and occasional synaptic contacts (Fig. 17) with the smooth surface of the dendritic shaft. The distal dendrites of mossy cells are thinner and possess fewer spines than the proximal dendrites. In addition, these spines are smaller, simpler and are contacted by smaller axon terminals. These terminals also contain round synaptic vesicles and form asymmetric synapses but are smaller than those that contact the larger spines on the proximal dendrites. Occasional symmetric synapses are observed on these dendrites.

Fig. 20. Electron micrograph of the axon initial segment of the mossy cell shown in Figs 1 and 2. This axon is characterized by a dense undercoating and a fasciculation of microtubules (m). A small axon terminal (t) forms a symmetric synapse (arrow) with this initial segment. × 23 000.

Fig. 21. Electron micrograph of two gold labelled axon terminals from a mossy cell that form asymmetric synapses (arrows) with an unidentified hilar dendrite. × 42 000.
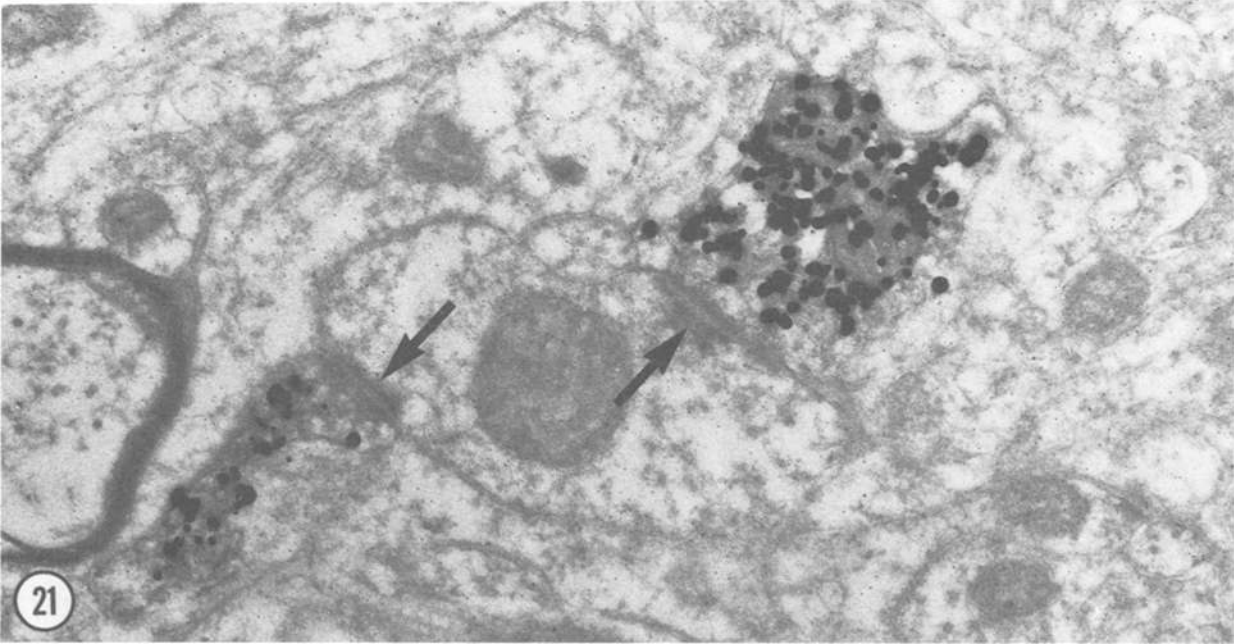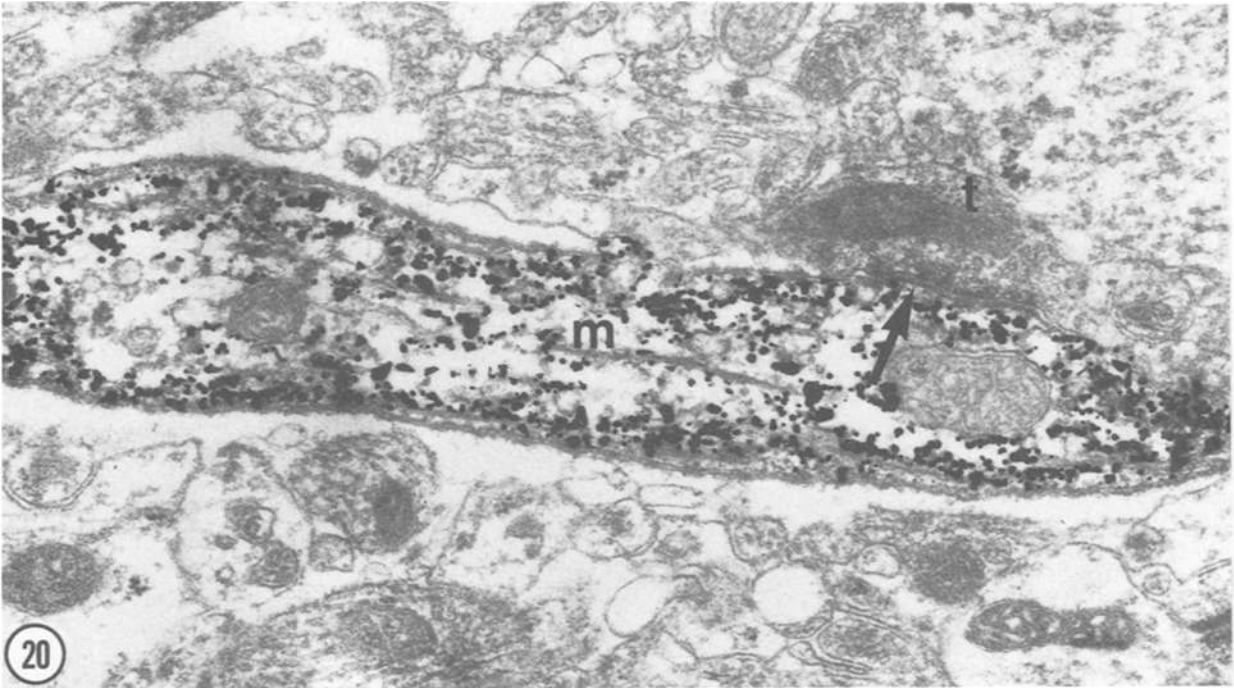
The axon initial segment of mossy cells arises from a distinct axon hillock region of the cell body and contains the typical axolemmal dense undercoating and fasciculation of microtubules. A few small terminals form symmetric synapses with it (Fig. 20). Where the axon acquires its myelin sheath, it loses its Golgi labelling. However, a few impregnated axon collaterals of mossy cells have been observed in the hilus and their terminals formed asymmetric synapses (Fig. 21).

Discussion

In the present study, the mossy cells of the dentate gyrus have been examined in developing and mature brains. They were analysed at the light and electron microscopic levels in Golgi, HRP, HRP–Golgi and Golgi–EM preparations. The results have demonstrated the unique development, connections and ultrastructural features of the mossy cells.

Development
The mossy cells are readily identified in Golgi preparations of the 1-day-old hilar region due to their distinctive morphology. They lie in a region in which granule cell proliferation and migration is taking place throughout much of the first 2 postnatal weeks (Schlessinger *et al.*, 1975; Bayer, 1980). Both the dendrites and axons of the mossy cells have an immature appearance at this age. The ipsilateral axon has numerous varicosities and several thin collaterals even though many of the commissurally projecting axons have already reached the contralateral hippocampal formation by the day of birth (Cowan *et al.*, 1981). The immature mossy cell dendrites are characterized by numerous growth cones and filipodia. While it would be of interest if the filipodial processes were the predecessors to the thorny excrescences, this does not seem to be the case. Few growth cones and filipodia are seen on mossy cells from 7-day-old brains, and this is at least 2 days prior to the emergence of the thorny excrescences.

We have shown that the thorny excrescences of the mossy cell appear at a rather late stage. They begin to appear around postnatal day 9 and are commonly observed around day 14. This is a similar time course as that for the excrescences on the proximal dendrites of the hippocampal pyramidal cells (Amaral & Dent, 1981), despite the fact that mossy fibres establish synaptic contact with the pyramidal cell dendrites as early as postnatal day 1. These data suggest that the mossy fibres may play a role in inducing the development of the complex spines, although this relationship awaits experimental verification. In contrast, the typical pedunculate spines on the distal dendrites of the mossy cells are first observed between postnatal days 3 and 5, and thus they appear several days before the development of the thorny excrescences. This differential temporal pattern would indicate that spine formation is not a unitary event but depends, perhaps, on the establishment of appropriate synaptic contacts.

The general dendritic orientation of the immature mossy cells is similar to the adult pattern. However, it is more common to observe a mossy cell dendrite extending into

the granule cell and molecular layers in the neonatal hippocampus. It is not clear whether this difference can be attributed to some regressive remodelling of the mossy cell dendritic tree or is simply due to an artifact of the Golgi technique. Intracellular filling of mossy cells with HRP would resolve this issue.

Retrograde tracer

We have used a silver-intensified HRP procedure because this allows substantial dendritic detail to be observed in the retrogradely labelled cells. Although a variety of cell types were retrogradely labelled following injections of HRP into the contralateral dentate gyrus, numerous mossy cells were consistently identified. In these preparations, the clusters of thorny excrescences were clearly observed on the proximal dendrites of the mossy cells and the pattern of dendritic arborization was reminiscent of that displayed by the mossy cells in Golgi preparations.

In these HRP preparations, the individual mossy cells appeared to be more heavily labelled than the other labelled hilar cells, even those of equal size. This finding might indicate that the mossy cells have a more extreme terminal plexus in the contralateral dentate gyrus than the other hilar cells. It is now clear that the commissural connection is somewhat heterogeneous. A small population of GABAergic neurons (Ribak *et al.,* 1985) and a small population of somatostatin-containing neurons (Zimmer *et al.,* 1983) are known to project to the contralateral dentate gyrus. The GABAergic neurons appear to terminate in a different manner from the majority of commissural fibres in that they form symmetric synapses (Ribak *et al.,* 1985). Since these terminals are sparse, the GABAergic neurons may account for some of the more lightly retrogradely labelled cells. There is at present little indication of a transmitter candidate for the mossy cells because their size and shape does not match the variety of peptide-containing and GABA-containing cells in the hilar region.

The combination of Golgi impregnation with retrograde transport of HRP confirmed the results obtained with the intensified HRP procedure: both mossy cells and other types of hilar cells identified from the Golgi staining contained HRP reaction product within their somal cytoplasm.

Electron microscopy

The combined Golgi–EM method facilitated an ultrastructural analysis of the somata, dendrites and axons of mossy cells. Important features of these structures were described to provide a basis for the identification of these neurons in conventional EM preparations. In addition, this analysis characterized the afferent axon terminals that contact mossy cells.

The somal features of mossy cells include large, round or oval nuclei and a perikaryal cytoplasm enriched with typical organelles. Although somal spines form asymmetric synapses, the smooth surface of the mossy cell forms symmetric synapses with axon terminals. Neuronal somata with similar features were described for the first of two types of commissural neurons of the hilar region in the dentate gyrus (Seroogy *et al.,*

1983). In fact, that cell type had thick and spiny dendrites, but the typical spiny nature of mossy cell dendrites was not described because reconstructions were not made from serial sections. Thus, these findings are consistent with the present retrograde tracer results which show that mossy cells are commissural neurons.

Light microscopic Golgi studies have shown mossy fibre tufts in apposition to mossy cell dendrites (Amaral, 1978). Our EM results confirmed this relationship and demonstrated that numerous synaptic contacts are formed by mossy fibres with mossy cells. In fact, most of the large axon terminals with round synaptic vesicles and numerous asymmetric synaptic contacts with the somal and dendritic spines of mossy cells are probably mossy fibres derived from the granule cells of the dentate gyrus; terminals with similar features were described as mossy fibres in the hilus of the dentate gyrus and the CA3 region of the hippocampus (Blackstad & Kjaerheim, 1961; Hamlyn, 1962; Blackstad, 1963; Laatsch & Cowan, 1966a). This synaptic feature for complex spines of mossy cells confirms the postulate of Lorente de Nó (1934) that the thorny excrescences observed at the light microscopic level represent the postsynaptic element of mossy fibre synapses.

The portions of mossy cell dendrites that are located in the superficial hilus located about 100–150 μm beneath the granule cell layer in the limiting subzone lack these large spines or thorny excrescences. This region also lacks the typical, large mossy fibre tufts (Cajal, 1911), although smaller versions of the mossy fibre expansion are observed in this region as well as small varicosities on the subgranular collaterals of mossy fibres (Blackstad, 1963; Claiborne et al., 1983). It is interesting that the thin spines of mossy cell dendrites in this area are contacted by terminals with features similar to those from the proximal axon collaterals of granule cell axons which were identified in Golgi–EM preparations and shown to make numerous synapses with the basal dendrites of basket cells (Ribak & Seress, 1983). Together with the information about complex spines, these data indicate that most of the somal and dendritic surfaces of mossy cells are contacted by axons of granule cells.

Another group of terminals form symmetric synapses with the somata and axon initial segments of mossy cells. These terminals are probably derived from GABAergic local circuit neurons in the hilus of the dentate gyrus because numerous GABAergic neurons exist in this region (Seress & Ribak, 1983) and because EM studies have shown that GABAergic terminals in the hippocampal formation form symmetric synapses (Ribak et al., 1978; Kosaka et al., 1984; Somogyi et al., 1983). Such a distribution of symmetric synapses is similar to, though less dense than, that described for pyramidal cells.

Unfortunately, the axons of mossy cells are difficult to examine in Golgi–EM preparations because they generally fail to impregnate due to myelination that occurs 50–100 μm away from the soma. Nevertheless, some gold-labelled terminals of mossy cells were observed and seen to form asymmetric synapses in the hilus. This type of synapse is considered to be excitatory and is the same type that is most frequently labelled in studies of the commissural pathway (Blackstad, 1965; Laatsch & Cowan, 1966b; Hjorth-Simonsen & Laurberg, 1977; Kishi et al., 1980; Seress & Ribak, 1984).

In conclusion, the mossy cell is a prominent cell type in the hilus and receives a major portion of its input from granule cells. Since this input is located on proximal and, most likely, distal dendrites of mossy cells, it could in fact have a dominant effect on the activity of the cell. In contrast, the pyramidal cells of CA3 receive granule cell input on restricted portions only of their apical and basal dendrites. Therefore, mossy cells are probably more easily influenced by granule cell activity than are pyramidal cells. Furthermore, the results of the present study demonstrate clearly that the mossy cells give rise to a portion of the commissural projection. It has also been established, with the use of double labelling techniques, that each commissurally projecting neuron gives rise to an ipsilateral association collateral (Swanson et al., 1981). Therefore, the mossy cell is in an ideal position to sample the output of numerous granule cells and to provide feedback about granule cell activity to both the ipsilateral and contralateral dentate gyrus.

Acknowledgements

References

AMARAL, D. G. (1978) A Golgi study of cell types in the hilar region of the hippocampus in the rat. *Journal of Comparative Neurology* **182**, 851–914.

AMARAL, D. G. & DENT, J. A. (1981) Development of the mossy fibers of the dentate gyrus. I. A light and electron microscopic study of the mossy fibers and their expansions. *Journal of Comparative Neurology* **195**, 51–86.

BAYER, S. A. (1980) Development of the hippocampal region in the rat. I. Neurogenesis examined with ^{3}H-thymidine autoradiography. *Journal of Comparative Neurology* **190**, 87–114.

BERGER, T. W., SEMPLE-ROWLAND, S. & BASSETT, J. L. (1981) Hippocampal polymorph neurons are the cells of origin for ipsilateral association and commissural afferents to the dentate gyrus. *Brain Research* **215**, 329–36.

BEROD, A., HARTMAN, B. K. & PUJOL, J. F. (1981) Importance of fixation in immunohistochemistry: use of formaldehyde solutions at variable pH for the localization of tyrosine hydroxylase. *Journal of Histochemistry and Cytochemistry* **29**, 844–50.

BLACKSTAD, T. W. (1963) Ultrastructural studies on the hippocampal region. *Progress in Brain Research* **3**, 122–48.

BLACKSTAD, T. W. (1965) Mapping of experimental axon degeneration by electron microscopy of Golgi preparations. *Zeitschrift für Zellforschung und mikroskopische Anatomie* **67**, 819–934.

BLACKSTAD, T. W. & KJAERHEIM, A. (1961) Special axo-dendritic synapses in the hippocampal cortex: electron and light microscopic studies on the layer of mossy fibers. *Journal of Comparative Neurology* **117**, 133–59.

CAJAL, S. R. y (1911) *Histologie du Système Nerveux de l'Homme et des Vertébrés* Tome II. Paris: Maloine.

CLAIBORNE, B. J., AMARAL, D. G. & COWAN, W. M. (1983) Intracellular filling of rat dentate granule cells with HRP: analysis of mossy fiber collaterals. *Society for Neuroscience Abstracts* **9**, 220.

COWAN, W. M., STANFIELD, B. B. & AMARAL, D. G. (1981) Further observations on the development of the dentate gyrus. In *Studies in Developmental Neurobiology* (edited by COWAN, W. M.), pp. 395–435. New York: Oxford University Press.

FAIRÉN, A., PETERS, A. & SALDANHA, J. (1977) A new procedure for examining Golgi impregnated neurons by light and electron microscopy. *Journal of Neurocytology* **6**, 311–37.

FREUND, T. F. & SOMOGYI, P. (1983) The section – Golgi impregnation procedure. I. Description of the method and its combination with histochemistry after intracellular iontophoresis or retrograde transport of horseradish peroxidase. *Neuroscience* **9**, 463–74.

GALLYAS, F. (1971) A principle for silver staining of tissue elements by physical development. *Acta morphologica Academiae scientiarum hungaricae* **19**, 57–71.

HAMLYN, L. H. (1962) The fine structure of the mossy fibre endings in the hippocampus of the rabbit. *Journal of Anatomy* **96**, 112–20.

HJORTH-SIMONSEN, A. & LAURBERG, S. (1977) Commissural connections of the dentate area in the rat. *Journal of Comparative Neurology* **174**, 591–606.

KISHI, K., STANFIELD, B. B. & COWAN, W. M. (1980) A quantitative EM autoradiographic study of the commissural and associational connections of the dentate gyrus in the rat. *Anatomy and Embryology* **160**, 173–86.

KOSAKA, T., HAMA, K. & WU, J.-Y. (1984) GABAergic synaptic boutons in the granule cell layer of rat dentate gyrus. *Brain Research* **293**, 353–59.

LAATSCH, R. H. & COWAN, W. M. (1966a) Electron microscopic studies of the dentate gyrus of the rat. I. Normal structure with special reference to synaptic organization. *Journal of Comparative Neurology* **128**, 359–96.

LAATSCH, R. H. & COWAN, W. M. (1966b) Electron microscopic studies of the dentate gyrus of the rat. II. Degeneration of commissural afferents. *Journal of Comparative Neurology* **130**, 241–62.

LIPOSITS, Z., GÖRCS, T., GALLYAS, F., KOSARAS, B. & SÉTÁLÓ, G. (1982) Improvement of the electron microscopic detection of peroxidase activity by means of the silver intensification of the diaminobenzidine reaction in the rat nervous system. *Neuroscience Letters* **31**, 7–11.

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.

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., SERESS, L., SEROOGY, K. B., PETERSON, G. M. & OERTEL, W. H. (1985) The hippocampal commissural pathway contains a GABAergic inhibitory component. *Anatomical Record* **211**, 160 A.

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.

SCHLESSINGER, A. R., COWAN, W. M. & GOTTLIEB, D. J. (1975) An autoradiographic study of the time of origin and the pattern of granule cell migration in the dentate gyrus of the rat. *Journal of Comparative Neurology* **159**, 149–76.

SERESS, L. & RIBAK, C. E. (1983) GABAergic cells in the dentate gyrus appear to be local circuit and projection neurons. *Experimental Brain Research* **50**, 173–82.

SERESS, L. & RIBAK, C. E. (1984) Direct commissural connections to the basket cells of the hippocampal dendate gyrus: anatomical evidence for feed-forward inhibition. *Journal of Neurocytology* **13**, 215–25.

SEROOGY, K. B., SERESS, L. & RIBAK, C. E. (1983) Ultrastructure of commissural neurons of the hilar region in the hippocampal dentate gyrus. *Experimental Neurology* **82**, 594–608.

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 synaptic terminals with special reference to the axon initial segment of pyramidal neurons. *Journal of Neuroscience* **3**, 1450–68.

SWANSON, L. W., SAWCHENKO, P. E. & COWAN, W. M. (1981) Evidence for collateral projections by neurons in Ammon's horn, the dentate gyrus, and the subiculum: a multiple retrograde labeling study in the rat. *Journal of Neuroscience* **1**, 548–59.

VONEIDA, T. J., VARDARIS, R. M., FISH, J. E. & REIHELD, C. T. (1981) The origin of the hippocampal commissure in the rat. *Anatomical Record* **201**, 91–103.

WEST, J. R., NORNES, H. O., BARNES, C. L. & BRONFENBRENNER, M. (1979) The cells of origin of the commissural afferents to the area dentata in the mouse. *Brain Research* **160**, 203–16.

ZIMMER, J., LAURBERG, S. & SUNDE, N. (1983) Neuroanatomical aspects of normal and transplanted hippocampal tissue. In *Neurobiology of the Hippocampus* (edited by SEIFERT, W.), pp. 39–64. London: Academic Press.