A STUDY OF THE OLFACTORY PATHWAY.

by

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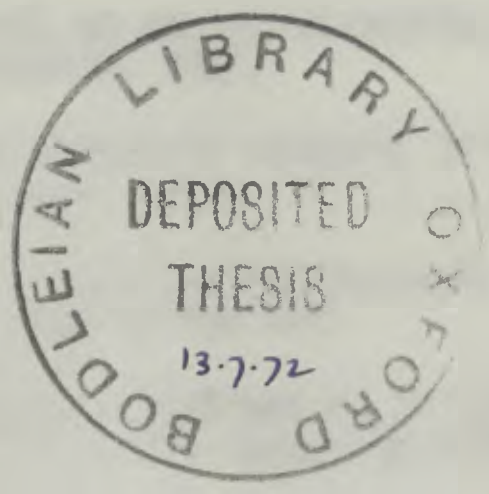

being a thesis submitted in fulfilment of the requirements for the degree of Doctor of Philosophy in the University of Oxford.

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List of Abbreviations used in Photographic Illustrations

a axon
c cell soma
cy cytoplasm
d dendrite
da transneuronal degeneration of an axon
dc transneuronal degeneration of a cell soma
dd transneuronal degeneration of a dendrite
de desmosome
dg transneuronal degeneration of a gemmule
dp transneuronal degeneration of the peripheral process of a granule cell
ds transneuronal degeneration of a spine
dt transneuronal degeneration of an axon terminal
e extracellular space
ep external plexiform layer
er granular endoplasmic reticulum
et external tufted cell
f glial filaments
fi fibrillar part of nucleolus
g gemmule
gj gap junction
gl glial cytoplasm
gm glomerulus
go Golgi apparatus
gr granular part of nucleolus
h degenerating centrifugal fibre terminal
ip internal plexiform layer
is axon initial segment
k degenerating tufted cell collateral terminal
l glial lamella(e)
ly lysosome
m mitochondrion

mi mitral cell layer
my myelin sheath
n nucleus
no nucleolus
on olfactory nerve layer
ot orthograde degeneration of an axon terminal
p pedicle
pg periglomerular cell
pr periglomerular region
r nuclear rodlet
ro robosomal rosette
s spine
sa superficial short-axon cell
sj sac junction
t axon terminal
to glial tongue
u unmyelinated olfactory nerve fibre
v vacuole
z nuclear indentation

Arrow-heads indicate synaptic specialisations from the post synaptic side.

Double arrow-heads indicate persisting post-synaptic membrane thickenings.

CHAPTER 1

GENERAL INTRODUCTION

"What is great in man is that he is a bridge and not a goal."

Friedrich Nietzsche.

GENERAL INTRODUCTION

The complexity of the central nervous system of higher animals has never been in doubt, but it has perhaps been brought home more forcibly in recent years by virtue of the extremely detailed knowledge that has been obtained by electron microscopic studies on many regions of the mammalian brain; ultrastructural studies seem to provide information that is closer than any previous morphological investigations to the basic function of neurons in information processing. This is due, as much as anything, to the fact that the resolution of this type of microscope is sufficient to show what appears to be the morphological equivalent of Sherrington's functional concept: the "synapse"; indeed, this ultrastructural entity is now so accepted as being identical with the physiological synapse, that the same name is used for both. Moreover, recent electron microscopy has shown that several ultrastructural subtypes of synapse may be discerned, and these have been tentatively related by some authors to the functional properties of excitation and inhibition. The inevitable corollary of the high magnification provided by the electron microscope is that only limited areas of brain tissue may be examined and this makes the use of light microscopic information and material imperative to provide the context for assessing and interpreting ultrastructural findings.

Several approaches are currently being used to determine the principles of information processing in the central nervous system, and in many parts of the brain; electron microscopy is a relatively recent technique for study of this kind, and it has so far only been

systematically applied to a few sites such as the retina, lateral geniculate body and cerebellum in an attempt to understand these principles. The olfactory bulb is a highly suitable site for these studies, for many reasons, some of which it shares with the aforementioned sites. It is a clearly laminated and quite discrete structure; the few basic neuron types have processes whose distribution is well known from classical histology. It has a few well-defined afferent and efferent pathways, and no fibres pass through it to or from unrelated nuclei; both the bulb and its pathways are easily accessible for anatomical and physiological approaches in several species including the macrosmatic rat and rabbit. The olfactory bulb is probably the most accessible first relay in any sensory pathway and may serve as a model for other sensory relays. It is essentially a simple structure, having the same fundamental architecture throughout, and has been considered as a simple cortical structure (Shepherd, 1963b); through an understanding of its function, it may thus be possible to comprehend the basic coding principles of neuronal organisation. Finally, since the physiology of olfaction is still an obscure subject, more precise anatomical knowledge of the neuronal organisation of its pathway could help to clarify concepts regarding its function.

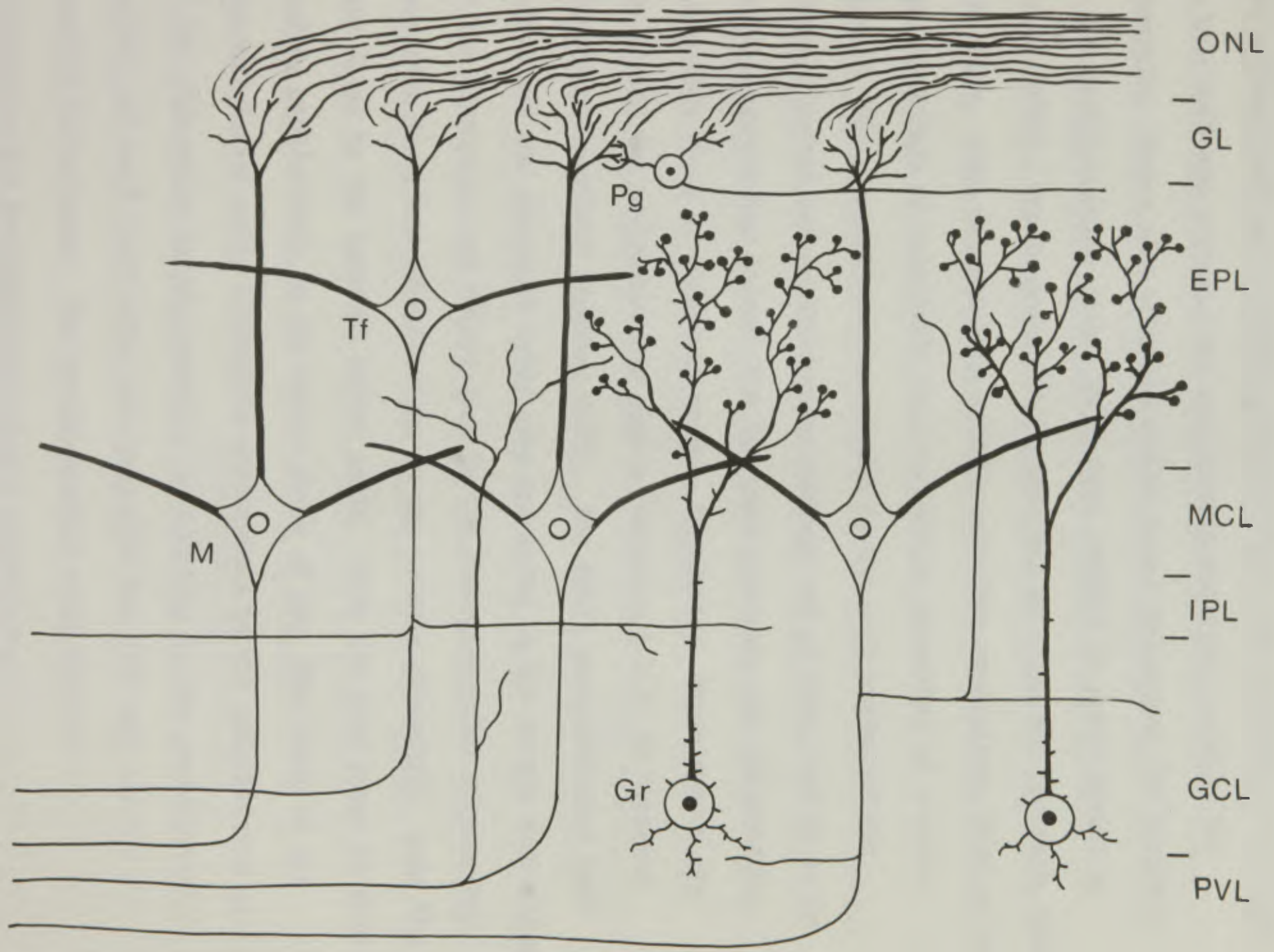
Light Microscopy

The histology of the olfactory bulb at the light microscopic level has been known in considerable detail for many years, and particularly

since the studies by Golgi (1875), Cajal (1890,1911,1955) and their contemporaries (van Gehuchten & Martin, 1891; Blanes, 1898). On the basis of previous studies on the layering of the bulb as seen with the Nissl staining procedures (e.g. Schwalbe, 1881), these authors used the Golgi method and its modifications to analyse the neurons, their processes and the glia of the bulb in the great detail that this new technique then offered them. Several summaries of the overall picture of the bulb that emerged from these early studies are available (e.g. Cajal, 1955) & (Figure A); to further clarify the present study on the glomerular layer it may be useful first to outline in some detail the concept of this layer held by the classical histologists, particularly the Madrid group. This is not to replace the reading of the valuable and highly detailed original descriptions, notably those by Cajal (1890,1911,1955) and Blanes (1898), which provide authoritative and careful light microscopic descriptions, but rather to link these with our own Golgi material, which has been studied with a view to correlation with electron microscopic data.

The olfactory receptor axons, after passing through the cribriform plate of the ethmoid bone, form a layer on the surface of the olfactory bulb from which they penetrate into the subjacent layer of glomeruli; their ramifications within the glomeruli are precisely delineated by the boundaries of these structures and never extend beyond them. Each nerve fibre "breaks up successively into short, flexuose, relatively stout and extremely varicose secondary branches, and always terminates freely with an olive-shaped or rounded knot" (Cajal, 1890). The same

Fig. 4 A schematic diagram, modified from Cajal (1911) to show the layers of the olfactory bulb and their principal component neurons and neuronal processes (short-axon cells are not included). Olfactory nerve fibres pass from the olfactory nerve layer (ONL) into the glomeruli (GL), where they contact the dendritic arborisations of the mitral (M), tufted (Tf) and periglomerular (Pg) cells. The mitral and tufted cells send their axons in the lateral olfactory tract towards the cerebral hemispheres via the periventricular layer (PVL); the dendrites of these cells pass in the external plexiform layer and are here related to the peripheral processes of the granule cells (Gr), whose cell bodies and deep dendrites lie deep to the mitral cell layer (MCL), in the granule cell layer (GCL). The peripheral processes of the granule cells do not reach the glomerular layer, nor do the lateral, secondary dendrites of the mitral and tufted cells. Collateral fibres arising from the mitral and tufted cell axons arise in a subdivision of the granule cell layer, just deep to the mitral cell layer, known as the internal plexiform layer (IPL). The axons of the peri-glomerular cells run laterally, restricted to the glomerular layer.



A

glomeruli also contain the thick expansions of the mitral and tufted cell primary dendrites, which break up on entering the glomeruli into a dense tuft or tree-like arborisation; the branches which make up this tuft are varicose and are distributed solely within the glomeruli. There also arborise within these structures the dendrites of the periglomerular cells, which were studied in great detail by Blanes (1898), who described their branches as "fine and varicose, but not spiny. Some nevertheless exhibit hair-like expansions, that is to say, coverings of relatively long appendages sprouting at a sharp angle." The glomeruli are surrounded by the cell bodies of the external tufted and periglomerular neurons and of glia, and it is in the interglomerular spaces that the axon terminals and collaterals, other than those of olfactory nerves, terminate; these spaces are now termed the periglomerular region (Chapters 4 & 5) or "stratum granulosum externum" (Andres, 1965). The small periglomerular cell bodies are the commonest type, some appearing to lie on the very edges of the glomeruli, and others deep in the interglomerular spaces, and they give dendritic arborisations to one or more glomeruli; some lie very close to the external tufted cells, which are also found in this region, particularly in the deeper parts of it. The axons of the external tufted cells distribute collaterals to the neighbouring parts of the glomerular layer, generally terminating in the periglomerular region, and send their main axon deep into the bulb and towards the cerebral hemispheres; the periglomerular cells and all their axonal branches to the periglomerular region around several adjacent glomeruli.

The collaterals of the mitral and deep tufted cells do not appear to reach the glomerular layer.

The more recent studies of Valverde (1965) have largely confirmed and extended these earlier studies, but he introduced the concept that the tufted cells were periglomerular cells, displaced inwards, rather than outwardly displaced mitral cells; this view has not been confirmed in this study (Chapter 3), but his excellent drawings showing individual neurons in the bulb are a splendid addition to the drawings of the classical histologists.

The nomenclature of the cells of the olfactory bulb has somewhat altered with the passage of time and it may be useful to clarify the terms at this juncture. Cajal (although his practice varied from time to time) used the designation external tufted cell for those tufted cells lying just deep to or within the glomerular layer, middle tufted for those in the superficial two-thirds of the external plexiform layer and internal tufted for those just superficial to the mitral cells. External or superficial granule cells have more recently become known as periglomerular cells; to avoid confusion with the granule cells of the deeper layers, only this latter term will be used here, despite its looseness. To the third type of cell discovered in the glomerular layer we have assigned the term 'superficial short-axon cells'; in addition to its descriptive value, this brings them into line with the third type of the deeper layers described by Golgi, Cajal, Blanes and van Gehuchten, with which they appear to be analogous.

It is against this background of light microscopic knowledge

that studies have been made on the olfactory bulb using the electron microscope; the highly comprehensive nature of the data on the different cell types were particularly valuable in determining more precisely the synaptic connections established by the various neuronal processes in the bulb. A functional analysis of any neuronal relay can only be achieved in full with a suitably detailed map of the anatomical connections in the region, both intrinsic and extrinsic; by defining the principal connections, it should be possible to make certain suggestions about function and to exclude some of the possible interpretations of electrophysiological recordings. It is with these aims in view that the present studies have been made.

Electron microscopy

Apart from the study by Andres (1965) on the structure of the whole olfactory bulb, using the electron microscope, other workers have tended to concentrate on certain particular features such as the reciprocal synapses characteristic of this site (Hirata, 1964; Rall, Shepherd, Reese & Brightman, 1966; Price, 1968; Hinds, 1970) or on details of particular cells (Price & Powell, 1970a, b,c,d). The latter authors have investigated the connections of the mitral, granule and deep short-axon cells of the rat olfactory bulb, as well as demonstrating the mode of termination of the centrifugal pathways to the deep layers of the bulb. The present study, primarily on the rat, covers the glomerular layer, the site of synaptic contact between the olfactory nerves and the mitral cell and other dendrites. The recent electron

microscopic research on the olfactory bulb in this laboratory has separated into studies on two sets of layers - deep and superficial - a division that appears to have some functional significance and which is discussed in this thesis. My colleague, Dr J.L. Price, has in effect concentrated upon the granule cell, mitral cell and external plexiform layers and these findings are presented in his thesis and publications (Price and Powell, 1970a-d).

Experimental light microscopy

Experimental anatomical studies on the glomerular layer are relatively few at light microscopic level: the termination of the centrifugal fibres in the periglomerular region has been described by several authors (Cragg, 1962; Powell & Cowan, 1963; Powell, Cowan & Raisman, 1965; Heimer, 1968; Price & Powell, 1970c) and it appears from these that the centrifugal fibres from the nucleus of the horizontal limb of the diagonal band (Price & Powell, 1970a) are the only axons that reach the glomerular layer from the cerebral hemispheres. Experimental anatomical studies on the olfactory nerve projection to the bulb are fewer still, but show a broad area to area distribution from mucosa to bulb (Le Gros Clark, 1951; Le Gros Clark, 1957); recently, silver degeneration techniques have shown a well circumscribed distribution of termination from individual nerve fascicles running from the mucosa to the bulb, and suggest a degree of intraglomerular specificity as well (Land, Sager and Shepherd, 1970).

Physiology

From the physiological point of view, studies on the organisation of the olfactory bulb have been limited by a lack of specific knowledge of the intrinsic neuroanatomy of the site (for example, the early studies of Baumgarten, Green & Mancia, 1962; Green, Mancia & Baumgarten, 1962; Yamamoto, Yamamoto & Iwama (1963); Phillips, Powell & Shepherd (1963); Shepherd (1963a,b)); as information has become available, considerable advances have been made in our understanding of bulbar organisation and function (e.g. Rall, Shepherd, Reese & Brightman, 1966). The early studies did, however, define the basic parameters of function - the olfactory nerve input, the mitral cell responses and factors affecting these, as well as indicating the possible pathways for extrinsic effects (Yamamoto et al, 1963). The combined anatomical, physiological and mathematical approach of Rall, Shepherd, Reese and Brightman (1966), and Rall and Shepherd (1968) suggested a basis for understanding the function of the reciprocal synapses found in large numbers in this site (Hirata, 1964; Andres, 1965; Reese and Brightman, 1965). Nicoll (1969,1970b) has confirmed and extended these findings. Naturally, studies have tended to concentrate on the deeper layers of the bulb on account of the better segregation of different cell types and their processes as compared with the glomerular layer, and the greater ease of recording in these sites. Shepherd (1971) has briefly reported some findings in studies on the glomerular layer, and these are encouraging in their relationship to the findings to be described in this thesis, to which the author has

been able to relate them. Interpretation of results is necessarily difficult in this part of the bulb and it is evident that a detailed knowledge of anatomical connections will greatly facilitate the interpretation of electrophysiological observations; it is hoped that the work presented here may contribute at least some of this necessary knowledge. As an illustration of the importance of anatomical and physiological correlations in the olfactory system, the recent findings of Lohman and Mentinck (1969) and of Nicoll (1970a) on the tufted cell projection in the lateral olfactory tract have clarified an important issue that has been uncertain since the time of Cajal. Cajal (1911, 1955) tentatively suggested that the fibres of the tufted cells passed in the anterior commissure and this concept was given further support by Allison (1953); thus the central projections of the mitral cells (by the lateral olfactory tract to the pyriform cortex etc.) and the tufted cells appeared to be distinct. However, with more direct methods Lohman and Mentinck (1969) (silver degeneration methods), and Nicoll (1970a) (electrophysiology) have demonstrated that the course and termination of the mitral and tufted cell axons outside the bulb are the same.

The work of MacLeod and his coworkers (Leveteau and MacLeod, 1966, 1969; Leveteau, Daval and MacLeod, 1972), has helped to elucidate the nature of glomerular function in terms of the olfactory stimuli, although much remains obscure in this important field. Although many workers have attempted to define the stimuli giving rise to patterns of activity in the olfactory nerves, these have been largely unsuccessful

and an account of them would not be relevant here in view of this unfortunate difficulty.

Of the physiological data that is available, the nature of synaptic influences upon given cells are of the greatest importance in the anatomical correlations and these will be summarised briefly here, while receiving more detailed attention elsewhere: Olfactory nerves are clearly excitatory (Yamamoto et al 1963; Shepherd, 1963a, 1971). Mitral cell dendrites (Rall and Shepherd, 1968) and their axons (Nicoll, 1970b; Beidenbach and Stevens, 1968) are excitatory, as are those of the tufted cells (Nicoll, 1970a). Periglomerular cells (Shepherd, 1971) and granule cells (Rall and Shepherd, 1968) appear to be inhibitory. Yamamoto et al (1963) and Callens (1965) proposed an additional inhibitory interneuron acting through the granule cells in the deeper layers, and this could correspond anatomically with the deep short-axon cells; the superficial short-axon cells have not as yet been studied. Centrifugal fibres appear to be excitatory to granule cells (Dennis and Kerr, 1968); Fibres from the contralateral anterior olfactory nucleus, running in the anterior commissure also appear, by an interpretation of the results of Leveteau et al (1972), to be excitatory to granule cells (see Discussion).

Presentation of Results

The Material and Methods used for all the studies will be described in some detail in Chapter 2. The succeeding three chapters (3-5) describe the light microscopy and electron microscopy of the normal

structure of glomerular layer in the rat olfactory bulb, dealing respectively with the neuron types, and the glomerular and peri-glomerular neuropil. These are followed by a short chapter on an unusual finding in the monkey olfactory bulb and its relation to the findings in the rat. The subsequent four chapters are concerned with experimental observations after section of the olfactory nerve and the lateral olfactory tract and after making small lesions within the substance of the olfactory bulb; the studies after olfactory nerve damage are divided into two - the first relates to the degeneration of the presynaptic terminal and the fate of the post-synaptic thickening in the rat, and the second is a study (almost entirely in the rabbit) of the ultrastructural features of transneuronal degeneration after the same lesion. The experimental findings are correlated with the observations on normal material, with a view to determining the synaptic characteristics of all the processes in the glomerular layer. The last chapter of results is a presentation of the use of Golgi-impregnated material for electron microscopy in the olfactory bulb. Since this thesis is principally concerned with the anatomical organisation of the bulb, the general discussion will concentrate specifically on points arising from the thesis in this particular context. Points of significance to degeneration studies are discussed in the specific chapters. Each chapter has its own discussion so that the results are brought together and considered in stages, whereas the general discussion gives broader consideration to certain aspects of the study. Physiological correlation is emphasized throughout, and attempts have been made to

give some functional meaning to the anatomical results; these are consistent with physiology as far as it is known, though there is naturally a degree of speculation beyond this.

CHAPTER 2

MATERIAL AND METHODS

"Technik ist alles."

INTRODUCTION

Electron microscopy has become such a widely used technique that it is felt unnecessary to give in detail all the chemical solutions and methods, when these are standard practice in any electron microscope laboratory. In the following sections, attention is paid to special features of methodology used in this study. The animals used were predominantly rats, but rabbits and monkeys were also used (Chapters 6 and 8). Rats were operated on, or perfused for normal material, at a relatively young age (4-8 weeks), so as to avoid the complication of spontaneous degeneration that is a well-known hazard of working with older animals. Rabbits were aged between 5 and 7 weeks at the time of operation, and the monkeys used were young adults.

1. PREPARATIVE PROCEDURES

a) Normal Light microscopy (Chapter 3)

Several sets of sections of paraffin embedded, Nissl stained material were available for study; these were stained with thionin and cut at 25µm. For Golgi impregnations, normal rats, aged between four and seven weeks, were used; the basic Golgi-Kopsch technique (Colonnier, 1964b) was used principally, with slight modifications.

Fixation. Either the standard method for electron microscopy was used (see later section) or a fixative solution containing 5% glutaraldehyde and 2.4% potassium dichromate in phosphate buffer, was given by perfusion.

Staining. a) material is stained for 5 days in the glutaraldehyde-

dichromate mixture above.

b) the blocks are washed well in several changes of distilled water, rinsed with silver nitrate (0.75%) and then left for a further five days in the silver nitrate solution. They are then washed again in distilled water. Repeated impregnation may then be carried out by repeating a) and b) twice in the same order, washing carefully with distilled water between each fresh solution giving blocks two to three days in each stain. All solutions should be made up freshly and the pots kept in the dark during staining; about 50ml of each solution is used for a pair of olfactory bulbs, which are put through as single blocks for light microscopy.

Embedding is with Low-viscosity Nitrocellulose and mounting with XAM mountant and a cover slip. Sections were cut in the sagittal and coronal planes at 75-100µm. The method employed here appears to avoid fading of sections, over long periods of time.

b) Golgi-Cox (Chapter 8)

The brain was exposed under anaesthesia, the animal decapitated and its skull placed in the Golgi-Cox fixative; after an hour the brain was removed from the skull, cut into blocks and placed in fresh Golgi-Cox fixative. These were left for 8 weeks, then embedded in L.V.N. and cut at 150µ; these sections were processed according to the method given by Sholl (1953).

c) Experimental Light Microscopy (Chapter 10)

Perfusion for experimental light microscopy was carried out after

intrinsic lesions of the bulb (4 or 5 days after the operation) with normal saline and 10% formaldehyde; after further fixation, the olfactory bulbs were cut at 25µm on a freezing microtome in sagittal or coronal planes. A 1 in 10 series was stained by the method of Wiitanen (1969) to locate the lesion and determine the approximate extent of the degeneration at the glomerular level; all sections of the part of the bulb affected were then stained by the methods of Fink and Heimer (1967) and of Nauta and Gygax (1954), to determine more exactly the extent and nature of the degeneration.

d) Electron microscopy (Chapters 3-10)

For perfusion, it has been the practice to cool the animal under Nembutal anaesthesia to 15-20°C before perfusing it with fixative solution at about the same temperature; although this procedure may have little effect on the quality of the fixation, it allows time in case of any mishaps during preparation, tending to avoid the complication of asphyxic change. A very short preliminary wash with buffered saline (White's) is followed by a brisk perfusion with 150-200ml of fixative (1% glutaraldehyde with 4% formaldehyde in 0.1M phosphate buffer) after which a very slow perfusion rate is used for a further 200ml of fixative; the formaldehyde is made up from paraformaldehyde with heating to 60-70°C. A prior injection of 1% sodium nitrite (0.1ml) and heparin (0.2ml) causes dilatation of cerebral blood vessels and prevents clotting. Perfusion is via the left ventricle and aorta, using a wide-bore glass cannula with bulb, and at a hydrostatic pressure at or just

above the animal's normal blood pressure. The brain is left in the skull for some hours before being removed; care must be taken in removing the olfactory bulbs, as the olfactory nerves attach themselves firmly to the underlying bone - it is usually possible to sever the nerves close to the cribriform plate with fine watchmakers' forceps. The brain is immersed in fresh fixative solution for at least six hours.

Blocks of olfactory bulb are then cut (a minimum of six blocks from each bulb for the rat), given a very brief rinse with 10% sucrose in phosphate buffer and post-fixed with 2% osmium tetroxide, also in phosphate buffer; dehydration is in ascending alcohols, with block staining (1% uranyl acetate in 70% ethanol) at the 70% stage. After immersion in epoxypropane, followed by a 50:50 mixture of epoxypropane and embedding mixture, the blocks are embedded individually in plastic capsules using Araldite or Epon/araldite mixture. The blocks are allowed to polymerise in 40°C (24 hours) and 60°C (48 hours) ovens. 2µm sections are then cut off the trimmed block, mounted and stained with the method of Richardson, Jarett and Finke (1960) - 50:50, 1% methylene blue in 1% borax and 1% aqueous Azure II - for the orientation of the block; in some cases, these 2um sections were left unstained for phase contrast microscopy. An area or 'mesa' for study is marked out and trimmed; this should have a maximum size of 0.5 by 1mm, but for routine work, 0.5mm by 100µm is a good size; with smaller mesas, material is improved in reproducibility, and longer series of sections may be cut without interruption.

For ultrathin sectioning, the knife scoring angle used is 45° and

the clearance angle of the microtome 1-2°; cutting speed range is 1.6-2.0mm per sec. for smaller blocks and slower speeds for larger ones. Section staining is in 5% uranyl acetate in 50% ethanol at 40°C in a wet chamber, for 30 mins; the grids are then washed in warm ethanol and dried. Reynolds (1963) lead citrate is used for further section staining; it is important after glutaraldehyde fixation, to dilute this stain 1 in 5 with 0.01N Na OH to prevent the granular appearance caused by heavy lead deposition. Staining is at room temperature in a chamber containing N NaOH, to avoid precipitation with CO_2. The grids are rinsed in distilled water and dried.

For <u>serial sections</u>, small regular mesas must be used in order to obtain long series, and these are mounted on formvar-coated slit grids (slit - 1mm by 2mm). A stock solution of polyvinyl formol formvar (1%) is made up in ethylene dichloride and stored in a flask at 0°C. The coating concentration determines the thickness of the film and it is necessary to achieve a balance between strength of the film and a minimal loss of resolving power; a 0.2% solution in ethylene dichloride is optimal. A 3" by 1" slide is cleaned in acid alcohol and Teepol and is then dipped into a coplin jar containing the formvar solution (at room temperature) for 3-4 seconds. The excess is drained and one side wiped clean; the film is allowed to dry and is then scored with a razor blade, just inside the edges and about ½" inside the limits of the coating. The slide is breathed on, and inserted at about 45°, coated side up, into a dish of clean distilled water. The film is thus released to float on the surface of the water; slit grids are carefully placed

on the central portion of the film and pressed down lightly to ensure adhesion; a wire frame is brought under the film, so as to support it on all sides and the whole is removed from the water at an angle; it is allowed to dry under cover. The grids may be picked off with forceps, as needed. This technique has been discussed at some length since its correct use is an absolute essential for good, repeatable serial sections.

The smallness of many of the profiles in the glomerular layer made such serial sections a significant and imperative part of this study, and large numbers of series were used for the greater part of the routine analysis of material. Two major classes of serial sections were used: the first had average dimensions of 500µm by 50µm and each uninterrupted series consisted of 50-120 sections; the second type were about 100µm and series of between 100 and 200 sections were obtained, uninterrupted.

e) Intrinsic Lesion Material for electron microscopy (Chapter 10)

Blocks of this material were taken so that the centre part of the dorsal surface, containing the lesion, was included in a single block; this block was cut on the ultramicrotome after embedding, until the lesion could be seen in a 2um section, stained by the method of Richardson, Jarett and Finke (1960). Ultrathin sections including part of the lesion and the adjacent glomerular layer were then cut and serial sections mounted. After study of these serial sections, the next 50µm of the block was discarded before a further series of sections was taken

for electron microscopy; different areas in relation to the lesion were also taken at the different levels. This procedure was followed until the end of the lesion was reached; the distribution of terminal degeneration in the glomerular layer in the region of the lesion could thus be determined.

f) Combined Golgi-E.M. Material (Chapter 11)

Golgi-impregnated material, preferably perfused with the primary staining fluid of the Golgi-Kopsch technique described above, and in blocks of typical electron microscopic dimensions (see above), is stained in 2% osmium tetroxide and embedded in Araldite; this is allowed to polymerise slowly at room temperature, or after 1 day in a 40°C oven only. When the blocks are hard enough to be gripped for cutting on a sledge microtome (several days), sections are cut at 20-25um with the knife edge steeply angled to the axis of travel. These sections are mounted in castor oil and a coverslip placed over them. They may then be examined and appropriate cells for study found. The dimensions of the sections are such that much of the dendritic fields of cells at the glomerular level is included, but not much extra stained material in the vicinity of the cell, which may confuse study with the electron microscope. The cells chosen are then photographed and/or drawn under high power, and their site in the section noted. The cover slip is then removed and the castor oil washed off with epoxypropane; the section may now be re-embedded by immersion in a drop of fresh Araldite in the lid of a Beem capsule; it is then firmly pressed down by a flat-

surfaced, preformed and already polymerised Araldite block, which is in the remainder of the capsule. The whole is then polymerised at 60°C.

The composite block may then be examined under a dissecting microscope and a normal electron microscopic 'mesa' made around the cell, avoiding as much other stained material as possible. This facilitates study and prevents excessive blunting of the microtome knife, allowing longish series of sections to be cut at a time. After cutting down the mesa with 2um sections to remove the fine surface layer of Araldite, ultra-thin sections are cut through the mesa and collected; these are examined so as to ascertain whether or not the cell has been reached, and more sections cut in serial order, until the cell or its processes appear. All sections are mounted on single-slit formvar-coated grids which allow easy serial study (see above); the sections are stained with uranyl acetate and lead citrate on the grid.

2. OPERATIVE PROCEDURES

a) Olfactory mucosa removal (Chapter 7 & 8)

This relatively crude but selective operation has the advantage that a large number of fibres may be destroyed without opening the cranial cavity; its principal disadvantage is the relatively high mortality, due to the nature of the lesion and the young animals (4-8 weeks) that have to be used to reduce the likelihood of 'atrophic rhinitis'. Anaesthetics with minimal or short-lasting respiratory depression are essential, and Avertin (tribromoethanol with amylene hydrate) gives good results; 0.5ml Avertin is mixed with 25ml normal

saline and 2ml 95% ethanol. A 200gm. animal is given 1ml mixture intraperitoneally and a further 0. -0.5ml after 5 minutes if necessary. The skin over the nasal bones is divided anteroposteriorly and the pericranium separated; the dividing line between nasal and frontal bones is noted and the nasal bone of one side removed with small bone forceps (a drill must not be used, as it drives spicules of bone into the respiratory tract). The dorsal mucosa is removed with watch-makers' forceps and the whole area of olfactory epithelium is stripped off, especially on the septum and the undersurface of the cribriform plate. Cotton wool wrapped around forceps is a convenient tool. In this way most of the medial aspect of the olfactory bulb and the anterior part may be denervated. It is difficult to effect a complete posterior denervation, and some of the conchae may also escape damage. There is considerable bleeding and measures should be taken to remove blood as quickly as possible until it abates. Penicillin powder may be sprayed into the wound and the skin incision closed; the animal is then allowed to survive for a variable period of time.

b) Lesions of central pathways (Chapter 9)

For lesions of the centrifugal pathways, the temporal approach of Powell, Cowan and Raisman (1965) should be used, making the lesion far back in the white lateral olfactory tract, to avoid damage to the anterior olfactory nucleus. More anterior lesions involve this nucleus and may be used to study the distribution of its fibres in the bulb, but it must be borne in mind that the centrifugal pathway has also been involved: damage to the anterior olfactory nucleus gives rise to degeneration in

the fibres passing in the anterior commissure to the opposite bulb (see Lohman & Heimer). In all cases it is advisable to use young animals (4-6 weeks) to avoid the complication of degenerative changes that occur in older animals. The rostral ends of the cerebral hemispheres were embedded in paraffin, sectioned in the coronal plane and stained with thionin to determine the exact site and extent of the lesion.

c) Intrinsic lesions (Chapter 10)

The limits of the frontal bones covering the olfactory bulbs are noted after an incision has been made in the overlying skin. The bone covering one bulb is removed using a drill until a thin translucent layer is left, which may be removed carefully using watchmakers' forceps under a dissecting microscope; this avoids surface damage by the drill. The dura over the midpoint of the dorsal surface is carefully divided and a small hole made in the pia-arachnoid over the intended site of the lesion. A fine needle is then manipulated so as to make a small lesion in the underlying bulbar tissue, of appropriate depth: this may be a point lesion or a short line running in coronal or sagittal planes. It should be emphasised that the lesion must be placed in such a way that its site can be located after fixation, in the case of electron microscopic material, as the small size of the lesion may mean that it is not evident on the bulbar surface. The wound is sprayed with penicillin and the skin incision closed; the animal is allowed to survive for a variable length of time. In the present experiments the

lesions were placed in the dorsal, dorso-lateral and dorso-medial aspects of the bulb for light microscopy, and at the centre and top-most point of the dorsal surface for electron microscopy.

CHAPTER 3

THE NEURON TYPES OF THE GLOMERULAR LAYER OF THE OLFACTORY BULB

"Observation and Experiment are essential ways of testing our theories. They may thus be regarded as belonging to the critical discussion of our theories."

Karl Popper.

INTRODUCTION

The work of Cajal and his group showed the existence of two neuron types intrinsic to the glomerular layer - the external tufted cells and the superficial granule cells. The electron microscopic study described here confirms the existence of the external tufted cells, cells analogous to mitral cells, as well as that of many smaller granule-like, or periglomerular cells, lying in between the glomeruli; in addition, a third type of neuron intrinsic to the glomerular layer has been distinguished, which has features distinct from the other two (Fig. 7). The ability to differentiate a third neuron type with the electron microscope made it necessary to identify such a cell in Golgi-impregnated material; a third type has indeed been characterised (Fig. 4) and its light microscopic features have been confirmed in turn by further electron microscopic evidence.

This Chapter is concerned with the morphological and synaptic features of the cell somata of the cells lying in the periglomerular region and of the large mitral cell primary dendrites, as well as those parts of the dendrites and axons of these cells that may be seen in continuity with the cell somata (or primary dendrite) with the electron microscope; the light microscopic features of these cells are also defined. In the following Chapters (Chapters 4 & 5) the neuropil of the glomeruli and the periglomerular regions is described and the various profiles in these regions identified according to their cells of origin, on the basis of criteria established here.

RESULTS

TUFTED CELLS

Light microscopy

As far as can be determined, the internal tufted cells entirely correspond with the mitral cells, apart from their more superficial positions and smaller size (18-25μm), and they will not be further considered here. The middle and external tufted cells show a further gradation of size and position, becoming smaller the closer they are to the surface of the bulb; the external tufted cell bodies have a mean diameter of 10-15μm while the middle tufted are intermediate between these and the mitral and internal tufted cells, according to position. The external tufted cells, which are fusiform or ovoid, lie in the periglomerular region, most commonly in its deeper parts; the middle tufted cells, lying in the external plexiform layer, tend not to be as elongated as the external tufted cells, but still retain an essentially oval form. In Nissl-stained sections, the somata of these more superficial tufted cells (external and middle) appear similar to the mitral and internal tufted cells showing marked Nissl substance, which is particularly noticeable in the apices of these cells, although the amount varies according to size. Their nuclei are generally pale and have a thin fringe of chromatin material and a smallish nucleolus; their perikarya are relatively large.

In Golgi-Kopsch preparations (Fig. 1), the external tufted cells are seen to have a large primary dendrite which may enter the glomerulus

Fig. 1 Camera lucida drawings of neurons impregnated with the Golgi-Kopsch method. A & B. External tufted cells with glomerular arborisations which are more limited than those of mitral cells, but do show varicosities. Axons are marked (a) and cell A also shows the beginning of a second stem dendrite.

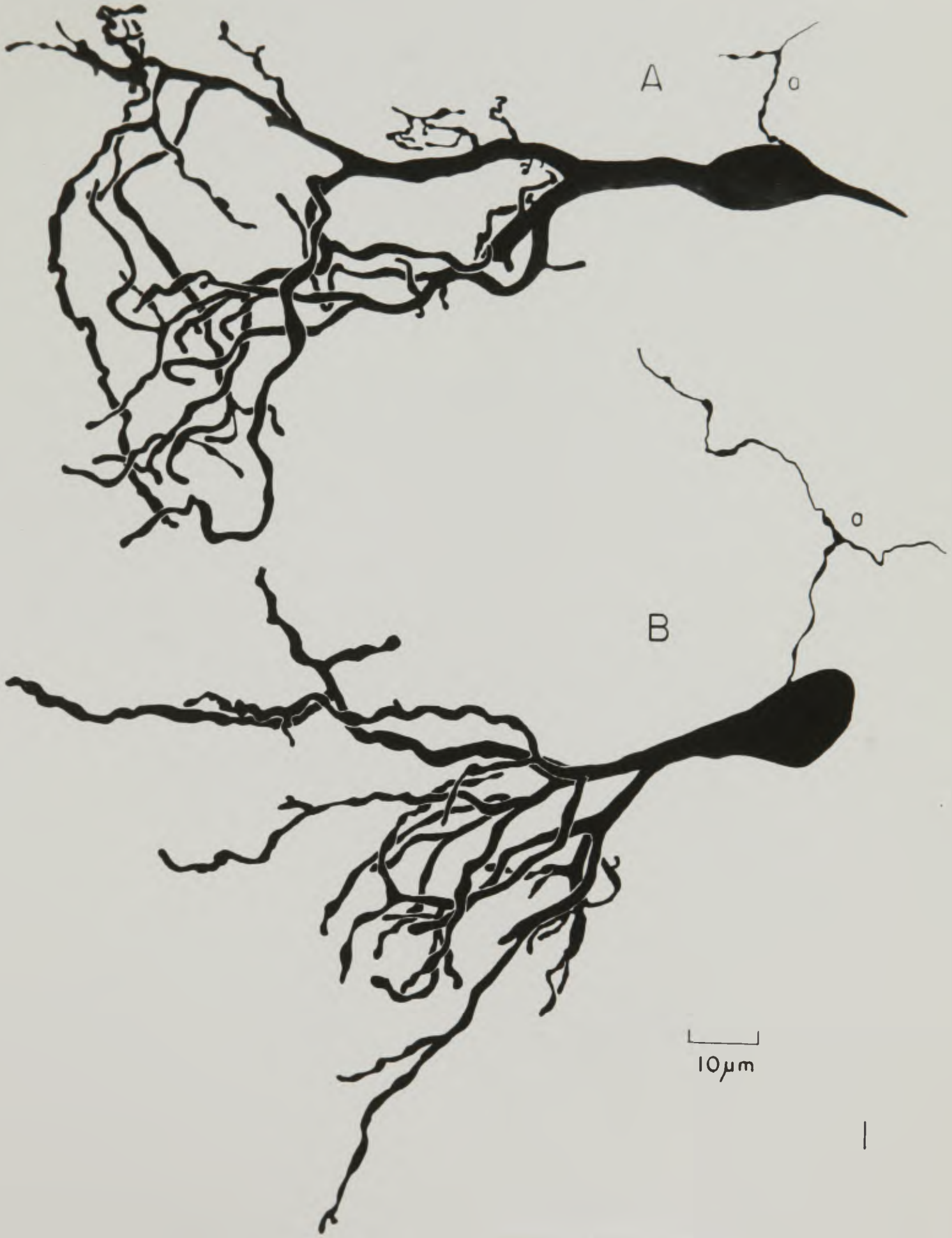
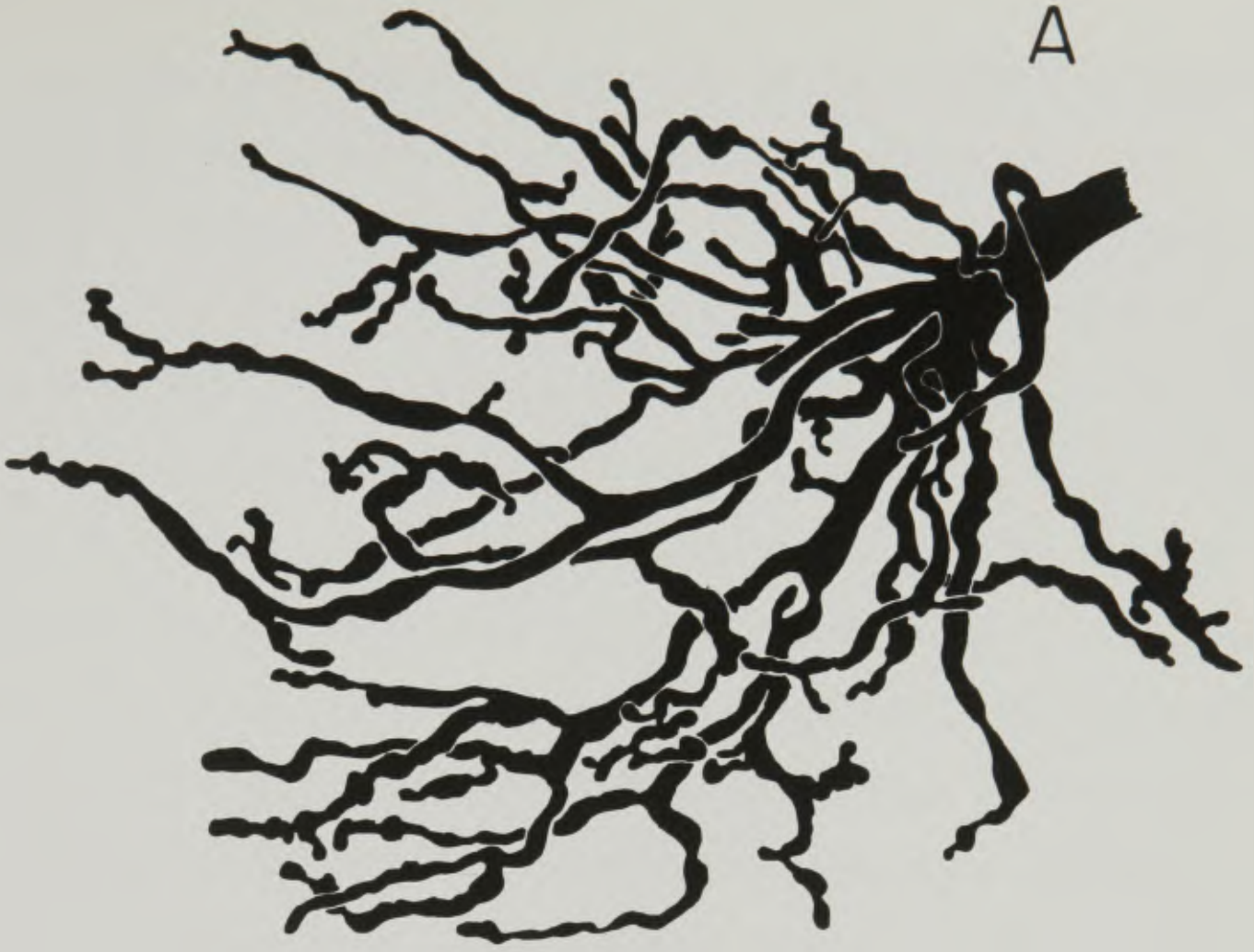
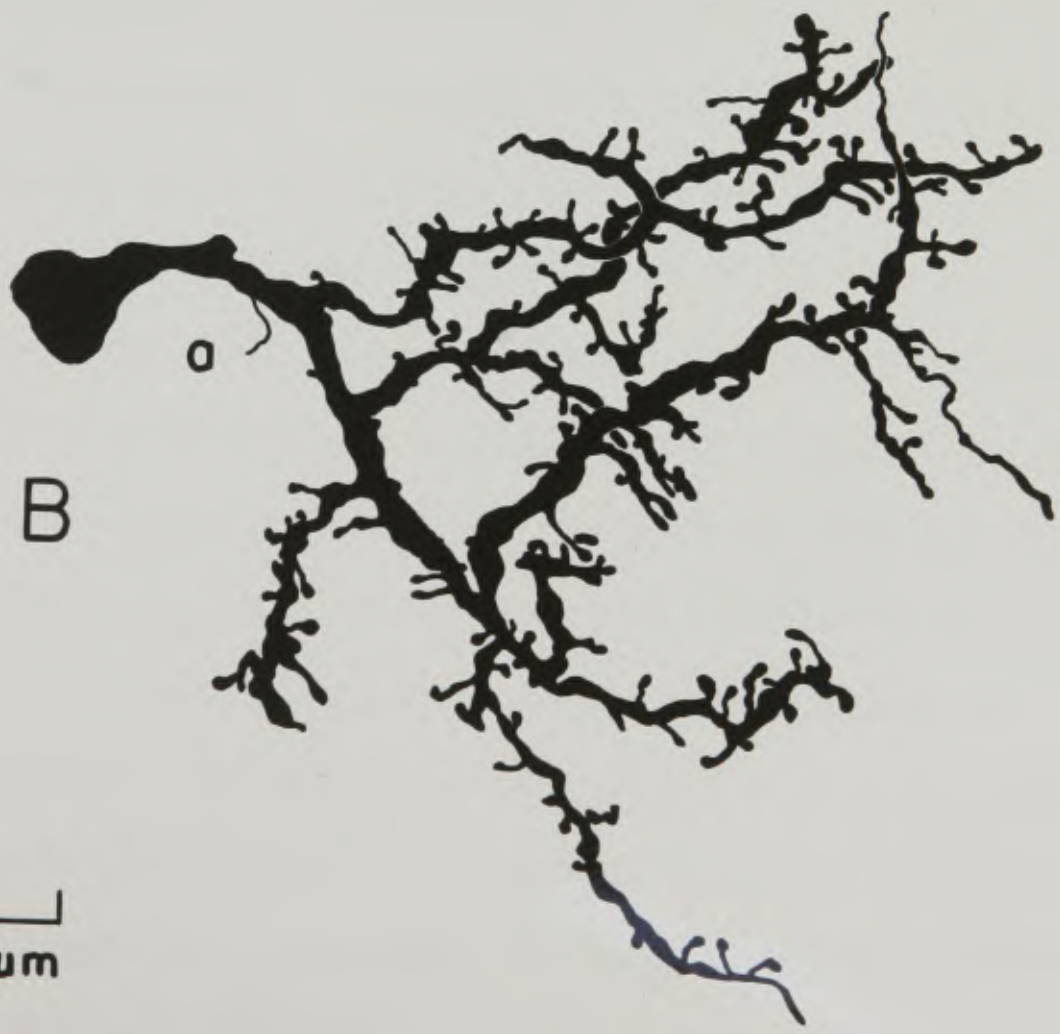

A
B
10µm

Fig. 2 Camera lucida drawings of neurons impregnated with the Golgi-Kopsch method. A. Glomerular arborisation of a mitral cell primary dendrite showing extensive ramification and varicosity of terminal dendrites. B. Periglomerular cell with spiny glomerular arborisation and only partially impregnated axon (a).


A


a
B
10 µm

from any angle, depending on the situation of the cell body in relation to its glomerulus, and which branches repeatedly within it. Only rarely do the external tufted cells show secondary dendrites, which, if present, lie in the superficial part of the external plexiform layer or the deep part of the periglomerular region; the middle tufted cells have secondary dendrites arranged in a similar manner to those of the mitral and internal tufted cells. The arborisations of the primary dendrites of the external tufted cells are generally simpler and less tortuous than those of the mitral or deep tufted cells and the branches arise at a more acute angle than those of the latter; in this way they tend to fill a triangular segment of the glomerulus, situated opposite the site of entrance of the primary dendrite (compare Figs. 1 and 2A). In most other respects they are similar to the mitral cell arborisations (see below): the dendrites become increasingly varicose as they branch and decrease in size; they do not show typical spines, although some of the terminal branches may at times take on a spine-like morphology (Fig. 1A). The axons of the external tufted cells are fine, emerging from the soma or initial part of a dendrite, and after pursuing a tortuous course in the periglomerular region and giving off several collaterals to this layer, they pass into the external plexiform layer; they may show a few beadings at irregular intervals.

The glomerular arborisations of the <u>mitral</u> and <u>deep tufted</u> cells are very characteristic (Fig. 2A). Studies at high magnification show that their branches are more profuse and have a greater tendency to be varicose, particularly the terminal branches, than those of the external

tufted cells; therefore the arborisations of these deep cells form a dense network which extends over almost the whole glomerulus. This seems to be also partly due to the wider angle of branching of their dendrites so that, instead of the rather fan-shaped arborisation of the external tufted cells (Fig. 1), these take on a more spherical outline (Fig. 2A).

Electron microscopy

The tufted cells are characterised by their large size relative to other somata at the same depth, the cytoplasmic abundance evident at their apices, and marked granular endoplasmic reticulum (Figs. 7,8,9). In view of the uneven distribution of the cytoplasm around the nucleus, identification on the basis of cytoplasmic volume alone is insufficient, since several cells are cut through their centres, at right angles to the axis of the oval; also there may be little endoplasmic reticulum in the thin portions of the cytoplasm (Fig. 8). The tufted cells may be readily distinguished with the electron microscope, however, by virtue of the reciprocal synapses commonly found on their cell somata and dendrites (Figs. 9,18,19), similar to those on mitral cells (Hirata, 1964; Andres, 1965; Rall et al, 1966; Price & Powell, 1970d); this primary criterion has been used for defining equivocal cell somata and thus serial sections have been an invaluable aid (Figs. 18,19).

The nuclei of tufted cells are pale relative to those of periglomerular cells but the nuclear material stands out more clearly than

that of short-axon cells and often has a patched appearance (Fig. 7); they may show slight nuclear indentations, commonly opposite the base of the primary dendrite, but these are rarely deep or complex (Fig. 8). The nucleoli are well delineated and show a clear distinction into granular and fibrillar components; a very fine layer of chromatin material surrounds the nucleus, giving these cells a very characteristic appearance at low magnifications.

The cytoplasm of the external tufted and middle tufted cells is pale, but rich in granular endoplasmic reticulum and this is most commonly found as multiple parallel stacks in the apices of the cell somata (Fig. 9); large complexes of Golgi apparatus are characteristic, notably at the bases of dendrites and sometimes quite distant from the cell soma. Ribosomal rosettes are common and are especially concentrated in the nuclear indentations. In the larger tufted cells, some of the mitochondria may present a slightly blown up appearance (Fig. 8); several large dense lysosomes and lipoprotein bodies are very often present in the cytoplasm of these cells, although rarely present in such numbers in other cell types. Some cells of this type are surrounded by several thin glial lamellae, similar to those observed on their dendrites (Fig. 19).

The main dendritic shafts of the tufted cells are smooth in outline and of wide diameter, and have a characteristically pale appearance resembling the wider primary dendrites of the mitral and internal tufted cells; they arise from one or both sides of the cell soma and appear to be drawn out from them (Fig. 8). The neurotubules

of the tufted cell dendrites are typically very regularly arranged. These dendrites show many reciprocal synapses with gemmules of granule cell peripheral processes or of periglomerular cell dendrites, in the external plexiform and glomerular layers respectively. One or more thin glial lamellae may cover the surface of the dendrites in the periglomerular region and superficial part of the external plexiform layer, except at regions of synaptic contact, and this covering usually remains more or less intact until their entry into the glomeruli (Figs. 16,17). The origin of these lamellae is certainly glial and has been unequivocally determined in many cases by noting continuity with obvious glial profiles containing filaments or glycogen granules or with the glial somata (Fig. 17); nevertheless some periglomerular cell processes may also sometimes become extended into thin sheets (Reese & Brightman, 1970). On occasion the same glial cell has been seen to ensheath bundles of olfactory nerve fibres at the surface and to form typical glial lamellae around the dendrites of mitral or tufted cells in the periglomerular region (Fig. 17). These probably correspond with the glial cells of the olfactory nerve layer described by Blanes (1898) as having stout, granular peripheral expansions and several long smooth and unbranched fibres that pass centrally in between the glomeruli; the latter are clearly demonstrated by the Weigert method and may well be responsible for the formation of this myelin around this dendritic segment in the monkey (Chapter 6). It is possible, however, that the interglomerular glial cells that Blanes describes also contribute to these lamellae.

At the point of entry into the glomeruli the tufted cell dendrites branch, and around this region of primary glomerular branching, characteristic unidirectional symmetrical synapses are found on to the dendrites from definite axon terminals containing large flattened vesicles (Fig. 20); these terminals may be identified as being those of periglomerular cell axons (see below). Reciprocal synapses with periglomerular cell gemmules are also found on this periglomerular part of the dendrite. The reciprocal synapses, which the tufted cells show on their somata and dendrites are exactly comparable to those of the mitral cells (Figs. 9,18,19). In the external plexiform layer these synapses are formed with the gemmules arising from the peripheral processes of granule cells, which are present up to the deep edge of the glomerular layer; in this region and more superficially the gemmules of periglomerular cells are also found and participate in reciprocal synapses with the tufted cells and their processes, the synapses showing the same polarities as those with the granule cells. The tufted cells always show an aggregation of spherical vesicles in their dendrites or somata at the points of synaptic contact that are directed away from the profile and the membrane thickenings are always of the asymmetrical type (Figs. 9,18); the gemmules, on the other hand, regularly contain flattened vesicles of the large type (Price & Powell, 1970b) and synapse back on to the tufted cell with symmetrical membrane thickenings (Figs. 9,19). These polarities are a constant feature of the reciprocal synapses, wherever they occur, and have been confirmed by serial sections. Flat

sacs, occupying half or the whole of the presynaptic position have also been noted on the tufted cell side of the reciprocal synapses in the somata (Fig. 18) or large dendrites, similar to those described for mitral cells (Hirata, 1964; Price & Powell, 1970d). Tufted cell somata and stem dendrites very rarely receive any other synapses than those from gemmules and from the axon terminals of periglomerular cells; occasionally, however, asymmetrical synapses are found on to the cell somata or stem dendrites from axon terminals containing spherical vesicles (Chapter 5). In the unusual instance of a tufted cell soma that extends partially across the border between the glomerular and periglomerular regions, it may receive an asymmetrical synapse from an olfactory nerve terminal, but these terminals are not found in the periglomerular region (Chapter 4).

Occasionally somato-somatic and dendro-somatic synapses have been observed to occur between external tufted cells and periglomerular cells (Fig. 24); the synaptic specialisations orientated from the tufted cell soma or dendrite are the same as those of the tufted cell half of the reciprocal synapse with gemmules and have spherical vesicles and an asymmetrical thickening. A slight 'symmetrical' membrane thickening may be found adjacent to this type of synapse polarised in the opposite direction, with a flat sac of smooth endoplasmic reticulum on the periglomerular cell side (Fig. 25), similar to that described by Hirata (1964) and Price & Powell (1970d). It is noteworthy that most of the somato-somatic contacts of this sort that have been found, occur on, or close to, the axon hillock of the tufted

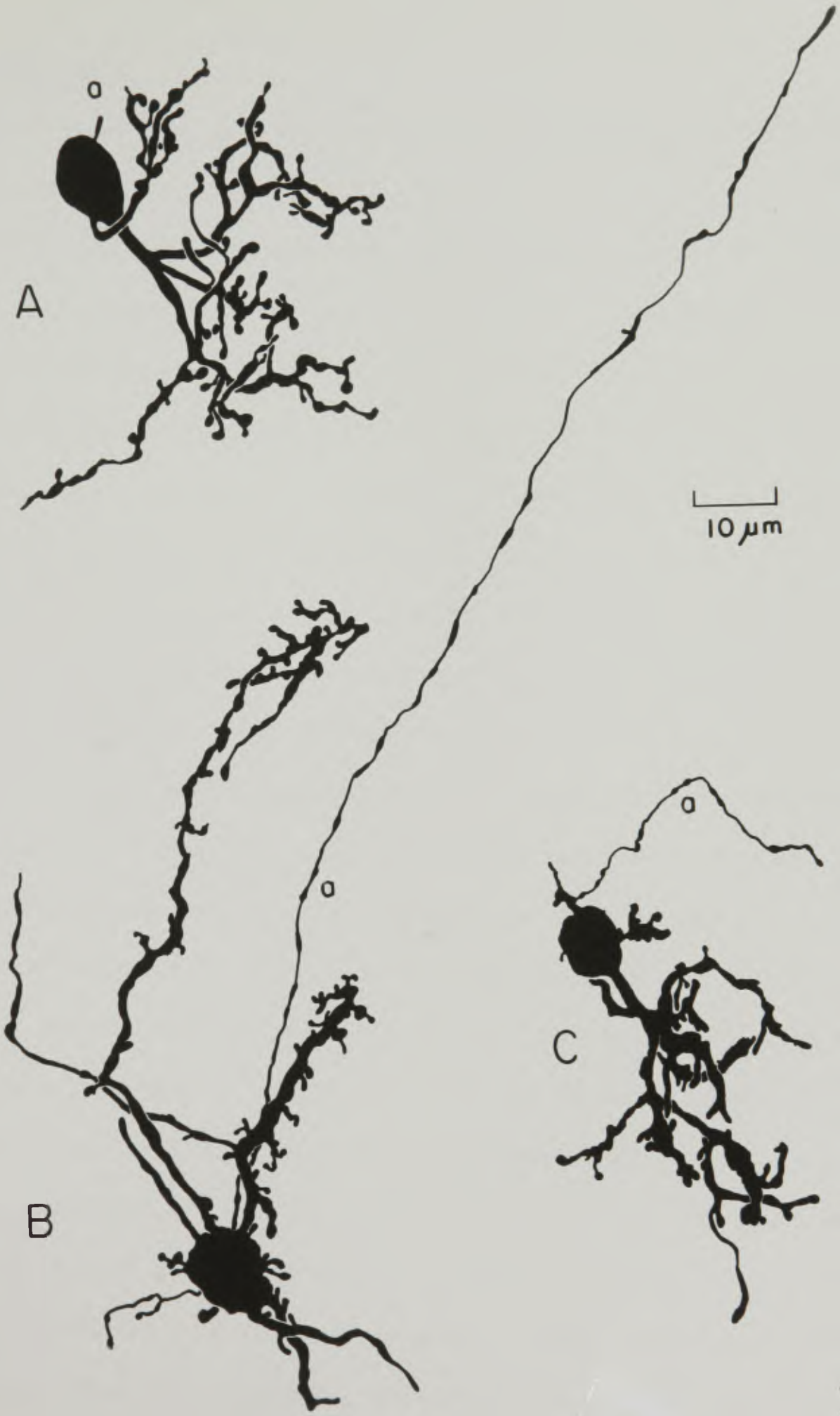
cell involved. The regular presence of spherical vesicles in the cell somata (Figs. 18,24) and dendrites of tufted cells in relation to synaptic specialisations strongly suggests, by the morphological corollary of Dale's principle (Eccles, 1964), that all regions of these cells involved in synaptic transmission will have such spherical vesicles, notably the axon terminals; similarly, the regularly asymmetrical membrane specialisations of dendro-dendritic and somato-somatic synapses may be considered as a constant feature of all the processes of these cells.

The axon hillock of tufted cells (Fig. 8) is characterised by an absence of Golgi apparatus and rough endoplasmic reticulum, as well as by the presence of several neurotubules, often aggregated. The latter pass into the initial segment of the axon which shows typical features of this region (Palay, Sotelo, Peters & Orkand, 1968; Peters, Proskauer & Kaiserman-Abramof, 1968; Westrum, 1970) that is, aggregated tubules, many free ribosomes, plasma membrane undercoating and dense material in the surrounding extracellular space; dense-cored vesicles, smooth agranular vesicles and alveolate vesicles are also common, the latter often budding off the plasma membrane. These axon initial segments may emerge from any part of the cell soma of the tufted cells or occasionally from the initial part of their dendrite. Synapses on to the axon initial segments are rare but, when present, they are always of the symmetrical type, the presynaptic vesicles of the axon terminal being the large flattened type characteristic of periglomerular cells; reciprocal synapses with gemmules are found on initial segments of

middle tufted cells, and this constitutes a difference from the mitral cells (Price & Powell, 1970d), but all tufted cells are similar to mitral in that reciprocal synapses also occur on the axon hillock.

The large, smoothly outlined mitral and deep tufted cell dendrites (Fig. 16) pass from the external plexiform layer, through the deep aspect of the periglomerular region and into the glomeruli; their pale cytoplasm is very characteristic and the neurotubules are very regularly arranged throughout the dendritic profile. They have reciprocal synapses with the gemmules of the granule and periglomerular cells, in the external plexiform and glomerular layers respectively; the polarities and characteristics of these are exactly similar to those described for the deeper parts of the mitral cells by Price & Powell (1970d), and for the tufted cells above. Like the dendrites of the tufted cells, these dendrites are often covered by several thin glial lamellae, particularly as they pass through the superficial part of the external plexiform layer and periglomerular region (Fig. 16). Around their point of primary glomerular branching they receive symmetrical synapses from periglomerular cell axon terminals containing large flattened vesicles, but do not give a return synapse; very occasionally the shaft of the mitral cell primary dendrite may receive an asymmetrical synapse in the periglomerular region from an axon terminal containing spherical vesicles. These dendrites also make asymmetrical dendro-somatic synapses with periglomerular cell somata (Fig. 23).

Fig. 3 Camera lucida drawings of neurons impregnated with the Golgi-Kopsch method. Periglomerular cells: A shows spiny glomerular arborisation and axon stub. B shows typical length of axon, but has two limited glomerular arborisations, somatic spines and periglomerular dendrite dendrites. C demonstrates an irregular glomerular/and a short beaded axon.


o
A
10 µm
o
B
o
C

PERIGLOMERULAR CELLS

Light microscopy

These spherical or occasionally ovoid cells surround the glomeruli together with the other glomerular layer neurons and many glial cells of similar Nissl appearance; the periglomerular cells are also similar in this material to the granule cells of the deeper layer. They range in diameter from 5 to 8µm and have dark nuclei with a clumped fringe of chromatin and relatively large, dark and uneven nucleoli; their perikarya are thin and unstained. Golgi-impregnated material (Figs. 2A,3,5) shows that these cells may give rise to dendritic arborisations in more than one glomerulus, as noted by Blanes (1898), though one field may greatly exceed the others in extent (Fig. 3,A,B); they may also have short dendrites which extend into the periglomerular region (Fig. 3B). Their glomerular arborisations rarely fill the glomeruli but seem to contribute in most cases to a circumscribed part of them, similar to those of the tufted cells. Of those that arborise within the glomeruli many show very varicose secondary and tertiary branches, from which spring a large number of spine-like appendages of variable shapes and sizes and which may have very long pedicles (Figs. 2B,3A); it is considered that these long-pedicle appendages are the 'hair-like appendages' of Blanes (1898), as they often appear to have no head or else this head is so far from the dendritic shaft that it may seem separate. Other cells show very irregular, rather than varicose, dendrites, both within and between

the glomeruli, which are more like glial processes, although these too may branch and give rise to some spines in the glomeruli (Fig. 3B,C). Somatic spines (Fig. 3B) are regularly seen, as are spines on the initial parts of dendrites.

The axons of the periglomerular cells are not always evident in Golgi-stained material, even after repeated impregnations, a fact also noted by Blanes, who considered this to be an impregnation defect. In view of the difficulty of staining these cells at all, and indeed of impregnating the axons of any of the cell types of the glomerular layer, such an explanation would seem fair; however, the absence of an axon in the granule cells, to which the periglomerular cells are in many ways analogous, suggests that the possibility that <u>some</u> periglomerular cells may be entirely lacking in an axonal process cannot be excluded. Serial electron microscopic sections have always revealed an axonal process, tending to rule out this possibility, but the inevitably low sampling prevents a final judgment on this point. Some cells show a short stub of axon in Golgi material which probably corresponds to the initial unmyelinated segment of axon (Fig. 3A); when it has been possible to trace the axon for greater distances, it has been of variable length, though it rarely reaches beyond three or four glomeruli and shows much beading at irregular intervals (Fig. 3B,C). These periglomerular cell axons may occasionally branch and their course is predominantly in the periglomerular region, although they may pass into the edges of the glomeruli.

Electron microscopy

Periglomerular cells are the commonest neuron type in the glomerular layer but may appear similar to some glial cells (Fig. 7); for this reason, only those cells that show synaptic specialisations can be certainly identified as periglomerular cells. Their dark nuclei almost fill the cell somata, leaving a very thin layer of cytoplasm (Fig. 10) which only expands opposite the origins of the cell processes. The chromatin material at the edge of the nucleus is markedly clumped into large and uneven masses which extend deeply into the nucleus, where they generally merge with the nucleolus; nuclear rodlets are occasionally found (Fig. 24), but are small and ill-defined, being very much rarer in the rat than in the rabbit (see Siegesmund, Dutta & Fox, 1964; Estable-Puig & Estable-Puig, 1970). The bulk of the nucleus has an irregular, patchy appearance, as a result of considerable local clumping of the nuclear material; at several points deep indentations may extend into the nucleus, which may be quite complex (Fig. 10). The thin cytoplasmic shell surrounding the nucleus is darker than that of other neurons and contains many ribosomes arranged into rosettes, but very little granular endoplasmic reticulum. Most of the perikaryal organelles are concentrated around the bases of the dendrites where a few segments of endoplasmic reticulum may be seen, rarely organised into stacks, but regularly surrounded by a particularly large number of ribosomal rosettes; large Golgi complexes and most of the somatic mitochondria are encountered in these regions. On some occasions, the periglomerular cell soma may be

enveloped in a glial wrapping of one or more thin lamellae, which only stop at the sites of emergence of the cell's processes; these have been noted by Brightman (1968) and differ from those of neuronal origin, also observed in this study, described by Reese & Brightman (1970).

The dendrites are of two broad types, although intermediate forms do exist: these are large, pale dendrites (Fig. 22) and thin, darker ones; both have a rather uneven outline, particularly those of the thinner class, giving them an appearance similar to, but never as marked as, that of glial processes. Cytoplasmic organelles extend for a considerable distance into the large periglomerular cell dendrites, and Golgi apparatus is regularly seen at the base of these dendrites though less commonly in relation to the thin dendrites. The bases of the thin dendrites are more commonly associated with endoplasmic reticulum and many free ribosomes, the latter extending well into the dendritic shafts in many cases. Neurotubules are irregularly disposed and flexuouse, notably in those of the thinner dendrites; they are widely spread in the large dendrites but seem more concentrated in the thinner ones. These latter processes may become extended into thin sheets of cytoplasm, without tubules, similar to glial sheets (see Reese & Brightman, 1970), and may partially enclose a tufted or mitral cell dendrite, from which they may receive an asymmetrical synapse (see Chapter 5); no return synapses from these sheets have been seen. Many spines arise from the cell soma and the large dendrites of these cells and have variable appearances; most

somatic spines are short or sessile protrusions with a characteristically grey or flocculent cytoplasm and may contain a few cisternae, probably representing spine apparatus, and a number of vesicles (Figs. 11,12). Some somatic and most dendritic spines are larger with longer pedicles, and often contain many large flattened vesicles, a few cisternae, mitochondria and a variable number of ribosomes, free or as rosettes. The vesicles in the somatic and some of the proximal dendritic appendages are not related to synaptic contacts directed away from the periglomerular cell; a similar finding has been reported for the spines of the granule cell deep dendrites (Price & Powell, 1970a).

Somatic and dendritic spines of periglomerular cells most commonly receive asymmetrical synapses from pale axon terminals containing spherical vesicles, which are never olfactory nerve terminals (Fig. 11); synapses may on occasion be from a mitral or tufted cell dendrite or a tufted cell soma. The bases of these spines and the heads of others may also receive symmetrical synapses from axon terminals containing flattened vesicles, either of the large or small type (Fig. 12), and from gemmules of periglomerular cells containing large flattened vesicles. These two axon terminal types showing symmetrical membrane thickenings frequently synapse on to the cell somata or dendritic shafts of periglomerular cells, the terminals with small flattened vesicles predominating, and are a very common feature of profiles of this type of cell (Figs. 10,12); asymmetrical synapses from axon terminals containing spherical vesicles are seen less frequently on main dendritic shafts or cell somata, but are more common

on the finer dendritic shafts (the various axon terminals are described in detail in Chapter 5). The cell soma and either type of dendrite may, however, receive an asymmetrical synapse from a tufted cell soma or a mitral or tufted cell primary dendrite, as described in the previous section (Figs. 23,24), but a sac and limited membrane thickening orientated away from the periglomerular cell soma are the only possible synaptic structures that have been seen indicative of a reciprocal synapse (Fig. 25); a similar sac and membrane thickening may be associated with the region of contact between a periglomerular cell soma and an adjacent terminal containing spherical vesicles - probably a tufted cell recurrent collateral. Such sacs have been seen in continuity with granular endoplasmic reticulum (Fig. 25) and are similar to those found opposite one half of the tufted or mitral cell component of the reciprocal synapses on their cell somata (Fig. 18) (Price & Powell, 1970b,d). Extracellular material is present in the intercellular cleft similar to that found in relation to more typical synaptic structures; no vesicles have been seen in relation to this apparent synaptic structure, despite the fact that vesicles are regularly found in somatic spines (Fig. 11). The large periglomerular cell dendrites may make synaptic contact from their shafts (Fig. 22) or by means of a spine-like appendage, the gemmule, with the primary dendrites of mitral or tufted cells; these synapses are regularly characterised by symmetrical membrane thickenings and large flattened presynaptic vesicles. The somato-somatic, somato-dendritic, and dendro-dendritic synapses (Figs. 22-24) in the two directions between

the somata or primary dendrites of mitral or tufted and periglomerular cells are not always reciprocal in the immediate vicinity of each synaptic specialisation, although the reverse synapse may occur between the processes of the same two cells at some distance (Chapter 4).

The initial segments of the periglomerular cell axons are difficult to identify as they show few of the typical characteristics of these processes; this may be a feature of small neurons (Fig. 21). The initial segments are thin, of fairly regular outline and contain a few neurotubules, which only rarely appear to be aggregated and are never markedly so. Many free ribosomes are present, however, and limited plasma membrane undercoating may occasionally be discerned; multivesicular bodies, dense-cored vesicles and coated vesicles, and flattened agranular vesicles are common, and seem characteristic of this type of axon initial segment. Synaptic contacts are regularly found on to these structures, usually two or three per process; they are always of the symmetrical type, though the presynaptic terminal may contain either small or large flattened vesicles (Fig. 21). Small irregular cisternae may be found to lie in the immediate post-synaptic cytoplasm, probably analogous to the cisternal organ in the initial segments of other cells (Peters et al, 1968; Westrum, 1970; Kemp & Powell, 1971a). The axon hillock is identifiable in the periglomerular cells by the absence of Golgi apparatus and the presence of a limited amount of granular endoplasmic reticulum and many ribosomes, free or in rosettes; this region is not generally very marked since the axon initial segments of these cells appear to arise abruptly from the soma

surface.

The regular presence of vesicles of the large flattened type (see Price & Powell, 1970b) in the somatic and dendritic spines (Fig. 11), main dendritic shafts (Fig. 22) and their gemmules and axon initial segments of periglomerular cells is strong evidence that this type of vesicle is to be found in all parts of these cells, particularly those related to synaptic contacts - i.e. the finer dendritic branches in the glomeruli and their gemmules and the axon terminals of these cells; similarly, the symmetrical membrane thickening at synaptic contacts appears to be a regular feature of synapses orientated from periglomerular cells.

SHORT-AXON CELLS

Light microscopy

In Nissl-stained material the short-axon cells may be discerned in the periglomerular region as spherical or slightly ovoid, but have larger somata with a more irregular outline than the periglomerular cells. They also have characteristically pale nuclei with a very thin fringe of chromatin and a small rounded nucleolus; their perykaryon is larger than that of the periglomerular cells but shows much less Nissl substance than that of tufted cells. These cells are less common than the other two neurons of the glomerular layer, and resemble the short-axon cells of the granule cell layer, although they are rather smaller, being 8-12μm in diameter. In Golgi-impregnated material

Fig. 4 Camera lucida drawings of neurons impregnated with the Golgi-Kopsch method. Short-axon cells. A and D distribute their dendrites to the periglomerular region adjoining several gomeruli, while B and C have dendrites forming a 'clasp' around a single glomerulus. A, C and D show a few spines and the dendrites of B and C are notably varicose; C and D show characteristic lengths of axon. Note that C is at a lower magnification than the other three.

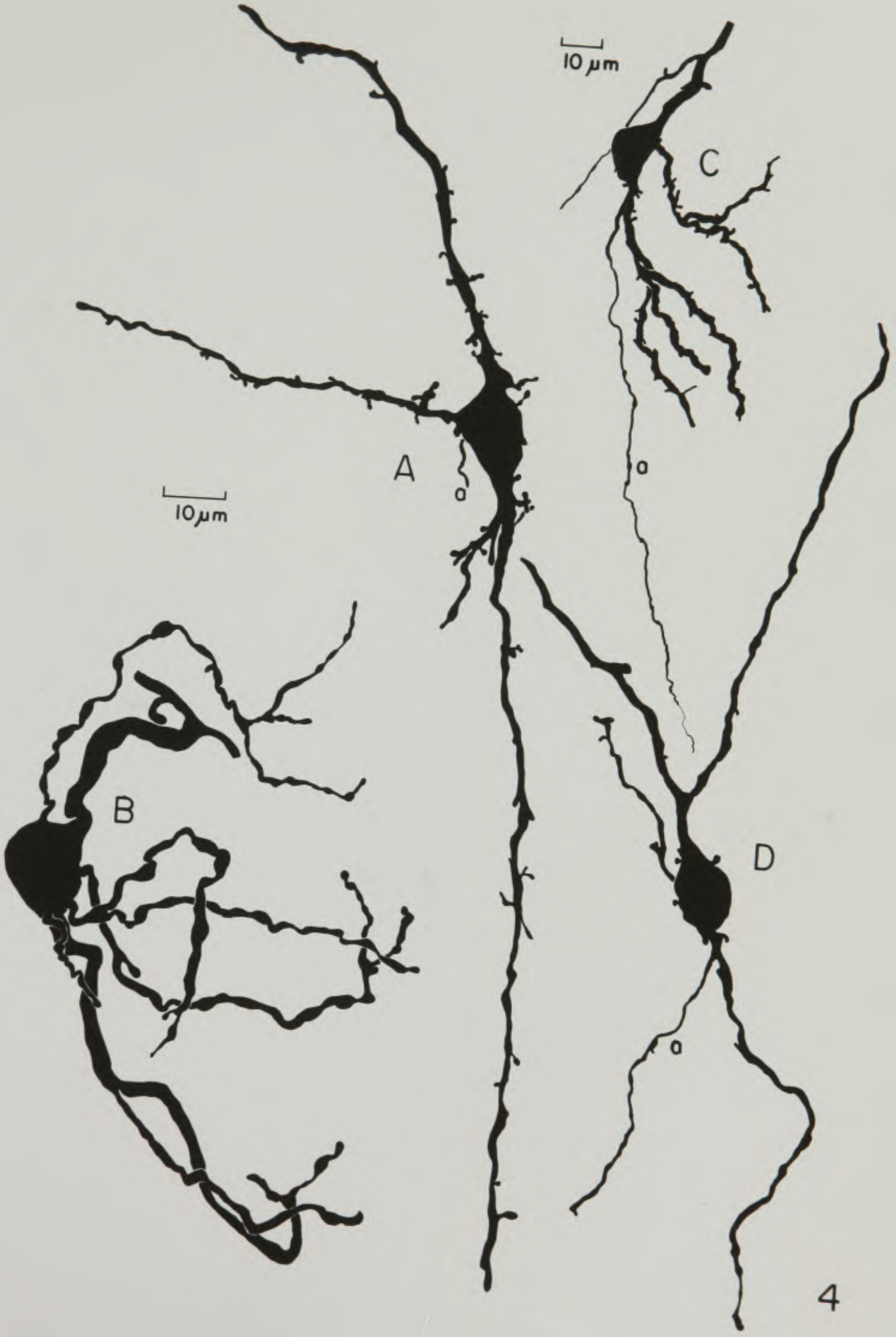

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Fig. 5 Photomicrograph of part of a periglomerular cell impregnated with the Golgi-Kopsch method, showing characteristic appendages.

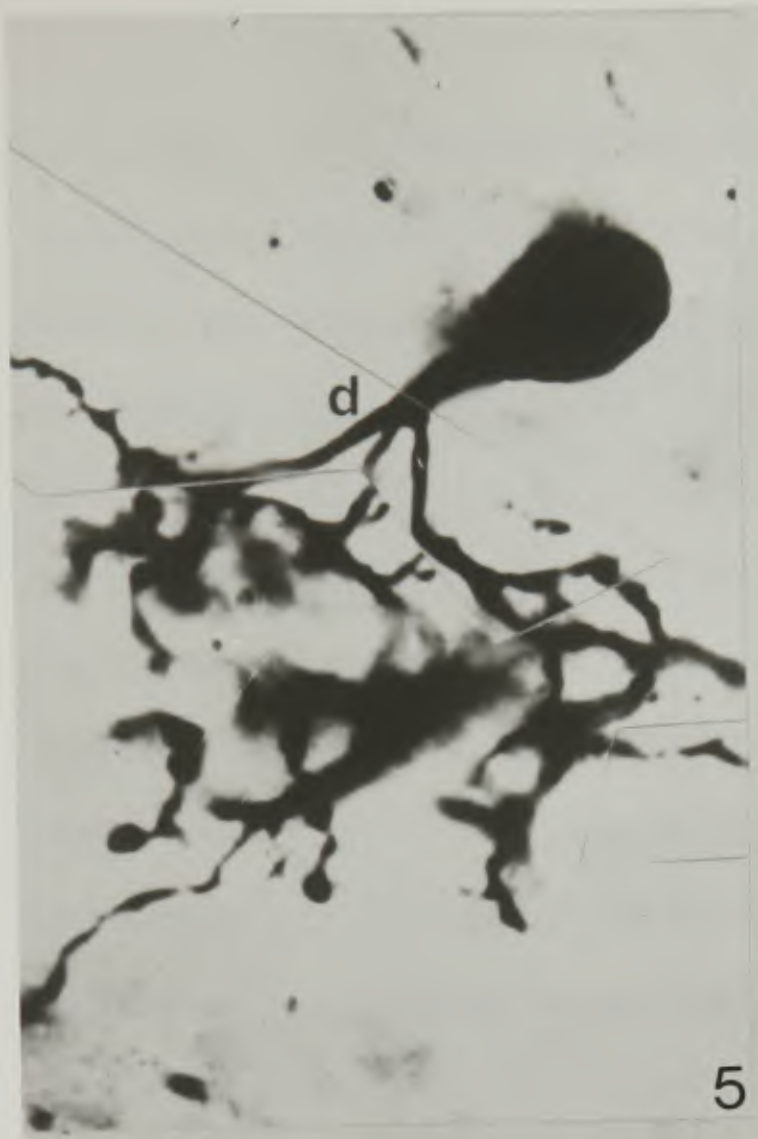
X1,620

Fig. 6 Photomicrograph of part of a superficial short-axon cell impregnated with the Golgi-Kopsch method, showing varicose dendrite and axon.

X900

Fig. 7 Low magnification electron micrograph of the periglomerular region showing the three neuron types of the glomerular layer - external tufted, periglomerular and superficial short-axon cells - as well as a glial cell.

X6,600



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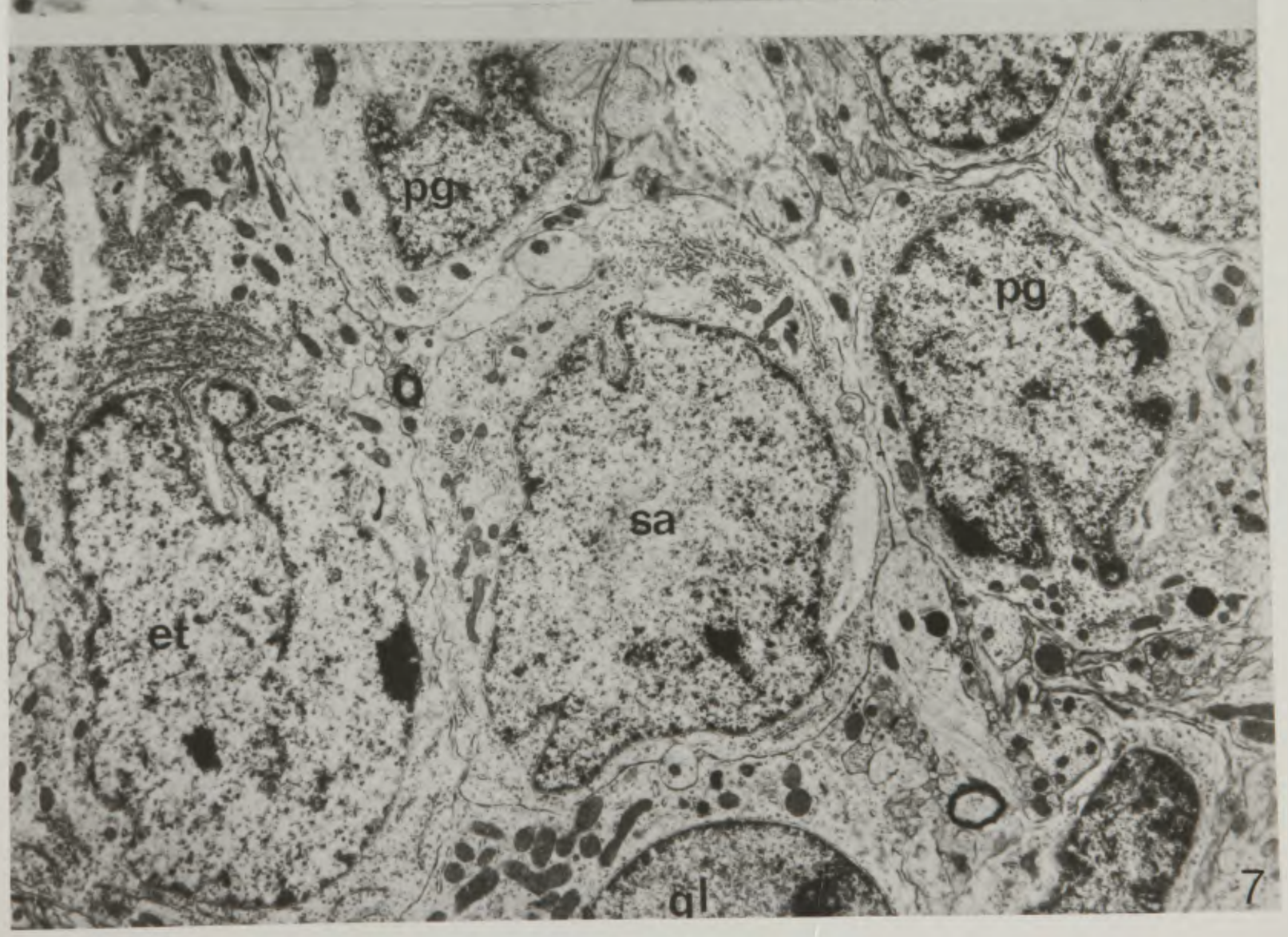

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(Figs. 4,6) they are particularly distinguished by their dendritic field, which is entirely periglomerular, the dendrites weaving in between the glomeruli or lying deep to them (Fig. 4A,D); others seem to surround a single glomerulus with a clasp-like formation of dendrites (Fig. 4B,C); the dendrites of the superficial short-axon cells are characteristically stout. They branch very infrequently, unlike the other glomerular layer neurons, and have a typically varicose or irregular outline (Fig. 4B,C), and, while some show dendritic and somatic spines (Fig. 4A,C,D) others seem free of spines; these differences may reflect a further subdivision of dendritic types similar to that described for the short-axon cells of the deeper layers. The axons of the superficial short-axon cells, when they are stained, are short, on average shorter than those of the periglomerular cells (Fig. 4C,D): they rarely traverse more than one or two glomeruli, and pass around or through the edges of these structures; they tend to be beaded but show little branching.

Electron microscopy

The superficial short-axon cells, which are less common than periglomerular or external tufted cells, may be found lying at any point around the glomeruli in the periglomerular region; they are predominantly spherical and appear of the same order of size as the external tufted cells (Fig. 7). They are characterised by pale nuclei and a moderate amount of cytoplasm, intermediate between the other two types, which is fairly evenly distributed around the nuclei (Figs. 7,13). The nuclear material is irregular, but less formed in patches

than that of tufted cells (Fig. 7), and the chromatin around the edge is thin; nucleoli are well-defined. Nuclear indentations are common and may be deep and quite complex, containing many ribosomes and some granular endoplasmic reticulum. The granular reticulum in the bulk of the cytoplasm is arranged into small groups of two or three parallel stacks and never forms the large complexes typical of the tufted and mitral cells. Golgi apparatus is found at the bases of dendrites, while ribosomes, mitochondria and other typical neuronal inclusions are frequent throughout the cytoplasm. The dendrites of the superficial short-axon cells are large and many cytoplasmic organelles may be found in their initial parts; neurotubules often appear rather irregularly disposed in the dendritic cytoplasm, apparently in relation to surface irregularities and varicosities, unlike the well-ordered array of tubules in the smooth dendrites of tufted and mitral cells. The short-axon cell dendrites are always directed into the periglomerular region, in which they may follow tortuous paths, and are of irregular shape, becoming increasingly varicose at a distance from the cell soma, as is seen in Golgi-impregnated material. Spines are seen on cell somata and dendrites, but are rare compared with those of periglomerular cells; they are characteristically small and contain a few irregular cisternae in a flocculent cytoplasm, and sometimes small mitochondria.

Although these cells sometimes appear similar to external tufted cells, they may be readily distinguished by the absence of reciprocal synapses on their cell body and dendrites, as confirmed by serial

sections; in order to ascertain this fact, several series of consecutive sections were examined, in which the whole cell soma and part of the dendrites could be observed (Fig. 13). Each series consisted of between 100 and 200 sections, and each short-axon cell, after tentative identification as such, was rigorously examined for synaptic contacts and other distinguishing features. Agranular vesicles were never found in the cell somata or somatic spines of these cells; another criterion for identification that emerged from studies of long series of sections, is the common appearance of asymmetrical synaptic specialisations directly on to the cell soma, from pale axon terminals containing spherical vesicles (Figs. 14,15). Direct synapses of the asymmetrical kind on to the cell somata of the other two cell types are very rare, but are characteristic of the superficial short-axon cells. Symmetrical synaptic contacts are also commonly found on these cell somata and main dendrites and are made by axon terminals containing either small or large flattened vesicles. The main dendritic shafts receive all synaptic types found in the periglomerular region (thus excluding olfactory nerve terminals), and by far the most common are the asymmetrical type; the spines arising from cell somata and dendrites also receive synapses from this latter type of terminal. Dendrites of short-axon cells contain few, if any, vesicles, and these are irregular structures, and are never aggregated or related to synaptic structures off the dendrites.

An unusual and characteristic feature of the short-axon cell somata, and to a lesser extent of their dendrites, is the presence of

small cytoplasmic protrusions around synaptic terminals of the asymmetrical thickening type; these may seem at some points to encircle the terminal, but they have never been observed to receive a synapse from it. The cytoplasm of these protrusions is typically flocculent but is otherwise empty of organelles. It is possible that these structures bear some relationship to spines, in view of their similar cytoplasm and the fact that asymmetrical terminals characteristically terminate on to the somata of the other cell types of this region by means of somatic spines; it may therefore be necessary to include them into the broadening concept of spine types (Jones & Powell, 1969a), as a stage less than sessile.

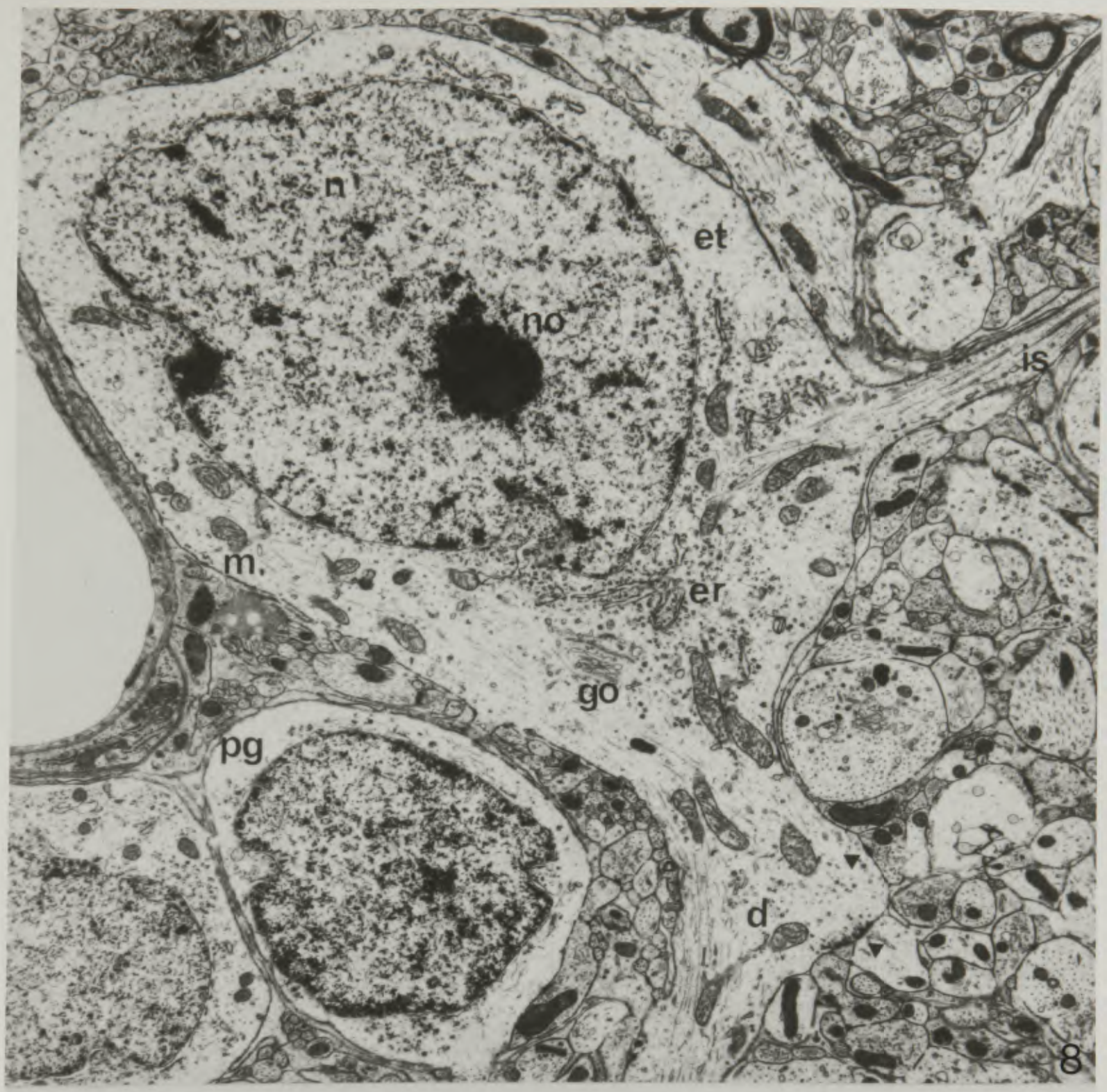
The axon hillocks of short-axon cells are not at all marked in the majority of cases and show little, if any, funnelling of cytoplasm (Fig. 13); where present, they contain granular endoplasmic reticulum and many ribosomes, but no Golgi-apparatus. The initial segment regularly emerges abruptly from the cell soma (Fig. 13). It shows the typical features of this region, the aggregation of neurotubules and coating of the plasma membrane being very marked; dense cored, alveolate and agranular vesicles are common, the latter showing considerable variety. Synapses are found on to these initial segments and are of the symmetrical type with large flattened pre-synaptic vesicles.

Fig. 8 External tufted cell soma showing initial part of dendrite and initial segment of axon.

X12,000

Fig. 9 Detail of external tufted cell, showing characteristic stacks of endoplasmic reticulum; note also the two components of the reciprocal synapse in relation to two different gemmules, and adjacent sac junction.

X21,500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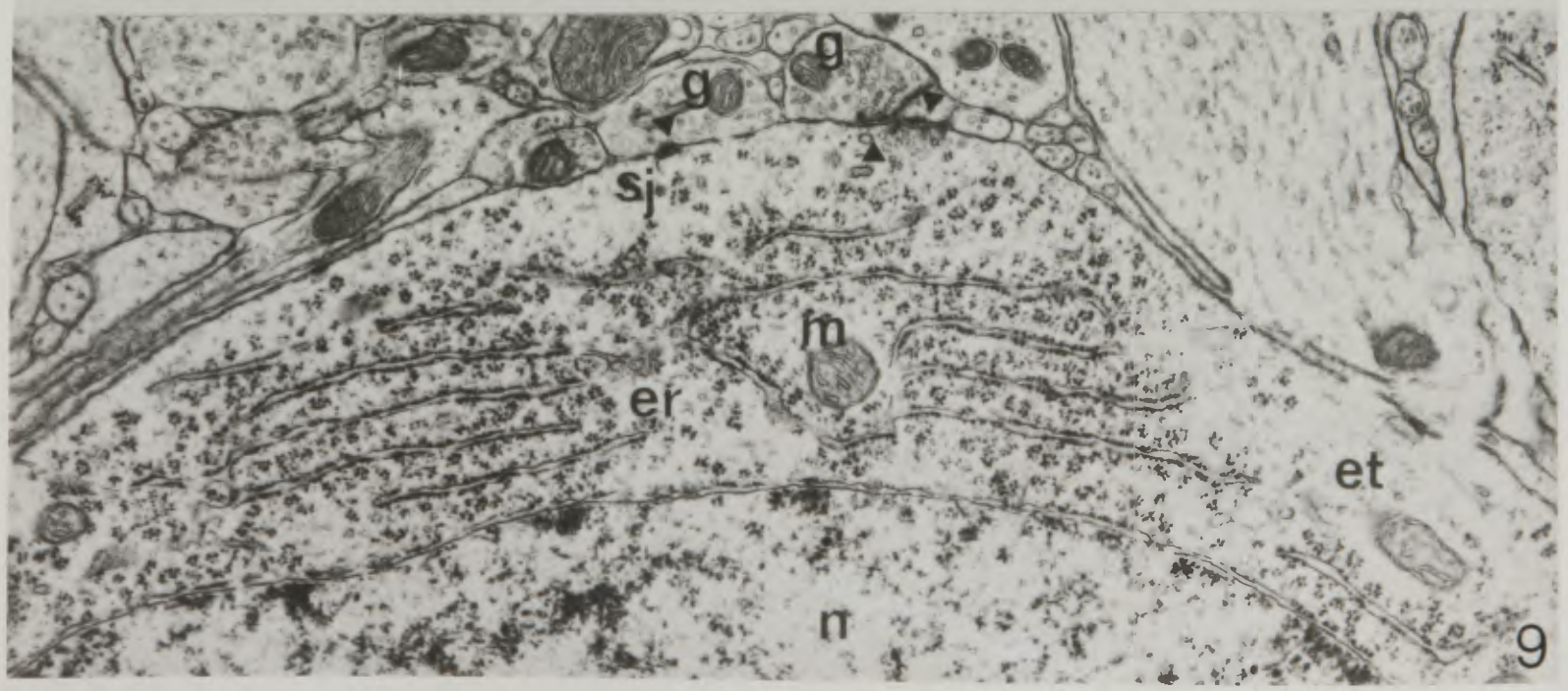
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Fig. 10 Periglomerular cell soma showing marked nuclear indentation and receiving a symmetrical synapse; note paucity and darkness of cytoplasm.

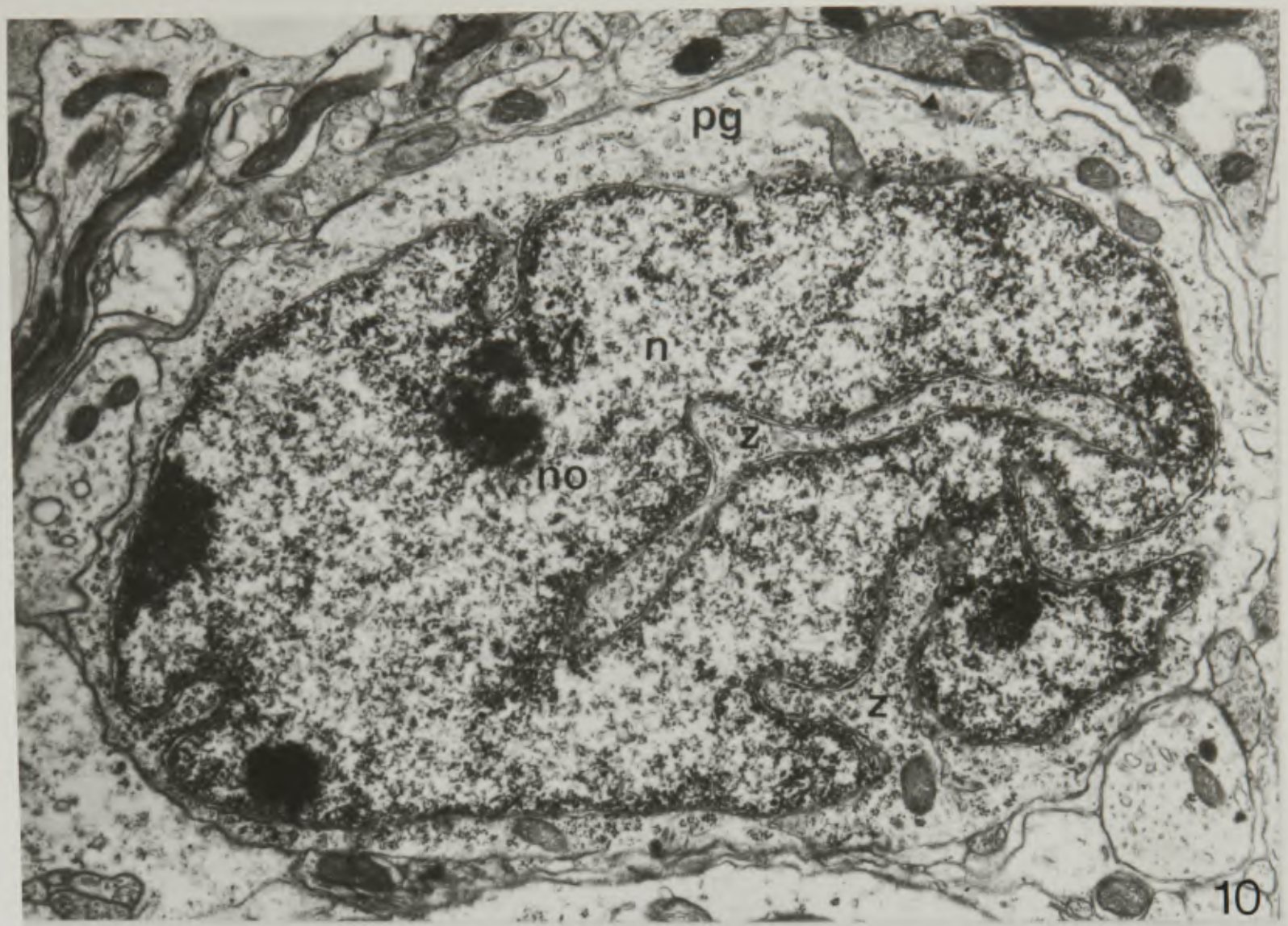
X22,000

Fig. 11 Somatic spine of periglomerular cell, containing large, irregular, flattened type vesicles, which receive an asymmetrical synaptic contact from an axon terminal containing spherical type vesicles. Note also evidence of symmetrical contact, outside the plane of this section, from an axon terminal containing small flattened type vesicles.

X53,500

Fig. 12 Axon terminal containing small flattened vesicles (compare adjacent terminal with spherical vesicles) synapsing with symmetrical thickenings onto a somatic spine and the cell soma of a periglomerular cell.

X53,500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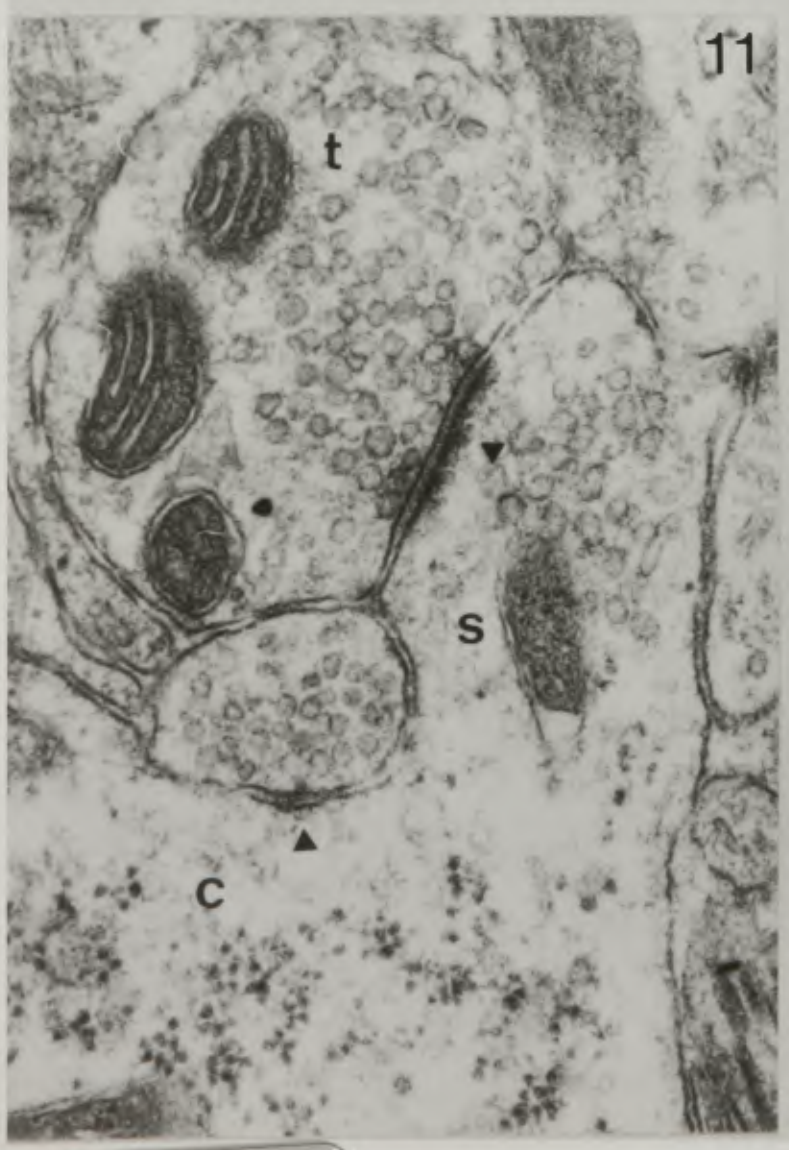
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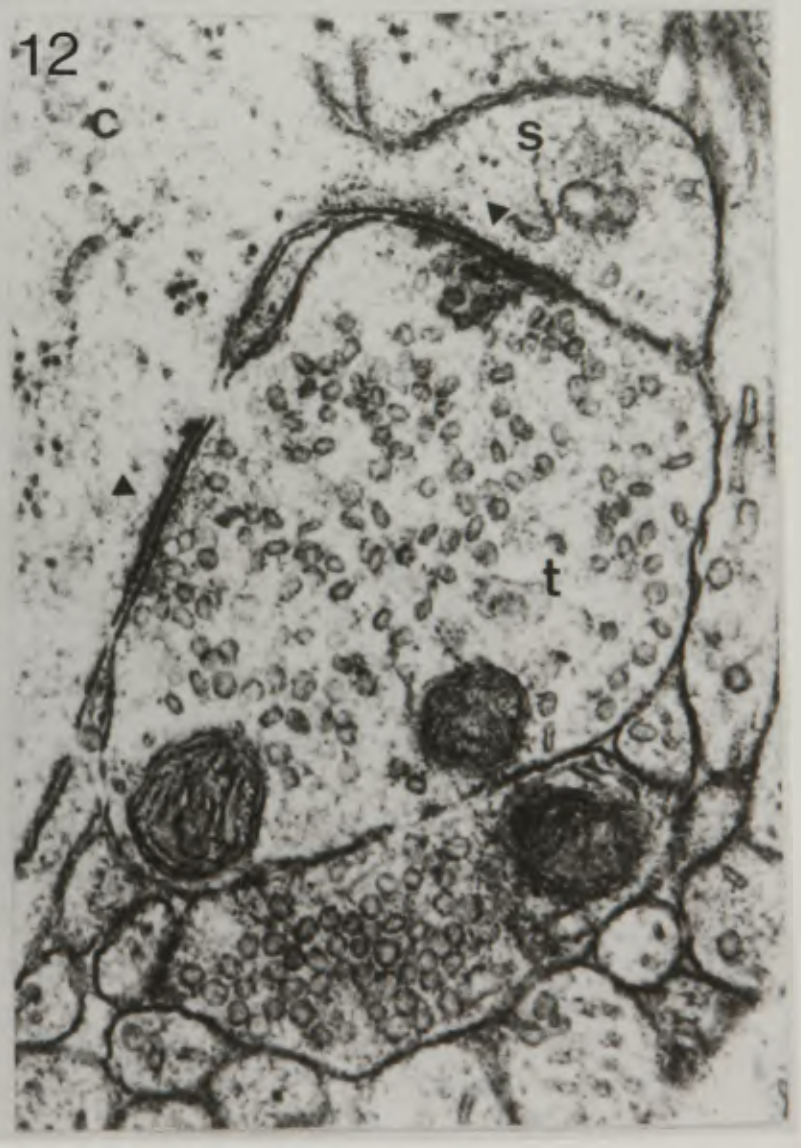
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Fig. 13 Superficial short-axon cell soma showing several nuclear indentations and axon initial segment. This cell was traced throughout in serial sections to ensure correct identification.

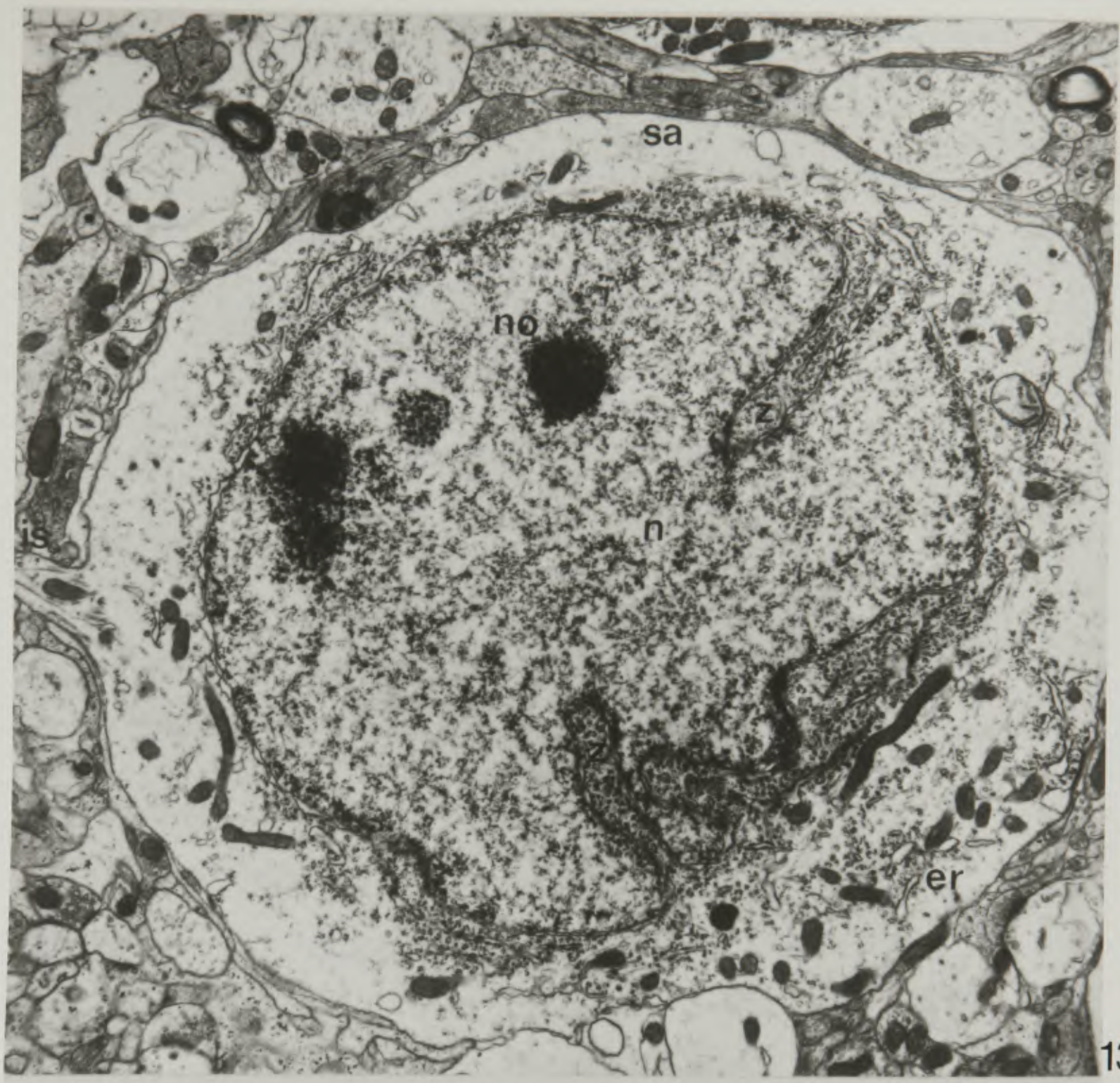
X15,000

Fig. 14 Asymmetrical synapse onto the soma of the same cell as in Fig. 13, from an axon terminal, containing spherical vesicles, that also contacts a vesicle-filled appendage; the large flattened vesicles of this appendage identify it as deriving from a periglomerular cell.

X50,000

Fig. 15 Asymmetrical type synapse onto short-axon cell soma from axon terminal containing spherical vesicles.

X67,000



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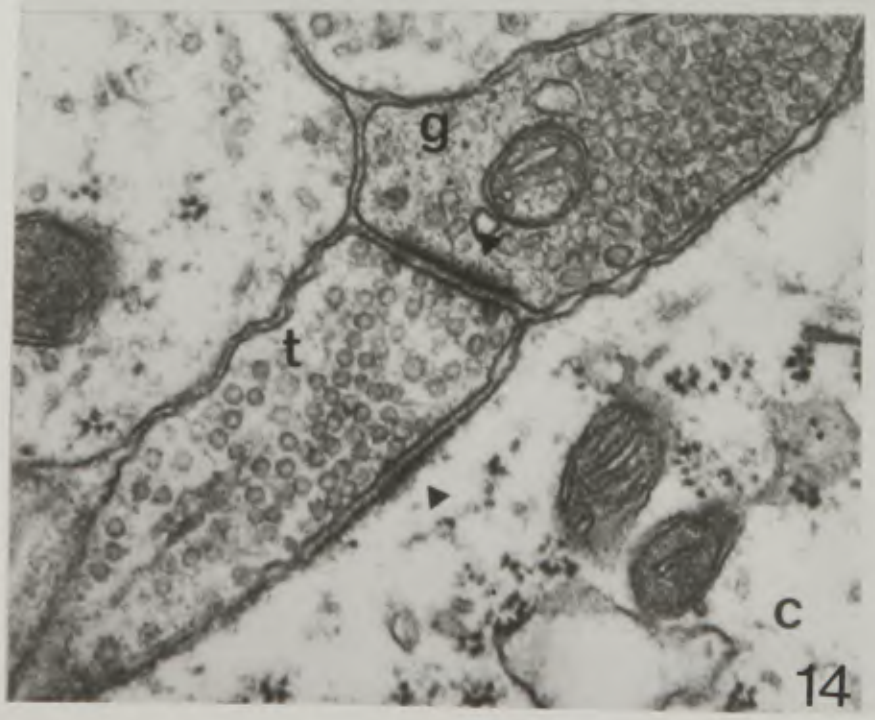

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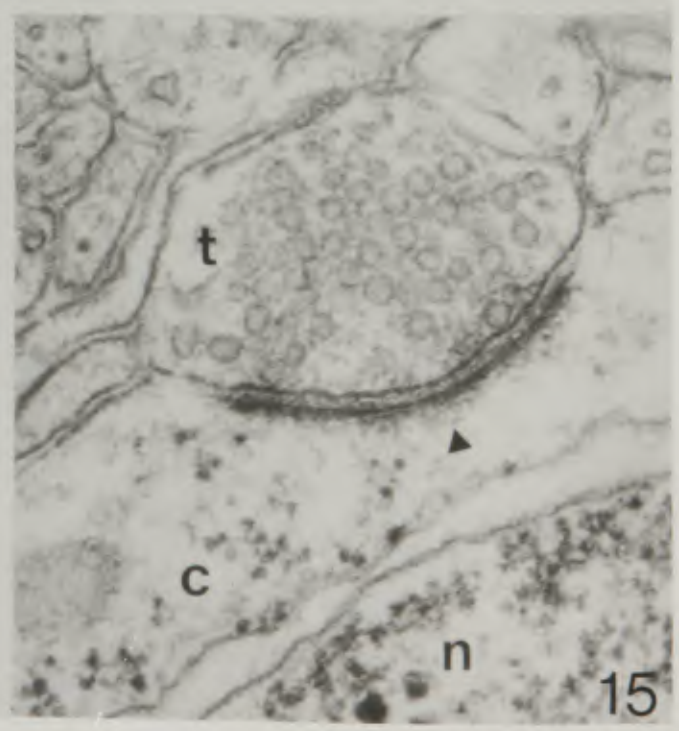

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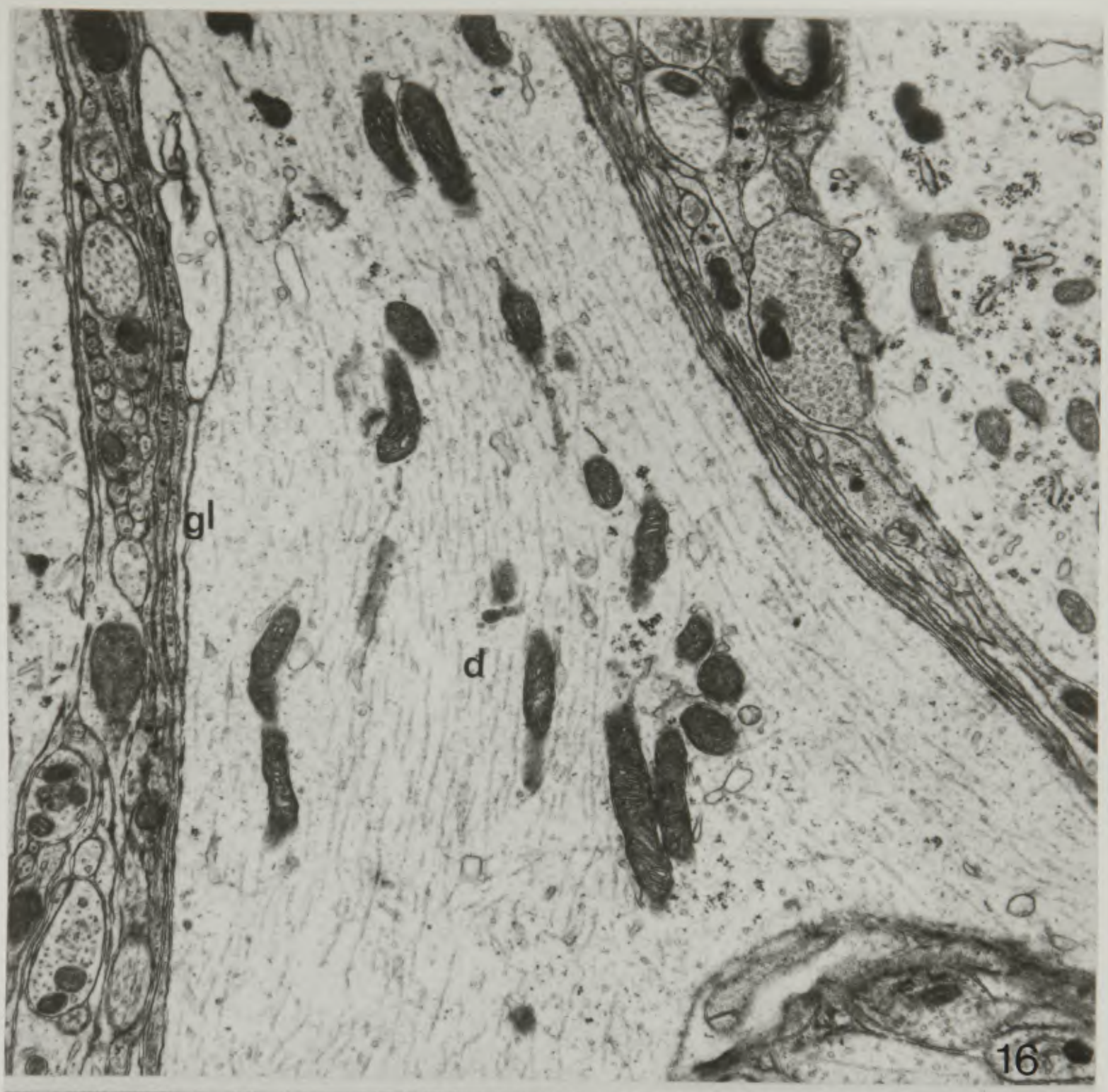
Fig. 16 Mitral cell primary dendrite at the point of primary glomerular branching, covered by several thin glial lamellae; the pale swollen profile may be a sheet derived from a periglomerular cell thin dendrite.

X18,000

Fig. 17 Glial profile giving rise to a thin lamella that surrounds a tufted cell primary dendrite, approximately in the middle of the glomerular layer. The same glial cell also surrounds a bundle of olfactory nerve fibres running from the olfactory nerve layer towards a glomerulus.

X45,200



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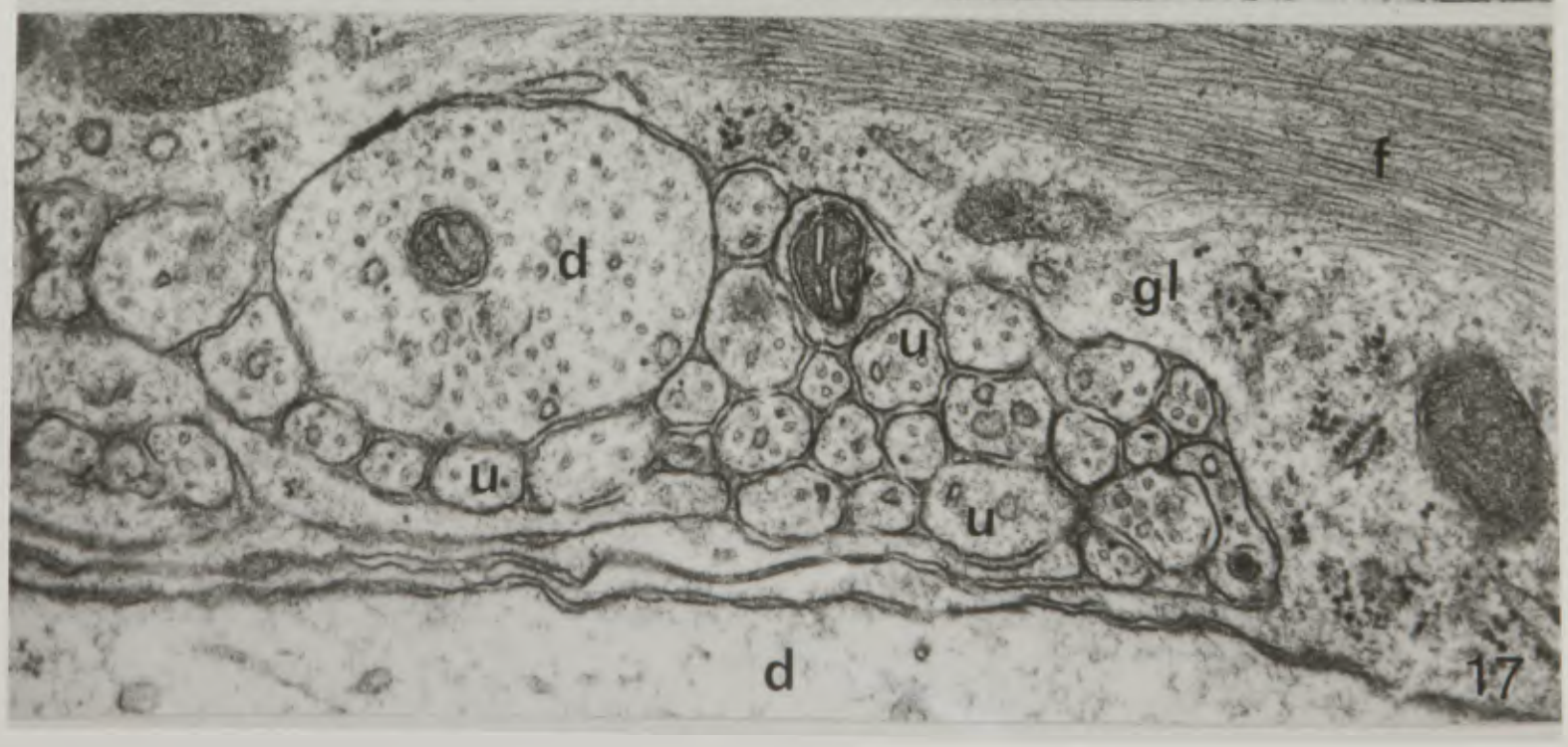

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Fig. 18 Part of a series of sections through a reciprocal synapse on an external tufted cell soma. Synaptic specialisations orientated from the soma showing spherical vesicles, a flat sac of endoplasmic reticulum and an asymmetrical membrane thickening. See also Fig. 19.

X48,000

Fig. 19 Further section from same series as Fig. 19, showing the return synapse from the gemmule onto the cell soma; this has large flattened vesicles and a symmetrical membrane thickening. Note glial lamellae passing over the gemmule, which continue over the cell soma.

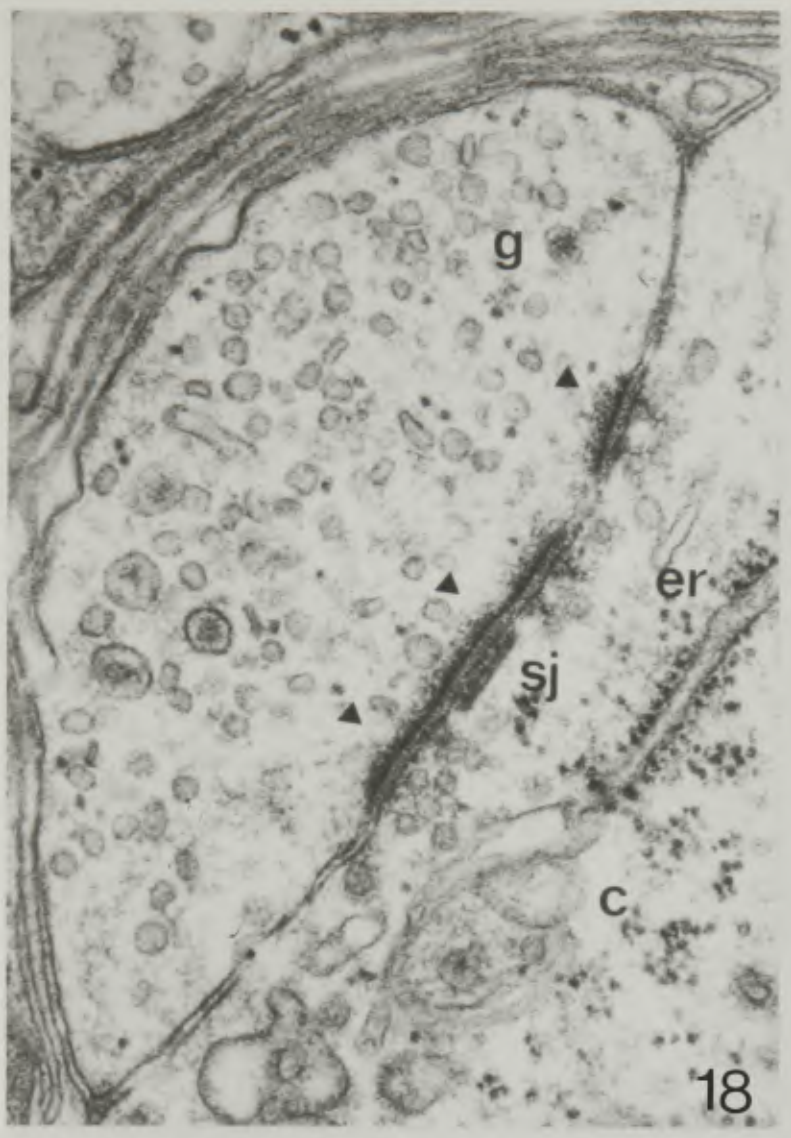
X48,000

Fig. 20 Axon terminal of periglomerular cell, containing large flattened vesicles, synapsing with a symmetrical thickening onto the stem dendrite of a tufted cell; no return synapse was found in serial sections, and the profile was continuous with a length of preterminal axon. Golgi apparatus is commonly found in the initial parts of tufted cell dendrites.

X53,500

Fig. 21 Synapses onto axon initial segment of periglomerular cell, showing large flattened vesicles and symmetrical thickenings. Note that periglomerular cell initial segments show little evidence of neurotubular aggregation, and have limited plasma membrane undercoating.

X40,000



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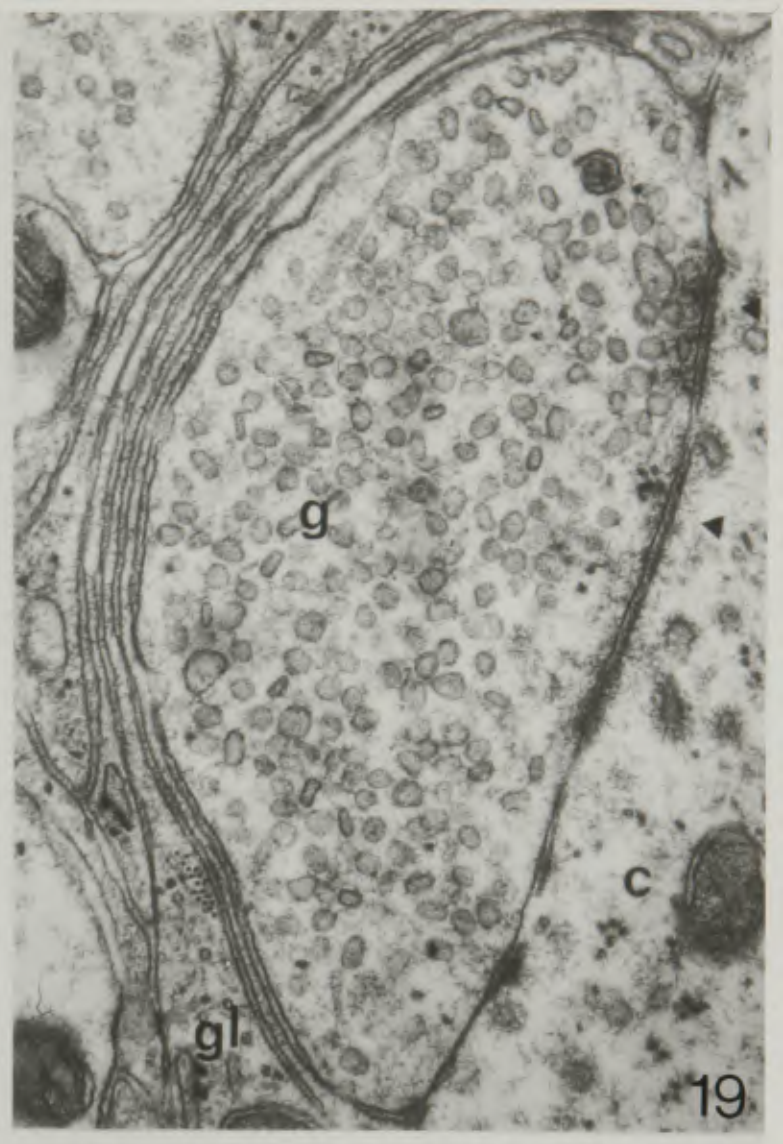

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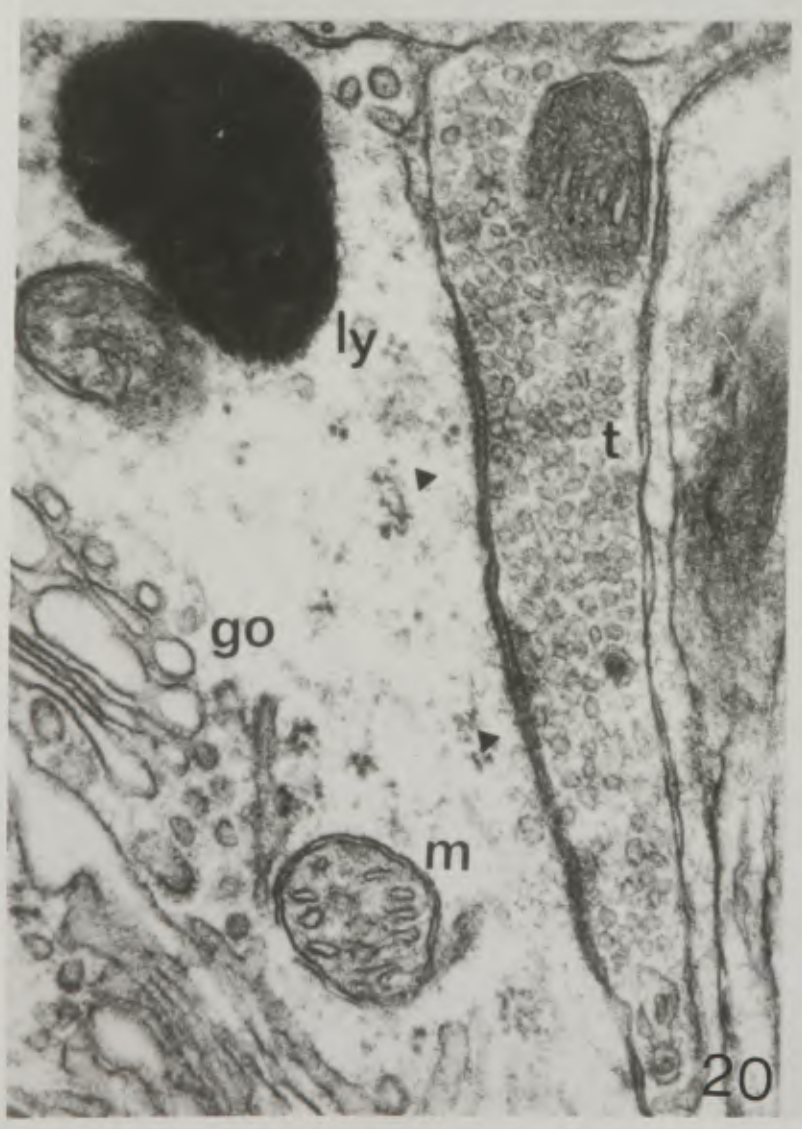

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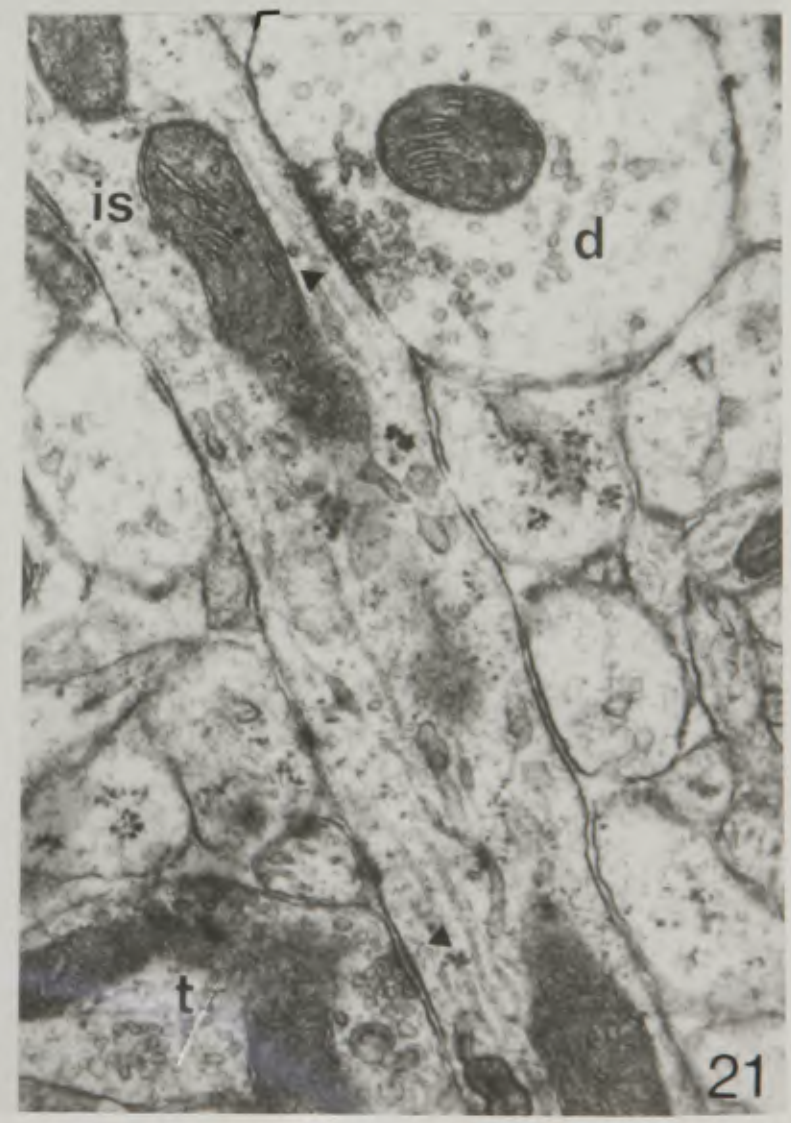

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Fig. 22 Dendro-dendritic synaptic contact showing large flattened vesicles and symmetrical thickening; the profile making this synapse was traced back to its cell of origin, a periglomerular cell, and the identity of the vesicle type and thickening type confirmed in serial sections. No return synapse was found but the post-synaptic process is probably the dendrite of a mitral or tufted cell.

X53,500

Fig. 23 Dendro-somatic synapse from the stem dendrite of a mitral cell onto the soma of a periglomerular cell; spherical presynaptic vesicles and asymmetrical membrane thickening.

X53,500

Fig. 24 Somato-somatic synapse from an external tufted cell soma onto a periglomerular cell soma; spherical presynaptic vesicles and asymmetrical thickening. The periglomerular cell shows an intranuclear rodlet.

X42,800

Fig. 25 Somato-somatic sac junction from periglomerular cell to external tufted cell; sac of endoplasmic reticulum situated 'presynaptically' and an equivocal membrane thickening, probably symmetrical in comparison with adjacent profiles.

X53,500

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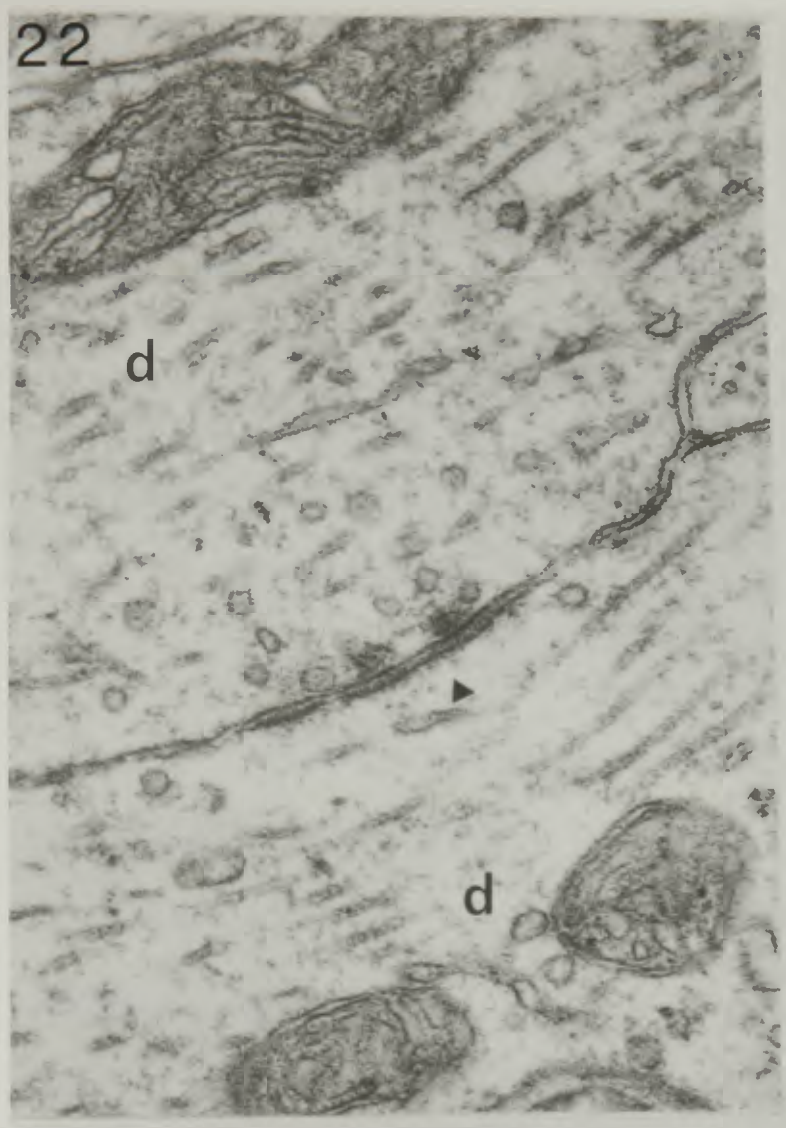

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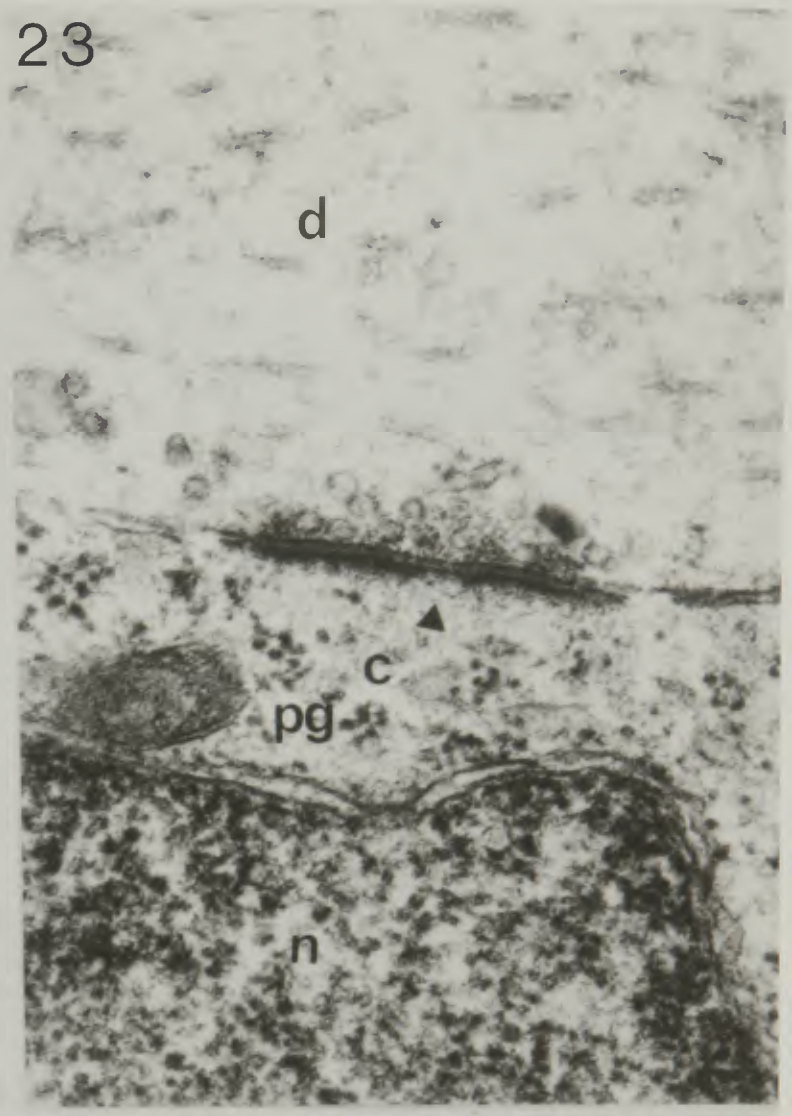

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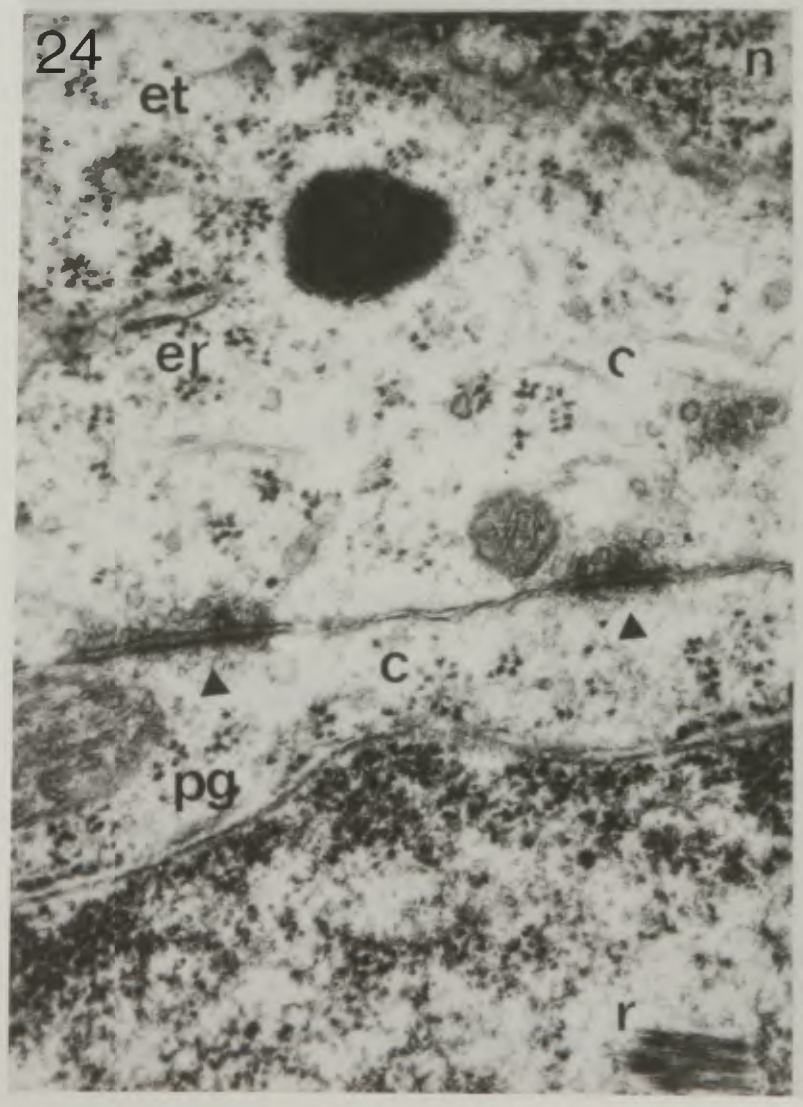

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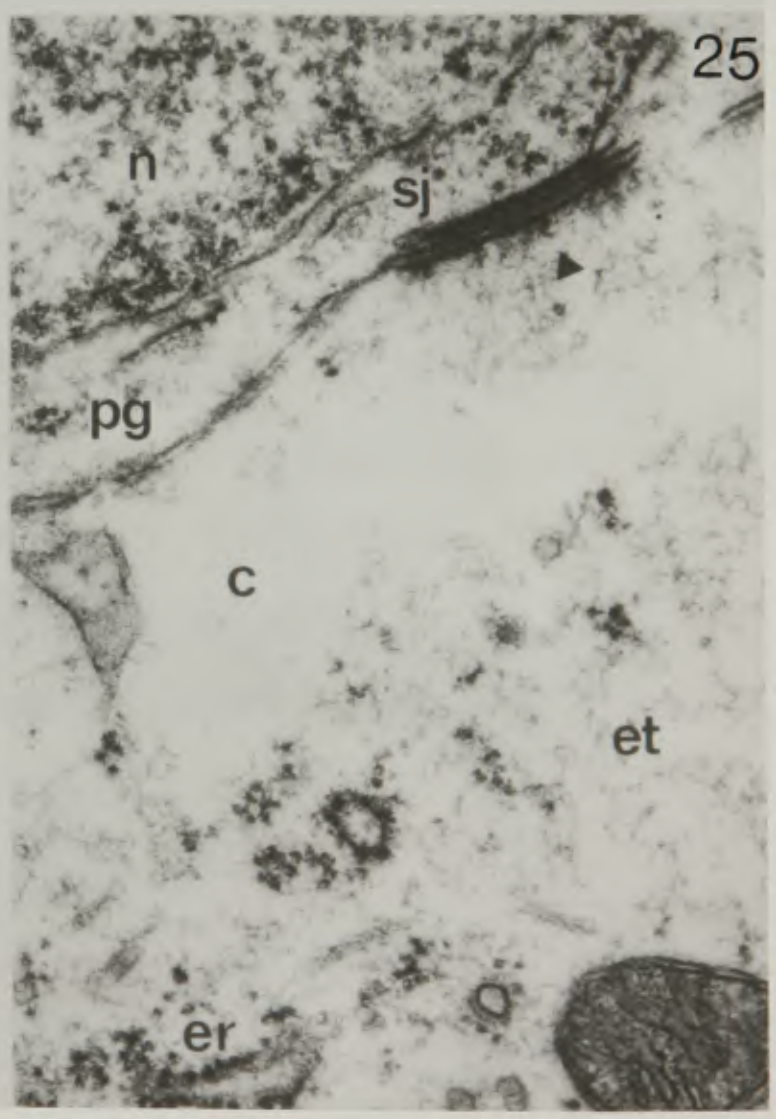


DISCUSSION

In this Chapter the cell types of the glomerular layer of the olfactory bulb have been described from observations with Nissl staining, Golgi impregnation and electron microscopy. It has been shown that, in addition to the two neuron types intrinsic to this layer described by the classical histologists - the external tufted and periglomerular cells, a third type may be recognised - the superficial short-axon cell. These three types have been characterised according to several morphological criteria with each method, notably electron microscopy, with a view not only to distinguishing the cell bodies and initial parts of their processes but also to providing means of identifying these processes at a distance from the somata, when demonstration of continuity with an identifiable cell is not possible. Although this study has been carried out on the rat, and the descriptions and figures in this and the following Chapters have been drawn exclusively from this species, the bulbs of the rabbit and to a lesser extent the cat and monkey have also been examined; as far as can be assessed, these bulbs are similar to that of the rat but with certain differences in general morphology (Chapter 6); further slight differences may emerge, but the broad organisational principles appear to be the same in the four species.

The discovery of a third cell type in the glomerular layer and its subsequent confirmation with several techniques is a clear demonstration of the value of correlative studies with electron microscopy

and Golgi impregnation. The additional detail provided by electron microscopic examination strongly suggested the existence of a third neuron type at this level, and light microscopic studies confirmed the existence of a distinct cell type which might correspond to it; the Golgi-impregnation characteristics of this neuron further aided its electron microscopic characterisation and particularly that of its processes. The relative rarity of these cells, together with the difficulty of impregnating cells in the glomerular layer, would not have permitted their identification using the Golgi technique alone, as evidenced by their neglect in the careful and meticulous works of Cajal and Blanes and other classical histologists. However, some illustrations by these, notably Golgi (1875), who considered them to be glial, and van Gehuchten & Martin (1891) as well as some of those by Blanes (1898) indicate that these authors had seen such cells but had insufficient evidence to distinguish them from glial or periglomerular cells. Furthermore, the possibility that short-axon cells may develop later than the other types (Hinds, 1968) would have tended to militate against their being regularly observed by the Madrid histologists, who both preferred to use very young animals. Nevertheless, a personal study of some of Cajal's original preparations (in the Cajal Museum of the Institute Cajal, Madrid) did reveal some cells of this type in preparations from older animals. It is felt therefore that the superficial short-axon cells were not described by these histologists for reasons of rarity and an obvious caution against the possibilities of incomplete impregnation or of false

identification of glia (the latter was already a source of contention in the bulb as regards the granule cells). In view of the very strong electron microscopic evidence that is now available, as well as considerable Golgi evidence for the existence of an additional cell type, we feel justified in putting forward the superficial short-axon cell as a definitive neuron type of this layer.

The presence of a short-axon cell in the glomerular layer has greatly extended the already significant parallels between this and the deeper layers, comprising the granule and mitral cell layers (equivalent to the periglomerular region) and the external plexiform layer (broadly equivalent to the glomeruli), so that each of the intrinsic neurons of the glomerular layer has a counterpart with similar features in the deeper layers. Thus the external tufted cells seem analogous to the mitral and deep tufted cells, and periglomerular cells to the granule cells and the superficial short-axon cells to the deep short-axon cells. Each glomerular neuron shows a broadly similar morphology to its deep counterpart, but is generally smaller and shows a different orientation of its processes - glomerular or non-glomerular - rather than predominantly vertical or horizontal. The type, density and relative proportions of nucleus and cytoplasm and the distribution of organelles within them are strikingly similar in the analogous cell types of the two groups of layers (see Price & Powell, 1970a,d). Although there is no overlap between the distribution of processes of granule and periglomerular cells, nor of the two short-axon cell types, each pair being limited by the deep aspect of the glomerular layer, the

tufted and mitral cells represent more of an overlapping continuum from superficial to deep, both receiving glomerular activity in their terminal dendritic tufts.

Certain notable differences between the cells of the two groups do exist, however, The granule cell (Price & Powell, 1970a) has no axon, but rather two types of dendritic processes - the deep dendrites, which are varicose and give rise to typical spines, and the peripheral processes that give rise to some spines and to gemmules which enter into reciprocal synapses. The periglomerular cell on the other hand does possess an axon, but its dendritic arborisation within the glomeruli appears to be analogous to the granule cell peripheral processes in bearing some spines and many gemmules. The periglomerular cells may also show a more glial-like dendritic process having an irregular outline, which often becomes drawn out into thin sheets, of which the granule cell appears to have no counterpart. The analogy between the peripheral process of the granule cell and the large glomerular dendrites of the periglomerular cell is further strengthened by the regular presence of Golgi apparatus in the cell soma at the bases of these processes and not opposite the axon or glial-type dendrites of the periglomerular cell or the deep dendrites of the granule cell (Price & Powell, 1970a). The nature of the dendritic processes in the olfactory bulb will be further considered in Chapter 5.

The external tufted cells have various special characteristics in which they differ from mitral and deep tufted cells - these are the extent of their glomerular arborisations, their relative lack of

secondary dendrites and the additional distribution of their collaterals. It has already been noted that the glomerular arborisations of the external tufted cells are characteristically wedge-shaped, with the result that they cover only a portion of the glomerulus, while the deep tufted cells and mitral cells have arborisations that fill the glomeruli with an almost spherical distribution. Such a difference could be related to the fact that the mitral and the tifted cells of the external plexiform layer always enter from the deep aspect of the glomeruli; by extending throughout the glomeruli they subserve, these cells must receive afferent impulses from olfactory nerves terminating in all parts of the glomerulus, while a given glomerulus is 'covered' by several external tufted cells whose dendrites arise from different points around it. It is interesting to note in this respect that Land, Eager & Shepherd (1970) show that degeneration of olfactory terminals within the glomeruli after localised lesions of the nerves may be restricted to part of a single glomerulus. Secondly, the external tufted cells are unusual in that they rarely have secondary dendrites, only those cells on the edges of the external plexiform layer ever showing them, although one cell may show two primary dendrites, each with a separate glomerular arborisation. The fact that the secondary dendrites of mitral and deep tufted cells are related exclusively to the reciprocal synaptic contacts with granule cell gemmules (Price & Powell, 1970d) indicates that these secondary dendrites are specialised to establish such contacts; their absence in many of the external tufted cells further supports this view, since

the primary dendrites of these cells are able to establish a considerable number of similar contacts with the periglomerular cells, the glomerular analogues of the granule cells, by the nature of the glomerular formations. The distribution of axon collaterals of the tufted cells to the glomerular layer is a further difference between them and the deep cells, which distribute collaterals only in the granule cell and external plexiform layers (Cajal, 1955; Valverde, 1965). Thus, in spite of the many striking similarities between mitral and tufted cells, the latter and particularly the external tufted cells, do form a distinct group in the extent and levels of their connections; the observation of reciprocal synapses on the initial segments of middle tufted cells, indicates that these too may form a partially separate group.

The concept, introduced by Valverde (1965), that the tufted cells are inwardly displaced periglomerular cells rather than outwardly displaced mitral cells (as Cajal considered) has not been borne out by this electron microscopic study: no periglomerular-type neurons have been observed in the external plexiform layer, while the characteristic tufted neurons lying in this layer are all of the mitral type in general cytological features and particularly in the possession of reciprocal synaptic arrangements with granule cell gemmules, showing the same types of polarity as exhibited by the mitral cells. Similar cells, the external tufted cells, in the periglomerular region have also been demonstrated to show mitral-type features; Cajal's classification of the mitral and tufted cells in all layers of the olfactory

bulb as a single basic group has thus been confirmed at electron-microscopic level and is a striking tribute to his insight.

Three types of vesicles may be distinguished in normal aldehyde-fixed material of the glomerular layer of the bulb as in the deeper layers - spherical vesicles, and large and small flattened vesicles (see Price & Powell, 1970b). It should be noted that for the brains in which flattening was less marked, probably for reasons of varying osmolarity of fixative, processes could be identified by the greater irregularity of shape shown by the flattened-type vesicles as compared with the regular spherical type. The presence of synaptic vesicles related to membrane thickenings in the cell bodies and dendrites of the mitral and tufted cells and in the dendrites of periglomerular cells as well as that of exactly similar vesicles in the spines of the latter, has considerably aided the identification of vesicle-containing profiles at a distance (Chapters 4 & 5); large flattened vesicles are characteristic of periglomerular cells and their processes, like the granule cells, while spherical vesicles are found in the processes of mitral and tufted cells. Axon terminals containing small flattened vesicles are also found in the glomerular layer and this type of terminal, recognised by site and mode of termination, only degenerates after intrinsic lesions of the glomerular layer (Chapter 10), but no dendrites have been found containing this type of vesicle. Since the distribution of these small flattened vesicle axon terminals corresponds to that of the superficial short-axon cell axon known from Golgi impregnation evidence, they may be identified as being those of the

short-axon cells by a process of elimination. A similar line of reasoning has identified the terminals containing small flattened vesicles in the deeper layers as being those of the short-axon cells of the granule cell layer (Price & Powell, 1970b & d).

The observation of somato-somatic, dendro-somatic, and dendro-dendritic synapses between cell types identifiable by their somata has assisted the identification of the processes involved in similar synaptic arrangements on the distal dendrites in the neuropil. Such synapses are only found between mitral or tufted and periglomerular cells and the synaptic membrane thickenings are characteristic - those from mitral and tufted cells being regularly asymmetrical and those directed from periglomerular cells being symmetrical. It can therefore be deduced that dendrites showing such polarities in reciprocal or serial synaptic arrangements are those of the same cells. Such an observation, to which no exceptions have been found, would confirm an analogy between the periglomerular cell dendrites and granule cell peripheral processes and of the tufted cells with the mitral cells. On this basis we cannot agree with the interpretations of Hinds (1970) as to the polarities of the reciprocal synapses. The false interpretation stems from his identification of the cell shown in Fig. 5 of his report as a periglomerular cell, whereas in our consideration there is little doubt, even from the small part of the cell body shown, that it is an external tufted cell, by virtue of its general cytology and its synaptic specialisation; indeed many exactly similar arrangements have been observed in this study in which the neuronal soma

involved was always identifiable as an external tufted cell, while no such relationships are found in the case of periglomerular cells.

The presence of vesicles in the dendrites of many of the cells of the olfactory bulb and their presynaptic position has been known for some time (Hirata, 1964; Andres, 1965; Rall et al 1966; Price, 1968; Price & Powell, 1970a-d) and although initially considered to be a peculiarity of this region, it is emerging that neither the presence of vesicles nor a presynaptic role are the entire preserve of axon terminals in several other parts of the central nervous system (e.g. Dowling & Boycott, 1966; Sétáló & Székely, 1967; Lund, 1969; Ralston & Herman, 1969; Famlighetti, 1970). Certainly in the olfactory bulb, where the majority of profiles contain vesicles, it can be difficult on occasion to know whether one is dealing with axons or dendrites; in the glomeruli, the position has been greatly simplified by knowledge obtained from past and present Golgi studies on the distribution of axons and dendrites in this layer (Chapter 4). Criteria have to be established nevertheless by which vesicle-containing dendrites and axon terminals may be distinguished. In the primary analysis of a region, there is no substitute for the examination of serial sections to determine the identity of the neuropil profiles; continuity with a preterminal axon, a dendritic shaft, a cell soma, an axon initial segment or with a profile that contains some recognisable feature, is the most straightforward and certain way of identifying it. Naturally, information on the distribution of processes from light microscopy is the essential adjunct to such a study. Eventually, from

these serial studies criteria will emerge for distinguishing between profiles, very often allowing their identification in single sections, although confirmation by serial examination is still valuable as a continuing check.

Dendritic profiles commonly show ribosomal rosettes, many cisternae of agranular endoplasmic reticulum, often associated with mitochondria, and their shafts have a variable number and density of neurotubules, that are fairly evenly spread through the profile; few, if any, neurofilaments are found. Ribosomal rosettes are frequent, and their presence is a strong indication of dendritic nature, although single ribosomes may be found in axons. Vesicles, when present, are unevenly distributed in dendrites, either closely associated with the presynaptic membranes of a synaptic specialisation, or aggregated at branch points of the dendritic shaft; they are also found in small groups which are not associated with branching or synaptic contacts orientated away from the dendrites (e.g. in spines). Most dendritic profiles, including appendages thus have a relatively inhomogeneous distribution of vesicles. Axon terminals or en passant boutons, on the other hand, rarely show evidence of ribosomes, and when present, these are never aggregated into rosettes; few cisternae or mitochondria are seen and neurotubules are either absent (terminal) or restricted to one side of the profile (en passant); neurofilaments are commonly associated with the tubules. Vesicles are evenly spread through the profile in most cases tending to cover the whole, except in those regions containing neurotubules. Despite the slightly unsatis-

factory nature of these criteria, the majority of profiles in the bulb neuropil may now be classified as axonal or dendritic.

If vesicle-containing and presynaptic dendrites are shown to be widespread in the nervous system, then some anatomical and physiological work may need re-interpretation in the light of such a demonstration. There is no doubt that the presence of synaptic specialisations orientated away from dendrites of otherwise typical neurons, notably those with axons such as the mitral, tufted and periglomerular cells, suggests that such a peculiarity is not solely a feature of cells that lack axonal processes, like the granule and amacrine cells of the bulb and retina. However, it could be suggested that these presynaptic dendrites represent a form of cell process that is intermediate between axon and dendrite, with some properties of each (see Chapter 5). That such an intermediate process exists could emerge from a physiological examination of these unusual cells in the olfactory bulb; certainly their physiological properties pose a considerable problem and must be evaluated before the exact significance of their participation in the neuronal organisation within any nucleus can be assessed. It should be emphasized, however, at this juncture, that although some previous studies have focused upon the unusual aspects of the organisation of the olfactory bulb neurons, these aspects exist not in isolation, but in a larger context which includes straightforward axo-dendritic and axo-somatic arrangements; the analysis of the mode of combination of all these features underlies the essential purpose of this study, which is to produce a comprehensive anatomical circuit diagram of the cells

of the olfactory bulb.

The large number of vesicle-containing processes which enter into synaptic contact within the glomerular layer has had further repercussions in that it has provided additional evidence for the correlation first made by Colonnier (1968) between type of vesicle and type of membrane thickening. It can be stated unreservedly that the correlation between spherical vesicles and asymmetrical contacts, and between flattened vesicles of both types and symmetrical thickenings has been entirely confirmed in every case in which synaptic contacts have been examined. Serial sections have proved invaluable for ensuring that the type of thickening being examined is cut in such a way as to show its genuine characteristics; this is particularly important because the periphery of an asymmetrical thickening often appears to be 'symmetrical'.

The demonstration of 'sac junctions', in which a flat sac, continuous with endoplasmic reticulum lies presynaptic to part or whole of a membrane thickening of either type, extends the distribution of such apparently synaptic structures to the tufted and periglomerular cells, in addition to those already described for the mitral and granule cells (Hirata, 1964; Price & Powell, 1970b,d). Although the synaptic nature of these sac junctions is difficult to ascertain, the similarity of the membrane specialisations and the presence of an undoubted layer of extracellular material between the membranes (of the type associated with typical synaptic structures) suggests that these are specialisations of a similar type. The facts that sac

junctions usually occur with the appropriate membrane thickening polarities as those of typical dendro-dendritic synapses, and may even share the presynaptic site with vesicles (in the mitral and tufted cells), all indicate that the flat sac of endoplasmic reticulum situated presynaptically may be analogous to, or a substitute for, presynaptic vesicles. Nevertheless, it must be noted that the similar appearance of cisternae opposite persistent post-synaptic membrane thickenings (Chapter 7) cannot be taken as evidence of functional contact; indeed the possibility remains that at least some of the above 'sac junctions' are cases in which a degenerating terminal has left behind its post-synaptic structures. The similarity of the 'presynaptic cisternae' in the two cases could be taken to suggest that some of these apparent synaptic structures have been left over, developmentally, and the sac or cisterna has been induced opposite it in a similar way to the apposed process after long-term degeneration. These observations on sacs or cisternae in relation to membrane thickenings are clearly impossible to assess on solely anatomical grounds; thus while in some cases they would seem to be functionally related to synaptic specialisations (in mitral and tufted cells), their presence in other situations may represent either a form of functional contact of a non-synaptic nature, or else a non-functional synaptic contact. Whether or not the sacs have any relation to the derivation of vesicles cannot be stated on this evidence.

The mitral and tufted cells present a site in which the questions of dendritic integration (Rall, Burke, Smith, Nelson & Frank, 1967;

Diamond & Yasargil, 1969) and dendritic spike generation may be studied (Rall & Shepherd, 1968); this point has already been considered in an earlier paper (Price & Powell, 1970d) but new observations on the glomerular components of these cells may be able to throw further light upon these problems. The main primary dendrites of the mitral and tufted cells enter the glomeruli by passing through the periglomerular region and then branching several times within the former. Around the point of primary branching and within the periglomerular region these large dendrites are covered by several layers of thin glial lamellae; also in this region they receive the symmetrical synaptic contacts of the periglomerular cell axons, additional to the reciprocal dendro-dendritic synapses characteristic of all but the most distal parts of the mitral and tufted cell dendritic trees. The extensive glomerular arborisations of the stem dendrites receive the massive and excitatory input from the olfactory nerves (Yamamoto, Yamamoto & Iwama, 1963; Shepherd, 1963) in addition to receiving the probably inhibitory dendro-dendritic synapses from periglomerular cells; the point at which the post-synaptic affects of such inputs will converge will thus be at the region of primary glomerular branching. Clearly any inhibition applied at this level, mediated by the periglomerular cell axon terminals, could be highly effective in reducing or preventing any excitatory effect from spreading beyond the glomeruli; similarly, any excitation will have a strong facilitatory effect. If, as is possible (Rall & Shepherd, 1968; see also Spencer & Kandel, 1961), this is a region of dendritic spike generation, similar functionally to

the axon initial segment, local inhibition will be powerful in preventing spike initiation. The role of the glial lamellae in relation to dendritic function is less straightforward: it may serve to insulate this critical region of integration from local 'noise' or may perform a role similar to that of a myelin sheath, leading to some form of facilitated conduction through the periglomerular region; the formation of a myelin sheath around this specific segment of dendrite in the monkey (Chapter 6) may add support to this interpretation.

It is interesting to consider the significance of the probable origin of this glial wrapping of the large mitral and tufted cell dendrites in view of developing ideas on a dynamic role of glia in the central nervous system. If, as is strongly indicated, some of the lamellae originate from deep processes of the glial cells whose peripheral processes wrap around the bundles of incoming olfactory nerve fibres, then these cells occupy a position closely related to both input and output of the glomerular relay; in this way they could be considered similar to the Müller cells of the retina (Miller & Dowling, 1970), although the contact of the bulbar glia with the neurons is more intimate. The observation by Cajal (1890) and Blanes (1898) that these cells are stained by the Weigert method indicates a myelin-like role for their processes, and the observation on myelination at this site in the monkey further suggests such a role. The further possibility exists that, like the Müller cells of the retina, these glia act as sinks or electrodes for K^+ ions, and may as such play an

active role in the relay system in the glomeruli: thus the peripheral processes of this glial cell type will be subjected to a large potential change when the olfactory axons in the bundles are active, and this may be transmitted as a slow glial potential to the large mitral and tufted cells dendrites as they leave the glomeruli having received synaptic contact from the incoming olfactory axons. The striking anatomical similarity of the olfactory nerve bundles to the fibre bundles of the optic nerve or Necturus, on which much work on the ionic properties of neuroglia has been done (see Kuffler & Nicholls, 1966) is certainly suggestive that the anatomical relationship in the two sites may have similar ionic consequences.

Finally, it should be noted that the somata of the cells of the glomerular layer may lie very close together or even with large areas of plasma membrane directly apposed; in addition, the three major types are often situated in close proximity to each other (Fig. 7). These small cells may be difficult to record from singly with present techniques, and potentials recorded in the periglomerular region are likely to be derived from several cells of different types, operating variously in a single locality. In view of this and the extensive interconnections of the various processes detailed here and in the following Chapters (4 & 5), electro-physiological studies should be interpreted with caution; it is hoped that the findings presented here may allow certain possibilities to be excluded and the remaining interpretations to be tested.

CHAPTER 4

THE NEUROPIL OF THE GLOMERULI OF THE OLFACTORY BULB

"Each synapse is an apparatus for co-ordination; it introduces 'a common path'."

Sir Charles Sherrington.

INTRODUCTION

Electron microscopy is the only technique at present available that is capable of providing detailed and definitive information about the synaptic connections established in any nucleus; it is limited, however, by an essentially two-dimensional approach and therefore large numbers of profiles may be seen, comprising the neuropil of the nucleus, which are not in visible continuity with their cells of origin. Even long series of serial sections, as used extensively in this study, cannot in most cases trace processes this far. As a result, criteria must be established by which individual profiles may be recognised, regardless of the plane of sectioning, as belonging to a given group and, in turn, to a particular cell type; such criteria include overall morphology, cytoplasmic organelles and synaptic relationships. In the first instance, groups of profiles in the neuropil are characterised; correlative studies with the Golgi method at light microscopic level and studies on the cell bodies with the processes that may be seen in continuity with them using the electron microscope, allow certain deductions to be made about which group of profiles in the neuropil correspond to which cell type. These must in turn be rigorously examined and other possibilities carefully excluded, before they may gain acceptance, and have their properties as a group studied with a view to drawing up a scheme for each whole cell. Certain unusual features of the olfactory bulb have aided this analysis and the deductions have been repeatedly confirmed,

both with normal electron microscopy and with combined Golgi-E.M. studies on single cells (Chapter 11). In the present chapter and that following the principal components of the neuropil of the glomerulus and periglomerular region respectively are described in terms of the criteria established in the previous chapter on the cell types of this layer; although some profiles occur in both regions, they are described with the region in which they predominate.

RESULTS

Subdivisions & Components

The neuropil of the glomerular layer of the olfactory bulb may be subdivided into two main zones - the glomeruli themselves, and the periglomerular region or stratum granulosum externum (Andres, 1965). These regions, although they interdigitate at their mutual boundaries around the edges of the glomeruli, forming an intermediate zone, are quite different in their synaptic arrangements. The primary distinguishing features are the presence of the dark olfactory nerve terminals within the glomeruli and the presence of neuronal somata in the periglomerular region; the limits between the two zones of neuropil are never as distinct under the electron microscope as they are under the light microscope, since the broadly spherical appearance of the glomeruli in the latter is determined by the presence of the cell somata, while the outline of the neuropil surrounding them is more

irregular at the higher magnification. In the intermediate zone, the large dendrites, as they penetrate into the glomeruli, seem to retain, for short distances, some of the synaptic arrangements as well as the 'grain' characteristic of the periglomerular neuropil, so that these rather conical protrusions are, in turn, surrounded by typical glomerular neuropil. Where it is possible to do so, these components of the intermediate zone will be considered as either glomerular or periglomerular, according to their characteristics; however, in order to describe the distribution of processes, particularly axon terminals, this intermediate zone will be considered as a separate entity.

The neuronal processes of the olfactory bulb are unusual, although probably not unique, in that many of the dendrites as well as all axon terminals contain large numbers of agranular vesicles and both types of process can be either pre- or post-synaptic. Thus while dendrites of medium to large calibre are distinctively dendritic in their morphology, vesicles being almost entirely restricted to the immediately presynaptic regions, the finer dendritic branches and particularly the dendritic appendages are more difficult to identify with certainty; frequent ribosomal rosettes and cisternae of endoplasmic reticulum in profiles are useful, though probably not absolute criteria for dendrites (see Chapter 3). The irregular outlines and the sparsity or absence of neurotubules in the finer dendrites, as well as the presence of vesicles over a large part of the profiles, make it essential to define the distribution of dendrites and axons

with light microscopic techniques as far as it is possible; with the electron microscope, it is important to investigate serially the continuities of equivocal profiles until their identity can be established definitively. For the former we have used our own material to supplement the detailed and accurate evidence of Cajal (1890; 1911) and Blanes (1898) on this point. It is extremely fortunate that, on account of their disagreement with Golgi (1875) as to the nature of dendritic (protoplasmic) expansions, these authors devoted considerable study to the question of axon and collateral distribution to the glomeruli and the interglomerular (periglomerular) spaces. Golgi considered that protoplasmic expansions (such as those of the mitral and tufted cells) served solely nutritive function and that nerve impulses from the afferent olfactory axons pass by means of a 'nerve net' to recurrent collaterals of tufted cells and thence via their main axons into the olfactory tract. The observations of both Golgi and the Madrid group showed that the olfactory axons terminate within but do not transgress the limits of the glomeruli, but the latter authors could find very few other axons indeed, of either tufted or periglomerular cells, terminating within or passing through the glomeruli; they also pointed out that those of the mitral cells do not even reach the glomerular layer. The tufted and periglomerular cell axons typically pass just deep to the glomerular layer in a thin, more or less well-defined layer and terminate between the glomeruli in the periglomerular region, or at the very edges of the glomeruli (Blanes, 1898). Again, both groups were agreed that the dendritic or

protoplasmic expansions of the mitral, tufted and periglomerular cells all possess large glomerular arborisations; the close, and numerically large, contact between these processes and the olfactory axons led the Madrid histologists to consider that this was the site of transmission of the olfactory impulses. Our own observations, both of Golgi-impregnated and electron microscopic material entirely bear out the observations of Cajal and Blanes on the distribution of axonal and dendritic processes, although the axons of periglomerular and short-axon cells may be found just within the edges of the glomeruli proper, as defined by the presence of the olfactory nerve terminals. This, together with the evidence obtained by studies of serial sections with the electron microscope, shows that the great majority of vesicle-filled profiles that lie within the glomeruli themselves, other than the olfactory nerve terminals, are genuine dendrites or the dendritic appendages of mitral, tufted and periglomerular cells.

It should be emphasised here that the examination of many long series of serial sections (100-200 sections) provided information of enormous value in elucidating the organisation of the neuropil of the glomerular layer, which could otherwise have been deduced only from rare fortunate planes of section (e.g. Fig. 40) and other indirect procedures. Not only the identification of processes, but detailed knowledge of the relationships between synaptic contacts and dendritic location could be relatively easily discovered by this apparently laborious, but extremely rewarding mode of study. Because it is difficult to do justice to serial examination by illustration, much

of the detailed information gained by this technique is described in the text, the illustrations often providing part of a serial set. Where possible, other contacts established outside the plane of section shown will be detailed in the legends.

In the previous Chapter, vesicle and synaptic thickening types associated with each type of cell were defined as follows: tufted cells contain spherical vesicles and synapse with asymmetrical thickenings; periglomerular cells contain large flattened vesicles and synapse with symmetrical thickenings; and short-axon cell axons contain small flattened vesicles, and their synaptic thickenings are also of the symmetrical type. In addition, the glomerular layer has the considerable advantage that the olfactory nerves, in their pre-terminal and terminal parts, are characteristically electron-dense (Andres, 1965) and may easily be distinguished in normal material, although their identity naturally had to be confirmed in an experimental manner by removing the olfactory mucosa (Chapter 7).

Thus, from a detailed knowledge of the light microscopic distribution of the cells and their processes, and on the basis of various electron microscopic criteria, a very large number of isolated profiles may be identified as either axonal or dendritic, and also as belonging to a particular cell type. Further confirmation of the various cellular identities of profiles in the neuropil has been achieved by the use of combined Golgi-E.M. material, in which a single stained cell may be examined under both light and electron microscopes, and its morphology and synaptic relationships established and confirmed

(Chapter 11); such direct observations are in complete agreement with the results obtained in this study by more indirect procedures. Certain distinctions may not be definitively assessed in normal material however: the mitral and tufted cell dendrites, having undergone their primary branching within the glomeruli, are indistinguishable, since they have the same electron microscopic morphology and the same vesicle and synaptic types; they appear to have no essential differences in structural organisation and synaptic arrangements and seem therefore to fulfil a very similar function at glomerular level, as originally proposed by Cajal, although the extent of the arborisation over the glomerular volume does differ (Chapter 3).

Synaptic Patterns

A further problem which has been encountered in this study of the glomeruli has been largely a question of definition and terminology. Most of the previous studies on the reciprocal synapses of the olfactory bulb (Hirata, 1964; Andres, 1965; Rall et al, 1966; Price and Powell, 1970a,b,d) have dealt with those occurring in the external plexiform layer, between the gemmules of granule cells and the primary and secondary dendrites of the mitral and deep tufted cells. Reese and Brightman (1965) and Hinds (1970) have also noted their occurrence in the glomeruli. Our studies on the glomerular layer have shown that both the gemmules and the dendritic shafts of periglomerular cells may participate in reciprocal synapses with the dendrites of mitral

and tufted cells; the inclusion of the dendritic shafts in such arrangements, as well as the occasionally uncertain distinction between dendrites and gemmules within the glomeruli, raises the question of how close two synapses of opposite polarity (between the same two cells) have to be, in order to be considered as reciprocal. Although the point may appear pedantic, it may have considerable physiological implications to determine whether or not every dendro-dendritic synapse has an immediately adjacent return synapse; the dendritic localisation of synaptic arrangements appears to have considerable importance, particularly in view of the observations and hypothesis of Diamond, Gray and Yasargil (1970). Clearly, a reciprocal arrangement in relation to a single gemmule or short segment of dendrite may be termed a reciprocal synapse, but when the two components of the 'synapse' are widely separated, for instance one on the dendritic shaft and the other on a gemmule of a single cell, the issue is less clear. A 'reciprocal arrangement' may be said to occur, but, with some considerable distance along the dendritic membrane between the two components, it is difficult to justify the use of the singular term 'reciprocal synapse' to this type of organisation. Although it is not possible to define the exact proportions of 'reciprocal synapses' and 'reciprocal arrangements' in the glomerular layer, it is certain from data obtained by serial sections that both types exist, though the former are much more common; that this is so might indicate that the reciprocal arrangement, occurring at some distance, does fulfil a similar or even the same physiological purpose

as the reciprocal synapse. However, it is unwise to state that all dendro-dendritic synapses within the glomeruli are reciprocal, although it can be strongly suggested that all pairs of dendrites entering into dendro-dendritic synapses are reciprocally related to each other.

A similar and related problem arises over the term 'serial synapse', especially when used in the context of dendro-dendritic contacts (cf. Hinds, 1970). Single or even serial sections may reveal contacts of the type A $\rightarrow$ B $\rightarrow$ C, but to regard this group as a 'unit' is dangerous, since this may only be a part of a unit (if such a species exists), the rest being missed by the sectioning (for instance A $\rightarrow$ B $\rightleftarrows$ C $\leftarrow$ A), or it may be a chance relationship abstracted from the context of the whole dendritic tree. On the other hand, it is equally dangerous to assert, having demonstrated the occurrence of the second arrangement, that the observation of an A $\rightarrow$ B $\rightarrow$ C relationship necessarily indicates the presence of the other components. As in the problem of reciprocality, the issue at stake becomes: How wide a region must be examined to determine the anatomical or physiological unit? Clearly no simple answer exists at the present time, but the problem cannot be ignored on that account. With the proviso that these may represent artefacts of the approach used, 'serial synapses' and other synaptic patterns will be described as such only when they have been observed repeatedly and confirmed by serial examination to have the stated arrangement. Synapses are only considered to be part of a single pattern if they are directly related to an appendage or lie on the dendritic shaft within about 500nm of

the base of the appendage, measured along the membrane; most cases examined occurred well within this rather arbitrary limit. Some of these patterns of organisation are certainly common and may be considered as characteristic, although variable; their occurrence and frequency varies considerably according to exact situation in the glomerulus or periglomerular region, and thus on the various parts of the dendrites concerned.

The Glomeruli

The shape and size of the glomeruli may vary considerably but they are generally spherical, oval or pearshaped and have mean internal diameters ranging from 50-120μm. With the electron microscope, the glomerulus is characterised by the dense mass of olfactory nerve terminals and preterminals which outline the pale dendrites of mitral, tufted and periglomerular cells; the latter may be single or grouped into islets of varying size, surrounded by the olfactory nerve terminals and consisting of several different dendritic profiles. Apart from a few glial cells, no cell somata lie in the glomeruli; very occasionally a neuron may appear to lie within the glomerular limits, but serial examination regularly shows it to be protruding from the edge of the periglomerular region 'behind' the plane of section. Although it is far from constant for complete, a glial wrapping, often composed of several thin lamellae, can be seen partially enclosing the glomerulus and separating it off from the periglomerular region; it is absent around the entry of the olfactory nerves and the main den-

drites, as well as at other parts of the glomerular-periglomerular boundary.

Olfactory nerves

The olfactory nerve fibres form a distinct layer around the olfactory bulb and have been well described (Gasser, 1956; de Lorenzo, 1957; Andres, 1965; Berger, 1969). The mode of entry of the fibre bundles into the glomeruli varies considerably in site, direction and distribution. Although most of the bundles enter from the superficial aspect of the glomeruli where there are few cell bodies other than those of glia, some bundles may run in between the glomeruli, up to about midway across the glomerular layer, before penetrating into the glomerular neuropil and spreading out in their preterminal portions. The bundles may cross each other in various directions as they enter the glomeruli, but once they have crossed the boundary they rarely remain as distinct bundles (Fig. 26). The preterminal and terminal parts of the olfactory nerves are characteristically electron-dense, although the axons in the olfactory nerve layer are not distinguished in this way, the darkening of fibres occurring approximately as they enter the glomeruli and begin to lose their glial sheathing (Fig. 27). These glial processes which surround the fibre bundles in a very characteristic manner in the olfactory nerve layer (Gasser, 1956; de Lorenzo, 1957; Andres, 1965; Berger, 1969), are shed at the glomerular limits, at which point the glial relationships of the glomeruli begin, relating rather to portions of dendrite than to the

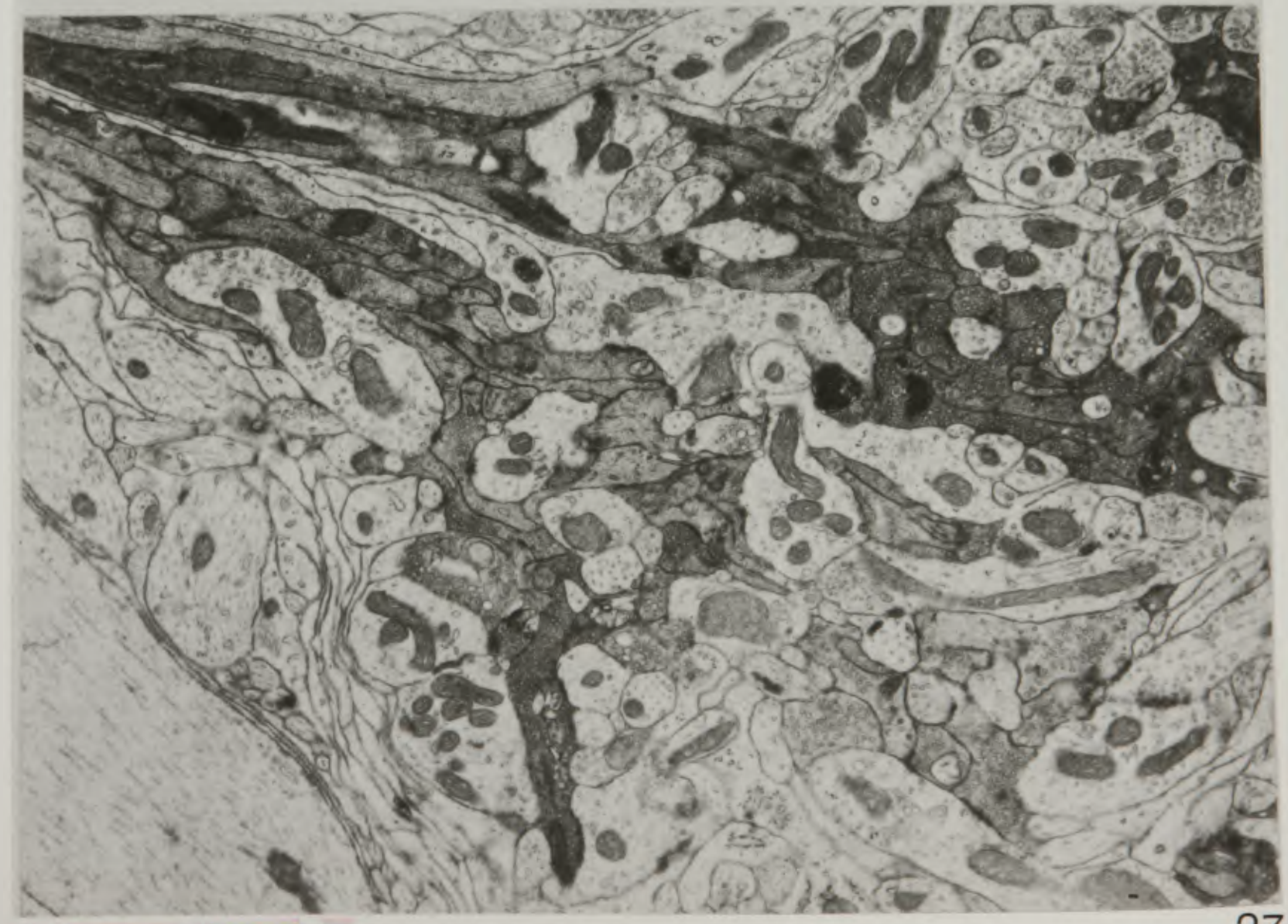
Fig. 26 Photomicrograph of several bundles of olfactory nerve fibres entering a glomerulus, impregnated with the Golgi-Kopsch method; note the clear delimitation of the glomerular boundaries by these fibres and their characteristically tortuous courses and beaded outlines.

X1,500

Fig. 27 Low magnification electron micrograph showing the initial expansion of a bundle of olfactory nerve fibres, intermingling with and synapsing onto the pale terminal dendritic profiles of the glomerulus; some olfactory terminals are showing spontaneous degenerative changes.

X12,000

26



27

afferent axons.

As can be seen from the drawings of Golgi-impregnated material of Cajal (1911), van Gehuchten & Martin (1891), and Valverde (1965), many of the olfactory fibres run for considerable distances within the glomeruli, giving off short branches, and showing many beadings or varicosities (Fig. 26); others appear to terminate more sharply with fewer branches. All of these fibres, after their relatively straight and regular courses in the olfactory nerve layer, become tortuous and extremely irregular on entering the glomeruli (Fig. 27). Sometimes they splay out and give the appearance of a bunch of flowers. However with the electron microscope it is difficult to see the entire ramification of a single bundle as its own tortuous nature may confuse the pattern of distribution within the glomerulus. As a general rule it may be said that once the fibre bundles have lost their integrity within the glomeruli, it is probably the organisation of the dendritic branches, particularly those of the mitral and tufted cells that determine the course and gross appearance of the olfactory nerve fibres in their preterminal and terminal parts, which appear to fill in the interstices formed by these larger structures.

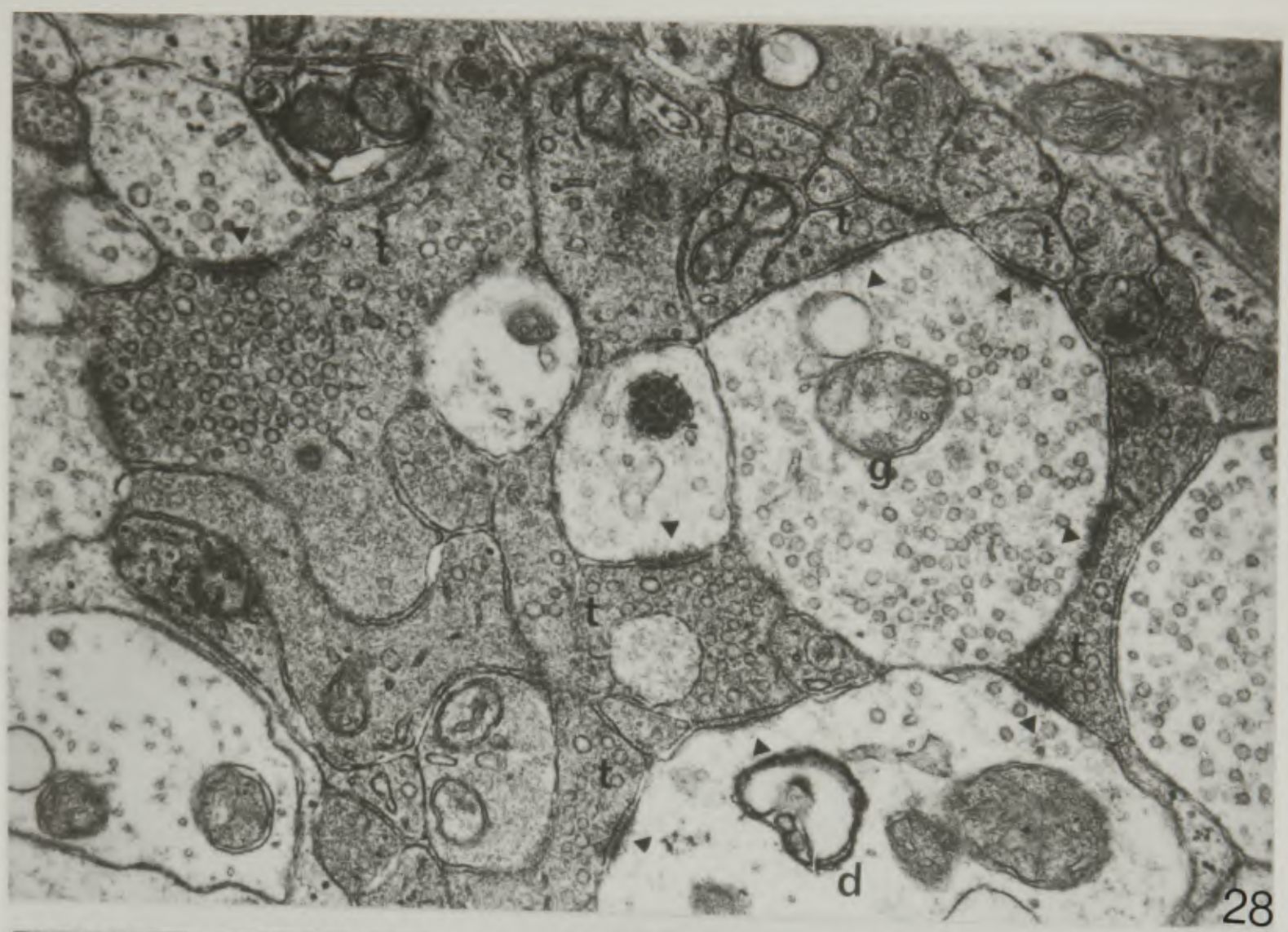
The preterminal and terminal parts of the olfactory nerves are extremely irregular (Figs. 27,28,31), and may become very attenuated in places. 'En passant' synaptic boutons, showing a few neurotubules are common (Fig. 30) as would be expected from the evidence of Golgi-impregnated material; they may be found to be separated from further

Fig. 28 Group of olfactory nerve terminals making asymmetrical type synaptic contacts with pale dendritic profiles, some of which contain vesicles; note the unusual dark granular cytoplasm of the axon terminals and their spherical vesicles.

X33,800

Fig. 29 Synaptic pattern of the glomerulus involving the terminal part of a tufted or mitral cell dendritic tree, two gemmules of periglomerular cells (containing many large flattened vesicles) and several olfactory nerve terminals. The central dendritic profile is running into the plane of section and gives off two spine-like branchlets, each of which receives an asymmetrical synapse from an olfactory nerve terminal and a symmetrical one from a gemmule; one of the gemmules receives similar synaptic contacts from the same olfactory nerve terminals at the sites indicated (arrow) determined by serial sections. The dendritic shaft makes an asymmetrical synaptic contact with spherical vesicles onto one of the gemmules and onto the third gemmule, several sections further on.

X50,000



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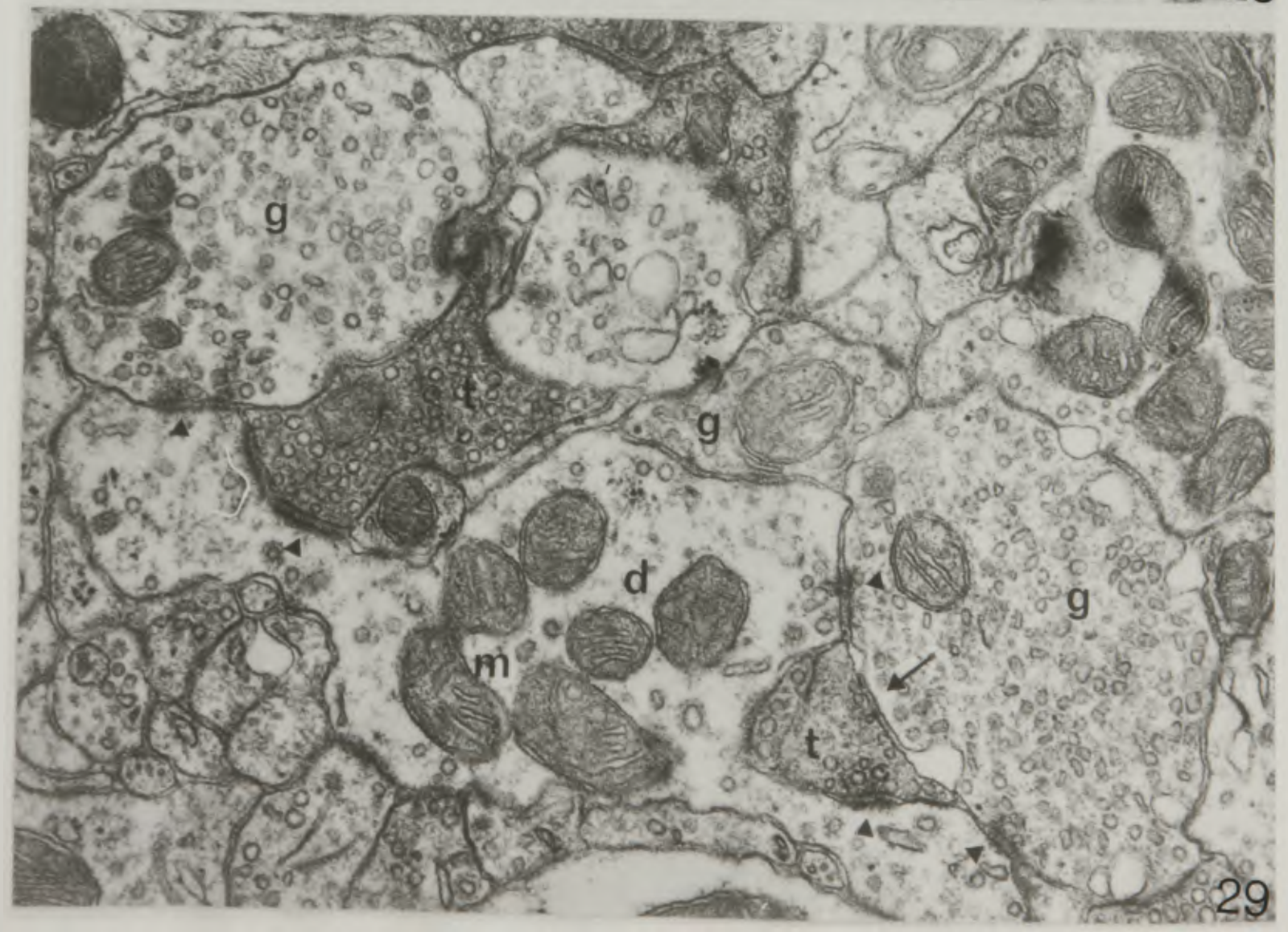

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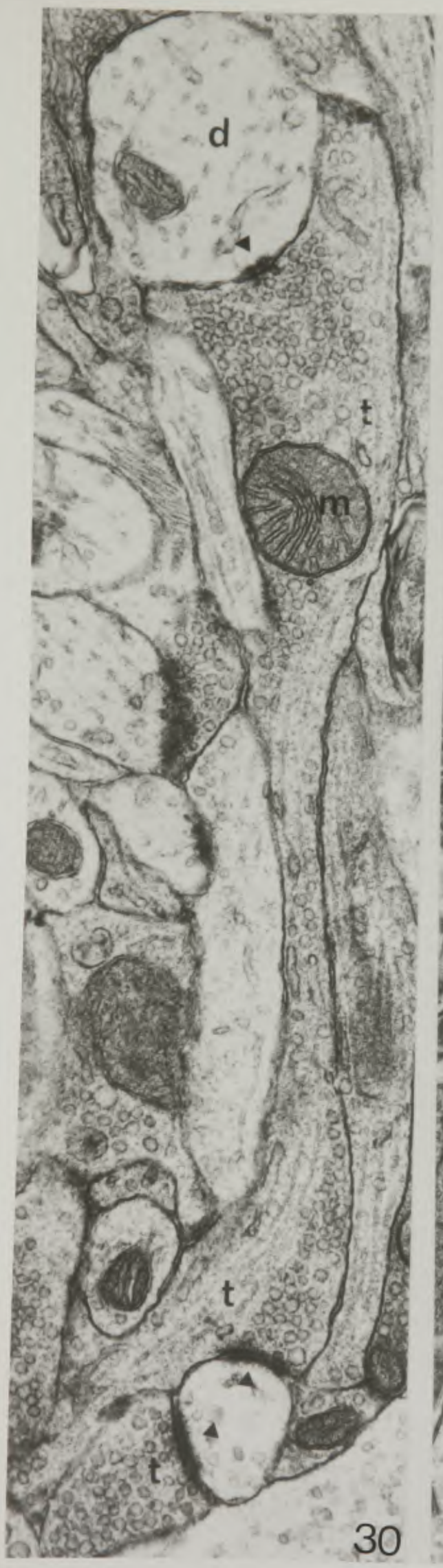
Fig. 30 Olfactory nerve fibre with two en passant synaptic boutons that make synaptic contact with dendritic profiles; note the presynaptic aggregation of vesicles and the length of the linking preterminal axon.

X36,400

Fig. 31 Varicose dendrite of tufted or mitral cell in the glomerulus, receiving many synaptic contacts from olfactory nerve terminals and making an asymmetrical synaptic contact with spherical vesicles onto a gemmule of a periglomerular cell.

X20,600



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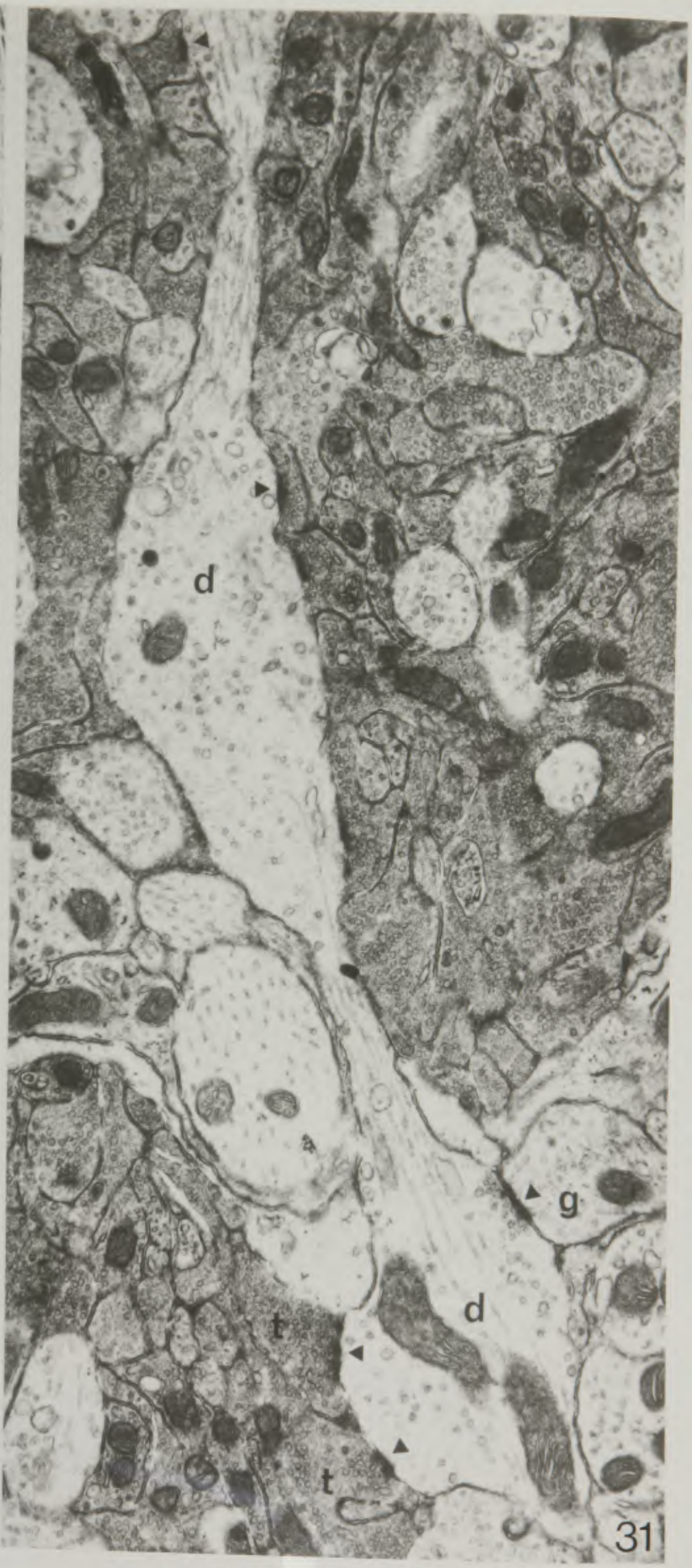

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en passant or terminal boutons by considerable lengths of axon. The most notable and unusual feature of the olfactory fibres is their dark, rather granular cytoplasm and although the actual density may vary, not only from brain to brain but also from fibre to fibre, it is a constant and distinguishing feature of them (Figs. 27-31,33,34,39). It may be interesting to discover the biochemical nature of this dark, osmiophilic material which has only been found in the olfactory and vomeronasal nerve terminals, and which may give some clue to the functional properties of these nerves. The terminal and en passant boutons are packed with spherical vesicles in the region of their synaptic thickenings and which often lie very close to each other within the dense matrix (Figs. 28,30). Small mitochondria and cisternae of various shapes and sizes are present, as well as a few coated, or alveolate, vesicles, first noted by Andres (1965). The groups of terminals lie packed together, only separated by a thin gap of extracellular space, and their peculiarly irregular outlines may give rise to the appearance of whorls and other unusual formations (Figs. 28,31). The olfactory terminals make typical synaptic contacts of the asymmetrical type, showing presynaptic dense projections, extracellular material and a dense post-synaptic membrane thickening extending as a granular web into the cytoplasm of the post-synaptic dendrite (Figs. 28-31,33,34,39). The olfactory nerves terminate predominantly upon the fine dendritic branches of mitral, tufted and periglomerular cells, but a few may be found on most parts of the glomerular arborisations of these dendrites, regardless of size or position; they may even

Fig. 32 Periglomerular cell dendrite in the glomerulus giving rise to a gemmule, showing symmetrical synaptic contact onto a mitral or tufted cell dendrite; the vesicles in this brain were not markedly flattened.

X29,000

Fig. 33 Periglomerular cell dendrite in glomerulus giving rise to a gemmule showing a thin pedicle and a large number of flattened vesicles; the gemmule makes a symmetrical synapse onto a tufted or mitral cell dendrite, which also receives an olfactory terminal synapse. There is evidence of two olfactory terminal synapses, onto the gemmule and its pedicle, that take place on other sections of the series.

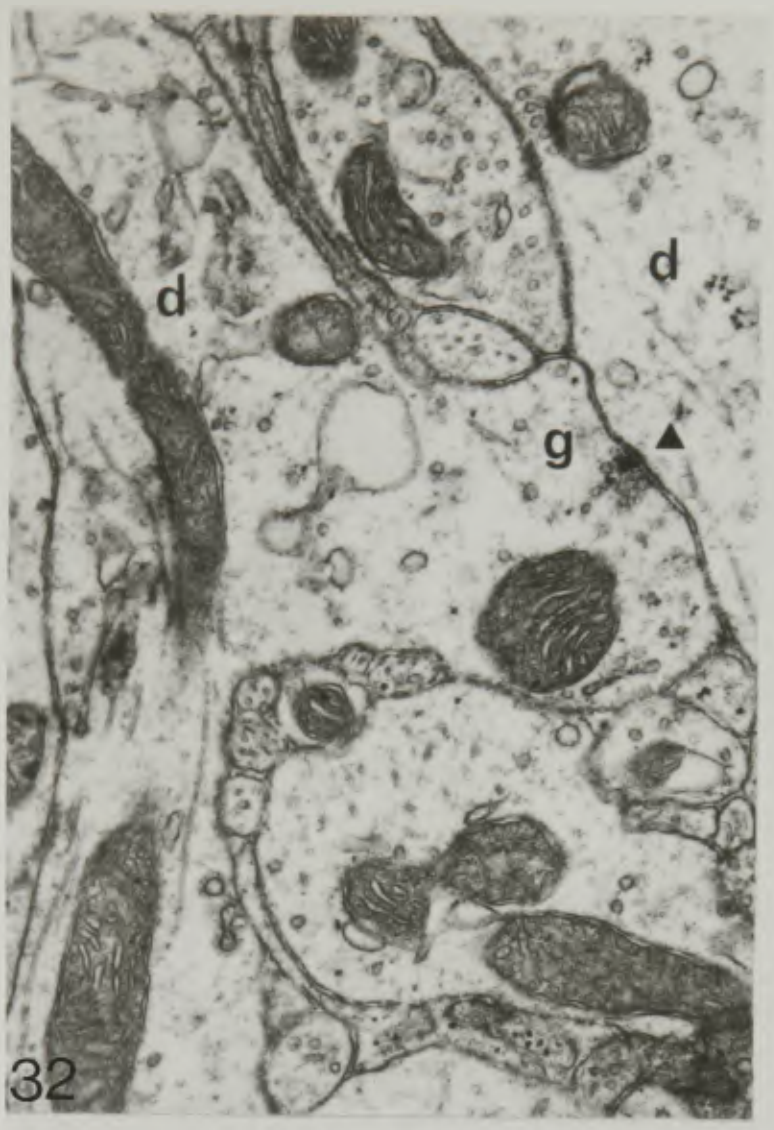
X32,000

Fig. 34 Sessile gemmule arising from a thin periglomerular cell dendrite, receiving an olfactory nerve synapse and making a symmetrical contact onto a tufted or mitral cell dendrite profile. Note ribosomal rosettes and large flattened vesicles; the same olfactory terminal synapses onto the tufted/mitral dendrite in another section.

X36,000

Fig. 35 Gemmule arising from the large shaft of a periglomerular cell stem dendrite in the intermediate zone and receiving asymmetrical synaptic contact from a non-olfactory terminal. Note the large number of large flattened type vesicles.

X45,000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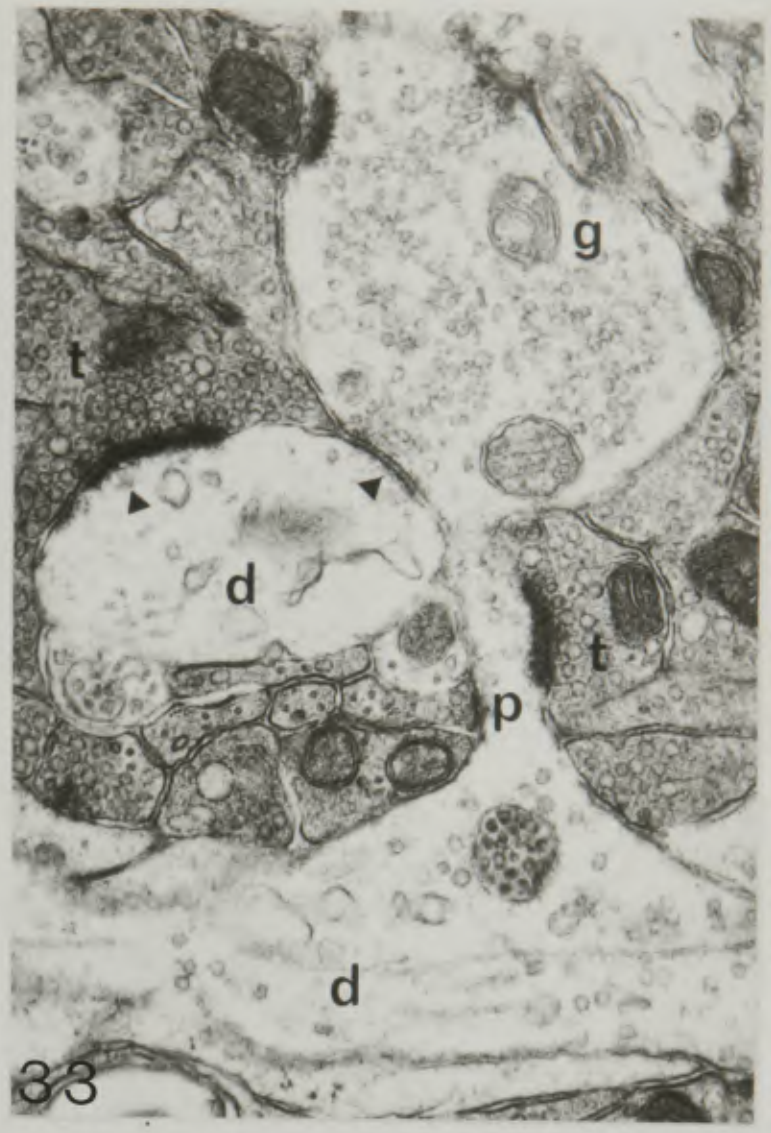
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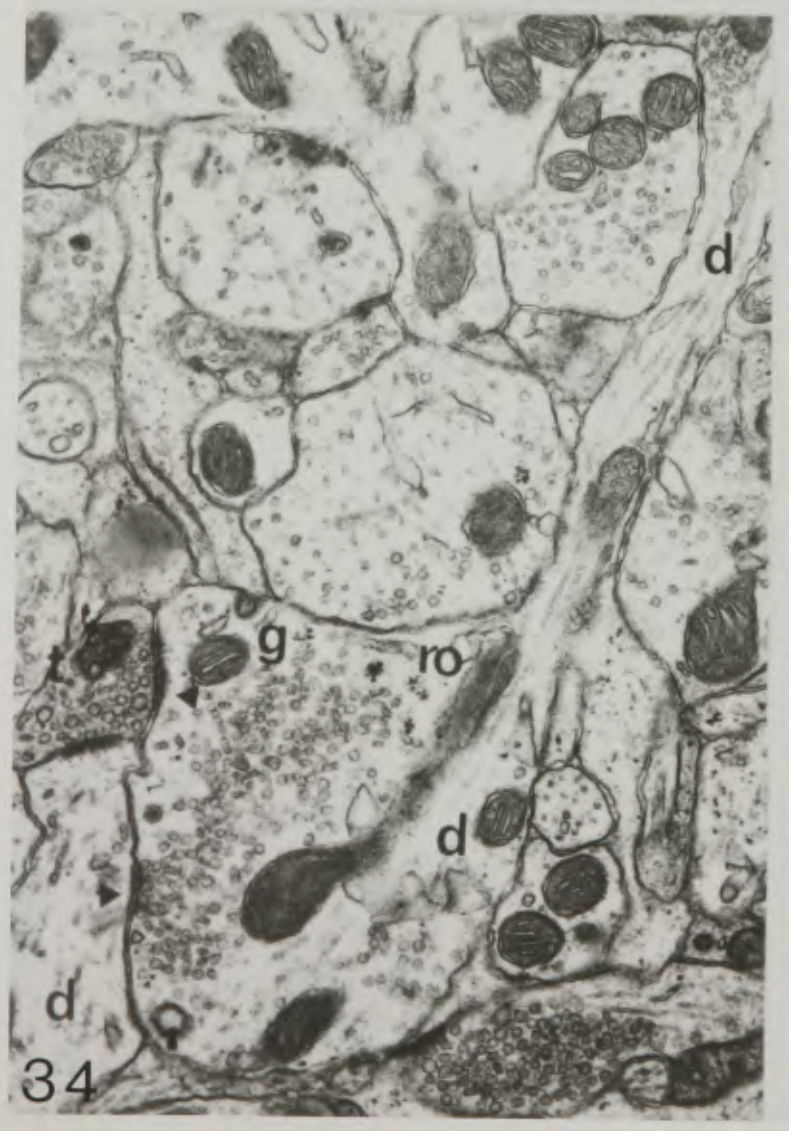
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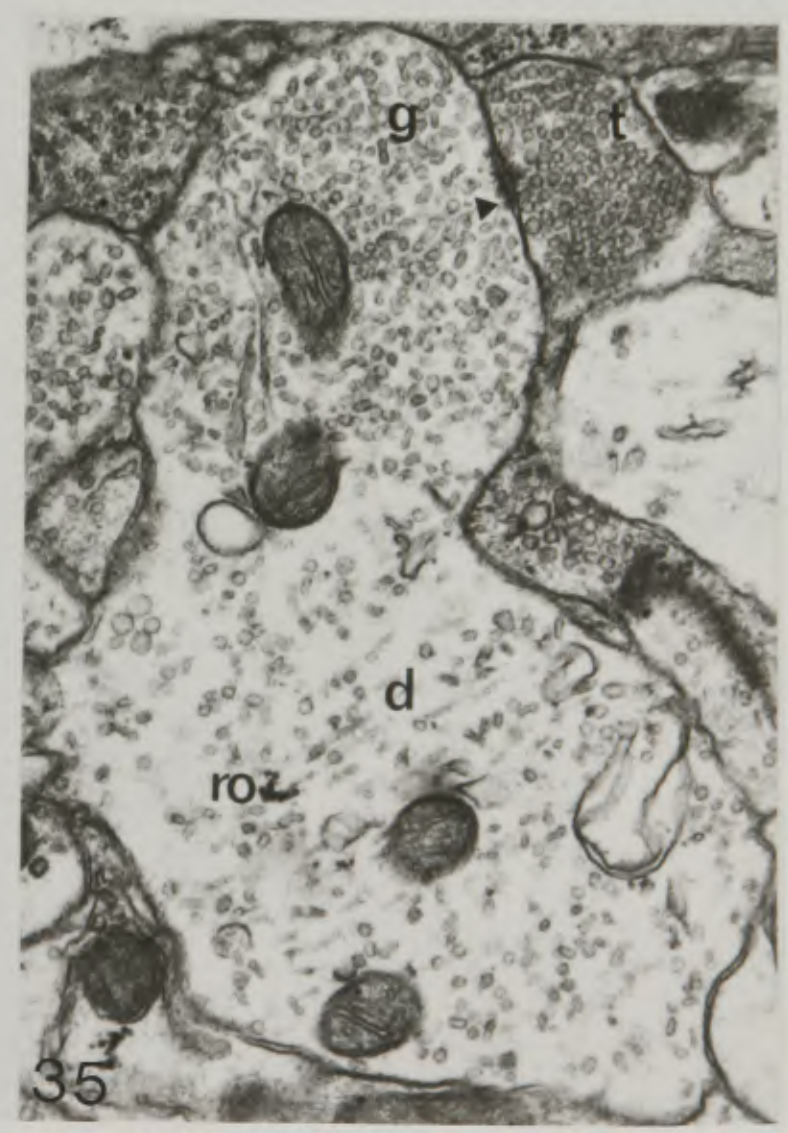
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synapse on to a cell soma of a tufted or periglomerular cell, if it extends into the glomerulus. A single olfactory nerve fibre makes synapses on to several dendrites which may belong to any of the cell types distributing to the glomeruli (Figs. 28-30). The olfactory axons do not, however, receive any synaptic contacts from axons or dendrites at any point in their bulbar course; desmosomes may, however, be found between pairs of fibres, preterminals or terminals (Berger, 1969).

Mitral and tufted cell dendrites

The dendrites of mitral and tufted cells, which arborise within the glomeruli, show many similarities in light microscopic morphology, although they differ in their distribution and amount of branching (Chapter 3). With the electron microscope there is little to distinguish the glomerular dendrites of the two cell types distal to their primary intraglomerular branching; proximal to this, size and position of entry into the glomeruli serve as criteria to distinguish partially the external tufted from the mitral and deep tufted cells. Mitral and deep tufted cell dendrites enter from the deep half of the glomerular outline, sometimes to one side, having passed through the periglomerular region, while external tufted cell dendrites may enter the glomeruli at any point, generally close to the position of their cell body. Examination of serial sections has clearly borne out the original interpretation of Cajal that the two cell types form a single group, in terms of morphology and types of synaptic connections; this applies especially to the glomeruli, in which they both receive and make contact

With the same processes in the same manner. For this reason and on account of the difficulty of distinguishing between them, they will be considered together.

The pale dendrites of the mitral and tufted cells may be found in the glomerular neuropil cut at all angles and at all stages of branching. The largest profiles tend to lie in the deeper parts of the glomeruli, probably representing the first glomerular branches of the mitral and deep tufted cells. One of the most striking features of all these dendrites is the regularity of their outline, up to the finest branches, so that they appear roughly spherical when cut transversely and oval when cut obliquely. Longitudinally cut, the mitral and tufted cell dendrites may appear broadly cylindrical with even outlines if they are of medium or large calibre (2-5μm), or varicose (Fig. 31) if small (0.5-1μm). As a general rule, the dendrites become increasingly varicose as they diminish in size, but the extent of the varicosity and the proportion of dendrite showing it, varies considerably from one to another; such an observation is borne out by the light microscopic evidence (Chapter 3). The large straight portions of the dendrites of mitral or tufted cells have a characteristically regular array of neurotubules in a pale cytoplasm containing relatively few mitochondria and other organelles; neurofilaments are occasionally found in these dendrites, but are usually evident only in the smaller dendrites. The neurotubules and the occasional neurofilaments in varicose dendrites show a very characteristic funnelling into the thin segments, either side of which they

fan out to become very sparsely arranged in the varicosities. The terminal portions of the dendrites may be long, and are often very thin processes (0.2µm), typically surrounded entirely by olfactory nerve terminals and receiving synapses from them; these parts of the dendrites are often strikingly electron-dense in comparison with their parent processes, sometimes achieving a similar order of density to that of the olfactory terminals themselves. The neurotubules of these fine portions are very closely packed, rather similar in appearance to the thin parts of varicose dendrites but not showing any tendency to dilate distally. On other occasions the mitral and tufted cell dendrites end in rather irregular processes (Fig. 29) that resemble spines in their slightly flocculent cytoplasm and lack of neurotubules, but do not show any sign of spine apparatus or cisternae nor the outline typical of spines; neurofilaments may also be found entering these structures from the parent process. Rarely these small appendages are seen to be gemmules filled with spherical vesicles and making synaptic contact on to periglomerular cell processes. Such spine-like terminal parts probably represent the final branchlets, while the long thin portions containing neurotubules are the termination of the most distal dendritic stems - a view consistent with the Golgi-stained appearance. Mitochondria and cisternae of agranular endoplasmic reticulum are found throughout the dendritic trees of the mitral and tufted cells and both are most evident in the finer dendritic branches (Fig. 29); ribosomes, free or as rosettes, are also common. A distinctive feature of dendrites of both cell types is the

presence of spherical agranular vesicles, usually found as small clusters, but occasionally as larger groups, and most often related to a synaptic thickening orientated away from the dendrite (Figs. 29,31,36-38,40,45). All these components, notably the mitochondria and vesicles, may become concentrated into unusually large groups at the branch points of the smaller dendrites.

The membrane thickenings associated with the mitral and tufted cell dendritic synapses are regularly asymmetrical in type (Figs. 29, 31,36-38,45) and show an aggregation of the spherical vesicles pre-synaptically (see Price & Powell, 1970d), although no other vesicles may be evident in the rest of the dendritic profile. All parts of the dendritic trees of these cells may show such synaptic specialisations except the fine terminal portions and the thin parts of the varicose dendritic segments. The post-synaptic structures may be identified in most cases as being the dendrites, spines or gemmules of periglomerular cells by the presence of large flattened vesicles (Figs. 29,31,36,37,45) and from the study of continuity in serial sections (Fig. 38); on the rare occasions when, in single sections, no characteristics allow the post-synaptic profile to be distinguished, it may be reasonably assumed that these are the dendritic processes of the periglomerular cells. As discussed previously, these synapses are commonly related to a nearby synapse in the reverse direction from the same process, forming the relationship known as the reciprocal synapse (Figs. 29,36,37,45); the return synapses are invariably of the symmetrical type, with large flattened vesicles aggregated pre-

Fig. 36 Reciprocal synapse in the glomerulus between tufted or mitral cell dendrite, showing spherical vesicles and asymmetrical thickening, and a periglomerular cell gemmule showing large flattened vesicles and a symmetrical thickening, confirmed in serial sections.

X47,500

Fig. 37 Reciprocal synapse between periglomerular cell dendrite and tufted or mitral cell dendrite, showing the same polarities as Fig. 36; another synapse from the tufted/mitral dendrite, onto a gemmule, is also seen. Note particularly the clear distinction between types of thickening.

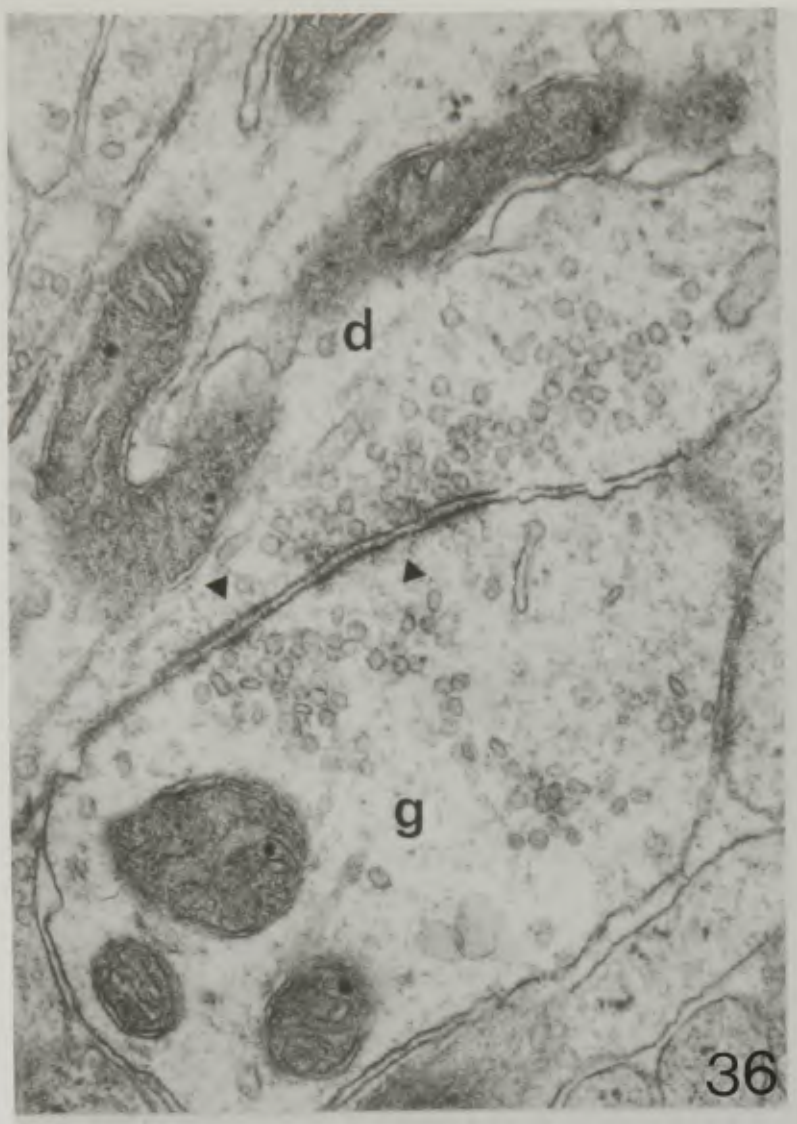
X45,600

Fig. 38 Synaptic pattern showing asymmetrical synapse from tufted or mitral cell dendrite onto a periglomerular cell spine which also receives a symmetrical synapse from a small flattened vesicle-containing axon terminal. Note that these vesicles are significantly smaller than those in the dendrite.

X45,600

Fig. 39 Synaptic pattern in glomerulus showing a tufted or mitral cell dendrite receiving an axo-dendritic contact from an olfactory terminal (asymmetrical) and a symmetrical contact from a periglomerular cell dendrite (note ribosomal rosettes), containing large flattened vesicles. This periglomerular cell dendrite receives symmetrical synaptic contact from an axon terminal containing large flattened vesicles (i.e. a periglomerular cell terminal).

X52,000



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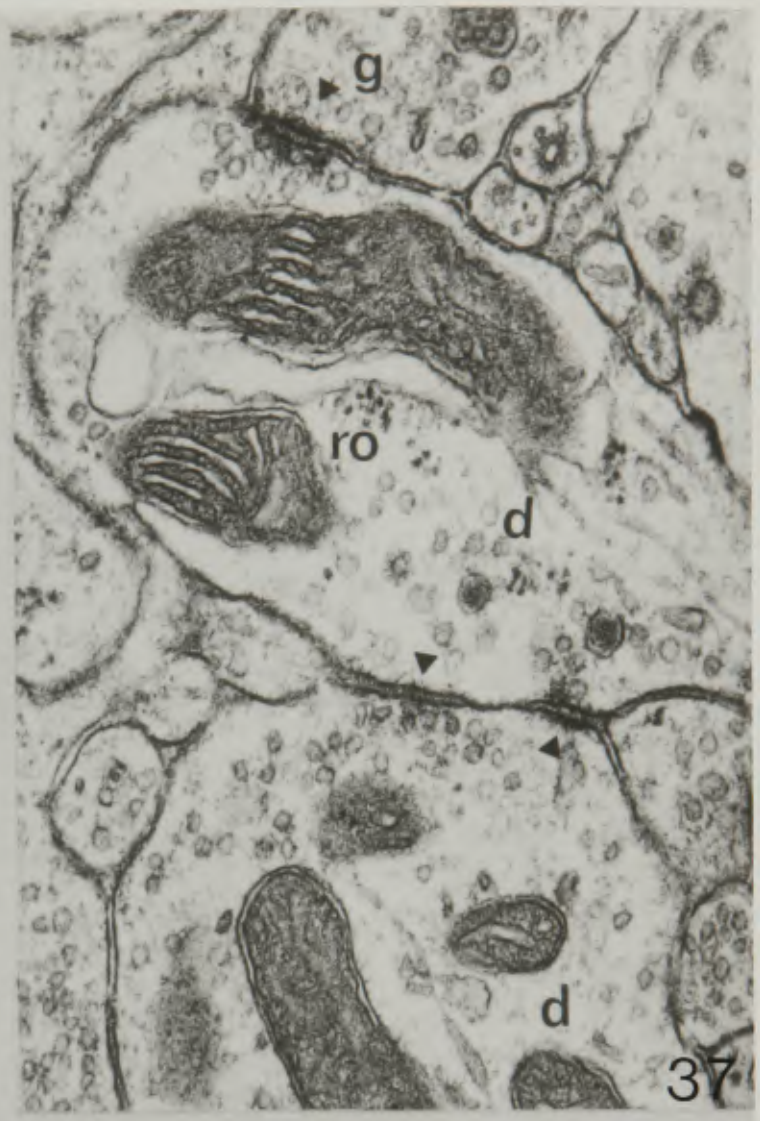

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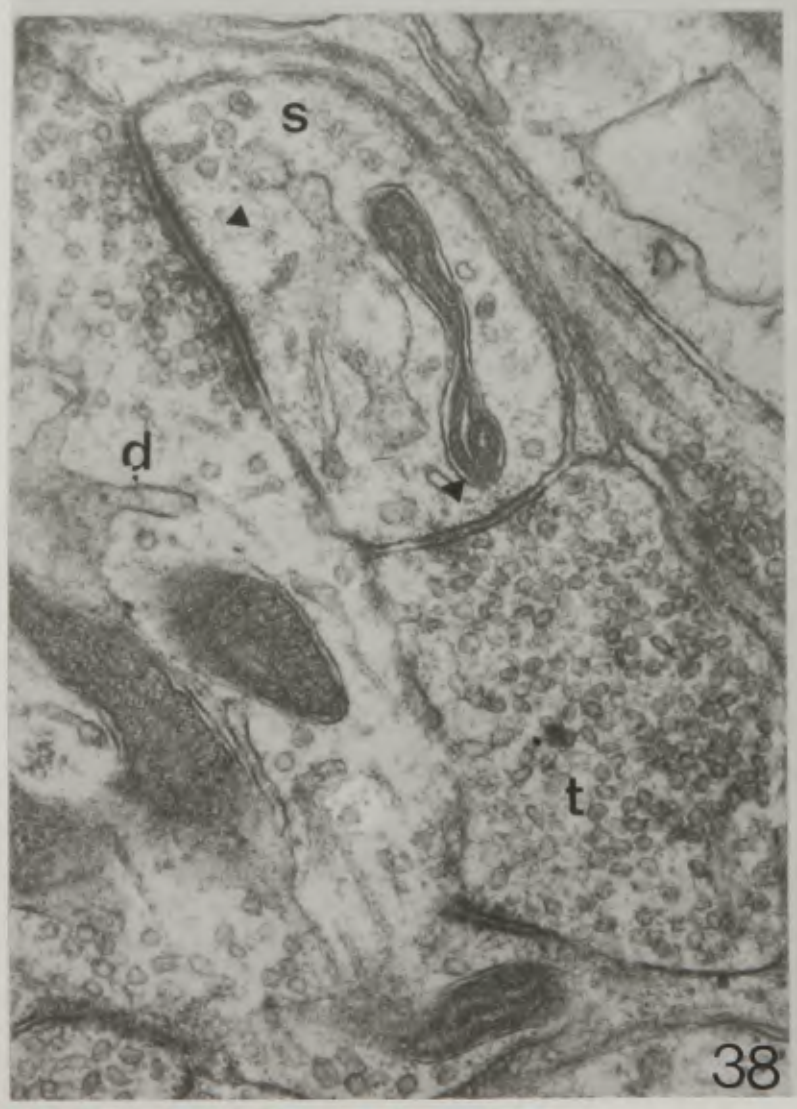

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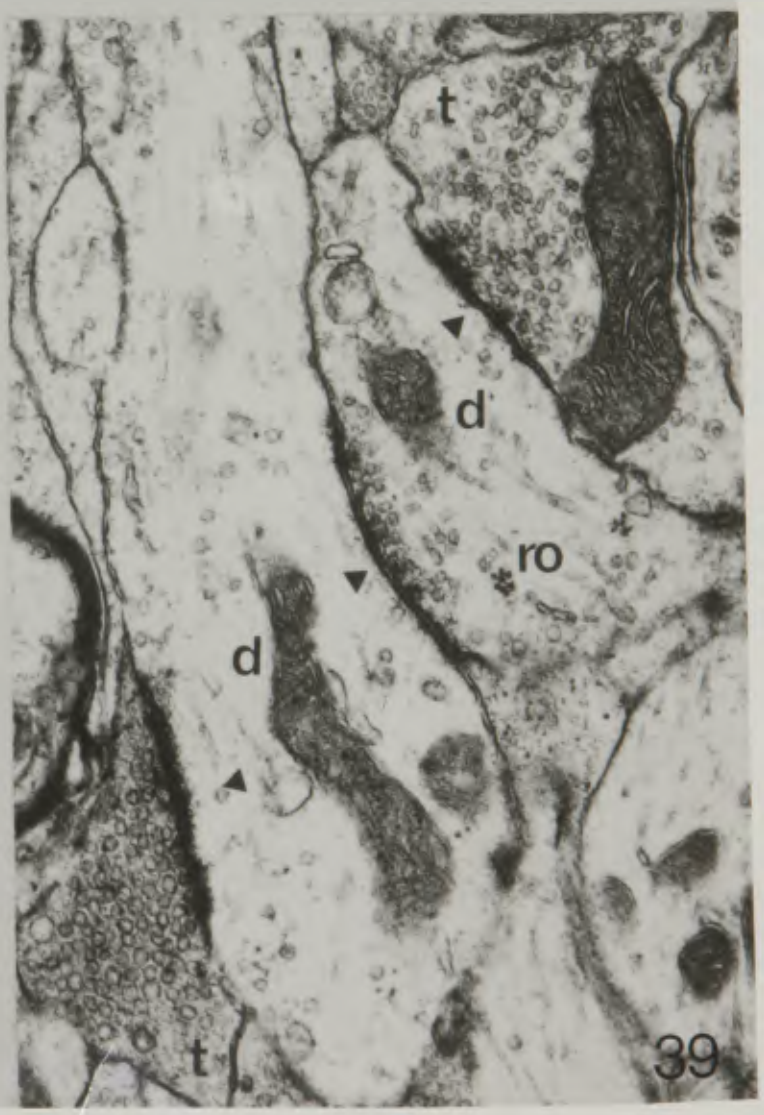

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synaptically. Small desmosomes (Fig. 47) are sometimes found close to reciprocal synapses, between the profiles involved, and sometimes located in between the two opposite membrane specialisations; these desmosomes are characterised by a smaller cleft width than that of synaptic structures and an even distribution of dense material in each profile. Less commonly 'gap junctions' are seen (Figs. 45-47) (Brightman & Reese, 1969; Sotelo & Palay, 1970), which are always between mitral or tufted and periglomerular cell processes and close to a reciprocal synapse and desmosome. The other source of synaptic contacts on to the glomerular arborisations of the mitral and tufted cell is, of course, the olfactory nerve terminals, which are found in greater density upon the dendrites of medium to small calibre, but may also be found on larger segments. Often a mitral or tufted cell dendritic profile is surrounded by many olfactory terminals, all of which make synapses on to the dendritic shaft (Fig. 31).

Periglomerular cell glomerular dendrites

The dendrites of periglomerular cells are extremely variable, not only from one cell to another or from one dendrite to another, but also within a single dendrite; the major variables are their outline, their appendages, the arrangement of neurotubules and the presence of vesicles. Golgi-impregnated material demonstrates to some extent the first two factors (Chapter 3 & Figs. 41-44), although it is difficult to illustrate the remarkably tortuous course of the processes within the glomeruli by either drawings or photographs. Once the main stem

Fig. 40 Serial synaptic arrangement between tufted/mitral and periglomerular cell dendrites in the glomerulus; these synapses are undoubtedly part of a more complex inter-relationship between these dendrites involving reciprocal synapses. A periglomerular cell dendritic shaft, that gives rise to a large vesicle containing gemmule, synapses with symmetrical thickening onto a mitral/tufted dendritic shaft; the latter in turn synapses onto the spine or gemmule of a periglomerular cell dendrite, whose shaft makes a symmetrical synaptic contact onto another mitral or tufted cell dendrite. Note the large flattened vesicles of the periglomerular profiles and the greater irregularity of cytoplasmic contents and outline as compared with the spherical vesicles of the mitral/tufted cells and the regular tubules and outline of these profiles.

X33,200

Figs. 41-44 Photomicrographs of gemmules and spines of periglomerular cells, impregnated with the Golgi-Kopsch method.

Figs. 41, 43, 44: X3,300

Fig. 42: X3,100

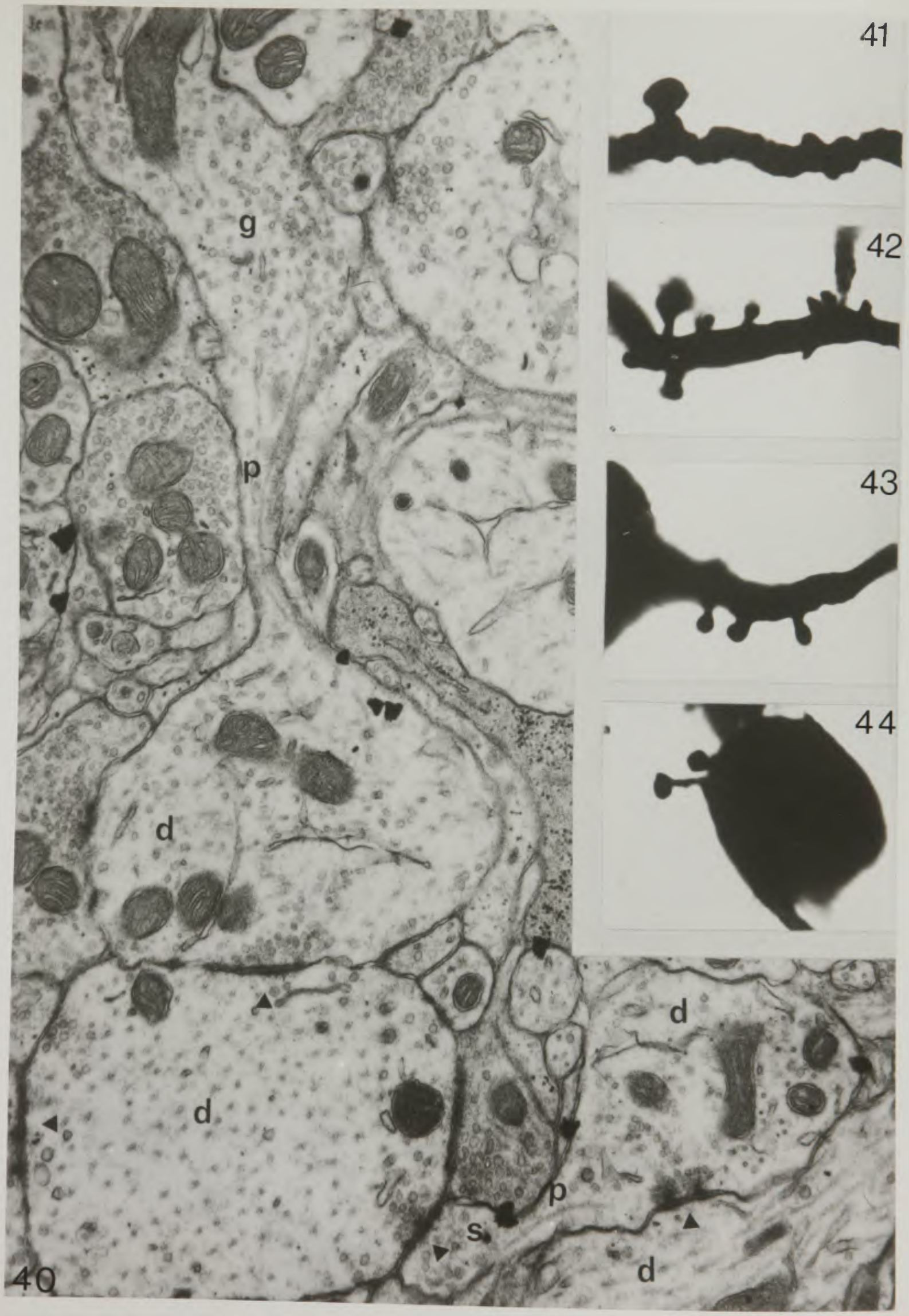
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Fig. 45 Mitral or tufted cell dendrite in the glomerulus showing reciprocal synapse with periglomerular cell dendrite (left) and gap junction with a similar process (right). Note the characteristic polarities of the reciprocal synaptic specialisations, the periglomerular cell profile showing large irregular vesicles compared with the spherical vesicles of the mitral/tufted dendrite. A detail of this gap junction is shown in Fig. 46.

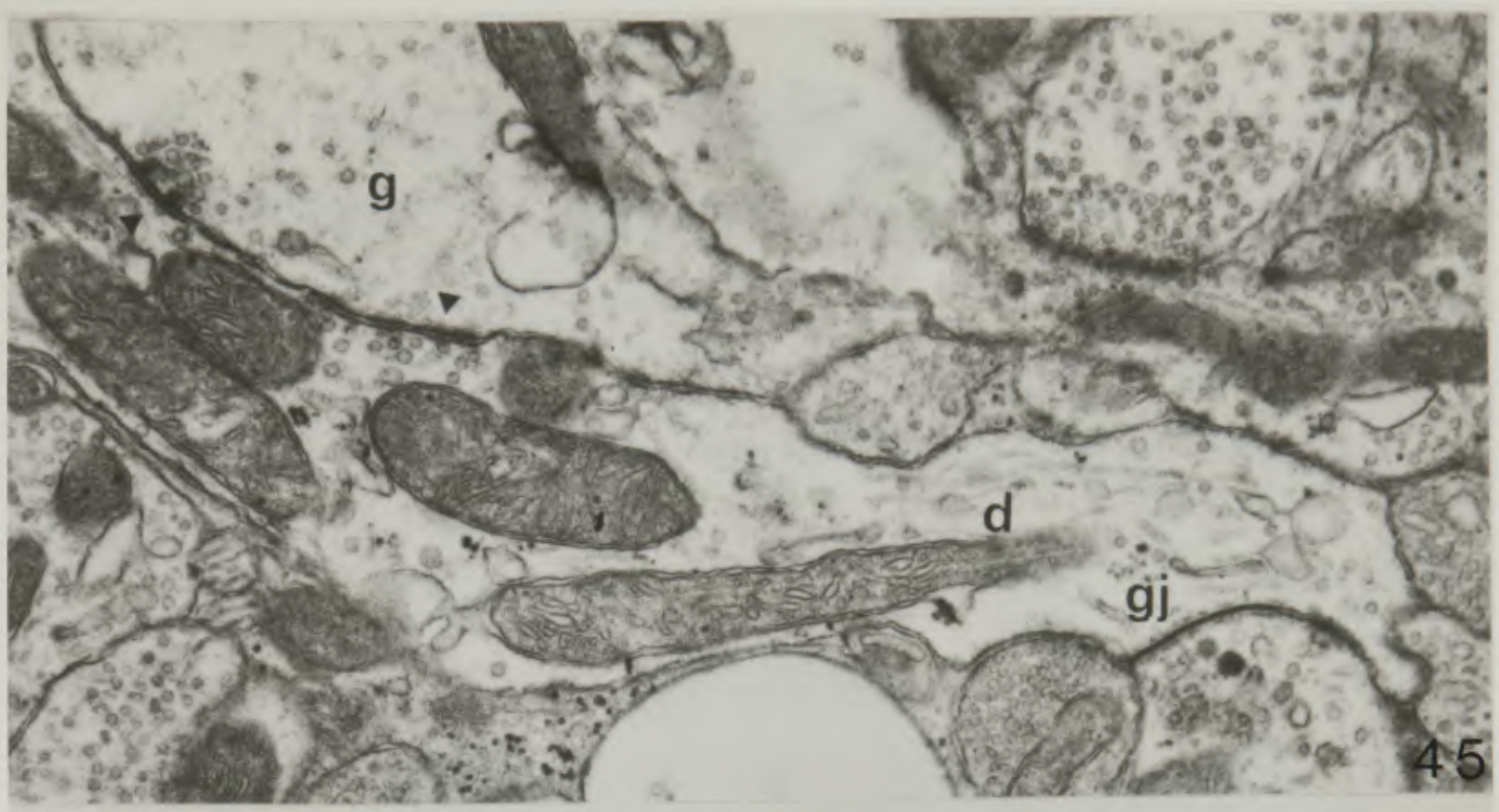
X32,000

Fig. 46 Gap junction from Fig. 45. Note the characteristic laminar appearance of the membranes (although the central gap is not clearly resolved in this example) and the typical arrangement of dense material in the cytoplasm of the neuronal profiles.

X113,000

Fig. 47 An unusual combination of four types of membrane specialisation found on profiles in the glomeruli. A desmosome, cut rather obliquely and showing a large tail of dense material resulting from the curve of the central dendritic profile and an adjacent gap junction. A symmetrical synaptic thickening from a gemmule adjoins the asymmetrical membrane thickening derived from an olfactory nerve terminal (arrow), which has degenerated due to a lesion in the olfactory mucosa, and has become partly apposed to the gemmule and partly to a glial profile. All identities were confirmed by serial sections.

X74,000



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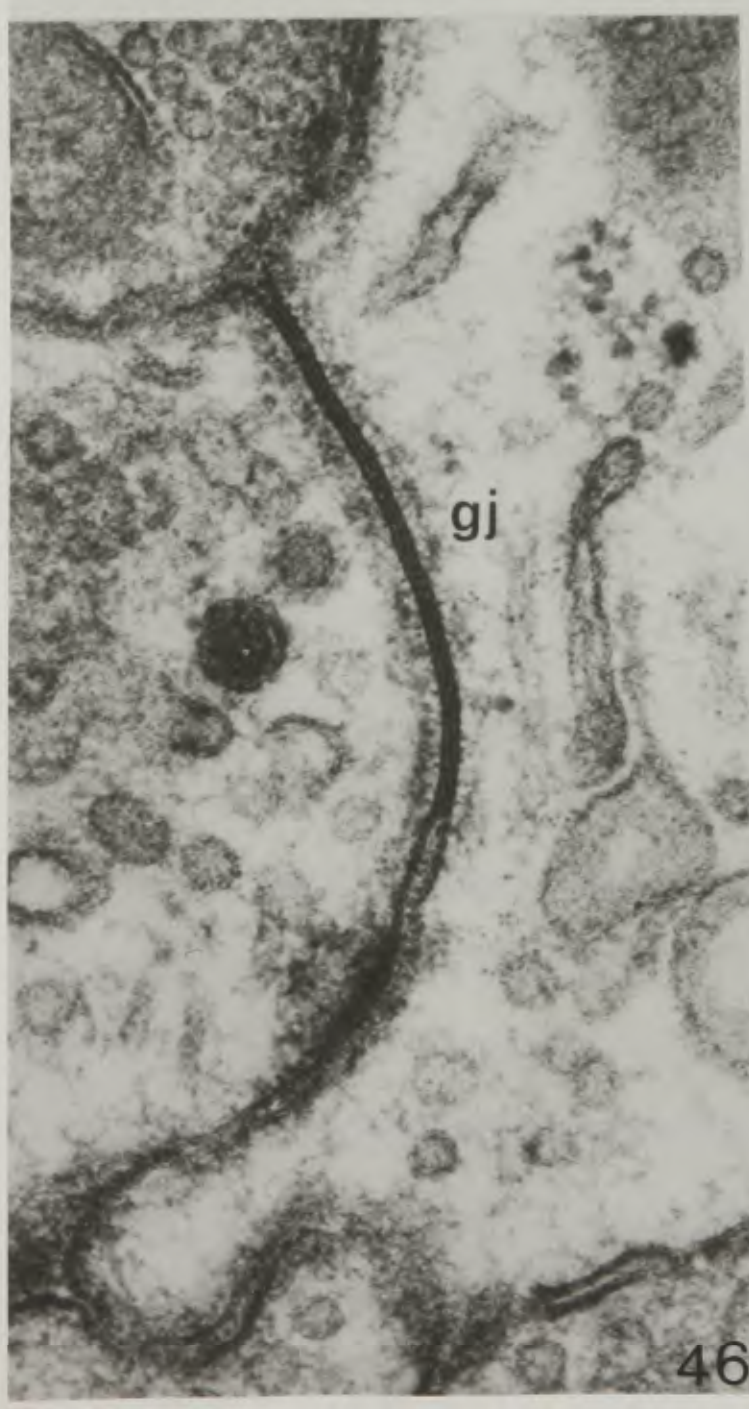

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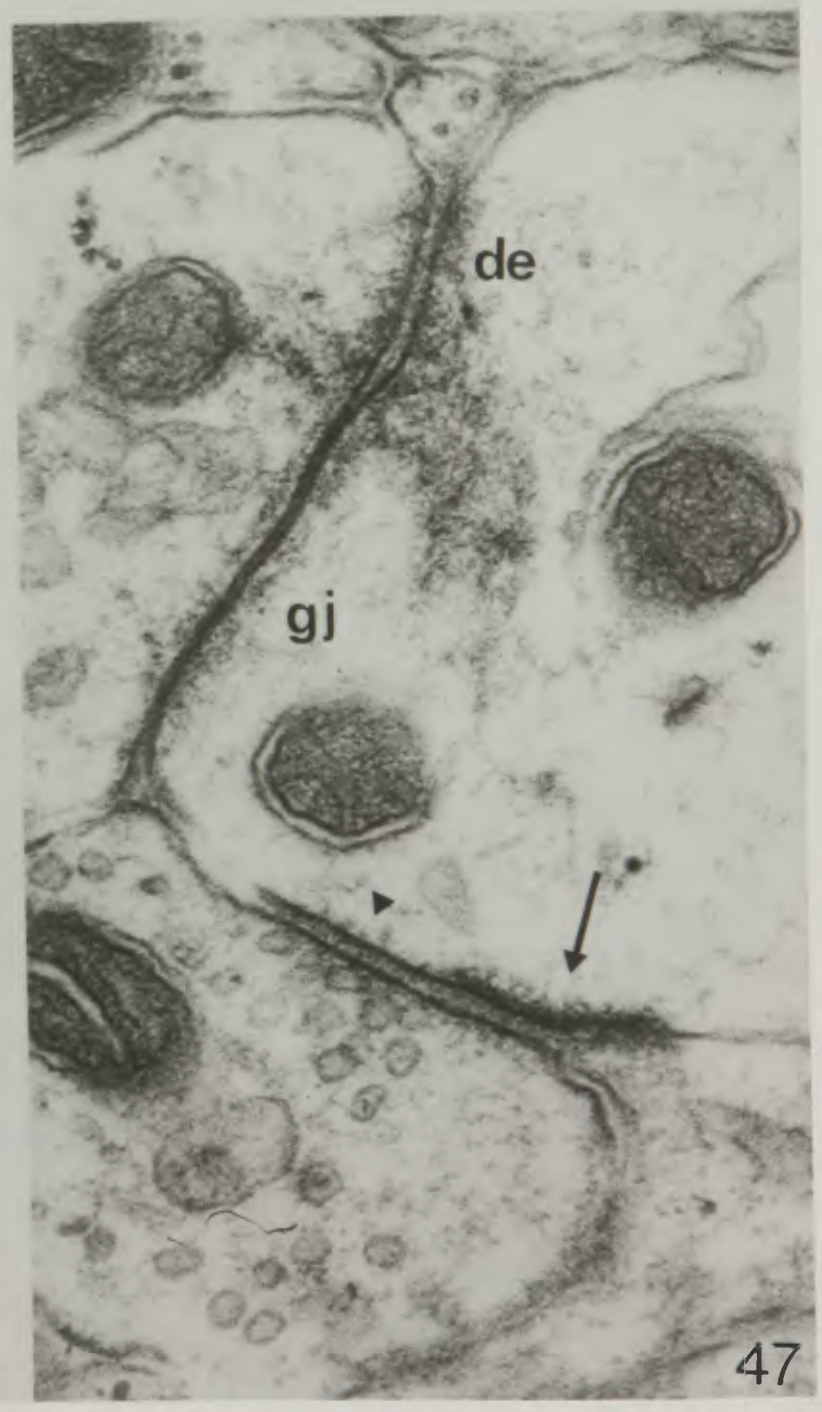

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dendrites have crossed into the glomerular neuropil, they begin to branch and to follow very irregular paths; the extremely irregular and often varicose nature of these dendrites means that they take on many varied and unusual shapes in single electron microscope sections. In addition, the presence of appendages of various shapes and sizes (Figs. 32,35,40-44) and of protusions from the dendritic surface may greatly confuse the assessment of what course the dendritic shaft is taking. As a result of these factors it is generally impossible to be certain what part of the dendritic tree is being examined to the extent that can be achieved for the mitral and tufted cell dendrites; moreover, the appendages may sometimes appear to be larger than their parent processes (Figs. 33,34,40) and may even show considerable morphological complexity, including branching. The terminal parts of the dendrites are typically very thin processes, similar to those of the mitral and tufted cell dendrites, but contain fewer neurotubules and more vesicles. The appendages of periglomerular cell dendrites - spines and gemmules - frequently emerge from the dendritic shafts with extremely attenuated pedicles (Figs. 33,40,42,44) which may be long and contain a few neurotubules and are consequently difficult to follow; at other times they appear more like large outgrowths of the dendritic surface, without any pedicle or tendency to be pinched off (Figs. 32,34,35). Gemmules are most commonly encountered in single sections as roughly spherical structures (Figs. 29,31,36), and the pedicles may be difficult to demonstrate, even with serial sections, on account of their remarkable thinness.

Neurotubules are irregularly disposed in the dendrites of periglomerular cells in all but the largest dendrites, and even in these they never show the regular array typical of the mitral and tufted cells (Figs. 32,40). The more distal dendritic shafts have neurotubular arrangements whose degree of organisation and position seem to vary according to the irregularity of the surface of the dendritic profile. Vesicles are in far greater abundance than in the mitral or tufted cells, being particularly concentrated in the gemmules, dendritic protrusions and the varicosities or broad irregularities (Figs. 29,32-36,40), but are also observed in smaller quantities in main dendritic shafts and in spines (Figs. 33-35,37,40). In the largest shafts, they are only seen clustered around synaptic specialisations orientated away from the dendrites, but in much of the dendritic tree they may be found as large groups often filling the dendritic profile or that of its appendages. This characteristic vesicle-filled appearance of the periglomerular dendrites and their appendages led Andres (1965) to consider them as axonal where they made synaptic contacts of the reciprocal type, but there can be no doubt that the pale vesicle-filled profiles making reciprocal synapses within the glomeruli are dendritic, as determined from serial sections and Golgi material. The vesicles in these dendrites are always of the large flattened type, and are quite distinct from the spherical vesicles in mitral or tufted cell dendrites, although, as discussed previously, the degree of flattening may vary from brain to brain. Extensive cisternae of agranular endoplasmic reticulum are a common feature of

periglomerular cell dendrites and appendages and their characteristic distribution within the profile is a distinguishing feature of these cell processes, particularly the gemmules: ribosomes, often arranged in rosettes, are also common - these two latter features, together with the uneven distribution of vesicles within a given profile, are useful criteria for establishing or confirming the dendritic nature of equivocal processes.

The dendritic appendages of periglomerular cells are of two main classes - spines and gemmules: according to the convention of Rall et al (1966), the term gemmule is reserved for those appendages of spine-like morphology that not only receive synapses, but also give off synapses, generally in a reciprocal manner; spines are defined as those structures of spine-like morphology which receive but do not make synaptic contacts, even if they do contain vesicles. Clearly, serial sections are necessary to confirm these identities and some processes may not be certainly identifiable as one or the other. Nevertheless, it has been possible by use of serial sections to demonstrate the existence of both sorts of appendage in the glomerular and periglomerular regions; most of those in the glomeruli are gemmules, but a few spines may be observed, while the reverse is the case in the periglomerular region. Gemmules tend to be larger structures, and contain a greater concentration of vesicles than spines. Otherwise the two classes of appendage are broadly similar. Spine apparatus is rarely evident in either process, although some small cisternae related to dense material have been observed, which may be

analogous to it. Both types of process show the characteristic flocculent cytoplasm of spines, in which the vesicles appear to be embedded. Ribosomes, cisternae and mitochondria, the latter two often in close relation to each other, are common features of the periglomerular cell dendritic appendages; various dense-cored and alveolate vesicles may also be found, though they are generally few. On very rare occasions, however, the gemmules may show a considerable number of large dense cored (70-120nm) vesicles, in addition to the large flattened vesicles. While the pedicles, particularly of gemmules, often show neurotubules, these structures do not persist for any distance into the swelling or head of the appendage.

The dark olfactory nerve terminals make synaptic contacts of the asymmetrical type with all parts of dendritic shafts and the gemmules of the periglomerular cells, but not with their spines (Figs. 29,34). Mitral and tufted cell dendrites make dendrodendritic contacts with the dendritic shafts, gemmules and spines, and these are always of the asymmetrical type (Figs. 29,31,36-38,40,45). The gemmules and also the dendritic shafts make return synapses on to these mitral and tufted cell dendrites, forming reciprocal synapses; the dendritic shafts do not always make a return synapse in the vicinity, although they may do so at some distance, forming a reciprocal arrangement (see above). These return synapses, from the periglomerular cells on to the mitral or tufted cells, are always of the symmetrical type; the presynaptic vesicles are the large flattened type, characteristic of the periglomerular cell processes (Figs. 29,

32-37,40,43). In addition to the olfactory and dendro-dendritic arrangements, the gemmules, spines and dendritic shafts may occasionally receive three other types of synapse, which are generally in the intermediate zone and arise from axon terminals. The two commonest are both of the symmetrical thickening, flattened vesicle type: predominating are those with small flattened vesicles, which are very similar to those predominating on the cell somata and dendrites of the periglomerular cells, and which have a characteristically medium electron-dense cytoplasm with ill-defined and sometimes rather sparse vesicles (Fig. 38); these terminals have been identified as those of the superficial short-axon cells (Chapter 3). Terminals containing large flattened vesicles (Fig. 39), like those of the same type on to the cell somata, have a paler cytoplasm than those of the short-axon cells; they show evenly distributed vesicles, few cisternae, few or no ribosomes and receive no synapses themselves, distinguishing them from gemmules and identifying them as the axon terminals of periglomerular cells. Both symmetrical type synapses tend to be found on the sides or the bases of spines or gemmules, or on to the dendritic shaft. The third, asymmetrical type of synapse is generally found on to the heads of the spines and the sides of the gemmules (Fig. 35) or, more rarely, the dendritic shaft. These latter axon terminals may be tufted cell collaterals or centrifugal fibre terminals and will be more fully described, together with the other axon terminals, under the periglomerular neuropil (Chapter 5).

Synaptic Patterns within the glomeruli

As discussed above, the varieties of serial synaptic arrangements that may be found are considerable, particularly according to the approach used in defining and studying them. It has emerged from studies of serial sections that two basic patterns of synapses are common and, although variations do exist, involving the absence of one component or another, or a partial combination of the two patterns (e.g. Fig. 39), they appear to have a sufficiently regular relationship to be picked out. It should again be emphasised that a large number of synapses occur in relative isolation from other arrangements, and can only be considered serial within the context of a large part of the dendritic tree. Both of the synaptic patterns centre around the reciprocal contacts between the mitral or tufted cell dendrites and the periglomerular dendrites or gemmules. The first and most common is formed by additional local synaptic contacts from olfactory nerve terminals on to both of the processes entering into the reciprocal synapse (Fig. 29); these contacts may be made by a single olfactory terminal synapsing on to both profiles, or by several. It is estimated that about half the reciprocal synapses that have been serially examined within the glomeruli show this pattern. The second type is generally found in relation to the larger parts of the mitral and tufted dendritic processes in the intermediate zone; it consists of a reciprocal synapse with the addition of a symmetrical synaptic contact on to the periglomerular element or on a closely adjacent part of the dendrite (Figs. 38,39). In the majority of cases

this synapse is made by a medium-dense axon terminal containing small flattened vesicles (short-axon cell terminal) (Fig. 38) but may be a terminal showing large flattened vesicles in a pale cytoplasm (periglomerular cell terminal) (Fig. 39). The relative frequency of this type of arrangement is less easy to determine, since the distances between the reciprocal synapse and the symmetrical contact may vary considerably but about a quarter of reciprocal synapses examined serially show a synapse of this type in the near vicinity. In addition, it should be noted that since both dendritic elements of the reciprocal synapse may make further reciprocal synapses with other processes, probably of different cells of origin, long serial arrangements may at times be traced relating to two or sometimes more of each type of dendrite (Fig. 40).

DISCUSSION

This chapter has been concerned with the neuropil of the glomeruli, its morphology, and the identities and connections of its constituent profiles; it comprises the axon terminations of the olfactory nerve fibres and the dendritic arborisations of the mitral, tufted and periglomerular cells, with small peripheral contributions from the intrinsic axons of the glomerular layer. The connections may be summarised as follows:- The olfactory axons terminate in large numbers on the glomerular arborisations of the mitral, tufted, and periglomerular cells. Reciprocal synaptic relations are established between mitral

and periglomerular and between tufted and periglomerular cell dendrite trees. Olfactory axons terminate on to both processes involved in a reciprocal synaptic arrangement, often closely adjacent to the region of dendro-dendritic synaptic contact; other axon terminals (from short-axon and periglomerular cells) may also come into close synaptic relation with the periglomerular cell component of the reciprocal synapses. It should be emphasized that the identity of the periglomerular cell component as being the gemmule or dendrite of these cells, as opposed to an axon terminal, was defined in several ways. Firstly, from the predominantly dendritic contribution of the bulbar neurons to the glomeruli, established by Cajal (1911) and Blanes (1898), and confirmed by our own material; secondly, by the careful examination of many long series of electron microscopic sections, in which these processes were traced, often for long distances, into continuity with identifiable dendritic profiles; thirdly, by the presence in these processes of organelles considered characteristic of dendrites - ribosomal rosettes, and large agranular cisternal complexes. Although each line of argument on its own is only suggestive, the combined evidence is unequivocal. The particular value of serial examination of electron microscopic sections cannot be underestimated, since it was only through this that the confirmation of classical histology was possible on the one hand, and the establishment of criteria for dendritic and axonal processes in the bulbar neurons could be effected, on the other. Similarly, it was a result of serial studies that the synaptic properties of the periglomerular cell axon

terminals could be shown to be different from those of its dendrites and appendages in having only one-way synapses (see Chapters 3 & 5). Furthermore, the distinction between gemmules and spines can only be made by means of serial sections, and in this way their distribution determined; clear-cut criteria to distinguish between the two kinds of appendages are, however, difficult to obtain, but it appears that olfactory nerves only terminate upon gemmules or dendritic shafts and never on spines.

The periglomerular cell gemmules represent a further stage of synaptic complexity to those of the granule cell peripheral processes, which receive centrifugal and other axon terminals that are excitatory to granule cells, because in the glomeruli the mitral/tufted cell component of the reciprocal synapse is also locally excited by the same (olfactory) axon terminal or type of axon as that exciting the periglomerular cell gemmule. The reciprocal synapses of the glomerular layer thus pose a considerable biophysical problem by combining dendro-dendritic (reciprocal) and axo-dendritic (olfactory) synapse in close proximity on both processes; indeed, until the question of spine potentials has been more fully investigated, it is difficult to reason fully on the functional nature of a single input acting on two reciprocally connected dendrites. It is certain that the extent to which the gemmules are affected by or are independent of the activity in their parent dendritic shaft must determine their mode of function (see Diamond, Gray & Yasargil, 1970), as will the proportion of the excitatory, olfactory input (Yamamoto, Yamamoto & Iwama, 1963;

Shepherd, 1963a) on to each cell of the reciprocal synapse; similarly, it is difficult to assess the relative importance of the synaptic events in the gemmules to the dendritic potentials arising in the whole dendritic tree, and thus their contribution to the axonal activity of these cells. In both cases the extent to which the pedicle isolates the gemmules from the dendritic shaft will be critical in determining the degree of interaction; this in turn depends upon the core resistance that the pedicle presents, and although pedicle dimensions are variable, the extreme thinness of many of these structures might be taken to suggest that this resistance is large (Diamond et al 1970). Such considerations would imply the possibility that the gemmules on long thin pedicles act in relative isolation from their parent shafts in terms of electrical activity and may even be independent, the shaft only being in metabolic continuity; in such a case, it would be the shaft synapses and spine synapses that contribute principally to the production of a spike in the periglomerular cell axon. It should be noted that those reciprocal synapses occurring on the dendritic shaft or on gemmules with little or no pedicle would, however, be affected by, and would affect, the activity in the main shaft of the dendrite. It is important to note in relation to this hypothesis of spine and gemmule isolation that the gemmules of the granule cells are the sole morphologically evident sites of output from these cells; they have a profuse input on to their conventional deep dendrites, which is known to be functionally active (see Price & Powell, 1970,a,b,c). The more general problem

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of the nature of the periglomerular cell dendrites that give rise to the gemmules and of the peripheral processes of the granule cells will be discussed in the following chapter.

From the observations presented in this chapter, the glomerulus may be seen to represent a region of considerable transfer of information between the olfactory axons and the mitral and tufted cells which, by virtue of their central connections, may be considered as relay neurons, and the periglomerular cells, one of the two intrinsic interneurons of this layer. The two sets of dendritic arborisations from relay and inter-neurons then interact by means of reciprocal dendro-dendritic arrangements; then, such information as summates in the soma/IS of the periglomerular cells will be transferred laterally by these axons to the cells of neighbouring glomeruli and notably to the large primary branches of the dendrites of the relay neurons of these glomeruli. The characteristic vesicles and synaptic membrane thickenings of these periglomerular cells may be taken to suggest that these are inhibitory in their action at both dendritic and axonal synapses, like the granule cells (Price & Powell, 1970b) and other cells making similar contacts elsewhere in the central nervous system (see Uchizono, 1965,1968; Gray, 1969 for review).

The glomerulus, being a roughly spherical structure, appears to be ideally suited to the task of bringing about the extensive inter-connections of roughly twenty-five thousand olfactory axons and the dendritic arborisations of well over a hundred cells (figures for rabbit from Allison and Warwick, 1949). Although the full extent to

which individual neurons are interconnected within the whole glomerulus cannot be assessed with present electron-microscopic techniques without massive serial reconstructions, it is quite evident that each mitral and tufted cell dendrite within a glomerulus receives a large proportion of the olfactory nerve input to that glomerulus and is related by dendro-dendritic synapses to several periglomerular neurons, which are also receiving the same or a similar input from the olfactory nerves. Clearly the location of the olfactory nerve synaptic inputs on to the dendritic trees of these three cell types must be critical in determining the properties of each relay neuron and interneuron in relation to this input and, more particularly, the mode of function of their reciprocal contacts; thus it is likely that each dendrite, arborising from a different point of entry into its glomerulus, will receive a different pattern of olfactory input, on the assumption that the olfactory fibres entering a single glomerulus are not a functionally homogeneous population. How these potentially specific and subtle variations in neuron input relate to their role in the relay network is a problem for physiological investigation; the type of connections that have been demonstrated here, should limit the interpretative possibilities involved in assessing any physiological results.

Glomeruli in the Central Nervous System

The term 'glomerulus', derived from the Latin for a small ball, is presently defined as "a compact tuft or tangled mass of branching

processes" (British Medical Dictionary); it has become widely used in the descriptive anatomy of the central nervous system, particularly that deriving from electron microscopy. It may be useful at this juncture to consider the implications of the various uses of the term and the similarities and differences occurring in different localities; this question has also been reviewed by Akert & Steiger (1967) in which they also consider glomerular structures in insect nervous systems. As these authors point out, the earliest application of the term to the central nervous system (in the last century) was in relation to the termination of the olfactory nerves in the olfactory bulb; more recently, however, the term has been applied to much smaller structures, of which the most notable in vertebrates are those in the cerebellum (Gray, 1961; Palay, 1961; Mámori and Szentágothai, 1966; Eccles, Ito and Szentágothai, 1967), thalamus (Peters & Palay, 1966; Szentágothai, Mámori & Tömbol, 1966; Majorossy, Réthelyi & Szentágothai, 1965; Jones & Powell 1969), substantia gelatinosa (Réthelyi & Szentágothai, 1968) and trigeminal nucleus (Dubner & Gobal, 1969).

The olfactory glomeruli, nevertheless, present an altogether different order of size to these other glomeruli, the range of diameters in the rat being 50-120μm (internal), while figures of 7-20μm may be taken as the average value for the cerebellar and thalamic glomeruli in the cat. In addition, the components involved in the olfactory glomeruli are much smaller, so that a single section through one glomerulus may contain several thousand profiles, while a large

thalamic or cerebellar glomerulus may contain less than one hundred. It is clear, therefore, that the multitude of processes concerned in a single olfactory glomerulus, with many thousands of olfactory axons and the dendritic arborisations of 80-100 cells, must make this a region of more complex interaction and integration than that occurring in the other smaller glomeruli, which typically relate to either a single dendrite or axon terminal. Despite such a gross numerical disparity, it is interesting to note certain principles that are common to the organisation of neurons and their processes in the various glomeruli. Although each site presents slight variations in the components involved, it is a common feature of all glomeruli that they represent a complex synaptic interaction of three, or sometimes four major sets of processes - an incoming axon and a dendrite (olfactory bulb, cerebellum, thalamus, spinal cord, trigeminal nucleus), and the processes of an interneuron - axon (thalamus, spinal cord, trigeminal nucleus), dendrite (olfactory bulb, thalamus) or both axon and dendrite (cerebellum). It may be noted here, however, that the nature of the interneuronal processes in some sites may be brought into question by the changing criteria for dendritic and axonal structures (e.g. Ralston & Herman, 1969; Chapter 5).

A clear delimitation of at least one of the processes involved seems to be another feature common to glomeruli: in the olfactory bulb the olfactory nerves never extend beyond the glomeruli (Cajal, 1890), nor make synaptic contact anywhere but within these structures; in the cerebellum and lateral geniculate, the mossy fibres and optic fibres

terminate exclusively within the glomeruli (Eccles et al, 1967, Szentágothai et al, 1966). The presence of glial lamellae around the small glomeruli, and the partial glial wrapping of the olfactory glomeruli are similar features, although the latter cannot be said to be as constant or as well-defined a feature as the former; the glial relationships of the olfactory glomeruli, being such large structures, are more complex and less easily abstracted. It is notable, however, that the processes of single glial cells may be in close relation both to the incoming olfactory axons and the outgoing mitral/tufted cell dendrites of a glomerulus, perhaps fulfilling a similar role to the glia wrappings of the smaller glomeruli (Chapters 3 & 6).

Evidently, the distinctive characteristic of glomeruli in the central nervous system whatever their size is a synaptic interaction between several sets of mutually interconnected processes; variations in the size and the array of the glomeruli, and in the types of process involved or the synaptic patterns they establish, represent the individual features of any relay - indeed it is notable that many of the glomeruli occur in sensory relay nuclei. The olfactory glomeruli, which are undoubtedly the most extensive and complex glomeruli, in terms of all four features just listed, may be expected to have somewhat different functional properties to the smaller glomeruli described elsewhere in the nervous system. It is not possible to consider the olfactory glomeruli as a group of many smaller glomeruli on account of the extensive interconnections between the dendritic arborisations and

the wide distribution of synapses made by a single olfactory nerve fibre or bundle. On the contrary, it may be just this property of containing so many interconnected neuronal processes that is the clue to understanding the functional principles on which the organisation is based.

CHAPTER 5

THE NEUROPIL OF THE PERIGLOMERULAR REGION OF THE OLFACTORY BULB

INTRODUCTION

This chapter continues the analysis of profiles and their interrelationships in the neuropil of the glomerular layer of the olfactory bulb, describing those components in the periglomerular region that have not received detailed consideration in the two previous chapters. Since many of the profiles may be traced by serial examination into continuity with the nearby cell somata, some of the processes in this region are described with their cell bodies of origin. The anatomical arrangements emerging from these three chapters will be summarised and their implications discussed.

In the previous two chapters the characteristic distribution of processes between the periglomerular region and the glomeruli has been described and criteria for the identification of neuronal profiles in the neuropil according to cell type and process type have been established. Since all types of dendrites pass through the periglomerular region, some giving rise to appendages, and because it is the site of termination of almost all the non-olfactory axons and their collaterals, the definition of profiles in single sections is less easy than in the glomeruli; however, profiles are generally more easily traced into continuity with an identifiable process using serial sections, as most dendrites are large enough to show typical morphological features. Identification of a profile according to cell type may be achieved by tracing it into continuity with the cell soma of origin, by the presence of the various classes of vesicles, and on the

basis of certain characteristic morphological features shown by some types of dendrite. Nevertheless, the additional contribution of the dendrites of the superficial short-axon cells (Chapter 3) to the neuropil of the periglomerular region, makes the problem considerably greater. An additional difficulty for normal identification of processes is caused by the presence of the centrifugal fibre terminations in this zone; like their deeper counterparts (Price & Powell, 1970c) these fibres terminate with spherical vesicles and asymmetrical thickenings (Chapter 9), and are therefore of the same type as the tufted cell recurrent collaterals, which also terminate in this region. The centrifugal fibres are the only extrinsic axonal connections of the glomerular layer, additional to the olfactory nerves, so that they can be distinguished from the tufted cell collaterals by experimental analysis; the distribution of the two types of axon is very similar and no consistent means of distinguishing between them in normal material has been found.

RESULTS

The periglomerular region

The periglomerular region consists of the interstices between individual glomeruli and between these glomeruli and the olfactory nerve and external plexiform layers. Although it shows considerable variation in its breadth and extent around different glomeruli, it is generally most marked where it adjoins the external plexiform layer

and least evident in its superficial border with the olfactory nerve layer; it is often absent from this where the olfactory nerves make their entry into the glomeruli. The bulk of the periglomerular region consists of the somata of the intrinsic neurons of the glomerular layer, which have been described previously, together with the processes that may be found in continuity with them (Chapter 3); however, the remainder of the periglomerular region is filled with a characteristic neuropil. It contains parts of the dendrites of all the cell types intrinsic to this layer, as well as those of the mitral cells, and includes the whole course of the dendrites of the superficial short-axon cells (Chapter 3); it is also the principal site of termination of all axons intrinsic to the glomerular layer, and of the centrifugal fibres, which are the only axons of extrinsic origin, other than the olfactory nerves themselves, to reach this layer. The olfactory nerve fibres are never found in the periglomerular region, although they may interdigitate with the periglomerular neuropil in the intermediate zone. From the evidence obtained with Golgi-impregnated material, it is clear that most of the dendrites of the periglomerular region are large primary branches, passing on their route to the glomeruli, but some short fine periglomerular cell dendrites also appear to have entirely periglomerular courses; the electron microscope has shown that most of the typical spines of the periglomerular cells lie in this region, as well as some gemmules.

In view of their preponderance in the periglomerular region, it is felt appropriate to describe the morphological characteristics of

the non-olfactory axon terminals in this section, although these profiles have been encountered and briefly described in the previous chapters. The main dendrites and their spines and gemmules, on the other hand, have been fully described in these places and will not be given detailed consideration here; only those dendritic components exclusive to the periglomerular region will be considered separately from the point of view of their general morphology and synaptic relations: i.e. the dendrites of the short-axon cells and the thin periglomerular dendrites of the periglomerular cells.

Tufted cell recurrent collateral and centrifugal fibre terminals

By far the most common axon terminal type in the periglomerular region is characterised by spherical vesicles and an asymmetrical synaptic thickening (Figs. 51-54,60,65,66,69). As intimated previously, there are two candidates for the origin of this terminal: first, the recurrent collaterals of the external tufted cells that are known to course and terminate in this region (Cajal, 1911; Blanes, 1898) and which by the morphological corollary of Dale's principle (Eccles 1964) would be expected to make synaptic contact by means of the same vesicle and thickening characteristics as the dendrites and cell soma of origin (Chapter 3). Such an interpretation is confirmed by studies of rabbit material in which transneuronal cell degeneration has occurred, involving the axons of these cells (Chapter 8), as well as by studies on rat material with intrinsic lesions (Chapter 10). However, the centrifugal fibres are known also to terminate in the periglomerular

Figs. 51-54 These micrographs show examples of terminals with spherical vesicles and asymmetrical synaptic thickenings in the periglomerular region.

Fig. 51 Terminating onto the spine of a periglomerular cell; note attenuated pedicle; the spine was not connected to the dendrite in either of the adjacent sections.

X53,500

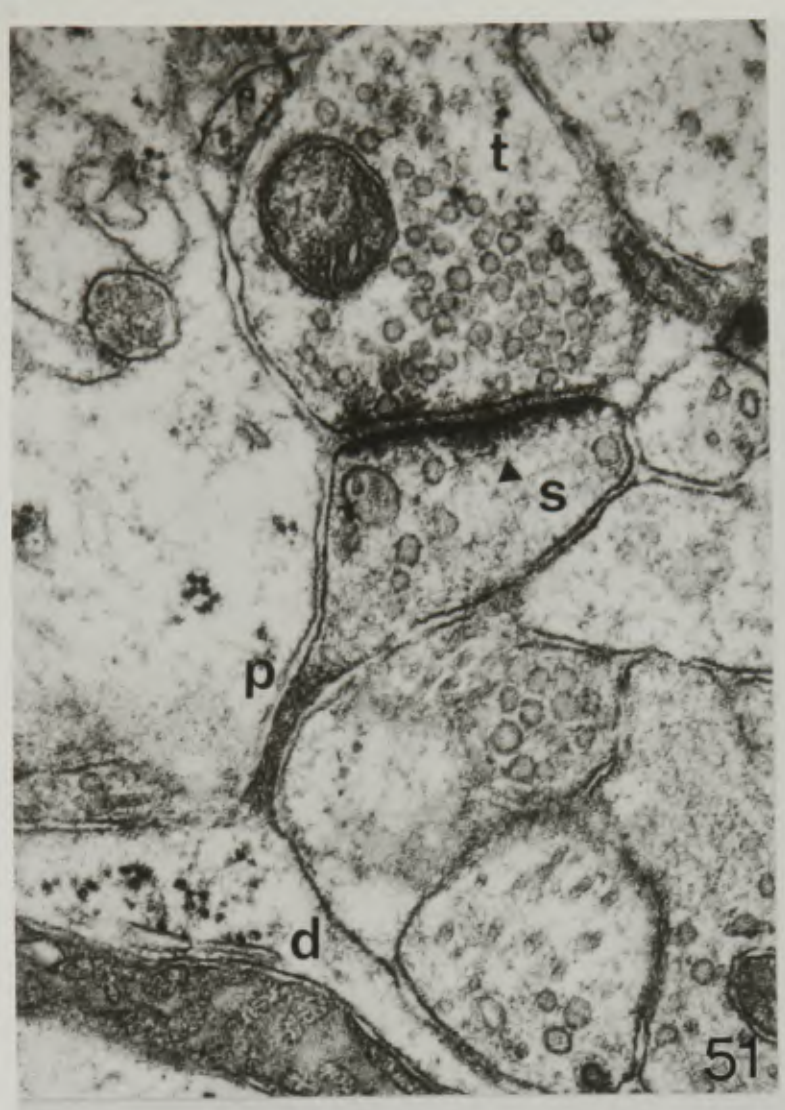
Fig. 52 Terminating onto two profiles that may be identified as spines of periglomerular cells from serial sections; note the absence of neurotubules, and the flocculent cytoplasm.

X42,700

Fig. 53 Terminating onto a profile filled with large flattened vesicles, probably the gemmule of a periglomerular cell.

X68,500

Fig. 54 Terminating onto the dendritic shaft of a periglomerular cell dendrite; compare the dendro-dendritic synapse opposite, from a mitral or tufted cell dendrite, noting the different distribution of vesicles in the two presynaptic profiles.



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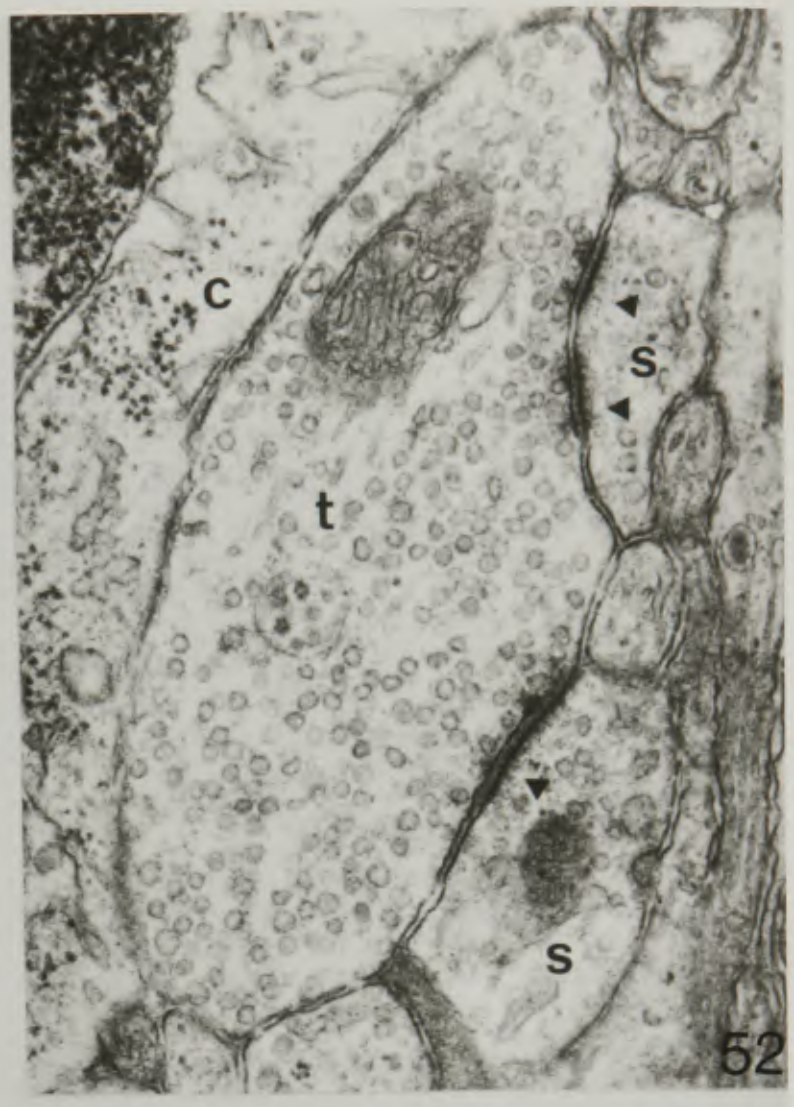

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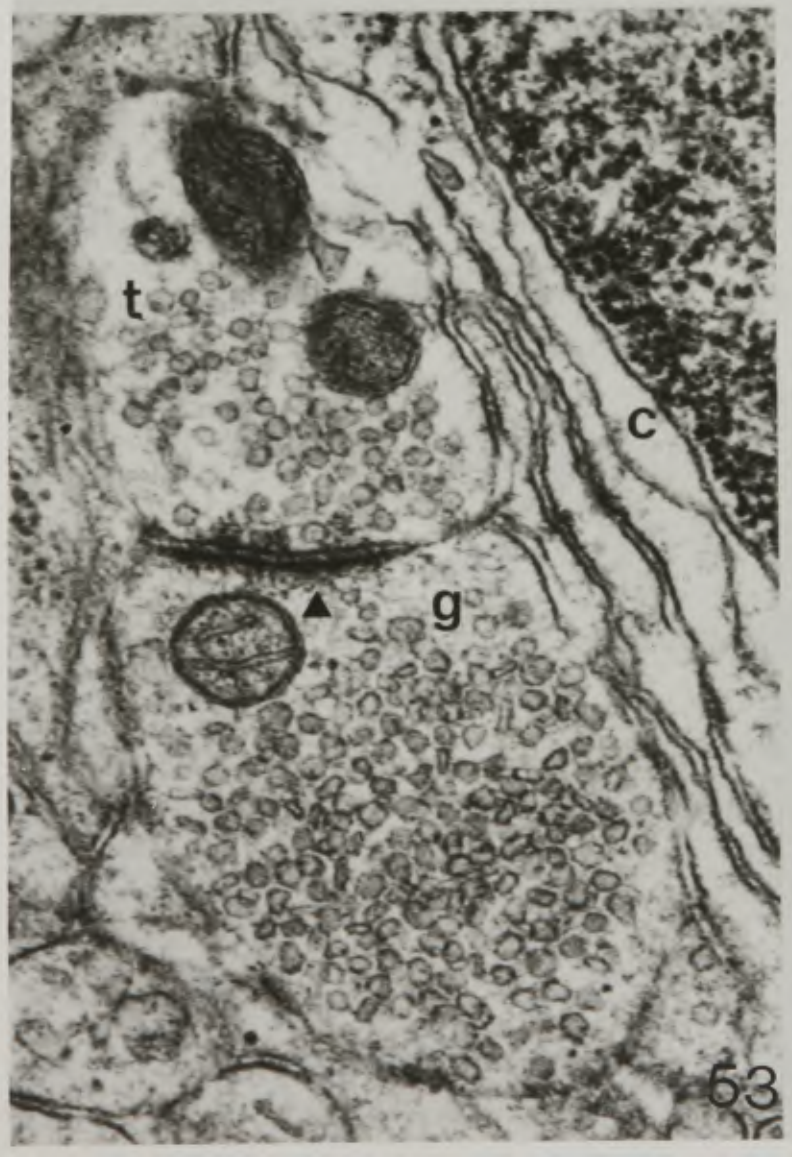

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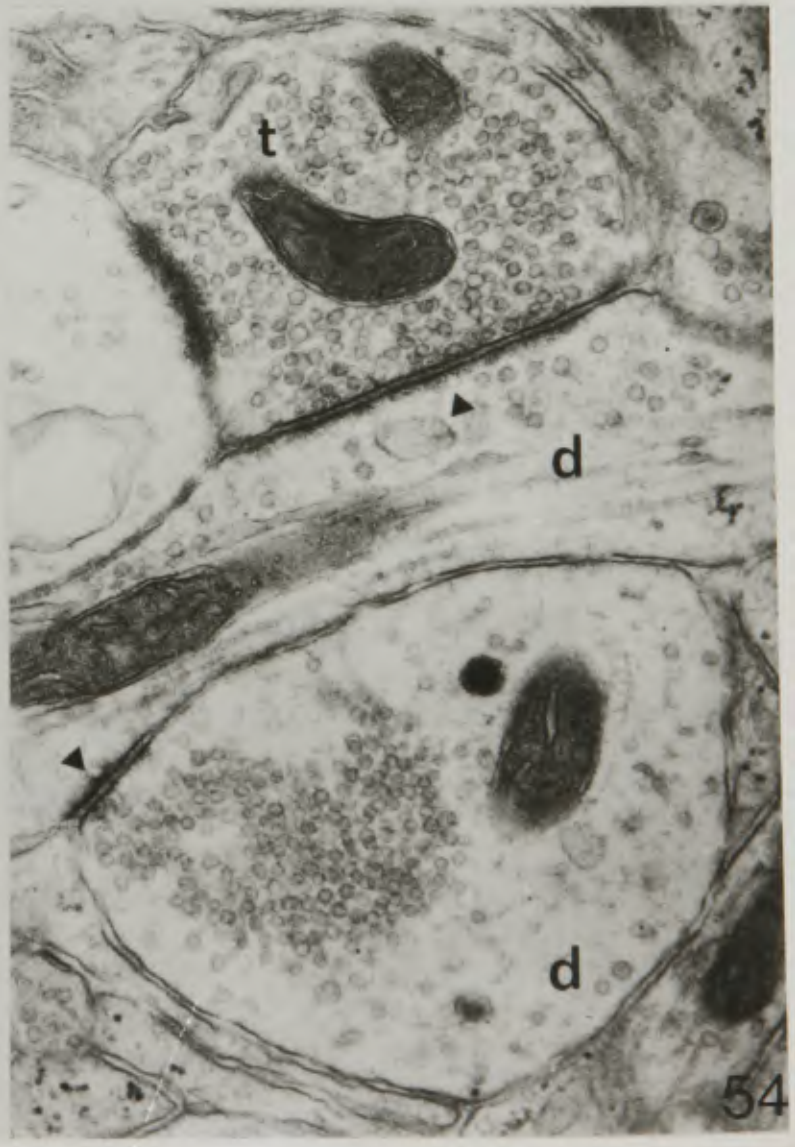

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region (Cragg, 1962; Powell, Cowan & Raisman, 1965; Price & Powell, 1970a); it has been shown that these fibres terminate with spherical vesicles and asymmetrical thickenings both in the external plexiform layer (Price & Powell, 1970a) and the glomerular layer (Chapter 9).

The size of terminals containing spherical vesicles varies considerably, although it is not possible to correlate their size with their identity; they generally have a pale cytoplasm with many vesicles, fairly evenly distributed throughout the profiles. The vesicles are not tightly packed in the cytoplasm in most instances; some aggregation of synaptic vesicles around the membrane thickening and presynaptic dense projections are usually evident. The synaptic membrane thickenings associated with these terminals are invariably asymmetrical, whatever type of profile they synapse on to; a large web of dense material extends within the post-synaptic profile opposite the region of membrane thickenings and the extracellular cleft material (Figs. 51-54,66). Small desmosomes may be found closely adjacent to the thickenings: these are characterised by a cleft width smaller than that of synaptic thickenings and a literally symmetrical distribution of dense material extending into both pre- and post-synaptic profiles (Fig. 69). Small mitochondria, multivesicular bodies and occasional cisternae may be found in the cytoplasm of these terminals; ribosomes are very infrequent and are never seen organised into rosettes. Many of these axon terminals have been seen in continuity with their preterminal axon, which is fine, contains a few neurotubules, and is pale or only slightly electron-dense; sometimes

neurotubules may be seen in part of the terminal indicating an en passant situation. Very rarely, an asymmetrical synapse has been observed from the node of Ranvier of a myelinated axon.

The spherical vesicle-containing terminals of the periglomerular region most commonly make synaptic contact with spines (Figs. 51,52) or occasionally gemmules (Fig. 53) of periglomerular cells; the latter two processes may both contain large flattened vesicles, but only the gemmules enter into synaptic (reciprocal) contact with mitral or tufted cell dendrites, in addition to receiving the terminal contact. Both spines and gemmules have been seen arising from the large dendrites of periglomerular cells (Fig. 51), but only spines from their cell somata (Chapter 3); some spines also arise from the dendrites of the short-axon cells and receive these synaptic contacts (Fig. 65). Axo-dendritic contacts are seen direct on to the shafts of periglomerular cell dendrites (Fig. 54) and, characteristically in groups of several large terminals, on to the shafts of short-axon cell dendrites (Figs. 60,65); occasionally isolated terminals of this type may make synaptic contact with mitral or tufted cell main dendrites in the periglomerular region. Axo-somatic contacts are also made, generally by smaller terminals, directly on to the somata of short-axon cells, and very rarely on to those of periglomerular and external tufted cells. Each terminal may make more than one synaptic contact, either with different processes or on occasion with both a spine and its parent process. These terminals do not receive synaptic contacts, although in a few instances a sac junction has been seen

between a periglomerular cell soma and the axon terminal. The predominant influence of these two types of asymmetrical axon terminal, the tufted cell recurrent collateral and the centrifugal fibre, can thus be seen to be exerted upon the periglomerular and particularly short-axon cells, both of which are interneurons having connections restricted to the glomerular layer.

Periglomerular cell axon terminals

It is only possible by the use of serial sections to identify a profile containing large flattened vesicles as an axon (Figs. 55, 56,70) and thus to distinguish it from a periglomerular dendritic gemmule (Fig. 69); some criteria can then be established for application to a single section examples. Axon terminals may be differentiated by demonstrating continuity with a preterminal axon rather than with a dendrite by means of a pedicle; and by the fact that they make synaptic contact with symmetrical thickenings, but receive no synapses themselves, particularly not from the processes they synapse on to, in a reciprocal arrangement. The vesicles are evenly distributed in the cytoplasm which is typically pale (Fig. 56), but may appear denser if the vesicles are tightly packed (Fig. 55). Ribosomes are rare and do not appear in rosettes, while the latter are common in gemmules; small loose cisternae are sometimes present but never show the clear and complex arrangements characteristic of dendritic profiles. Neurotubules are only present in cases of en passant synapses; a few multivesicular bodies, large dense-cored vesicles

Fig. 55 Axon terminal containing large flattened vesicles and making symmetrical synaptic contact onto the primary stem dendrite of a tufted cell; serial sections showed no evidence of a return synapse from the dendrite.

X53,500

Fig. 56 Axon terminal containing large flattened vesicles, synapsing with a symmetrical thickening onto the soma of a periglomerular cell.

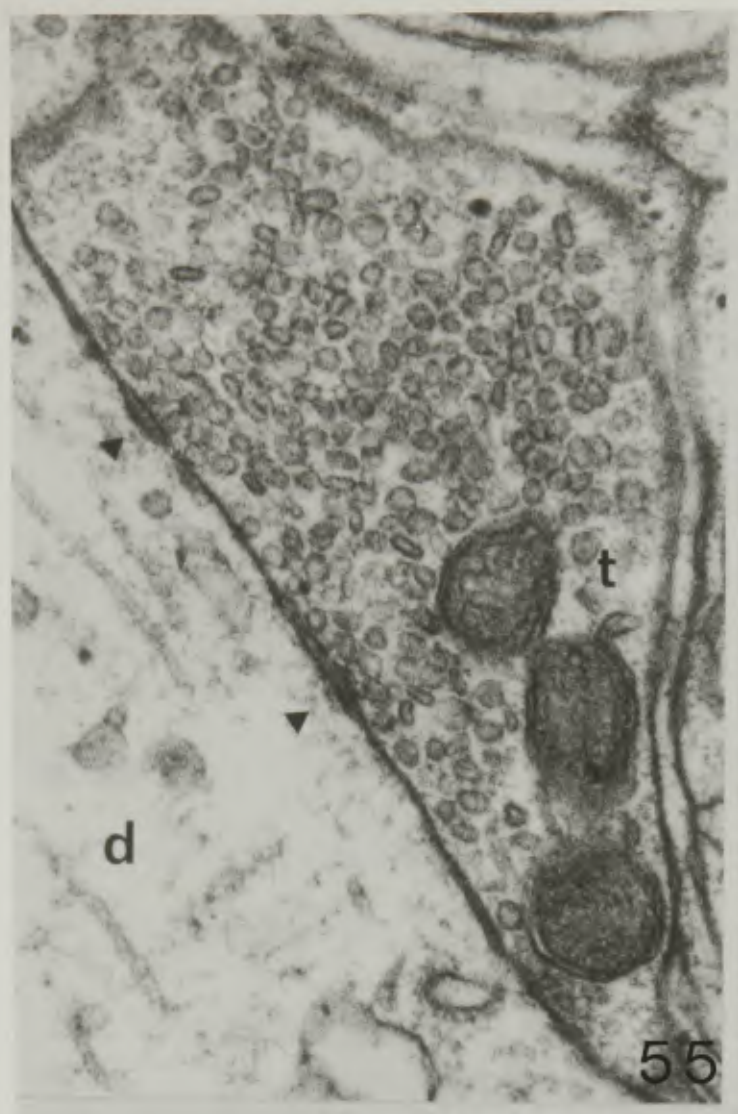
X53,500

Fig. 57 Axon terminal containing small flattened vesicles and synapsing with a symmetrical thickening onto a dendritic shaft; an adjacent axon terminal contains large flattened vesicles.

X53,500

Fig. 58 Axon terminal containing small flattened vesicles terminating with a symmetrical thickening onto a small profile, probably part of a spine; another terminal of the same sort synapses onto a profile containing large flattened vesicles, derived from a periglomerular cell.

X53,500



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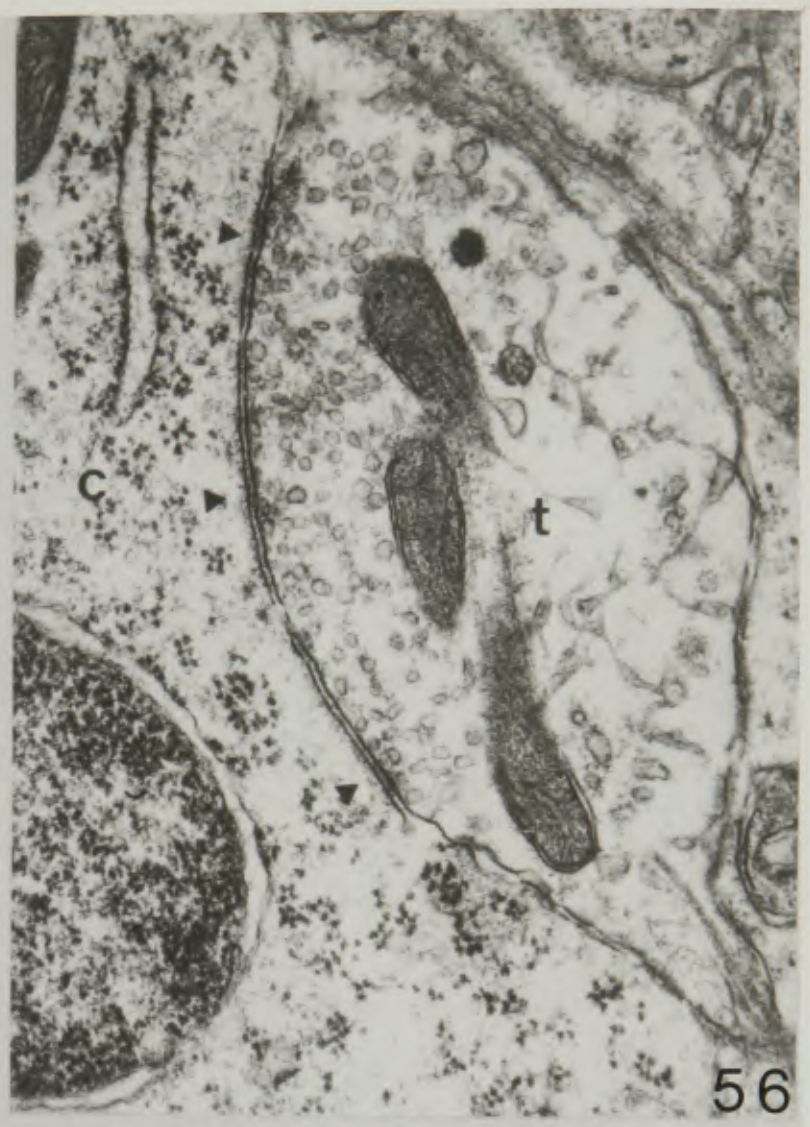

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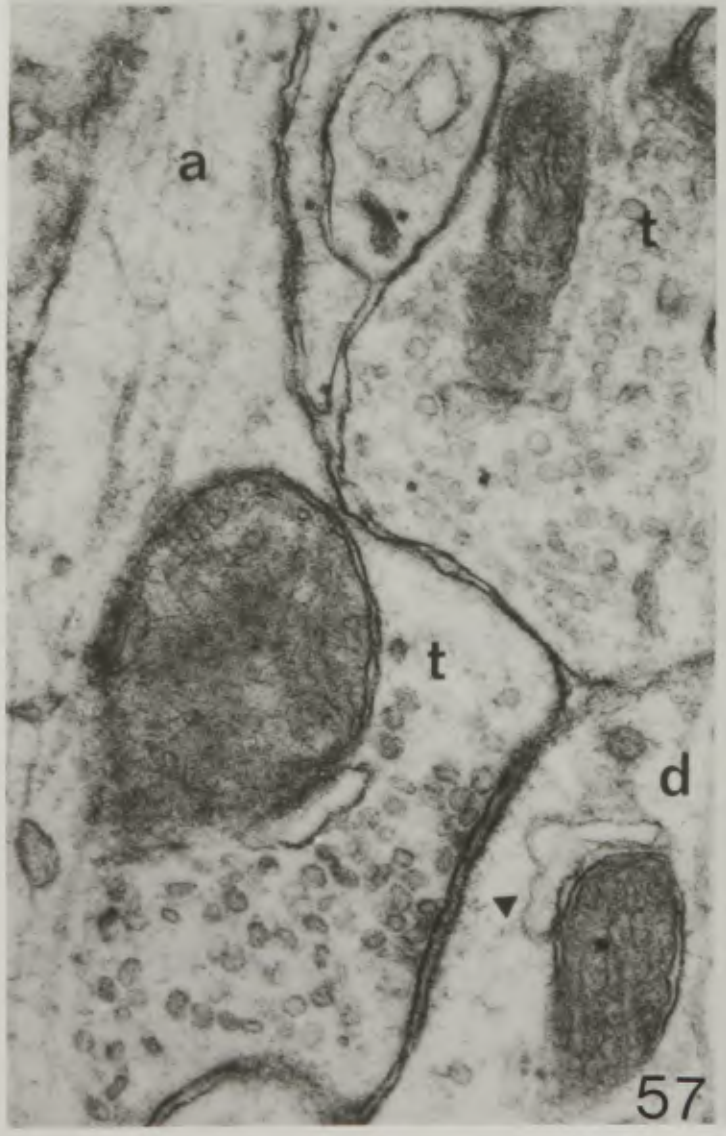

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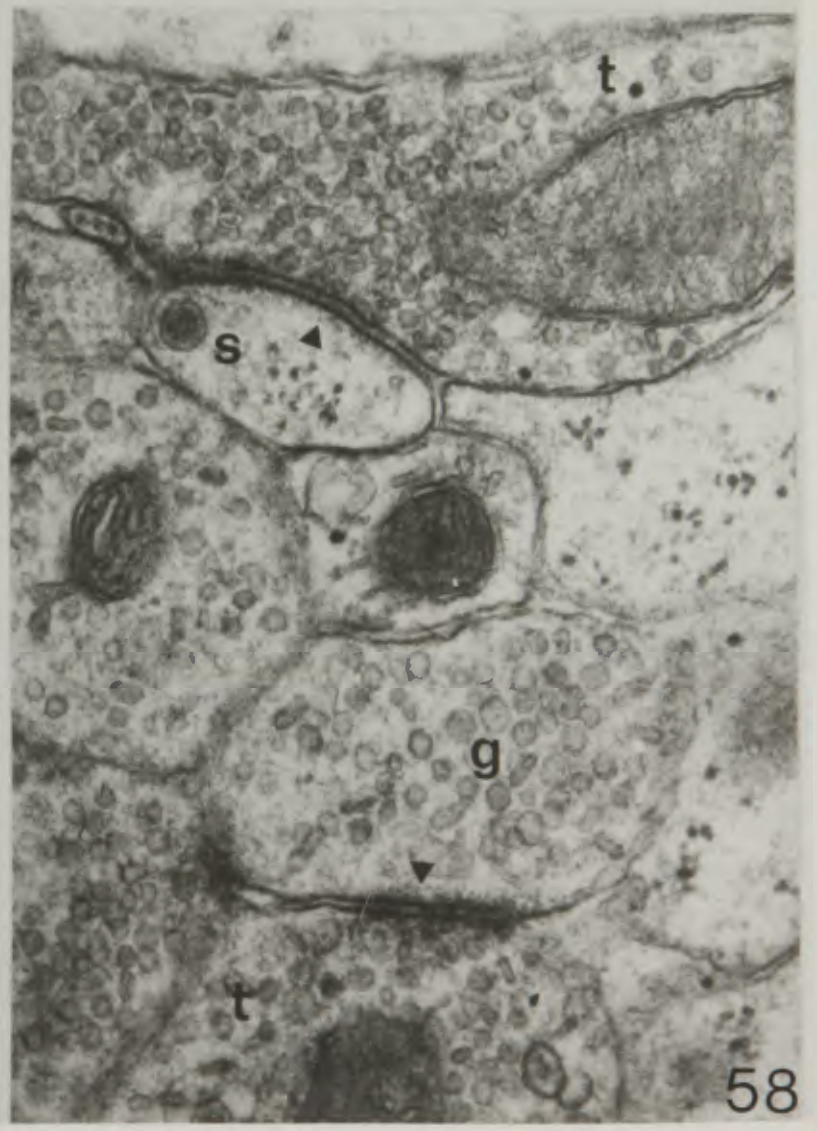

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(70-120nm) and mitochondria are also seen in such axon terminals. These terminals always make synaptic contact by means of symmetrical membrane thickenings and show presynaptic dense projections, aggregation of large flattened vesicles and a plate of extracellular cleft material (Figs. 55,56,70); the post-synaptic membrane density lies almost entirely on the membrane itself and scarcely extends beyond it in most instances. Small desmosomes may be found adjacent to the synaptic thickenings. The size of these terminals varies considerably, but many are quite large.

The most characteristic site of termination of these profiles is on the large dendrites of the mitral and tufted cells (Fig. 55), usually in the region of their first branching and lying just within the periglomerular region (Chapter 3). This type of terminal is also found on to the somata and dendrites of both periglomerular (Fig. 56) and short-axon cells and their initial segments; they may also be found, though more rarely, on to the spines (Fig. 58) and gemmules of periglomerular cells, typically at the side or the base of the appendage (Fig. 70). From their distribution in the periglomerular region and their vesicle and membrane thickening characteristics, these axon terminals are identified as those of the periglomerular cells and may be seen to have a distribution that extends to all the cell types involved in the glomerular layer.

Short-axon cell axon terminals

The axon terminals containing small flattened vesicles are very

characteristic, mainly on account of the small size of the vesicles, which is very striking when, as is common, the profile is adjacent to one containing spherical or large flattened vesicles (Figs. 57,58, 63,67,69,70); also, since there is no dendritic process containing large numbers of such vesicles, they represent a clear-cut group, all of which are either en passant or terminal axon boutons. Many of these boutons are seen to be of medium electron-density (Fig. 58) and of a rather granular cytoplasm but others, which are paler and may contain a few neurotubules, are probably en passant; although the size of the terminals varies, they never reach the size of the larger periglomerular cell axon terminals. The vesicles are usually found crowded and evenly dispersed within the terminal profile, but on some occasions they appear sparser and also rather ill-defined in outline, almost as if they were always just behind the plane of section (Fig. 70). Small mitochondria and occasional small cisternae are the only obvious cytoplasmic inclusions additional to the vesicles, but very rarely a single free ribosome may be seen. Preterminal axons containing neurotubules and/or neurofilaments in a slightly electron-dense cytoplasm have regularly been observed in continuity with these terminals (Fig. 57). Synaptic thickenings are invariably of the symmetrical type exactly similar to those described in the previous section for the periglomerular cell axons; small desmosomes may also be seen in relation to these contacts (Fig. 69).

The great majority of these small flattened vesicle terminals synapse on to the somata, spines, gemmules, dendrites and axon initial

segments of periglomerular cells, identifiable by the presence of large flattened vesicles, dendro-dendritic contacts and somatic characteristics; their situation is most commonly on the soma, and dendritic shaft (Figs. 57,63) or close to the base of an appendage (Figs. 58,69,70). A smaller proportion terminate upon the soma and basal dendrites of the short-axon cells (Fig. 67). These axon terminals, which have been identified as those of the short-axon cells (Chapter 3), are seen to exert their main influence upon the periglomerular cells, but additionally feed back on to their own type; they never establish direct contact with mitral or tufted cells.

Periglomerular cell thin dendrites

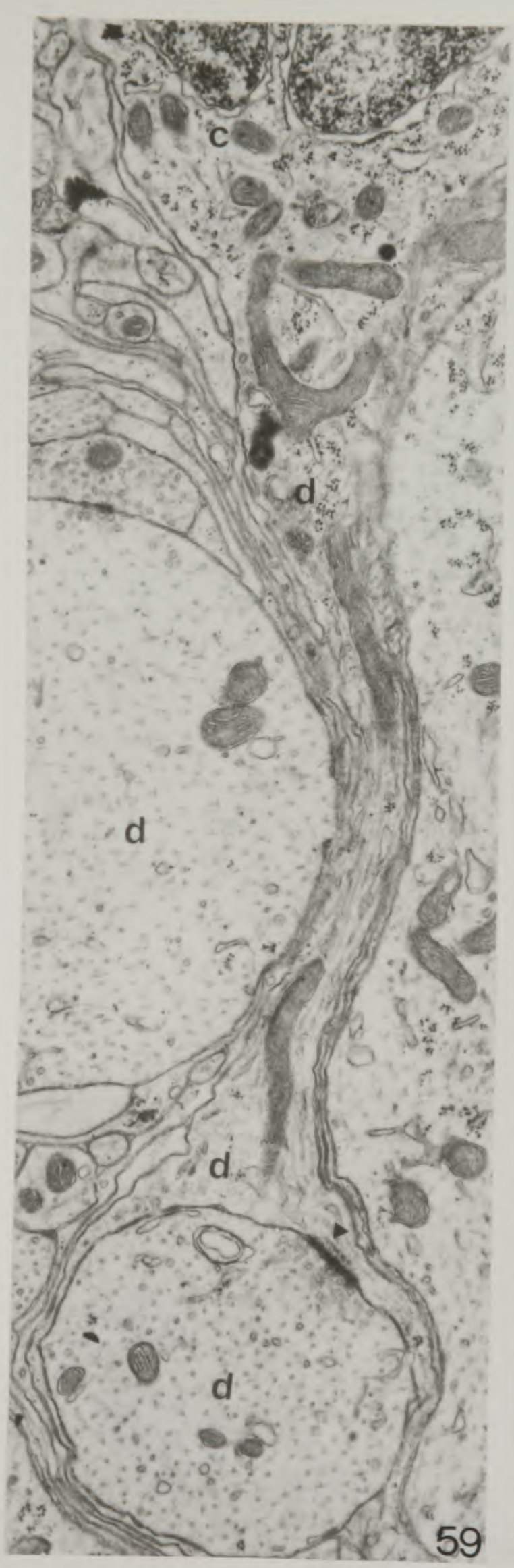
The thin class of dendrites belonging to the periglomerular cells are only found lying in the periglomerular region; they are generally short, very irregular in outline and follow a tortuous course (Figs. 59,61-64). Similar dendrites have been observed in Golgi-impregnated material with these characteristics (Chapter 3); in both this and electron microscopic material they are seen to have small branchlets or twigs of cytoplasm, although spine-like appendages are not seen. They lack the typical pale electron microscopic appearance of the larger periglomerular cell dendrites and have a granular, medium electron-dense cytoplasm, regularly containing a large number of ribosomes, both free and in rosettes; mitochondria, complex cisternae, multivesicular bodies, alveolate agranular and granular vesicles are common inclusions. Neurotubules are characteristic in their apparent

Fig. 59 A thin dendrite arising from the soma of a periglomerular cell splitting into sheets that enclose a tufted cell dendrite, from which it receives a synapse. A detail of an adjacent section is shown in Fig. 61.

x20,600

Fig. 60 Large short-axon cell dendrite running in the periglomerular region and receiving many asymmetrical synaptic contacts onto the shaft from axon terminals containing spherical vesicles.

x24,500



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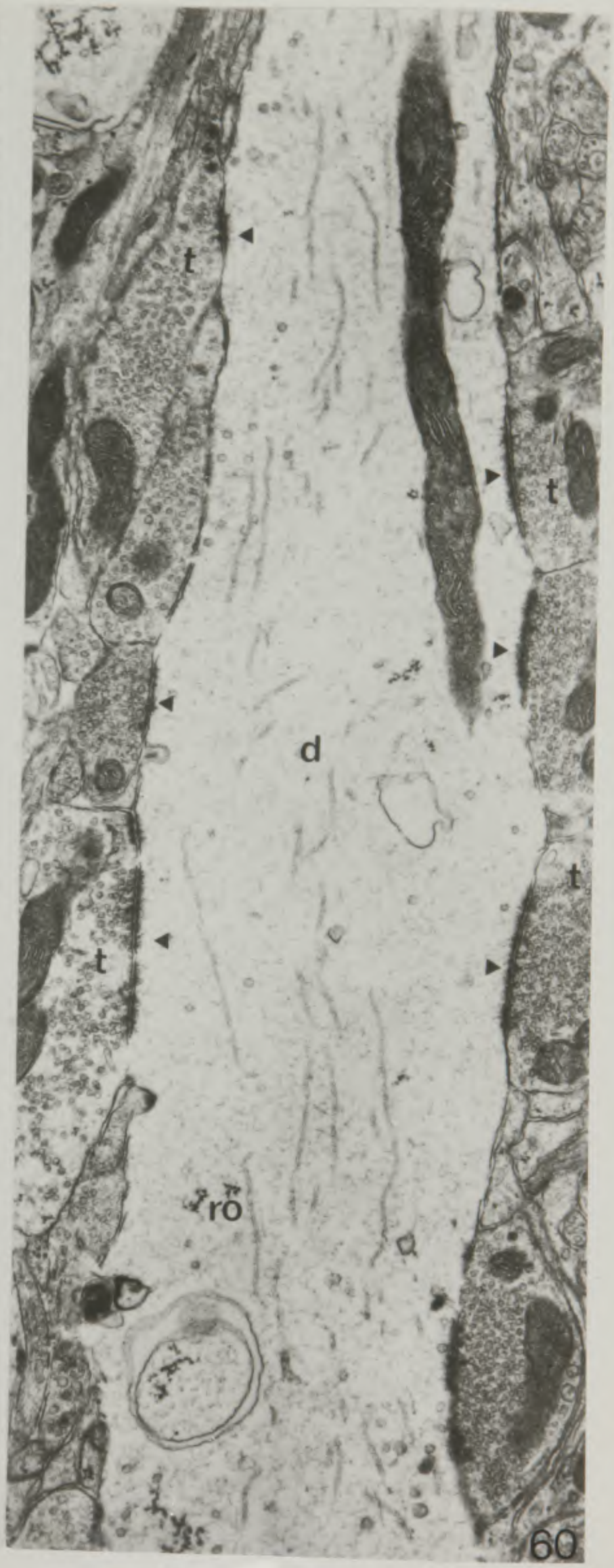

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Fig. 61 Detail of section adjacent to that shown in Fig. 59 showing the synapse from the tufted cell dendrite onto the thin perigloserular cell dendrite; note the spherical vesicles, markedly asymmetrical thickening and sub-synaptic formation of Taxi (arrow). The dendrite becomes extended into thin glia-like sheets; some of the other sheets surrounding this may well be glial in origin (see Fig. 64).

X45,200

Fig. 62 Dendro-dendritic synapse from a tufted or mitral cell dendrite onto a thin type perigloserular cell dendrite with Taxi formation and many ribosomes in the post-synaptic profile.

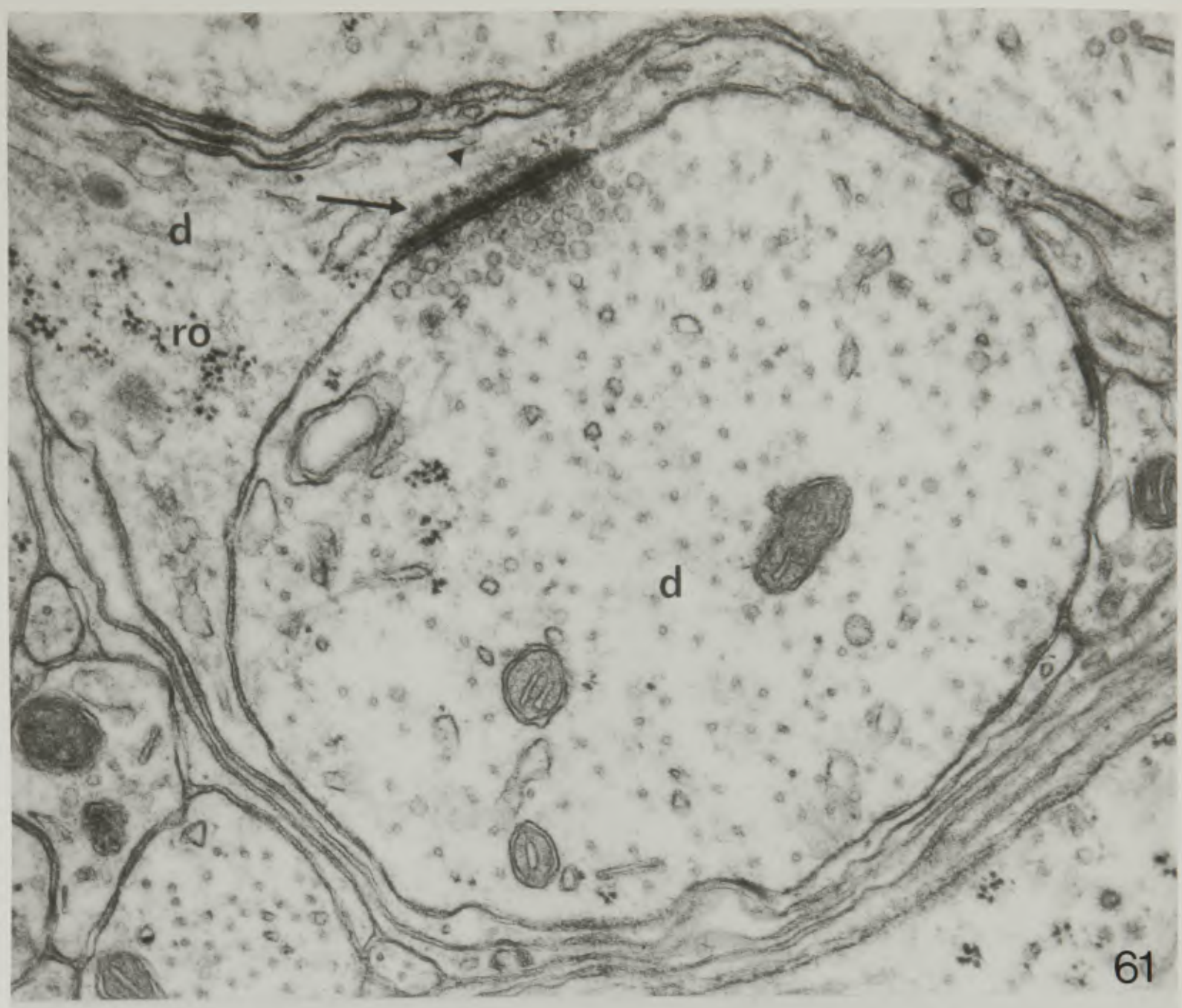
X45,500

Fig. 63 Symmetrical synapse from an axon terminal containing small flattened vesicles (compare adjacent profile containing spherical vesicles) onto a perigloserular cell thin dendrite close to the cell soma.

X45,500

Fig. 64 Longitudinal section of a tufted cell primary dendrite making synaptic contact onto a glia-like sheet derived from a perigloserular cell thin dendrite. Compare the morphology of this with the surrounding lamellae of glial origin, containing filaments and glycogen granules.

X45,200



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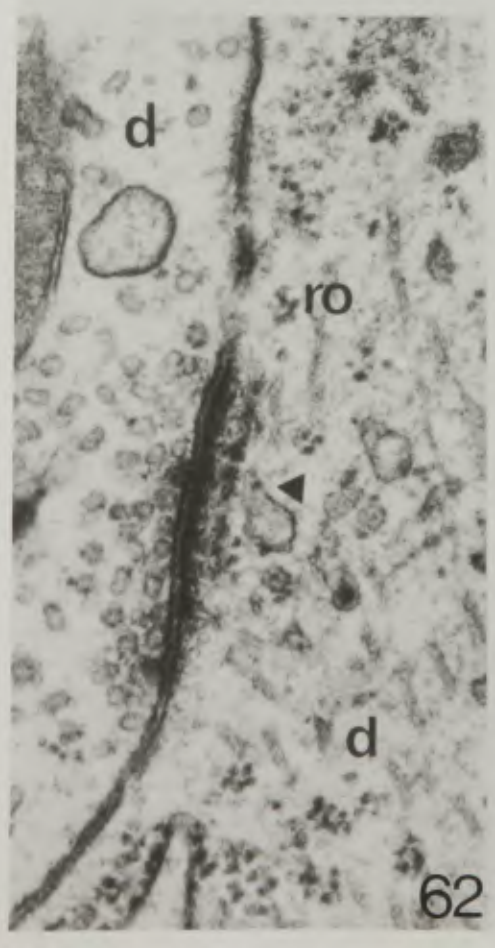

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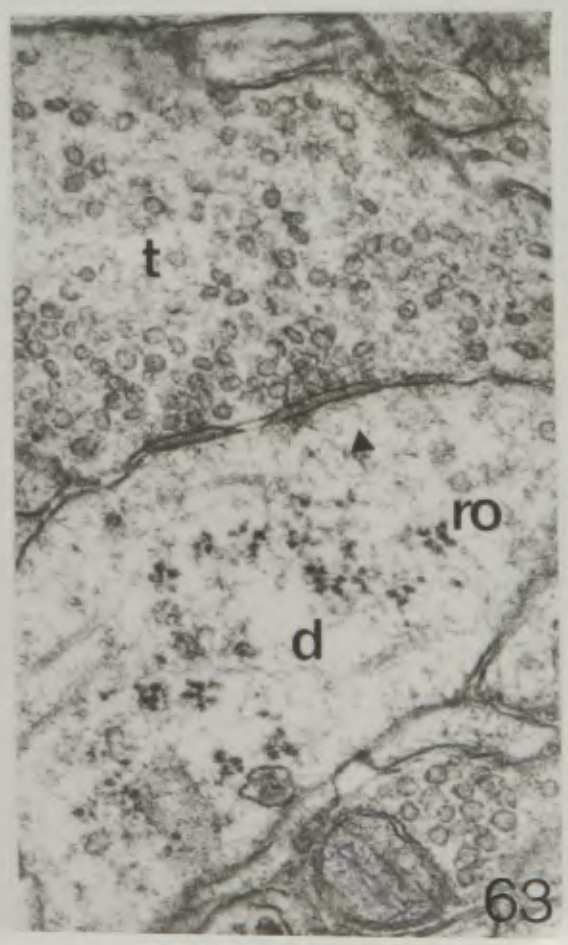
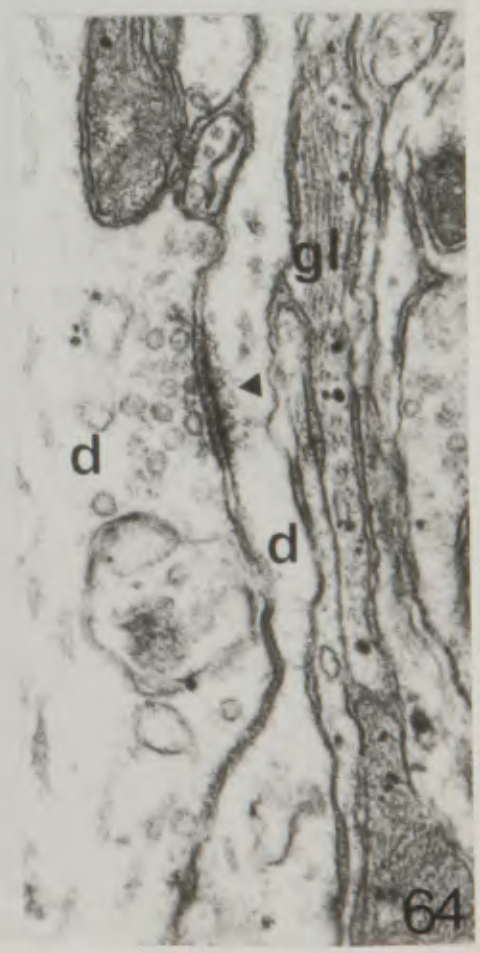


63



64

disarray and irregularity of course, and may be totally absent in some parts of the cytoplasm. Some of these dendrites have been seen to extend into thin glial-like sheets, which partially surround mitral or tufted cell dendrites (Figs. 59,61,64); they often contain little but a few ribosomes and cisternae in a rather paler cytoplasm than that of the parent process, and occasionally a few vesicles and small mitochondria. It should be emphasised that although these periglomerular cell processes, appearing like glia, surround part of the mitral or tufted cell dendrites, they do so for short distances and are not to be confused with the glial lamellae previously described around these large dendrites (Chapter 3). They are independent of these and the lamellae of glial origin either pass over them (Fig. 64) or start at each side, as if they were gemmules or axon terminals making synaptic contact with the large dendrite; they may be distinguished from the glial lamellae by their somewhat greater width, paler cytoplasm and the presence of vesicles and other organelles.

Most commonly these periglomerular cell dendrites receive one-way synaptic contacts from the mitral or tufted cell dendrites around which they wrap, never showing any indication of a return, reciprocal synapse (Figs. 59,61,62,64). Closer to the cell soma they may also receive synapses from any of the three axon terminal types of the periglomerular region (Fig. 63), but these are generally sparse and may not be present. In the post-synaptic regions of the main dendrite a slight aggregation of ribosomes (Figs. 62,63) (c.f. Bodian, 1964),

and sometimes a few vesicles or cisternae are commonly observed; such a relationship has not been noted in relation to any other synaptic contacts. Neither these dendrites nor their processes have been observed to make synaptic contacts away from them.

Short-axon cell dendrites

These large dendrites of the short-axon cells course entirely in the periglomerular region, becoming increasingly varicose the further they are from the cell soma, as shown by studies of Golgi-impregnated material (Chapter 3) (Figs. 60,65-67). They are typically seen as large segments of slightly uneven outline, at times covered by several axon terminals, none of which are olfactory nerve terminals; in transverse section they are seen as having a somewhat angular outline due to the apparent impress of the axon terminals making synaptic contact (Fig. 66). Neurotubules have a slightly irregular course, but never as markedly so as those of the periglomerular cell dendrites. Cisternae, mitochondria, multivesicular bodies and ribosomal rosettes are commonly found in these processes sometimes more concentrated beneath surface irregularities. Varicose dendrites belonging to these cells have been observed and these receive several synaptic contacts. Although a few isolated vesicles may be found in these dendrites, they are of variable shape and size and are not aggregated; no synaptic contacts orientated away from the short-axon cell dendrites have been found despite a careful search using serial sections. The vesicles are commonly related to multivesicular bodies and may bear some func-

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Fig. 65 Axon terminals making asymmetrical synaptic contacts onto a spine and the shaft of a short-axon cell dendrite that had been traced back to its cell soma of origin.

X71,200

Fig. 66 Axon terminals making asymmetrical synaptic contacts with a varicosity of a short-axon cell dendrite, cut in transverse section.

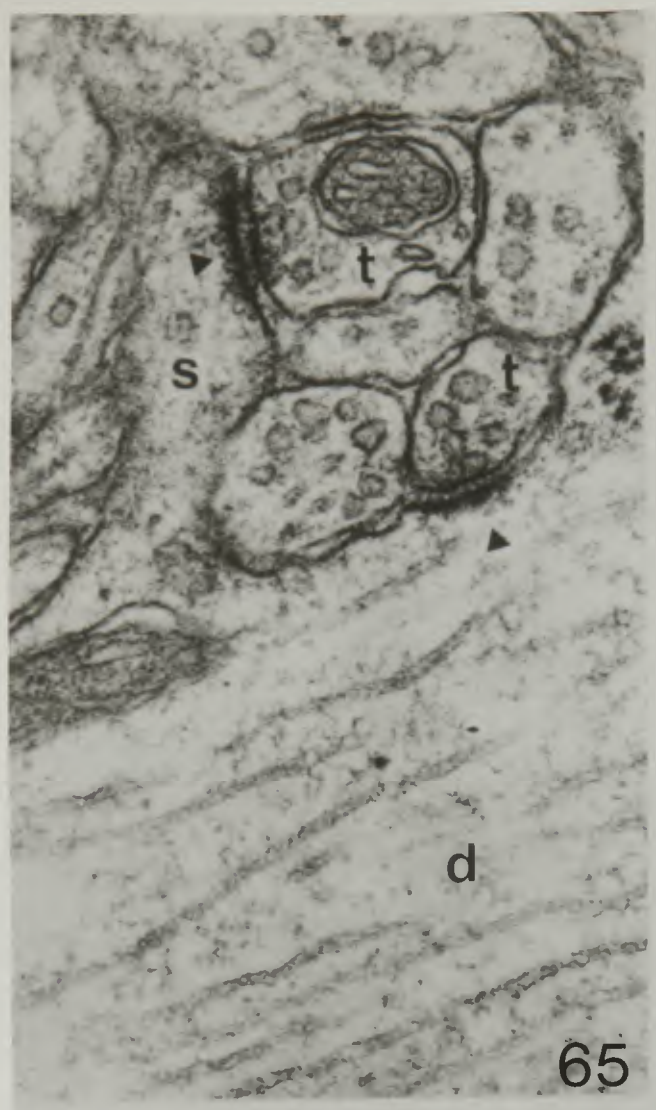
X40,000

Fig. 67 Axon terminal containing small flattened vesicles synapsing with a symmetrical thickening onto the dendritic shaft of a short-axon cell, close to the cell soma.

X75,00

Fig. 68 Part of a synaptic pattern in the periglomerular region, showing a dendro-dendritic asymmetrical synapse from a tufted or mitral cell dendrite to a periglomerular cell gemmule (note distribution of large flattened vesicles in the latter), which in turn synapses with a symmetrical thickening onto a periglomerular cell somatic spine.

X42,700



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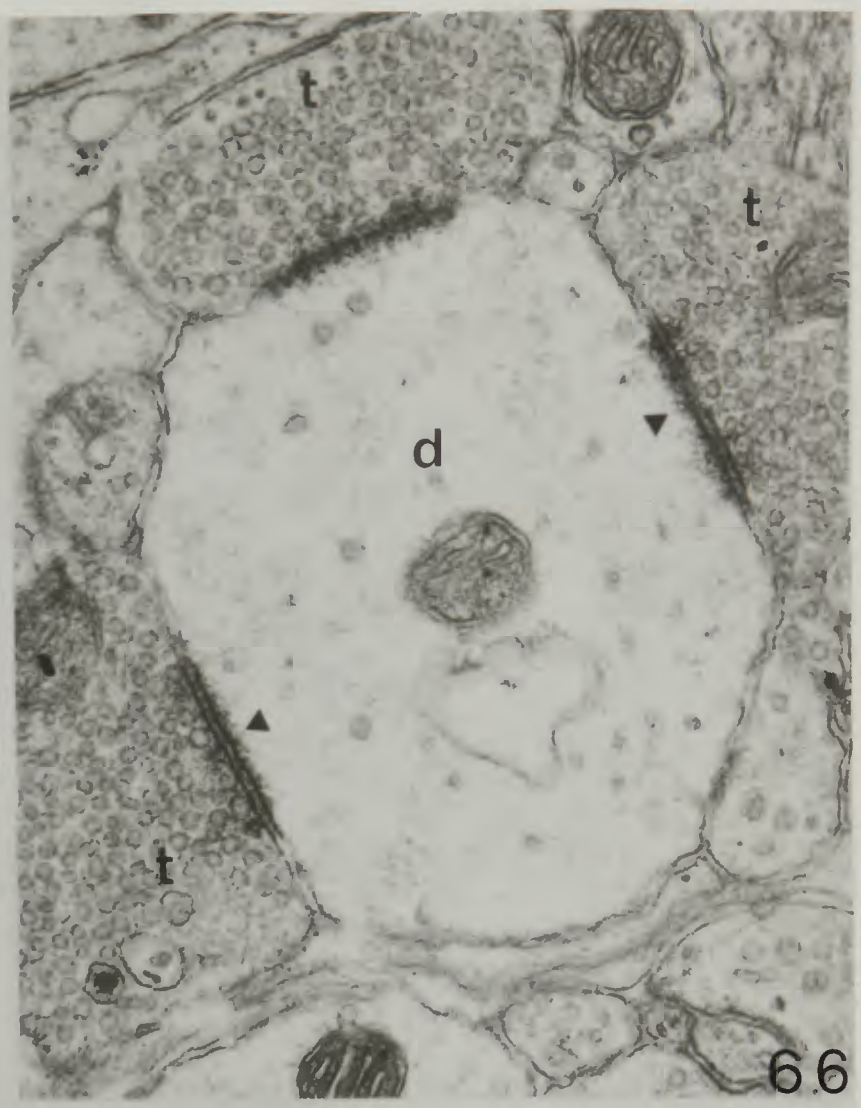

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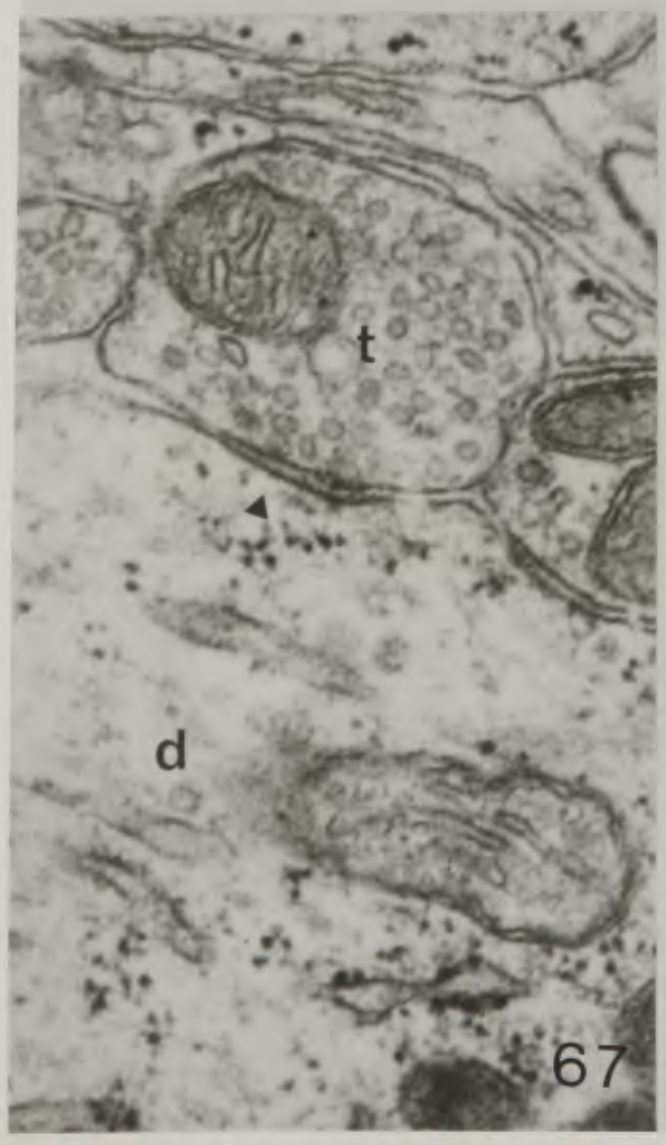

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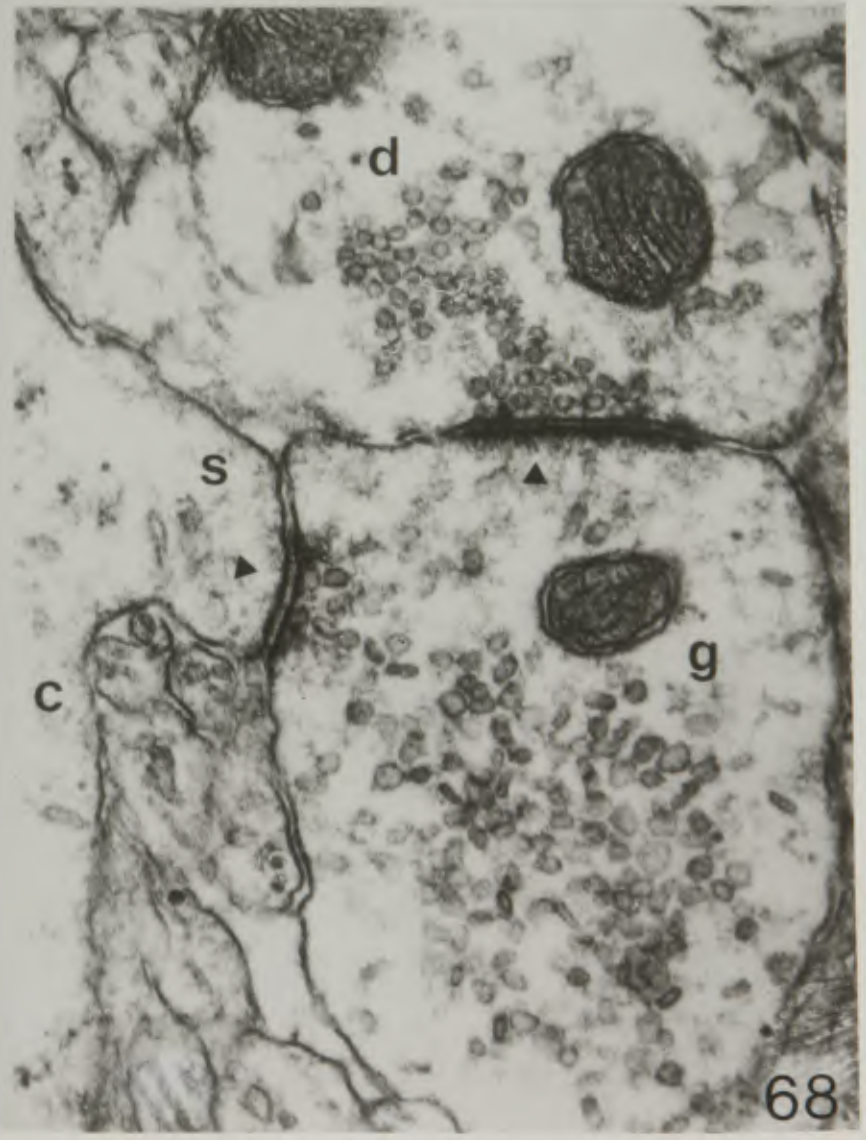


68

tional relationship to them. Spines are infrequently seen arising from these dendrites (Fig. 65), as expected from Golgi impregnation evidence; they are of typical appearance and, like those of the periglomerular cells, only show a few cisternae in association with dense material, resembling spine apparatus, but contain no vesicles.

Synaptic contacts on to the dendritic shafts of the short-axon cell dendrites may be from any of the three axon terminal classes found in the periglomerular region. Those with spherical vesicles and asymmetrical thickenings are by far the most common and are characteristically large in size (Figs. 60,66); surface protrusions, similar to those observed on the cell somata may be seen on either side of the terminal and may partially surround it. Spines always show the asymmetrical type of contact from these spherical vesicle-containing terminals (Fig. 65). Symmetrical synaptic contacts are usually made by large periglomerular axon terminals containing large flattened vesicles, but some may be found, especially close to the cell soma, containing small flattened vesicles (Fig. 67).

Synaptic patterns

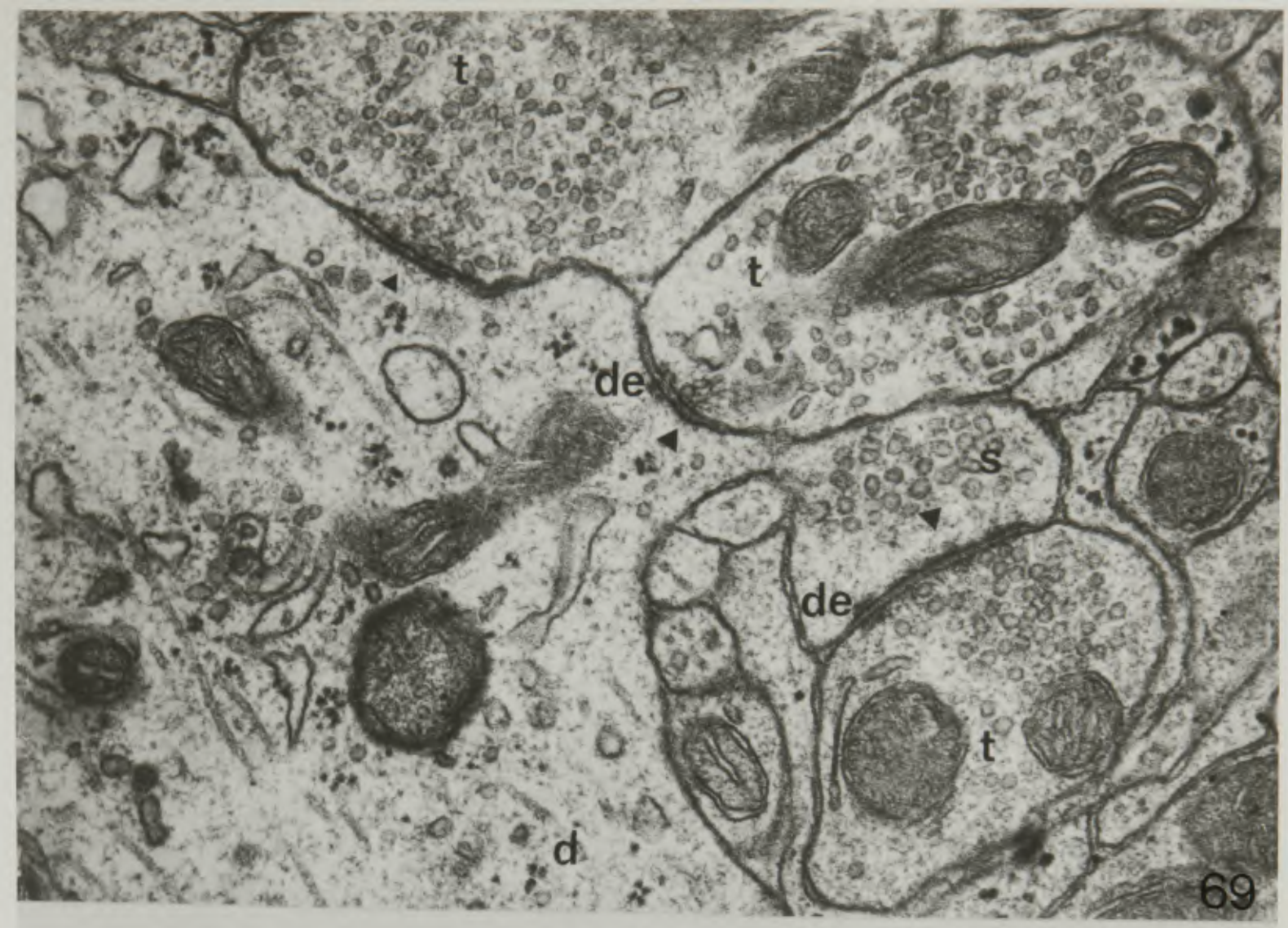
Three common synaptic patterns exist in the periglomerular region - two serial and the third related to spines. The first two are usually centred around the reciprocal synapse, although the periglomerular cell to mitral/tufted cell component of this may be missing on occasion (Fig. 68); to the reciprocal synapse is added an axon terminal containing small or large flattened vesicles and synapsing with a symmetrical

Fig. 69 Part of a synaptic pattern on a periglomerular cell dendrite and spine, traced from its cell soma of origin, and identifiable by the large flattened type vesicles in the spine. The latter receives an asymmetrical contact from an axon terminal containing spherical vesicles, with a desmosomal contact; the base of the spine and the dendritic shaft receive symmetrical contacts from axon terminals containing small flattened vesicles, one of which also has an associated desmosome. The latter terminal also made symmetrical contact with the base of another spine derived from the same cell.

X48,300

Fig. 70 Periglomerular cell dendrite with spine, the head of which receives an asymmetrical synapse, probably from a mitral or tufted cell dendrite; the side of the spine receives a symmetrical contact from a terminal containing small flattened vesicles, characteristically indistinct. The adjacent shaft receives a symmetrical contact from a periglomerular cell process containing large flattened vesicles.

X48,300



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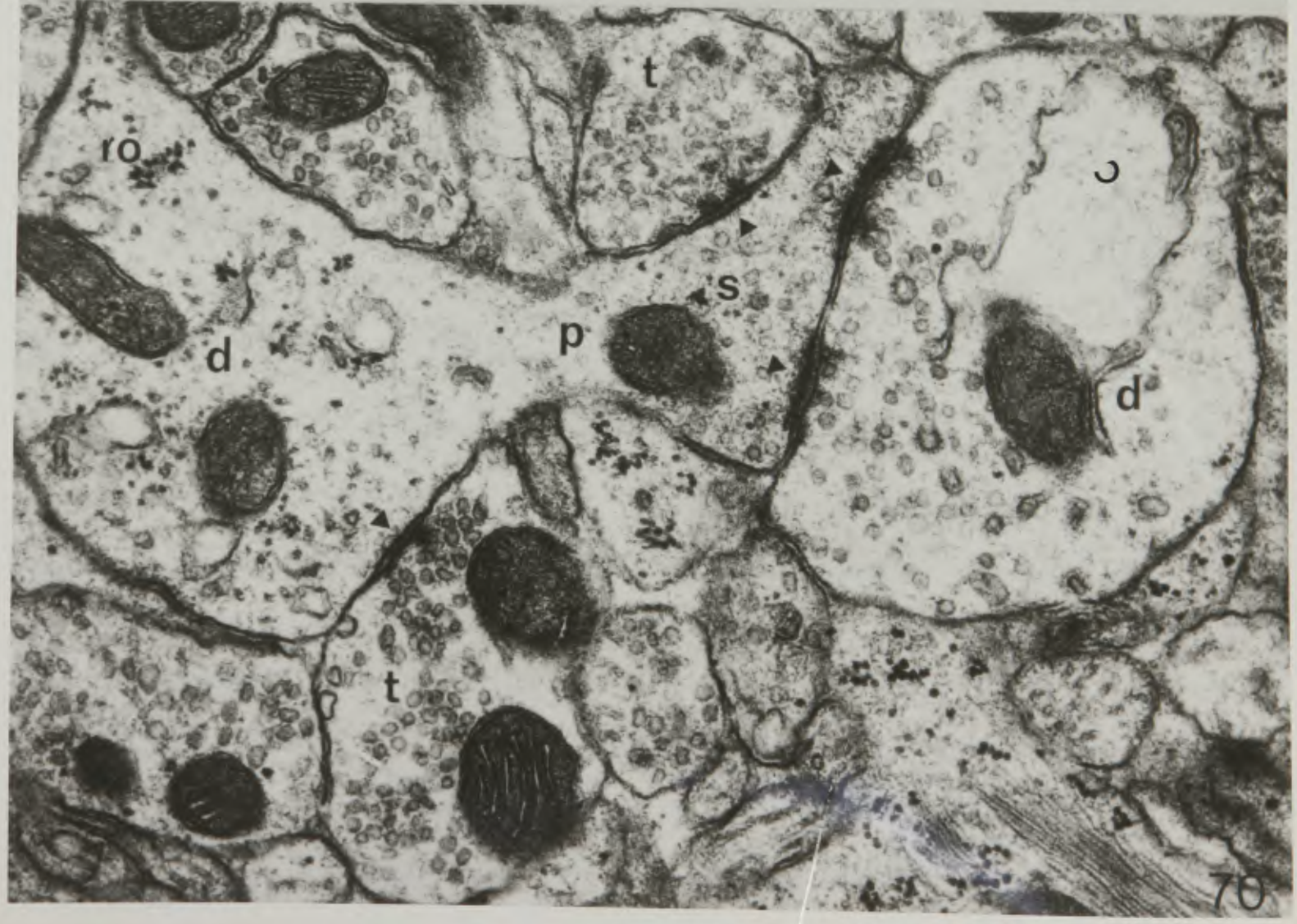


70

thickening on to the periglomerular cell component (as in the glomeruli), or a spherical vesicle/asymmetrical thickening axon terminal, also on to the periglomerular cell component (similar to that found in the external plexiform layer on granule cell gemmules: Price & Powell, 1970b,c). These two arrangements involve the axon terminals of the short-axon or periglomerular cells on the one hand, and the tufted cell recurrent collaterals or centrifugal axons on the other. In the periglomerular region the synaptic patterns involving small flattened vesicles (from short-axon cells) or spherical vesicles in the axon terminals are the most commonly encountered. The third type of synaptic pattern is related to the spines of the periglomerular cells: the head of the spine receives an asymmetrical synapse from an axon terminal containing spherical vesicles, while the side, base or closely adjacent part of the parent dendrite of the spine, receives one or more symmetrical synapses from processes containing either type of flattened vesicle (Figs. 69,70). Over half the spines examined serially showed multiple synaptic patterns and others may have done so outside the range of sections covered, but there are certainly a proportion of spines that do not; the problem of the extent of the membrane to be considered as belonging to a unit has been considered in the previous chapter.

DISCUSSION

In this chapter the neuropil of the periglomerular region of the olfactory bulb has been described, including the non-olfactory axons

and those dendrites that are solely distributed within this region. It may be useful to summarise briefly the connections between the various cell types that have been described in this and the preceding two chapters before considering them in the light of other studies on the deeper layers and other parts of the brain, as well as in terms of the possible functioning of the olfactory bulb as a relay system. In the glomeruli (Fig. 48), the mitral and tufted cells, which form a single group and never enter into direct dendritic relationship with each other, receive synapses from the olfactory nerves (not shown) and periglomerular cell dendritic processes, making return, reciprocal synapses with the latter (Fig. 48); they receive axon terminal synapses in the periglomerular region from periglomerular cell axons and occasionally tufted cell collaterals and/or centrifugal fibres. Periglomerular cells (Figs. 48,49) receive all inputs to the glomerular layer - olfactory and centrifugal (Chapter 9), as well as intrinsic connections from all synapsing processes of the glomerular layer, except periglomerular cell dendrites. With the notable exception of the olfactory nerve terminals, the short-axon cells (Fig. 50) receive synapses from all types of axon terminal and predominantly those of tufted collaterals. The axons of the periglomerular cells, the collaterals of the tufted cells and the centrifugal fibres show little specificity of terminal distribution, in terms of cell types, as they synapse with the processes of all types, although in varying proportions and at sites which are likely to have varying significance to the whole soma-dendritic tree. The axons of the short-axon cell show a greater

Fig. 48 Schematic diagram to show the dendro-dendritic relationships between the mitral or tufted cell and periglomerular cell arborisations within the glomeruli; the triangular cell body represents the tufted cell but, from the stem dendrite distally, the figure also represents the mitral cell dendritic arborisation. The mitral/tufted cell dendrites show spherical vesicles and synapse with asymmetrical thickenings while those of the periglomerular cell show large flattened vesicles and symmetrical thickenings; axon terminals of periglomerular cells synapse with the latter specialisations onto the stem dendrites. Although not shown, the olfactory nerve terminals may synapse onto any dendritic profile within the glomerulus; "a" indicates the axon of each cell, in this and the two figures following. In all these schemes the synaptic contacts, their relationships to each other and sites on the dendritic tree are based on evidence from many micrographs, but all variations are not shown.

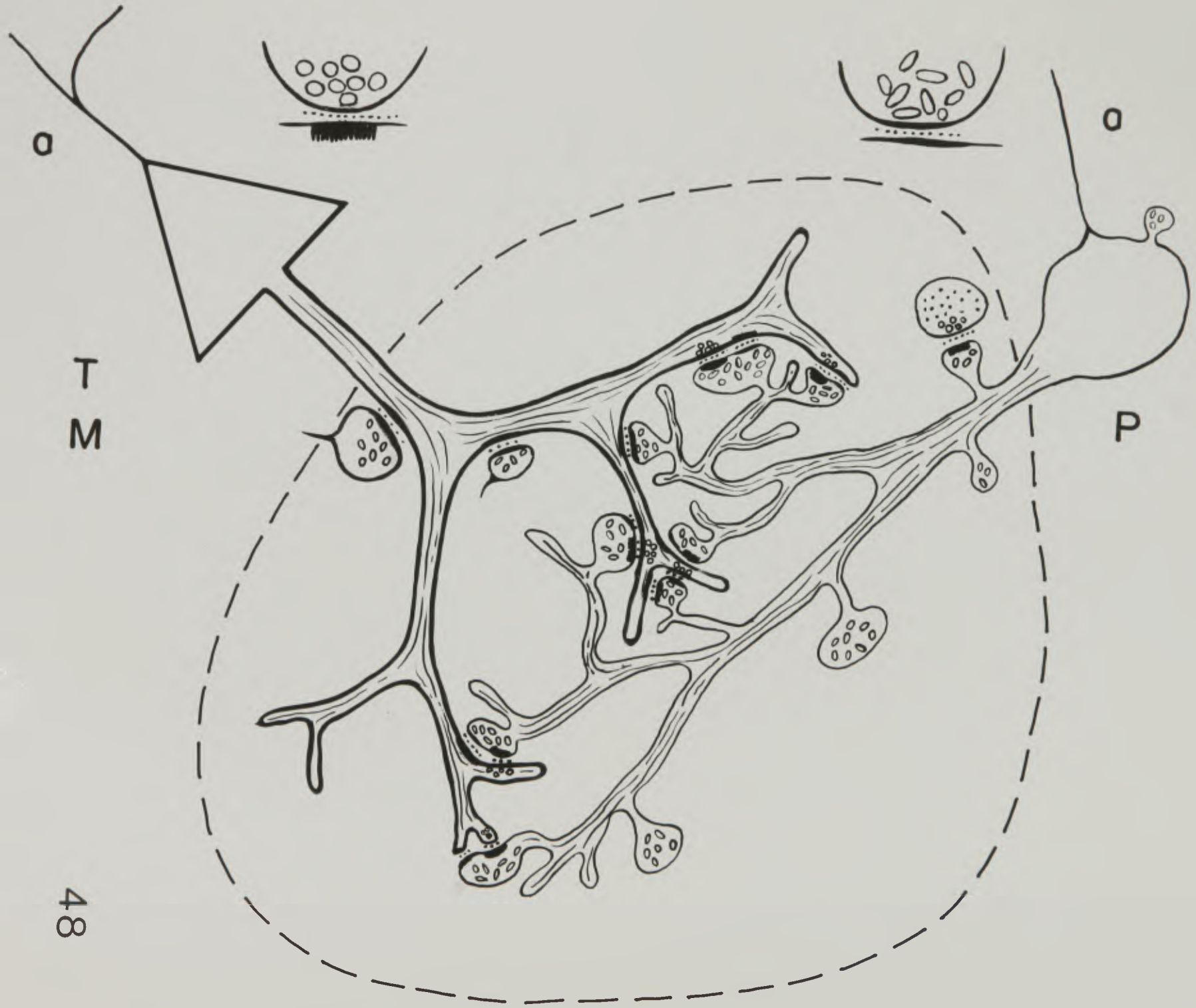

a
a
T
M
P

Fig. 49 Schematic diagram to show the additional, axonal relationships of the periglomerular cell shown in Fig. 48. The three types of axon terminal are indicated as follows.

A. Spherical vesicles and asymmetrical thickening.

B. Large flattened vesicles and symmetrical thickening.

C. Small flattened vesicles and symmetrical thickening.

A derives from tufted cell recurrent collaterals or from centrifugal fibres; B derives from periglomerular cell axons and C from superficial short-axon cell axons.


A
B
C
D
C
B
A

Fig. 49 Schematic diagram to show the additional, axonal relationships of the periglomerular cell shown in Fig. 48. The three types of axon terminal are indicated as follows.

A. Spherical vesicles and asymmetrical thickening.

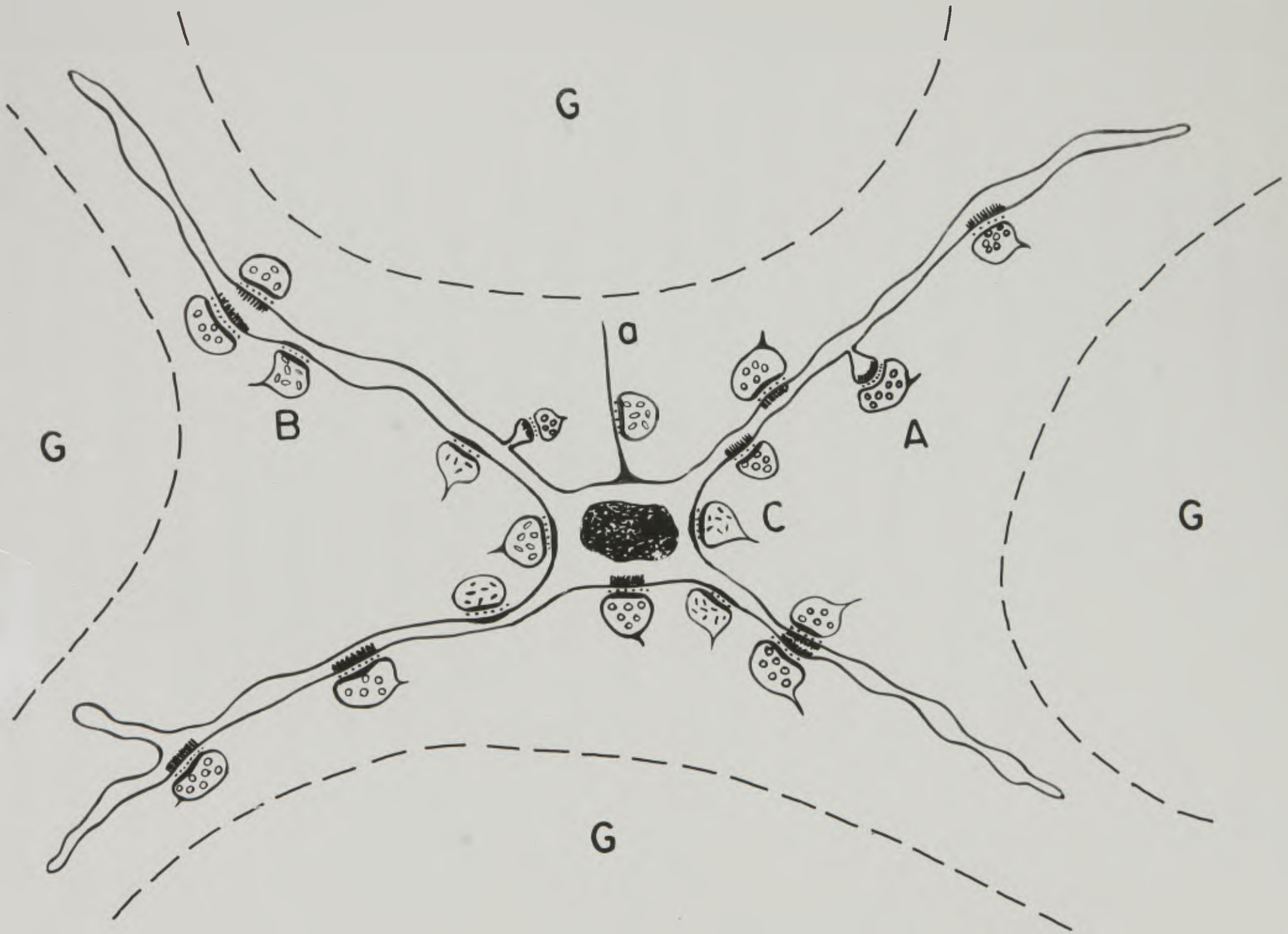
B. Large flattened vesicles and symmetrical thickening.

C. Small flattened vesicles and symmetrical thickening.

A derives from tufted cell recurrent collaterals or from centrifugal fibres; B derives from periglomerular cell axons and C from superficial short-axon cell axons.


A
B
C
a
C
B
A

Fig. 50 Schematic diagram to show the synaptic relationships of the superficial short-axon cell; symbols are the same as in the previous two diagrams.


G
a
G
B
A
C
G
G

degree of specificity, making synaptic contact with the periglomerular cells and to a lesser extent with cells of their own type; the small flattened vesicle terminals of short-axon cells have not been seen synapsing with mitral or tufted cell dendrites.

The connections that have been established in this study of normal material of the rat olfactory bulb are summarised in Table 1. Vertical columns are processes that make synaptic contact, and horizontal rows are the categories of receptive process for these synapses. In this way it can be seen on to what cells and processes a given axon, synapsing dendrite or cell soma make synapses by following the columns; on the other hand, if one wants to know the input to a given receptive process or cell, the rows may be followed. The processes synapsing with asymmetrical thickenings and spherical vesicles are currently considered to be excitatory, and those with flattened vesicles and symmetrical thickenings inhibitory. It should be clearly realised that the number of positive signs for a given relationship is intended to give only an approximate indication of the proportion of each input and should not be considered as a definitive quantitative assessment; however, the numbers have been adjusted so that both vertical and horizontal columns give the relevant proportions: for instance, the mitral cell glomerular arborisation receives rather more olfactory terminals than periglomerular dendritic synapses; the olfactory nerve terminals synapse primarily on to the glomerular arborisations of mitral and tufted cells and the dendritic shafts of periglomerular cells, but do also contact gemmules, to a lesser extent

Table 1. *Matrix of synaptic connexions in the glomerular layer*

Matrix of known synaptic connexions between neuronal processes in the glomerular layer of the olfactory bulb. o = No evidence of contact; + = definitive evidence of contact (number indicates proportion of each input); (+) = rare observed contacts.

Receiving synapse		Cell processes making synaptic contact							
		Asymmetrical thickening/spherical vesicles					Symmetrical thickening/flattened vesicles		
				Tufted cell			Periglomerular cell		
Cell	Process	Olfactory nerve terminal	Mitral cell dendrite	dendrite	soma	*SV/AT axon terminal	Dendrite/ gemmule	Axon terminal	Short-axon cell axon terminal
Mitral cell	Glomerular arborization	+ + + +	o	o	o	o	+ + +	o	o
	Periglomerular shaft	o	o	o	o	+	+ +	+ +	o
Tufted cell	Glomerular arborization	+ + + +	o	o	o	o	+ + +	o	o
	Periglomerular shaft	o	o	o	o	+ +	+ +	+ +	o
	Soma	(+)	o	o	o	+	+ +	o	o
	Axon initial segment	o	o	o	o	o	o	(+)	o
Periglomerular cell	Dendrite	+ + + +	+ +	+ +	o	+ +	(+)	+	+ +
	Spine/gemmule	+ + (only gemmules)	+ + +	+ + +	+ +	+ + +	(+)	+	+
	Soma	(+)	+	+	+	+	o	+	+ +
	Axon initial segment	o	o	o	o	o	o	+	+
Short-axon cell	Dendrite/spine	o	o	o	o	+ + +	o	+ +	+
	Soma	o	o	o	o	+ +	o	+ +	+
	Axon initial segment	o	o	o	o	o	(+)	+	o

* 'SV/AT axon terminals' are those pale axon terminals in the periglomerular region that synapse with asymmetrical thickenings and spherical vesicles; the group comprises tufted cell recurrent collaterals and centrifugal fibres. For details of use of Table see text.

(very rarely these contacts are seen on to tufted or periglomerular cell somata).

In the scheme that emerges from this analysis of the connections of the various cell types in the glomerular layer, the tufted cells, which may be considered as relay cells together with the mitral cells by virtue of their central connections (Lohman & Mentinak, 1969; Nicoll, 1970a), are contacted primarily by olfactory nerve fibres and by processes of periglomerular cells, but also by centrifugal axons and tufted cell collaterals; the periglomerular cell may therefore be seen as the primary interneuron acting upon the relay neurons and receiving all available inputs, including feed-forward olfactory, and mitral/tufted dendrodendritic. The short-axon cell, which receives principally external tufted cell collaterals and centrifugal fibres, as well as interneuronal connections, acts only by way of the periglomerular cells; thus it may be considered as a secondary interneuron, acting on relay neurons only through the mediation of the primary interneurons and receiving information predominantly of a feedback nature, with no feedforward component from olfactory nerve fibres.

Although there are few clear specificities in the connections of the glomerular layer, most variation being in the proportion and site of any particular input, such specificities as there are, seem to be clear indications of specificity of function; it is these that provide a clue to the nature of the organisational principles involved. Since the information provided by the methods used here can only relate to cell types and not to individual cells, much greater specificity may be

found for the latter, although the broad outline gained from type studies must necessarily provide the framework for such individual specificity of connections.

The question of the morphological basis for excitation and inhibition has been repeatedly raised in recent papers and the relevant evidence is well known (e.g. see Eccles, 1964; Uchizono, 1965; Uchizono, 1968; Gray, 1969); this problem is very relevant to studies such as these, in which the clear differences in vesicle shape and associated membrane thickenings are used as criteria for different cells of origin. Although the correlation between spherical vesicles and asymmetrical thickenings with excitation, and flattened vesicles and symmetrical thickenings with inhibition cannot be regarded as proven, there are many indications in its favour. Its tenets will therefore be assumed in the following discussion of this work on the olfactory bulb; if, however, the suggestion is clearly and unequivocally disproved in some way, then although discussion of functional implications must become less valuable, the observations themselves should still be valid. Certainly the fact that the olfactory input and relay neuron output are of the spherical vesicle/asymmetrical thickening type would be consistent with already available physiological evidence (e.g. Yamamoto, Yamamoto & Iwama, 1963; Shepherd, 1963a; Rall & Shepherd, 1968; Biedenbach & Stevens, 1969; Shepherd, 1971); similarly the presence of flattened vesicles and symmetrical thickenings in the periglomerular and short-axon cells would be consistent with current views that interneurons tend to have inhibitory actions (although

there are well-defined exceptions to this outside the main sensory pathways). The polarities of reciprocal synapses further support the correlation for the mitral/tufted and granule/periglomerular cells in the light of anatomical, physiological and theoretical studies (Rall et al, 1966; Rall & Shepherd, 1968; Nicoll, 1969; Price & Powell, 1970b; Shepherd, 1971). However, the broad terms inhibitory and excitatory could turn out to be confusing in that the mode of action of any synapse may well be modified by its site on the post-synaptic process; it may be wiser to consider them as locally hyperpolarising and depolarising influences. It could be suggested that the type of specialisation on the post-synaptic process is the primary determining factor in these influences, and that vesicle shape, although presumably reflecting transmitter identity, may be only secondarily associated with post-synaptic events.

The analogies between the cell types of the glomerular layer and those of the deeper layers have already been discussed (Chapter 3), but their synaptic organisation may now be reviewed in the light of the findings presented in the last two chapters. The mitral cells and all tufted cell groups form a single class, characterised by their common olfactory input, their central axon projections via the lateral olfactory tract, and their synaptic characteristics. Both subgroups of the relay class - mitral/deep tufted and external tufted receive periglomerular cell dendritic and axonal connections within the glomeruli and periglomerular regions, but only the dendrites of the first group are related to the granule cells, by reciprocal synaptic

contacts. Within this deep group, the mitral cells probably receive more granule cell contacts than the internal and middle tufted cells, and the glomerular arborisations of these subgroups would suggest that the deeper, larger cells may also receive more periglomerular cell (and olfactory) influences; the extent of the secondary dendrites of these cells will also vary the proportion of granule cell contacts. The observation that some (middle) tufted cells may enter into reciprocal contacts on their axon initial segments may indicate a possible further subdivision of the primary relay class.

The periglomerular cells and granule cells have notable similarities and differences, a consideration of which may be instructive; both types of cell possess a gemmule-bearing dendrite or process - that is, the peripheral process of the granule cell and the glomerular dendrite of the periglomerular cell - which enter into reciprocal synaptic relationship with the mitral or tufted cells. Spines are also found on both the granule cell peripheral process, in its passage through the granule cell layer, and on the periglomerular cell dendrite, in the periglomerular region. A major and significant difference is that the periglomerular cell has a true axon, while the granule cell has none, but exhibits, in addition, typical spine-bearing dendrites (deep); the periglomerular cell has, further, a glia-like type of process, which does not give rise to appendages. The synaptic characteristics of the two granule type cells are very similar, each having the same class of large flattened vesicles and showing symmetrical membrane thickenings in synaptic contacts orientated from the cell;

the type and arrangement of synapses on to the two cells are similar, although the origins of axon terminals synapsing on to them are different.

From this comparison of the two cells a fundamental point emerges - the gemmule-bearing or synapsing process of the granule cell is the more axon-like of this cell's processes (Price & Powell, 1970a), while the morphologically equivalent process in the periglomerular cell is the more dendritic of this cell's process; yet both of these gemmule-bearing processes receive and give off synapses of a reciprocal nature as well as receiving synapses from axon terminals of various origins. In view of this dual role of the granule cell peripheral process and the periglomerular cell glomerular dendrite, it would seem necessary to propose that these structures represent processes intermediate between axon and dendrite, together with the dendrites of the mitral and tufted cells, the dendrites described in the superior colliculus (Sétálé & Székely, 1967; Lund, 1969) and other sites (Ralston, 1968; Ralston & Herman, 1969; Guillery, 1969; Famiglietti, 1970). It might perhaps be opportune therefore to give such processes a separate name which frees them from the rigid division, originating in classical light microscopy, between axon and dendrite. Since any additional distinction could become equally dangerously rigid, we propose a preliminary terminology that may be extended and more closely defined by further and broader physiological and anatomical studies. The characteristic morphology of dendrites and axons in light and electron microscopy makes some retention

of these terms useful, so we propose that dendrites which receive but do not give rise to synapses be termed Class A Dendrites; processes of dendritic general morphology which both receive and give off synapses would then be called Class B Dendrites. It must be emphasised that this distinction and classification is intended as an aid to understanding and description and as a preliminary to further, functionally orientated classifications.

There can be no doubt as to the existence of Class A dendrites, and numerous descriptions may be found in the literature dealing with many regions (notably the cerebral cortex); in the olfactory bulb, the dendrites of short-axon cells and the deep dendrites of granule cells fall into this category (it should be noted that the observation of vesicles in a dendrite is not, on its own, evidence that a synapse arises from it, and cannot be used as a criterion for a Class B dendrite). The Class B dendrites are found in cells without axons - amacrine and granule cells - but may also be found in cells having typical axons - mitral, tufted, periglomerular cells. It seems likely from these examples that a cell may either possess Class A dendrites and an axon (e.g. short-axon cells of the olfactory bulb), Class B dendrites and an axon (e.g. periglomerular cells) or Class A and Class B dendrites (e.g. granule cells). It is evident that Class B dendrites could be further subdivided according to the presence (e.g. periglomerular cells) or absence of appendages (e.g. mitral and tufted cells). It is quite possible that the Class B dendrites have been seen before, in addition to these sites in which they have been distinguished as synapsing

dendrites, but have been described as axons on account of their synaptic behaviour or their appearance in a particular plane of section; for instance, some component of the glomeruli of the thalamus (Majorossy et al, 1965; Jones & Powell, 1969b). Although the observation of a glia -like process or thin dendrite on some periglomerular cells could be taken to suggest that these form a further class of dendrites, it is felt that there are not sufficient precedents of such a dendritic type to allow classification as a third class at the present time.

In terms of their apparent role in the organisation of the olfactory bulb and their relationship to the relay neurons, the granule cells occupy the same primary interneuronal position as the periglomerular cells, while both deep and superficial short-axon cells appear as secondary interneurons, in view of their principal connection with the granule and periglomerular cells respectively. The periglomerular and granule cells receive predominantly extrinsic connections, but are also related to local interneurons and relay neuron collaterals; the granule cells appear to be the main site of termination of extrinsic fibres of central origin, while the periglomerular cells receive essentially the same olfactory nerve input as the relay neurons, in addition to a small centrifugal input (Chapter 9). It is interesting to note that by means of the secondary dendrites of the mitral cells and to a lesser extent from the branching of their own peripheral processes, the granule cells are connected with a large group of mitral cells by reciprocal contacts. The periglomerular cell Class 3 dendrites, on the other hand, relate only to the relay neurons in the one or two

glomeruli that they serve, which is clearly a more localised effect; however, these cells also have a wide effect by means of their axons, which act on the relay dendrites of up to four glomeruli distant (Blanes, 1898; Cajal, 1911; Chapter 3) will be recalled that these axons are situated in a potentially powerful site for inhibition (Chapter 3); furthermore, a spread of four glomeruli is the same order of magnitude as the length of the mitral cell secondary dendrites (Price & Powell, 1970d). Thus, apart from the centrifugal fibres to the glomerular layer, the central extrinsic pathways to the bulb acting through the granule cells, will tend to have a wide effect; the olfactory extrinsic fibres will have a localised effect directly on the relay neurons and by dendro-dendritic contacts through the periglomerular cells, and a wide effect by means of the periglomerular cell axon-dendritic connections. Both influences may be made more effective by means of the return dendro-dendritic synapses from the mitral cells and tufted cells. Thus, if, as is likely, the two elements of the reciprocal synaptic contacts are simultaneously excited by olfactory nerve volleys, then they will probably act simultaneously upon each other, depending on local conditions; in this way, the periglomerular element would perform immediate local inhibition on the relay neuron, after the primary depolarisation due to the olfactory nerve volley, and the relay neuron element would act to reinforce the effect of the same olfactory input to the periglomerular cell, in order to give rise to a wider effect by means of an axon spike discharge in the periglomerular cell. It is clear however that such an analysis is

an oversimplification, since it does not take into account ongoing activity in relay and interneurons, and can provide little clue to the events occurring through several synapses and interneurons, both locally and at a distance; it may at least provide a starting point for investigation of the functioning properties of the relay.

Although it has not been possible to analyse the connections of the deep short-axon cells in detail (Price & Powell, 1970d), certain parallels with their superficial counterparts emerge, in terms of their broad organisation and synaptic relationships. Recurrent collaterals of mitral and tufted cells act on the deep short-axon cells and collaterals of external tufted cells on the superficial short-axon cells; the extrinsic connections of central origin characteristic of both sets of layers make limited contact with these cells, which also receive the axon terminals of local interneurons (but no contact from granule cells). Collaterals, which are the main input to the short-axon cells, are derived from a wider area than the interneuronal input to these cells; the collaterals will carry information as to the effectiveness of the various afferents to the relay neurons in causing an axonal spike discharge in the latter. Such information will act by means of the granule cell Class B dendrites or the periglomerular cell Class B dendrites and axons, having been mixed with local activity as well as more generalised centrifugal input in both short-axon and granule or periglomerular cells; a small direct excitatory effect will also be mediated by the direct collateral contacts on to the mitral/tufted primary dendrites.

The significance and functional nature of the synaptic patterns described in these chapters is at present difficult to assess without detailed biophysical knowledge on spine function and local dendritic integration; such problems are currently being investigated in several laboratories (Rall & Shepherd, 1968; Diamond et al, 1970). The striking proximity of several synapses of particular types and the regularity of their occurrence makes it highly probable that these arrangements have a special functional role and are not chance occurrences; the criteria used to establish a synaptic pattern and its extent have been discussed in a previous chapter (Chapter 4). The reciprocal synapse itself has been widely discussed and its functional capacities assessed (Rall et al, 1966; Rall & Shepherd, 1968; Price & Powell, 1970; Shepherd, 1971) and will not be further considered here. However, the close relation of the reciprocal synapse to other synapses in several of the synaptic patterns observed in the glomerular layer could be taken to suggest that these patterns relate to reciprocal synaptic function itself and represent a means of local modification of this activity. This will in turn be affected to a greater or lesser extent by the general activity in the dendrite; it is the extent and nature of these modifications and interactions that are in doubt.

As discussed in the previous chapter, activity in a gemmule or spine may be more or less isolated from that in the parent dendritic shaft; otherwise transfer of potentials from one to the other may be by means of a spike potential arising in the appendage itself or by

electrotonic spread. These various theoretical alternatives make it difficult to assess the possible functional implications of, for instance, a symmetrical synapse situated on the shaft of a dendrite opposite the origin of an appendage, as opposed to one placed at the base of the pedicle. It is interesting to note that small 'glomeruli' of about ten profiles may occur in the periglomerular region, and are surrounded by a glial wrapping. These may represent the functional subdivisions of dendritic activity and include the whole of a synaptic pattern unit, but it is not possible to verify this in most cases. Thus although there can be little doubt as to the functional importance of the observation of these various synaptic patterns, just what form this takes is still quite obscure, and must remain so until more data is available on the interrelationships of different parts of dendritic trees.

Although this analysis of the structure and connections of the glomerular layer of the olfactory bulb is not yet complete, further knowledge being available from experimental studies on the distributions and location of various processes (Chapters 7-10), a clear picture is already emerging of the tremendous complexity and information capacity that lies in the cellular organisation of the olfactory bulb. It is interesting to note that this complexity has its origins in several diversities: a diversity of <u>types of process</u>, of <u>modes of cellular interrelationships</u> and of <u>synaptic arrangements</u>. Thus the olfactory bulb, so long regarded as a simple relay system, turns out to be a highly complex neuronal network, and may serve to indicate the order

of complexity that may be expected from regions already recognised as intricate. Some features shown by the bulb seem to be characteristic of the peripheral parts of sensory pathways; the extent to which they can serve as a model for other pathways and more central regions remains to be determined. Any variations in the mode of interaction at a given site may reflect an essential functional difference in that site, in terms of the type of neuronal transformation involved.

CHAPTER 6

MYELINATED DENDRITIC SEGMENTS IN THE MONKEY OLFACTORY BULB.

INTRODUCTION

The long primary dendrites of the mitral cells of the olfactory bulb are unbranched as far distally as the glomeruli, in which they arborise profusely among the incoming nerve fibres; it has been noted from studies on the rat (Chapter 3) that the portion of the primary dendrite passing from the superficial part of the external plexiform layer through the periglomerular region up to the primary glomerular branching point is covered by several thin cytoplasmic lamellae of glial origin. Observations on this site in the monkey have demonstrated that these dendritic segments have become myelinated in this species.

RESULTS & DISCUSSION

The monkey olfactory bulb is about twice the size of that of the rat and shows the same layers; its electron microscopic features and synaptic relations appear broadly similar to the rat (Chapters 3-5). An immediately striking difference, however, is the presence of a thin myelin sheath specifically located around the segment of the large mitral and tufted cell primary dendrites in the most superficial part of the external plexiform layer and in the periglomerular region (Figs. 71-73,75-77); this site corresponds well with the portion of these dendrites that is covered by several thin glial lamellae in the

Fig. 71 Low magnification view of a mitral cell primary dendrite passing from the periglomerular region into the glomerulus; note the thin myelin sheath. Monkey.

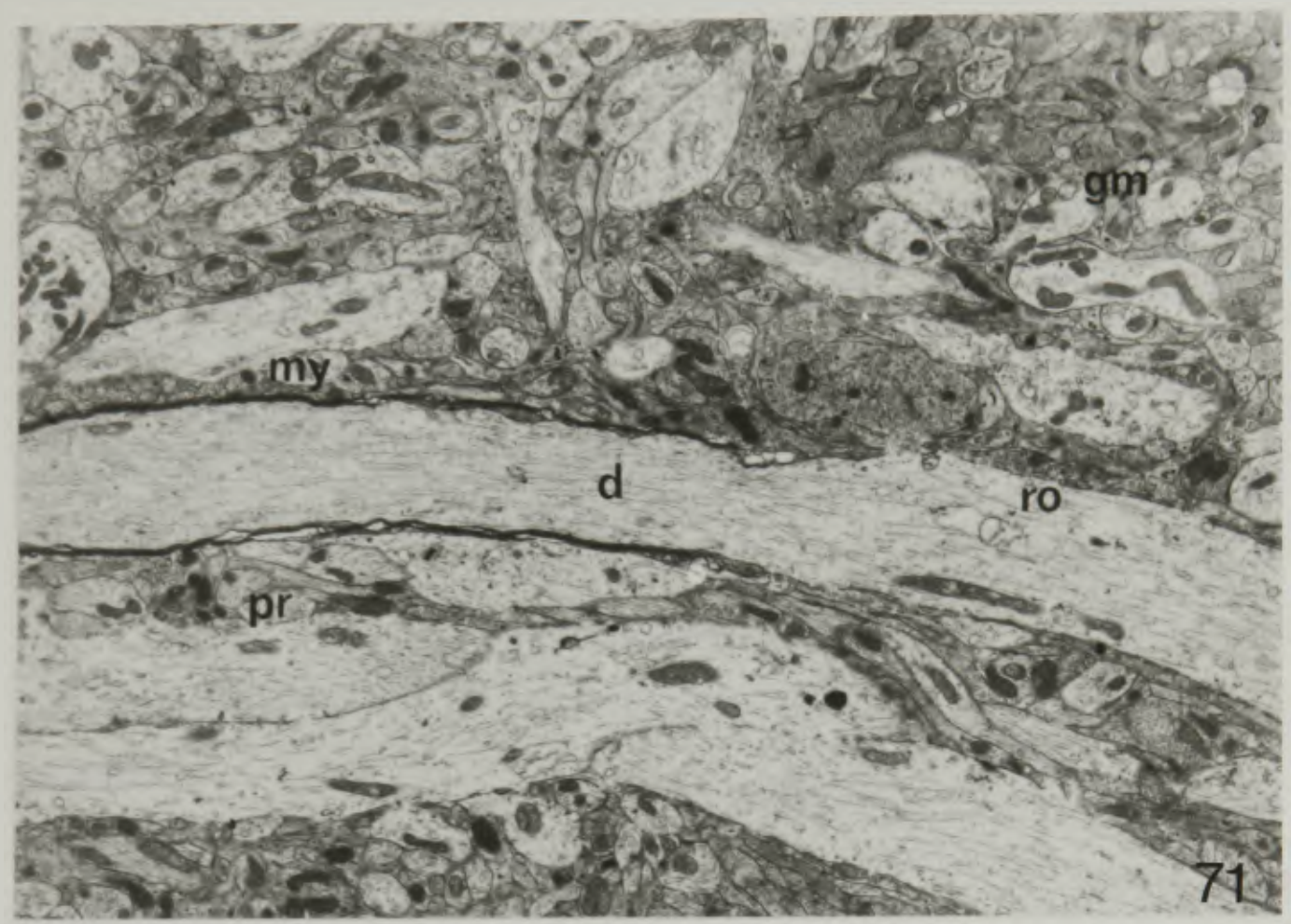
X5,500

Fig. 72 Detail of the myelin sheath of a tufted cell primary dendrite showing marked inner glial tongue and inner nesaxon (arrow). Monkey.

X67,000

Fig. 73 Myelin sheath of a mitral cell primary dendrite with inner and outer glial tongues and end expansions of glial cytoplasm. Monkey.

X53,500



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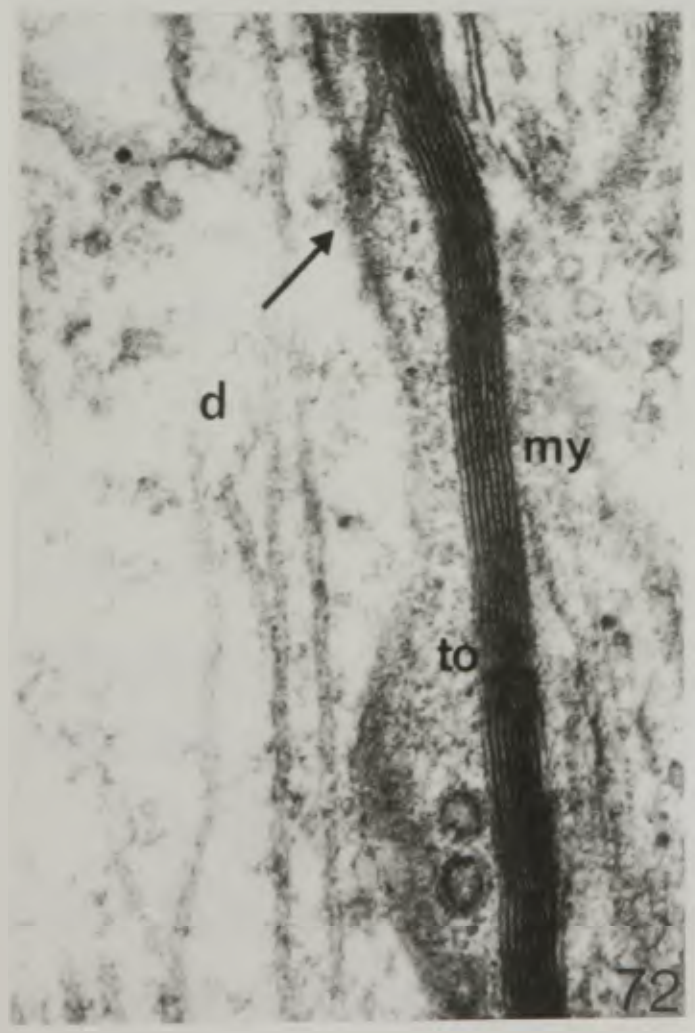

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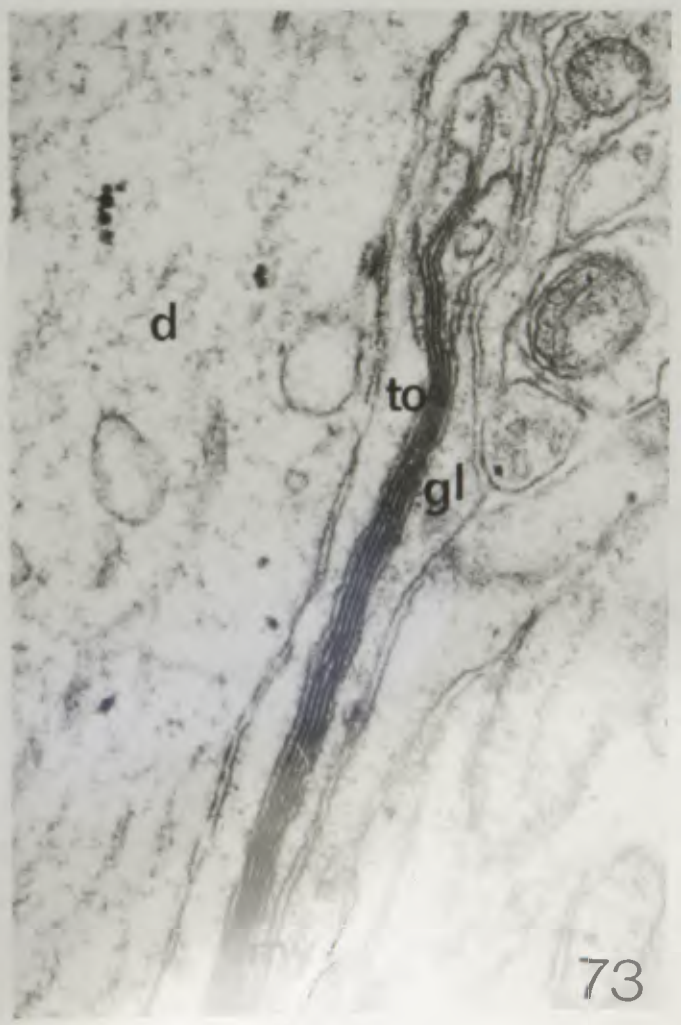

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rat (Fig. 74) and rabbit. Several features of these myelinated processes show that they are genuine dendrites: (a) their clear continuity, both proximally and distally, with lengths of unmyelinated dendrite, recognisable by frequent dendro-dendritic reciprocal synapses characteristic of mitral and tufted cell dendrites or, in the case of the latter, with their parent cell somata; (b) by their site and orientation, these dendrites being the only processes to pass from the external plexiform layer into the glomeruli; (c) by virtue of their diameter (4-10µm) - a size never reached by any axon in the olfactory bulb; (d) by other morphological features - ribosomal rosettes, cisternae, regular neurotubules and few filaments (Figs. 73,75-77). These myelinated dendritic segments have been repeatedly observed in stained light microscopic sections and by phase contrast microscopy; their specific location is very evident with these methods, which have the additional advantage that much larger areas can be examined at a time. Very occasionally an external tufted cell soma shows thin myelination, extending for a short distance over the dendrite, reminiscent of the specific myelination described by Rosenbluth (1962a); it may be pointed out that the cell somata of these external tufted cells are in an exactly analogous position with respect to their glomerular arborisations as the myelinated dendritic segments of mitral and deep tufted cells are to theirs.

With electron microscopy, the myelin shows 4-8 period lines (Figs. 72,73,76,77), which is thin in comparison with that of an axon of equivalent diameter; the ratio of internal to external diameters

Fig. 74 Glial lamellae surrounding mitral cell primary dendrite in the rat periglomerular region; note the glial cytoplasm within each lamella. Rat.

X53,500

Fig. 75 'End feet' of myelin at the end of a myelinated segment of a tufted cell primary dendrite; note the large amount of glial cytoplasm outside the myelin. Monkey.

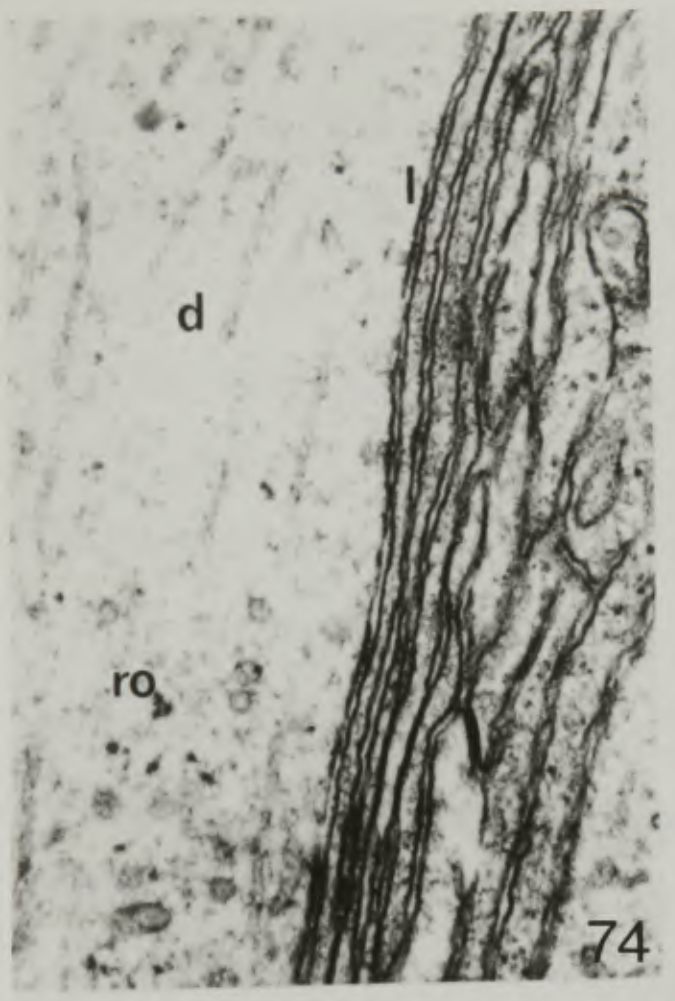
X47,500

Fig. 76 Myelinate segment of mitral cell primary dendrite, showing characterically regular array of neurotubules. Monkey.

X45,000

Fig. 77 Myelinated segment of mitral cell primary dendrite showing ribosomal rosettes. Monkey.

X45,000



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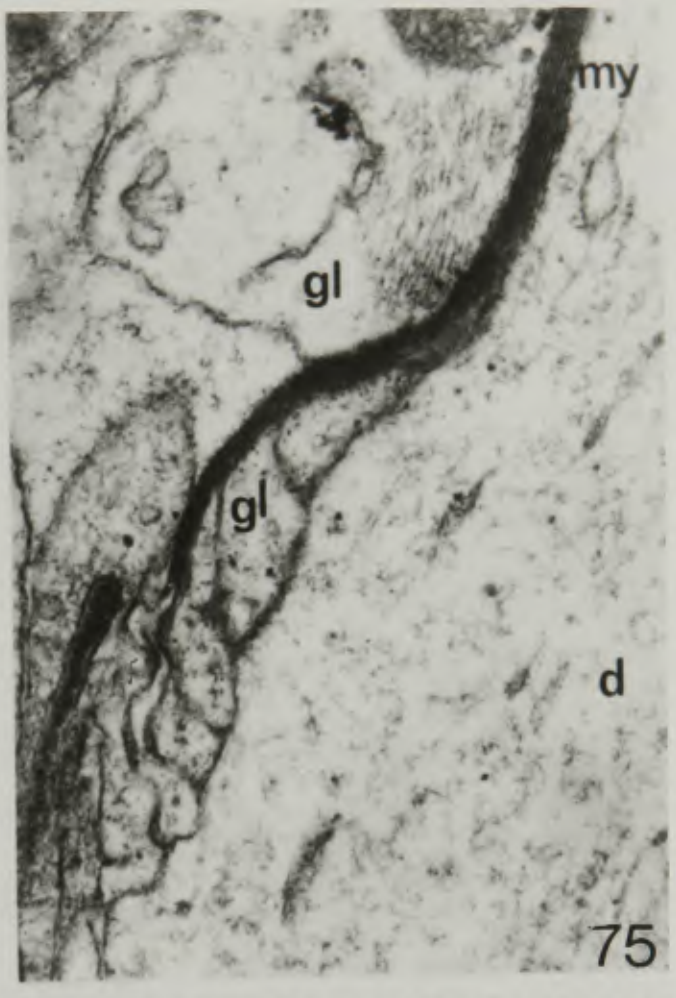

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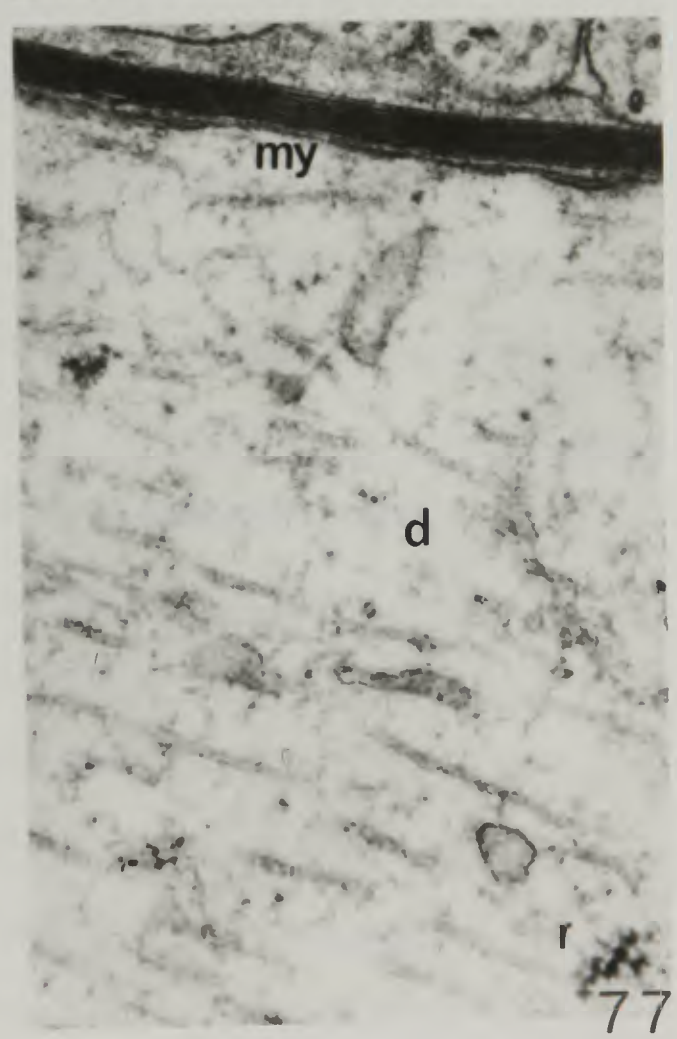

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(g) ranges from 0.90 to 0.95. The segments observed have been 20-60um in length. Typical feet of glial cytoplasm are seen at each end of the segment (Figs. 73,75) and are occasionally followed by a second segment of myelination; unlike most axonal myelin in the central nervous system (Peters et al, 1970), the myelin sheaths of these dendrites are surrounded by much glial cytoplasm, which may contain glycogen granules or filaments (Figs. 73,75). Most unequivocal examples of myelinated dendritic segments have been identified in longitudinal section, but they are often recognisable in other planes of section. It is notable that reciprocal synapses with gemmules frequently occur closely adjacent to the end of the myelinated segment; these gemmules may at times become ensheathed by the myelin.

The origin of this myelin is of some interest: Blanes (1898) noted that the glia of the olfactory nerve layer send long processes in between the glomeruli into the external plexiform layer and may be stained by the Weigert method. It is probable that the glial lamellae or myelin surrounding the mitral and tufted cell primary dendrites are derived to some extent from these glia, whose peripheral processes surround the olfactory nerves. The observation of myelinated dendritic segments is relevant both to the morphological criteria for electron microscopic identification of profiles in the central nervous system and to the functional properties of these unusual dendrites; it also points to the importance of species differences, even within mammals. Electron microscopy has already shown that it is difficult to retain the strict classical division between axons and dendrites (notably by

the observation of dendro-dendritic synapses in several sites); it has, on the other hand, made it possible to establish additional criteria for distinguishing between different types of neuronal process. Until now, the myelination of a process in normal material has been accepted as evidence of its axonal nature; such a simple view is clearly no longer tenable. That the glial lamellae (Fig. 74) of lower mammals are replaced by myelin in the monkey olfactory bulb would be explained either in terms of a possibly altered glial role in the primate, or by the increased length of the primary dendrites (mitral cell 250-300um, rat; 350-450um, monkey). Present views on the myelination of axons suggest that the myelin of these dendritic segments would be insufficient insulation to assist conduction in processes of such size, but the highly specific location seems to indicate that it is of significance in dendritic function. It may be noted that the segments are situated just proximal to the glomerular arborization, on which they receive their olfactory (and apparently only olfactory) input (Price & Powell, 1970d) (Chapters 3-5). The examination of different sites (for example, the hippocampus) and species may clarify the morphological and biophysical significance of such myelinated dendritic segments.

CHAPTER 7

A STUDY OF TERMINAL DEGENERATION IN THE OLFACTORY BULB OF THE RAT

"A theory belongs to science if it is in principle refutable."

Karl Popper.

INTRODUCTION

The ultrastructural features of terminals undergoing orthograde degeneration after axonal section have become of considerable importance in determining the distribution and mode of termination of afferent pathways to nuclei of the central nervous system; since the early work showing filamentous (e.g. Gray & Hamlyn, 1962; Colonnier and Guillery, 1966; Mugnaini and Walberg, 1967) and dark (Colonnier & Gray, 1962; Walberg, 1965; Alksne et al, 1966) degeneration, degeneration of various types has been described in many different sites. In view of the possibility of darkening due to poor fixation (Cohen & Pappas, 1969) or transneuronal degeneration (Chapter 8) or other sources, the importance of showing a broad sequence of degenerative changes by using several survival periods for each type of lesion has become essential, to ensure the accuracy of such experimental techniques. This study is concerned with the sequence of degeneration in the terminals of the fine olfactory nerve fibres in the olfactory glomeruli of the rat. In addition, the features of the degenerative process are extended to the point at which the presynaptic terminal disappears entirely, leaving behind the post-synaptic complex (See also: Gray & Hamlyn, 1962; Hunt & Nelson, 1965; Westrum, 1966, 1969; Sotelo, 1968; Westrum & Black, 1968, 1971; Lund, 1969; Mouren-Mathieu & Colonnier, 1969; Conradi, 1969; Lund & Lund, 1970; Price & Powell, 1970c). Instances of this persistence of post-synaptic specialisations in long-survival material after other lesions affecting

the glomerular layer axon terminals are also included, although the features and sites of the degenerating terminals will be described more fully elsewhere (Chapters 9 & 10).

The olfactory nerves, arising from the bases of the olfactory receptor cells in the nasal mucosa, have several unusual characteristics which make them suitable for the study of terminal degeneration over long survival periods; they are among the finest nerve fibres in the nervous system, ranging from about 80-300nm (Gasser, 1956; Andres, 1965) and run in bundles of varying numbers to the outer, olfactory nerve layer of the olfactory bulb and finally into the glomeruli in which they terminate. Their preterminal and terminal portions within the glomeruli are very characteristic, and possibly unique in the central nervous system, in having an extremely electron dense cytoplasm under normal conditions (Andres, 1965; Chapter 4). Furthermore, each glomerulus (mean diameter 80-120μm - rat) received about 25,000 fibres (estimate for rabbit, Allison, 1953). Thus a high density of terminals from a single fibre pathway may be studied with the electron microscope in a relatively small area, and these are as easily identified in normal as in experimental material; they have the additional advantage that the fibres may be experimentally interrupted without opening the cranial cavity.

RESULTS

It became evident during the course of this study that the olfactory

nerve terminals presented a rather variable time course of degeneration, even in a single animal, although a broad scheme for the majority of degenerating terminals at any survival time could be presented. The proportion of terminals undergoing a stage of degeneration either in advance of or beyond that shown by the general mass of terminals was approximately the same for any survival time, indicating that these are degenerating in a similar way and a similar sequence, but are doing so at a different rate from the others, or at the same rate but a different starting time. This latter phenomenon may be due in part to spontaneous 'atrophic' degeneration of the fibres which started prior or subsequent to the operative procedures or else an experimentally induced degeneration, caused by secondary vascular damage to the mucosa not removed at operation. Normal rats aged more than six weeks often showed stages of degeneration in the olfactory nerve terminals exactly comparable to those described here, indicative of spontaneous or 'atrophic' degeneration of axons. Finally the range of terminal degenerative changes found in a given brain might be related to fibre length (Vaccarezza, Reader, Pasqualini & Pecci Saavedra, 1970) or some other physical parameter, although this would be difficult to demonstrate in this system. In view of this variation in the time course of the degenerative process, the events occurring in the terminals will be described as five stages, which represent the major characteristics of the degenerative process, and which correspond to overlapping groups of survival times after olfactory mucosa removal.

Normal Olfactory Nerve Terminals

The terminals of normal olfactory nerves have been described in detail elsewhere (Andres, 1965; Chapter 4), but a general outline of their morphological features and connections will be given for comparison with descriptions of the degenerating terminals. The fine nerve fibres become characteristically electron-dense in their preterminal and terminal portions in the glomeruli, due to a marked granularity of the axoplasm (Fig. 78). During their course through the glomeruli, the olfactory nerves take on many unusual external forms, rarely showing the rounded appearance typical of many other axon terminals, but seemingly packed together in the available space between the dendrites in the glomeruli. They have many en passant boutons linked by varying lengths of preterminal axon, in addition to their terminal boutons; the size of the boutons varies considerably, a small proportion being rather larger than the rest. Neurotubules, neurofilaments, mitochondria and other organelles may be found in all these regions of the olfactory nerves and large numbers of spherical vesicles are found in association with synaptic membrane thickenings orientated away from the terminals. These vesicles appear to be embedded in the dense axoplasm and tend to fill the terminal portions of the axons or to fill those parts of the axon forming the en passant bouton; as noted by Andres (1965), alveolate vesicles are present in small quantities in the cytoplasm of the olfactory nerves. The synaptic membrane specialisations are typical of the asymmetrical type of Colonnier (1968) (Fig. 78): the presynaptic membrane itself is slightly

Fig. 78 Normal olfactory nerve terminals, showing characteristically dark granular cytoplasm, spherical vesicles and asymmetrical synaptic membrane thickenings.

X42,800

Fig. 79 Stage I degeneration of olfactory nerve terminal, showing swelling and pallor of terminal and swelling and distortion of vesicles. 3 days survival.

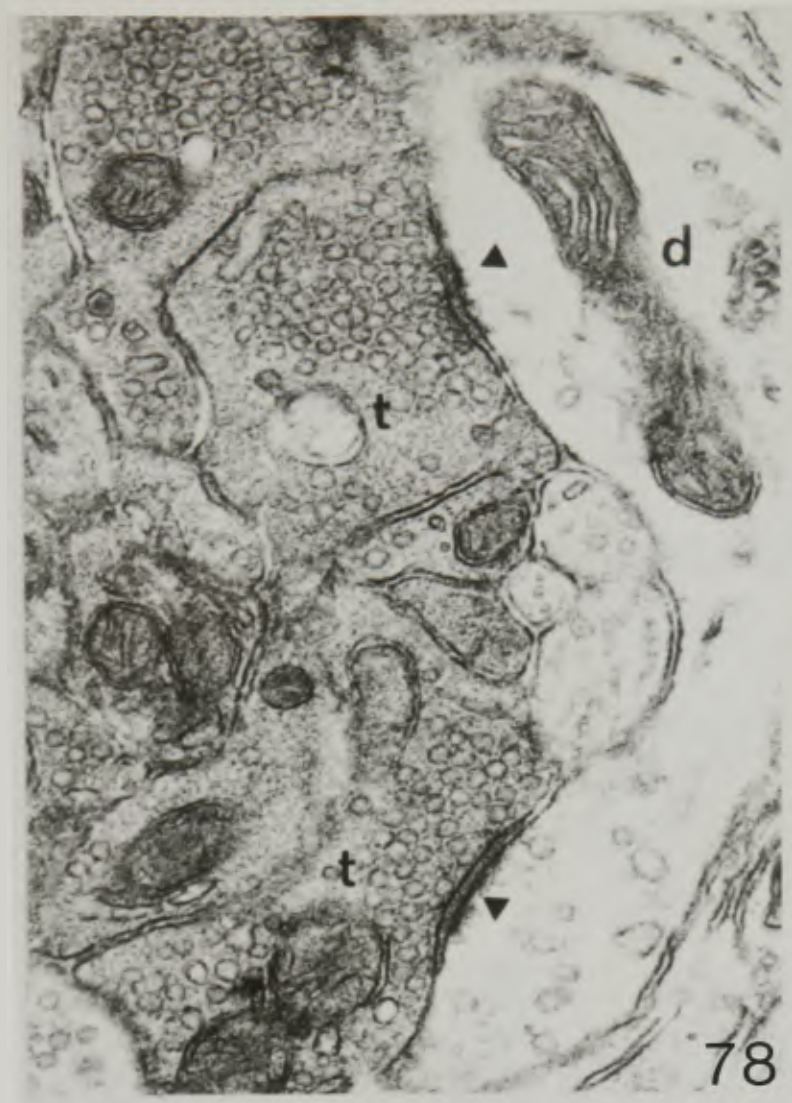
X42,800

Fig. 80 Stage I degeneration of olfactory nerve terminal after intrinsic lesion, showing markedly swollen vesicles and greyish cytoplasm. 5 days survival.

X42,800

Fig. 81 Filamentous degeneration (Stage II) of large olfactory nerve terminal with dense membrane-bound inclusions. Note magnification compared with previous Figs. Intrinsic lesion, 5 days survival.

X33,000



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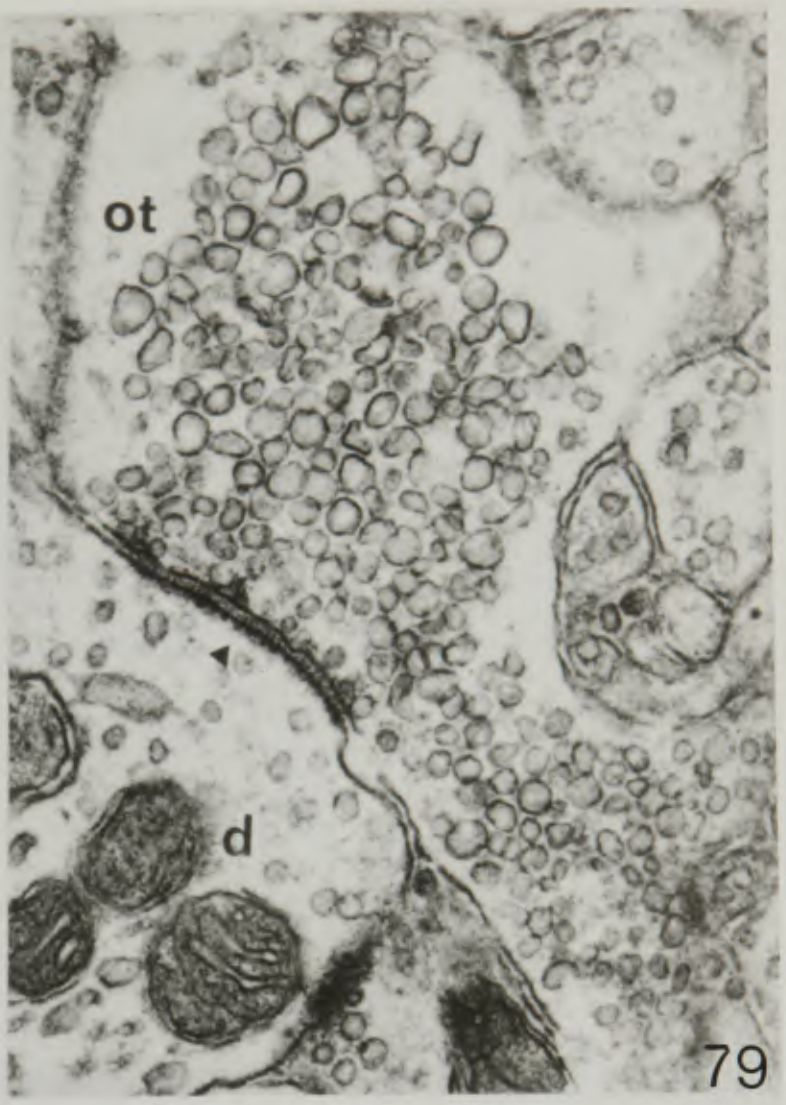

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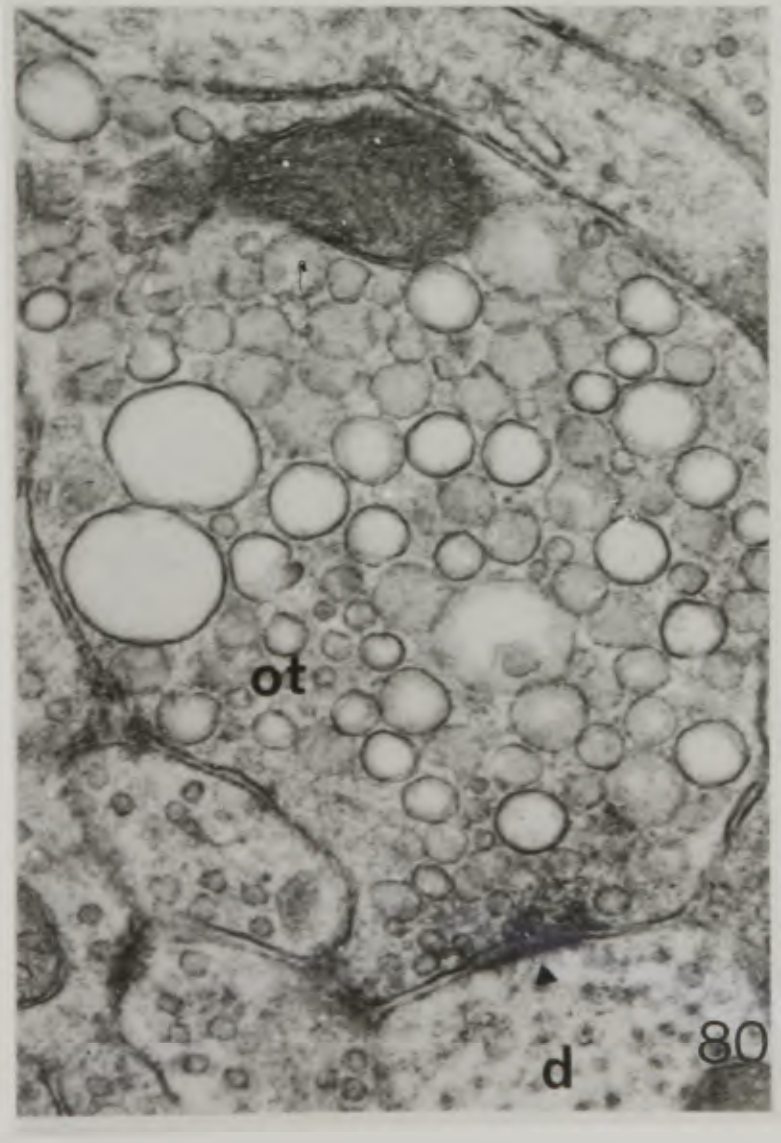

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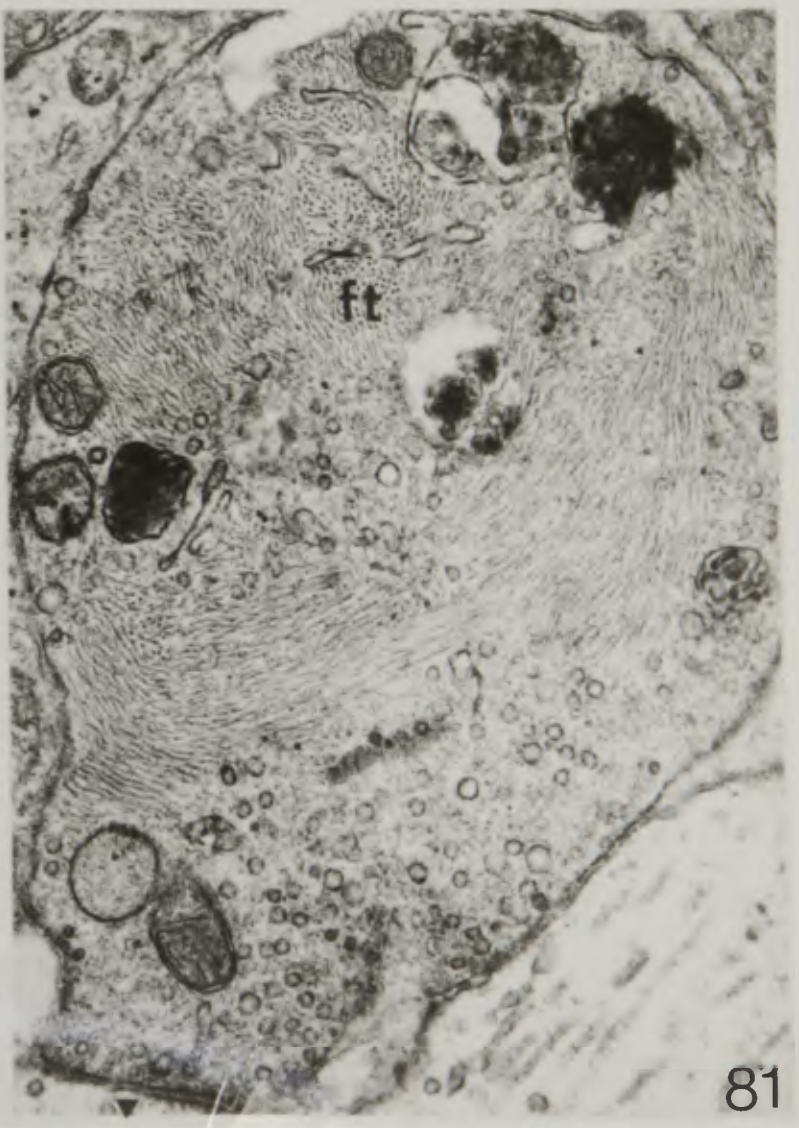

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thickened and occasional dense projections may extend from this membrane into the granular axonal cytoplasm. In the cleft between pre- and post-synaptic membranes is a layer of extracellular dense material in the form of a single row of electron-dense granules; this row tends to lie slightly closer to the post-synaptic membrane than to the pre-synaptic but often appears to lie half-way between the two (Gray & Guillery, 1966). The post-synaptic thickening is not only on the membrane itself but extends deeply into the post-synaptic process as a web of electron-dense material. Both pre- and post-synaptic membranes can be resolved so as to show unit membranes better than adjacent membranes and this is probably partly due to the apparent rigidity and relative regularity of the plasma membranes at the site of the thickening; it is possible that this property is conferred on the membranes by the extracellular material adhering tightly to both membranes (see below). The two thickened membranes and extracellular material always correspond exactly in length and situation, although the post-synaptic web may decrease in depth towards the edge of the region of contact; alveolate vesicles, often in continuity with the plasma membrane, may be seen in the post-synaptic process, lying adjacent to the membrane thickening (Waxman & Pappas, 1969). The post-synaptic profiles are the dendrites or dendritic appendages of mitral, tufted and periglomerular cells, most commonly their fine terminal parts, and these may contain vesicles and make synaptic contacts with other dendrites (Chapters 3 & 4).

Stage I. (1-5 days)

In the first identifiable stage of degeneration, the olfactory nerve terminals have become swollen, tending to take on more rounded and regular forms than is shown by normal terminals (Figs. 79,80). Their cytoplasm, though usually granular and greyer than that of nearby dendrites, has become appreciably paler than normal, as if the granules had become separated by more clear regions. Most striking about the appearance of these terminals however, is the very swollen appearance of some of the vesicles (Figs. 79,80); they may be so swollen as to exceed the thickness of the electron-microscopic section (40-50 nm), giving rise to a so-called glassy appearance. Many of these vesicles not only swell but take on distorted shapes (Fig. 79), lending a highly abnormal appearance to the terminal. Some vesicles are less swollen or distorted or may even appear to have a normal size and it is notable that the vesicles closest to the synaptic thickening or plasma membranes are the more normal in size, while those in the centre are the most altered, there being a broad gradient of change between these two extremes (Figs. 79,80). The distribution of vesicles in the terminal is also more irregular than normal, with large regions of cytoplasm showing no evidence of vesicles. Other cytoplasmic inclusions, notably mitochondria, show no tendency to swell or alter in any way at this stage, but complex membranous structures may appear in the cytoplasm of some terminals. Alveolate vesicles, present in normal terminals, may often be discerned in the axoplasm of degenerating terminals. In some larger terminals, fila-

mentous degeneration occurs (Fig. 81), but this is rarely seen in smaller terminals; it may take the form of a few isolated bundles of filaments or else a large circular whorl of them surrounding a central group of mitochondria and cisternae (see Gray & Guillery, 1966). Although more irregularly dispersed than in most other terminals at this stage, the vesicles are also swollen and distorted, and the changes in these terminals are equivalent to those occurring in the majority of non-filamentous degenerating terminals.

Stage II. (3-7 days)

The olfactory nerve terminals, now less acutely swollen, but still considerably larger than normal terminals, appear denser than in the first stage, generally about the same as, or a little less dense than normal terminals (Figs. 82,83). Their outline has lost the very rounded form of Stage I in most cases and is often as irregular and indented as that of normal terminals. The vesicles of these terminals are still very swollen, only those at the very periphery seeming normal in size and shape; the extent of their dilatation is rather less however, and many of them are more compressed and distorted into unusual shapes. Other organelles still appear normal but complex membranous inclusions are increasingly frequent; in a few cases, mitochondria may seem slightly paler than usual and a little swollen (Fig. 82). A few groups of glycogen-like granules may be found in the degenerating olfactory terminals at this stage, similar to those seen in glia during terminal degeneration (Fig. 83). The terminals

Fig. 82 Stage II degeneration of olfactory terminals, showing swollen vesicles in fairly normal sized terminals with granular cytoplasm; one terminal is undergoing filamentous degeneration. 5 days survival.

X42,800

Fig. 83 Stage II degeneration of olfactory terminal showing swollen vesicles in dense cytoplasmic matrix with glycogen-like granules. 5 days survival.

X53,400

Fig. 84 Stage III degeneration of olfactory terminal: a shrunken profile, still having slightly swollen vesicles, with intact synaptic membranes. 12 days survival.

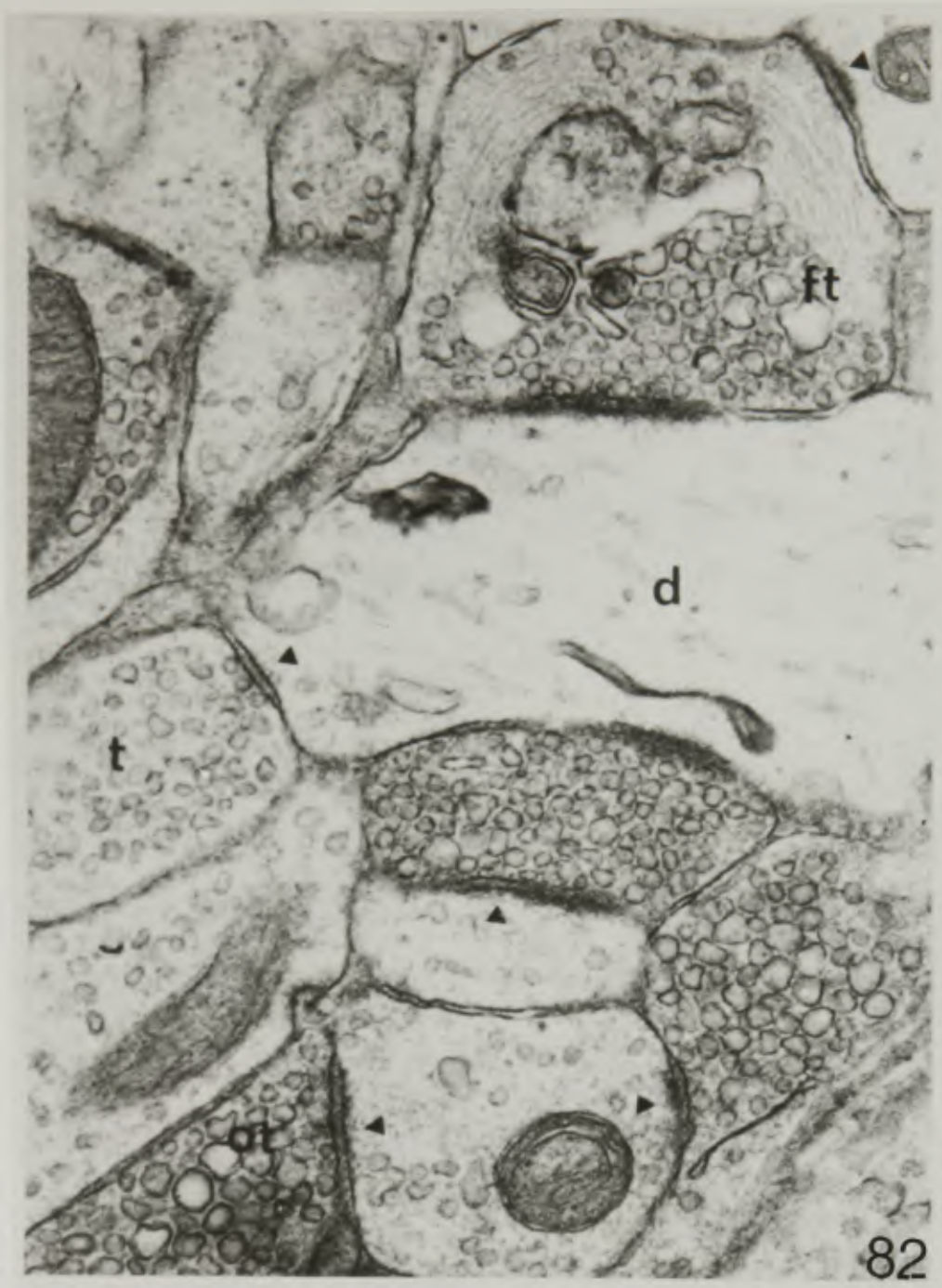
X53,400

Fig. 85 Stage III degeneration of another, larger, olfactory terminal with extreme darkening of the presynaptic terminal, distortion of vesicles, and pallor of a mitochondrion; the terminal is synapsing onto a peri-glomerular cell gemmule showing large flattened vesicles. 7 days survival.

X60,000

Fig. 86 Stage III degeneration in an olfactory terminal, showing dense matrix, packed vesicles and lamellated structure. 12 days survival.

X53,400.



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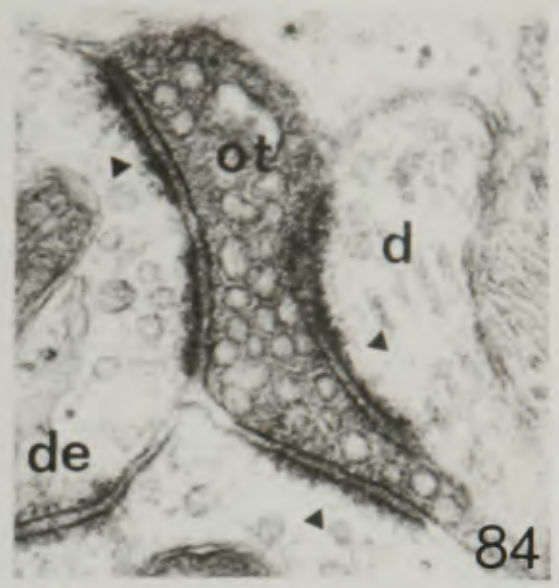

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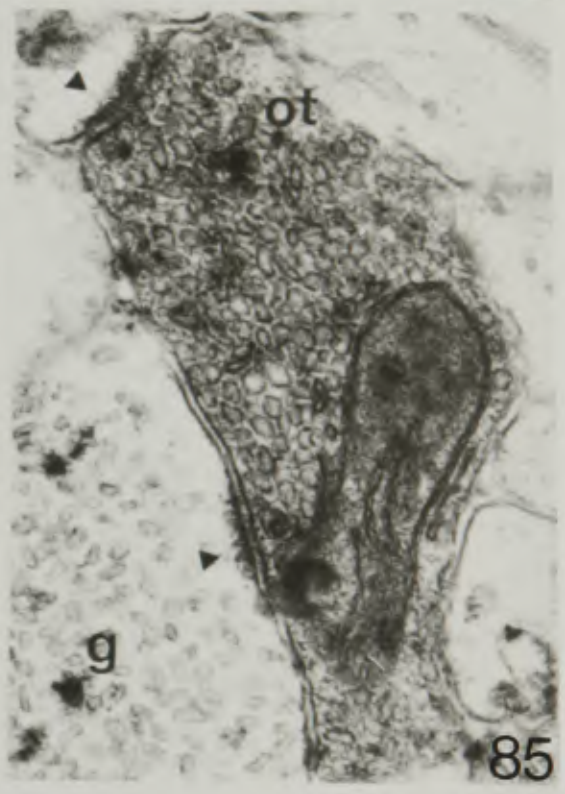

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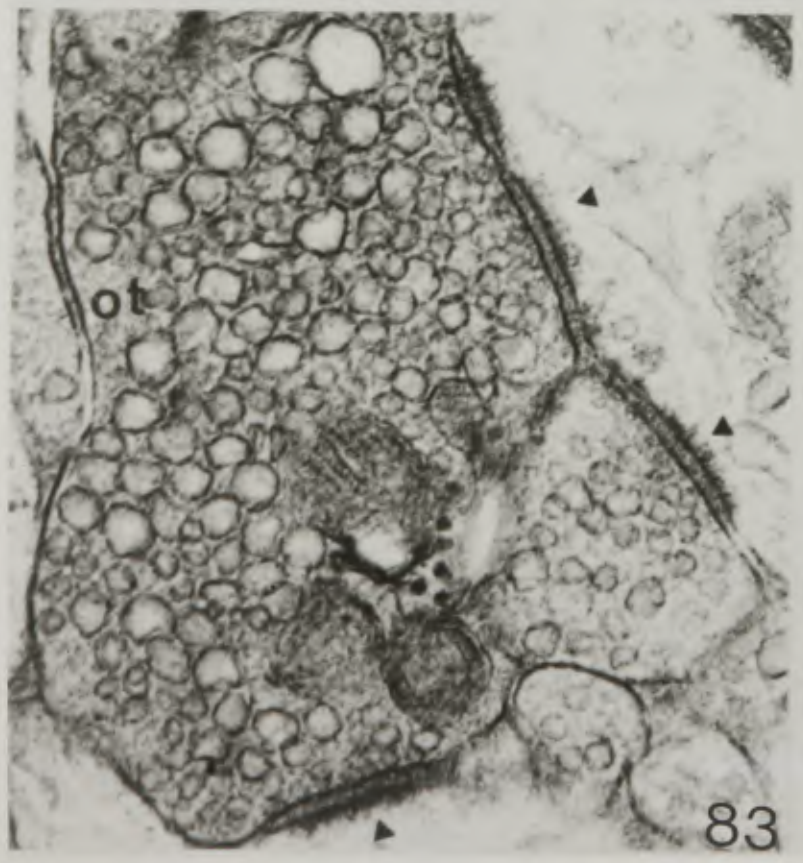

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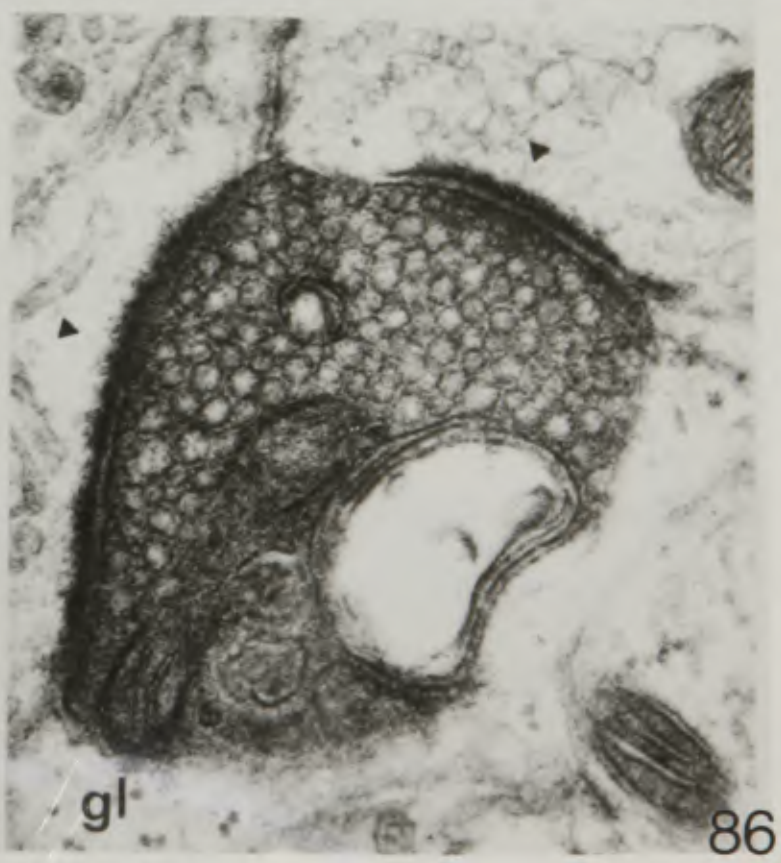


86

that are undergoing filamentous degeneration show marked whorls of filaments which are most evident at this time (Fig. 82); in all other features they are analogous to the other terminals, particularly the characteristics of their vesicles and the density of their cytoplasm, although dense bodies or membranous complexes seem more common (Fig. 81). The outline of these large terminals tends to be rounder than that of the smaller non-filamentous terminals.

Stage III. (5-12 days)

By this time the terminals have shrunk to a smaller size than normal, and the organelles are packed closely together (Figs. 84-86). The cytoplasm has become very electron-dense, much more so than that of normal terminals; mitochondria are typically pale, and apparently swollen with widely separated cristae (Fig. 8), but sometimes there is no detectable alteration in mitochondrial appearance. Vesicles are closely packed together and although some terminals show almost spherical vesicles at this stage (Fig. 86), it is more common to find them taking on unusual and distorted shapes (Figs. 84,85). Membranous complexes with large vacuoles (Fig. 86) within them are marked in some terminals which may also contain dense bodies and lysosomes. Filamentous degeneration has not been observed at an equivalent stage, so it must be assumed that the terminals undergoing this type of degeneration lose their neurofilaments before this stage, and become dark and shrunken in much the same way as the other terminals. No changes can be observed at this stage, or any previous one, in the

membrane specialisations of the synaptic structures of pre- or post-synaptic processes.

Stage IV. (7-20/40 days)

The last stage in the degeneration of the presynaptic terminal is very characteristic and may last for a considerable time in the case of a few terminals, although the majority pass through it fairly rapidly. The terminal has shrunk down in such a way that it tends to take on a rather circular outline in most planes of section, and consists of a dense mass of cytoplasm in which a few distorted membranous profiles may be discerned (Figs. 87-89). Vacuoles may appear in the cytoplasm of the terminals at this stage as well as large dense lysosomes. The degenerating terminal is still attached to the extracellular material and post-synaptic membranes in the regions of synaptic contact and it is opposite these that the small remaining amount of cytoplasm in the presynaptic terminal is situated; it is common to see the degenerating profile entirely surrounded by synaptic attachments. In many cases, the presynaptic membrane is ill-defined and difficult to resolve into unit membranes, although the post-synaptic membrane may still be resolved with ease (Figs. 87,88); the presynaptic membrane tends to lose its thickened appearance. The integrity of the extracellular material is unaffected and it remains firmly attached to the presynaptic membrane without any evidence of increased cleft width.

In some cases of severe degeneration of this type, the presynaptic terminal fragment appears to have drawn away from a part of the post-

Fig. 87 Stage IV degeneration of an olfactory terminal, showing loss of cytoplasmic detail and indistinct presynaptic membranes; one post-synaptic membrane is partially apposed to a dendritic profile. 40 days survival.

X62,300

Fig. 88 Stage IV degeneration of an olfactory terminal much like Fig. 87 but with very indistinct presynaptic membranes; the neuronal process to which part of one post-synaptic thickening is apposed also receives a synapse from the same presynaptic terminal, suggesting shrinkage of the latter. 41 days survival.

X62,300

Fig. 89 Stage IV degeneration of an olfactory terminal lying opposite only half of its post-synaptic thickening, the other half of which is apposed to a neuronal profile. 12 days survival.

X53,400

Fig. 90 Stage V of degeneration or persistence of post-synaptic thickening in the absence of a presynaptic terminal. The thickening is apposed to an obviously dendritic profile that shows no pre-synaptic specialisations; the thickening also shows a synaptic formation of Taxi. 40 days survival.

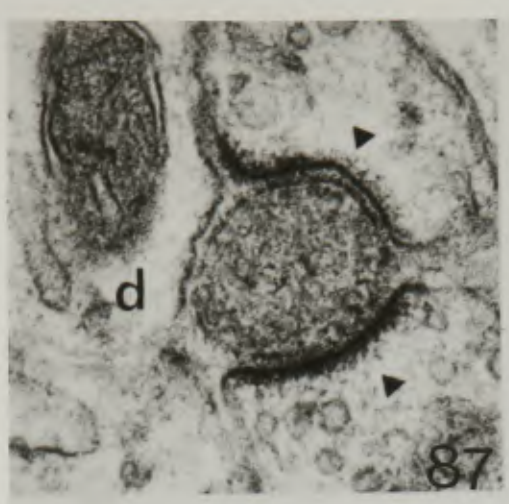
X66,900

Fig. 91 Persistence of two post-synaptic thickenings, both apposed to dendritic profiles, one of which shows a related cisterna. 40 days survival.

X75,300

Fig. 92 High magnification micrograph showing two persisting post-synaptic thickenings in reciprocal relation to each other; neither shows presynaptic specialisations, but there is an alveolate vesicle opposite one. Note the row of extracellular material in the cleft. 29 days survival.

X126,400



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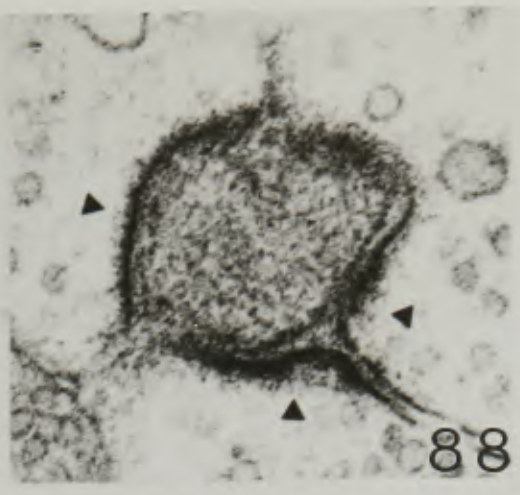

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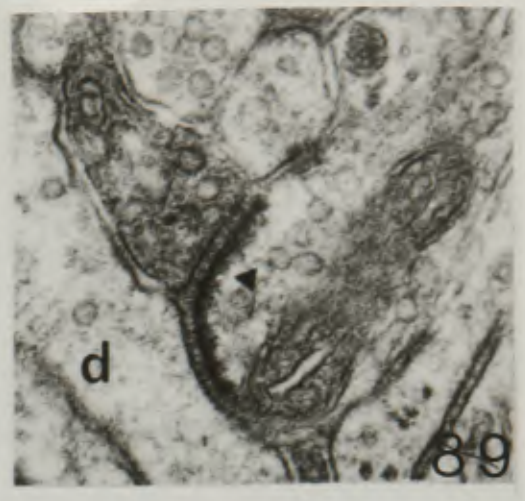

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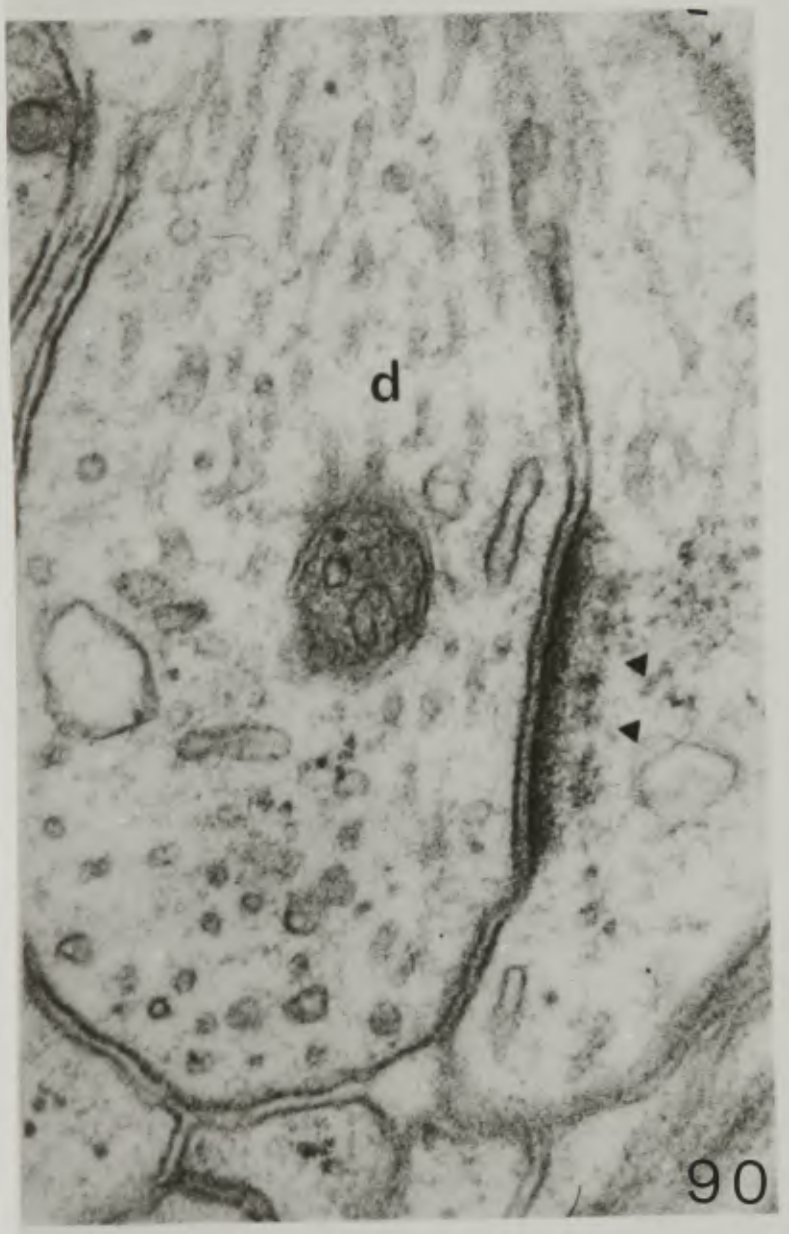

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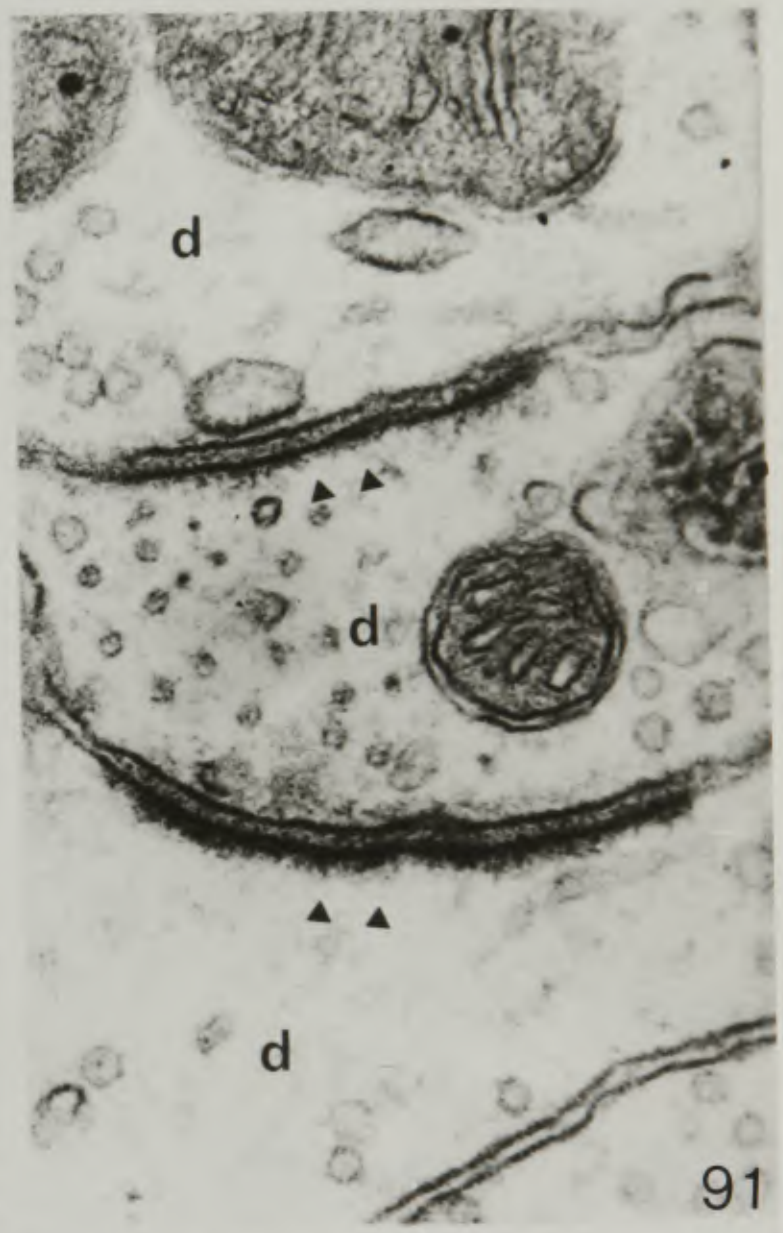

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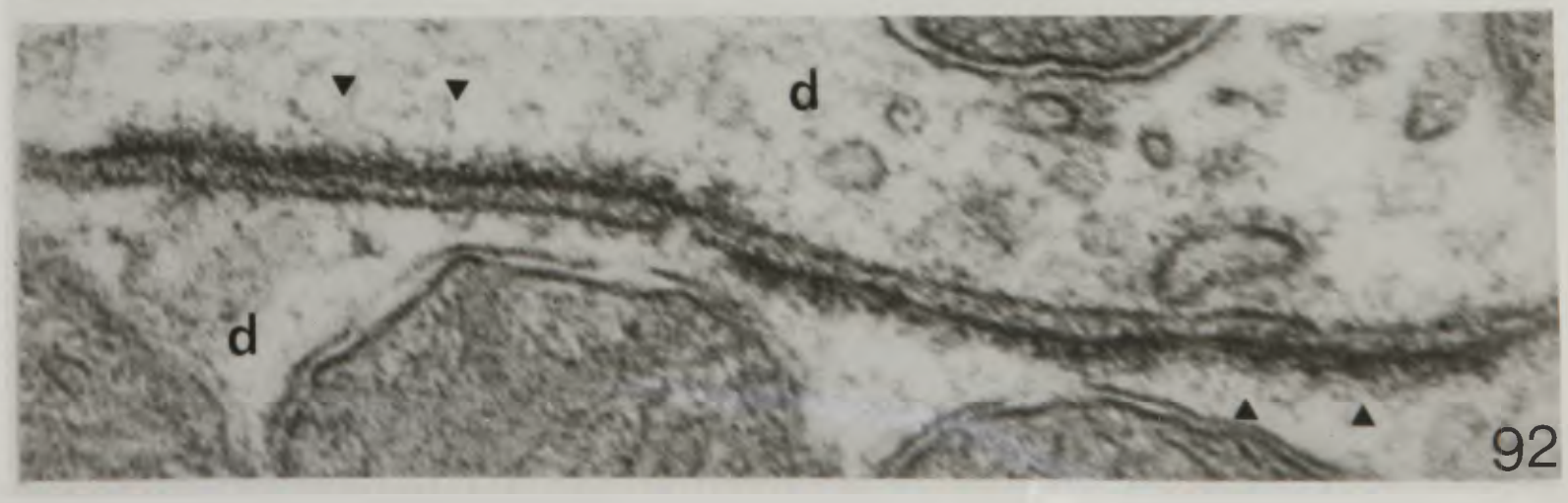


92

synaptic thickening and extracellular material, to be replaced by the apposition of the plasma membrane of an adjacent neuronal or glial profile, so that the terminal fragment and other profile seem to share the (apparently) presynaptic position related to a single post-synaptic complex (Figs. 87-89). There is never any significant gap between two such 'presynaptic' profiles where they lie against the post-synaptic specialisations; this may be taken to indicate that the extracellular material or post-synaptic thickening have a particular affinity for membranes, resulting in an apparent adhesion with any profile whether genuinely presynaptic, neuronal or otherwise.

Stage V. (after 9 days)

At this final stage in the orthograde degeneration of olfactory nerve terminals, the presynaptic terminal is entirely absent from the region of the synapse, leaving the post-synaptic thickening and extracellular material still in close relation to each other; in the apparent 'presynaptic' position lie other profiles, glial or neuronal, which show no signs of vesicular aggregation or of plasma membrane specialisations (Figs. 90-112). These newly apposed processes may be any of those normally present in the glomeruli, and the frequency with which a profile of a given identity appears in this position relates well to its frequency in that part of the glomerulus at that survival time; thus no specificity of apposition seems to be involved. On the basis of this and the appearance in Stage IV, when terminals shrink away from the postsynaptic complex to be immediately replaced by an

Figs. 93-101 show various features of persisting post-synaptic thickenings after the degeneration of olfactory nerve terminals; all examples are apposed to dendritic profiles.

Fig. 93 Shows apposition with an induced cisterna. 12 days survival.

X66,700

Fig. 94 Shows apposition with a flat sac induced opposite. 84 days survival.

X80,200

Fig. 95 The induced cisterna is continuous with granular endoplasmic reticulum. Atrophic rhinitis.

X53,400

Fig. 96 Two thickenings in reciprocal relation, one showing a cisterna. Although vesicles are present in one profile they are not aggregated to the region of apposition and there are no dense projections. Note also the "gap junction". 12 days survival.

X74,200

Fig. 97 The apposed dendritic profile shows both an alveolate vesicle and a cisterna. 29 days survival.

X85,000

Fig. 98 A group of thickenings, with an alveolate vesicle attached to the plasma membrane and a cisterna. 29 days survival.

X51,500

Fig. 99 Two thickenings apposed exactly to each other. Note the width of the cleft, the two rows of extra cellular material, the associated nerve fibre, and the two vesicle-filled post-synaptic profiles. 200 days survival.

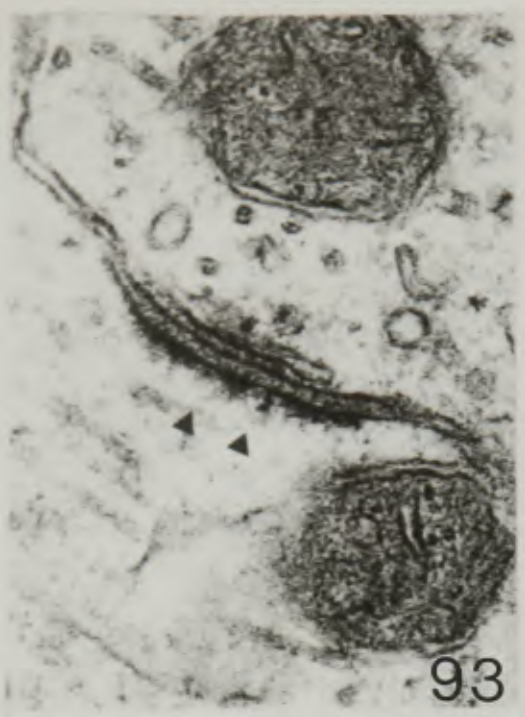
X66,900

Fig. 100 Two thickenings apposed to each other, one of which is much longer than the other; an alveolate vesicle appears to be related to the shorter thickening and the change of cleft width with double apposition is evident. 41 days survival.

X66,900

Fig. 101 Two mutually apposed thickenings, one slightly longer than the other; note the cleft width and the two clear rows of extracellular material.

X150,000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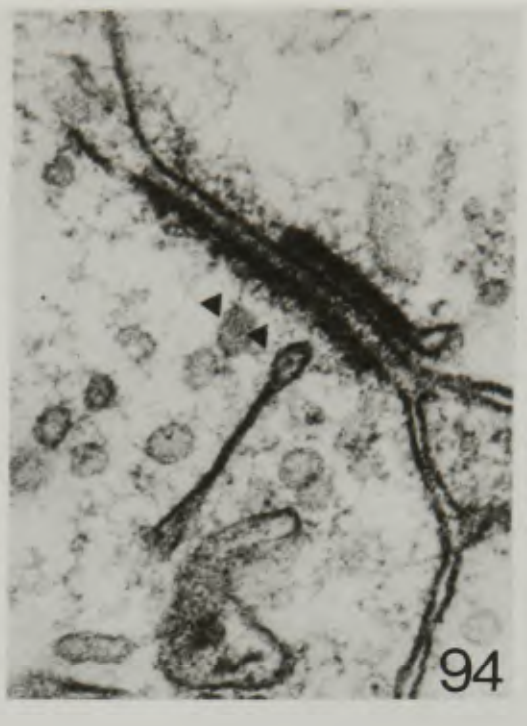
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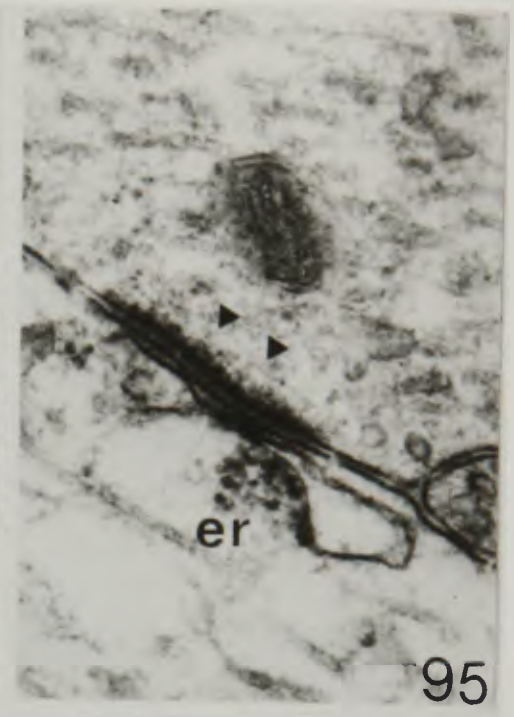

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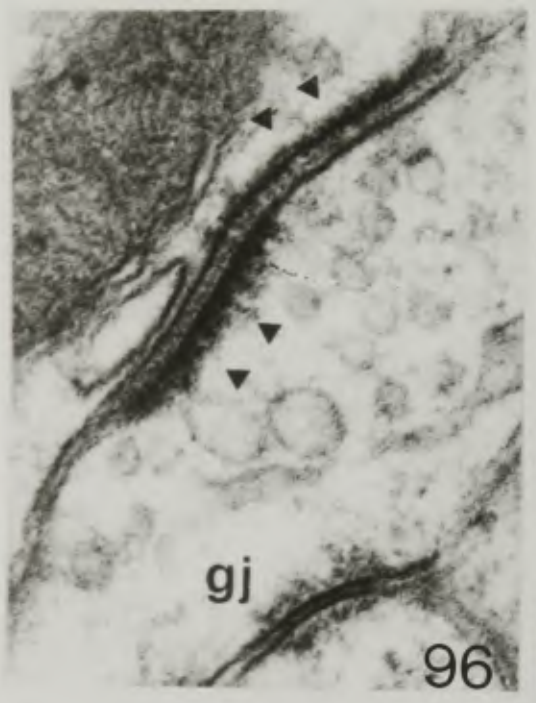

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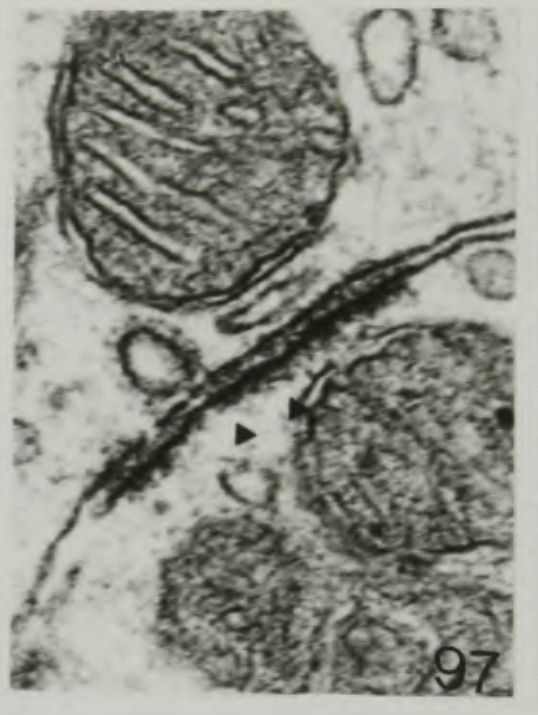
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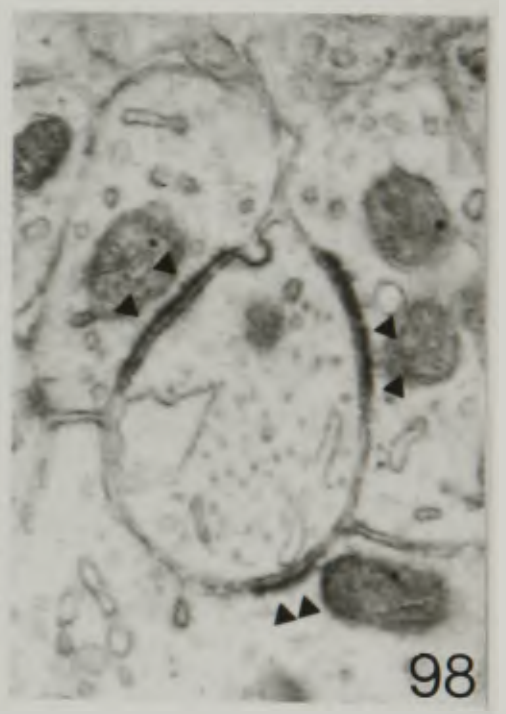
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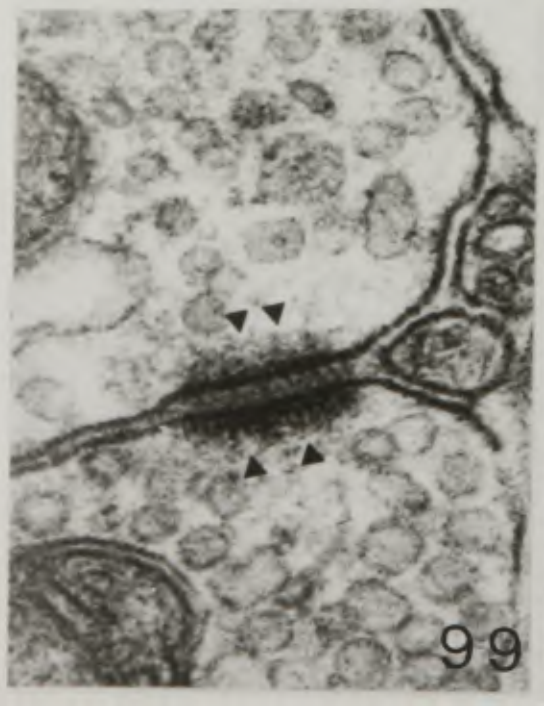
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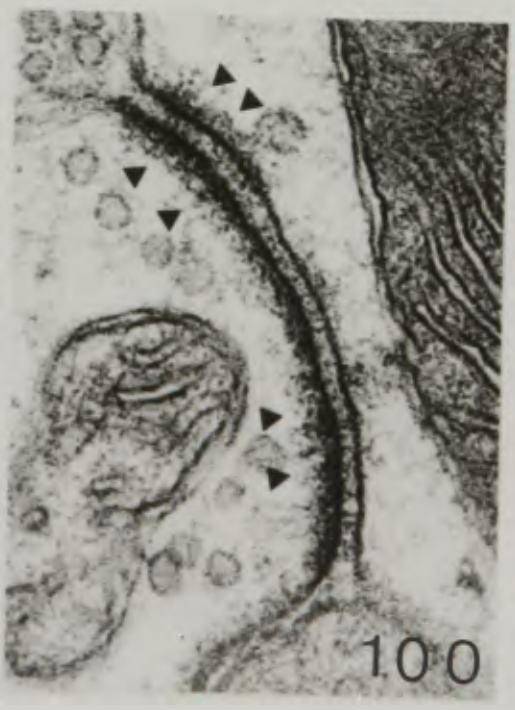
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101

Fig. 102 Low magnification view of several apposed dendritic profiles, demonstrating a large number of persisting membrane thickenings. 36 days survival.

X48,000

Fig. 103 Two persisting post-synaptic thickenings 'in series', showing well the relationship between the thickenings, cisternae and mitochondria. 150 days survival.

X61,100

Fig. 104 Single and double apposed thickenings, for comparison with the single thickening and desmosome in Fig. 105. 41 days survival.

X66,900

Fig. 105 Single persisting post-synaptic thickening, adjacent to desmosome; note the characteristics of the latter: less dense web, smaller cleft width, and no row of extracellular material. Compare with the double post-synaptic thickenings in Fig. 104. 40 days survival.

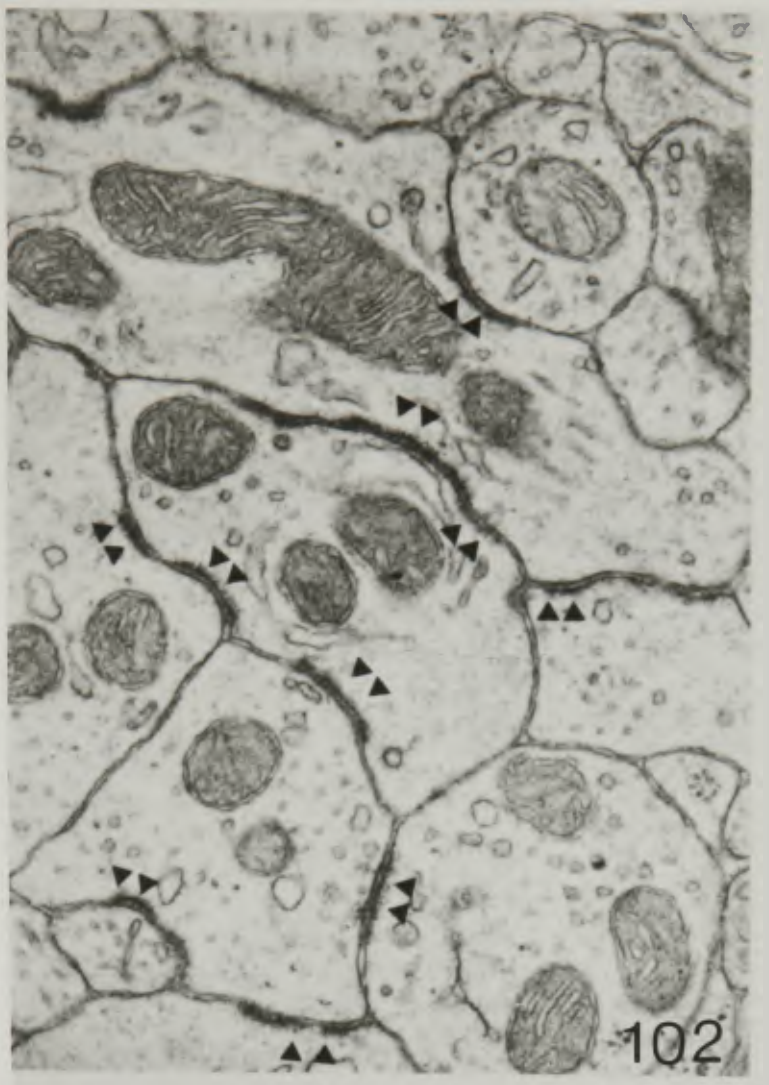
X66,900

Fig. 106 Persisting post-synaptic thickening apposed to two profiles, one of which is clearly glial, showing bundles of filaments. 9 days survival.

X80,000

Fig. 107 Apposition of a persistent post-synaptic membrane thickening to a thin glial profile containing glycogen-like granules. 41 days survival.

X55,400



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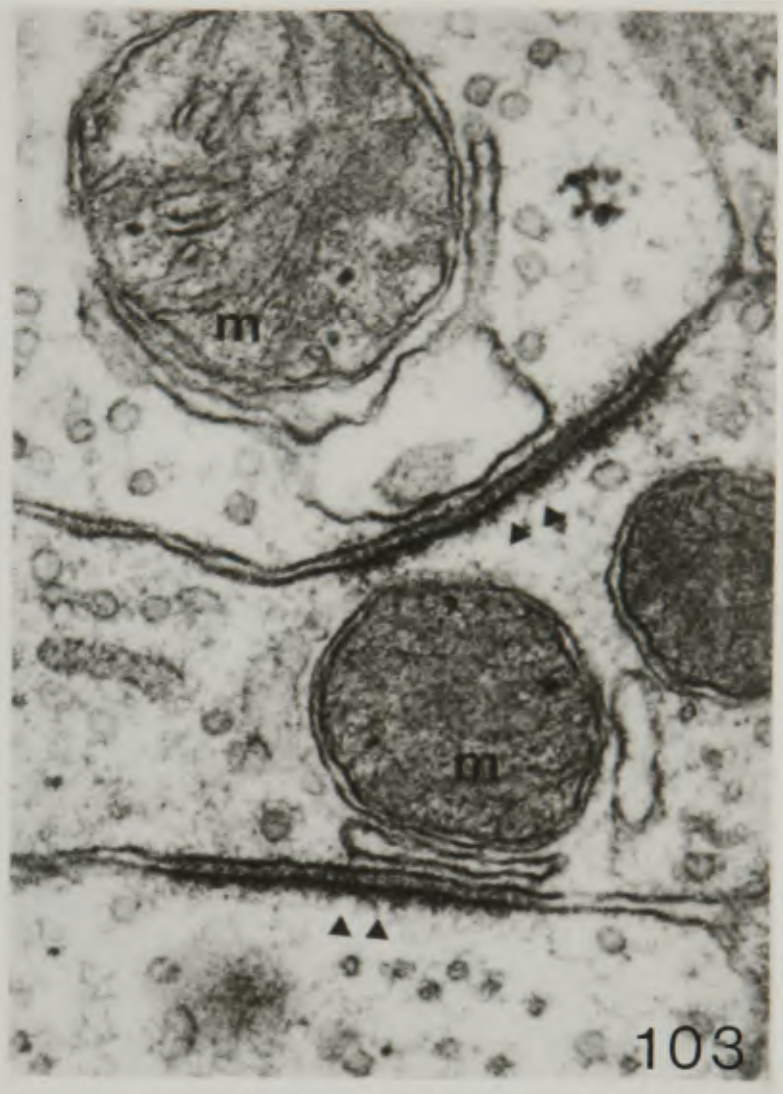

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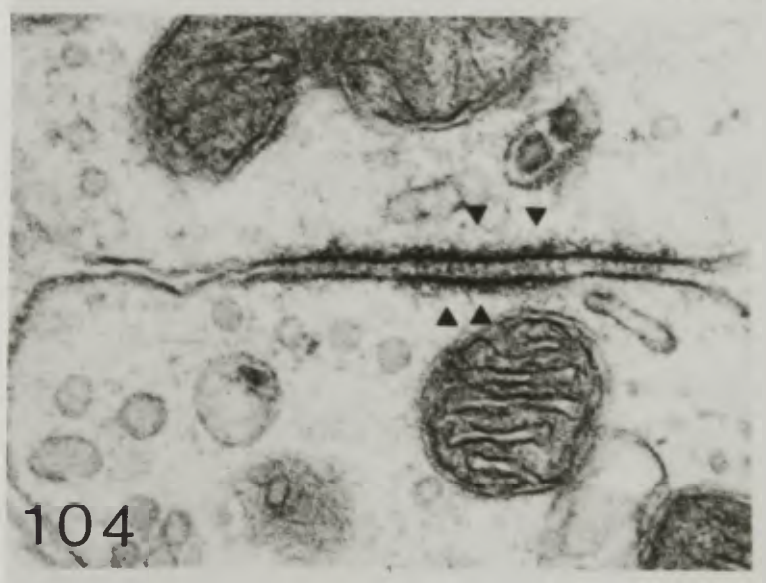

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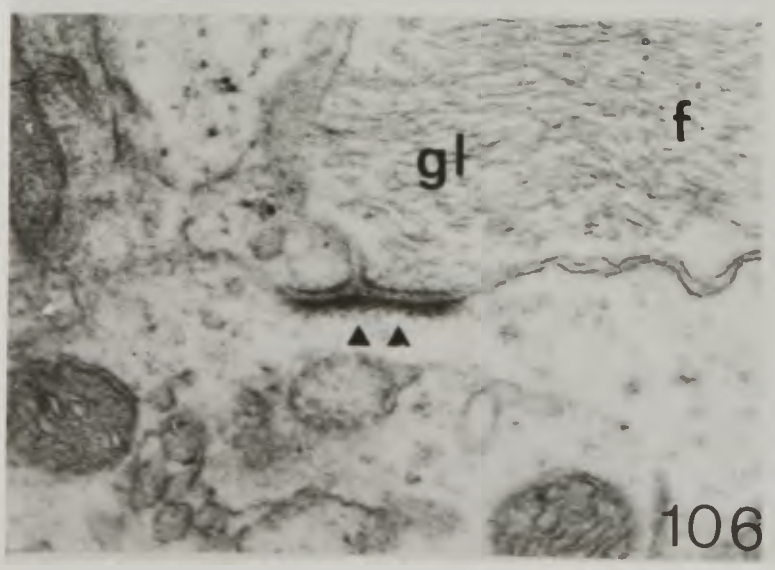

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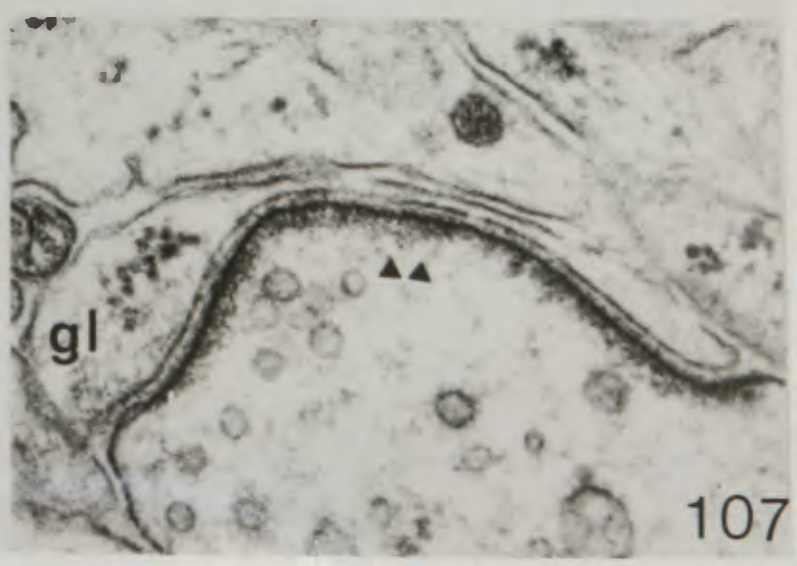

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Fig. 108 Post-synaptic thickening apposed to a mitral or tufted cell dendrite that is making synaptic contact with a periglomerular cell gemmule (which also receives a symmetrical synaptic contact from an axon terminal containing small flattened vesicles). There is no evidence of presynaptic specialisation in the dendrite opposite the persisting membrane thickening. 41 days survival.

X53,400

Fig. 109 Persisting post-synaptic thickening apposed to a mitral or tufted cell dendrite that is making two true synaptic contacts with other profiles, but which shows no presynaptic specialisation in relation to the apposed thickening. 40 days survival.

X61,200

Fig. 110 Persisting post-synaptic membrane thickenings apposed to a thin glial profile interposed between the two dendritic profiles. 84 days survival.

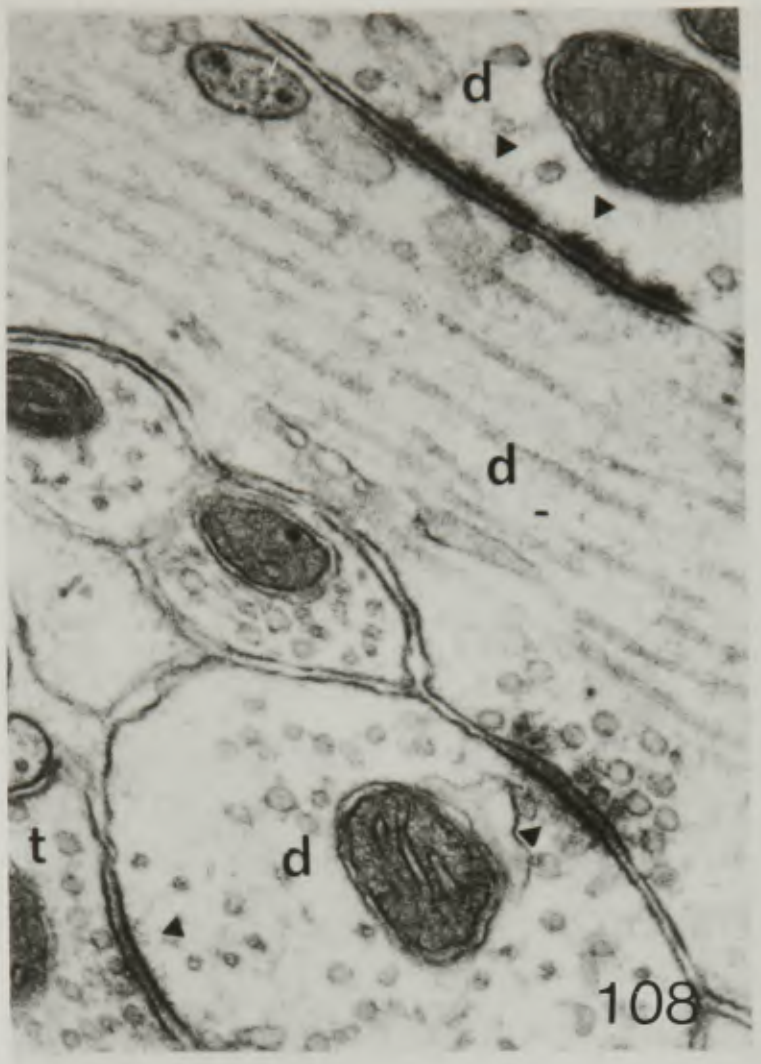
X53,400

Fig. 111 Two persisting post-synaptic membrane thickenings, one of which is apposed to a vesicle containing profile; none of these vesicles are aggregated presynaptically and there are no dense projections. 150 days survival.

X53,400

Fig. 112 A persisting post-synaptic thickening apposed to a mitral or tufted cell dendrite that is making a synapse nearby with another profile; there are no presynaptic specialisations related to the persisting thickening. 41 days survival.

X59,500



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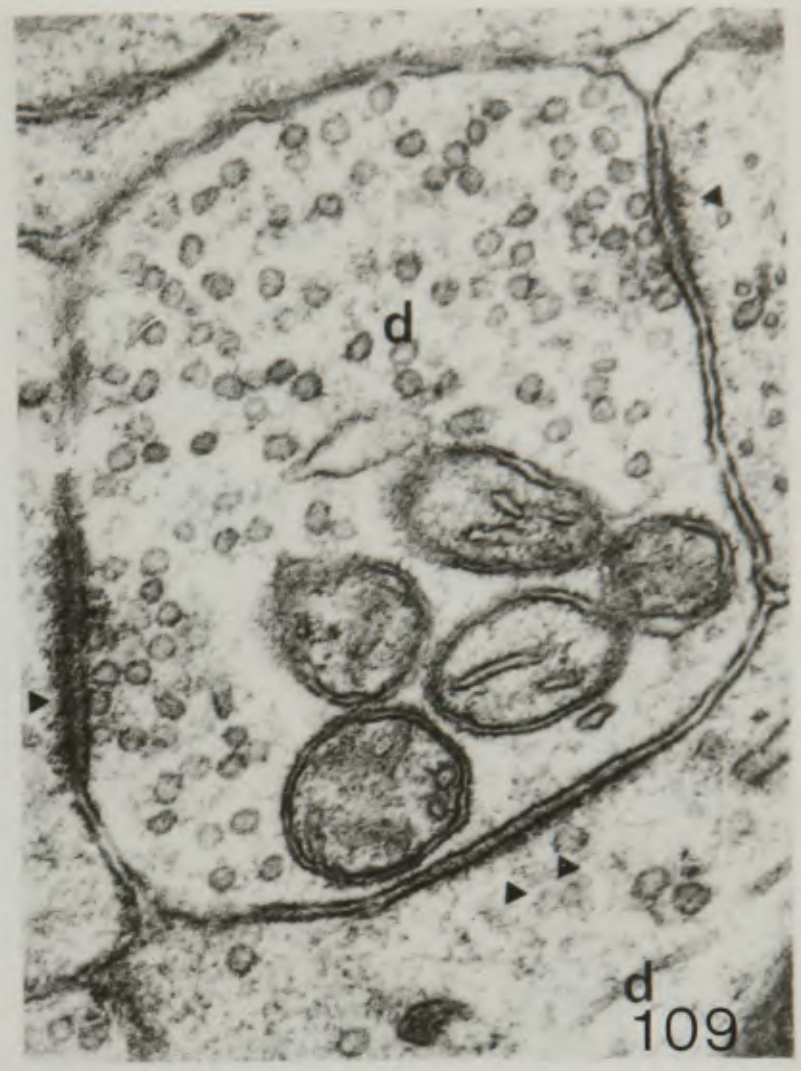

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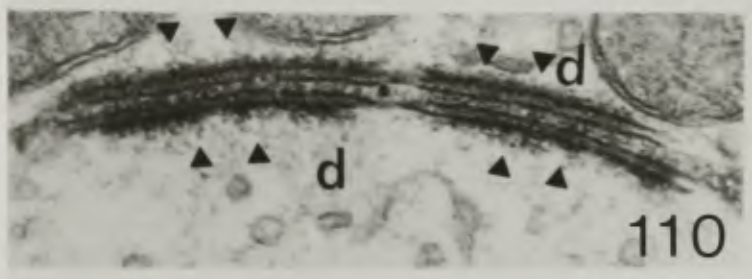

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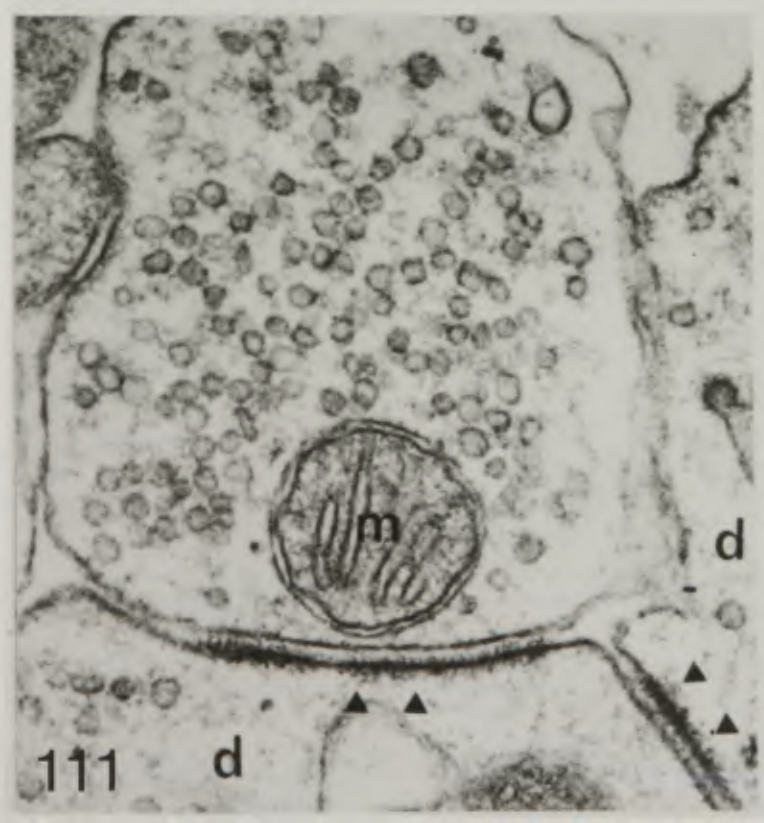

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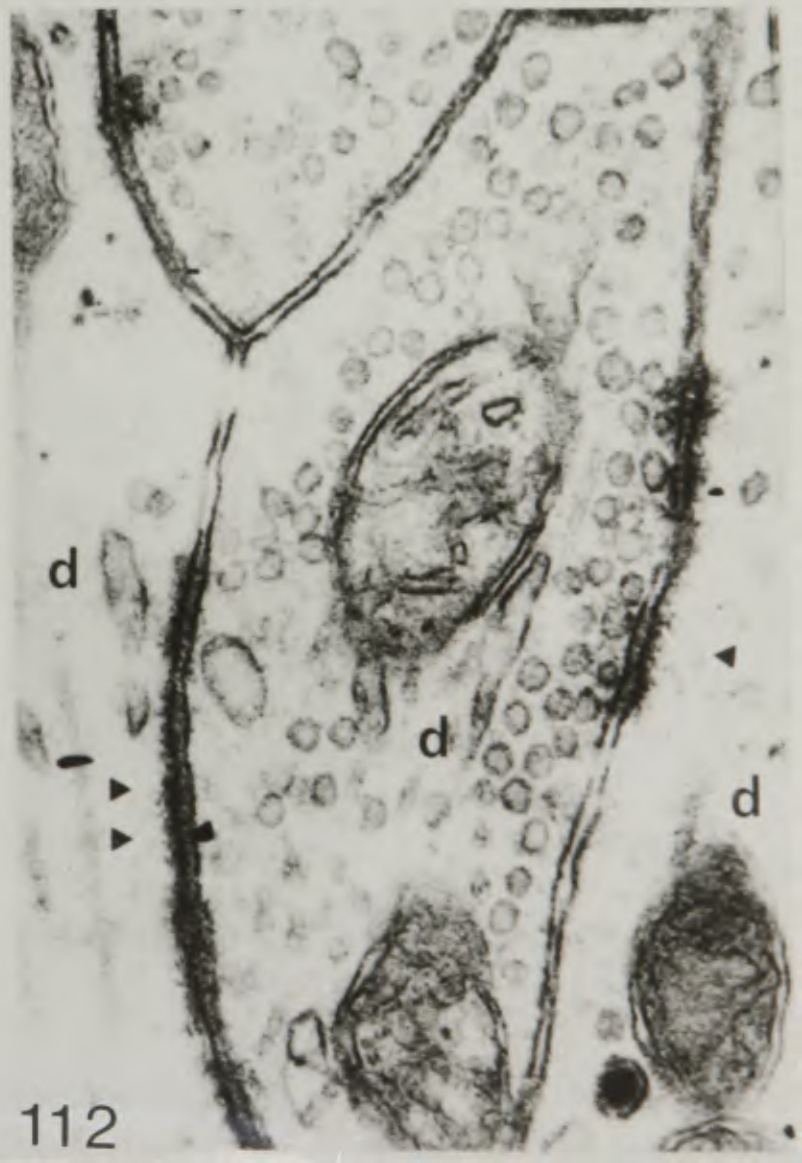


112

adjacent profile, it would seem reasonable to conclude that the nearest adjacent profile takes the 'presynaptic' position, regardless of its identity. In fact, it is quite common to find two profiles, neither of them olfactory terminals, apposed to the post-synaptic complex (Fig. 106). Further evidence will be adduced below in favour of the interpretation of the membrane thickenings as the post-synaptic specialisations left after the complete degeneration and disappearance of the olfactory nerve terminal. A persistent post-synaptic thickening shows exactly the same structure as that of a thickening post-synaptic to a normal olfactory nerve terminal, that is, with a marked web of material extending into the cytoplasm, typical of that associated with asymmetrical synapses; similarly, the extracellular material remains in close relation to this membrane thickening, and retains the same granular structure as before (Figs. 90-112). The newly-apposed plasma membrane may appear to be denser than the surrounding membranes but this seems to be an illusion created by the presence of the granular material and the post-synaptic thickening opposite, and by the regularity of line that the apparent adherence of the extracellular material endows on this part of the plasma membrane. It is notable that the extracellular material is never left "exposed" in the literal sense, but is always closely bound to some membranous structure, both pre- and post-synaptically.

Alveolate or coated vesicles, similar to those found close to the post-synaptic membrane or in the presynaptic terminal in normal material, are evident both in the post-synaptic process, adjacent to the thickening

(Fig. 100), and in the newly apposed process either opposite or close to the site of the post-synaptic thickening (Figs. 92,97,98,103). The latter may occur in an apposed glial profile as well as in a neuronal profile, so their appearance opposite a persisting thickening cannot necessarily represent a case of a coated vesicle associated with another persisting thickening nearby in the apposed process, as previously suggested.

Cisternae have been frequently observed in the cytoplasm of the apposed profile lying in close relation to the persistent membrane thickening, when this apposed profile is dendritic; these cisternae are typically loose, open agranular reticulum in the rat (Figs. 91,95, 96,97,98,102,103,112), and flat sacs of reticulum in the rabbit, though both species show various types of cisternae (Figs. 94,95). They are continuous in all cases with the endoplasmic reticulum of the dendrite; they dip from this towards the surface so as to lie in close proximity with the part of the plasma membrane which is directly apposed to the post-synaptic thickening (Figs. 95,98,103). In this way they are regularly seen to lie exactly opposite the membrane complex and to draw away from it on either side; this clear relationship has been demonstrated by the use of serial sections, which have been an essential aid to the verification of the identity of the persisting thickenings and the profiles to which they are related. Mitochondria, with which the agranular reticulum is often associated, may also lie close to these thickenings in the apposed profile, but this is not a constant relationship and is probably secondary to the cisternal apposition (Figs. 96,97,

103,111). There is no evidence in normal or short-survival experimental material of any cisternae situated so close to the plasma membrane in these sites, that is, apposed to the normal or early degenerating olfactory nerve terminals; the highly specific relationship of the cisternae to the membrane thickening apposition and not to any other part of the plasma membrane of the profile showing the thickening, is indicative of the direct nature of this relationship.

In order to establish the identity of the persistent post-synaptic thickenings, various primary criteria had to be fulfilled:-

(1) The presence of thickenings in sites and on processes known to receive olfactory nerve terminals in the normal animal.

(2) The numerical equivalence of olfactory nerve terminals with clear post-synaptic thickenings in normal material and persisting thickenings (plus any remaining olfactory terminals) in experimental material, in analogous areas of glomeruli. This criterion can only hold in the absence of transneuronal degeneration, as in the latter case the thickenings may disappear.

(3) The morphological equivalence of the post-synaptic thickening and extracellular material to those found post-synaptic to normal olfactory terminals.

(4) The absence of pre-synaptic dense projections and of a 'presynaptic' aggregation of vesicles in the apposed profile.

These criteria have been fulfilled in this material: the thickenings have been found only in the dendrites and dendritic appendages of mitral, tufted and periglomerular cells and solely within the limits of the

glomeruli, after olfactory mucosa removal (Figs. 90-112). Their distribution in these processes is exactly similar to that of olfactory nerve terminals, and the size and morphology of these structures are completely equivalent to the post-synaptic part of normal olfactory synapses. For descriptive, illustrative and quantitative evaluation of persistence, post-synaptic thickenings have satisfied the last criterion of the absence of normal presynaptic specialisations in the newly-apposed profiles (alveolate vesicles and cisternae in the apposed process may not be considered normal presynaptic specialisations in these sites).

In a quantitative study it was shown that the number of clear post-synaptic thickenings orientated from olfactory nerve terminals in normal glomeruli ranged from 6-11 per 100 μm^2. Equivalent areas from glomeruli of experimental animals at various survival times were examined and the sum of post-synaptic thickenings without presynaptic specialisations and those with normal or degenerating olfactory terminals, situated presynaptically, was determined. This figure, although varying slightly and tending to be marginally higher (on account of the higher dendritic density after olfactory nerve degeneration), was within the range of the figure for normal post-synaptic specialisations, at all survival times up to 150 days; after this time transneuronal degeneration may occur in the rat and the figure drops. During the first thirty days after olfactory mucosa removal the percentage of exposed membrane thickenings rises from zero, in the normal, to 70-80%; the remaining terminals were either undergoing late degeneration or had

not been affected by the lesion. A study on the length of the post-synaptic thickenings with and without presynaptic olfactory nerve terminals showed that the histogram of these in normal and experimental material was exactly comparable at all survival times. These quantitative studies show clearly that features of the apparent post-synaptic specialisations observed after olfactory nerve degeneration are compatible with an interpretation of these as the persisting post-synaptic specialisations, left behind after the shrinkage and disappearance of the presynaptic terminal.

Persistent post-synaptic thickenings may occur singly, or as groups in fairly close relation, and this fits well with the normal distribution of olfactory nerve terminal synapses. On occasion two thickenings, one in each of two apposed dendrites, may be seen either in an apparently reciprocal relation or else lying partly or wholly opposite each other (Figs. 92,96,99-102,104); (Westrum, 1969; Chapter 8); in the latter cases, the cleft between the two apposed thickenings is wider than that seen for single thickenings and shows <u>two</u> rows of granular extracellular material, lying parallel (Figs. 96, 99-101,104). The structure of such apposed thickenings is quite distinct from that of desmosomes (compare Figs. 104 & 105) for the following reasons:-

(1) The dense web of desmosomes (Fig. 105) is paler and less tightly packed than that of post-synaptic thickenings.

(2) The intercellular cleft of desmosomes (Fig. 105) is thinner than that of a single synaptic cleft, while apposed thickenings show a

wider cleft than the latter (Figs. 99-101, 104).

(3) The extracellular material in the cleft of desmosomes is less well-defined and arranged than that of synaptic clefts, never showing single rows of granules (Fig. 105).

(4) In many cases the two apposed thickenings do not exactly correspond in length or situation (Figs. 96,100,101,103), while the desmosomal thickenings are always symmetrically placed opposite each other.

It should be further emphasised that, for this analysis, only those thickenings that could be clearly resolved, preferably into unit membranes, were studied.

At the survival times studied, and indeed until the onset of transneuronal degeneration in both rat and rabbit (Chapter 8), no changes were observed in the structure of the post-synaptic thickenings (c.f. McMahan, 1967), the thickenings showing the usual variety due to planes of sectioning and other factors; very occasionally, notably in the larger dendrites, a subsynaptic formation of Taxi (Fig. 90) was evident, but this is present at a similar frequency in normal material. The loss of post-synaptic thickenings in transneuronal degeneration coincides with the loss of the terminal parts of the glomerular dendrites, on which most of these structures occur.

Role of glia

Reactive glia are seen at all stages of early degeneration after mucosa removal (Figs. 81-84,86), but are generally only in clear evidence

up to 16 or 20 days post-operatively. These glia are mostly astrocytic, containing bundles of filaments and sometimes glycogen granules in a typically pale cytoplasm, which may, however, have some granularity. Reactivity is very marked between 1 and 7 days, maximal at 7-9 days (Fig. 106) and then falls off fairly rapidly. These glia are often seen partially or totally enclosing degenerating terminals, fibres or dense axon fragments, but many degenerating terminals do not seem to have any glial profiles related to them (Figs. 79,80,82,84,85,87-89). Thus direct glial involvement does not seem to be essential for the degeneration and disposal of the presynaptic terminal, although it does occur for a proportion of terminals; this is notably true for the final stages (IV and V), in which the terminal is displaced from the post-synaptic complex and is replaced by another profile (Figs. 87-89,106). Neither the displacement nor the replacement necessarily involve direct glial apposition; in those cases where this does occur (Figs. 106,107,110), it may be simply due to the proximity of the glial profile to the region of the synapse. This does not exclude the possibility that glia are indirectly involved in the removal of all terminals from their post-synaptic sites, operating at a distance from the site of shrinkage. The observation of dendrites displacing the presynaptic terminal at stage IV, without any apparent glial apposition, is a clear indication that the separation of the presynaptic membrane from the extracellular material is not solely or necessarily the result of glial intervention. As the glial proliferation dwindles, more and more post-synaptic thickenings are 'occupied' presynaptically by

dendritic profiles, presumably becoming apposed during the retraction of the glial process, by a similar mechanism to that in the loss of the original presynaptic terminal.

Atrophic Rhinitis or Spontaneous Degeneration

Although the degeneration of olfactory nerves without experimental denervation is rare in the glomeruli of young animals, older animals (over eight weeks) may show increasing numbers of both degenerating olfactory terminals and persisting post-synaptic thickenings, at similar stages to those seen during the various survival periods after olfactory nerve section (Fig. 95). Such spontaneous degeneration or atrophic rhinitis has long been known to occur in the bulb, and the use of young animals is a common precaution to avoid this; that the course of this degeneration follows a similar pattern to that seen after mucosal lesions is not therefore surprising. Nevertheless, it makes use of young animals even more imperative if the synaptic organisation of a region susceptible to such degeneration is to be studied; this is particularly so in view of the apparent synaptic contacts found by apposition of persistent post-synaptic thickenings to neuronal profiles. Although these findings in no way invalidate the observation of dendro-dendritic synapses in the glomeruli of the rat (Reese & Brightman, 1965; Hinds, 1970; Chapter 4), they create a necessity for rigorously excluding the possibility of apposed persistent thickenings where an apparent synaptic structure is observed. Clearly a similar caution must be made in regard to the use of experimental material for the study

of normal synaptology.

Accessory Olfactory Bulb

A brief examination of normal and degenerate accessory olfactory bulbs, as well as the accessory bulbs of old animals, shows that the stages of degeneration of the dark vomeronasal nerve terminals are, in general, analogous to those seen in the olfactory nerve terminals of the main bulb (Figs. 113-120). The normal morphology of the vomeronasal terminals is exactly similar to that of their olfactory counterparts, with granularity in their axoplasm and tightly packed vesicles, although these terminals may not achieve the same degree of electron density as is seen in the main bulb (Fig. 113); similar whorls and other unusual conformations are formed by the terminal and preterminal parts of these nerves. The structure of the synaptic thickenings is exactly comparable to that seen in the asymmetrical thickenings of the olfactory nerve terminals. Swelling of vesicles and swelling and paller of the terminal are typical of early experimentally induced degeneration and early atrophic changes (Fig. 113) (the latter occur later and far more sparsely than in the main bulb); filamentous degeneration is also seen in some terminals. In addition, some very large terminals are observed in early degeneration, with a few swollen vesicles in a greyish granular, almost lattice-like, background, and these terminals may show many large dilated mitochondria (Fig. 120); this type of degeneration has not been seen in the main bulb. Darkening of the cytoplasm and shrinkage of the presynaptic

Figs. 113-120. The process of vomeronasal nerve terminal degeneration in the accessory olfactory bulb.

Fig. 113 A normal vomeronasal nerve terminal (top) next to one undergoing early (Stage I) degeneration, due to atrophic rhinitus; note the swollen vesicles and pallor of the terminal relative to the normal.

X44,700

Fig. 114 Late (Stage IV) degeneration, with terminal shrinkage, loss of cytoplasmic detail and presynaptic membrane definition. Atrophic rhinitis.

X84,700

Fig. 115 Persisting post-synaptic thickenings in apposed dendritic profiles; observe the increased cleft width at the site of double apposition. 110 days survival.

X66,900

Fig. 116 Persisting post-synaptic thickenings almost exactly apposed to each other; note also the fine nerve fibre adjacent to the apposition, probably representing the degenerating fibre of origin of the presynaptic terminal that has degenerated. 110 days survival.

X89,200

Fig. 117 Two apposed persisting thickenings, with associated nerve fibre, and alveolate vesicle in one profile. 110 days survival.

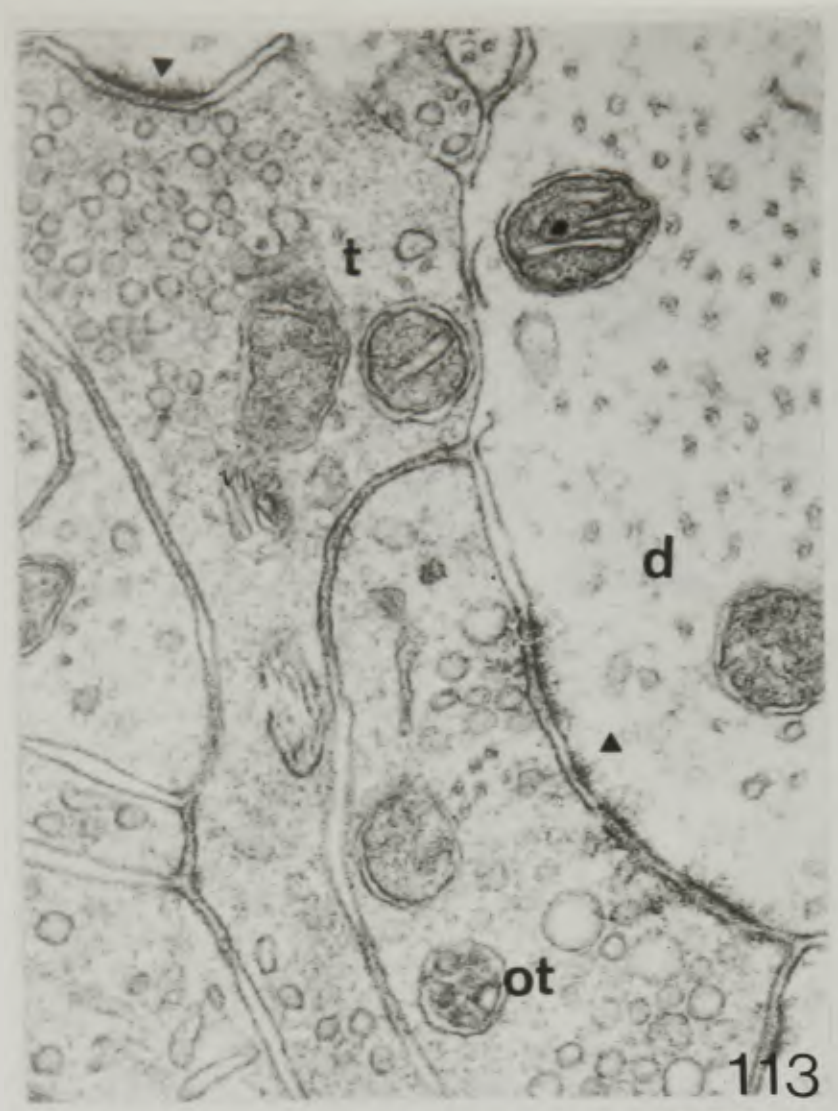
X106,800

Fig. 118 A large post-synaptic thickening, partly apposed to a glial profile, but also apposed to two neuronal profiles, neither of which is a vomeronasal terminal. 110 days survival.

X 66,900

Fig. 119 Persisting post-synaptic membrane thickening apposed to a glycogen containing dark glial profile. 110 days survival.

X53,400



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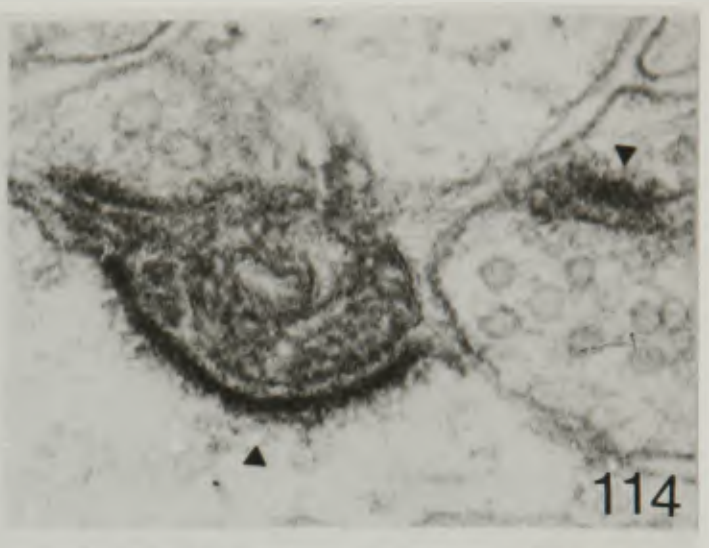

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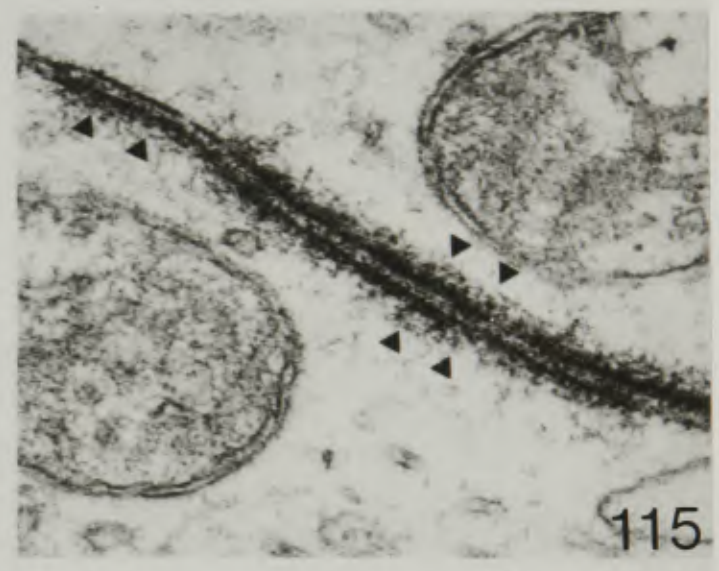

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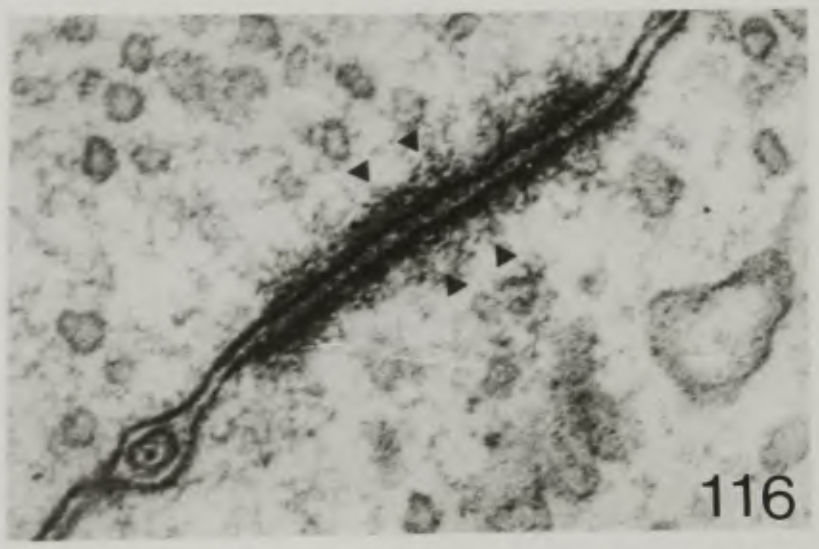

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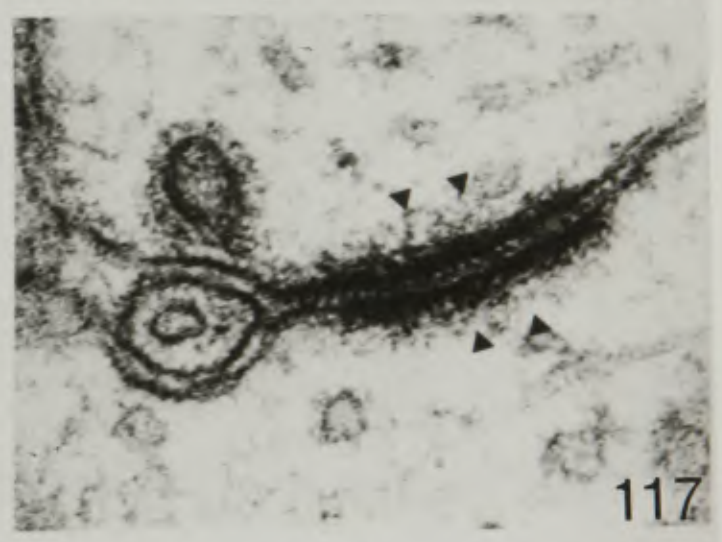

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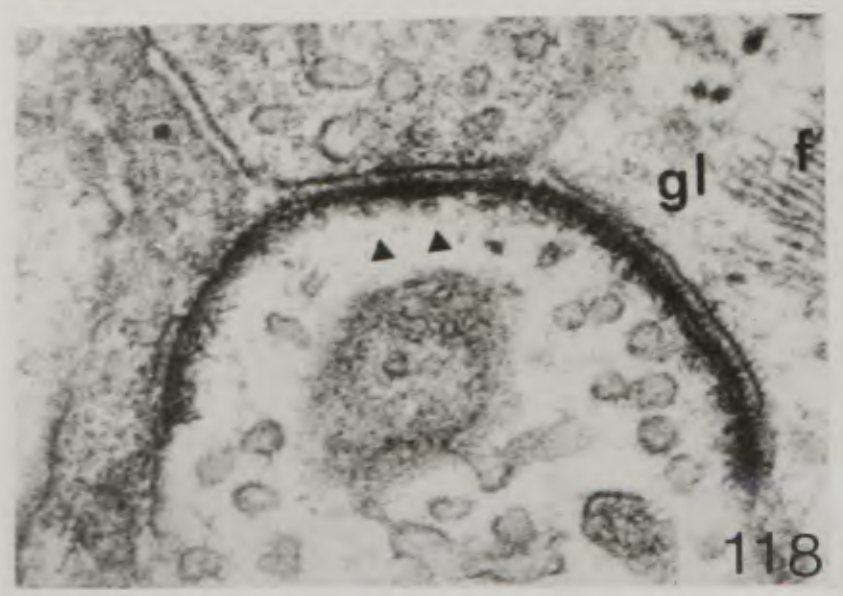

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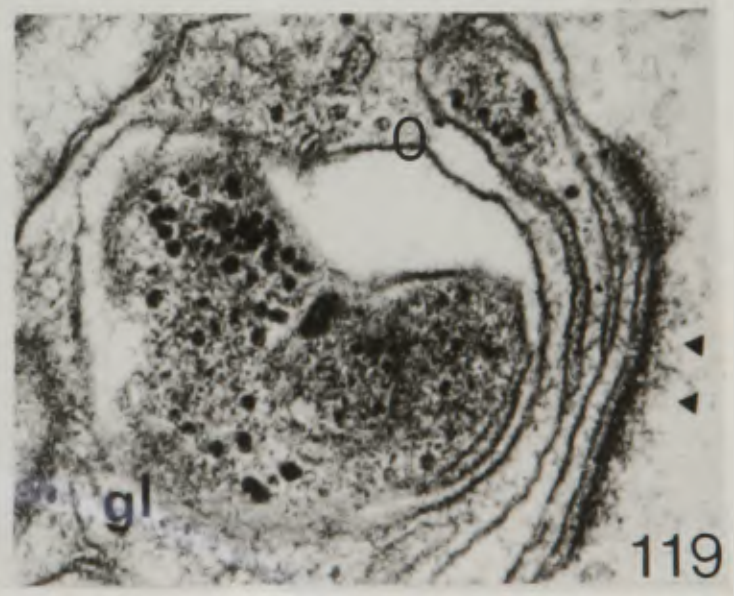

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profile are observed in later terminal degeneration, with a concentration of cytoplasm close to the post-synaptic membrane, and the presynaptic membrane tends to lose its definition (Fig. 114); vesicles are greatly reduced in number and distorted. Finally, persistent post-synaptic thickenings are observed in animals of long survival periods after lesions of the mucosa involving the vomero-nasal organ or nerve (Figs. 115-119), and very occasionally in old animals. The structure and distribution of those membrane thickenings correspond to the degeneration of the vomeronasal terminals and to the similar persisting thickenings in the main bulb.

Centrifugal degeneration in the long term

In material from rats in which the lateral olfactory tract has been sectioned caudal to the anterior olfactory nucleus, a few degenerating terminals may be seen in the periglomerular region (Chapter 9) corresponding to those described after similar lesions in light microscopic investigations (Cragg, 1962; Powell, Cowan & Raisman, 1965). After 7 or 10 days survival persistent post-synaptic thickenings may be found in the periglomerular region apposed to glia or dendrites; it should be noted that the periglomerular region does not receive olfactory terminals (Chapter 5), so this cannot be due to atrophic or spontaneous degeneration of these terminals. These membrane thickenings are of the asymmetrical type normally found in relation to centrifugal terminals and are distributed in processes known to receive centrifugal synapses. They correspond in their general features to those which have

been described after olfactory nerve lesions, and, more specifically, to those observed in the external plexiform layer of the bulb after lateral olfactory tract section by Price & Powell (1970a).

Material after lesions in the olfactory bulb

Brains have also been studied in which small lesions were placed by fine needles in the superficial layers of the dorsal aspect of the bulb (Chapter 10); the site and extent of the lesion was found in a 2 um thick section and electron microscope sections were taken from closely adjacent glomeruli. Degeneration of olfactory terminals is seen in these glomeruli and takes an exactly comparable course to that observed after olfactory mucosa removal, passing through all the same stages to leave the post-synaptic thickenings in the long term (Figs. 80,81). Although a similar spread of stages of degeneration can be seen at the various survival periods, the time course of the degeneration is slightly different, the predominant stage appearing rather earlier than after the mucosal lesion.

After such lesions, in which many intrinsic fibres have been cut in addition to the olfactory fibres, degeneration may be seen in other types of axon terminal, principally those lying in the periglomerular region. Most of these are the recurrent collaterals of external tufted cells, or else the axons of periglomerular or superficial short-axon cells, although in some cases (rostral to the lesion) the centrifugal fibres may also be interrupted. As described in Chapters 3 and 5, the terminals of the periglomerular cells and short-axon cells make synaptic

Fig. 120 Early degeneration of a vomeronasal nerve terminal in the accessory olfactory bulb, showing the unusual characteristics of degeneration in a minority of terminals - a grey granular matrix with many large pale mitochondria, a few normal sized, and a few swollen vesicles in an enormous terminal.

X30,000

Fig. 121 Persisting symmetrical post-synaptic thickenings on a mitral and a tufted cell periglomerular stem dendrite after an intrinsic lesion (5 days survival); both are apposed to the same dendritic profile (of a periglomerular cell). Note the alveolate vesicles, the extracellular material and the clear thickening on the post-synaptic membrane.

X66,900

Fig. 122 Persisting symmetrical post-synaptic thickening on the periglomerular part of a mitral cell primary dendrite, close to its primary glomerular branching; apposition is to a glial profile. Intrinsic lesion, 5 days survival.

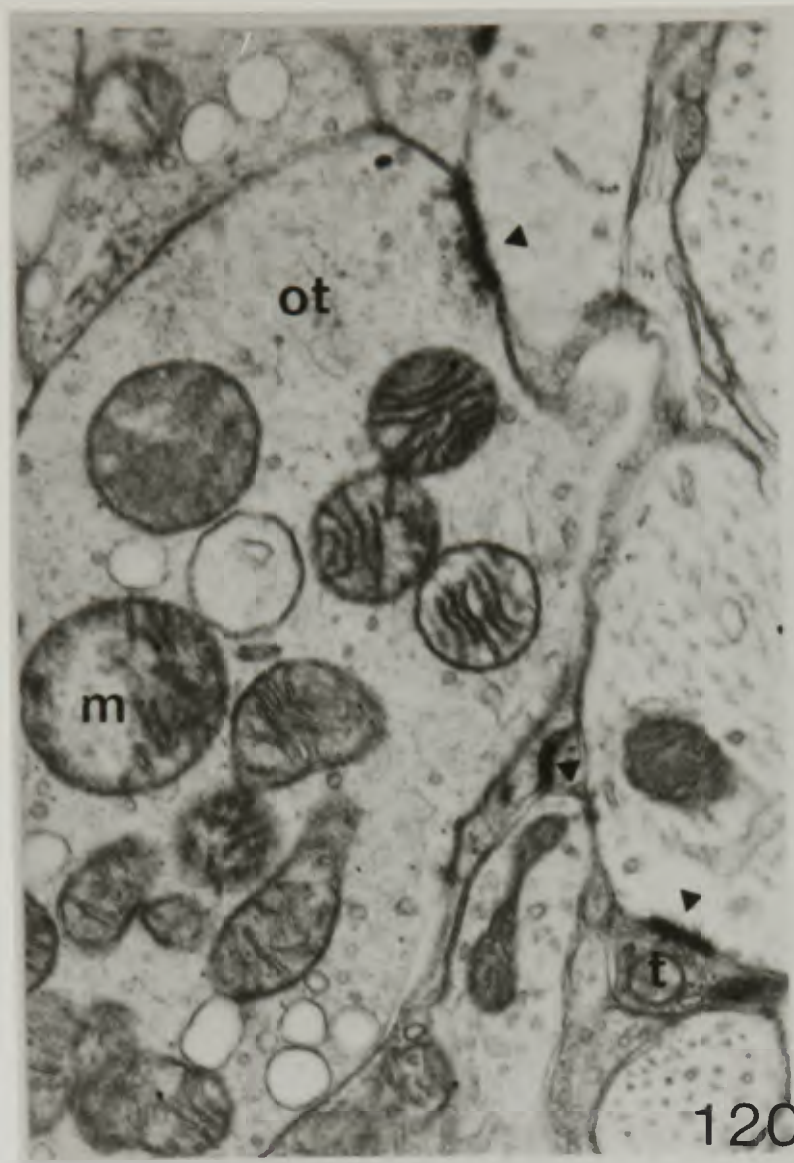
X66,900

Fig. 123 Persisting symmetrical post-synaptic membrane thickening on a mitral cell dendrite in its periglomerular course, apposed to a glial profile showing filaments and glycogen-like granules. Intrinsic lesion, 5 days survival.

X89,200

Fig. 124 Persisting symmetrical post-synaptic thickening on a mitral cell primary dendrite in the periglomerular region; note the alveolate vesicle. Intrinsic lesion, 5 days survival.

X71,300



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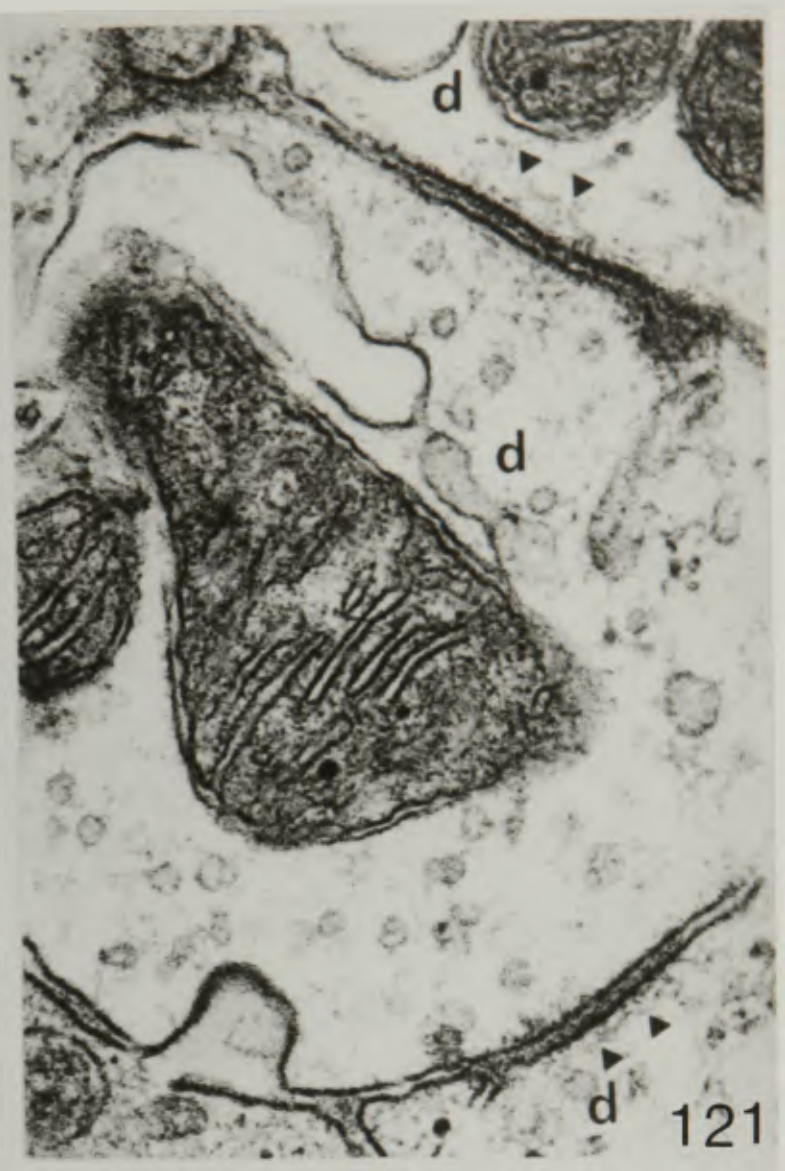

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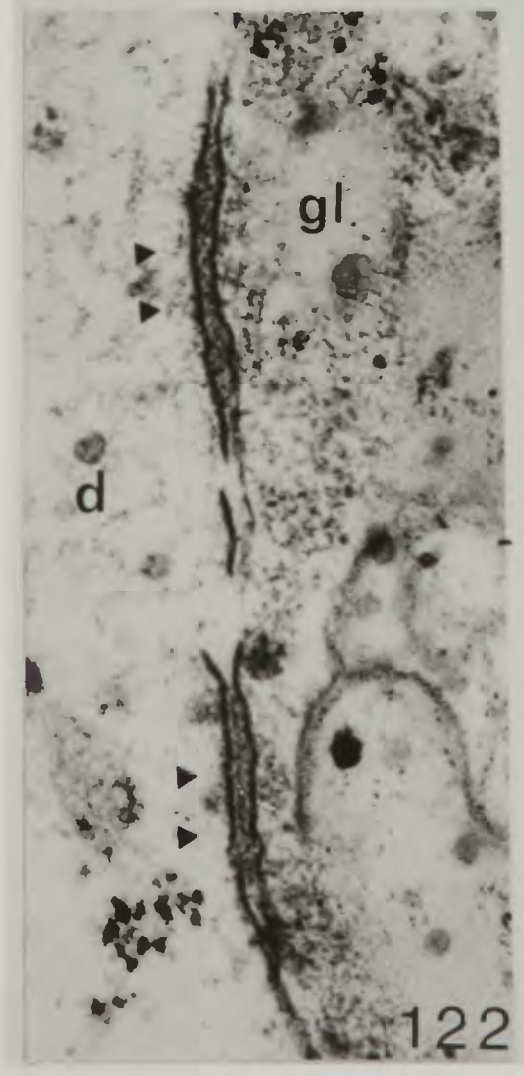

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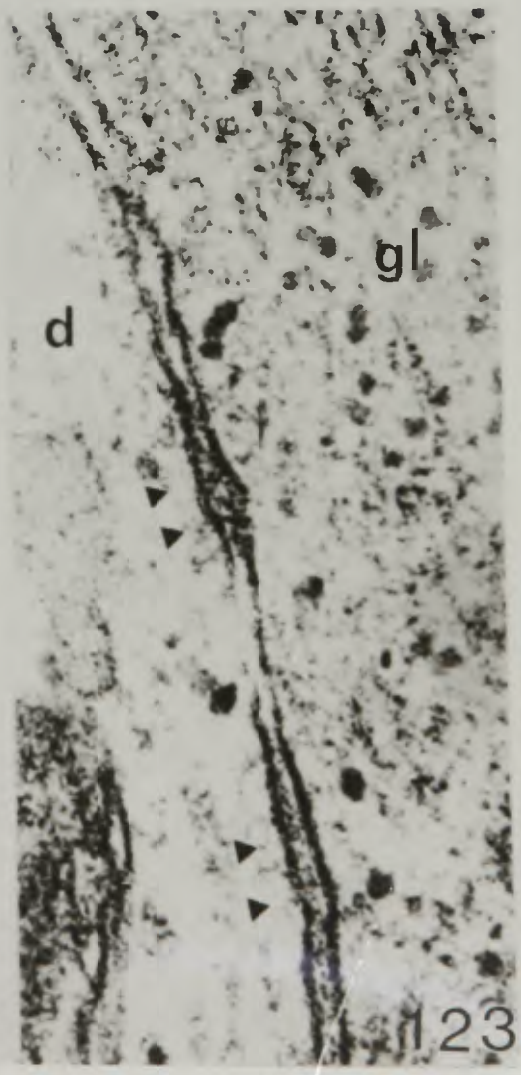

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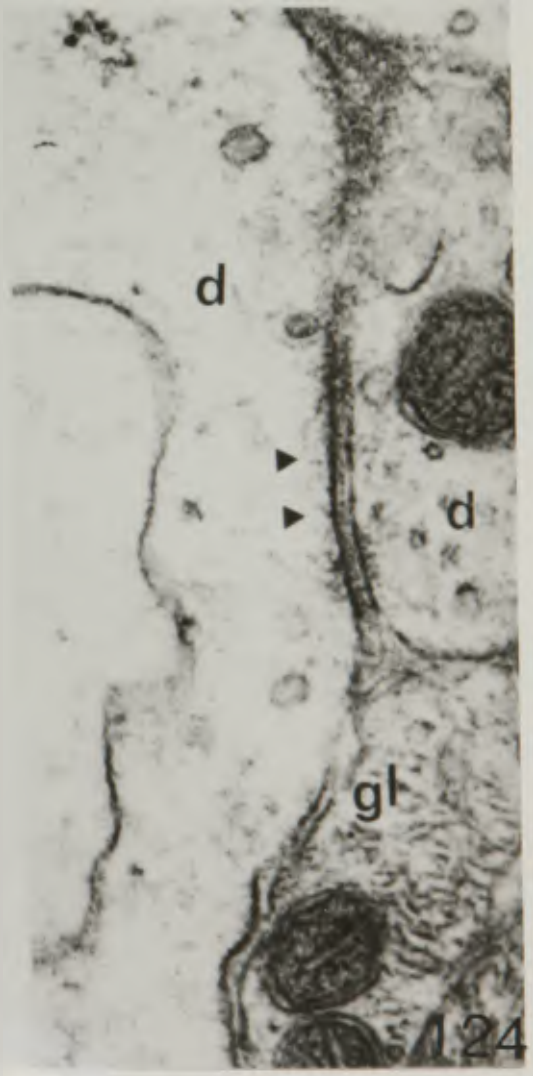

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contacts with symmetrical membrane thickenings. The former neurons make axonal contacts on to a very specific region of the mitral and tufted cell dendrites, in the vicinity of the point of their primary branching. In material from animals with such intrinsic lesions and after fairly long survival periods, post-synaptic thickenings of the symmetrical type may be seen in the same specific region of the tufted and mitral cell dendrites without presynaptic specialisations, but apposed to glial (Figs. 122,123) or dendritic (Figs. 121,124) profiles. These are found up to four to six glomeruli distant from the lesion, which corresponds to the usual length of the periglomerular cell axons known from evidence of Golgi-impregnated material (Blanes, 1898; Chapter 3). They show a slight thickening, situated mainly on the membrane of the mitral or tufted cell dendrite with no web extending into the cytoplasm, and a clear and distinctive row of granules in the intercellular cleft (Figs. 121-124). Since it is difficult to find unequivocal examples of these persisting symmetrical thickenings in other sites of termination of the axons of these cells (such as cell somata and other dendritic shafts), on account of low sampling and the less exactly predictable loci of termination, it can only be assumed that a similar process occurs after the degeneration of the presynaptic elements of these synapses.

On account of the distribution of recurrent collateral and centrifugal terminals, and the fact that they are distinguished from olfactory nerve terminals largely by the features of their presynaptic profiles, it is less easy to be certain, in this material, of the

persistence of their post-synaptic thickenings; asymmetrical thickenings do, however, appear in the periglomerular region after intrinsic lesions, a site which olfactory terminals never reach. These may be seen up to ten glomeruli distant from the lesion and in sites where normal and early degenerating terminals may be seen in normal and shorter survival material.

DISCUSSION

The sequence of degeneration in the olfactory nerves involves a transient swelling of both terminal and vesicles, followed by a darkening reaction and the shrinkage of the presynaptic terminal; some of the larger terminals undergo a filamentous type of degeneration prior to darkening. The terminals then shrink away from the post-synaptic membrane thickening and extracellular cleft material, which thus become apposed to other neuronal or glial profiles. In the rat, the post-synaptic structures remain unchanged until the onset of transneuronal degeneration (after about 200 days survival - Chapter 8). Similar post-synaptic thickenings persist after other lesions involving different axons terminating in the glomerular layer of the olfactory bulb, including those with the symmetrical type thickenings of Colonnier (1968); section of the vomeronasal nerves gives rise to a similar sequence of degeneration in the accessory olfactory bulb. In all cases, the presence of the post-synaptic structures appears to induce

the formation of subsurface cisternae and alveolate vesicles close to the plasma membrane of the apposed neuronal profile, and of alveolate vesicles in glia, in the vicinity of the apposed membrane thickening.

The early swelling associated with degeneration is a relatively recent finding, but several examples may be found in the literature (Cuénod, Sandri & Akert, 1970; Akert, Cuénod & Moor, 1971). Darkening in degeneration has been well documented in recent years by its wide use in experimental tracing of pathways (e.g. Colonnier & Gray, 1962; Walberg, 1965; Alksne et al., 1966) and it appears to be the common final stage of the degenerative process in presynaptic terminals; filamentous degeneration (Gray & Hamlyn, 1962; Colonnier and Guillery, 1966; Mugnaini & Walberg, 1967) seems to be associated with only certain types of terminal and may be due to a difference in terminal size rather than fibre origin, since only a small proportion of large olfactory nerve terminals showed this type of degeneration. The progressive shrinkage of the degenerating terminal and its indentation by surrounding profiles in the darkening stage has also been noted previously (e.g. Lund, 1969; Lund & Lund, 1970).

The persistence of the post-synaptic membrane thickenings after the degeneration of the presynaptic terminal has now been described in several sites and its features are broadly similar in all these regions. Gray and Hamlyn (1962) first noted their occurrence in the central nervous system after optic nerve lesions in the chick: two cases of persisting thickenings were described, both apposed to glial somata,

in the tectum; this observation has been confirmed and extended by Lund (1969) for the same site in the mammal. Westrum (1966, 1969) has described the persistence of membrane thickenings in the pyriform cortex after olfactory bulb lesions and has shown both glial and neuronal apposition; in the latter case, part of the apposed plasma membrane also exhibited a persisting thickening. Westrum and Black (1968, 1971) noted that asymmetrical post-synaptic thickenings remained apposed to glia in the spinal trigeminal nucleus after rhizotomy; they also showed partial apposition to glial and neuronal profiles and showed apposition of thickenings to axon terminals showing symmetrical synaptic thickenings. There was no way of determining whether this apposition was active reinnervation or passive apposition, however, In the sympathetic ganglion of the frog, Hunt and Nelson (1965) and Botelo (1968) have shown persisting thickenings, the latter observing that the cleft width between the thickening and apposed (glial) profile was equivalent to the normal synaptic cleft width. Persistence of membrane thickenings has been described in the paravisual cortex after contralateral cortical lesions (Lund & Lund, 1970) with both glial and neuronal apposition; other examples of persisting thickening in spinal cord and cerebellum have been given by Conradi (1969) and Mouren-Mathieu and Colonnier (1969). Terminal shrinkage and persistence of the post-synaptic membrane thickening during the final stage of degeneration of centrifugal fibres in the external plexiform layer of the olfactory bulb were described by Price and Powell (1970c). Although the post-synaptic specialisations are rather different at the

motor endplate, descriptions of post-synaptic specialisations remaining after denervation in this region with some degree of Schwann cell apposition have been given by Birks, Katz and Miledi (1960) and Miledi and Slater (1968).

There can be no doubt therefore that in many sites, post-synaptic structures may persist in the absence of the presynaptic terminals to which they were related; whether or not this is a general phenomenon is yet to be shown, but it is likely that transneuronal degeneration (Chapter 8), removal of spines or parts of dendrites with their terminals (Colonnier, 1964a; Mouren-Mathieu & Colonnier, 1969), or possibly reinnervation in certain sites (Raisman, 1969; Westrum & Black, 1971; Lund & Lund, 1971) may obscure it.

It is a significant observation that the extracellular material remains attached or related to the post-synaptic thickening after complete degeneration of the presynaptic terminal, so that the cleft width during apposition is the same as that of a normal synapse (Sotelo, 1968); it seems therefore to be an integral part of the post-synaptic complex. It should be noted that such an assertion can only be tested by determining the fate of the cleft material after causing degeneration of the post-synaptic process while leaving the presynaptic terminal unaffected; it is possible that this extracellular material remains attached to the remaining normal membrane. The apparent adhesive properties of the post-synaptic complex have already been mentioned; the observation of the structural integrity of synaptosomes (Gray & Whittaker, 1962) may be explained in terms of

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It is a significant observation that the extracellular material remains attached or related to the post-synaptic thickening after complete degeneration of the presynaptic terminal, so that the cleft width during apposition is the same as that of a normal synapse (Sotelo, 1968); it seems therefore to be an integral part of the post-synaptic complex. It should be noted that such an assertion can only be tested by determining the fate of the cleft material after causing degeneration of the post-synaptic process while leaving the presynaptic terminal unaffected; it is possible that this extracellular material remains attached to the remaining normal membrane. The apparent adhesive properties of the post-synaptic complex have already been mentioned; the observation of the structural integrity of synaptosomes (Gray & Whittaker, 1962) may be explained in terms of

adhesive properties of the post-synaptic complex, which seem to remain when the complex is in contact with any plasma membrane.

This leads to the question of the identity of the apposed profile and the role of glia in the last stages of terminal degeneration: the observations presented here, and those of Westrum (1969), Westrum and Black (1971), Lund and Lund (1970), and Price and Powell (1970c), have shown that the apposed profile may be either glial or neuronal; the evidence gained in the olfactory bulb has given clear indication that neuronal profiles may be apposed from the beginning of shrinkage of the presynaptic terminal. The neuropil of the glomeruli contains, apart from the olfactory nerve terminals, the dendrites of mitral, tufted, and periglomerular cells, glial profiles, and very few other axon terminals (Blanes, 1898; Chapter 4). Apart from glial proliferation in the early stages of degeneration, there is no numerical alteration in these components of the neuropil; the apposition of different profiles to persistent post-synaptic thickenings appears to be related simply to the frequency of occurrence of these particular processes in the region of the degeneration at that particular survival time, i.e. in a predominantly dendritic population, it is mainly dendrites that are apposed. Thus it is suggested that those profiles that lie on the side of a normal olfactory terminal opposite its synaptic thickenings become apposed to the post-synaptic complex that persists after complete degeneration of the presynaptic terminal. It has been noted that although glial apposition does occur, it does not seem to be obligate, and probably results from the adjacence of the

glial profile to the normal or early degenerate olfactory terminal.

In most animals, denervation was not complete and small groups of olfactory terminals remained normal; these terminals showed no evidence at any survival time either of proliferation or apposition, even to closely adjacent persisting thickenings and cannot therefore be considered as capable of actively reinnervating the post-synaptic sites (cf. Raisman, 1969). However, because many of the receptor cells from which the olfactory axons originate were destroyed at operation, the possibility of reinnervation from the original cell after axonal section has not been excluded; electron-microscopic evidence of regeneration of olfactory nerves has been claimed in Amblystoma (Winkelmann & Marx, 1969). Nevertheless, in cases of apposition to those dendrites which make synapses in the glomeruli (Class B dendrites - Chapter 5), there is no morphological evidence of presynaptic membrane specialisation or synaptic vesicle aggregation, even close to the sites of normal dendro-dendritic synapses in the apposed dendrite (Figs. 108,109,111,112); this suggests that the apparent synaptic contacts formed by apposition may be functionally different from normal synapses. That is to say, apposition of a persisting post-synaptic membrane thickening to a process that makes conventional synaptic contacts with other processes does not necessarily indicate functional synaptic contact, although it does not preclude it either; such equivocation serves to demonstrate that solely morphological criteria cannot be sufficient to demonstrate new functional synapses or reinnervation (cf. Raisman, 1969; Westrum & Black, 1971; Lund &

Lund, 1971). This important point may require clarification: any material that is examined at long periods after denervation may show persisting post-synaptic thickenings, many of which would be apposed to neuronal profiles. The evidence from the olfactory bulb shows that persisting thickenings are apposed to whatever process happens to be adjacent; naturally, a number of these are processes that normally make synaptic contacts with other profiles - i.e. Axons or Class B dendrites (Chapter 5). Simply to observe apposition to such processes cannot be considered definitive evidence of "reinnervation", for one cannot consider appositional contacts of exactly similar thickenings to glia and Class A dendrites (that do not normally make synapses) as new functional synapses. It is interesting to note that all reports that suggest the possibility of reinnervation mention that such "spare" post-synaptic thickenings were also seen (Raisman, 1969; Westrum & Black, 1971; Lund & Lund, 1971). Probably the only valid criterion for reinnervation anatomically is the observation of clear presynaptic specialisations in relation to such apposed thickenings. It should again be stressed that such reasoning is not designed to exclude the possibility of reinnervation, rather it is to show that other explanations exist for the observations of supposed reinnervation and that these latter cannot be considered valid proof for such a process. Similarly, the possibility of sprouting of remaining axons is not being questioned, but it should be noted that where such sprouting is observed the sprouts do not necessarily effect functional synaptic contacts. The results from the olfactory bulb also demonstrate

that, if experimental material is used incidentally for the study of other, normal synaptic relationships, then both pre- and post-synaptic specialisations must be evident for either qualitative or quantitative evaluation of this type of material.

The induction of cisternae and alveolate vesicles in the newly apposed neuronal profiles are significant in that they do demonstrate some degree of interaction between these two previously unrelated processes, although the nature of this interaction is difficult to interpret on solely morphological grounds. Two suggestions may be made as to the significance of the interaction: a) that the chemical components or physical properties of the post-synaptic complex lead to a reaction in the apposed profile and, in particular, its plasma membrane. b) that the cisternae are new or modified synaptic specialisations similar to the 'sac junctions' seen elsewhere in the bulb (Hirata, 1964; Price & Powell, 1970d; Chapter 3). These suggestions are not mutually exclusive, but the second must remain in doubt until there is definite evidence that sac junctions are active synaptically and to not merely represent a non-specific response to an unused post-synaptic thickening; that such subsurface cisternae (Rosenbluth, 1962b; Siegesmund, 1968) are common morphological features with diverse cytological functions is not unlikely. Alveolate vesicles have been related to the phenomenon of micropinocytosis, and the frequent observation of them in continuity with plasma membranes would support this; their ultimate fate is uncertain but some authors consider they contribute to the formation of multivesicular bodies (see

Peters, Palay & Webster, 1970). Waxman and Pappas (1969) showed that they are commonly found close to the plasma membrane surrounding the post-synaptic thickening of normal synapses in the central nervous system; they also demonstrated uptake of ferritin by these coated vesicles, and suggested that they were involved in a process "not immediately related to synaptic transmission". This suggestion would appear to be borne out by their relationship to persistent post-synaptic thickenings in the glomeruli; what their role is, however, is not clarified by these observations, but in this site at least, the post-synaptic complex seems to be an important factor in inducing their formation.

The observation of such a dynamic interaction between a persisting post-synaptic specialisation and other neuronal and glial profiles may shed some indirect light on developmental mechanisms. That this post-synaptic site is capable of inducing morphological, and therefore presumably functional, alterations in nearby processes may signify that it plays a far more active role in establishing synaptic connections during development than has hitherto been considered. If all post-synaptic sites are not occupied during development, and do not disappear, they could give rise to 'persistent' thickenings with induced cisternae, and may even explain the presence of 'sac junctions' in normal material (Chapter 5); it is significant that such junctions are commonly orientated away from cell somata. The question of trans-neuronal degeneration in relation to persisting post-synaptic thickenings has been discussed elsewhere (Chapter 8) but it should be noted that at

the onset of transneuronal change, most of the post-synaptic thickenings disappear together with the terminal parts of the glomerular dendrites; it is possible that a similar process may occur during normal development in the cases of most unoccupied post-synaptic sites.

CHAPTER 8

ULTRASTRUCTURAL FEATURES OF TRANSNEURONAL CELL DEGENERATION IN THE OLFACTORY SYSTEM

"When other philosophers rejected the evidence of the senses because these showed plurality and change, Heraclitus rejected their evidence because they showed things as if they possessed duration and unity."

Friedrich Nietzsche.

INTRODUCTION

The studies of Wiesel and Hubel (1963a,b) on functional deafferentation have stimulated fresh interest in transneuronal degeneration by their elegant demonstration of the physiological and anatomical changes in the neurons of the lateral geniculate nucleus and visual cortex consequent on light deprivation in the kitten. There have been many light microscopic descriptions of transneuronal degeneration using thionin-stained material (e.g. Cook, Walter & Barr, 1951; Matthews, Cowan & Powell, 1960); these have shown cytoplasmic and nuclear shrinkage and some alterations in the staining of the Nissl substance. The Golgi method has been used by several groups (Matthews & Powell, 1962; Jones & Thomas, 1962; White & Westrum, 1964; Globus & Scheibel, 1966; Valverde, 1967; Powell, 1967; Coleman & Riesen, 1968) to demonstrate alterations in the impregnation of neurons after deafferentation. There have been no complete studies of transneuronal changes with the electron microscope, however, though Smith, O'Leary, Harris and Gay (1964) and Mouren-Mathieu and Colonnier (1969) have noted some features. Although the primary cause of the degenerative change following deafferentation or sensory deprivation is probably of a biophysical or biochemical nature, the latter could give rise to secondary cellular alterations, distinguishable with this technique, other than cell shrinkage.

In view of the characteristic changes in the Golgi impregnation of the constituent neurons of the olfactory system after olfactory

nerve section, this site is very suitable for studying the ultrastructural correlates of these altered impregnation properties; the findings with the two techniques are complementary and their correlation may throw some light on the possible mechanisms of Golgi impregnation. The electron microscopic changes that were found seemed to correspond to some previous descriptions of 'dark neurons' in the central nervous system (Cammermeyer, 1962; Cohen & Pappas, 1969), so that this study has taken on a further significance in the elucidation of some of the different types of 'dark neurons' and their origin.

RESULTS

Rabbit Electron Microscopy

Light-microscopic studies on transneuronal degeneration have concentrated largely on size changes of cells, their nuclei and processes; the electron microscope, while in general unsuited to this kind of study on account of the thinness of the sections used and the limited areas studied, can provide more detailed qualitative data on the cytological changes involved. It is possible that the changes to be described here represent only one feature of the transneuronal degenerative process, being at this level the most distinct morphologically; however, the regularity of this characteristic degenerative change and the gradation in its severity found in different cells, as well as the close correspondence between the proportion of

affected cells and the extent of denervation within each brain, indicate that it is directly consequent upon the section of the olfactory nerves. Subtler alterations may occur in cells which appear normal in this kind of examination. Thus, although the published quantitative results of Matthews and Powell(1962) on mean cell areas would indicate that all mitral and tufted cells shrink, the areas of some individual cells remain within the normal range after degeneration. The electron microscopic findings show that roughly two-thirds of the mitral and tufted cells show the qualitative changes described below, while the rest appear normal, although they may be slightly shrunken.

The cellular changes described here have been seen at all the survival times studied and, apart from slight variations due to differences in the extent of denervation, there appears to be no significant alteration in the proportion of cells affected after 24 days. In all the material used, both from the normal and the operated sides, the material was regularly well-fixed and the cells that remained normal were well-preserved. However, because the onset of transneuronal degeneration is accompanied by a marked increase in the extracellular and glial volume in the glomeruli (Figs. 141,142,146) although the processes that remain seem adequately preserved, most material was taken from the deeper layers. In animals of 10-49 days' survival (aged 60-95 days), few or no cells on the normal side showed any cytological change similar to that on the operated side, and those that did could be related to some olfactory nerve degeneration at glomerular level, either spontaneous or possibly due to slight damage

of the nasal septum at operation. In the older animals of 70 and 90 days' survival (aged 120-135 days), more changed cells were seen on the normal side, although they were still considerably rarer than on the operated side; this would correspond with the observation that atrophic rhinitis occurs in animals of this age (Matthews & Powell, 1962).

While all the features described have been found in mitral, tufted, periglomerular, granule, and occasionally, short-axon cells, and show no variation according to type, for the reasons stated above, the mitral and granule cells will be used principally as examples; the mitral cells have the added advantage that they may be readily distinguished from glial or other cells by virtue of the reciprocal synapses on their cell body and dendrites (Andres, 1965) (Figs. 136, 139). It should be noted that sections from the normal and operated sides were carefully examined in each animal and were directly compared in the initial analysis. The typical features of the degenerative changes will be described for each part of the neuron, after which the variations found in more or less severely affected cells and the time of onset of each change will be indicated.

Soma

The most striking features shown by transneuronally degenerating neurons are an increased electron-density, apparently due to a concentration of the cytoplasmic and nuclear contents, and the loss of distinction between different parts of nucleus and cytoplasm (Figs.

125,127-131). The perikaryon regularly appears very shrunken in comparison with neighbouring, apparently normal cells, as well as with those of the normal side. Unlike the smooth outline of normal cells (Fig. 125), the boundaries of these cells are irregular, as if they had been scalloped by adjacent processes (Figs. 127-130). The nucleus also appears somewhat shrunken, the nuclear membrane often being detached and vacuolated in between the pores which usually remain intact, thus giving rise to a beaded appearance (Figs. 127,131). It should be noted that the normal mitral cells of the rabbit, unlike those of the rat, have marked nuclear indentations, and these remain despite the transneuronal change. The nuclear contents, which are normally clumped into islets of granular material, become densely packed and tend to lose all such local morphological differentiation (Figs. 130,131). The nucleolus however, as far as we are able to assess, seems unchanged in all but the most extreme cases; in a few very dense cells, the nucleolus does show a segregation of its granular and fibrillar components (Fig. 133) similar to that seen with actinomycin D treatment of kidney and liver cells (Bernhard & Granboulan, 1968). Intranuclear fibrillar rodlets (Masurovsky, Benitez, Kim & Murray, 1970) have been found in tufted and periglomerular cells more frequently than in normal material (Fig. 132).

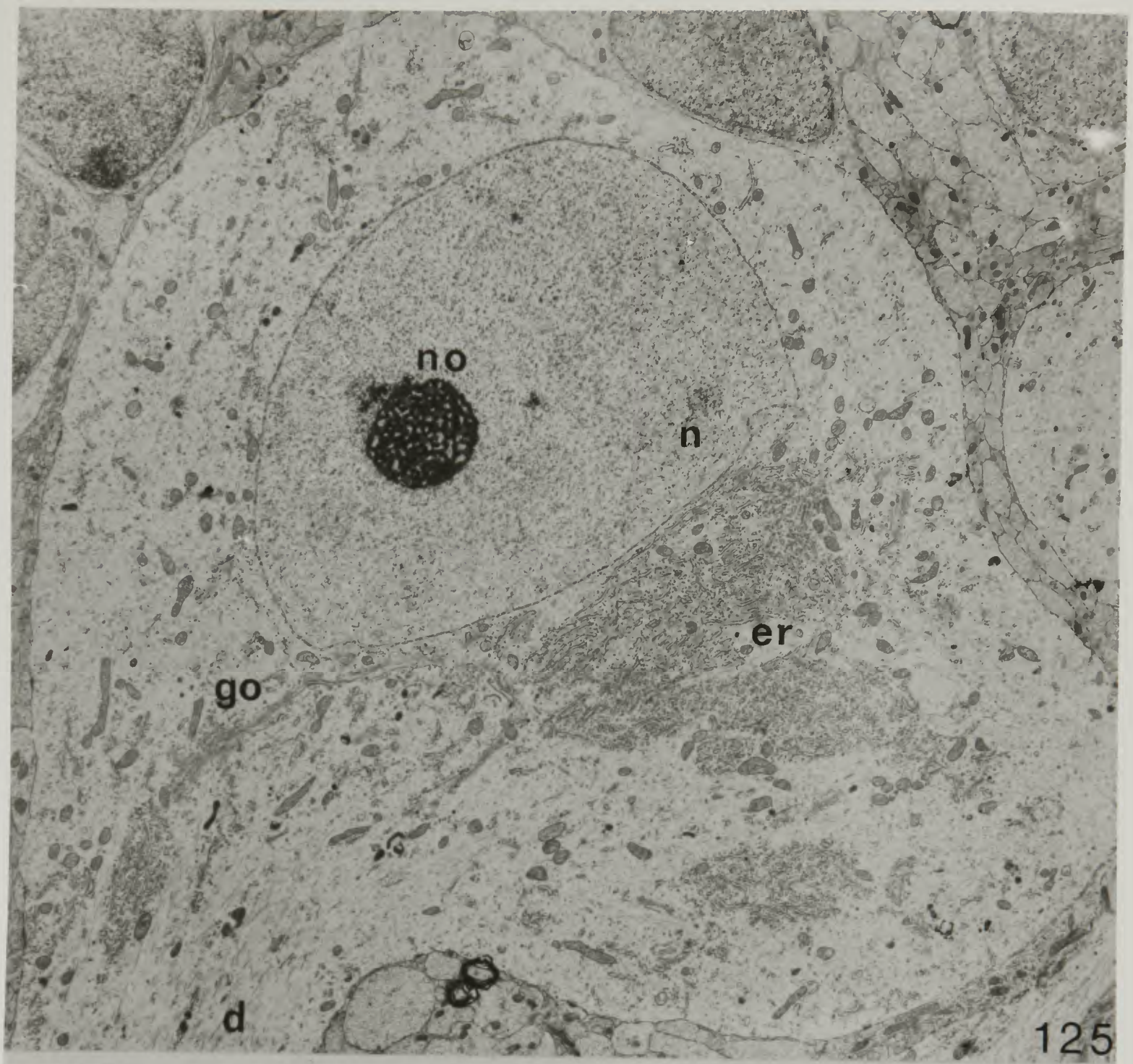
Cytoplasmic contents are tightly packed within a grey granular background, which seems to consist of a highly concentrated form of the fine dense material that is normally present in the cell (Figs. 127-131). Free ribosomes, lending a coarse grain to the cytoplasm, are

Fig. 125 Normal Mitral cell with beginning of primary dendrite.

X7,400

Fig. 126 Transneuronal degeneration of primary dendrite of Mitral cell in Fig. 127, passing between predominantly normal processes in deep part of external plexiform layer. 70-day survival.

X6,200



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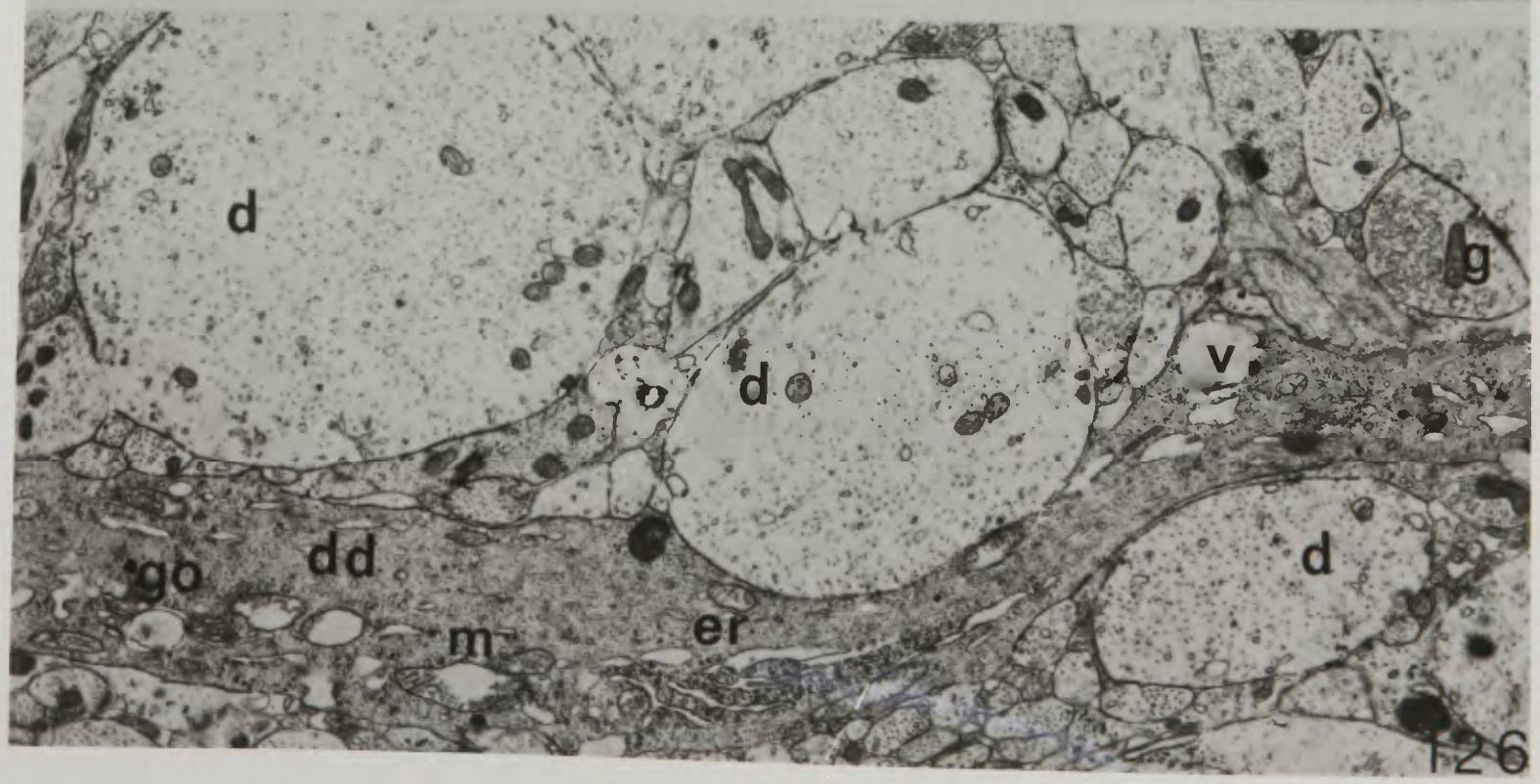

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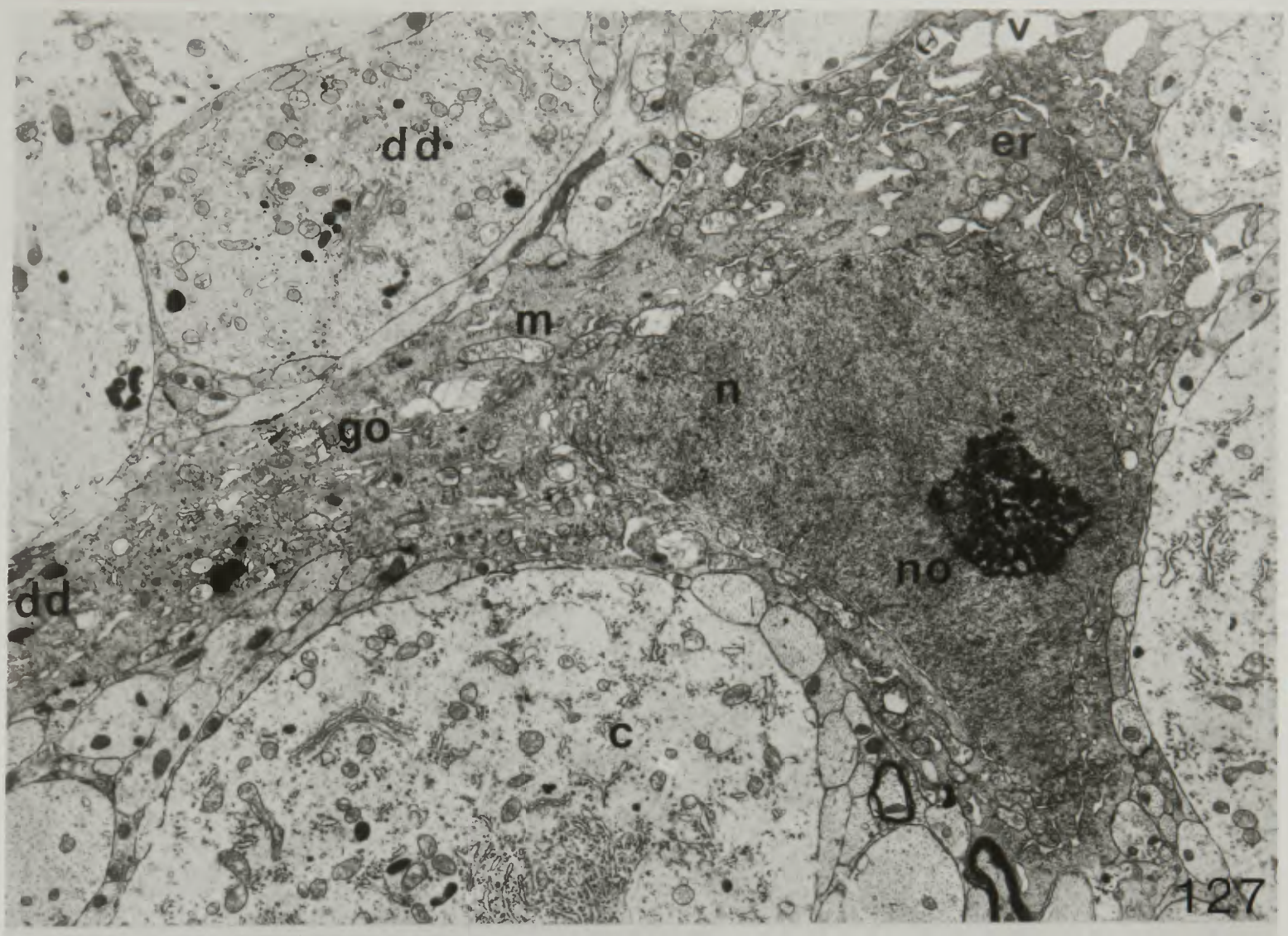
Fig. 127 Moderate transneuronal degeneration of Mitral cell, with beginning of primary dendrite. 70-day survival. (Note the lower magnification from Fig. 125).

X6,200

Fig. 128 Mild degeneration of Mitral cell. 70-day survival.

X7,000



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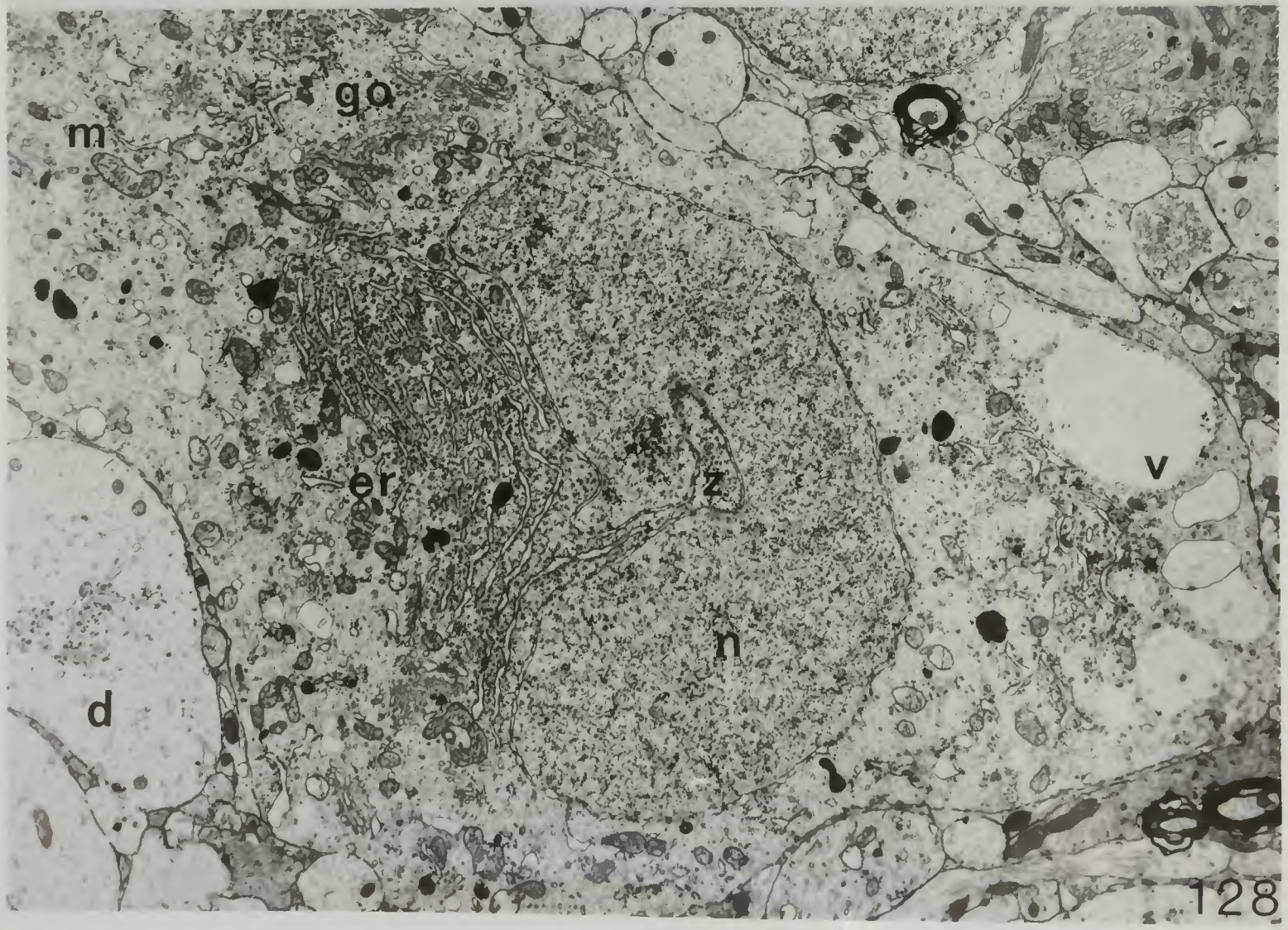

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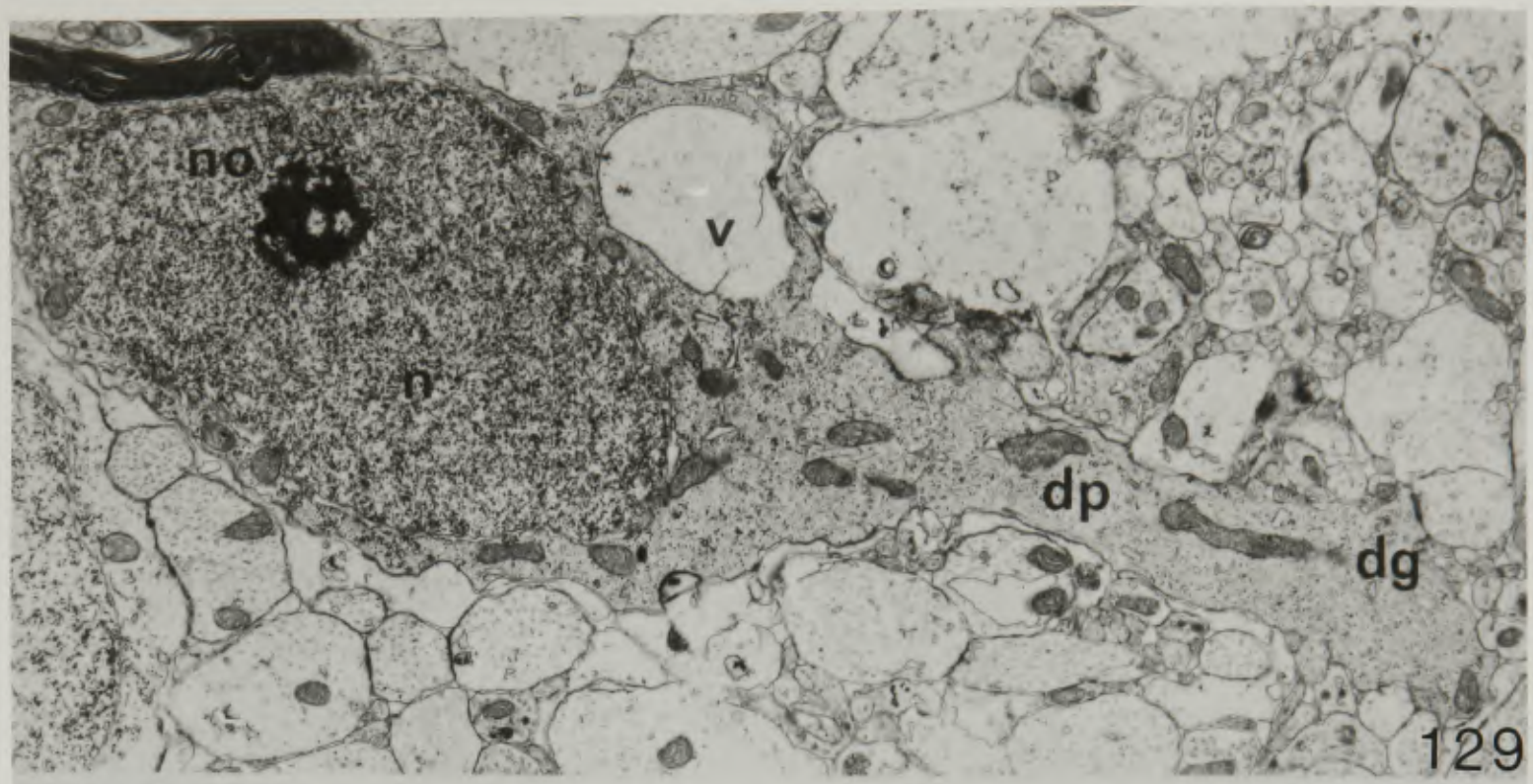
Fig. 129 Mild degeneration of Granule cell with peripheral process and gemmule receiving synapse. 70-day survival.

X9,200

Fig. 130 Severe degeneration of Granule cell with somatic spine (see Fig. 134). Note part of normal Granule cell (right) and mild degenerate granule cell (left). 49-day survival.

X15,700



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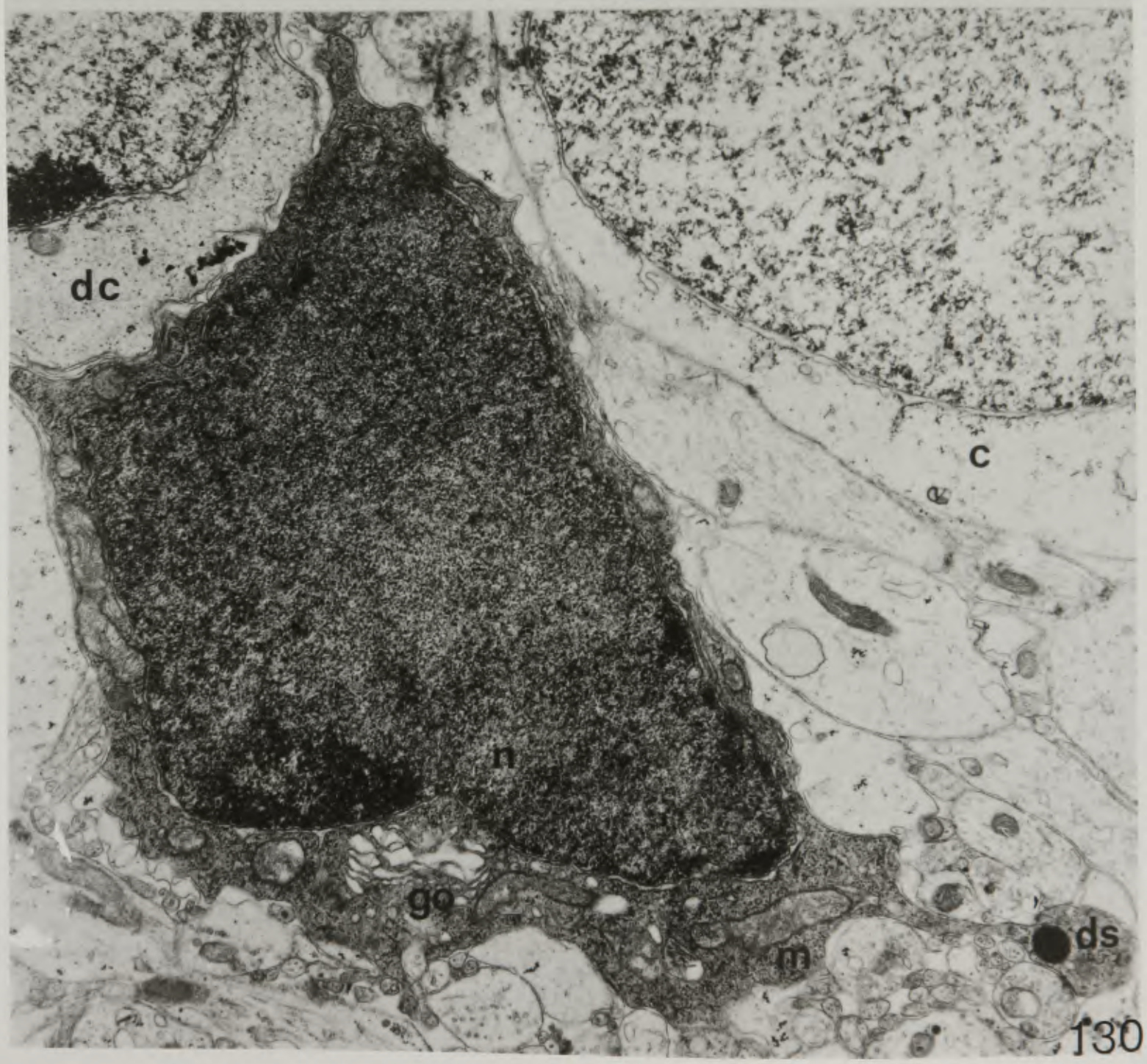

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Fig. 131 Detail of moderate degeneration in a Mitral cell, showing the characteristics of nucleus and cytoplasm. Note the intact pores in the nuclear membrane around the cytoplasmic indentation (arrows). 90-day survival.

X59,000

Fig. 132 Detail of an intranuclear fibrillar rodlet from a degenerating Tufted cell. 49-day survival.

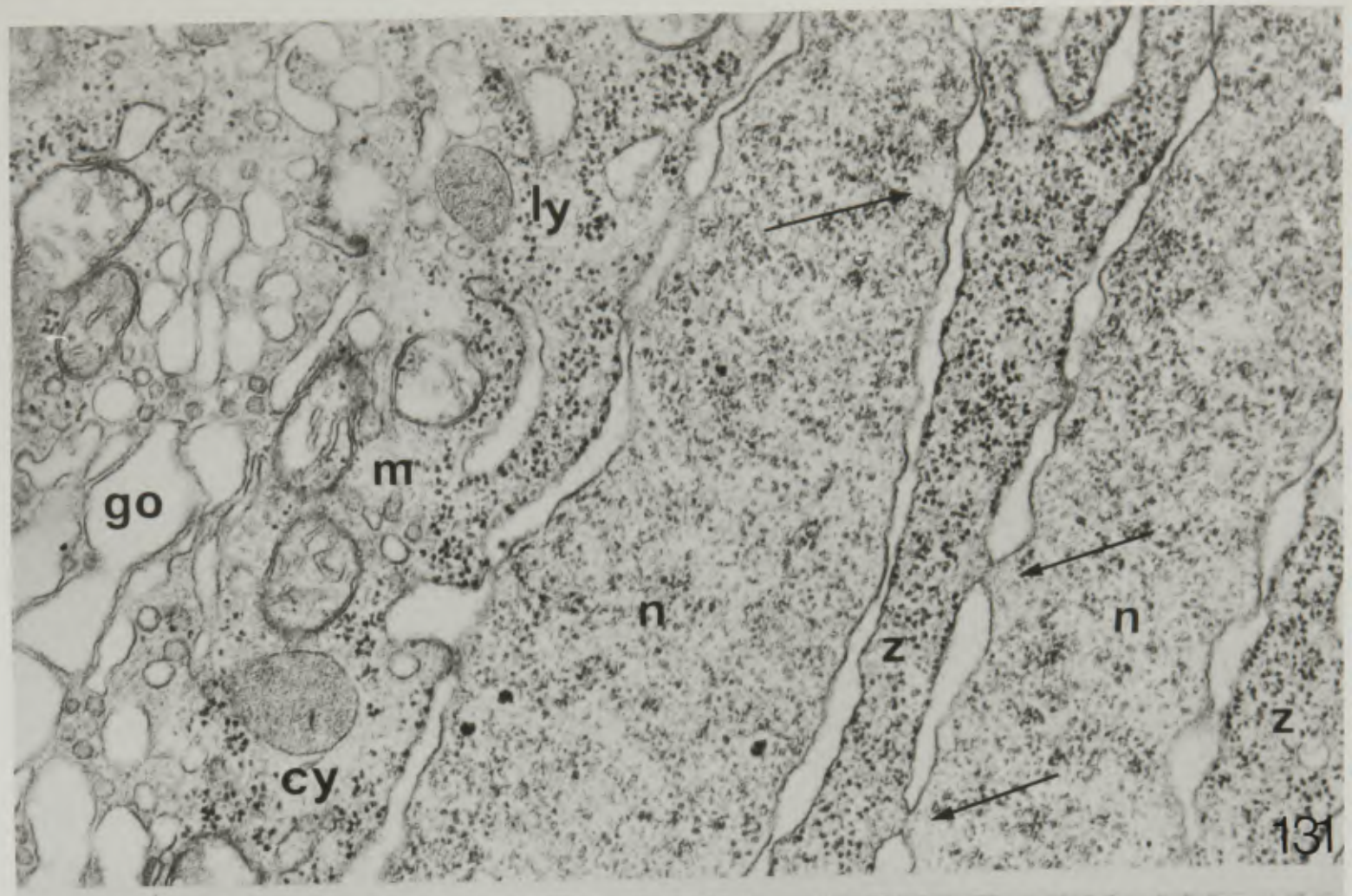
X54,900

Fig. 133 Detail of the Nucleolus in severe degeneration of a Mitral cell, showing segregation of nucleolar components (cf. Fig. 125). 49-day survival.

X26,000

Fig. 134 Detail of somatic spine of the Granule cell in Fig. 130, receiving a synapse from an apparently normal axon terminal. 49-day survival.

X35,600



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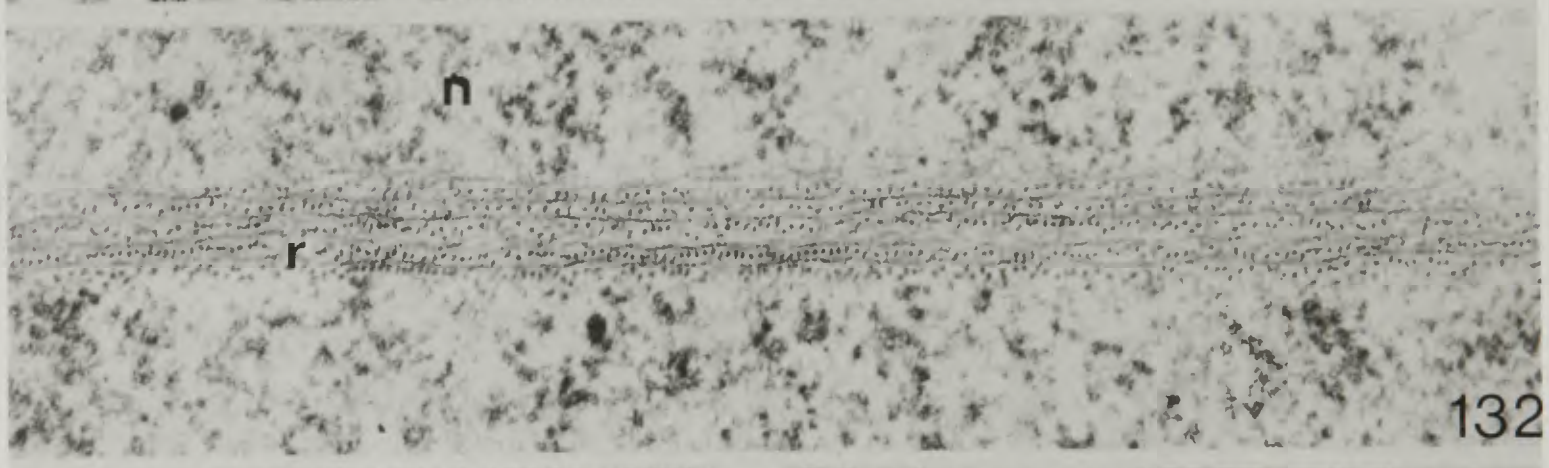

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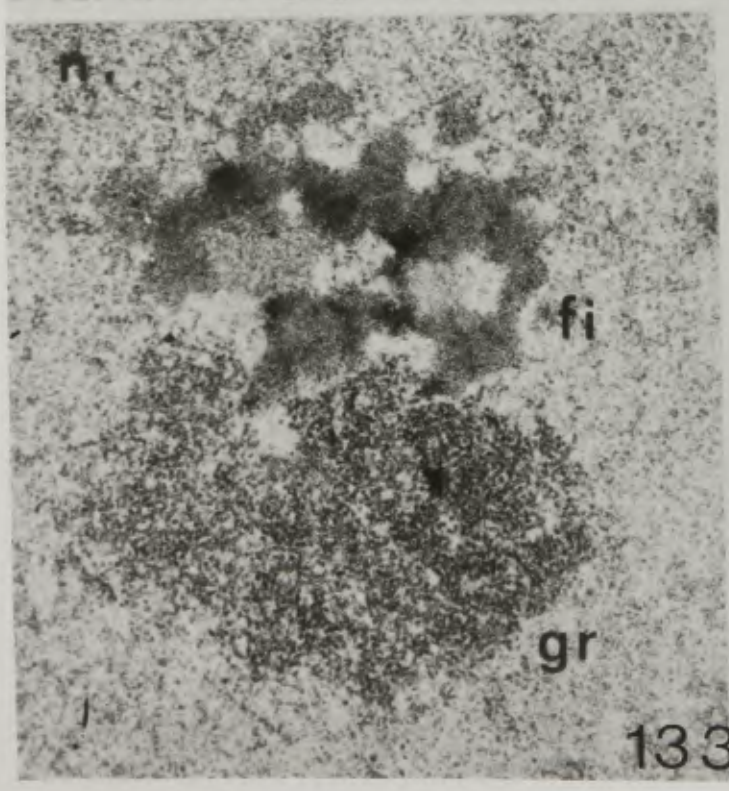

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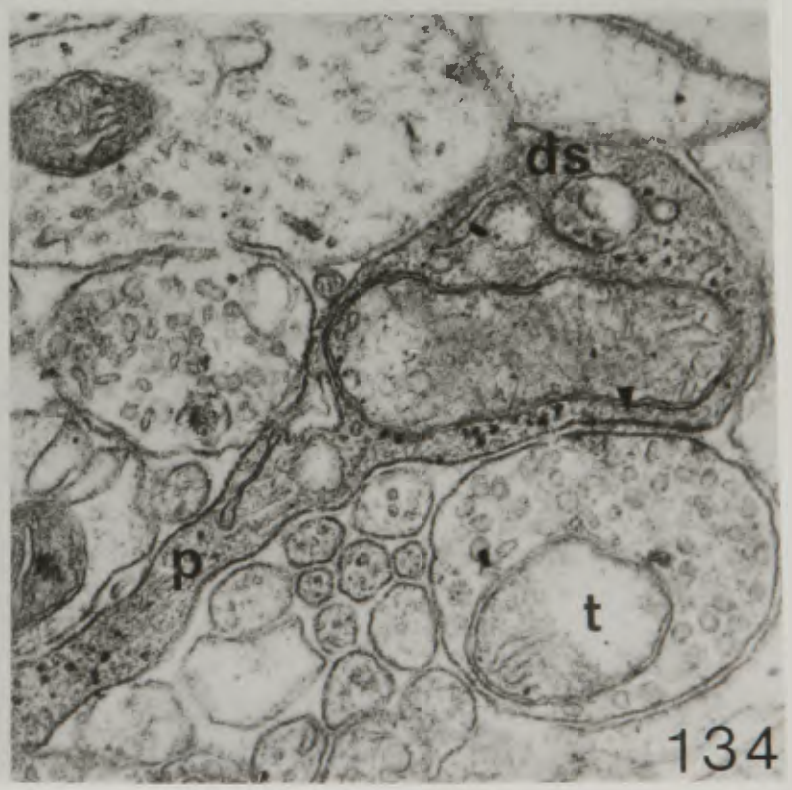


134

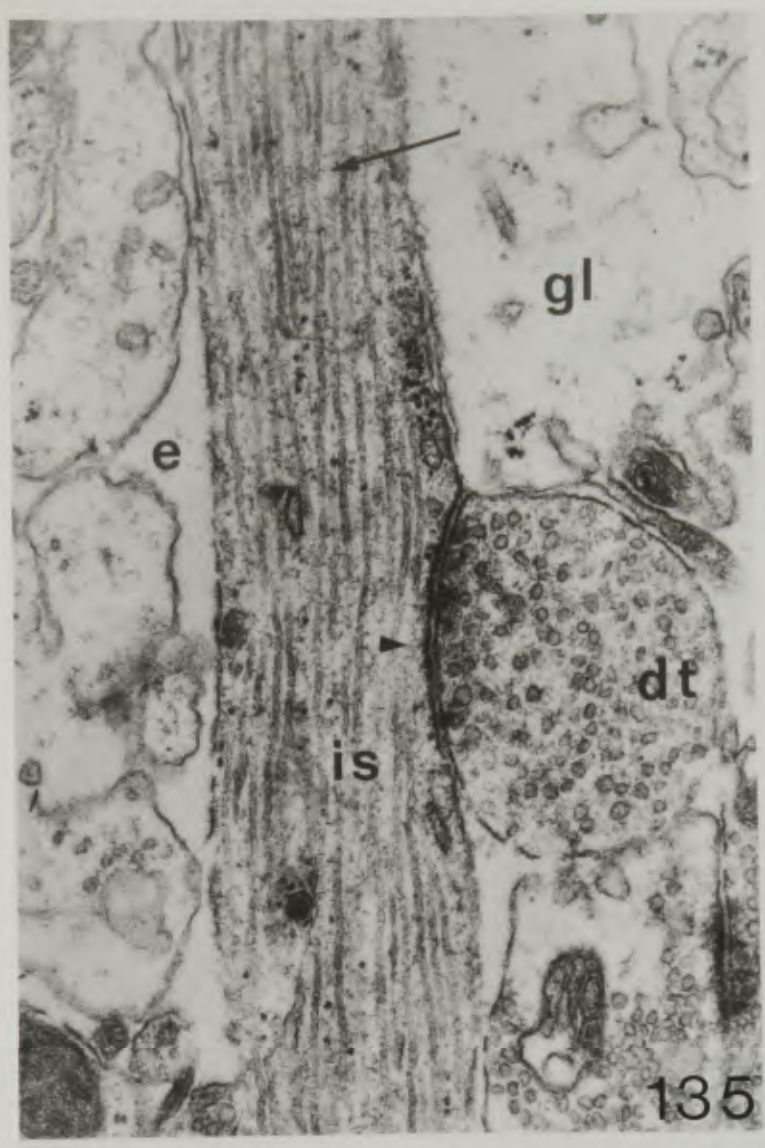
present in greater proportion while rosettes are rarely encountered; the granular endoplasmic reticulum seems to retain its attached ribosomes. All membrane-bound systems except lysosomes, that is mitochondria, endoplasmic reticulum, Golgi apparatus and multivesicular bodies, have a tendency to be swollen or vacuolated, the mitochondria showing a characteristic 'blown-up' appearance (Figs. 127,128,130,131), which is most marked in the large mitral cells; normal mitral cells do tend to show some blown mitochondria, but the disruption is more regular and more severe in transneuronally degenerating cells. Golgi apparatus and endoplasmic reticulum (Figs. 127, 130, 131) are found widely dilated in a manner never seen in normal cells. Fine granular lysosomes (Fig. 131), though present in normal neurons, are seen more frequently in degenerating cells, according to the severity of the change, but are never strikingly in evidence; dense lysosomes do not show any increase or morphological change. Large empty vacuoles often appear in the cytoplasm, particularly adjacent to the plasma membrane (Figs. 127-129). Synaptic structures will be dealt with in the following section.

Dendrites and appendages

Essentially the same kind of degenerative change has been found in the dendrites of the affected cells, including the peripheral processes of the granule cells, and if the cell body is affected then its processes are altered with a corresponding severity (see Figs. 126, 127). In the main dendritic shafts, which generally appear shrunken, there is

an increase in the fine granular background of the cytoplasm as well as in the proportion of free ribosomes, while rosettes are rare (Figs. 126,127,129,136-139,143,144); mitochondria and endoplasmic reticulum are swollen or dilated, as is any Golgi apparatus that may be present in the initial parts of dendrites (Figs. 126,127). But particularly characteristic of the dendritic change is the dense packing and altered appearance of the neurotubules (Figs. 126,136,143, 144); in severely affected cells they may be so densely packed that the intertubular spaces are no wider than the diameter of the tubules. The tubular outline, meanwhile, becomes more clearly defined and loses the web-like fringe that it normally exhibits; it may be that this fringe constitutes part of the fine granular background in dendrites. The clearer outline may be due to some swelling of the tubules in degeneration; however, as shown in Figs. 143 & 144, it is caused primarily by the fact that while the external tubular diameter remains approximately constant, the internal diameter increases. Fine granular lysosomes are present a little more commonly than usual. Varicose dendrites or varicose portions of dendrites shrink in such a way as to retain their varicose character, the connecting portions becoming extremely attenuated containing little more than tubules, and the varicosities remain comparatively paler, though revealing a typical cytoplasmic concentration (Fig. 142).

In the glomeruli, the olfactory terminals that have degenerated in the first 10-18 days after mucosa removal (Fig. 137) leave the post-synaptic thickening and extracellular material exposed, as described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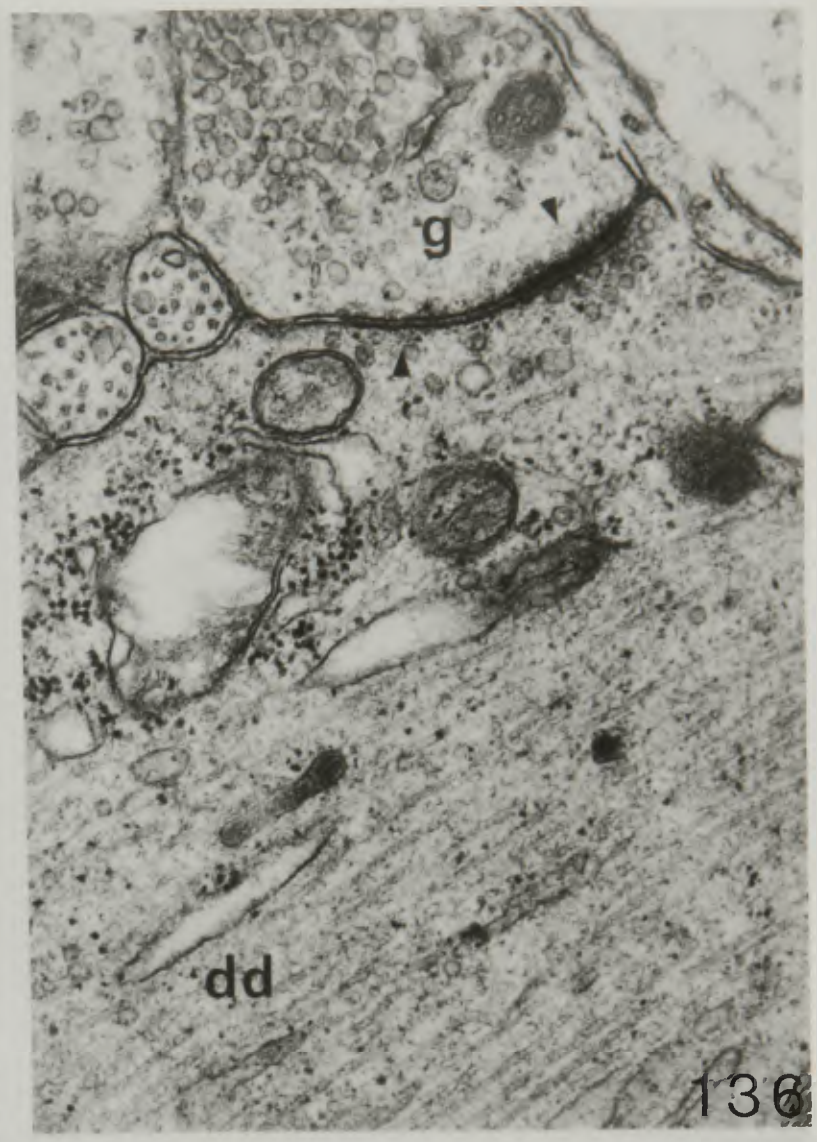
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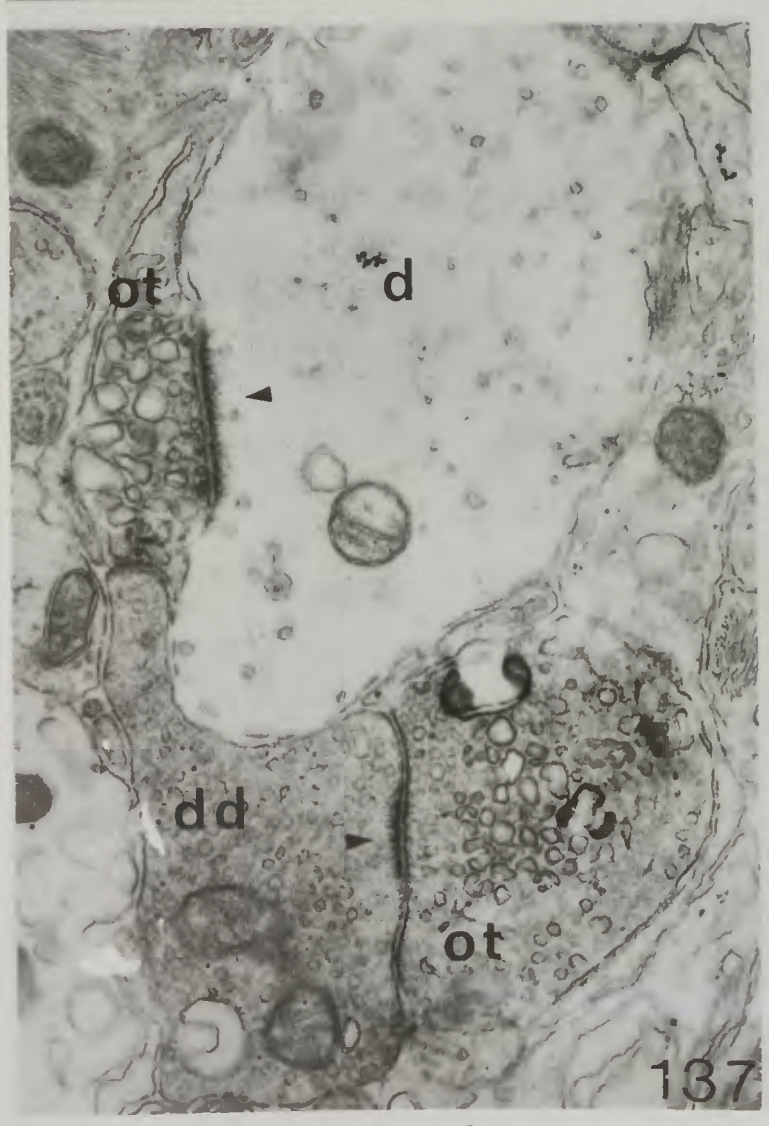
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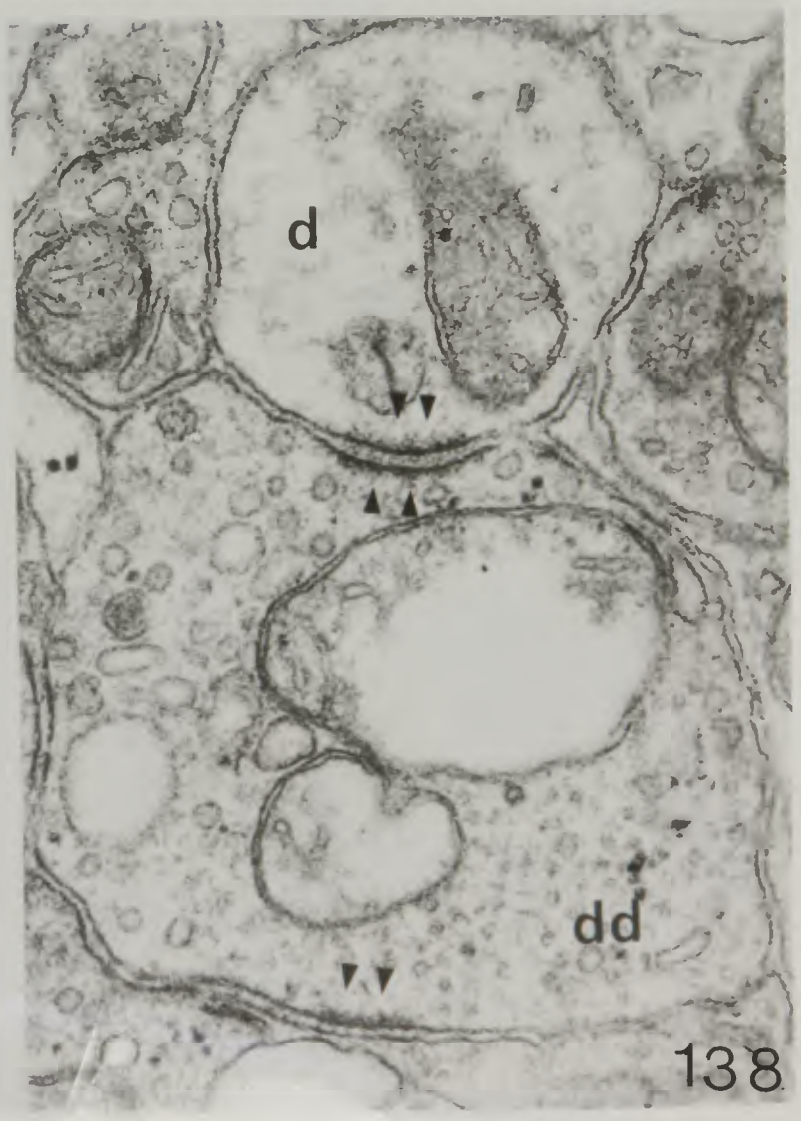
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Fig. 139 A normal gemmule in the external plexiform layer entering into a reciprocal synapse with a degenerating Mitral cell dendrite and receiving an asymmetrical synapse from a degenerating recurrent collateral. Synaptic polarities and axon degeneration were confirmed by serial sections. 70-day survival.

X24,700

Fig. 140 A degenerating dendrite in the external plexiform layer. Rat. 200-days survival.

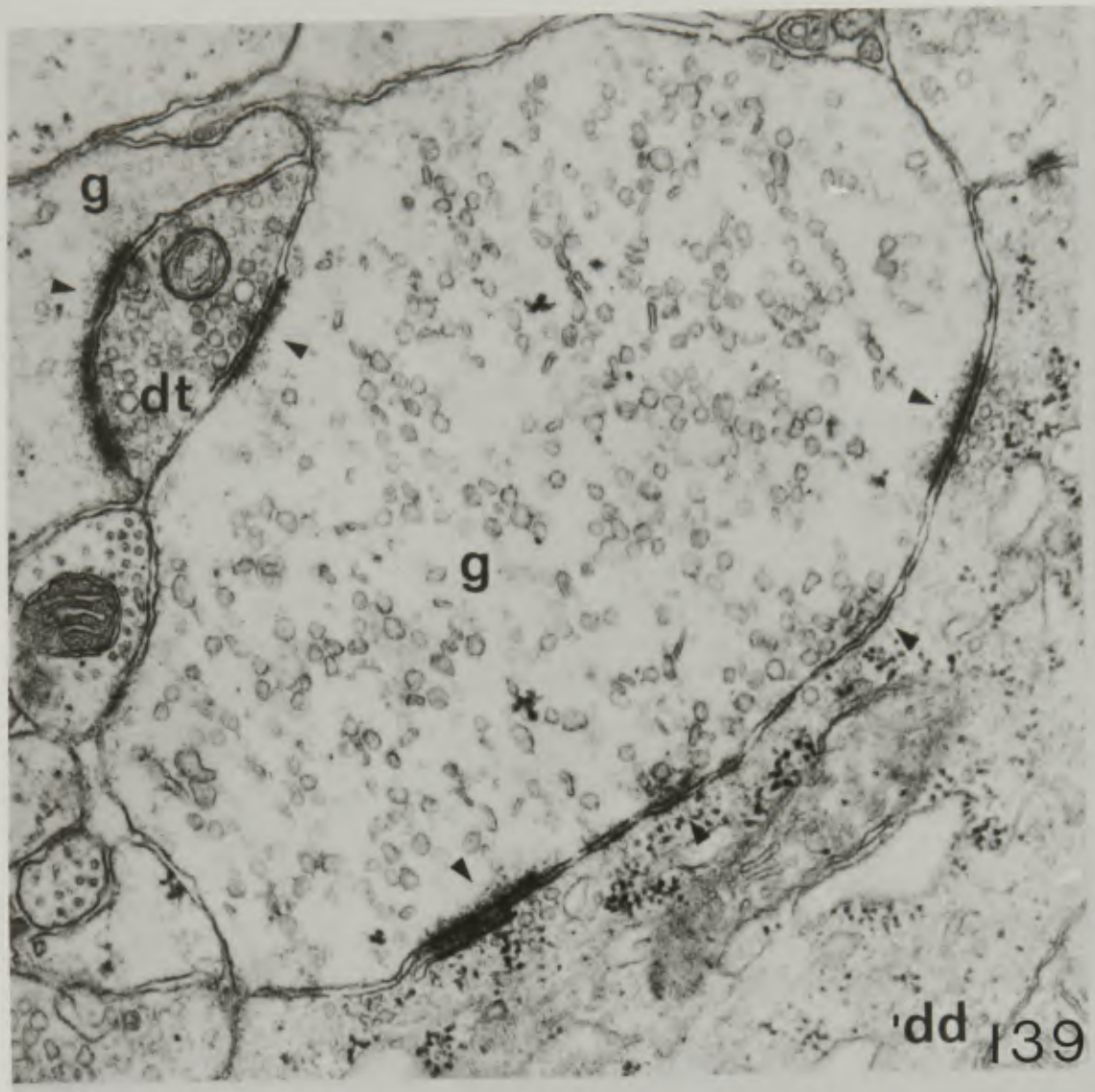
X33,000

Fig. 141 Fine degenerating terminal portions of dendrites in glomerulus, surrounded by marked extracellular space. 18-days survival.

X46,300

Fig. 142 Degenerating varicose terminal portion of Mitral or Tufted cell dendrite in glomerulus, with marked extracellular space. 70-day survival.

X36,000



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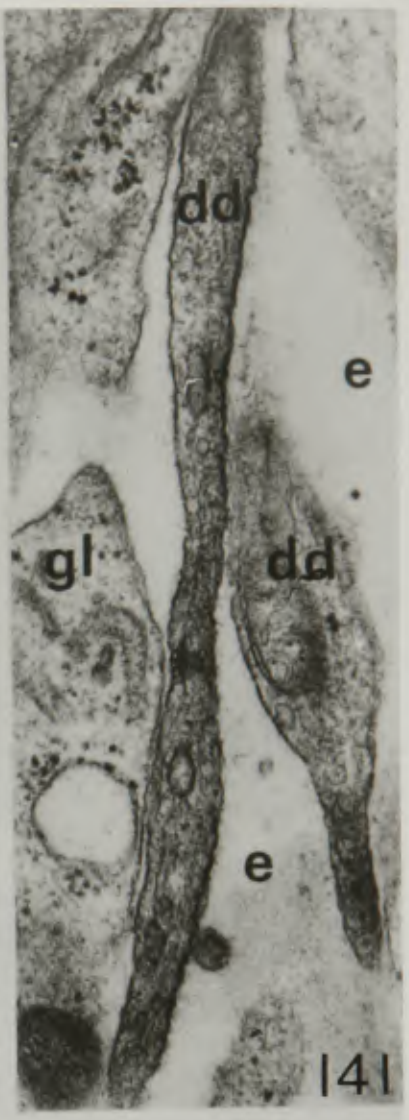

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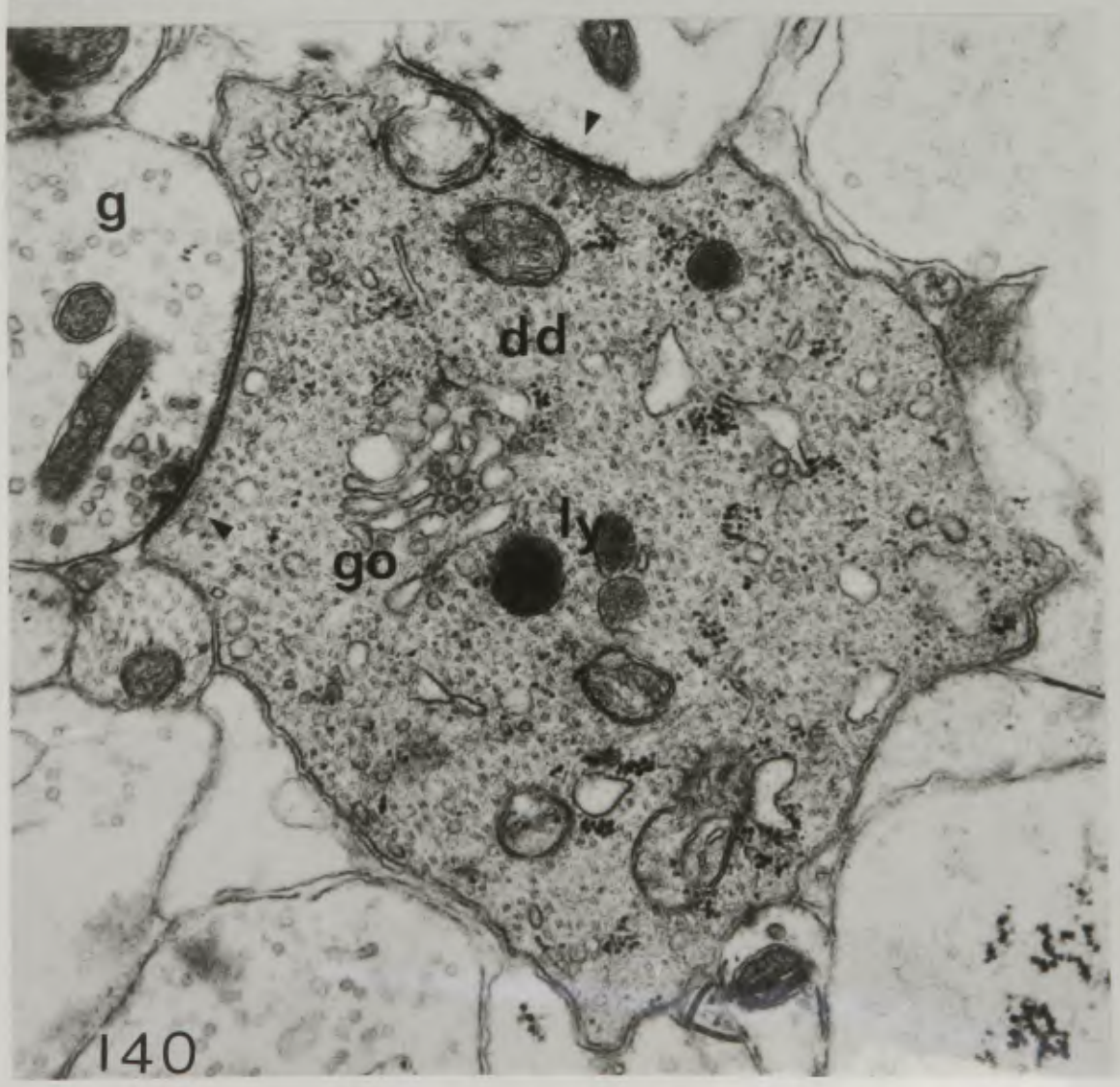

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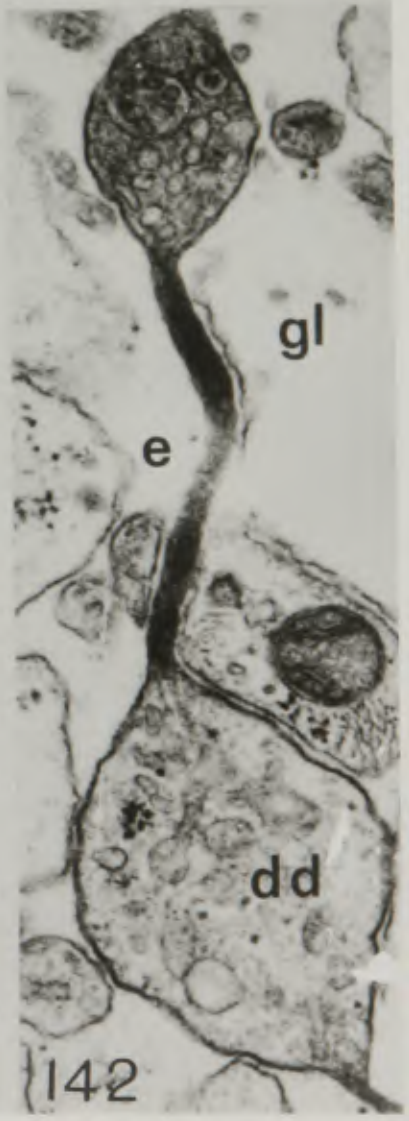

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Fig. 143 Transverse section of secondary dendrite of Mitral cell in moderate degeneration showing cross-section of neurotubules. 70-day survival.

X92,200

Fig. 144 Transverse section of secondary dendrite of Mitral cell from the normal side of the brain used for Figure 143.

X92,200

Fig. 145 Dark degenerating gemmule with reciprocal synapse in glomerular layer. 10-day survival.

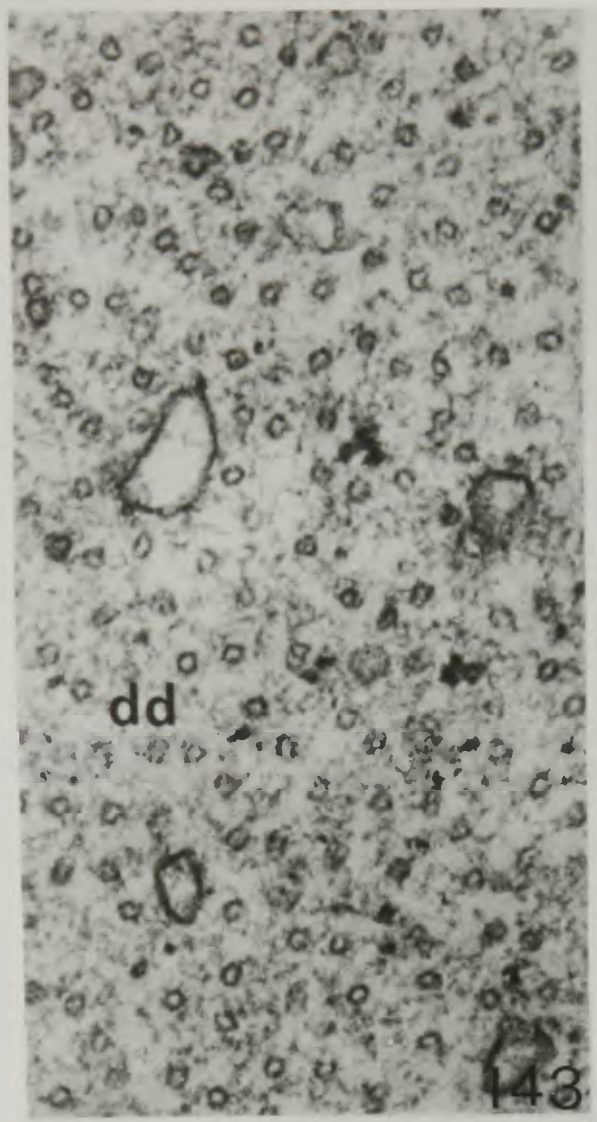
X44,000

Fig. 146 Pale degenerating gemmule with reciprocal synapse and attenuated pedicle in the glomerular layer. 24-day survival.

X32,600

Fig. 147 Degenerating dendrite in the Pyriform cortex with apparently normal asymmetrical and symmetrical synapses. 70-day survival.

X53,200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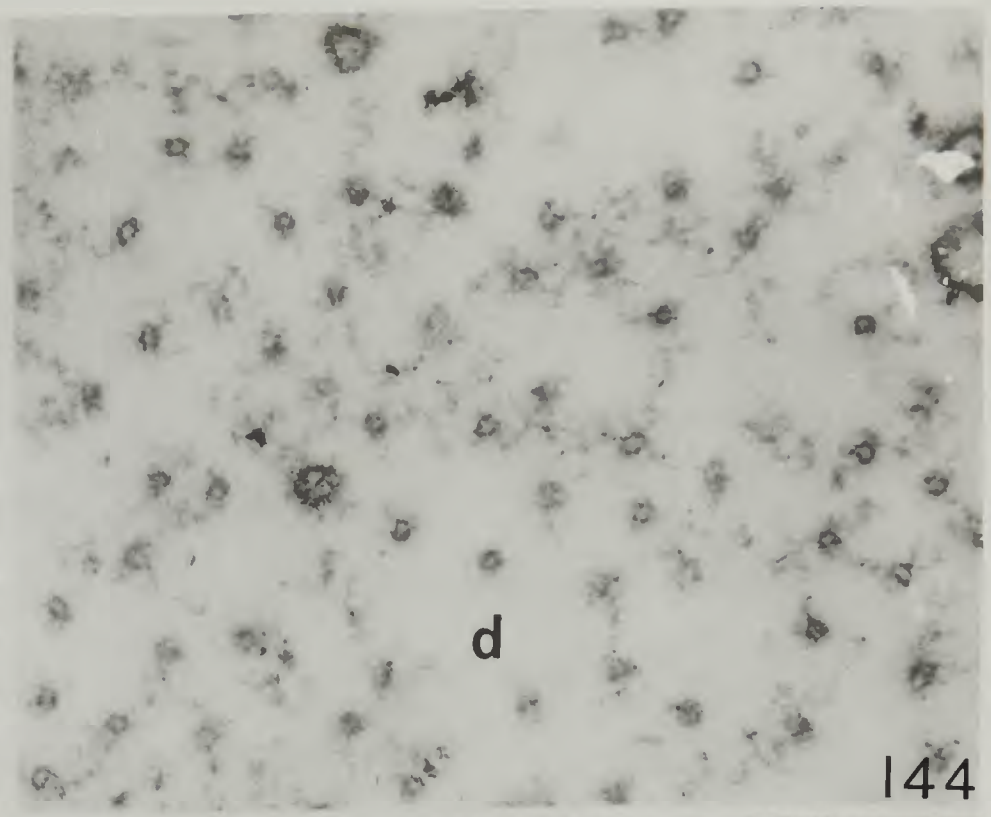
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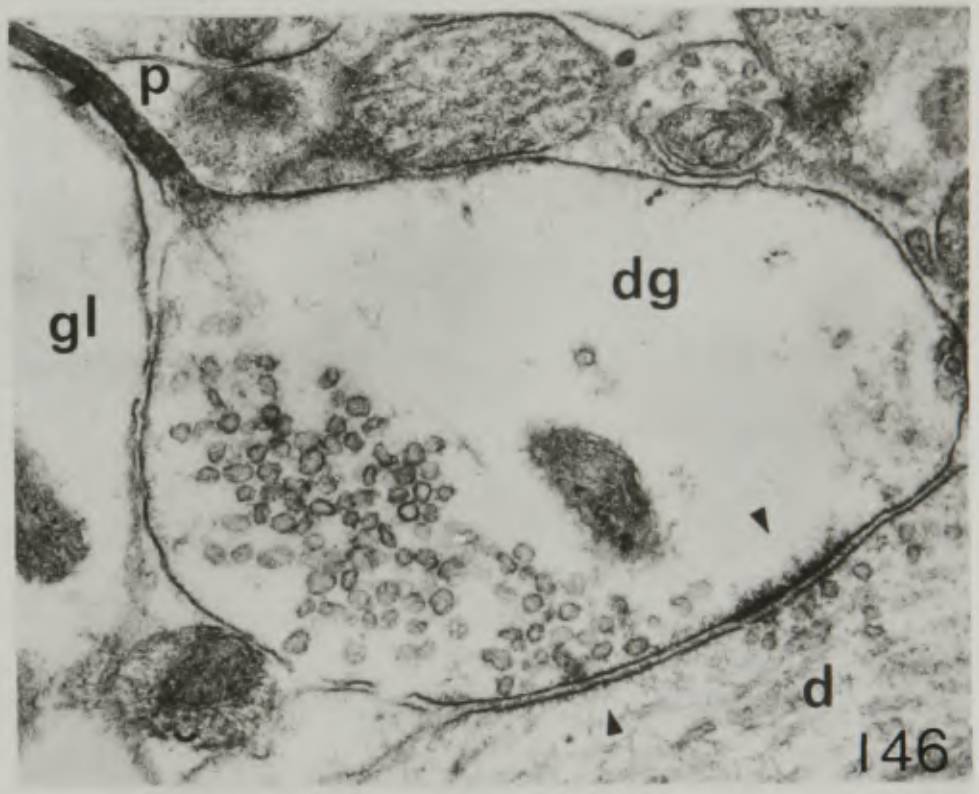
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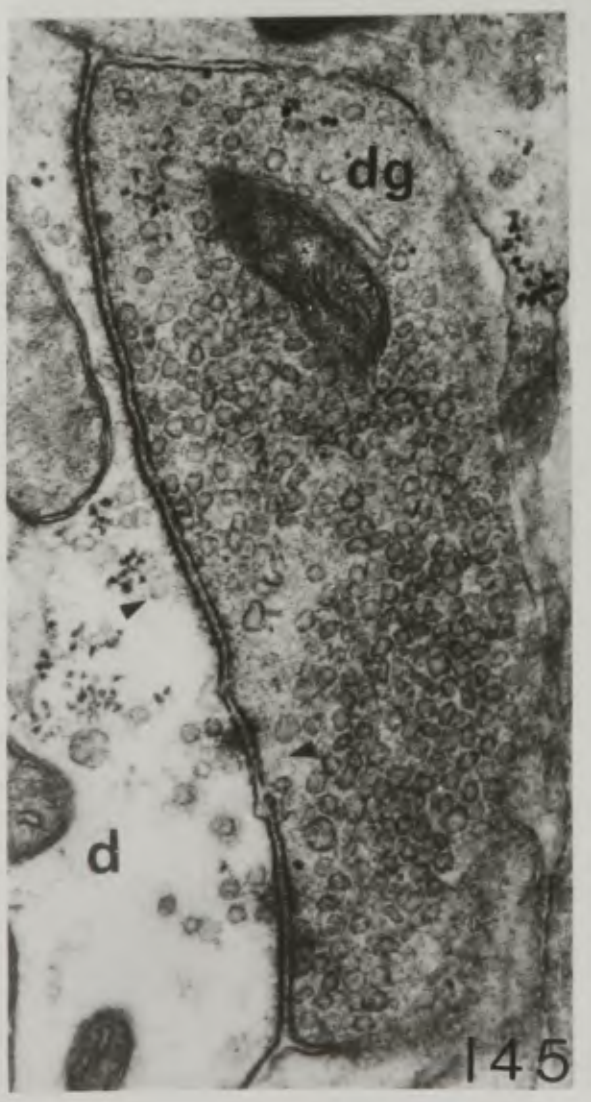
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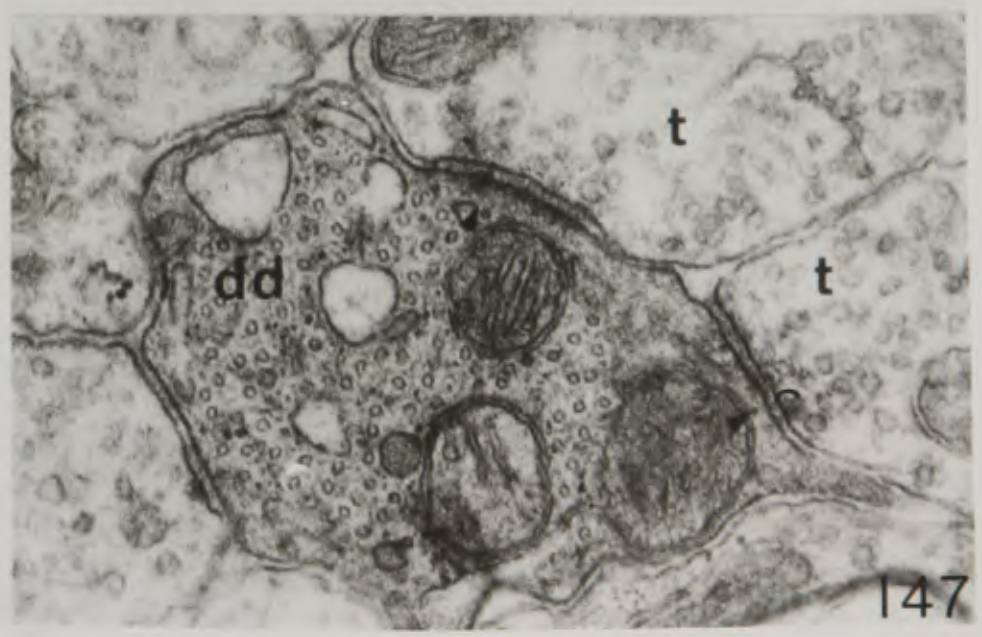
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in the rat (Chapter 7); degenerating dendrites with post-synaptic specialisations persisting have been found in the glomeruli of the rabbit (Fig. 138), but these are relatively rare. This paucity of membrane thickenings seems to be a reflection of the atrophy of the distal portions of the glomerular dendrites, which are only rarely encountered in material of more than 18 days' survival, being replaced by fine dark threads of cellular material (Figs. 141,142); these occasionally show signs of internal structure such as mitochondria or tubules but do not reveal any distinguishable post-synaptic structures.

In both somata and dendrites of all the transneuronally degenerating cells we have observed, regardless of the severity of the change, synaptic specialisations in relation to normal terminals or gemmules remain intact and unaltered. The reciprocal synapses of the mitral and tufted cells show exactly the same structure and arrangement as they do in normal material, including the presynaptic aggregation of spherical vesicles on the mitral tufted cell side, vesicles that are in all respects normal without any tendency to swell or to disperse into the surrounding cytoplasm (Figs. 136,139); the post-synaptic thickening of the gemmule to soma/dendrite synapse is unchanged, while the extracellular material and the cleft width of both synapses are the same as in normal material. Similarly all normal axon terminal synapses on to the dendrites and somata of any type of transneuronally degenerating cell are structurally unaltered. There is no evidence in our material of any change in the structure or staining properties of

the unit membrane of degenerating neurons (cf. Kruger & Hamori, 1970).

Spines and gemmules show the same gradations of change as the processes from which they arise (Figs. 129,130,134,145,149), and their pedicles shrink down to fine threads of electron dense material (Figs. 130,134,146). Ribosomes and vesicles (where present) become concentrated and the granular background becomes denser and less sparsely arranged; mitochondria, which are often found in these appendages, are sometimes distorted (Fig. 134), though spine apparatus seems relatively resistant to dilatation (Fig. 149). Synaptic specialisations are unchanged both in reciprocal synapses (gemmules) (Fig. 145) and in spines post-synaptic to normal axon terminals (Fig. 134). In a few cases, the gemmules swell and take on a watery appearance (Fig. 146), and in these many of the vesicles also swell; this has generally been found in conjunction with an extremely attenuated pedicle and may represent the degenerative isolation of the appendage from the parent dendritic shaft.

Similar changes have been found in somata, dendrites (Fig. 147) and spines (Fig. 151) of cells of the ipsilateral pyriform cortex in material of 49 days' survival and over. Dendrites and spines of the cortical neurons appear to degenerate without these changes necessarily affecting their parent stem dendrites or cell somata. The changes are sparse in comparison with those in the bulb but represent a trans-neuronal degeneration occurring across two synapses, as is presumably the case with the granule and short-axon cells, neither having primary contact with the incoming olfactory axons. Degenerating spines and

dendrites are sometimes found receiving synapses from apparently normal terminals (Fig. 147), indicating that the degenerative change in this region spreads along affected dendrites.

Axon Hillock and Initial Segment

The axon hillock and initial segments of the axons of normal mitral, tufted and short-axon cells are very similar to those described by Palay, Sotelo, Peters and Orkand (1968) and Peters, Proskauer and Kaiserman-Abramof (1968), while those of the small periglomerular cells show less clear differentiation of these regions. The axons of trans-neuronally degenerating mitral and tufted cells (Fig. 135) show a concentration of cytoplasmic constituents, similar to that described for the cell somata and dendrites with the result that it is difficult to be sure of any dense plasma membrane undercoating or tubular aggregation because of the increased cytoplasmic granularity and the dense packing of the neurotubules. It can be stated however that neither of these features is as marked as in normal cells and may even be absent, as is the extracellular granularity described by Peters et al (1968). The identification of the axon hillock and initial segment was therefore only certain in cases where the direction of the process was unequivocally that of an axon of these cells, and where serial sections revealed no reciprocal synapses on to the supposed initial segment. It has not been possible to trace one of these axons as far as the beginning of the myelin sheath. Free ribosomes, dense-cored vesicles and clear vesicles are common, as in normal initial segments,

though the former are rarely clustered in transneuronal degeneration; mitochondria and other membrane-bound organelles are swollen and disrupted. The neurotubules appear slightly swollen and stand out in a characteristic manner; synaptic structures, where these occur, are unaltered. It has not been possible to make unequivocal identifications of the axon hillocks or initial segments of transneuronally degenerating periglomerular or short-axon cells; granule cell peripheral processes may be considered as dendrites for the purposes of this study.

Axon and Terminal

The axons of mitral and tufted cells, after running in the lateral olfactory tract, terminate in the pyriform cortex (Cajal, 1955; Westrum, 1966), but before leaving the bulb they give off collaterals which terminate in the granule cell and external plexiform layers (mitral and tufted cell collaterals) and in the periglomerular zone of the glomerular layer (external tufted cells) (Cajal, 1955). A proportion of the myelinated axons of these cells in the olfactory bulb, lateral olfactory tract and in the pyriform cortex are altered at survival periods of 33 days and over (Fig. 148). They present a shrunken appearance and the cytoplasmic density increases, and tubules and filaments become more concentrated, lending a characteristic grain to these processes, similar to that described for early orthograde degeneration; mitochondria and multivesicular bodies swell and become disrupted. Occasionally these axons contain large vacuoles and dense lysosomes; free ribosomes are common, though they are rare in normal

myelinated axons. In severely affected axons the cytoplasm consists entirely of a granular osmiophilic matrix with glycogen-like granules, and the whole axon may be enclosed by a glial process showing reactive changes.

The processes that had been tentatively identified as axon collateral terminals from the examination of normal rabbit and rat material, as well as in relation to experimental studies in the rat (Price & Powell, 1970c; Chapter 10), were observed to alter in appearance in a similar manner to that shown by their supposed parent cells in undergoing transneuronal degeneration (Figs. 149,150). These morphological changes are quite distinct from those occurring in terminals after orthograde degeneration but, being subtler in nature, might have been considered artefactual or within an acceptable normal range. A double-blind trial was therefore carried out on the plexiform layer of the pyriform cortex; unlabelled sections from several blocks of ipsilateral and contralateral pyriform cortex from each brain were examined in random order and identified on the basis of axon terminal changes in this layer. The trial showed that the cortex from each side could be distinguished on this basis and that the axon terminals in the pyriform cortex do undergo characteristic changes, identifiable from 33 days after olfactory nerve section (Fig. 152). It is worth noting that the only block about which identification was uncertain was from a 70-day animal in which the spontaneous degeneration in the bulb of the normal side was quite marked.

The transneuronally degenerating terminals show a variable degree

Fig. 148 Degenerating Myelinated fibre in Pyriform cortex. 35-day survival.

X37,800

Fig. 149 Degenerating axon terminal of Tufted cell recurrent collateral terminating onto a degenerating Peri-glomerular cell spine in the glomerular layer. 18-day survival.

X30,800

Fig. 150 Degenerating terminal of Mitral or Tufted cell collateral in the Granule cell layer. 49-day survival.

X37,600

Fig. 151 Degenerating spine and dendrite in Pyriform cortex with apparently normal terminal synapsing onto it. Note glial engulfment. 70-day survival.

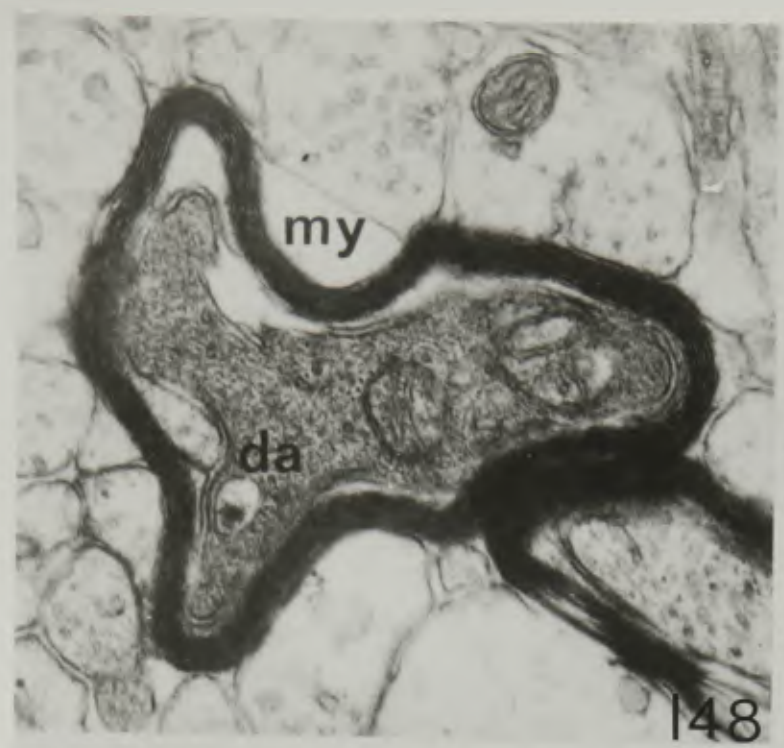
X45,400

Fig. 152 Degenerating axon terminal in Pyriform cortex. 33-day survival.

X29,800

Fig. 153 Normal terminal, spine and dendrite in Pyriform cortex.

X24,900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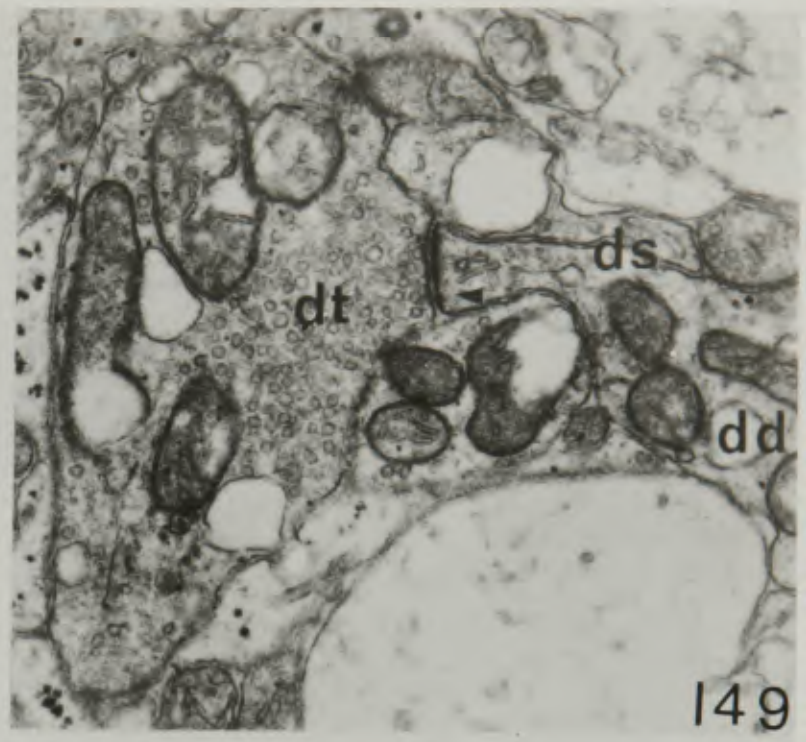
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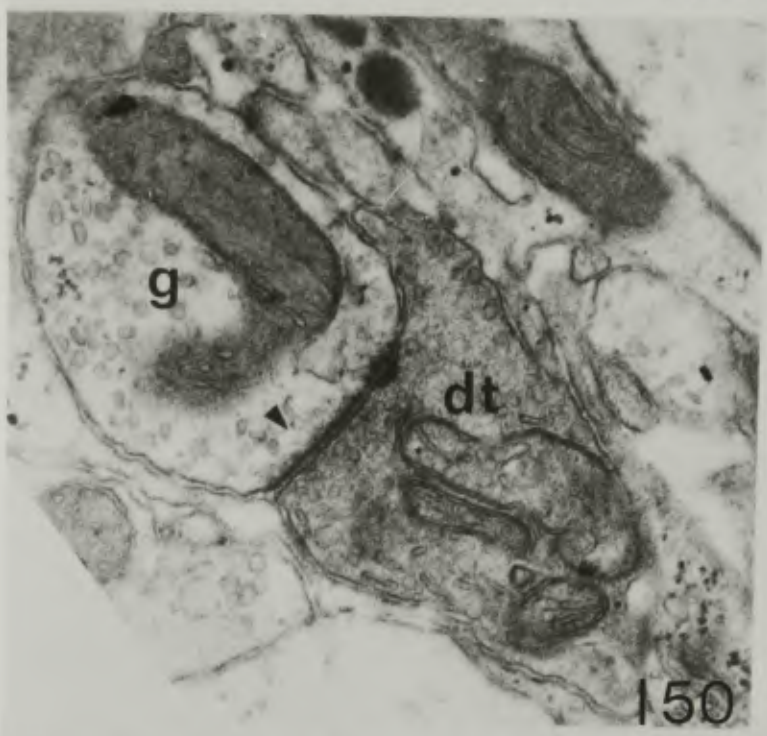
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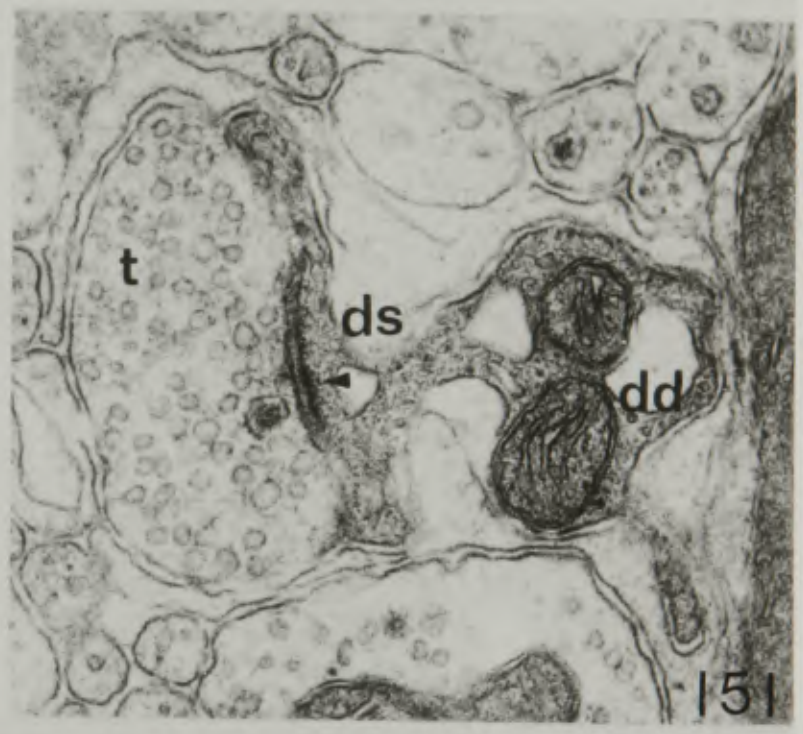
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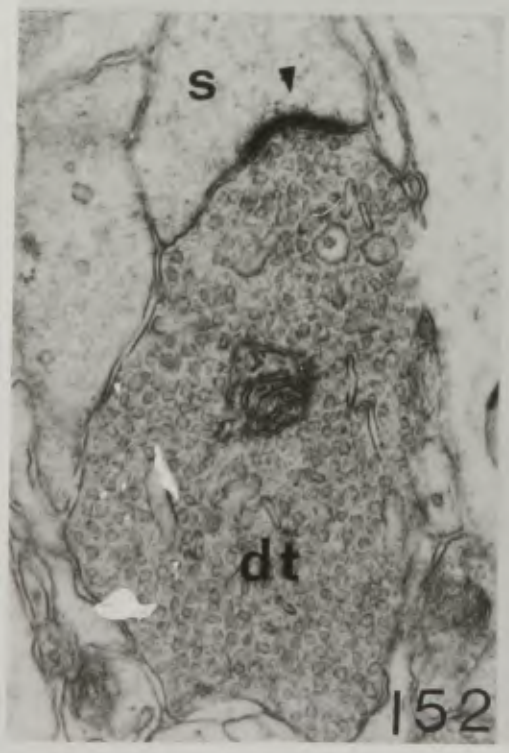
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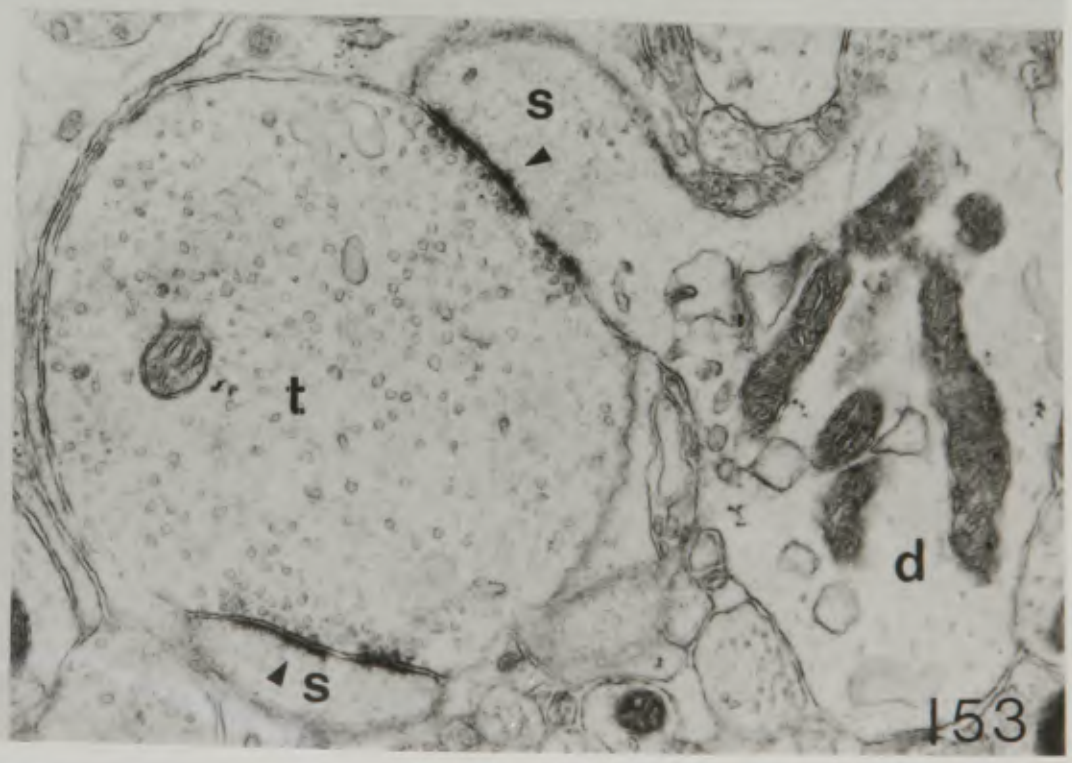
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of shrinkage and often take on an irregular outline; the vesicles in these terminals become crowded in the fine granular cytoplasm and lose their clearly defined boundaries, seeming on occasion to merge with the increased background granularity (Figs. 149,150,152). This change in texture is the most evident and the most constant change in the axon terminals of these cells, but where mitochondria are present they often swell (Fig. 149); some vesicles and cisternae also swell and become vacuolated (Fig. 150). In extreme cases the background becomes entirely opaque and glial processes may invaginate the terminal; the terminal and its vesicles then take on very distorted shapes. In other processes, dense granules are seen which may correspond with the glycogen granules seen in glia; free ribosomes are also occasionally found (Fig. 149). Synaptic thickenings remain unchanged in both pre- and post-synaptic processes at the survival periods studied, and there is no evidence of the complete removal of these terminals by the presence of bare post-synaptic thickenings. Where the preterminal axon is found in continuity with the terminal, it is very attenuated, containing little else but tubules, and has an irregular, wavy appearance.

These features of transneuronal degeneration of axons are the same in all parts of the olfactory bulb where collaterals terminate, as well as in the superficial half of the plexiform layer of the pyriform cortex, though the terminals in the latter show rather more mitochondrial and vesicular swelling. The axon terminals of periglomerular cells undergo the same type of change (Fig. 135), while the granule

cells have no typical axon (Cajal, 1955; Price & Powell, 1970a); so few degenerating short-axon cells have been found that it has not been possible to identify axon terminal changes for these cells, although it may reasonably be presumed that they are similar to those described above.

Mild and severe forms of degeneration

To avoid the possibility of comparing different portions of cells in different planes of section, the variation in degeneration will be described in cell somata although their dendrites and axons show similar variations. All survival times present a range of trans-neuronally degenerating cells, some of which show only slight differences from normal cells, others being severely altered; the majority, however, correspond with the above description of moderate degeneration, having only slight variations in density. This gradation of change may either represent an ongoing alteration over the survival periods studied, some cells changing at a greater rate than others, so that all types are seen at any time, or alternatively the degree of change in a given cell is a reflection of the extent of its deafferentation, so that partially deafferented cells show less severe changes than those that are totally deafferented. The evidence gained from the survival times covered in this study would tend to support the latter explanation. The degenerative change does not appear to spread from a particular part of the soma or dendritic tree, but all parts of a given neuron are affected with a corresponding severity, though the

axon terminal changes may be delayed (see below).

The characteristics of mild degeneration (Figs. 128,129) are a slight shrinkage of the perikaryon and a slight increase in the background granularity, as well as mitochondrial swelling and a tendency for pale vacuoles to appear near the plasma membrane; an increased proportion of free ribosomes is also common. Endoplasmic reticulum and Golgi apparatus may show no change and, though there is some loss of differentiation within the nuclear material, the nuclear membrane remains attached and the nucleolus appears normal. As the density of the granular background increases, so the cytoplasmic and nuclear alterations appear or become more marked until the typical appearance is achieved (Fig. 127).

The most severely affected neurons are extremely electron-opaque in both nucleus and cytoplasm, the latter to such an extent that, at low magnification it is sometimes difficult to distinguish the boundary between it and the nucleus. These cells may take on a stellate appearance (Fig. 130) by virtue of a marked indentation by the adjoining profiles and the presence of widely dilated vacuoles next to the plasma membrane. The swelling of membrane-bound systems is often absent, but instead they take on distorted shapes, though mitochondria retain their blown-up appearance. It is only in such severe cases of transneuronal degeneration that any gross segregation of nucleolar components into granular and fibrillar portions occurs (Fig. 133), indicative of a degenerative change in this organelle (Bernhard & Granboulan, 1968).

At most survival times, a small number of cells are found to have

the characteristic changes of membrane-bound systems, but show no signs of granularity or concentration in their cytoplasm; sometimes the cytoplasm, particularly of the larger dendrites, appears diluted, dendritic tubules becoming considerably more widely spaced than usual. The ribosomal rosettes of these cells are generally intact. Their nuclei often appear shrunken and the nuclear membrane has a marked tendency to vacuolate. These cells, which are not found on the normal side except occasionally in older animals, may represent a different type or stage of transneuronal degeneration; they could arise, however, as a consequence of an altered composition of the extracellular fluid.

Time course of the cellular changes

Up to 10 days after olfactory nerve section, the terminals of these axons degenerate; at 18 and 24 days the olfactory nerve terminals have disappeared and the glomerular arborisations of the dendrites of mitral, tufted, and periglomerular cells are shrunken and atrophied, and their parent cells show the characteristic degenerative changes in their main dendritic shafts, somata and axon initial segments. By 33 days the axons of these cells show clear changes in the olfactory bulb and pyriform cortex although some collaterals in the former are seen to degenerate earlier, perhaps representing those with shorter axons (Fig. 149). Granule cells are seen to degenerate at the same time as the other bulbar cells but dendritic changes are not seen in the pyriform cortex until 49 days after mucosa removal. This difference may reflect the additional connection between mitral/tufted cell dendrites and the

granule cell peripheral process, while the pyriform cortical neurons only have an axonal input from these relay cells; it indicates that the cortical cells are changed only after the appearance of morphological changes in the terminals of their afferent input and could result from these.

Glia and extracellular space

The glia of the deep layers of the olfactory bulb show little sign of active involvement in transneuronal changes, except in cases of severe degeneration of terminal processes, while those of the glomeruli, in addition to this, are only seen to participate in relation to the removal of olfactory nerve terminals (Fig. 137). However the volume of the glial cytoplasm of the glomerular layer and to a much lesser extent of the deeper layers, increases; this consists mainly of pale watery cytoplasm, often similar to extracellular space but for the sparse reticular background, the occasional mitochondrion or other inclusion, and the surrounding plasma membrane (Figs. 141,142,146). These glial processes may be acting to 'fill in' the spaces created by the loss or atrophy of the olfactory nerves and dendritic terminals of the cells with glomerular arborisations, possibly having taken up the fluid lost from these degenerating neurons. The less marked glial changes of deeper layers may reflect the qualitatively and quantitatively less severe degenerative changes in these layers. Similarly there is an increase in the extracellular space in the glomerular layer and the superficial part of the external plexiform layer, particularly around

the fine threads that are the remnants of the dendritic arborisations (Figs. 141,142). The changes in these elements of the neuropil may either represent the situation in the living animal or else a change in their composition and in the fluid balance between them and the neuronal processes, as revealed by their altered properties in the presence of fixative. This paradoxical finding of increased glial and extracellular volume in the shrunken olfactory bulbs seems to reflect the discrepancy between the neuronal shrinkage and the shrinkage of the bulb as a whole.

Rat electron microscopy

In rat material of 10-150 days' survival, apart from very occasional individual processes, no transneuronal change is seen; the post-synaptic processes retain their morphological integrity and are packed in the glomeruli without abnormal extracellular space. Many post-synaptic thickenings without pre-synaptic specialisations are found on dendrites and are apposed to other dendrites as described previously (Chapter 7). At 200 days a few glomeruli show transneuronal changes so that adjoining glomeruli are found with the typical appearance found in the rabbit glomeruli under these conditions on the one hand, and the typical appearance of earlier long-term rat glomeruli on the other. The alterations in the dendrites and somata of all cell types are exactly comparable to those described above for the rabbit (Fig. 140), showing similar gradations in severity and with the same glomerular appearance. The post-synaptic thickenings in the glomeruli decrease in

number and this may again be related to the degeneration of the fine terminal portions of the glomerular dendrites. No sign of transneuronal change has been found in the terminals of the axon collaterals of the mitral and tufted cells in this species, but this is probably due to either the low sampling of a small degenerating population or the fact that the cellular change has not yet reached the axon terminals.

Qualitative light-microscopic changes

In studying 2u sections, stained with methylene blue and Azure II (Richardson et al, 1960), for the orientation of blocks of this material for ultrathin sectioning, it was noticed that a variable number of the mitral, tufted, granule and periglomerular cells on the ipsilateral side were stained deep blue throughout their nuclei and cytoplasm, and seemed shrunken in relation to neighbouring cells. These were most evident in those parts of the bulb where denervation had been most complete (anterior and medial). These cells are the same cells as appear electron-dense, and show the same gradation of change, as determined by the study of sections with both methods, taken with 1u of each other (cf. Cammermeyer, 1962; Cohen & Pappas, 1969).

Golgi-Cox results

The same span of survival times have been studied on the rabbit as those used for electron microscopy; as described previously (Matthews & Powell, 1962; Powell, 1967), the cells of the olfactory bulb undergoing transneuronal degeneration are resistant to impregnation after the

removal of the olfactory mucosa. At 10 days there are fewer cells impregnated than on the normal side, but there is a reasonable proportion of all types. At 17 days the appearance of the two sides is strikingly different, with very few typical mitral, tufted or periglomerular cells stained and the granule cell population somewhat reduced; this situation persists unchanged up to 90 days. These authors also noted that several unusual cells appear in the mitral cell layer at the longer survival times; similar ones have also been found in the external plexiform layer. These have a rather stellate, but often indeterminate, form and seem at low magnification to have fine strands running from them in two primarily radial bundles, of deep and peripheral orientation. A careful examination of these cells at higher magnification shows that many of the finer processes are spiny, while others are varicose. Some cells are found with cell bodies very similar to normal mitral cells, if a little shrunken, and very occasionally these show several typical secondary dendrites in addition to the plethora of fine processes that surround them. After examination of electron-microscopic material from rabbits of the same survival times, the altered cells have been identified as degenerating mitral and internal tufted cells with their shrunken axons and dendrites, regularly surrounded by the peripheral processes of a group of granule cells lying deep to them, the cell somata of which may often be seen in continuity with the strands deep to the mitral cells. No glial cells additional to those normally present in the mitral cell layer have been found with the electron microscope that could correspond to these Golgi-stained cells, while the

indented or stellate appearance of the degenerating mitral cells is strongly suggestive that these are the impregnated cells.

As noted by Powell (1967), the neurons of the pyriform cortex at the longer survival times (49 and 90 days) showed a distinct thinning of their dendritic branching, particularly in the superficial part of the plexiform layer, and a slight reduction in the number of cells stained. The former effect corresponds with that described by Jones and Thomas (1962) after removal of the olfactory bulb. There was no clear evidence of a reduction in the number of spines on the remaining impregnated dendrites on the operated side (cf. White & Westrum, 1964), but the material was not adequate for a detailed study of the spines in this region.

All the differences between the operated side and the normal side of Golgi-stained material are distinct and occur regularly in all the brains studied; they cannot be discounted as reflecting the variability of the technique in view of their entire correspondence with electron-microscopic observations, as well as the sensitive way in which they reflect variations in the extent of denervation.

Studies on the rat with the Golgi-Cox method at short survival times after mucosal removal (10-100 days) show none of the qualitative changes seen in rabbits of the same survival periods. However similar changes are seen in material of 250 days' survival, thus confirming the electron-microscopic observations of the onset of transneuronal degeneration in the rat at 200 days survival.

DISCUSSION

The material

The olfactory bulb of the rabbit has proved to be a suitable site for the study of transneuronal cell changes at the electron microscopic level: the single high-density afferent pathway which may be interrupted without opening the cranial cavity, together with the well-defined laminar structure of the bulb, whose cell types may be readily identified, make this an excellent preparation. Although the use of young animals has largely avoided the complication of atrophic rhinitis and subsequent transneuronal changes on the control side, the limited appearance of such changes in the control side of older animals only, tends to confirm rather than confound the suggestion that the type of change described does represent true transneuronal degeneration.

The term 'dark cell' has been avoided in this chapter since its wide use has led to some confusion in the literature between quite different dark cell types in the CNS, both normal and pathological (see Cammermeyer, 1962; Cohen & Pappas, 1969); since the electron microscope can only provide information on electron density variations, to consider all cases of increased density or darkness as a fixation artefact, or as representing a non-specific pathological change, precludes the proper analysis of the detailed characteristics of different dark cell types. Though poor fixation may give rise to a certain type of dark cell or dendrite, their regular presence cannot be so discounted in material that is on all other criteria well-fixed. In the brains used for this study, fixation was of a good standard and the 'dark cells'

interpreted as transneuronally degenerating neurons were distributed in a manner closely related to the extent of the denervation in any part of the bulb. Although the dilatation and disruption of membrane-bound systems within the affected cells is likely to have occurred during fixation, it is specific to the degenerating cells and this suggests that it is a reflection of an increased fragility or susceptibility of these systems to the fixative, perhaps as a result of the altered osmotic properties of their cytoplasmic environment. In the rat material of 200 days' survival only a few glomeruli show changes; thus glomeruli adjacent to these undergoing transneuronal degeneration and subjected to exactly the same experimental treatment, show no evidence either of poor fixation or transneuronal change, but only the characteristic appearance of rat material of shorter survival periods.

The glial involvement in the degeneration of the terminal dendritic and axonal processes of some cells is strong evidence that the changes described are not simply produced at perfusion, as is the cell specificity of the change and its occurrence throughout the processes of any involved cell in the olfactory bulb. Cell death may be ruled out in the vast majority of cases, on account of the retention of certain features of cytological organisation, such as the granular endoplasmic reticulum and the vesicle clustering adjacent to the presynaptic part of dendro-dendritic synapses (Rall, Shepherd, Reese & Brightman, 1966). Apart from one clear example in a brain rejected from this study, there was no evidence of direct injury to the olfactory bulbs, either through the cribriform plate at operation or at any other stage. Since the blood

supply of the olfactory bulbs is separate from that of the olfactory mucosa, changes due to a vascular lesion may be discounted as may any question of infection; furthermore, the appearance of the bulbs is quite different from that found in ischaemia or after local bulbar lesions. We feel justified therefore in the conclusion that the changes described here represent true transneuronal degeneration, if only certain features of it. Mouren-Mathieu and Colonnier (1969) have identified similar changes in the Purkinje cell dendrites of the cerebellum after interruption of parallel fibres as transneuronal degeneration; swelling of membrane-bound organelles in the lateral geniculate nucleus was described by Smith, et al. (1964) from 7-100 days' after eye removal.

Possible origins of the morphological changes

Most of the observed cytological alterations are explicable in terms of a concentration of the cellular constituents, consequent on or giving rise to cell shrinkage. The changes in the distribution and arrangement of the nucleic acid components of both nucleus and cytoplasm are the only clear evidence of a modification of cellular organisation and activity (see Brattgård, 1952). It could be considered that the increase in free ribosomes is a reactive change (Kruger & Hamori, 1970) or else an indication of a reduction of cellular activity to a basal level. The apparent increase in neurotubules in dendrites and axons could similarly be considered as a reactive generation of these structures, but may solely reflect the concentration of cytoplasmic constituents. It is difficult to differentiate between these possibilities on

account of the variability of dendritic and axonal size and number of tubules, but there is no positive evidence at any level in favour of such a generation, such as sprouting, branching or division of tubules.

Transneuronal degeneration only appears to occur in certain cells in the central nervous system, particularly in the sensory pathways (see Matthews, et al., 1960) and may represent a sensitivity of neurons with particular properties to a fall in the level of this afferent, presumably excitatory, input. For this reason it is necessary to examine the different synaptic properties of the cells of the olfactory system that undergo transneuronal change, with a view to finding a common factor that is changed in all cases. If the correlation of vesicle shape and type of membrane thickening with functional activity holds true for the olfactory bulb (see Rall et al., 1966; Price & Powell, 1970b), the granule cells and periglomerular cells (Chapters 3-5) may be considered as inhibitory neurons. The only excitatory input to the mitral and tufted cells, therefore, is that from the olfactory nerve terminals in the glomeruli. For periglomerular cells, the olfactory axons are also the major excitatory input, but other excitatory influences are contributed by the axon collaterals of the tufted cells and by centrifugal fibres (Chapters 3-5). Intrinsic axons, including these collaterals, may only be affected secondarily and may not necessarily undergo transneuronal degeneration and centrifugal fibres are not affected at all. The excitatory influences on to granule cells include the axon collaterals and the dendrites of mitral and tufted cells, as well as terminals of centrifugal axons and those from the

anterior olfactory nucleus and the anterior commissure (Price & Powell, 1970c). Short-axon cells, both of the deep layers and the glomerular layer, have occasionally been seen to show degenerative changes at long survival periods; these cells receive a collateral input from mitral and tufted cells, together with other intrinsic and extrinsic (excluding olfactory) inputs. The pyriform cortex receives the axon terminals of mitral and tufted cells in the superficial third of the plexiform layer, but also receives other afferent inputs.

Thus of the cells of the olfactory system that show transneuronal changes, as seen with the electron microscope and with the Golgi technique, the mitral, tufted and periglomerular cells are directly deprived of the whole or a large part of their afferent (excitatory) input, although they retain other, mainly inhibitory inputs; all the other cells affected, which have no primary contact from the olfactory nerves, have a common input from the axons, collateral or terminal, of the mitral and tufted cells, which have themselves been to a large extent deafferentated. It may be seen, therefore, that although normal boutons may persist an affected cells, and in some cell types none are subject to orthograde degeneration due to experimental interruption, the balance of excitatory and inhibitory inputs has been altered in all cases, so that they may be unable or only rarely able to reach the threshold for producing a propagated impulse. This poverty of depolarising input or spike output could give rise to an alteration in cellular activity resulting in the morphological changes. The simplest explanation, therefore, is that the cellular change has an ionic basis, in which the

movement of ions involved in the depolarisation of the membrane and its subsequent recovery is essential to the normal fluid and ion balance between the cell and its surroundings. The altered appearance of the extracellular and glial space consistently associated with the glomerular arborisations of the degenerating cells may reflect the simultaneous alteration in the composition of the extracellular space and glial cytoplasm; the neurons showing a watery cytoplasm may also be related to this process. Alternatively, the reduction of spike generation at the initial segment may cause primary changes at the level of the cell soma, which are then reflected throughout the whole of the cellular ramifications, the axons being the last to be affected, although this mechanism cannot explain the changes in dendrites and spines of the pyriform cortex.

Whatever its cellular basis, the 'orthograde' transneuronal degeneration of cells across more than one synapse (see Powell & Mrulkar, 1962) represents a form of functional deafferentation in which the number of excitatory impulses are reduced in a given pathway, without any primary alteration in inhibitory or other excitatory inputs. The effects of functional deafferentation, without any neuron injury, have been described in the visual pathway of the kitten (Wiesel & Hubel, 1963a & b; Hubel & Wiesel, 1970) and the mouse (Valverde, 1967), and the former authors have noted cellular changes in the lateral geniculate nucleus at the light-microscopic level similar to those occurring in transneuronal degeneration of that nucleus after eye removal; Valverde has noted the loss of spines in the visual cortex, as seen with the Golgi method and

Coleman and Riesen (1968) have demonstrated a reduction in the number of stellate cell dendrites and in their length. It would be of interest to determine whether electron microscopic changes similar to those occurring in the olfactory system after olfactory nerve section can be found in the neurons of the geniculo-cortical pathway in conditions of light deprivation. Wiesel and Hubel (1963 a & b) have also shown that the geniculate neurons may be excited in the normal way, although their physiological effects at cortical level have been affected; this is consistent with the finding that the cells of the olfactory pathway maintain a level of cytological organisation, and may be considered capable of normal activity. This has been confirmed by the physiological study of the transneuronally degenerating olfactory bulb of the rabbit (Phillips, Powell & Shepherd, 1960, unpublished observations) in which the responses of the mitral cells to antidromic stimulation of the lateral olfactory tract were studied. The evoked potentials following the tract shock, were found to be of normal pattern and of normal or only slightly reduced amplitude; the correlation of wave pattern with histological depth was also normal. The conclusion could therefore be drawn that the mitral cells generate antidromic impulses in the normal manner and the mitral-to-granule excitatory synapses also operate normally. The findings also suggest that the granule-to-mitral inhibitory synapses are also capable of operating normally.

If the whole cell transneuronal degeneration in the olfactory bulb and lateral geniculate body and the more limited dendritic degeneration in the pyriform and striate cortices may be considered analogous, the

present observations could throw some light on the anatomical basis for the physiological alterations in cortical function described by Wiesel and Hubel (1963b). These authors note that the "abnormality is in the region of the synapse between the axon terminals of geniculate cells (those receiving input from the deprived eye) and the cortical cells on which these terminals end." In view of the fact that transneuronal degeneration of axons in the olfactory system is exactly comparable to the degeneration of their parent cell somata and dendrites, it could reasonably be considered that these axons too were excitable in the normal way, the 'degeneration' representing an altered functional state rather than cell death. On the other hand the selective transneuronal degeneration of some spines and dendrites of the pyriform cortical neurons without these changes necessarily occurring in the cell somata and other dendrites, may cause the degenerating components to lose their functional importance in the spike generation. Thus, although the altered dendrites and spines may be responsive to depolarising input, the other normal dendrites could have become 'dominant' in determining the cell's responses, due to a functional adaptation in the neuron to the altered dendritic input.

It has been noted that transneuronal changes occur at different rates in different species (Matthews et al., 1960). The same has been found in the olfactory system with both electron microscope and Golgi techniques - the rabbit shows cellular changes at 10 days after operation, while no changes are found in the rat with either technique until 200 days, suggesting that the rat is better able to maintain neuronal struc-

ture, and therefore presumably function, than the rabbit. Differences in the time course of transneuronal degeneration between different species have been observed in the visual system (Cook et al., 1951; Matthews et al., 1960). No obvious differences have been found in the neuronal organisation of the olfactory bulbs of the two species at electron microscopic level, nor in the time course of the orthograde degeneration of the olfactory nerves; there is no evidence of re-innervation in either rat or rabbit. It should be noted that both species have been operated on at an immature age (5-7 weeks) and that this may be a critical factor in determining the severity or even the occurrence of a degenerative alteration (Hubel & Wiesel, 1970).

It is a striking fact that in both rat and rabbit the onset of transneuronal cell change corresponds with a distinct decrease in the number of post-synaptic thickenings without presynaptic terminals, and of fine terminal parts of dendrites. Clearly it is not possible with these methods to determine which factor is the primary cause of the onset of cellular change, but it could be suggested that the presence of post-synaptic thickenings until the appearance of transneuronal change in the rat is directly related to the persistence of the fine dendritic terminals in the glomeruli of this animal and the absence of transneuronal change; this could be considered as a transient manifestation of denervation hypersensitivity. The loss of spontaneous activity in the region of the post-synaptic thickenings could be responsible for terminating the prevention of cellular degeneration by these elements.

Golgi studies

Experimental Golgi studies have shown that cells or parts of cells that have been subject to deafferentation to not impregnate with this technique or are resistant to it (Matthews & Powell, 1962; Jones & Thomas, 1962; White & Westrum, 1964; Globus & Scheibel, 1966; Valverde, 1967; Powell, 1967; Coleman & Riesen, 1968). The correspondence in time course between the ultrastructural changes and the failure of Golgi impregnation in the olfactory system undergoing transneuronal degeneration is significant in this regard. It should be noted that while the degenerative changes occur throughout the affected cells of the olfactory bulb, the changes in the pyriform cortex affect mainly dendrites and/or spines rather than whole cells; the latter observation has also been made in the visual cortex (Globus & Scheibel, 1966; Valverde, 1967; Coleman & Riesen, 1968), but reasons for the difference are obscure.

Two related explanations may be put forward in relation to the failure of Golgi impregnation:- 1. The traditional view of the Golgi technique has been recently restated (Schapiro & Vukovich, 1970); that is, that it only stains those neurons that are chemically, and therefore functionally different, at the time of fixation - i.e. those that have been active just before fixation. If transneuronally degenerating cells are inexcitable due to an insufficient number of functional excitatory synapses, they may never reach the chemical state necessary for impregnation. 2. The Golgi technique has been shown to be a cytoplasmic stain, by combined Golgi-electron microscopic studies (Blackstad, 1965; Stell,

1967; Chapter 11), and spares the membrane-bound systems. If the reaction is with the 'free' cytoplasmic component, the reduction of this in transneuronally degenerating cells could reduce the likelihood of their being impregnated.

The characteristic impregnation of the 'altered mitral cell' in transneuronal material, that is, the mitral cell and the granule cells with peripheral processes adjacent to the mitral cell body, bears on this problem. If the impregnation of these granule cells represents their functional activity, they would be acting to inhibit the mitral cell (Rall et al., 1966; Price & Powell, 1970b), yet this cell does stain. Alternatively they could be considered as 'trying' to excite the mitral cell, by a reduced activity which may also make them chemically different, the staining of the mitral cell, in spite of its cytoplasmic poverty, indicating the success of the attempt. A further explanation could be that the mitral cells are able to give rise to an impulse spontaneously and, in doing so, excite the granule cells via the dendrodendritic synapses on to their peripheral processes; the constant relationship of the impregnated granule cells to the soma of the altered mitral cell could be taken as suggesting a somatic rather than dendritic origin for the postulated impulse which affects primarily those granule cells whose gemmules synapse with the mitral cell soma. This anomalous finding on the altered mitral cells of transneuronal degeneration and their attendant granule cells points clearly to the inadequacy of our knowledge of the mechanism of the Golgi method, though it indicates that functional rather than simply morphological interpre-

tations of experimental material using this method may be necessary.

The cases of dendrites or their spines failing to impregnate while their parent processes remain normally stained (Jones & Thomas, 1962; White & Westrum, 1964; Globus & Scheibel, 1966; Valverde, 1967; Coleman & Riesen, 1968) are still open to several explanations, although the phenomenon is beyond doubt. Either the spine or dendrite undergoes a change similar to transneuronal degeneration without the same changes occurring in the parent process, and the Golgi stain fails on this account, whether it is functional or morphological or both; or the spine or dendrite is lost entirely, as has been considered by most observers (Globus & Scheibel, 1966; Mouren-Mathieu & Colonnier, 1969). The question of the age of the animal at operation is clearly critical in differentiating between failure of spine development and actual spine loss. It is felt that to try to differentiate between these possibilities is not advisable on the present evidence, although the observation of degeneration of spines which receive synapses from normal terminals in this material, indicates that the transneuronal changes in them cannot simply be ascribed to the degeneration of individual axon terminals.

The clear correspondence between Golgi and electron-microscopic observations on the olfactory systems of rabbit and rat, both in the time course and the nature of the cellular changes of transneuronal degeneration, shows the complementary nature of these very different techniques. The fact that transneuronal degeneration can be observed as a clearly defined phenomenon in both, each demonstrating quite distinct

characteristics in comparison with normal material, is strong evidence for the validity of both sets of findings.

The results of the studies on transneuronal cell degeneration with three techniques - Thionin and Golgi for light microscopy, and electron microscopy - require further correlation in respect of the proportion of cells of any type that are affected. The thionin results indicate a decrease in cell size for all cells of any type, although the ranges of normal and degenerate cell areas do overlap, suggesting that some cells could be entirely unaffected; Golgi-Cox impregnation however shows that all but the few 'altered' mitral and tufted cells are resistant to impregnation after denervation. Approximately one third of any type appear unaffected at electron microscopic level although they may have shrunken slightly without obvious qualitative change. The fact that most of the 'altered' mitral and tufted cells of Golgi preparations have atypical shapes and appear very shrunken suggests that those cells which are resistant to Golgi impregnation after deafferentation include the cells that seem least affected at electron microscopic level. Thus the subtlest transneuronal changes after deafferentation appear to be reflected most sensitively by the failure of Golgi impregnation; this in turn favours the suggestion that this failure is primarily due to a functional change rather than a morphological one.

Transneuronal and Spontaneous Degeneration

The occasional presence of similar dark degenerating neurons in other parts of the brain, notably in sensory systems, may be considered

in relation to these findings as representing the spontaneous degeneration of these neurons due to a reduction or loss of activity of peripheral or central origin. On the other hand, transneuronal degeneration could be regarded as an increased level of spontaneous degeneration brought on by deafferentation, either functional or pathological. The occurrence of 'dark profiles in apparently-normal central nervous system' (Cohen & Pappas, 1969), which may well have such an origin as discussed above, need not bring into question the application of experimental degeneration studies, although it may make these more difficult, for two main reasons:- firstly, on account of the clear distinction between the types of change taking place in orthograde and transneuronal axon terminal degeneration, which are only morphologically similar at the early stages of the former; and secondly, these spontaneous degenerative changes are rare in comparison with the terminal degeneration produced by the section of an afferent pathway, so that even if they are confused morphologically, they should not affect the large sampling of degenerating terminals that is essential for the electron-microscopic identification of the mode of termination of that afferent pathway. The use of young animals to avoid a high incidence of spontaneous degeneration and the study of orthograde degeneration at several survival periods to demonstrate a sequence of terminal changes should distinguish clearly between it and the spontaneous degenerative changes similar to those seen in transneuronal degeneration of axon terminals. Careful examination of the normal side and direct comparison of the two sides in each animal is obviously essential.

Experimental value of Transneuronal Degeneration

The occurrence of transneuronal degeneration has been used with the light microscope to determine the organisation of the projections of certain pathways, for example the retinal projection to the lateral geniculate body (Le Gros Clark & Penman, 1934). Similarly, cellular alterations of the kind described above are of great value as markers in the electron microscopic analysis of a part of the central nervous system: to be able to identify a specific cell or cell type throughout its extent without requiring continuity in a single or a limited number of planes for identification, greatly facilitates the predominantly two-dimensional approach that is inevitable with the use of the electron microscope; a similar suggestion has been made by Grant and Westrum (1968, 1969) in regard to the cellular changes occurring in retrograde degeneration. Clearly the question of cell specificity of the trans-neuronal change must be carefully considered in any region, but together with other criteria, normal and experimental, as well as Golgi evidence, this type of marking could be of considerable importance, particularly for the identification of intrinsic connections; in the olfactory bulb, for instance, this approach has considerably clarified the problem of the mode of termination of the recurrent collaterals of the mitral and tufted cells.

CHAPTER 9

THE TERMINATION OF CENTRIFUGAL FIBRES IN THE GLOMERULAR LAYER OF THE OLFACTORY BULB.

"This process of the central control of sensory impulses obviously has wide implications, for it must mean that what we ultimately perceive with our senses is not necessarily a faithful reproduction of external realities."

Sir Wilfred Le Gros Clark.

INTRODUCTION

In the systematic study of any part of the brain, it is necessary to define the origin of the different types of axon terminal that have been characterised, as far as possible, by ultrastructural studies of normal material. This may be achieved by interrupting the fibre pathways that give rise to these terminals; it is then generally possible to correlate the terminals that show degenerative changes with a profile type determined from normal material. Similarly, in order to discover the precise site of termination of an afferent pathway in a given region, in terms of the cell types and kinds of process that receive synaptic contacts from the terminals of this pathway, the area must be examined with the electron microscope at various intervals after interrupting the pathway. If possible, the lesion should be made in such a way as to avoid interrupting other pathways to the same nucleus, and a background of studies with the light microscope, using the silver degeneration techniques, is an essential basis for this more detailed investigation. Furthermore, in electron microscopic studies, the use of several survival times shows a sequence of degenerative changes in the terminals, that serves to exclude the possibility that these morphological alterations are due to some non-specific or secondary change (Cohen & Pappas, 1969; Chapter 8).

Light microscopic studies with silver degeneration methods indicate that the glomerular layer, as well as the deeper layers, of the olfactory bulb receives centrifugal fibres (Cragg, 1962; Powell & Cowan, 1963;

Powell, Cowan & Raisman, 1965; Heimer, 1968); these fibres have recently been shown to originate, along with the centrifugal fibres to the deeper layers, from the nucleus of the horizontal limb of the diagonal band (Price & Powell, 1970c). The axons run rostrally in the lateral olfactory tract towards the bulb and show a characteristic terminal distribution in the deep half of the external plexiform layer and in the periglomerular region. In a previous electron microscopic study, the mode of termination of these fibres in the deeper layers has been determined (Price & Powell, 1970c), but it was not possible, at that time, to be certain of their termination in the glomerular layer. After a longer study of the normal glomerular layer (Chapters 3-5), it has been possible to elucidate this superficial termination of the centrifugal fibres in experimental material, and these observations are presented here.

RESULTS

Studies with the silver degeneration techniques have clearly demonstrated that the degeneration at the glomerular level of the olfactory bulb after lesions of the centrifugal pathway has a <u>peri-glomerular</u> distribution; this is true both for caudally placed lesions in the lateral olfactory tract (Cragg, 1962; Powell & Cowan, 1963; Powell et al., 1965; Heimer, 1968) and for stereotactic lesions affecting the cells of origin of the fibres in the nucleus of the horizontal limb of the diagonal band (Price & Powell, 1970c). The ultrastructure of the glomerular layer has been studied recently

(Chapters 3-5) and this knowledge provides the background to the present study. On the basis of the distribution of neuronal somata, axons and dendrites, it has been possible to divide the glomerular layer into two main regions - the glomeruli themselves and the peri-glomerular region, with a thin, intermediate zone between them. The olfactory nerve terminals lie exclusively within the glomeruli, making synaptic contact with the glomerular dendritic arborisations of mitral, tufted and periglomerular cells; the great majority of the other types of axon terminate in the periglomerular region. In order to confirm the light microscope findings concerning the centrifugal fibres, the lateral olfactory tract was sectioned far caudally, and the glomerular layer examined after varying survival periods. Degenerating terminals were found in the periglomerular region, but several other terminals could not be definitively distinguished from normal or spontaneously degenerating olfactory nerve terminals lying on the periphery of the glomeruli or in the intermediate zone. So as to clarify the identity of these terminals, animals in which bilateral removal of the olfactory mucosa had been carried out several weeks before were studied after unilateral tract section (Figs. 154,156,158,160,162,165,167). In this way, the great majority of olfactory terminals had degenerated from most parts of the bulbs and degenerating centrifugal terminals could be clearly identified in the periglomerular region and intermediate zone of the bulb ipsilateral to the tract lesion; no comparable degeneration could be found on the control side, eliminating the possibility that the degeneration observed was in some way a result of the mucosal lesion

or of spontaneous degenerative change. By this means, the distribution and characteristics of degenerating terminals were confirmed and this information was then used to assist in distinguishing centrifugal fibre terminals from olfactory terminals after tract section, without prior mucosa removal (Figs. 155,157,159,161,164,166,168).

The most easily distinguished degeneration that could be seen was after a survival period of 6-7 days but at 5 days slight alterations were detectable; degenerating myelinated axons and terminals were confined to the periglomerular region or the intermediate zone, in accord with light microscopic observations. The terminals were of fairly regular outline, even during early degeneration and occurred singly, whereas groups of olfactory nerve terminals lie adjacent to each other and have very irregular outlines (Chapters 4 and 7). The synaptic specialisations of degenerating terminals were always of the asymmetrical type (Colonnier, 1968), similar to those found in the external plexiform layer of the bulb (Price & Powell, 1970c); thus these terminals can be identified with the pale terminals showing spherical vesicles and asymmetrical thickenings and located almost exclusively in the periglomerular region or intermediate zone known from normal material (Chapter 5). However, as only a small proportion of this type of terminal degenerates after complete lateral olfactory tract section, the normal population of this type must be a composite group consisting of centrifugal fibre terminals and others (see Fig. 1), presumably the recurrent collaterals of the tufted cells (Chapters 3 and 5).

Fig. 154 A typical cross-section of a short-axon cell dendrite, receiving asymmetrical synaptic contacts from 2 profiles, in this plane of section, and from a third in another plane. The terminal at the top of the figure is a normal terminal, with the characteristic appearance of axon terminals in the periglomerular region having spherical vesicles and an asymmetrical synaptic thickenings; in normal material this terminal could be either a tufted cell collateral or a centrifugal fibre terminal. This material is after a lesion involving the whole lateral olfactory tract and in this figure the two lower terminals are centrifugals undergoing early degenerative change and the top one is a normal tufted cell collateral. Combined lesions: 40/7 days survival.

X38,900

Fig. 155 Early degeneration of a centrifugal terminal which is synapsing onto the shaft of a short-axon cell dendrite. Note the darkening of cytoplasm and crowded appearance of vesicles. 6 days survival.

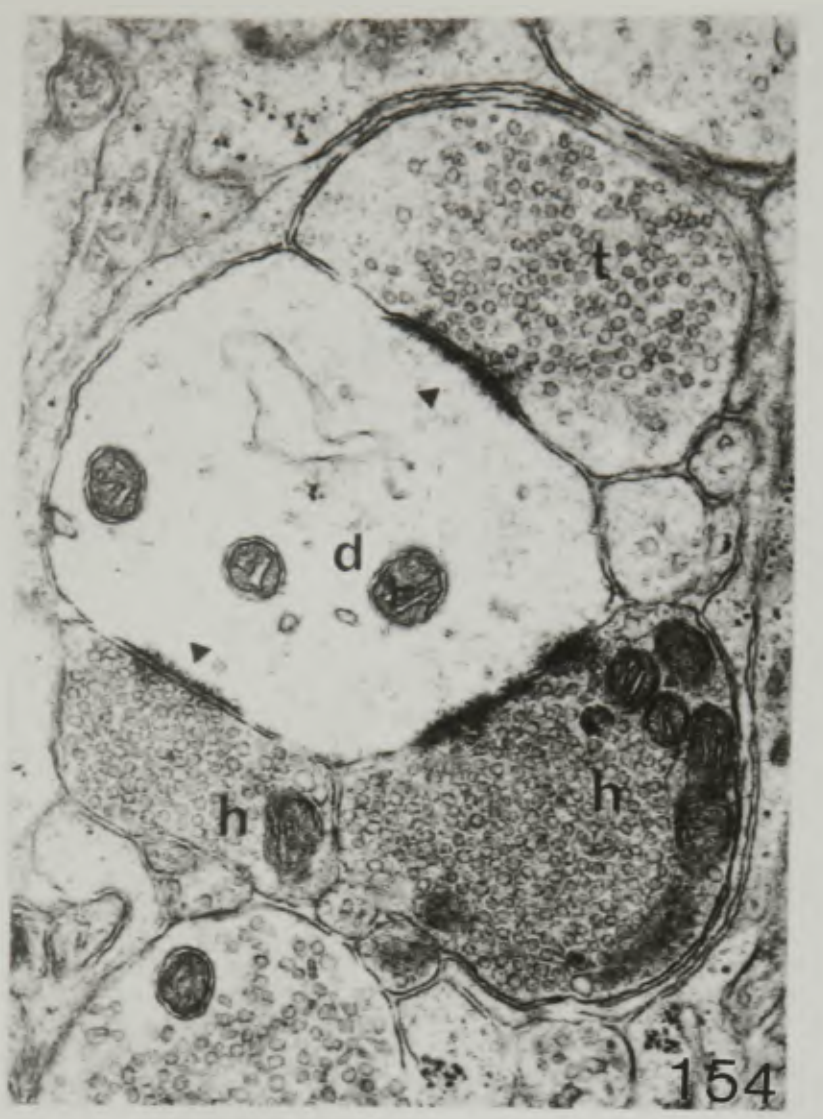
X53,400

Fig. 156 Early degeneration of a centrifugal terminal; note the gliosis and swollen appearance of some vesicles. Combined lesions: 60/5 days survival.

X66,900

Fig. 157 Early stage of degeneration in a centrifugal terminal in the intermediate zone; it is making synaptic contact with the gemmule or spine of a periglomerular cell. 6 days survival.

X61,100



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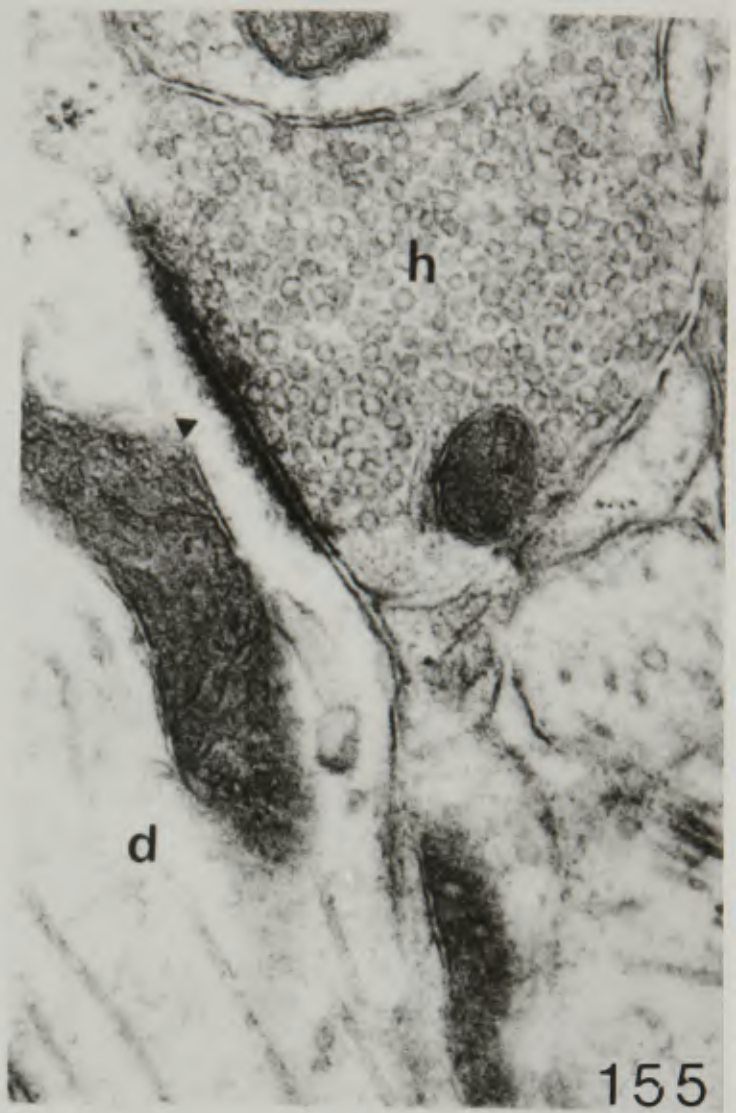

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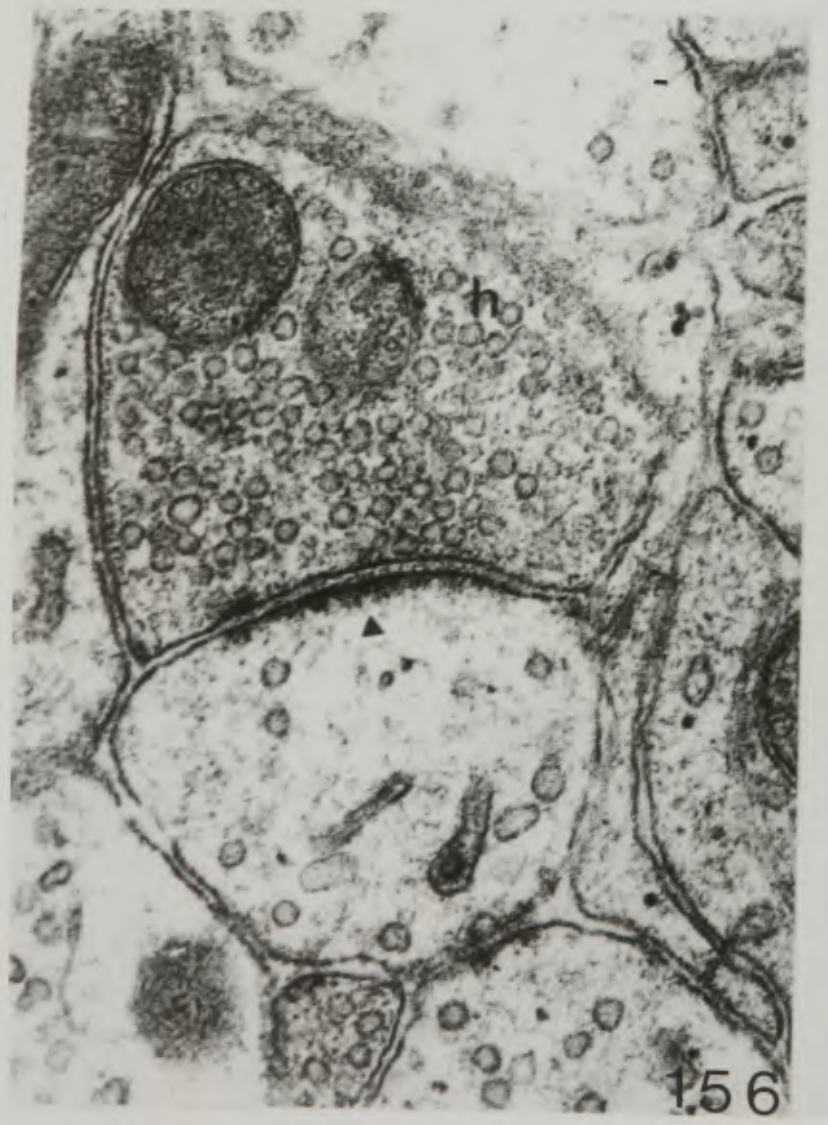

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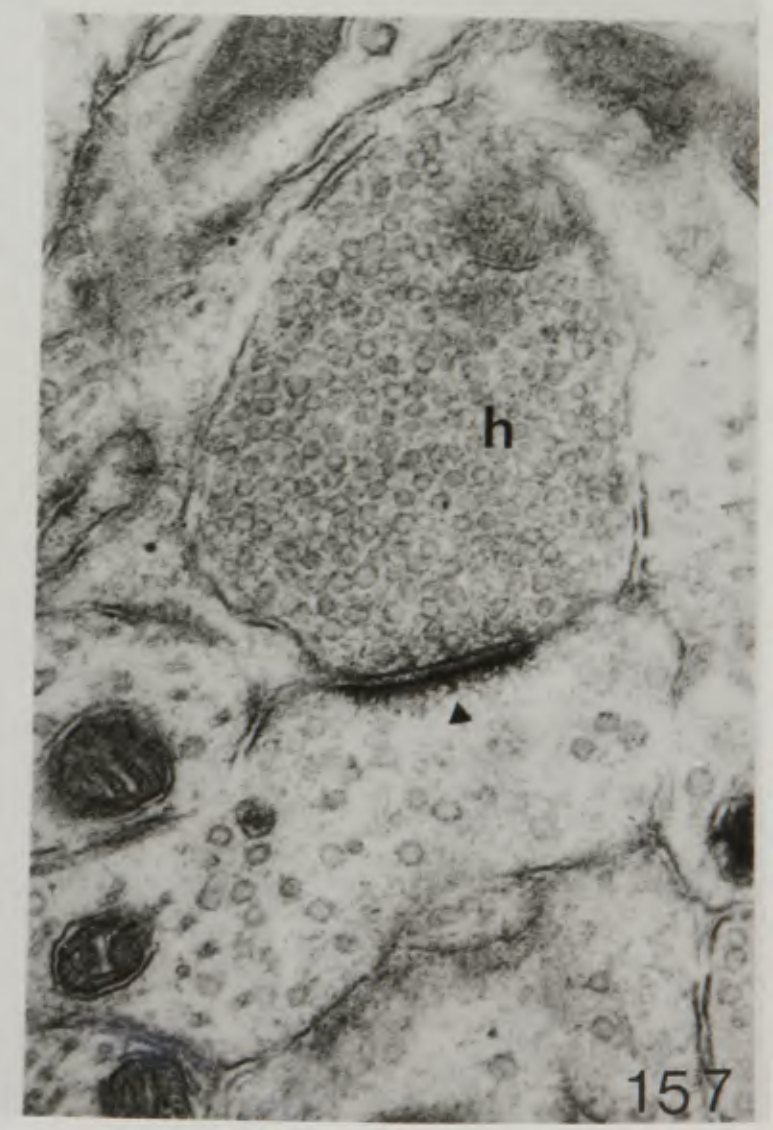

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Fig. 158 Degenerating centrifugal fibre terminal, synapsing onto the shaft of a tufted or mitral cell; note the crowded vesicles and darkened cytoplasm of the terminal and the reciprocal synapse of the mitral/tufted cell shaft with a periglomerular cell gemmule. The tufted/mitral cell dendrite contains spherical vesicles and makes symmetrical synapses, the periglomerular cell gemmule contains large flattened vesicles and has symmetrical synaptic contacts. Combined lesions: 60/5 days survival.

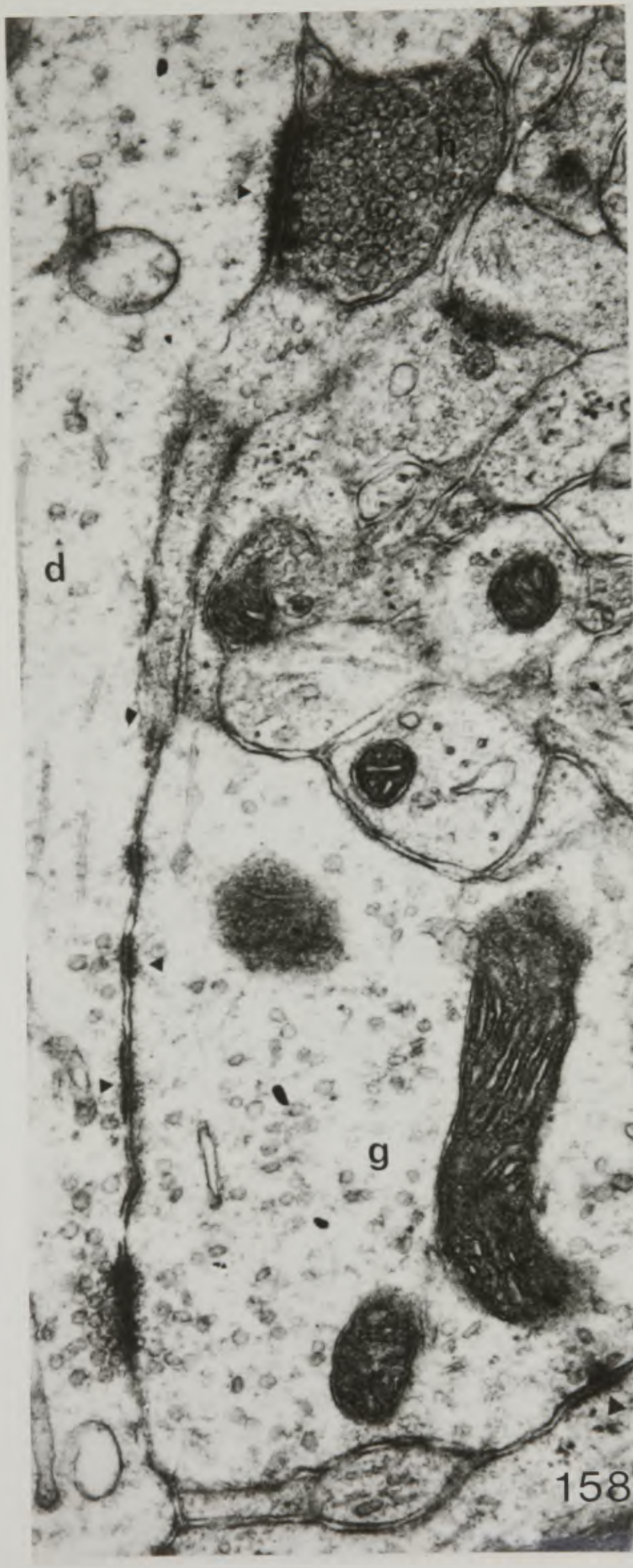
X45,700

Fig. 159 Degenerating centrifugal terminal in the intermediate zone, terminating on the shaft of a periglomerular cell dendrite which is passing into the glomerulus. The degenerating terminal has swollen vesicles and very dark granular cytoplasm; note the characteristic large flattened type vesicles of the periglomerular cell process. 7 days survival.

X53,400

Fig. 160 Degenerating centrifugal terminal terminating onto a periglomerular cell appendage, and showing a few swollen vesicles, including a dense cored vesicle. Combined lesions: 60/5 days survival.

X53,400



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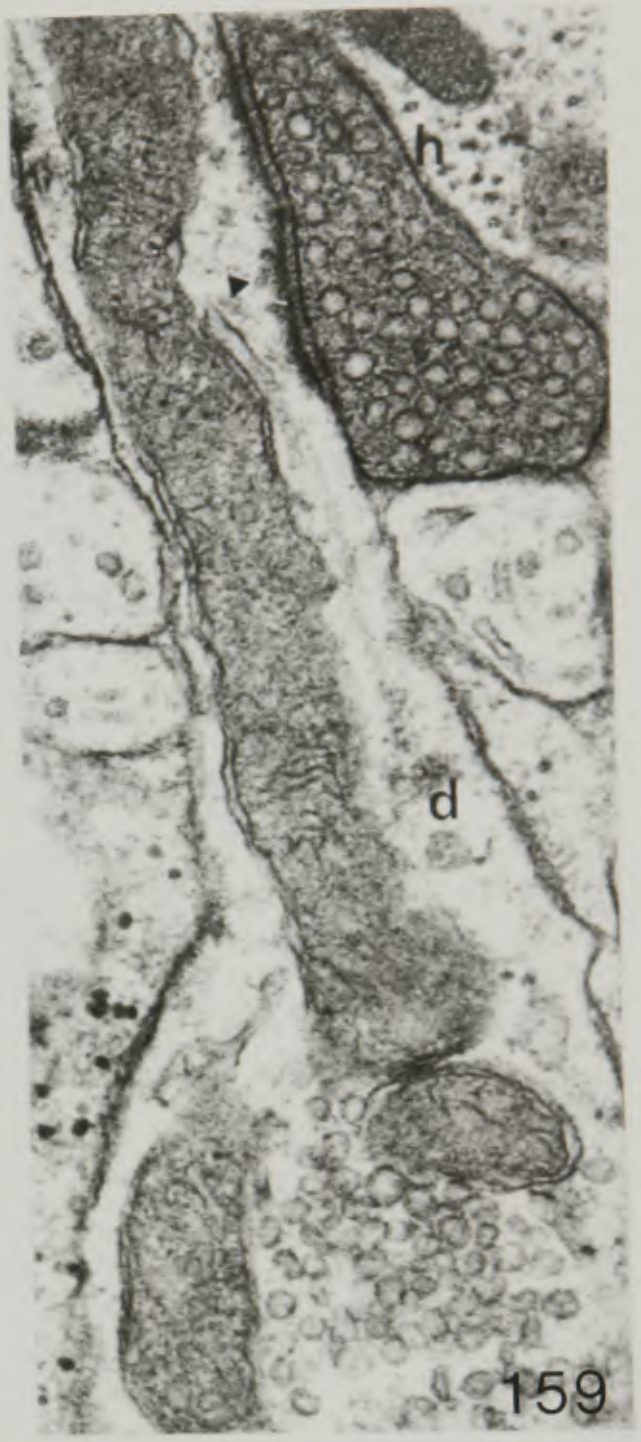

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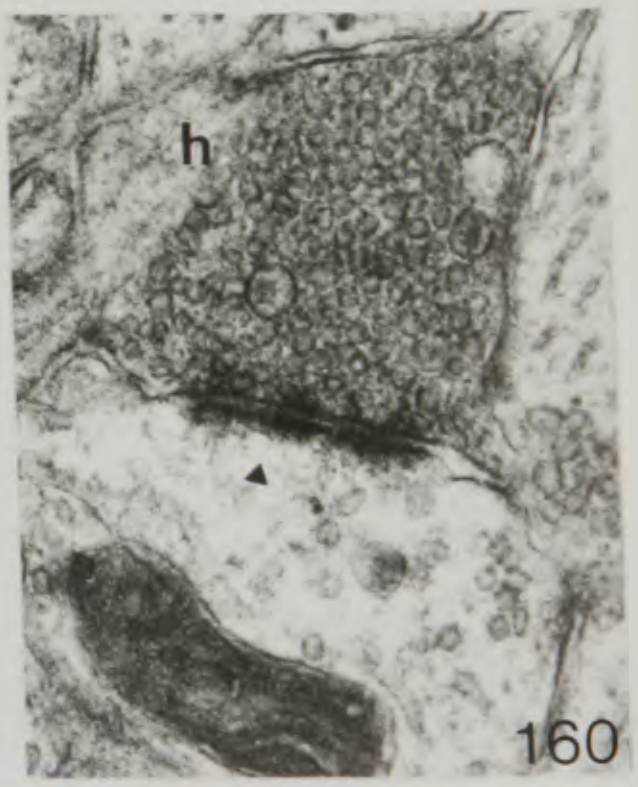

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Fig. 161 Degeneration of a centrifugal terminal that is synapsing onto a periglomerular appendage; in the degenerate terminal, vesicles are rather swollen and a mitochondrion is distorted in appearance. 6 days survival.

X64,100

Fig. 162 Late degeneration of a centrifugal terminal showing pale mitochondria, a swollen multivesicular body and marked gliosis. Combined lesions: 40/7 days survival.

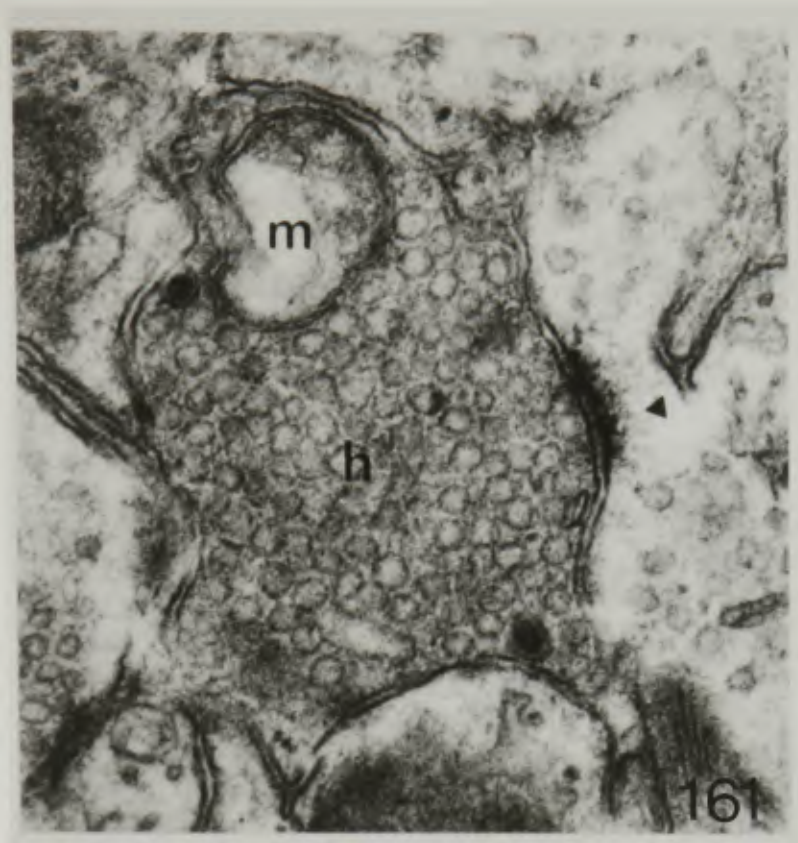
X55,400

Fig. 163 Degeneration of a terminal of an axon from the anterior olfactory nucleus in the superficial part of the external plexiform layer, making synaptic contact with two granule cell appendages. Note the distinct morphology and the multiple synapses characteristic of this type of terminal that, together with their position, enable their differentiation from centrifugal terminals. Lesion of the lateral olfactory tract involving the anterior olfactory nucleus. 6 days survival.

X53,400

Fig. 164 Degeneration of a centrifugal terminal that is synapsing onto the shaft of a thin dendrite from a periglomerular cell; note the dark granular cytoplasm of the terminal and the indentation of its outline. 6 days survival.

X76,300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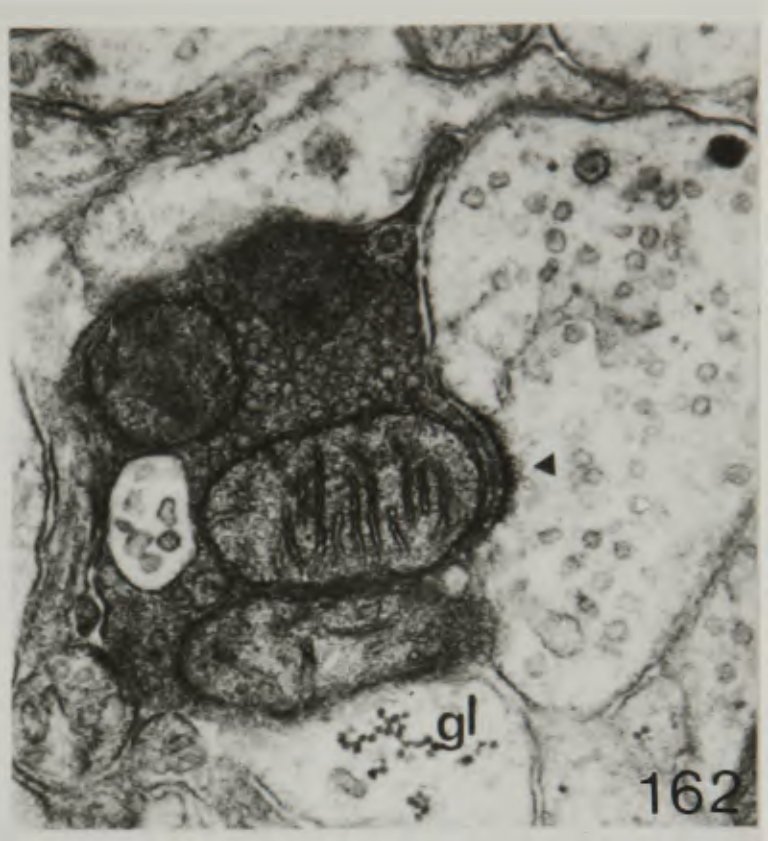
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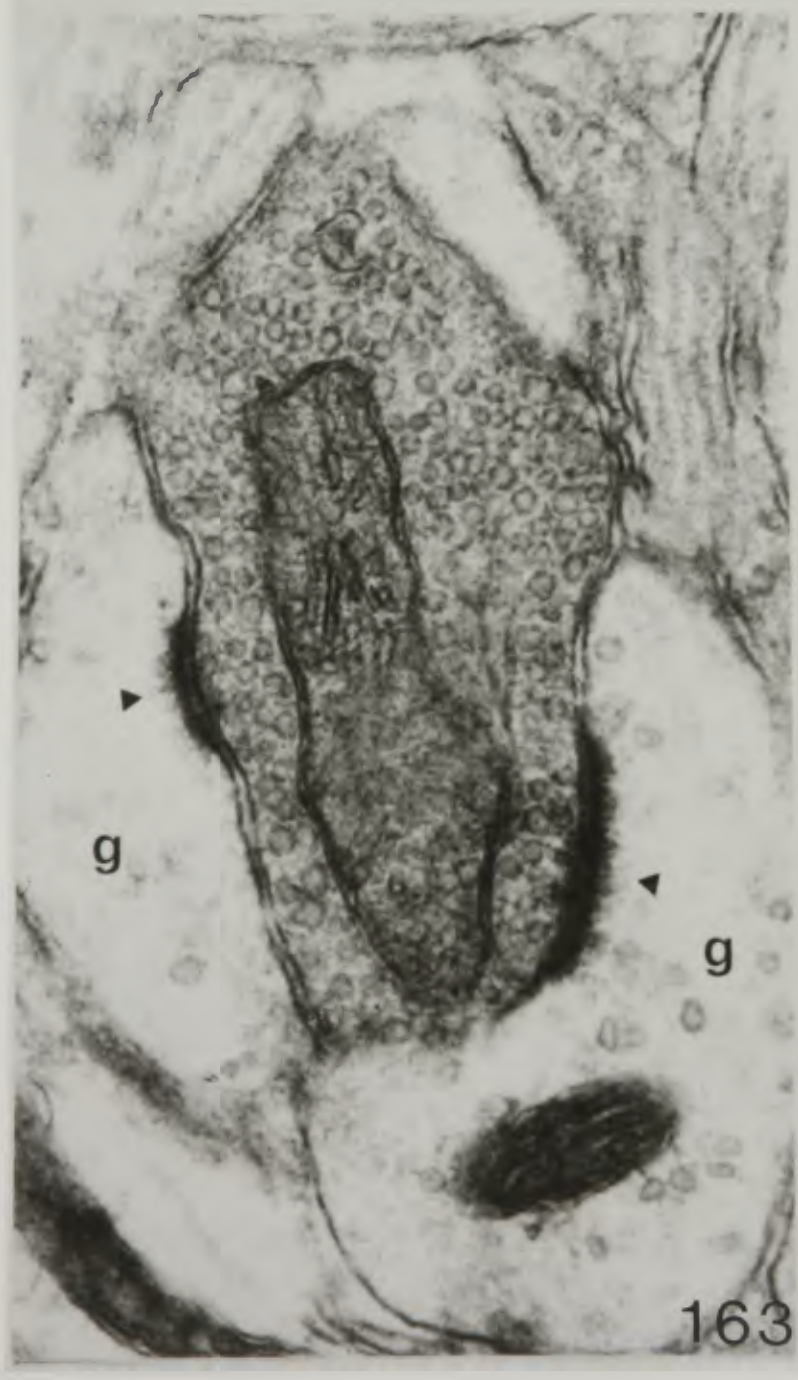
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Fig. 165 Degenerating centrifugal terminal with irregular shaped vesicles and an indented outline. Combined lesions: 40/7 survival.

X66,700

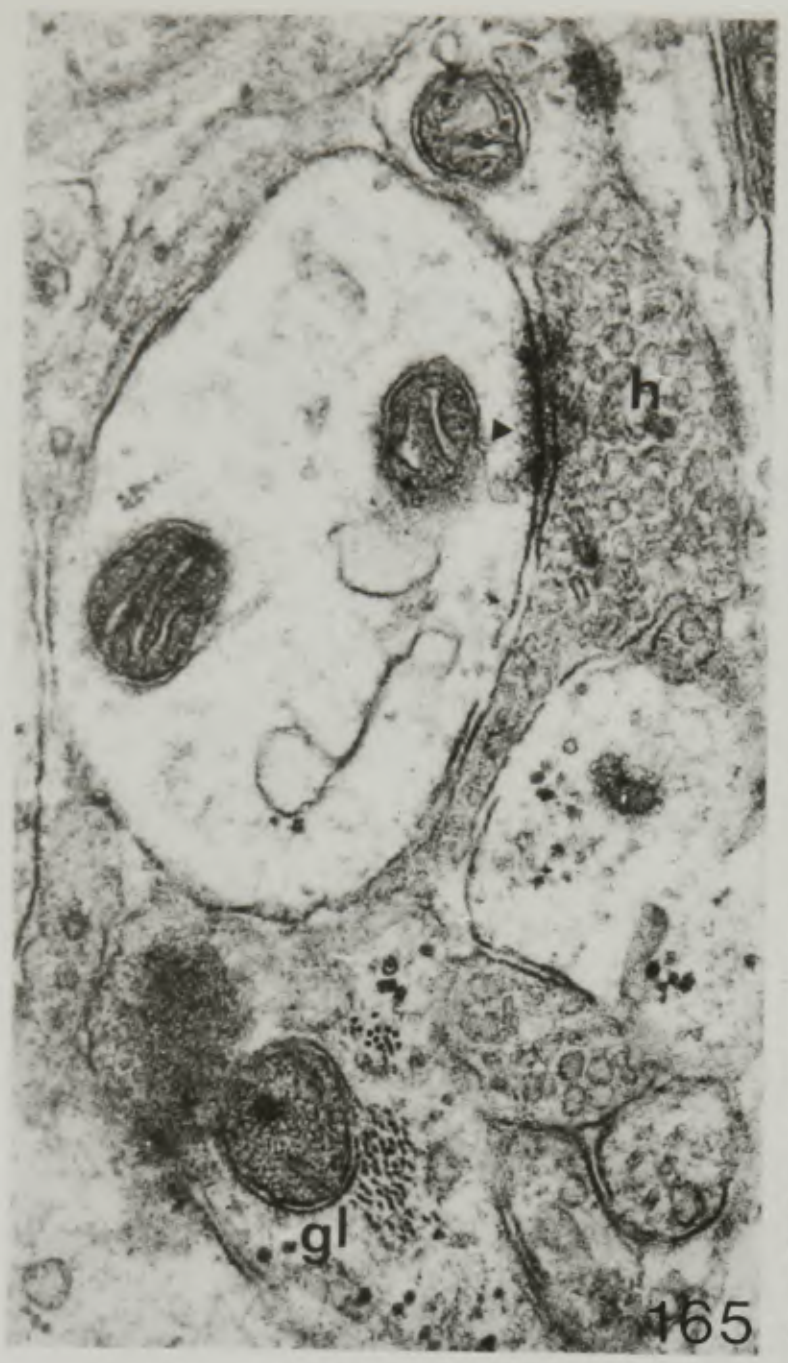
Fig. 166 Very late stage of degeneration in a centrifugal terminal showing vesicle distortion, mitochondrial swelling and distortion and marked gliosis, appearing to involve the post-synaptic spine. It was not possible with serial sections to determine whether or not the post-synaptic spine was still attached at any point to its parent process. 7 days survival.

X74,300

Fig. 167 A degenerating terminal of a centrifugal fibre being replaced at its post-synaptic thickening by a glial profile, while still remaining apposed to part of the post-synaptic thickening. Combined lesions: 40/7 days survival.

X64,100

Fig. 168 A post-synaptic thickening on the shaft of a peri-glomerular cell dendrite, showing the absence of the presynaptic terminal; the post-synaptic thickening is apposed to a glial and another, possible neuronal, profile, 10 days survival.



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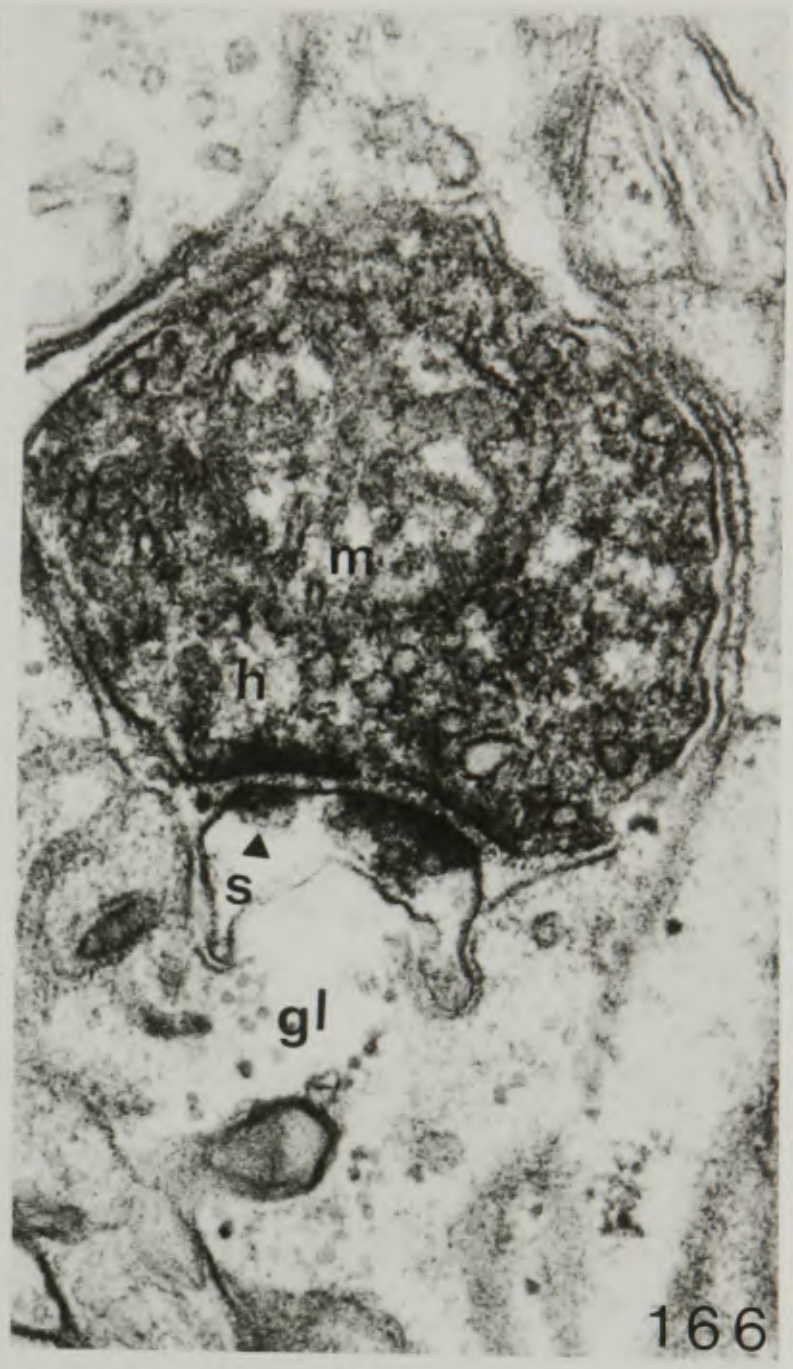

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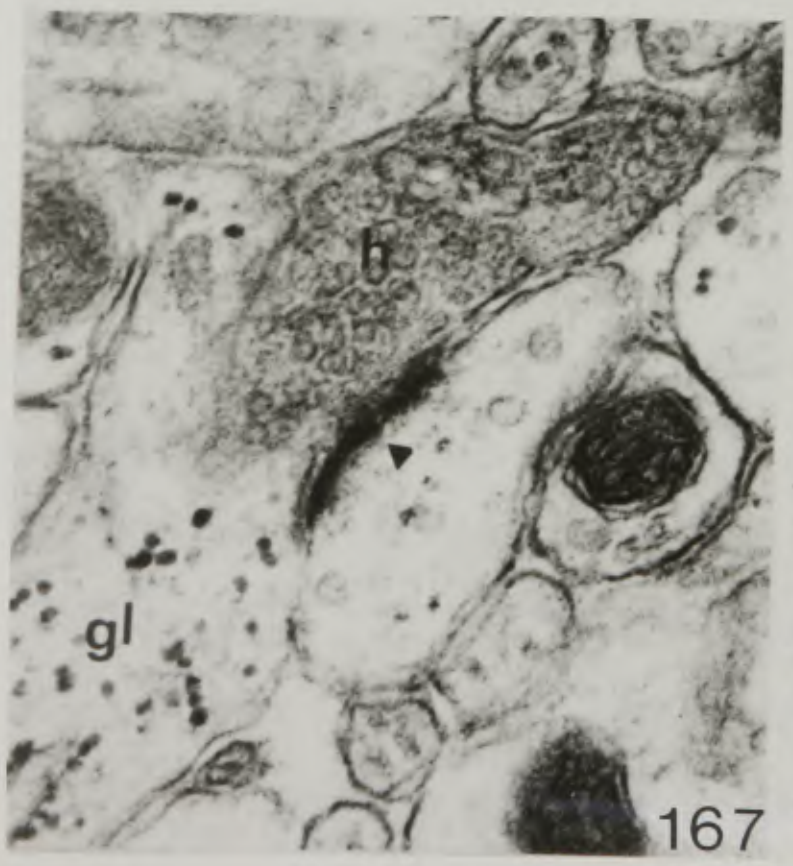

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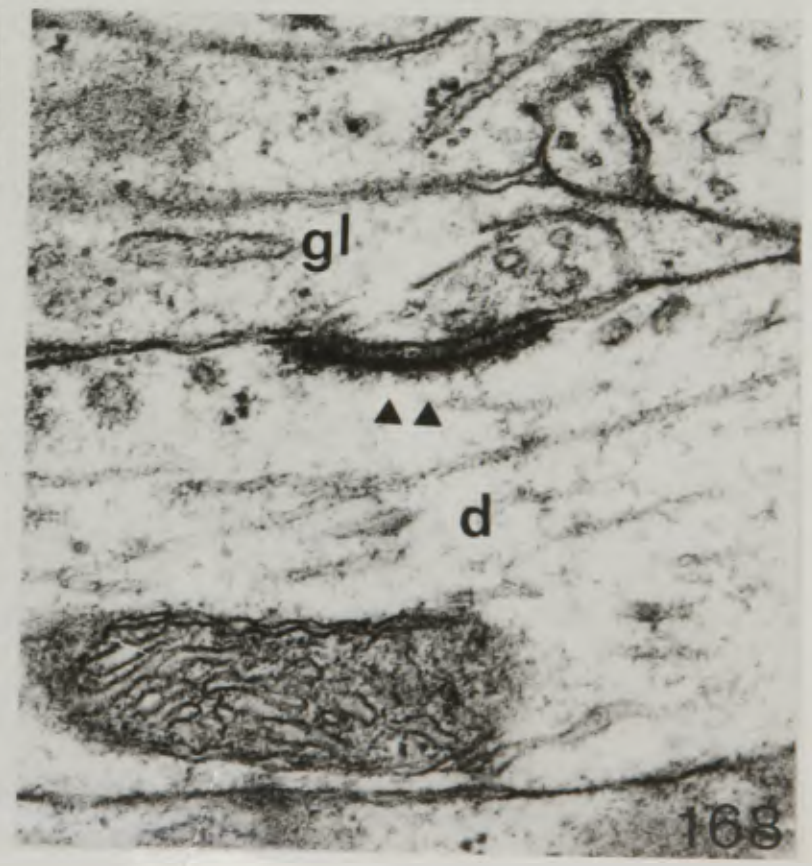

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The type of degeneration found after lesions of the lateral olfactory tract is of the typical darkening type (Figs. 154-167), with little sign of any early swelling of the kind observed in the olfactory nerve terminals (Chapter 7), except for a small proportion of swollen vesicles (Figs. 156,159-161). As the survival time increases, the terminals become more electron-dense and shrunken (Figs. 158-160,162,164,166), often indented by surrounding profiles (Figs. 164,165); vesicles become crowded together and are often distorted (Figs. 164-166). In the later stages, pale and swollen mitochondria (Figs. 161,162,166), multivesicular bodies (Fig. 162) and dense cored vesicles (Fig. 160) could occasionally be found. Gliosis was marked around these terminals even in the early stages (Figs. 156,159,161-162,165-167), and glycogen granules are often evident in the glial cytoplasm (Figs. 159,162,165-167). This is a striking difference from olfactory nerve degeneration, in which it was common to find no evidence of any glial proliferation around the degenerating terminals at all stages (Chapter 7). Such a difference may reflect a difference in the glial relationships of myelinated and unmyelinated fibres. Finally, the terminals are displaced from their post-synaptic thickenings between 7 and 10 days, most commonly replaced by glia (Figs. 167,168), but occasionally by neuronal profiles.

The number of degenerating terminals that can be unequivocally identified is small and this certainly reflects a genuine sparseness of distribution in the periglomerular region as compared with the deep half of the external plexiform layer. Nevertheless, study of a large

number of animals either with single lateral olfactory tract lesions only or with combined mucosal and tract lesions, at various survival times, leaves no doubt at all of their termination in this site and has given a clear idea of the cells and processes upon which they terminate. The fact that the centrifugal fibres terminate in the periglomerular region limits the possibilities of postsynaptic cells and processes, by excluding the glomerular arborisations of the mitral, tufted and periglomerular cells, and also the olfactory nerve terminals as suggested by Powell et al. (1965). The most frequent postsynaptic processes are the spines or gemmules of the periglomerular cells (Figs. 156,157,160-162), which are particularly recognisable by virtue of the large flattened vesicles they contain, their lack of neurotubules, and a rather flocculent cytoplasm. Degenerating terminals are also found quite frequently on the dendritic shafts of the periglomerular cells (Figs. 159,164,165,168) - either of the thin type, whose course is entirely periglomerular, and which contains a large number of ribosomes, or the larger stem dendrites passing through the periglomerular region and intermediate zone towards the glomeruli in which they arborise.

Some degenerating terminals have been found on large dendrites in the periglomerular region which show no sign of entering the glomeruli or of branching, and which contain no significant groups of identifiable vesicles (Figs. 154,155). The size and outline of these dendrites, as well as the morphological characteristics of their shafts, are consistent with their identification as those of short-axon cells (Chapters 3 and 5).

A few spines without vesicles in any plane of section have been found receiving centrifugal terminals and may be tentatively identified as the rare spines of the short-axon cell dendrites, with which some have been traced in continuity. Finally a few examples of degenerating centrifugal terminals have been found in synaptic relation to the periglomerular portion of the tufted and possibly the larger mitral cell primary dendrites, adjacent to reciprocal synapses of the appropriate polarities (Chapter 3) (Fig. 158). No evidence was found in this study of any significant variation in the number of centrifugal terminals in the glomerular layer in different parts of the bulb, as suggested by Cragg (1962).

In those brains in which there was evidence, in the histological controls, of damage to the anterior olfactory nucleus, no additional degeneration could be found in the glomerular layer. However, a number of very characteristic terminals in the superficial part of the external plexiform layer, showing asymmetrical thickenings, were undergoing degeneration (Fig. 163); these terminated on to the gemmules of granule cells and are clearly the most peripheral group of those described by Price and Powell (1970c) after similar lesions. It is important to note that these terminals never encroached upon the glomerular layer, despite the occasionally irregular contour dividing this layer from the most superficial external plexiform layer; nor was there any evidence that they made contact with any processes originating in the glomerular layer. Moreover, it was only after lesions that involved the anterior olfactory nucleus that any degeneration was found in the superficial half of the

external plexiform layer, in agreement with the observations of Price and Powell (1970c).

DISCUSSION

The termination of centrifugal fibres in the periglomerular region of the olfactory bulb which was described by workers using light microscopy with the Nauta technique (Cragg, 1962; Powell & Cowan, 1963; Powell et al., 1965; Heimer, 1968; Price & Powell, 1970e), has been confirmed by electron microscopy; no evidence has been found of any such fibres terminating in the superficial part of the external plexiform layer or in the olfactory nerve layer. The main cell type receiving centrifugal fibres at glomerular level is the periglomerular cell, but short-axon and tufted cells may also receive a small proportion; neither the olfactory nerve fibres nor their terminals show any sign of receiving synapses from centrifugal or any other axons. Price and Powell (1970c) considered that, although occasional dark terminals were observed after tract section, it was not possible to be certain that these were degenerating terminals of centrifugal fibres at the glomerular level, and several factors contributed to this caution: the distribution of profiles, particularly olfactory nerve terminals between periglomerular regions and glomeruli, was not known in detail at that time; also, the terminals of centrifugal fibres are sparsely distributed at this level, in comparison with the deep group, and are at times difficult to distinguish from normal, dark or spontaneously degenerating

olfactory nerve terminals. The true periglomerular distribution of the centrifugal fibre terminals considerably assisted their identification in the present study, since it has been shown that olfactory nerve fibres and their terminals are never found in this region (Chapter 5); however, the presence of olfactory terminals in the intermediate zone, where the periglomerular region interdigitates with the glomerulus itself, made identification of possible degenerating centrifugal terminals more difficult and necessitated the use of combined lesions of olfactory mucosa (long term) and lateral olfactory tract (short term). The centrifugals form a small proportion of the pale type of terminal found in the periglomerular region of normal material, which contain spherical vesicles and make synaptic contact by means of asymmetrical thickenings; the remainder of this population is made up by axons intrinsic to the bulb, the recurrent collaterals of tufted cells (Chapter 5).

This study has again demonstrated the value of correlative light and electron microscopy for defining the exact site of termination of an afferent pathway. It is notable that the Nauta-Gygax (1954) technique, which stains both fibres and terminals shows heavy degeneration in the periglomerular region after tract section whereas the Fink-Heimer modification (1967), staining predominantly terminal degeneration, shows much less degeneration. The paucity of degenerating terminals found with the electron microscope corresponds to the latter; a reasonable quantitative correlation of terminal degeneration has been obtained between the number of granules in a 25 um Fink-Heimer stained section

and the number of dark terminals in a series of 50 electron microscope sections (c. 50 nm), covering a 2.5 μm thickness.

Combined lesions of different pathways using long and short survival periods seem to be a valuable method for eliminating any confusion concerning their site of termination, either if they distribute in a similar part of the nucleus, or if lesions of one pathway inevitably involve the other, but not vice versa. An optimal survival time for the first lesion must be found which avoids late orthograde degeneration and precedes transneuronal degeneration or possible reinnervation or apposition (Raisman, 1969; Chapters 7 and 8); the optimum total survival time after mucosal lesions in the rat is 40-60 days. The study of several sites may be assisted by this technique, including the olfactory bulb itself where the exact site of termination of the axons from the anterior olfactory nucleus in the deeper layers cannot be ascertained since a lesion of this nucleus necessarily involves damage to the centrifugal and commissural pathways. Thus preliminary lesions of the latter pathways, followed, after a suitable interval, by lesions of the anterior olfactory nucleus may be of use in clarifying this question.

The centrifugal fibres are the only extrinsic axons of central origin that reach the glomerular layer; their distribution to this layer is quite separate from the projection to the deep external plexiform layer. The reason for this separation of the centrifugal pathway into two components is difficult to interpret; it may represent a pathway for centrifugal influence acting indirectly on the superficial tufted

cells, together with a concurrent effect on the deep tufted and mitral cell dendrites. The distribution of centrifugal axon terminals on to several cell types at the glomerular level, unlike the deep group, would indicate that the role of these superficial terminals may in fact be different from that of the deep group. The siting of terminals on the proximal part of the periglomerular soma-dendritic tree would similarly suggest a difference, in that their effect will tend to be mediated more by the periglomerular cell axo-dendritic synapses on to mitral and tufted cells than by the dendrodendritic synapses (Chapter 4); the granule cells can only act by the latter type of synapse, close to which the centrifugal terminals are situated (Price & Powell, 1970c). Thus, although there is a clear analogy in the interneuronal position of the periglomerular cells and granule cells with respect to the centrifugal axons and the relay cells, the functional nature of the interaction may be of a somewhat different type. In relation to the mitral cells, the effects of the superficial group of centrifugal fibres will be mingled with the primary integration of olfactory and inter-glomerular inputs whereas the deep group will act, via the granule cell gemmule, on to the proximal, or final dendritic level of the mitral cells. It is interesting to note that Cajal (1911) observed two levels of termination of the centrifugal fibres to the pigeon retina, and there is some electron microscopic evidence in support of this (Dowling & Cowan, 1966); these are a common one at the border of the inner plexiform and inner nuclear layers, and a rarer, at a deeper level, within the inner nuclear layer.

CHAPTER 10

EXPERIMENTAL STUDIES ON THE AXONS INTRINSIC TO THE GLOMERULAR LAYER OF THE OLFACTORY BULB.

INTRODUCTION

Previous studies have shown that axons derived from five different sources pass into and terminate in the glomerular layer of the olfactory bulb: the extrinsic axons are the olfactory nerves (Cajal, 1911; Andres, 1965; Chapters 4 & 7) and the centrifugal fibres (Cajal, 1911; Cragg, 1962; Powell, Cowan & Raisman, 1965; Chapter 9); the intrinsic axons are derived from the tufted cells (collaterals), periglomerular cells and superficial short-axon cells (Blanes, 1898; Cajal, 1911; Chapters 3 & 5). With the exception of the olfactory nerves, the great majority of these axons terminate in the periglomerular region or intermediate zone; many of their fibres pass in a more or less well-defined layer, just deep to the glomerular layer (Blanes, 1898), although others do pass through the neuropil. In attempting to determine the intrinsic organisation of the glomerular layer of the olfactory bulb with the electron microscope, much of the evidence has come from the study of normal material (Chapters 3-5); this has been because only the two extrinsic fibre pathways could be selectively interrupted (Chapters 7 & 9), the terminals of one of which, the olfactory nerves, could already be easily distinguished in normal material. After the interruption of both these pathways on the same side of a single brain, many axon terminals, presumably derived largely from intrinsic cells, still remained. In order to ascertain their identity and provide further information on their distribution, particularly in terms of the spatial relationship between adjacent glomeruli, lesions were placed in the

superficial layers of the olfactory bulb itself; the results of these experiments, using both electron microscopy and the silver degeneration methods of light microscopy, are presented in this report. A similar approach has been used in the study of the caudate nucleus (Kemp & Powell, 1971b).

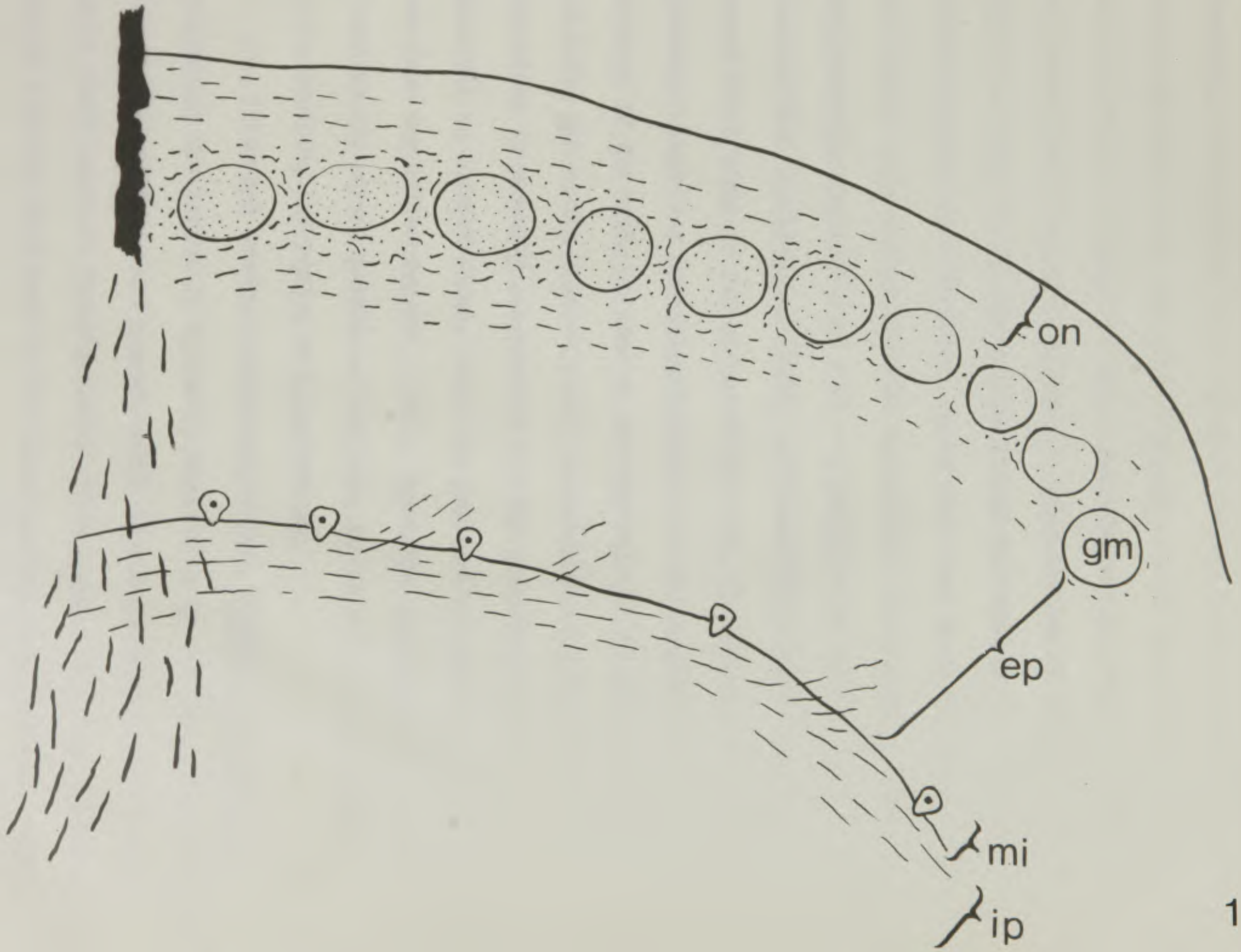
It is evident that a lesion placed directly into the substance of the glomerular and olfactory nerve layers will interrupt fibres of diverse types and of both intrinsic and extrinsic origins; for this reason, the interpretation of results is difficult and often requires qualification. It is therefore especially important to know the ultrastructure of the region under normal and experimental conditions as well as the evidence obtainable from Golgi-impregnation and silver degeneration methods. The same types of lesion were used for electron microscopy and for light microscopy, and the descriptions given here are restricted to those lesions that did not extend more deeply than the superficial part of the external plexiform layer. Estimates of spread are given in terms of the number of glomeruli traversed from the lesion so as to give an order of magnitude in functional terms; the average glomerular width is 80-120 μm.

RESULTS

Light Microscopy

The pattern of degeneration after superficial lesions, extending just deep to the glomerular layer, in various sites on the dorsal aspect of the olfactory bulb was essentially similar to all the brains studied(Fig.169A). T

Fig. 169A A schematic diagram to show the distribution of axonal and terminal degeneration in the different layers of the olfactory bulb after a small lesion (solid black) in the olfactory nerve (on) and glomerular (gm) layers. Fine intraglomerular degeneration represents olfactory nerve degeneration; periglomerular (.-.-.) degeneration is that of glomerular layer intrinsic axons (periglomerular, tufted and short-axon cells). The rows of fine fibre degeneration parallel to the laminae in the external plexiform (ep) and internal plexiform (ip) layers represent tufted cell collateral branches, while the coarser degenerating fibres perpendicular to the laminae are the main axons passing to the pyriform cortex. Mitral cell layer, mi.



169 A

lesions themselves (Fig. 169) appeared to affect between two and four glomeruli, which showed as characteristically dark and granular areas containing blood cells and glia with pale spaces in between; in a few brains there was evidence of more extensive damage due to vascular involvement and these were rejected. Degeneration of fibres and terminals was found in all directions around the site of the lesion, becoming sparser at a distance, up to about ten glomeruli distant in each plane; this was distributed both within the glomeruli and in the periglomerular regions (Fig. 170) - a distinction that is only really possible with the Wiitanen (1969) and Nauta-Gygax (1954) techniques. Beyond about eight or ten glomeruli distant, the appearance of the glomerular layer was indistinguishable from the normal pattern of staining of the bulb, as seen on the ventral and anterior aspects of the bulb, and on the control side; spontaneous olfactory nerve degeneration is probably responsible for the granular appearance of the glomeruli in control sites, while the periglomerular regions are typically free from any silver deposit. It is therefore particularly important to determine the normal pattern of staining in the bulb of each brain, before studying the region of degeneration.

The fibre and terminal degeneration seen <u>within the glomeruli</u> surrounding the lesion is typically densest up to six or seven glomeruli distant, and is then more sparse, until it becomes equivalent to the normal level found in the glomeruli. This degeneration may extend either more or less far than that in the corresponding periglomerular region and superficial part of the external plexiform layer and commonly shows a more

uneven distribution around the lesion; this presumably reflects the involvement of fibres passing in the olfactory nerve layer, and varies according to the exact position of the lesion on the bulbar surface. In cases where the lesion has been sectioned, the pattern and density of degeneration in the immediately surrounding glomeruli are exactly similar. The silver staining itself typically appears as fine granules or rows of granules (Fig. 170).

The <u>periglomerular</u> degeneration and that in the most superficial part of the external plexiform layer, which may be regarded as a part of the former in this context, is most dense for the first three to five glomeruli in each direction (Fig. 170); the appearance of the staining ranges from fine granules to rows of large granules, indicative of large single fibres. The finer staining continues up to eight to ten glomeruli from the lesion, at which point it disappears altogether; this outer zone of degeneration is very sparse compared with that occurring in the glomeruli - even within normal glomeruli. The orientation of fibres or rows of granules in the periglomerular region and superficial part of the external plexiform layer is predominantly parallel to the surface, and to the lamination of the bulb; from the immediate vicinity of the lesion some fibres, often appearing relatively large, pass perpendicular to the surface, through the mitral and granule cell layers and into the deep white matter. They may give off collateral fibres in the granule cell layer and there again run parallel to the laminae forming the internal plexiform layer (Fig.171); these collaterals spread up to 10 glomerular widths and rows of granules can be seen

Fig. 169 Low magnification light micrograph of the olfactory bulb, showing the site of entry of a small lesion that affected the glomerular and olfactory nerve layers only. Fink-Heimer Method.

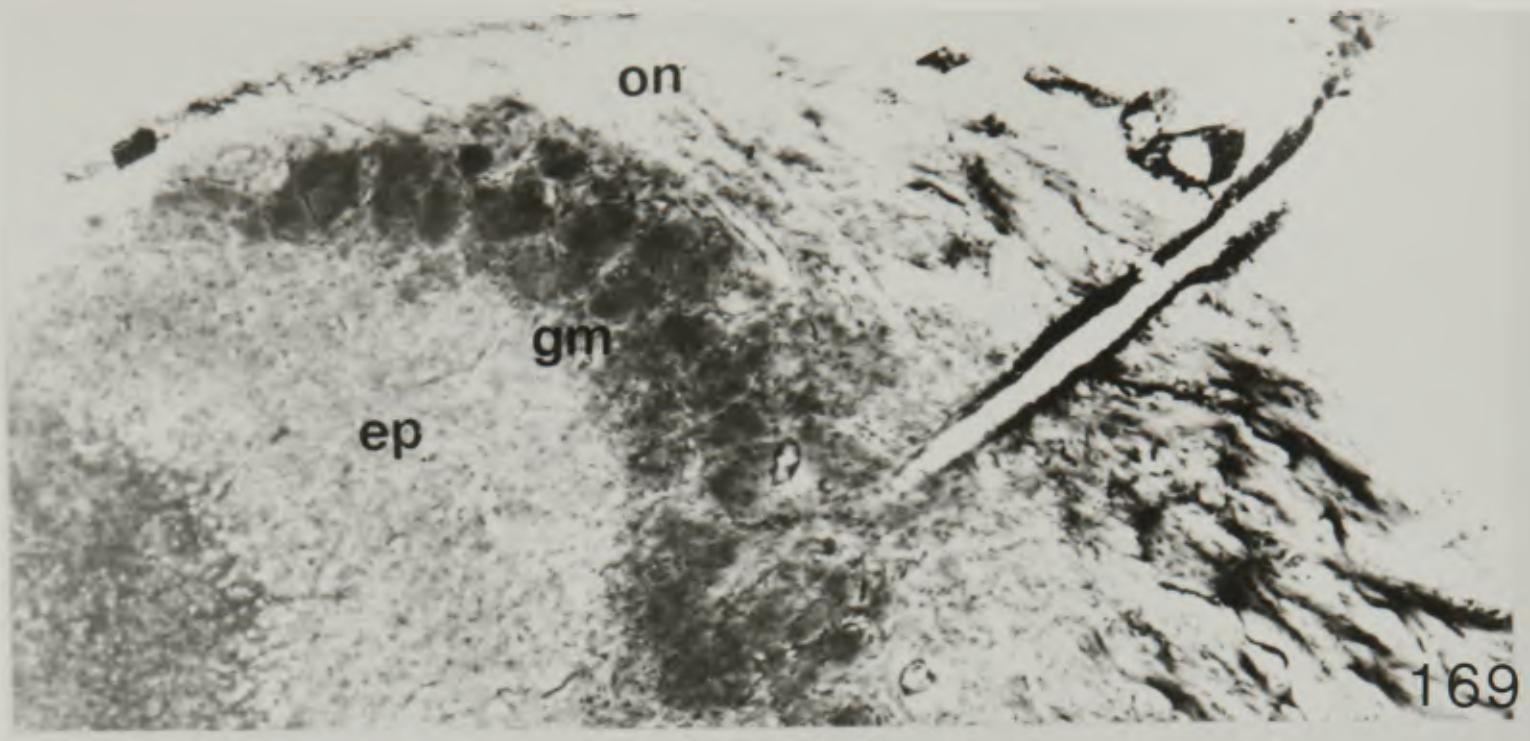
X60

Fig. 170 Degeneration of nerve fibres and terminals in the glomerular layer after a small intrinsic lesion, as seen with the Nauta technique.

X660

Fig. 171 Degeneration of nerve fibres and terminals in the internal plexiform layer, just deep to the mitral cell layer; the rows of silver granules represent the degeneration of tufted cell collaterals after a superficial lesion. Fink-Heimer technique.

X820



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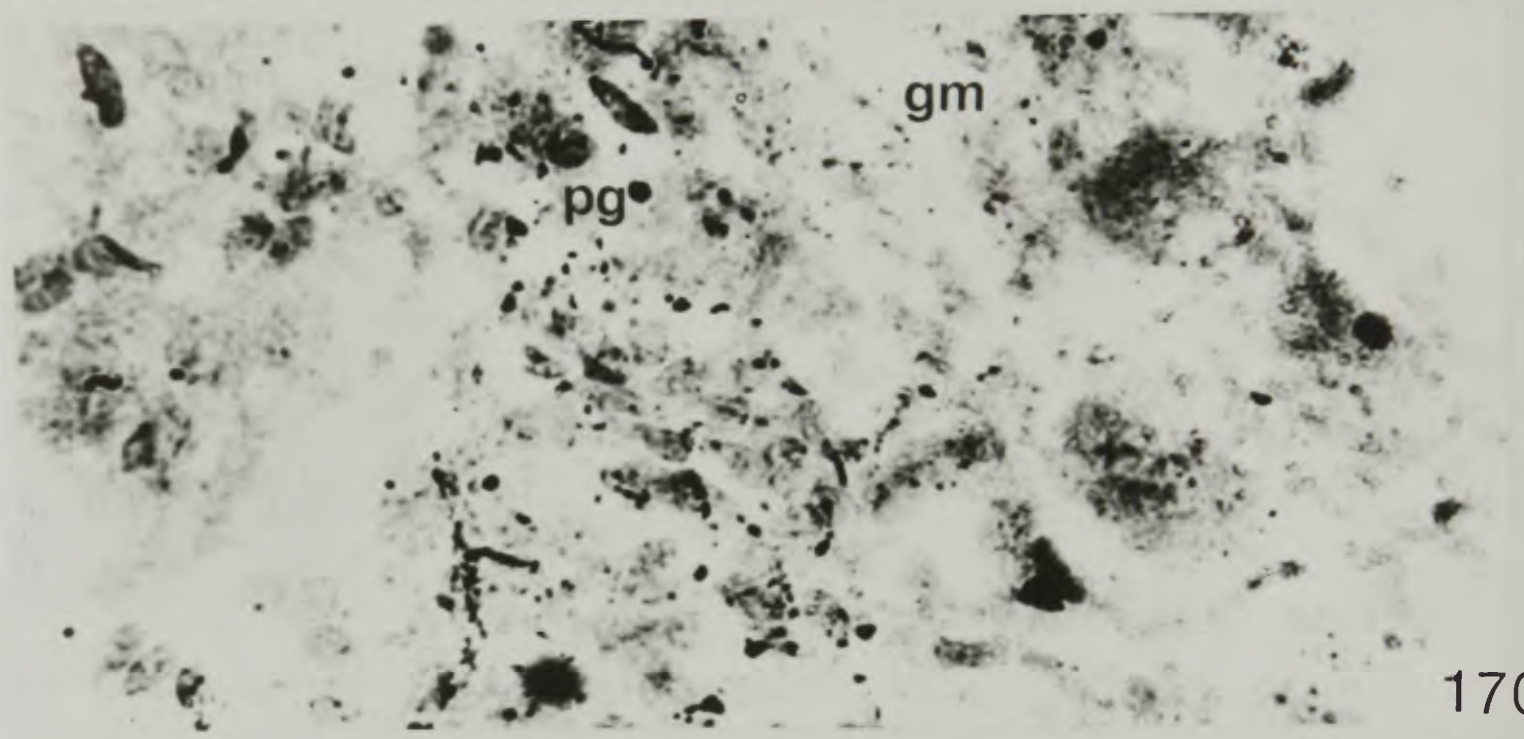

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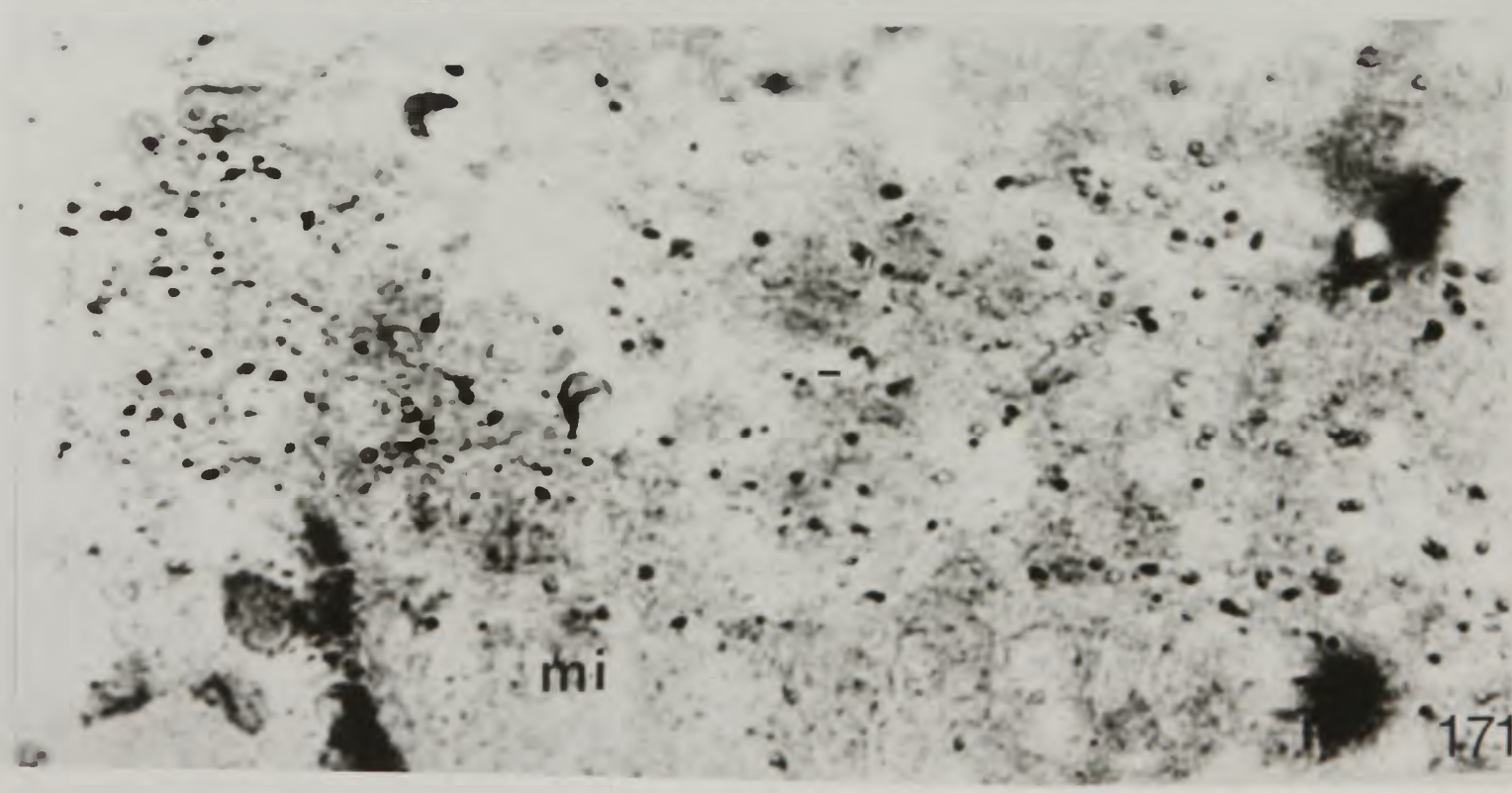

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curving superficially into the deep part of the external plexiform layer. These deeply running fibres are taken to be those of the external tufted cells, and occasionally they may be traced passing back into the pyriform cortex, although more extensive superficial lesions show this route more clearly as described by Lohman and Mentinck (1969).

Electron Microscopy

The lesions in the material used for electron microscopic examination were all placed in the middle of the dorsal surface of the bulb, so that they could easily be found after fixation, and blocks could be taken around the lesion; this limitation of site for the lesion seemed justified by the similarity of distribution of degeneration in light microscopy after lesions placed all over the dorsal surface and the dorsomedial and dorsolateral surfaces. Blocks from other parts of the same bulb were examined to detect any further spread of degeneration, and were also used as controls, together with the opposite side. In this way an impression of the spread of degeneration and its type could be obtained for each brain; the relative sparsity of degeneration after such intrinsic lesions made this mode of study essential to achieve a picture of the distribution of the degenerating terminal types in surrounding glomeruli. It is only in terms of the distribution of degeneration of the various types that the terminals of different cellular origins could be differentiated during degeneration.

Although the actual site of the lesions was often poorly fixed, the

surrounding regions were regularly well-fixed and showed no sign of asphyxic or secondary cellular degenerative change; extensive gliosis had occurred in the region of the damage, but beyond this, gliosis was only notable in relation to degenerating profiles (Figs. 172,174-176, 178, 181-183,186-188). Some "dark neurons" and neuronal profiles (Fig. 184) were observed in the immediate vicinity of the lesions, and these could be interpreted as those neurons that had been directly damaged by the lesion. Several survival periods were used, from 1 to 8 days, and the stages of degeneration seen in the axon terminals at each of these periods were variable, partly on account of the heterogeneity of the axons involved and partly due to their varying distances from the lesion to the terminals and from the cells of origin to the lesion. Thus although terminals were clearly undergoing sequential change, it is not possible to give a detailed account of the degenerative process in each terminal type. Swelling and pallor of terminals appeared to be an early change in most terminals, often accompanied by vesicle swelling (Figs. 172,173,177,181,182,185); shrinkage and darkening were commonly indicative of a later stage, with progressive fragmentation or loss of cytoplasmic contents (Figs. 174-176,178-180,183,186-188). Other features common to all degenerating terminals after intrinsic lesions included marked gliosis around the terminal (Figs. 172,174-176,178,181-183,185-188), glycogen-like granules and vacuolation within the terminal. The fate of the post-synaptic specialisations after intrinsic lesions has been considered in Chapter 7, as has the degeneration of olfactory nerves after intrinsic lesions; we will therefore confine ourselves to

describing the degeneration process in terminals with a periglomerular distribution.

Terminals with asymmetrical thickenings

These terminals, associated in normal material with spherical vesicles (Figs. 175,176), are derived from the recurrent collateral axons of the tufted cells and from the centrifugal fibres (Chapter 5). Although it is not possible to make a definite distinction between these two types of fibres in all cases, they do show a slightly different morphology and time course during degeneration, and their distribution appears on these grounds to be different; the identity of the two types of degenerating terminals with asymmetrical thickenings was deduced on a numerical basis - it is known from material with lesions in the lateral olfactory tract, in which the whole centrifugal pathway is interrupted, that the centrifugal fibre terminals are much rarer than the axon terminals with asymmetrical thickenings that do not degenerate (the tufted cell axon collaterals). The centrifugal fibres (Fig. 178) affected by intrinsic lesions have a more restricted distribution than the tufted cell collaterals - between two and four glomeruli from the lesion - and do not appear to degenerate caudal to the lesion. This suggests, along with light microscopic evidence, a fibre course roughly perpendicular to the surface and a terminal distribution running anteriorly and to either side. The axon collateral degeneration is particularly distinguished at shorter survival times on account of a marked swelling and pallor of the terminals (Figs. 172,173,177,185) -

a stage not typically seen during centrifugal fibre degeneration after these lesions or those in the lateral olfactory tract (Chapter 9). Subsequently, these terminals show a characteristic sequence of darkening and shrinkage as shown in Figs. 174-176,179 & 180. The distribution of collateral degeneration is the widest of all in the periglomerular region and degenerating terminals are seen in the periglomerular region, commonly up to six glomeruli distant from the lesion, and rather infrequently up to ten or twelve; an occasional degenerating terminal with an asymmetrical thickening could be found beyond this, but these were not a significant number in any brain. The pattern of degeneration appeared to be similar in all directions from the lesion; fibre degeneration could be observed in the periglomerular region and superficial part of the external plexiform layer up to about ten glomeruli from the lesion.

In terms of post-synaptic cells and processes, degenerating tufted cell collaterals were found on short-axon dendrites (Fig. 177), spines and somata (Fig. 179) and the dendrites and appendages of periglomerular cells (Figs. 173-175,180,185); degenerating centrifugal terminals could be definitely identified in this material on periglomerular dendrites and appendages (Fig. 178), and possibly on to short-axon cell processes. Such a distribution is in accord with the direct evidence from material after lateral olfactory tract lesions and with the reasoning based on studies of normal material. Moreover, the width of collateral distribution is in agreement with the estimates obtained from Golgi-impregnation studies (Cajal, 1911; Blanes, 1898; Valverde, 1965; Chapter 3).

Fig. 172 Early degeneration of a tufted cell collateral terminating onto the spine of a periglomerular or short-axon cell; note the swollen vesicles and pallor of the cytoplasm. 3 glomeruli from lesion. 5 days survival.

X66,900

Fig. 173 Early degeneration of tufted cell collateral terminating onto two periglomerular cell spines or gemmules, note the swelling and crowded appearance of the vesicles in the degenerating terminal. 4 glomeruli from lesion. 5 days survival.

X46,000

Fig. 174 Early degeneration of a tufted cell collateral terminal, synapsing onto a periglomerular cell appendage; marked gliosis and early terminal darkening. 3 glomeruli from lesion. 4 days survival.

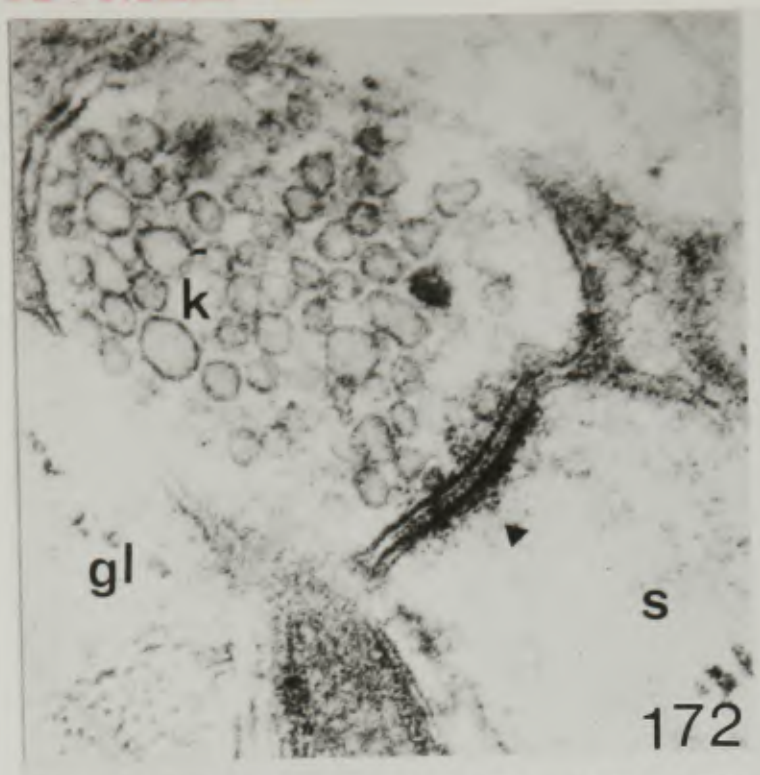
X53,400

Fig. 175 Degenerating tufted cell collateral terminal, showing swollen vesicles and darkening cytoplasm with some gliosis. Note the adjacent normal terminal; post-synaptic processes are from periglomerular cells. 5 glomeruli from lesion. 5 days survival.

X36,000

Fig. 176 Degenerating tufted cell collateral terminal, showing dark granular cytoplasm with indistinct vesicles and an irregular outline; a normal terminal lies nearby. 4 glomeruli from lesion. 5 days survival.

X51,400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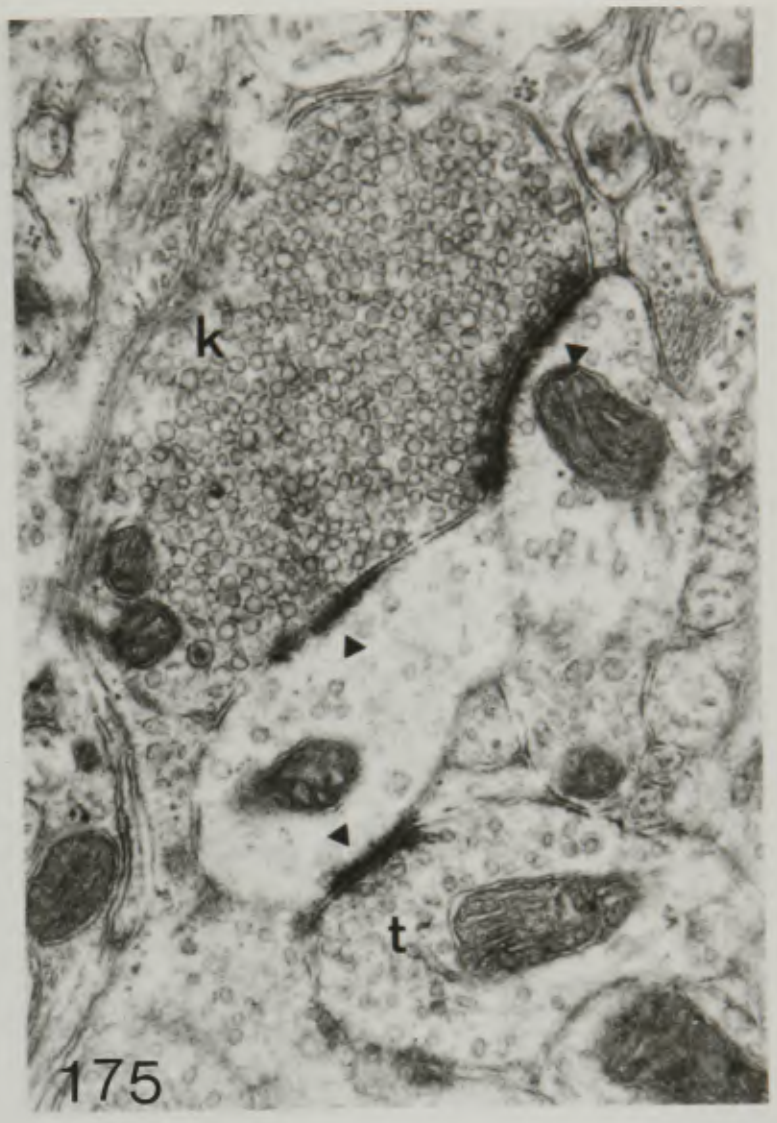
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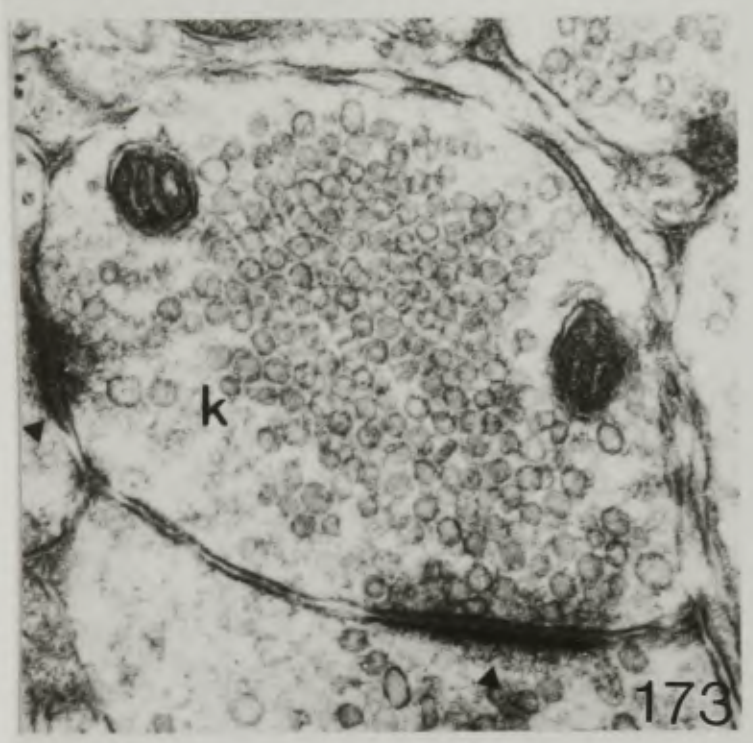
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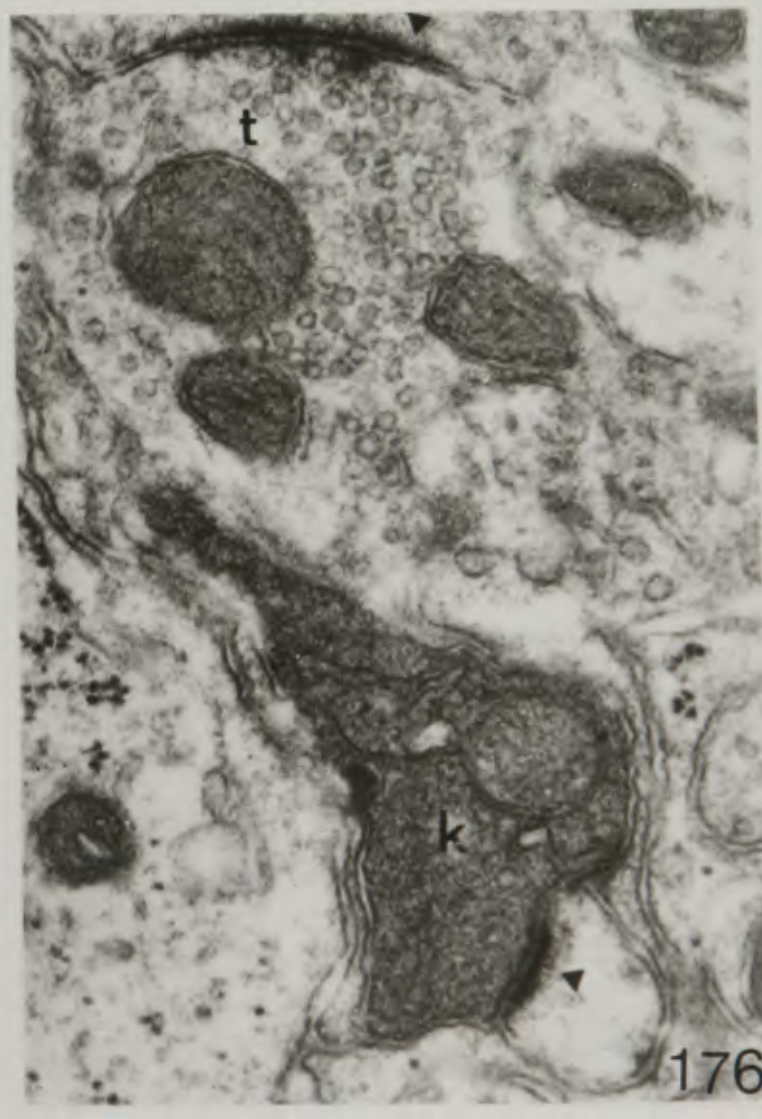
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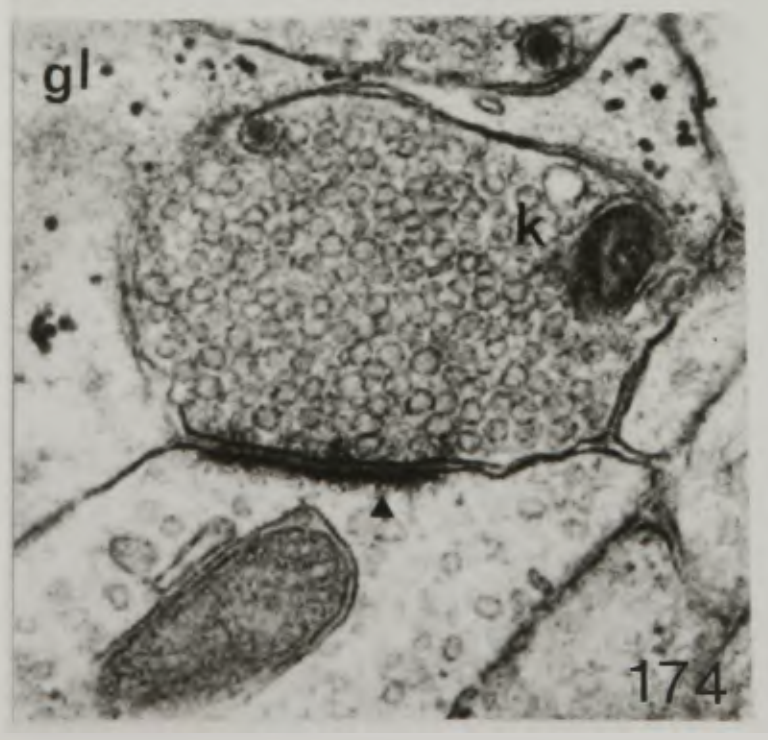
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Fig. 177 Early degeneration of a tufted cell collateral, which is terminating onto a varicose short-axon cell dendrite; note that the thickening is asymmetrical where it is cut in the plane of the synapse. 3 glomeruli from lesion. 5 days survival.

X53,400

Fig. 178 Degenerating terminal, probably centrifugal by virtue of its site and type of degeneration. 2 glomeruli from lesion. 5 days survival.

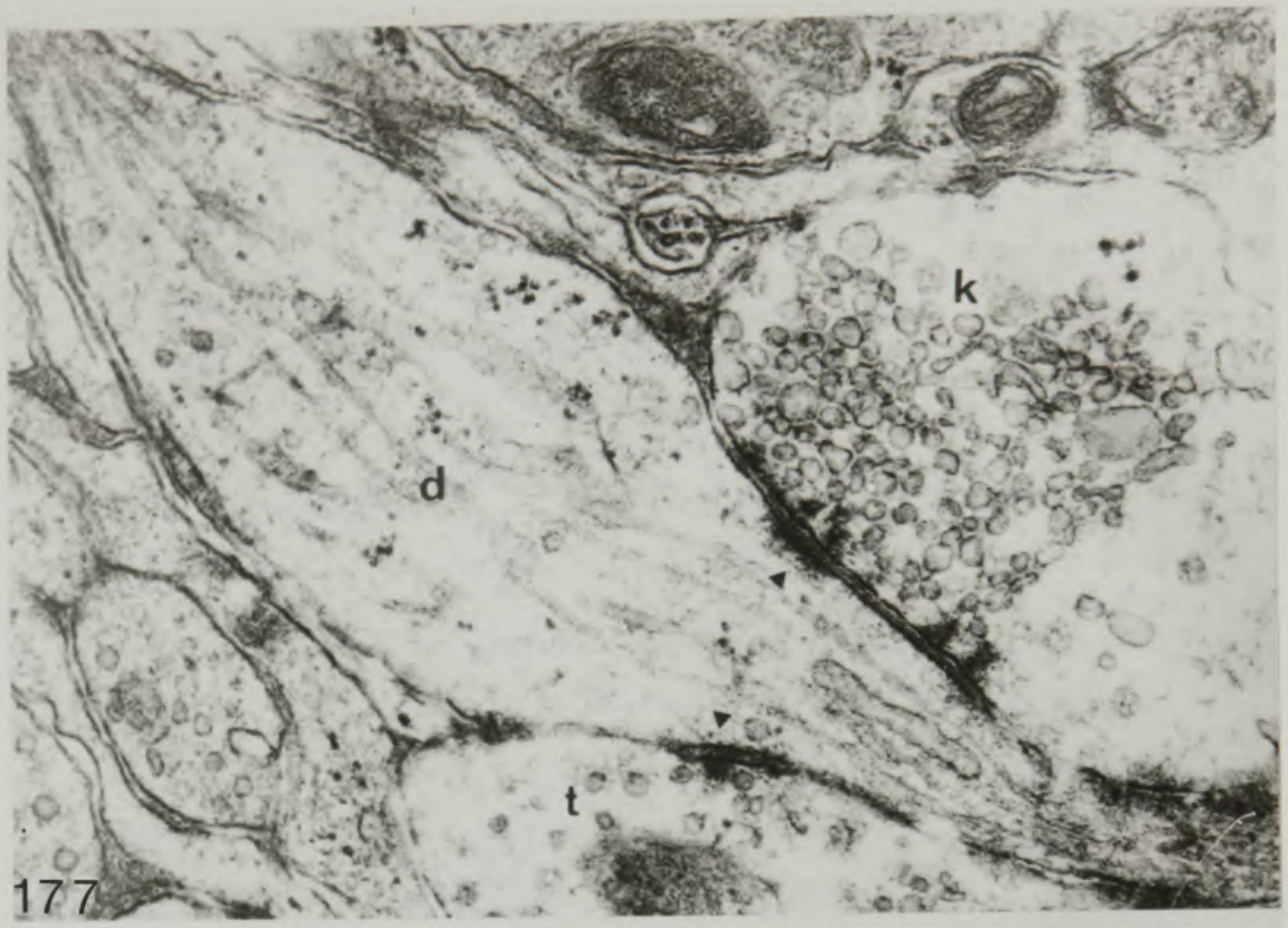
X53,400

Fig. 179 Degenerating collateral of tufted cell terminating on a short-axon cell soma; note the swollen vesicles, dark cytoplasm with fragmented material. 4 glomeruli from lesion. 4 days survival.

X61,100

Fig. 180 Degenerating terminal, probably the collateral of a tufted cell, terminating on a periglomerular cell dendritic shaft; note the swollen vesicles and dense fragmented mass in the terminal. 6 glomeruli from lesion. 5 days survival.

X40,000



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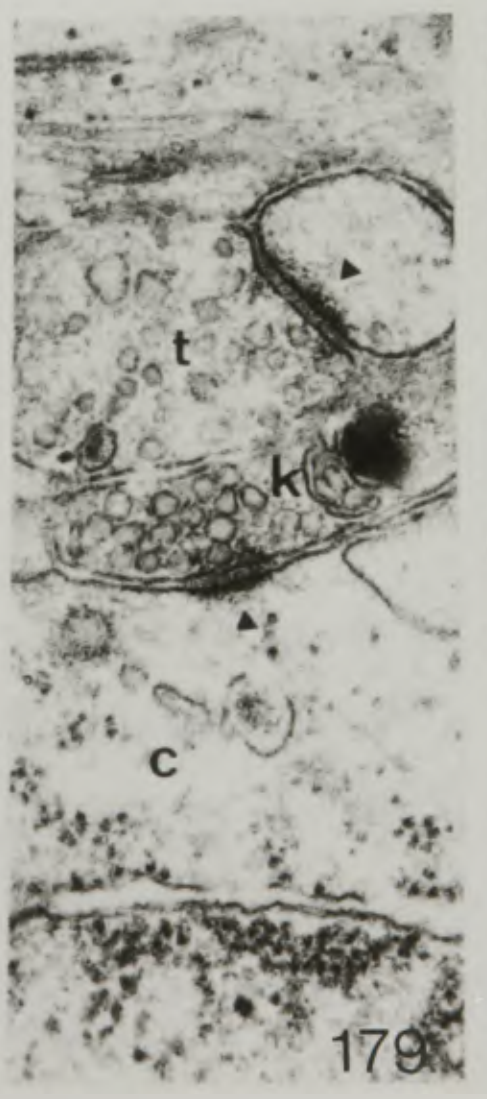

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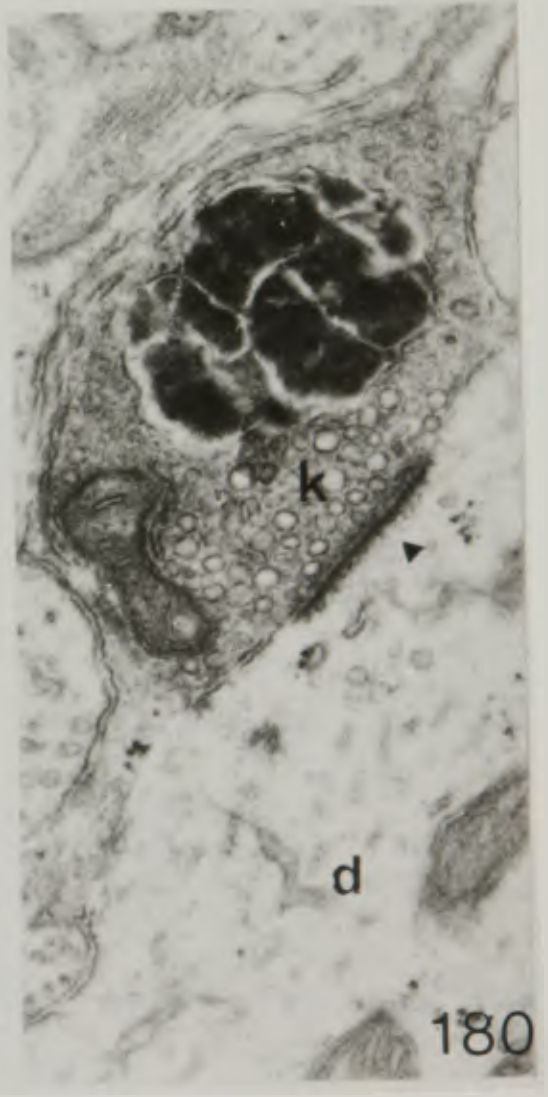

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Terminals with symmetrical thickenings

The axon terminals in this group are those of the periglomerular cells, associated with large flattened presynaptic vesicles in normal material, and those of the superficial short-axon cells, associated with small flattened vesicles. As can be seen in the matrix of synaptic connections given in Chapter 5, the only striking difference between the axon terminals of periglomerular and superficial short-axon cells is the termination of the former on to the shafts of mitral and tufted cells; other sites are common to the two groups and differences are liminal (the relative infrequency of short-axon cell initial segments makes this site of termination valueless for distinguishing these terminals). It is not at present possible to make a firm distinction between the two types of terminal on the basis of their morphology during degeneration: both types undergo a certain amount of terminal swelling and show pallor in the early stages (Figs. 181,182,186); later both darken and shrink and lose their cytoplasmic detail (Figs. 183,185,187,188).

Degeneration of terminals with symmetrical membrane thickenings (which only occurs after such intrinsic lesions) is densest around the two or three glomeruli adjacent to the lesion on all sides, and occurs on the cell bodies (Figs. 181,186-188), dendrites (Fig. 185) and appendages of periglomerular and short-axon cells, and on the main dendritic shafts of the tufted and mitral cells (Fig. 181), close to their point of primary glomerular branching. Further from the lesion,

Fig. 181 Early degeneration of a terminal with a symmetrical synaptic thickening, synapsing onto a periglomerular cell soma, and showing swelling of vesicles and of the terminal outline. 2 glomeruli from lesion. 5 days survival.

X61,100

Fig. 182 Early degeneration of periglomerular cell axon terminal, synapsing with a symmetrical membrane thickening on the primary dendrite of a tufted/mitral cell; note the greyish cytoplasm, slightly swollen vesicles and advanced gliosis. 4 glomeruli from lesion; 4 days survival.

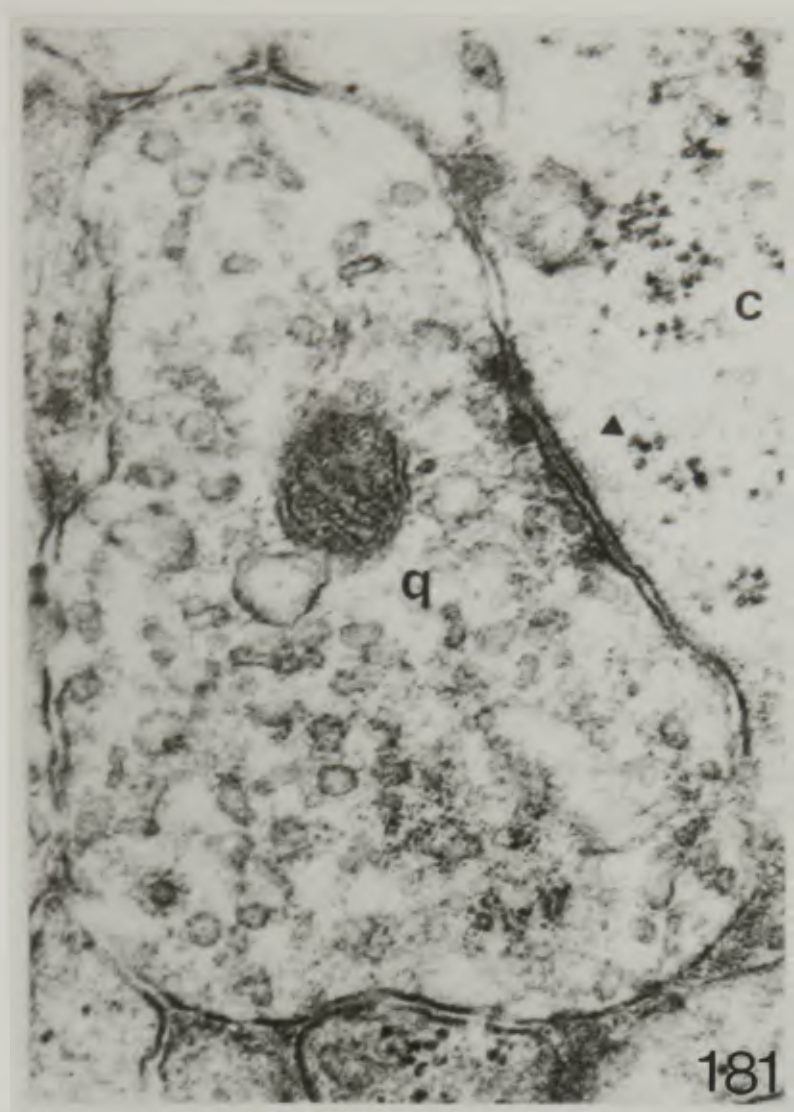
X61,100

Fig. 183 Degenerating terminal of a periglomerular cell axon, synapsing with a symmetrical thickening onto a tufted/mitral cell dendrite that itself synapses (with an asymmetrical thickening and spherical vesicles) onto another process (periglomerular cell dendrite). 2 glomeruli from lesion. 5 days survival.

X61,100

Fig. 184 Degenerating tufted or mitral cell dendrite in external plexiform layer, just deep and to one side of a lesion; observe the characteristic appearance of degeneration following recent direct damage to a cell, especially the distinguishing neurotubular 'grain' to the cytoplasm.

X47,500



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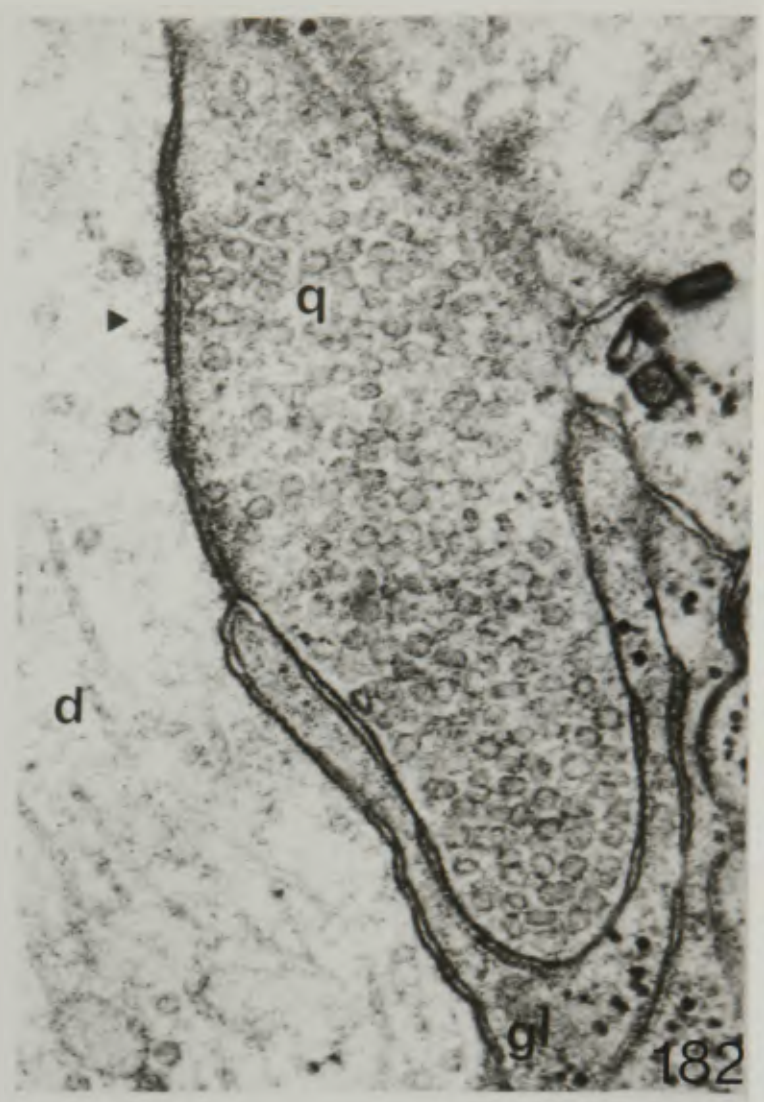

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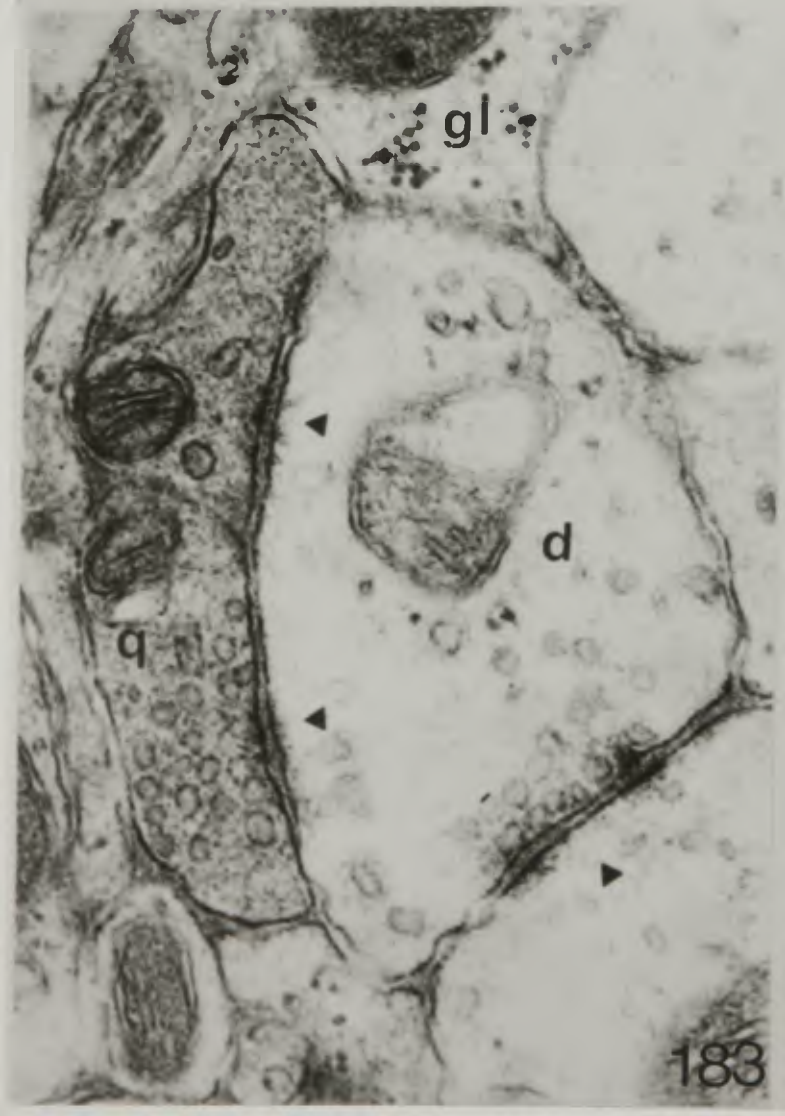

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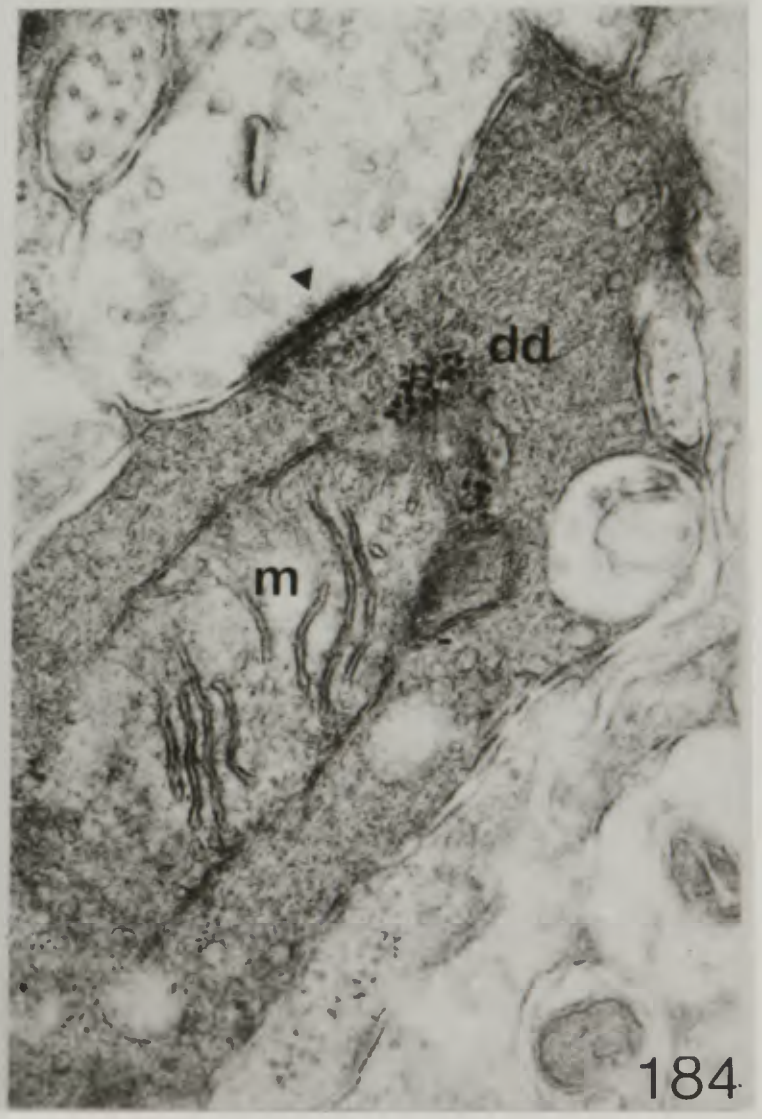

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Fig. 185 A group of degenerating terminals situated about 1 glomerulus from the lesion: the two with swollen vesicles are tufted cell axon collaterals, terminating onto the spines of periglomerular cell dendrites (one terminal is shown with asymmetrical thickening and spine in continuity with dendrite in this plane); one terminal having a symmetrical membrane thickening, shows darkening and gliosis (the post-synaptic process is the base of a periglomerular cell dendrite). 4 days survival.

X53,400

Fig. 186 A terminal synapsing with a symmetrical thickening onto a periglomerular cell soma; early degeneration, showing distortion of vesicles and gliosis. 2 glomeruli from lesion. 4 days survival.

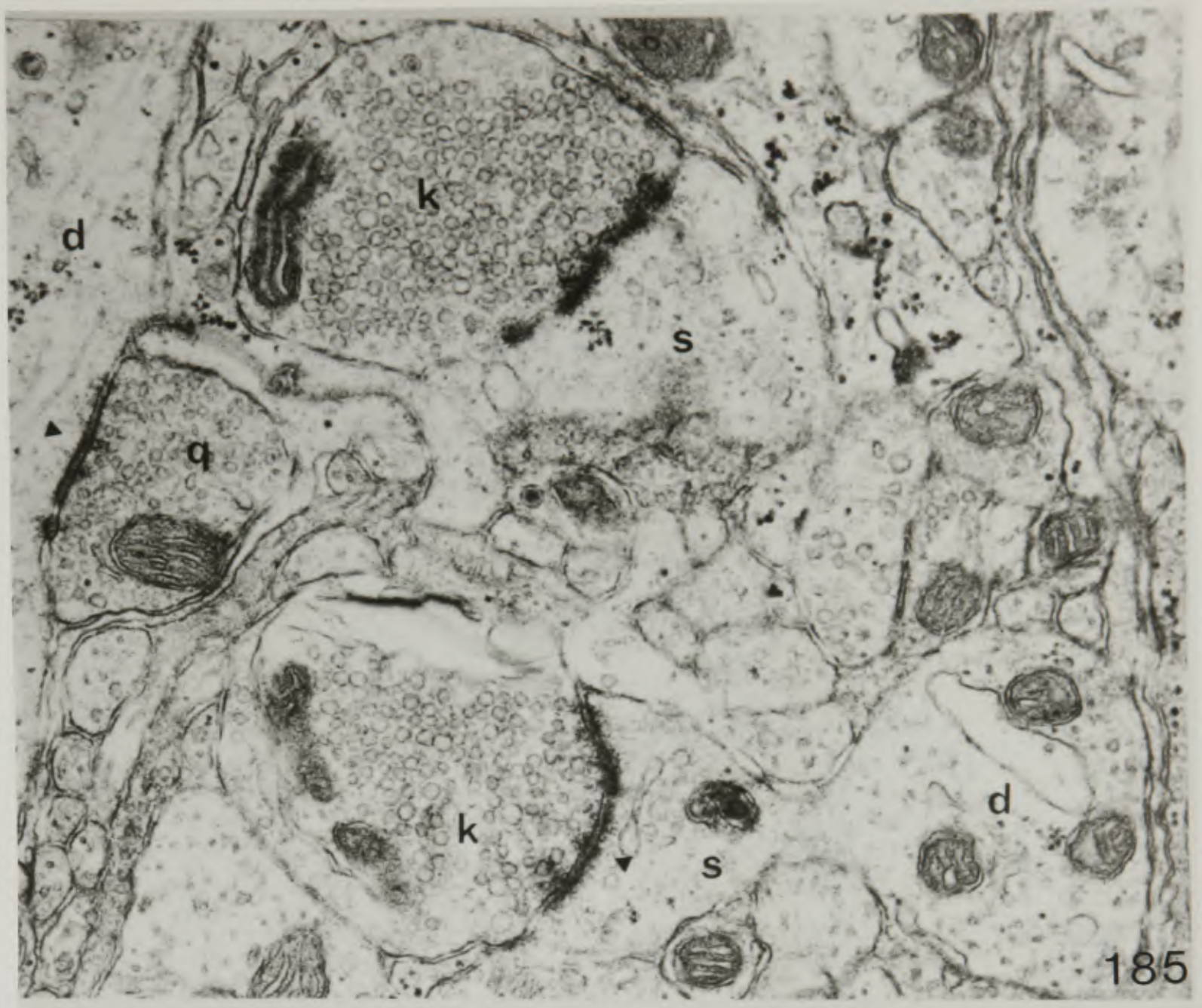
X53,400

Fig. 187 A degenerating terminal synapsing with a symmetrical thickening onto a periglomerular cell soma; observe the darkening of cytoplasm and crowding of vesicles as well as an adjacent asymmetrical thickening. 2 glomeruli from lesion. 5 days survival.

X43,000

Fig. 188 Degeneration of a terminal, synapsing with a symmetrical synaptic thickening onto a periglomerular cell soma, and showing darkening and fragmentation of cytoplasmic contents. 1 glomerulus from lesion. 4 days survival.

X39,900



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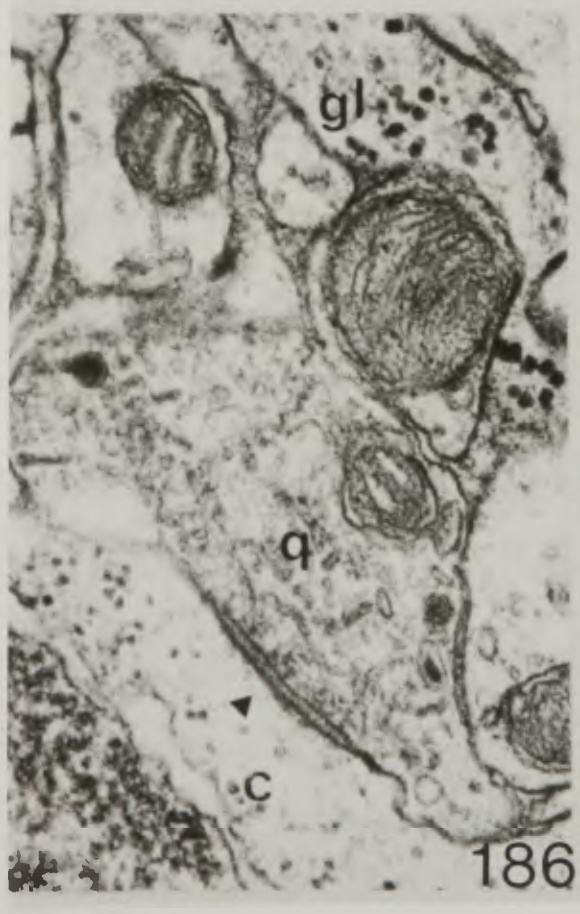

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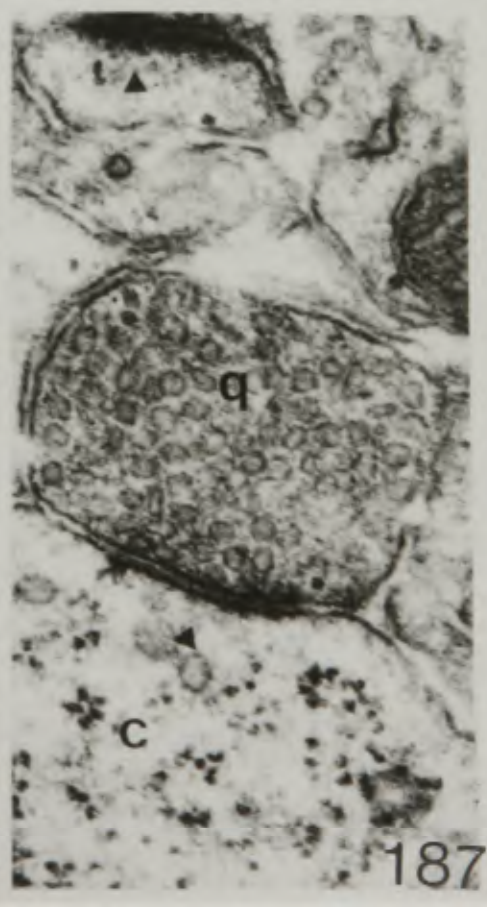

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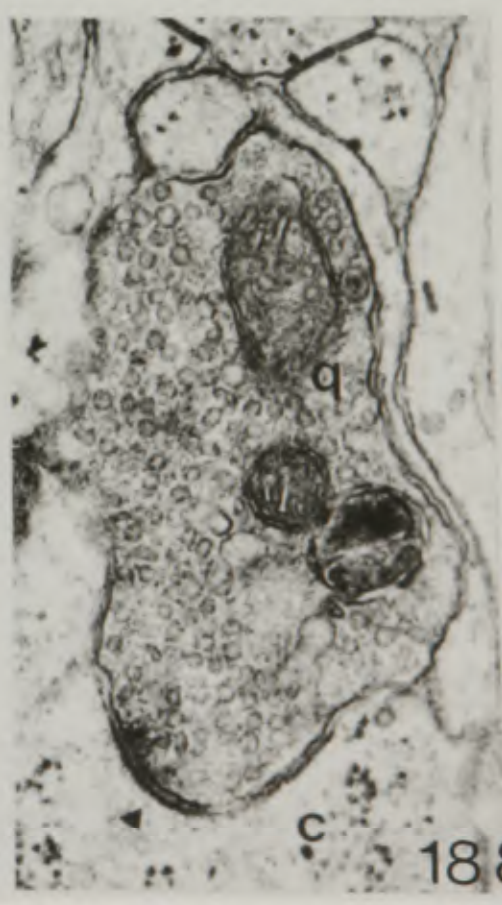

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up to five glomeruli distant, a smaller number of terminals may be found, notably still terminating on the mitral and tufted cell dendritic shafts as well as rather less frequently in the other sites. From this it may be deduced that the periglomerular cell axons extend further (five glomeruli) than those of the superficial short-axon cells (two to three glomeruli), as defined by evidence from normal material; this finding is in agreement with observations of Golgi-impregnated material (Chapter 3).

DISCUSSION

Intrinsic lesions have been made in the superficial layers of the olfactory bulb in the hope that they would provide material which is able to fulfil two major purposes: firstly, to confirm the interpretations of normal material in regard to the identity of axonal profiles; and secondly to obtain a measure of the extent of spread of the axons of cells at the glomerular level, as a background to a functional analysis of the neuronal connections. The latter observations must evidently be correlated, at the light microscopic level, with evidence from Golgi-impregnated material, whose capriciousness has led to some caution in using its results for definitive analysis; these two aims have been fulfilled in the present study. The bulb may not be entirely homogeneous in regard to its connections, and therefore it is unwise to attempt any absolute measures of spread; however, even from the study of a limited region on the dorsal aspect of the bulb, necessitated for

technical reasons, the spread of each of the various axons of the intrinsic neurons, relative to each other, may be obtained for the glomerular layer with the electron microscope. Estimates of spread are given in terms of numbers of glomeruli traversed so as to give a broad, functionally based, measure for each neuron type, but it must be emphasised that these are rough, maximum figures. Light microscopic studies on a greater sample of the bulbar surface indicate that there is little regional variation to be taken into account; it is likely, however, that the spread of olfactory nerve degeneration would be far greater after lesions in the antero-ventral bulbar surface, since they would involve many more fibres passing to distant parts of the bulb.

Accepting the limitations and provisos imposed on a study in which several types of axon have been interrupted, this method has provided evidence of the axonal distribution of the different cells in the glomerular layer of the bulb: the tufted cell collaterals extend furthest (6-12 glomeruli), periglomerular cells, less far (4-5 glomeruli) and superficial short-axon cells, least far (2-3 glomeruli); these estimates are consistent with those obtained from Golgi-impregnation (Cajal, 1911; Blanes, 1898; Valverde, 1965; Chapter 3) but provide more substantial evidence. Types of axon terminal that do not degenerate after extensive extrinsic lesions, do degenerate after intrinsic lesions, and this strongly suggests that they have an intrinsic origin. This is most evident for those terminals with symmetrical membrane thickenings, which only degenerate after intrinsic lesions, but many terminals with asymmetrical thickenings in the periglomerular region remain after large

lesions central to the bulb (Chapter 9). Thus, the ultrastructural picture obtained after intrinsic lesions is complementary to that found after extrinsic lesions; it also bears out entirely the deductions made concerning neuronal identification of terminal profiles from normal material (Chapters 3-5).

Intrinsic lesions provide a further means of distinguishing between the three neuron types at the glomerular level - in terms of the extent of their axonal spread; it also gives a clue to the functional relationships of these cells and their processes. Because of the limited spread of their dendrites, the tufted and periglomerular cells receive their major (olfactory) input in one or two glomeruli; in these glomeruli they have limited dendro-dendritic outputs, but they have more extensive <u>axonal</u> outputs (multiglomerular) - the tufted cell collaterals extending further than the axons of the periglomerular cells. The superficial short-axon cells have a relatively wide input of non-olfactory origin and since this is predominantly collateral, they probably have an even wider source of input than would appear from their dendritic tree; on the other hand their axonal output is relatively restricted, feeding exclusively on to local periglomerular cells. Clearly, the possible combinations are exceedingly complex and it would be wrong to try to work out the functional circuitry on purely anatomical distribution; however, there are strong indications from the latter for the kind of physiological role the various neurons may have.

The fact that each glomerulus, and its surrounding periglomerular region, is interconnected so extensively with neighbouring glomeruli has

wider functional implications: assuming that this mode of interconnection applies throughout the bulb, then the whole glomerular level is mutually interconnected. However this may function physiologically, it signifies that such odour specificity as reaches the olfactory bulb via the olfactory nerves must be fed in according to some overall pattern, so that these neuronal connections may have some value in terms of coding. Since it is not yet clear what kind of surround effects may result from these connections at any point around a given glomerulus, it is not possible to predict the kind of system that might underlie the olfactory input. Nevertheless, these results indicate that the level of odour specificity at olfactory nerve level may be considerably enhanced or modified in terms of other peripheral events at bulbar level; the nature of this modification depends in the first instance on the pattern of specific input to groups of neighbouring glomeruli and the way in which this signal radiates out in the various bulbar neurons.

The value of intrinsic lesions as a method for study in any region depends on several factors: the extent to which it is possible to analyse the site from normal material and experimental material with extrinsic lesions; the state of knowledge about the region from light microscopy; the feasibility of achieving suitably restricted lesions which may be located after fixation; and the possibility of making distinctions between several types of degenerating terminals in terms of morphology, time course, post-synaptic site, and spatial distribution, on the basis of the three previous factors. Naturally, each region of

the brain will have its problems and peculiarities, but the evidence obtained by this method in the olfactory bulb would indicate that, although limited in scope, it can provide information not definitively available in any other way.

CHAPTER 11

ELECTRON MICROSCOPY OF SINGLE CELLS STAINED BY GOLGI IMPREGNATION.

INTRODUCTION

This method seeks to combine the various advantages of the Golgi method and electron microscopy and to eradicate their individual disadvantages; naturally enough, the combination itself carries some of its own disadvantages, but there is no doubt that this approach is a most useful adjunct to the study of intrinsic organisation in a region. It allows a single cell, stained and identified at light microscopic level by the Golgi method, to be prepared for electron microscopy and examined in serial sections, particularly for synaptic interrelationships; in this way a direct correlation can be made between the identities and characteristics of cell types at the two distinct levels of study, which provide quite different and complementary information about the cell's properties. The stained cell is easily picked out under the electron microscope, enabling serial sections to be studied, without any doubt about whether one is still tracing the same profile. The method can be combined with experimental sectioning of axon pathways, as in the original study by Blackstad (1965).

The work of Blackstad (1965) and Stell (1965,1967) on the use and techniques for combining Golgi-impregnated material and electron microscopy in this direct manner, has been well reviewed recently (Blackstad, 1970). The present account deals with the methods and results from a study on some of the cells in the glomerular layer of the bulb, and which have been based upon the techniques already in use in this laboratory; certain of our experiences indicate some of the problems of the technique

in its present form, only some of which have been overcome. Combined Golgi-E.M. studies have also been described by Kolb (1970).

RESULTS AND DISCUSSION

Preservation of material is poor (Figs. 194-202), probably primarily on account of the fact that the silver nitrate has to be made up in distilled water to avoid precipitation; however, with a knowledge of the normal electron microscopy of the region, including profile outlines and orientations, most processes can be recognised. Knowing the definitive light-microscopic type (Figs. 189-193), its electron microscopic characteristics and relationships may be confirmed (or discovered). Synaptic structures, both pre- and post-synaptic, are easily picked out and the layer of extracellular material in the synaptic cleft may often be resolved, giving a clear indication of the extent of the synaptic specialisation (Figs. 197-202). Typing of synaptic specialisations however is not generally possible in this material. As reported by both Blackstad and Stell, the Golgi impregnation appears to be membrane-limited, occurring within the cytoplasm of cells, often sparing membrane-bound systems such as mitochondria or even neurotubules (Figs. 194,195), or else being contained within them, such as within endoplasmic reticulum. The characteristic appearance of the cytoplasm of Golgi-impregnated cells under the electron microscope is of extremely electron-dense areas, of a granular nature, surrounded by slightly less dense material; in between these areas are

empty regions of variable size (Figs. 194,195,197,201,202). The various manipulations suggested by Blackstad for preventing this loss of stain were unsuccessful in this study, so it must be concluded that it is a part of, or an inevitable consequence of the staining process itself. The rounded edges of the dense material indicate that they may be aggregations of cytoplasmic material that have taken up the silver complex.

We have not seen any staining of the extracellular space as described by Blackstad, but on the other hand we have seen similar thin stained regions, that have been identified as belonging to the thin cytoplasmic lamellae of glia by their site, arrangement and continuity with glial profiles that are also stained (Fig. 196). Like him, we have observed the sudden cessation of staining in a large neuronal profile (Fig. 195): this was the case of a tufted cell dendrite of 2-3 µm diameter, cut longitudinally and passing in the superficial part of the external plexiform layer. The stain stopped abruptly in a straight line running diagonally across the dendrite; moreover, a mitochondrion and several neurotubules passed through the line, being unstained on both sides, but only surrounded by dense material on one (Fig. 195). Such observations indicate that caution must be used in identifying and characterising cell processes in Golgi stained material. Finally, it may be noted that the technical procedures and examination needed to achieve satisfactory Golgi-E.M. material are very time-consuming and cannot at present be used for routine study; they are however of great value in confirming relationships and identities when

the groundwork from normal material has been completed.

Although studies have been made with this technique on several individual cells, the results on one cell, a middle tufted cell, will be reported here. This is partly to avoid repetition of observations that have been made on normal material with which the Golgi-E.M. results are in agreement, and partly on account of the difficulty involved in obtaining satisfactory material through a whole cell and its processes. Furthermore, the middle tufted cells are difficult to study as a group and this method provides an unusual opportunity to observe them. The cell was found in the light microscopic study of 100 µm Araldite sections, lying in the middle of the external plexiform layer, and sending four major dendritic branches peripherally and an axon deeply (Figs. 189-193); the latter divided relatively early to give a small collateral branch. One of the dendrites showed marked varicosities on light microscopy (Figs. 190,202) apparently before reaching the glomerular layer; since all the dendrites reached the glomerular layer and received contacts from olfactory nerves, this cell had no true secondary dendrites.

With the electron microscopic study of large numbers of serial sections the identity of the cell was confirmed and the distribution of processes found to correspond with the appearance on light microscopy, recorded photographically; this allowed orientation of the specimen. The cell soma and those parts of the dendrites in the external plexiform layer were found to show the reciprocal synapses characteristic of tufted cells in this site; the profiles with which they synapsed were difficult

Fig. 189 Light micrograph of a middle tufted cell, stained by Golgi impregnation, and showing a few other profiles in the surrounding neuropil. This cell was examined electron microscopically (see Figs. 194, 197-202).

X460

Figs. 190-193 Details of this cell in different focal planes.

X1150

Fig. 190 Showing one dendrite with branching and varicosities (arrow).

Fig. 191 Showing the three principal dendrites.

Fig. 192 Axon emerging from the cell soma. Note also the dendritic profile in the lower part of the field; this is the dendrite shown to cease staining suddenly in Fig. 195.

Fig. 193 The same axon as in the previous figure showing its point of branching.

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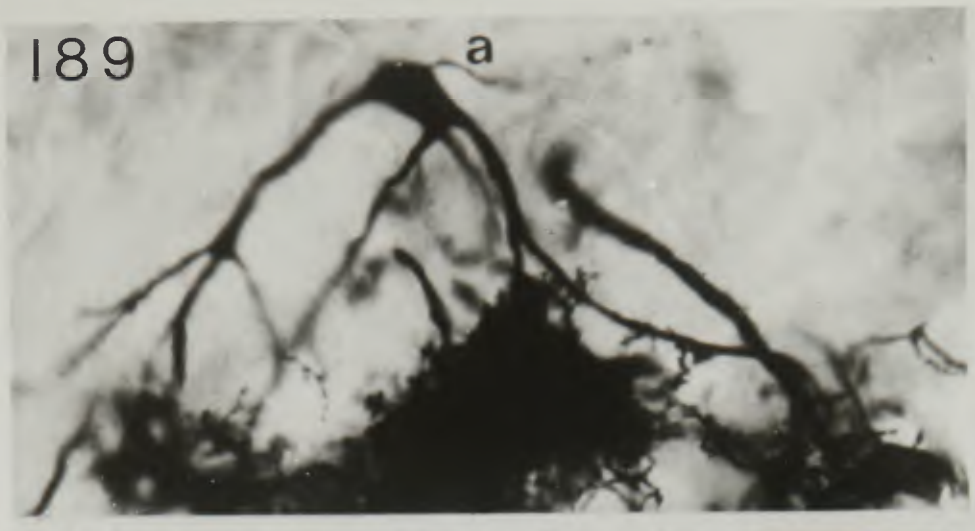

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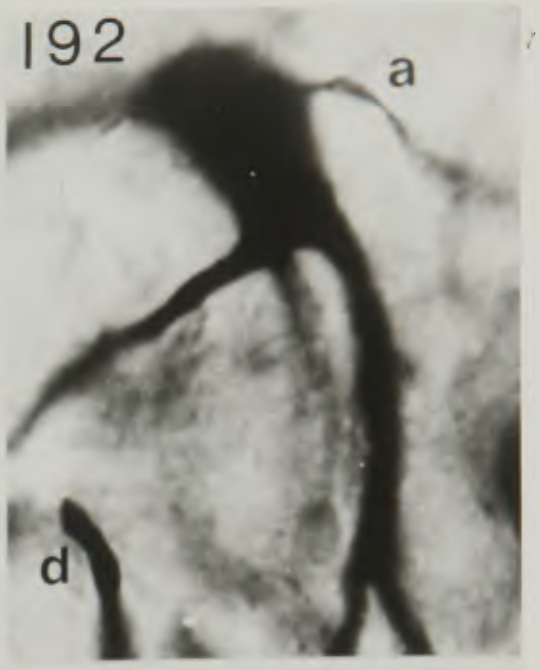

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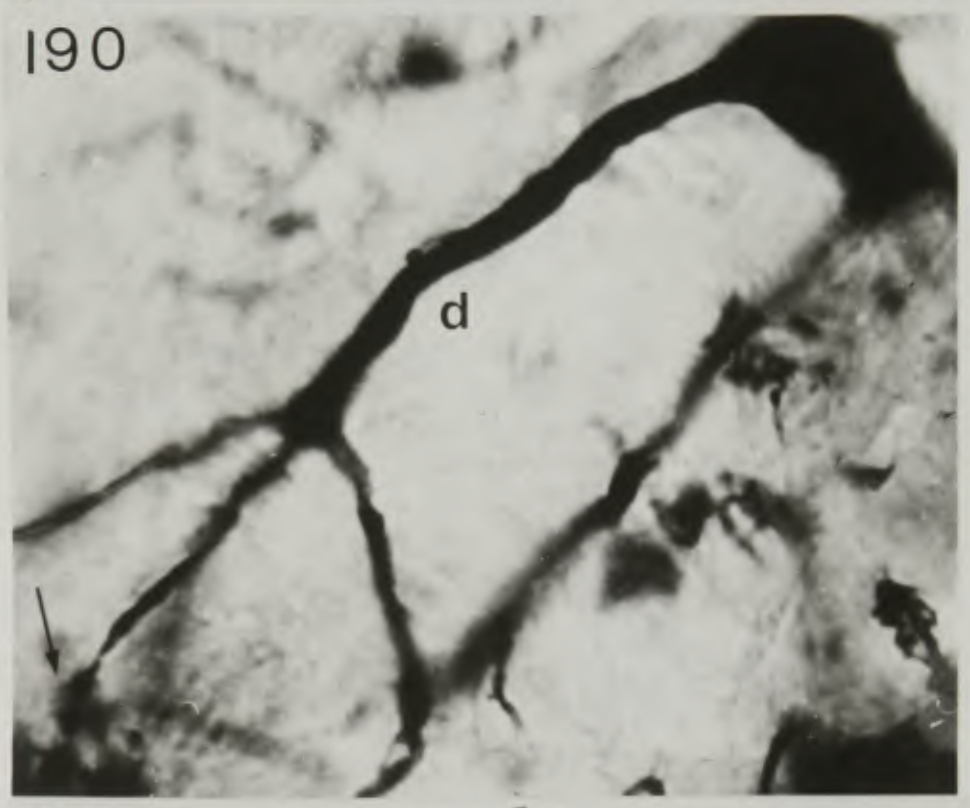

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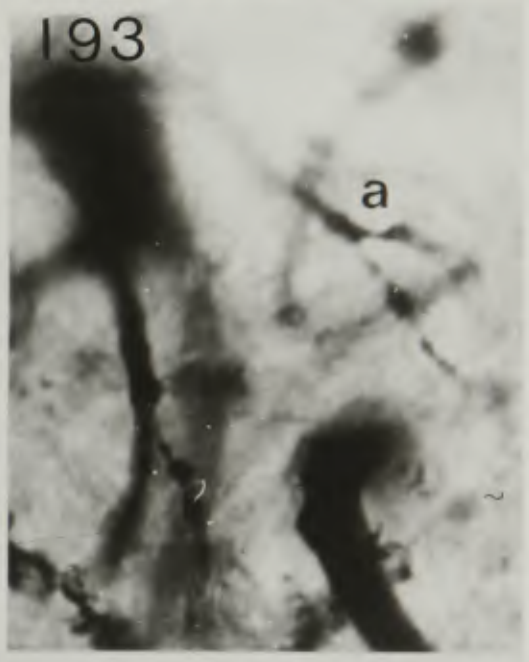

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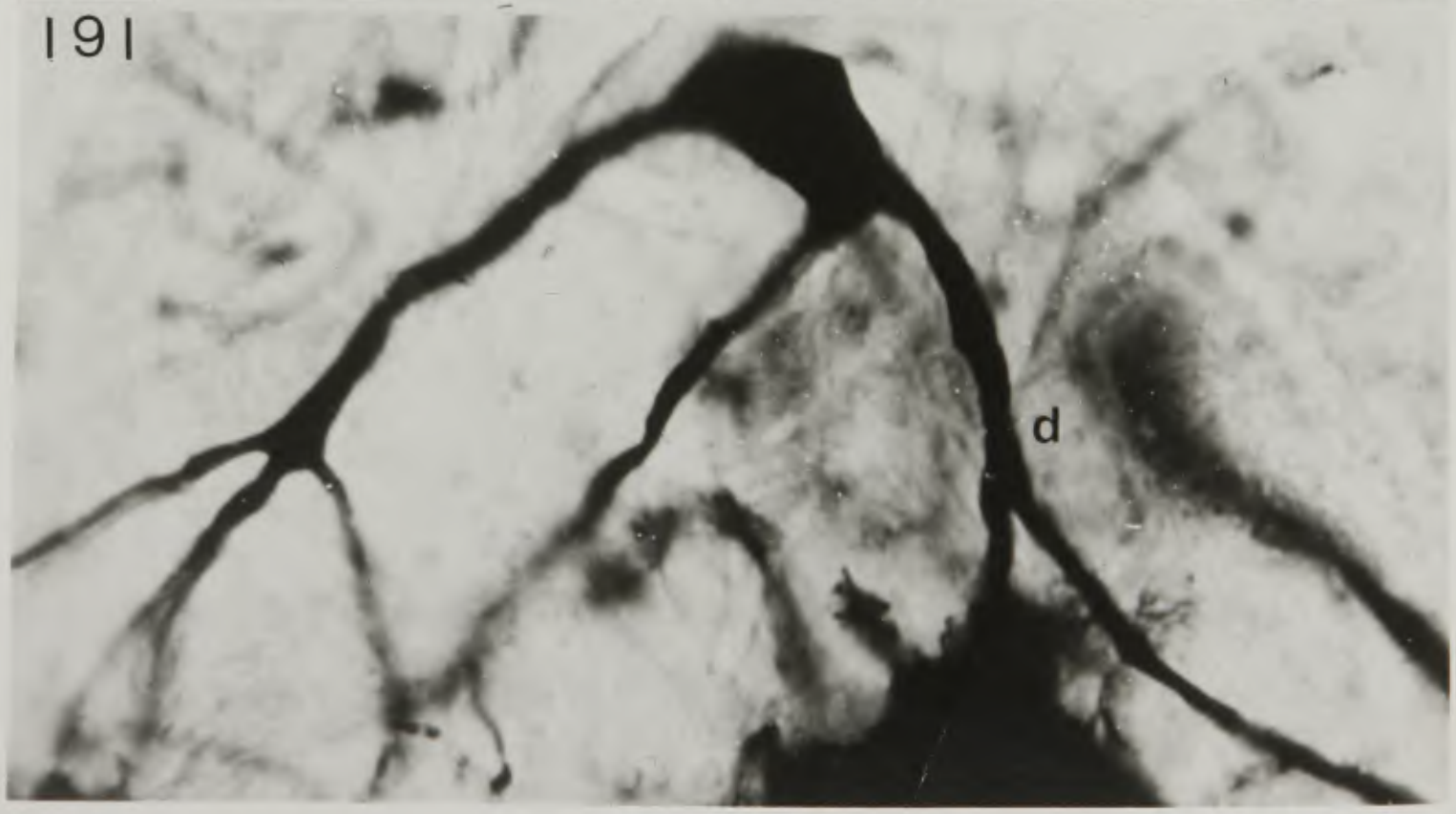


to identify on their appearance in this material, but were consistent with an identification as granule cell gemmules (Figs. 198,199,201,202) from correlation with normal electron microscopy of equivalent sites. Two of the dendritic branches terminated in one glomerulus and two in an adjacent one. It was not possible to identify periglomerular cell axon terminal contacts on these dendrites as they passed through the periglomerular region; this is because these contacts are relatively rare and can only be seen in favourable planes of section. Glial lamellae were found to surround these dendrites as they passed through the periglomerular region, however. Within the glomeruli the cell received contacts from many olfactory nerves (Fig. 200) and made large numbers of reciprocal synapses with presumed periglomerular cell profiles; the location of these synapses was entirely in accord with the results obtained from normal material on tufted and mitral cells as a group (Chapters 4 & 5). The varicose dendritic segment was noted and found to correspond with the typical varicosities identified in normal electron microscopy (Fig. 202); characteristic reciprocal synapses were found in relation to the varicose segment as well.

The axon and its collateral branch were traced through many serial sections, despite the tortuous courses they followed, which would have made conventional electron microscopy on these profiles difficult, if not impossible; in the Golgi-E.M. material, the densely stained profile stood out clearly from the unstained ones and made identification simple and the position relative to the cell soma was confirmed. No synapses were found in relation to the first part of the initial segment, but a

Fig. 194 Cell body of the middle tufted cell as seen with electron microscopy, showing nucleus, endoplasmic reticulum, mitochondria and the initial part of a dendrite. Note the large "empty" areas in nucleus and cytoplasm.

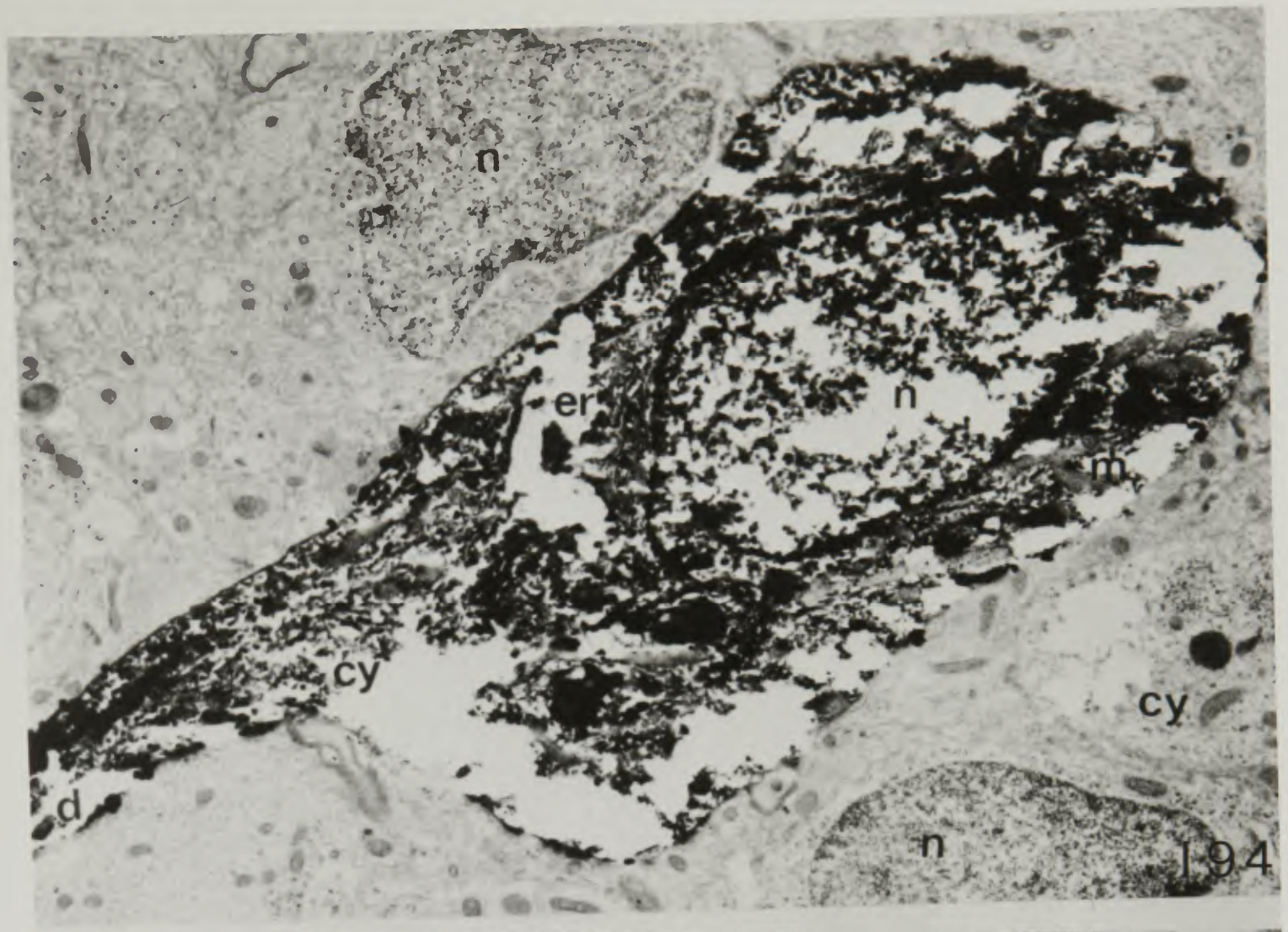
X8,100

Fig. 195 The sudden cessation of staining in a dendrite of another cell (see Fig. 192); note the rectilinear division between stained and unstained cytoplasm and the way in which a mitochondrion and the neurotubules pass across this borderline.

X39,900

Fig. 196 The glial lamellae surrounding a mitral/tufted cell dendrite, showing the way in which a single lamella may be stained by the Golgi impregnation.

X38,800



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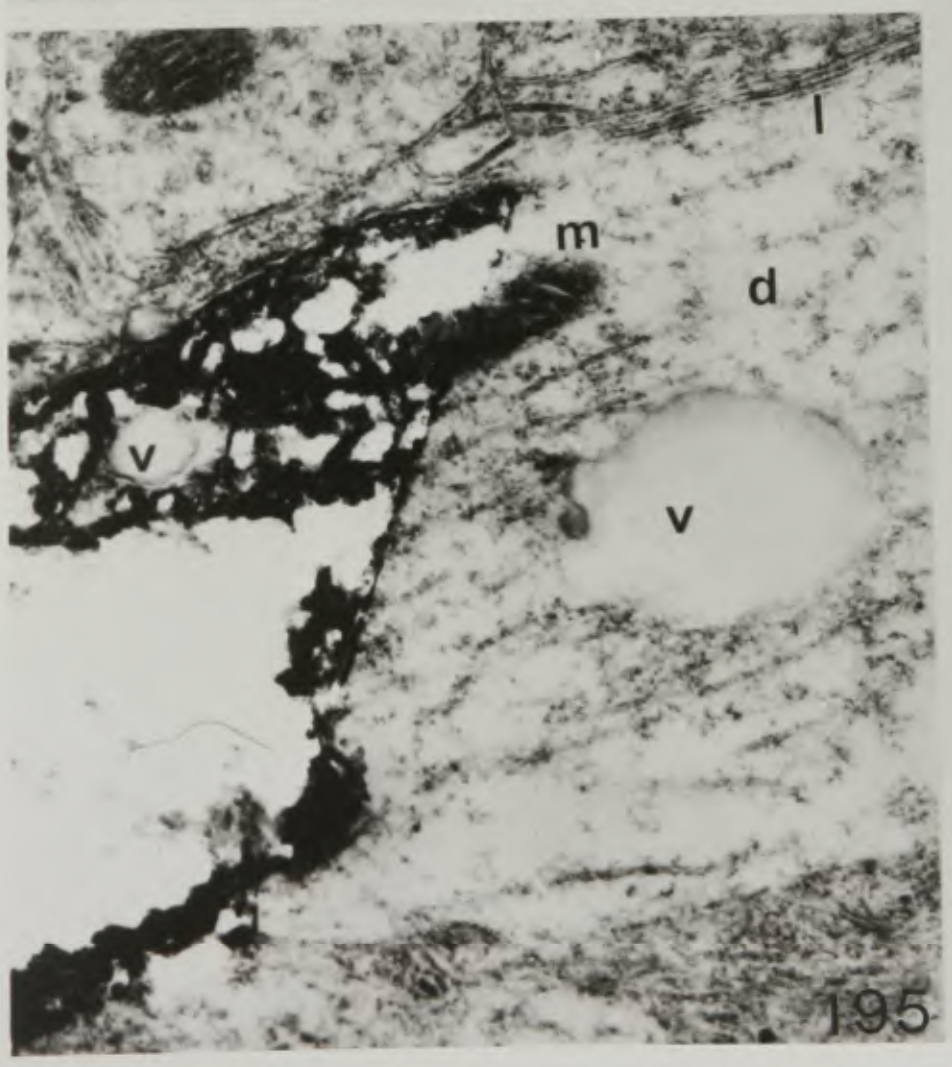

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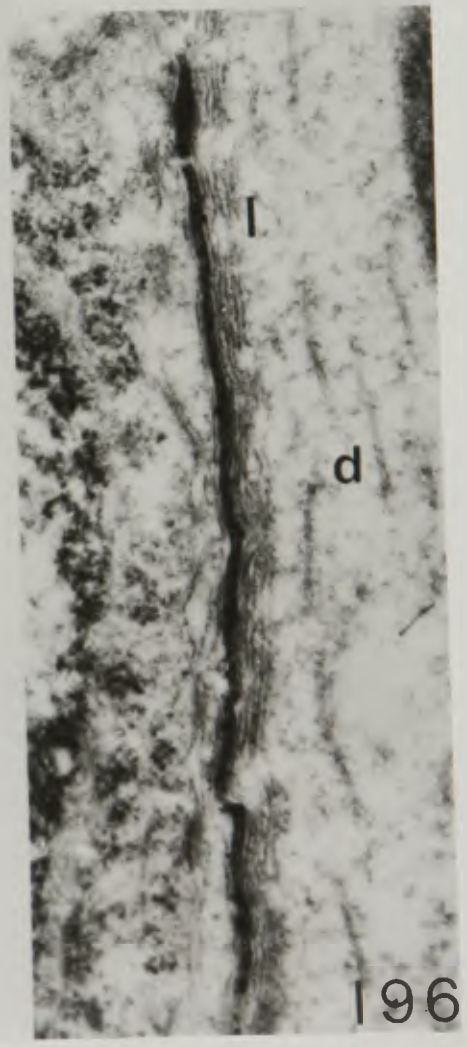

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Figs. 197-202 Synaptic specialisations and morphological appearances in the processes of the middle tufted cell under study.

Fig. 197 Reciprocal synapse between a presumed granule cell gemmule and the axon initial segment of the tufted cell.

X53,400

Fig. 198 Synapse orientated from the dendrite of the cell. Note the asymmetrical membrane thickening, and the just discernible vesicles, situated presynaptically.

X54,200

Fig. 199 Synapse, probably of the symmetrical type on the cell soma. Note the poor fixation of vesicles in unstained profiles, necessitating a careful correlation with normal material.

X53,400

Fig. 200 Dark olfactory nerve terminal synapsing onto the tip of a dendrite in a glomerulus.

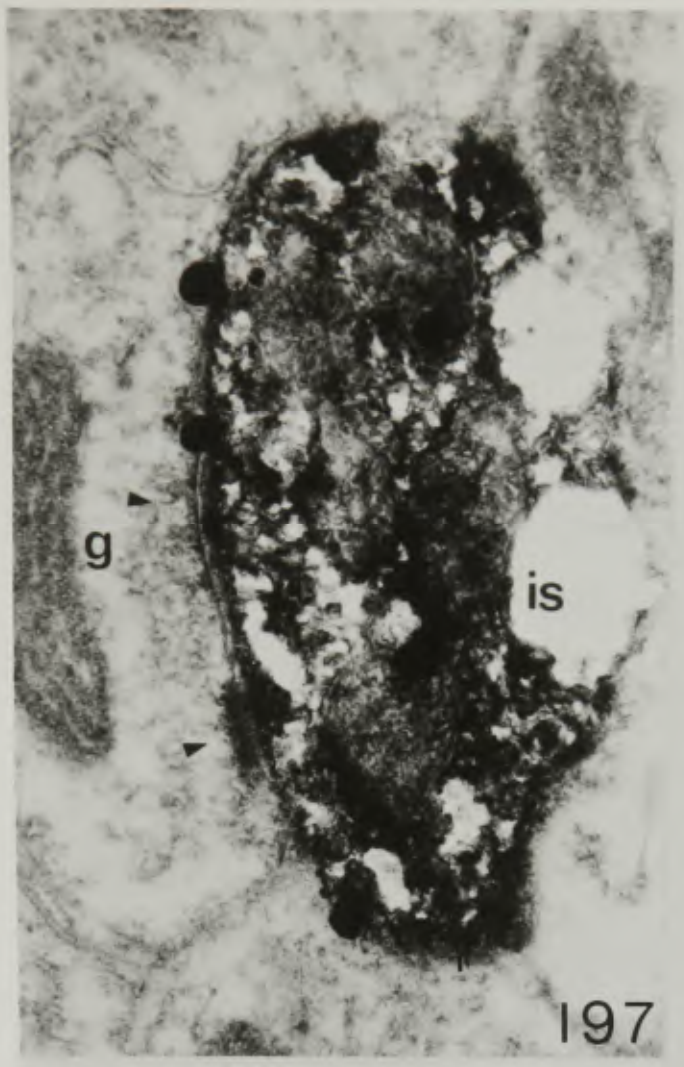
X53,400

Fig. 201 Dendrodendritic synapse from the tufted cell at a branch point; note the asymmetrical synaptic membrane thickening, but vesicles are not clearly evident.

X39,900

Fig. 202 Varicose part of dendrite corresponding to that shown in Fig. 190, with a synapse onto it from a presumed gemmule.

X53,400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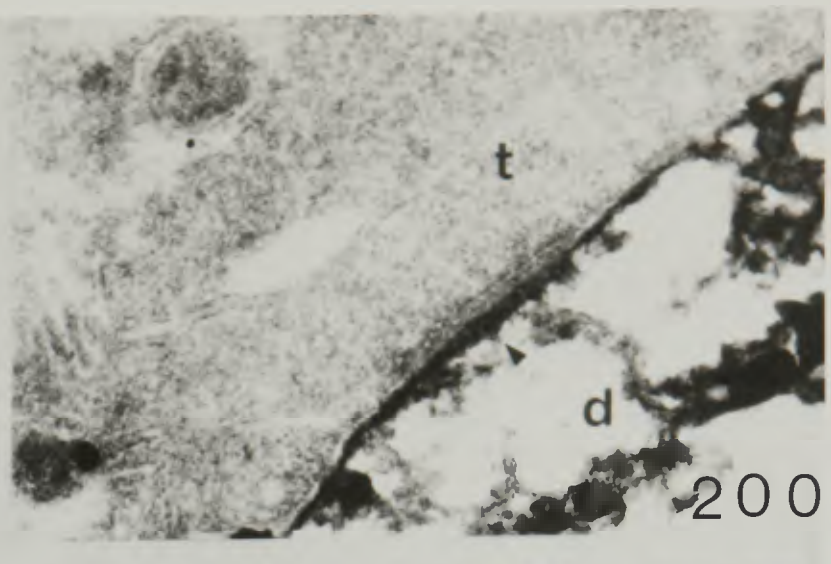
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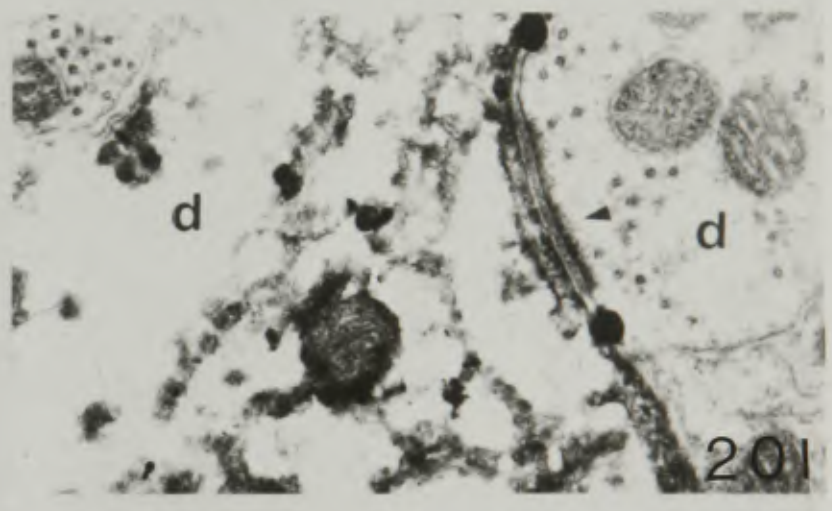
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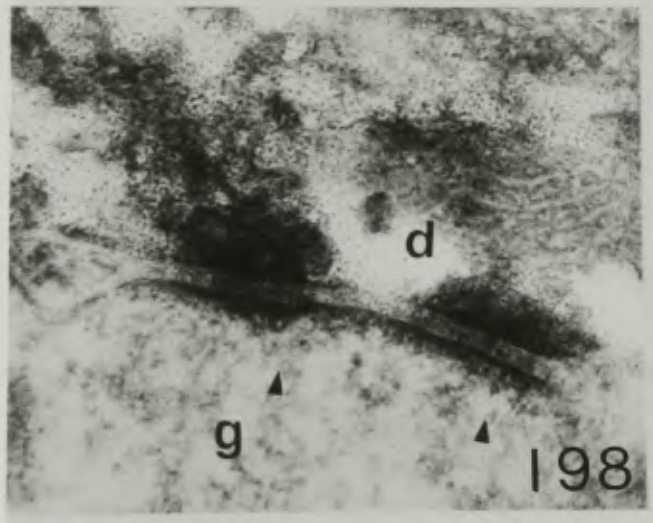
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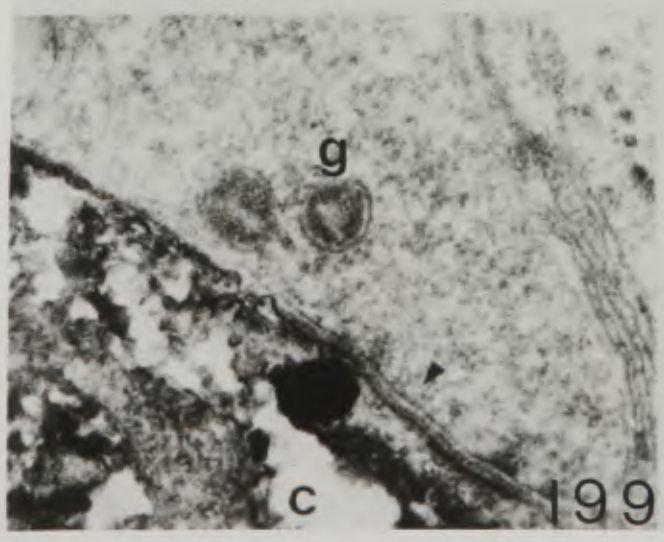
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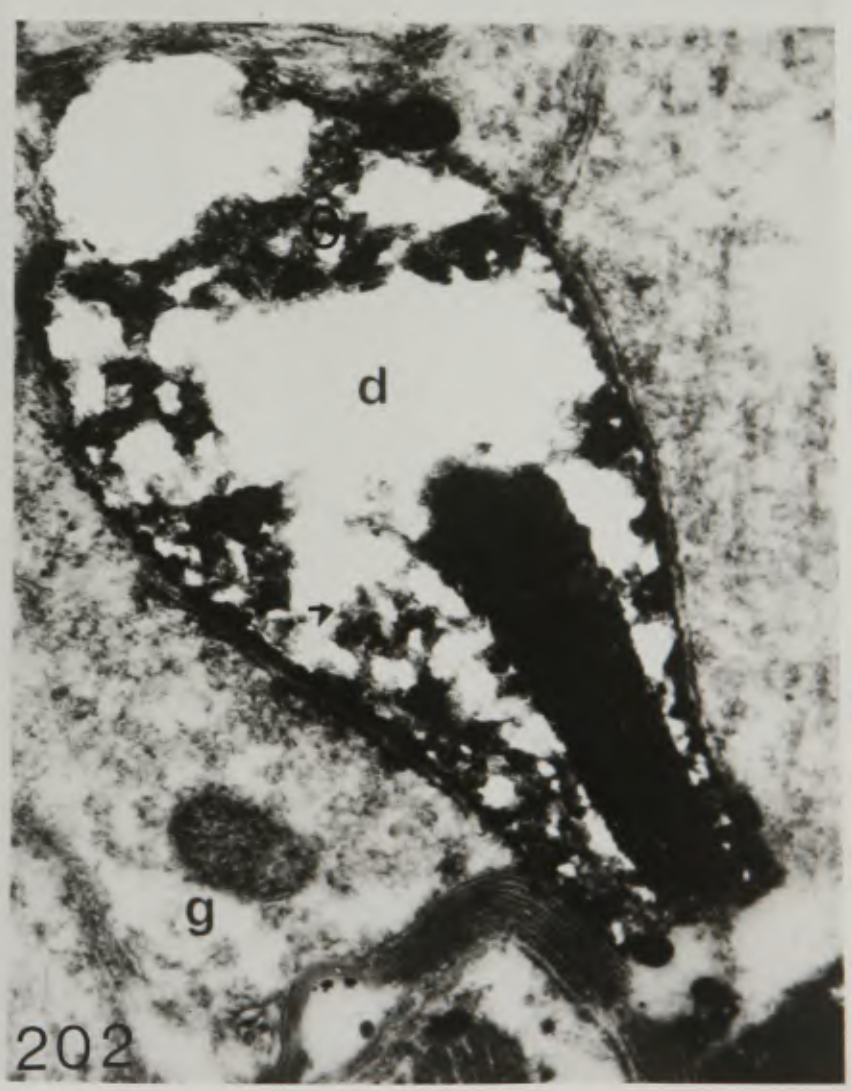
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reciprocal synapse with a presumed granule cell gemmule was found just prior to the point of branching (Fig. 197). This was a new and unexpected finding and must be presumed to be a feature characteristic of middle tufted cells, or at least of those tufted cells lying in the external plexiform layer, since no similar contacts have been found in detailed studies on the external tufted (Chapter 3) or mitral cells (Price & Powell, 1970d). This is the first definite example of combined axo-dendritic and dendro-axonic synapses in a reciprocal arrangement.

Olfactory nerves and periglomerular cells have been studied with this method; in the case of the former, single fibres have been stained, even when arising in a bundle of otherwise unstained fibres. Glial cells are often stained and are responsible for much of the finer network of apparent precipitate seen in light microscopy of Golgi-impregnated material. Very fine lamellae may be stained, as mentioned above, including single members of the group around the periglomerular segment of the relay cell dendrites; whether this indicates multicellular origin for these lamellae or simply only partial staining of one cell is uncertain. The capriciousness of the technique makes such distinctions difficult, particularly in view of the observation of the sudden cessation in staining in a thick dendritic shaft (Fig. 195).

While this technique has distinct limitations with respect to the time involved in preparation and study, the poor quality of the material and the difficulty in ensuring clear fields around the cells to be studied, it does have the great advantage of providing a direct correlation between light microscopy in a given cell. Since almost all electron

microscopy of the central nervous system is based on cell typing from light microscopy, such a method is clearly of value in drawing the two otherwise rather disparate techniques together. In many instances the cell profiles at a distance from the cell soma may be very difficult to identify as belonging to a particular type. These studies may well contribute to their identification not only directly, but also indirectly by providing criteria by which the profiles may be recognised in conventional electron microscopy. Synaptic characteristics of a single cell may be discovered and may be mapped out almost in their entirety if this is desired. Long axonal connections are clearly impossible to study by this method, but it may be useful in following the fine and tortuous axons for some distance from the soma, which is only rarely possible in conventional electron microscopy. A further use of the combined Golgi-E.M. technique would be in distinguishing between different types, derived on light microscopic criteria, on an individual or a group basis.

CHAPTER 12

GENERAL DISCUSSION

"It is useless for anatomy to establish connections if physiology does not allow us to elucidate the nature and direction of the impulses which pass through them, nor the role of the various cellular factors which are involved in the act of transmission."

Santiago Ramon y Cajal.

GENERAL DISCUSSION

In order to facilitate the discussion of the results presented in the previous chapters, a brief summary of the classical neuroanatomy of the olfactory bulb and of the present evidence from electron microscopic and other studies will be given; a section is then devoted to the enumeration of some of the data that is as yet not available. Following this, the broad principles emerging from the new knowledge concerning the bulb will be discussed under several general headings. Firstly, in terms of the ways that neurons and glia relate to each other anatomically, and some of the physiological and biophysical implications of these. Certain features of neuronal organisation will then be discussed, considering the bulb as a possible model for other nuclei. This will include a consideration of peripheral sensory pathways, especially such common features as may give some clue to similarity in the principles of the neuronal transformation of peripheral sensory information; finally, the bulb will be considered in relation to the cerebral cortex. Points that have been discussed in the individual chapters are only considered here when it is felt necessary to discuss them in a broader context or to extract different features.

CLASSICAL HISTOLOGY

Early histologists defined the layers of the olfactory bulb according to their characteristic differences in texture, which are indeed as distinct as any in the central nervous system. The most

commonly adopted system (Schwalbe, 1881, but using present terminology), gives the layers from superficial to deep thus: the olfactory nerve layer, the glomerular layer, the external plexiform layer, the mitral cell layer, the internal plexiform layer, the granule cell layer and the periventricular layer (Figure A). The advent of Golgi's technique for staining whole cells resulted in a tremendous advance in the understanding of the neurons of the olfactory bulb; although several distinguished neuroanatomists studied this site, it is the writings of Cajal and his group that have exerted the strongest influence on subsequent work; it is also the findings of this group that have been most extensively confirmed by different techniques.

The olfactory receptor cells, lying in the nasal epithelium, have thick dendritic processes that extend up to the surface and from their bases arise fine unmyelinated axons that segregate into bundles and pass, deep to the olfactory epithelium, through the cribriform plate of the ethmoid bone to form the outer layer of the olfactory bulb which overlies this bone. These axons terminate exclusively within the glomeruli, which lie just deep to the olfactory nerve layer (Figure A). Five distinct types of neuron were described in the bulb from studies with the Golgi method: mitral cells and tufted cells, periglomerular and granule cells, and short-axon cells (which lie in the granule cell layer). The mitral cells are the largest cells in the bulb and form a distinct layer, one cell thick in the rat and rabbit; the dendrites of these cells extend superficially - primary dendrites passing into the glomeruli in which they form characteristic tufts, and secondary dendrites

passing obliquely into the external plexiform layer only, and which do not arborise. Similarly the tufted cells, which are found at all levels between the mitral cell layer and the olfactory nerve layer, have primary dendrites extending into the glomeruli as tufted arborisations; some of the deeper tufted cells have secondary dendrites. Both these two cell types have long axons projecting to more central olfactory levels and they give off collaterals in the bulb in the internal plexiform layer to terminate in this, the granule cell and external plexiform layers; the tufted cells also send collateral branches to the glomerular layer. The granule cell layer consists principally of concentric rows of granule cells, which are unusual cells in that they have no axon; they have several deep dendrites lying in the granule cell layer and usually one branched peripheral process. The latter extends into the external plexiform layer, but does not reach the glomerular layer; both deep and superficial processes bear many spine-like appendages. The superficial counterparts of these cells, now termed the periglomerular cells, have glomerular arborisations similar to, but smaller than, those of the mitral and tufted cells, and they bear spine-like appendages; the periglomerular cells also have fine axons that run in, or just deep to, the glomerular layer. The short-axon cells that lie in the granule cell layer are rarer cells of intermediate size with dendrites restricted to the granule cell layer and axons distributing variously to the granule cell and external plexiform layers; several sub-types of short axon cell were described by Cajal and others according to dendritic and axonal distribution and

the presence or absence of spines (Cajal, 1955; Price & Powell, 1970d).

Broadly speaking, this scheme for the cells of the olfactory bulb has remained unchanged and without significant addition until the advent of electron microscopy in the last decade. Valverde (1965) included some excellent descriptions of the various cell types, but introduced the concept that tufted cells were a deeply placed form of periglomerular cells, a suggestion that has been completely invalidated by electron microscopic study. Silver degeneration techniques have enabled more detailed study of the projection pathways to and from the olfactory bulb (Cragg, 1962; Powell, Cowan and Raisman, 1965; Heimer, 1968; Lohman & Mentinck, 1969; Price & Powell, 1970e); the latter studies have been done in direct correlation with electron microscopic studies (Price & Powell, 1970c). The basic findings are that the tufted and mitral cells both project in the lateral olfactory tract to the pyriform cortex and other olfactory nuclei. Fibres pass to the bulb from the nucleus of the horizontal limb of the diagonal band and the ipsilateral anterior olfactory nucleus, as well as from the contra-lateral anterior olfactory nucleus via the anterior commissure.

ULTRASTRUCTURAL FINDINGS

The contribution of the electron microscope in recent years to our knowledge of the neuronal organisation within the olfactory bulb has been of paramount importance in that this is the only technique available by which definitive information may be obtained concerning the synaptic connections between different neurons. The following brief

summary of connections (Figs. 203,204; see also Table I, Chapter 5) within the olfactory bulb is derived from the various electron microscopic studies or normal and experimental material, which are broadly in agreement (Hirata, 1964; Andres, 1965; Reese & Brightman, 1965; Rall, Shepherd, Reese & Brightman, 1966; Price, 1968; Price & Powell, 1970a-d; Hinds, 1970; Reese & Brightman, 1970; Chapters 3-11). The detailed information on the identification of cells and processes and on their interconnections may be found in the original papers and only certain salient features will be brought out in this review, based upon the assumption that it is possible to distinguish the different cellular processes and relate them to the light microscopic types. It should be noted that by a combination of light microscopy and electron microscopy an additional cell type was discovered in the glomerular layer, the superficial short-axon cell; this has been characterised (Chapters 3 & 5) and seems in many ways to be the superficial analogue of the deep short-axon cells.

The relay cells - the mitral and tufted cells - whose features and connections are broadly similar, are the central elements in the neuronal organisation of this relay level in the olfactory system; they receive the primary olfactory input on to their most peripheral dendritic processes, the glomerular arborisations (Fig. 203). This input is, on morphological criteria (spherical vesicles and asymmetrical membrane thickening), excitatory (see below), and is, with rare exceptions, the only input of this type upon the relay cells. The long dendrites of the mitral and tufted cells are then subjected to influences

Fig. 203 A schematic diagram of the functional interactions in the glomerular layer of the rat olfactory bulb. The mitral, tufted and periglomerular cell glomerular arborisations are shown as quadrants of the glomerular circles; it should be remembered that many mitral, tufted and periglomerular cells contribute their dendritic arborisations to each glomerulus.

A SCHEMATIC DIAGRAM OF THE FUNCTIONAL INTERACTIONS IN THE GLOMERULAR LAYER.

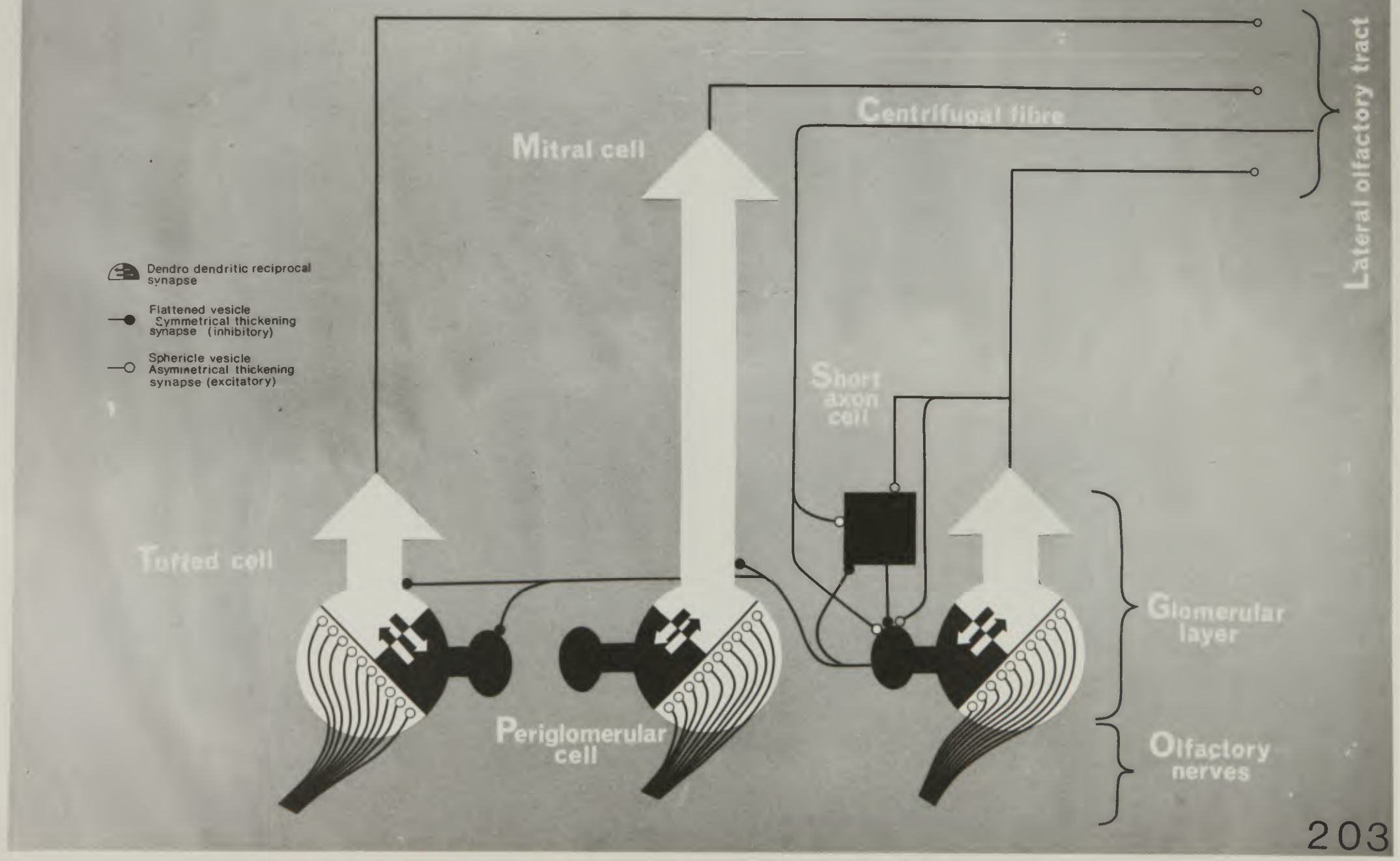
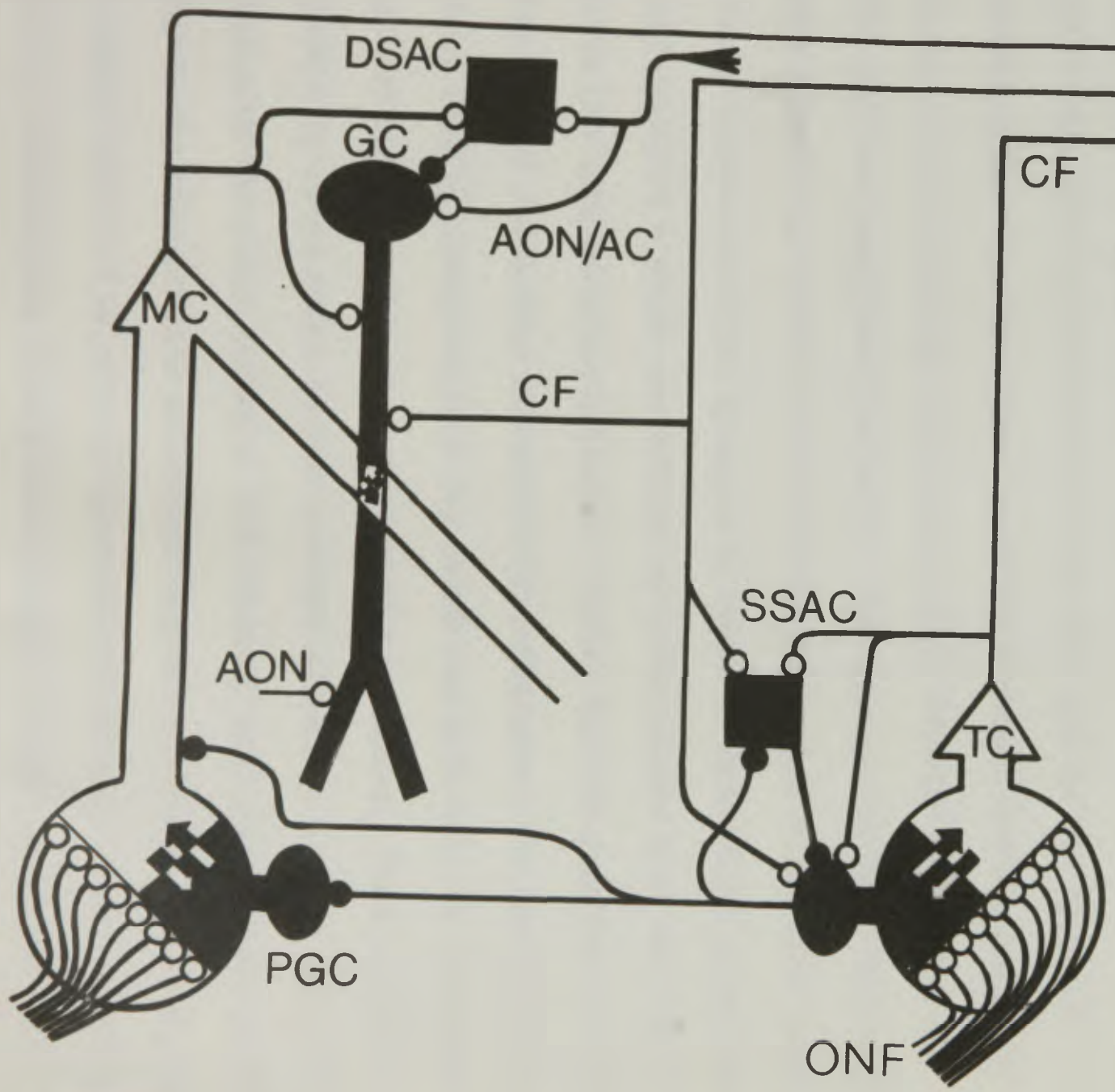


Fig. 204 A schematic diagram of the principal synaptic connections in the olfactory bulb of the rat. The glomerular arborisations of mitral, tufted and periglomerular cells are shown as quadrants of the circular glomeruli; it should be remembered however that many mitral, tufted and periglomerular cells participate in each glomerulus and that these and the other relationships in this diagram simply represent the types of synaptic connection occurring between the different types of neuron. FV/AT = flattened vesicles and asymmetrical thickening; SV/AT = spherical vesicles and asymmetrical thickening.



= Dendro-dendritic reciprocal synapses.

= FV/ST terminals (inhibitory)

= SV/AT terminals (excitatory)

LOT = lateral olfactory tract.

Axon Terminals:

AON = Anterior olfactory nucleus.

AC = Anterior commissure.

CF = Centrifugal fibre.

ONF = Olfactory nerve fibres.

Cells: TC = Tufted; MC = Mitral;

PGC = Periglomerular; GC = Granule;

SAC = Short-axon cell

(D = deep; S = superficial).

Schematic diagram of the principal synaptic connections in the olfactory bulb of the rat.

from the primary group of interneurons - the periglomerular and granule cells. The periglomerular cells act upon the relay cell types by means in dendro-dendritic reciprocal synapses within the glomeruli; at these synapses the interneuron appears to inhibit (flattened vesicles and symmetrical membrane thickening) the relay cell, and the relay cell to excite the periglomerular cell. The periglomerular cells also act on the relay cells at a slightly deeper level by means of their axon terminals - at the base of the glomerular arborisation; the synaptic type of these terminals is also inhibitory (Fig. 203). Those parts of the relay cell dendritic tree that lie in the external plexiform layer also take part in many reciprocal dendro-dendritic synapses with the granule cells (Fig. 204), exactly comparable to those with the periglomerular cells; the granule cells do not have axonal processes. Both granule and periglomerular cells make most of their dendro-dendritic synapses by means of spine-like appendages termed gemmules - which are defined as being spines that not only receive but make synaptic contacts. Having run the gauntlet of these three sets of inhibitory interneuronal influences, the depolarisation derived from olfactory stimuli may reach the cell body of the relay cells and may give rise to a propagated axon spike. This will be conducted by these axons to the cerebral olfactory centres and will continue in the relay pathway; but activity will also remain in the bulb, in the form of axon collateral excitation (Figs. 203,204), which is relayed both to the primary (granule and periglomerular) and the secondary interneurons (short-axon cells), predominantly the latter.

In the preceding account, the similarities of the two relay cell types have been emphasized, but certain noteworthy differences do exist, which may give some clue to the functional distinction between these two cell types. The distribution of the tufted cell somata allows their easy identification, being found between the superficial edge of the mitral cell layer and the deep edge of the olfactory nerve layer, and this is accompanied by a gradation in size from deep to superficial. The most superficial tufted cells will receive no contacts from granule cell processes, but only from periglomerular cells, while others will receive varying proportions of granule cell inhibition according to site. The more superficial tufted cells also have more limited glomerular arborisations within the individual glomeruli, occupying a wedge-shaped portion of these structures, while the mitral and deep tufted cells arborise throughout. The more superficial tufted cells have fewer, shorter secondary dendrites or none at all. Finally, only the axon collaterals of the tufted cells distribute to the periglomerular and superficial short-axon cells, whereas both types of relay cell distribute to the deep set of interneurons.

The periglomerular cells, the primary peripheral interneurons, receive synaptic contacts, in varying proportions and sites, from all the processes that make synapses at glomerular level; that is, the olfactory nerves, the dendrites of relay cells, the collaterals of tufted cells, the axons of superficial short-axon and other periglomerular cells and the excitatory terminals of the centrifugal fibres from the cerebral hemispheres. Similarly the granule cells, in the deeper layers

appear to receive synapses from the relay cell dendrites and all axon collaterals, the axons of deep short-axon cells and probably all types of fibre of central origin. These various inputs are distributed to different parts of the neurons. The periglomerular and granule cells have been termed the primary group of interneurons since they act directly on to the relay cells; this is a clear distinction from the short-axon cells which act on the relay cells only through the mediation of the primary interneurons.

The superficial and deep short-axon cells do not effect dendro-dendritic synapses, and they make axonal synapses by means of symmetrical thickenings and flattened vesicles (inhibitory). Their primary excitatory input appears to be from the collaterals of the relay cells. The superficial short-axon cells also receive centrifugal terminals, inhibitory influences from periglomerular cells and other short-axon cells. The connections of the deep short-axon cells are only incompletely understood; they certainly receive from collaterals and other short-axon cells, but the extent to which they receive contacts from more central nuclei is uncertain. This type of cell does not appear to receive dendro-dendritic contacts.

The centrifugal fibres from the nucleus of the horizontal limb of the diagonal band terminate at two levels (Fig. 204): in the deep half of the external plexiform layer, and in the periglomerular region, synapsing with a morphology suggestive of excitatory function. The similar terminals from the ipsilateral and contralateral (anterior commissure) anterior olfactory nuclei terminate, respectively, in the

superficial half of the external plexiform layer and the granule cell layer; it is not possible to assert that the ipsilateral nucleus does not project to the granule cell layer or deep part of the external plexiform layer, since lesions of this nucleus necessarily involve the anterior commissure fibres and the centrifugal fibres.

The foregoing account of the major synaptic connections in the olfactory bulb shows that electron microscopic investigations, in a site whose light microscopy has been well studied, can provide a detailed map or circuit diagram for the anatomical synaptic connections (Figs. 203,204), that lacks only a few items of information. Furthermore, persistent study of the bulb shows that all the observable synaptic connections between the various profiles in the various layers can be accounted for by the scheme presented; there is no evidence of any further subdivision of neuronal types on the basis of modes of synaptic connections or on other morphological features (with present techniques). This relatively complete picture of neuronal interconnections at the first relay level in the olfactory pathway therefore does allow certain suggestions to be made with regard to types of neuronal connection, both in a general context and in the specific context of sensory pathways, as well as providing a suitable background for electrophysiological study of this particular site. Before doing so, two additional features of the modes of cellular interaction will be noted and the most important pieces of information that are, as yet, lacking in our knowledge of the bulb delineated.

"Synaptic patterns" have been noted at several stages in this work;

these are groups of synaptic contacts acting in close proximity upon one or two dendritic profiles. They have been defined for the purpose of the olfactory bulb of the rat as being synapses occurring within 500nm along the dendritic shaft or relating to a single appendage and/or its adjacent dendritic shaft. This arbitrary figure was derived empirically from the observations on the bulb, and most of the examples of obvious synaptic patterns occurred within this limit. At this stage it is sufficient to note that such closely related synapses occur, in particular relation to various appendages, suggesting a degree of integration of input at these sites, and to emphasize that certain characteristic axon terminals and dendritic synapses are regularly found, forming different types of synaptic pattern (characteristic of different sites).

In addition to the various neuronal interrelationships, certain unusual features of glial cells have been noted in the course of these studies. These relate to certain glial cells that lie in the olfactory nerve layer; these cells are known from classical light microscopy to envelop the olfactory nerves with basal processes and to extend long thin processes inbetween the glomeruli into the external plexiform layer. Electron microscopy has shown that the basal processes do indeed wrap around groups of olfactory nerve fibres; the thin deep processes have been shown in some instances to form several thin glial lamellae around the stems of the mitral and tufted cells, just before their glomerular branching. The highly specific location of these unusual lamellae suggests some functional relationship, and in the monkey these segments

of the relay cell dendrites are surrounded by thin myelin sheaths, in place of the lamellae. It is presumed that they have the same cellular origin. The same glial cell is thus seen to surround both the incoming olfactory nerves and that part of the relay cells at which the olfactory input converges to pass into the primary dendritic shaft (i.e. as they emerge from the glomeruli).

DATA STILL NEEDED

In enumerating some of the more significant gaps in our knowledge of the neuroanatomy of the olfactory bulb, emphasis will be placed on those areas that would appear to be necessary for a complete understanding of this site as a relay and coding centre for olfactory information. The first of these is the basis for the organisation of the olfactory nerves into individual glomeruli; this is perhaps as much a physiological as an anatomical basis, but so far we have fragmentary evidence from either approach. Andres (1965) has noted that the fibre bundles appear to undergo reorganisation as they pass from the mucosa to the glomeruli; this implies that the glomeruli do not simply represent a spatial distribution over the mucosa, but that they are already arranged according to some functional specificity, possibly residing in the receptor cells. That this should be so is not surprising in view of the complexity of the bulbar organisation and its dependence on the glomeruli as the first unit of convergence and the basis for subsequent interactions. Land, Nager and Shepherd (1970) have been able to show areas of localised degeneration following small olfactory nerve

lesions with silver methods, but it is not yet clear whether the general application of this technique would provide the necessary overall information. The studies of Leveteau and MacLeod (1966,1969) have shown a certain degree of odour specificity at glomerular level but no clear aggregation of this over the bulbar surface. A second point relates to the extent of interaction of different relay cells and periglomerular cells within a single glomerulus. So far it is only possible to emphasize that extensive serial connections between different individual cells of each type have been observed and that these, in all probability, reflect a far greater number which cannot be seen even in the long sets of serial sections that have been used in some of these studies. Nevertheless this point is of crucial significance, together with the previous one, in assessing the exact functional role of the glomerulus in the integrative function of this site.

It has been difficult to identify the deep short-axon cell dendrites at a distance from the cell somata and so our knowledge of their connections is more fragmentary than that regarding their superficial counterparts; from the afferent terminals close to their cell somata, certain deductions can be made concerning their overall connections, and analogies with the superficial short-axon cells can also be drawn. The principal aim of recent studies has been to emphasize their existence, in the hope that they may be further characterised as techniques become available (Price & Powell, 1970d). A further gap in our knowledge is the extent of the distribution of the mitral and tufted cell collaterals: it is known from normal and experimental studies that tufted collaterals

distribute to all the layers of neuropil, but whether the mitral cell collaterals reach the glomerular layer or not is still uncertain. The only evidence is from light microscopy of Golgi preparations, which shows that these collateral branches of the mitral cells only reach as far as the superficial part of the external plexiform layer, but knowing the vagaries of the Golgi methods in the tracing of relatively fine axon projections over any distance, it would be ideal to obtain some more direct, preferably electron microscopic, confirmation of this. Uncertainty also surrounds the questions of the relative axonal contributions from the tufted cells at the various levels and of the mitral cells to the afferent input of the two sets of interneurons, particularly the deep set, and of the extent to which the deeper tufted cells send collaterals to the glomerular layer.

The exact location of the projections to the deeper layers from more central regions of the olfactory pathway, notably those of the anterior olfactory nucleus, are somewhat uncertain on account of the difficulty in achieving selective lesions of this nucleus, although additional sites of degeneration and denser zones allow inferences to be made on this point. Combined long- and short-term lesions of the various pathways should be a way of clarifying this point. Finally it should be noted that the glial relationships of the bulb have been largely ignored to date - only the most striking of these having been described at any length. Changing views of the significance of these cells in neuronal organisation strongly suggest that the glial organisation of these sites should be studied more closely. In the bulb, the light

microscopy of these cells has been exhaustively studied by Blanes (1898); there remains the technically exacting task of disentangling the often extremely fine glial profiles with electron microscopy.

In more general terms, two significant gaps in our knowledge exist: the quantitative aspects of the various connections; and the identity of the transmitter substances at the various synapses, characterised by conventional electron microscopy. Experimental histochemistry at electron microscopic level may well provide some clues and autoradiography has already been demonstrated to be of value in this kind of work (Matus & Dennison, 1971); otherwise there is the possibility of the less direct method of correlation with pharmacological studies, such as that of McLennan (1971) in which he shows that GABA (gamma amino butyric acid) is the probable transmitter at the granule cell to mitral cell synapses. It is to be hoped that one or more of these lines of research will provide more exact information on this important question.

NEURONAL STRUCTURE AND FUNCTION

Concepts arising from study of the olfactory bulb as a whole may be divided into two broad categories: the features of individual neurons, their synaptic specialisations in particular, and the features of the organisation of these neurons, with respect to their interneuronal relationships. It is at these two levels that electron microscopy can add significantly to the classical picture from light microscopy, principally through the apparent ability to visualise at this level the

morphological basis for the physiological synapse.

Dendro-dendritic synapses

Among the more dramatic discoveries in the olfactory bulb were the dendro-dendritic synapses (Hirata, 1964; Andres, 1965; Reese & Brightman, 1965; Rall, Shepherd, Reese & Brightman, 1966; Price & Powell, 1970b). Although these were initially regarded as a special feature of this site and the retina (Dowling & Boycott, 1966), more recent studies have revealed that, even if they do not occur as frequently, they may be found in many diverse sites, notably the thalamus (Ralston & Herman, 1968; Guillery, 1969; Famlighetti, 1970; Wong, 1970; Harding, 1971), superior colliculus (Sétáló & Székely, 1966; Lund, 1969), dorsal horn of spinal cord (Ralston, 1968), and motor cortex (Sloper, 1971). Another tendency has been to regard these synapses as an exclusive feature of amacrine-type cells (i.e. neurons lacking axons), despite the fact that the reciprocal nature of the synapses in both bulb and retina, shows clearly that the dendrites of other more conventional cells such as bipolar and mitral cells, also take part in such synapses and may thus be presynaptic. These facts together with the clear evidence for dendrites that do not make dendro-dendritic synapses (the short-axon cell dendrites and granule cell deep dendrites in the bulb, for instance) led to the suggestion (Chapter 5) that at least two separate dendritic types could be distinguished - Class A dendrites, that only receive synapses, and Class B dendrites, which both receive and make synaptic contacts (Chapter 5). It may well

be that additional, functional subdivisions of dendrites will emerge from further study, but such striking differences, even between the deep and superficial dendrites of a single cell (granule cell of the olfactory bulb), seem significant enough to divide in this way. Furthermore, the whole question of dendritic integration (Spencer & Kandel, 1961; Rall et al., 1967; Diamond & Yasargil, 1969; Diamond, Gray & Yasargil, 1970; Llinás & Nicholson, 1971) becomes even more crucial in Class B dendrites (where local effects may give rise to an output signal from them without necessary axon initial segment summation) than in the Class A dendrites; this means that different approaches to the two classes may be necessary in the biophysical evaluation of synaptic localisation.

The functional possibilities of reciprocal dendro-dendritic synapses have been widely discussed (Rall et al., 1966; Dowling & Boycott, 1966; Rall & Shepherd, 1968; Shepherd, 1971); a few points may be made here to highlight their possible significance. Since it appears that most, if not all, the dendro-dendritic synapses in the olfactory bulb are reciprocal, we will only consider the consequences of reciprocal interaction of dendro-dendritic synaptic contacts. The physiological activity of reciprocal dendro-dendritic synapses must depend on several factors, some arising from anatomical disposition and organisation with respect to their cells of origin and various inputs, and others relating to the functional state of the various neurons at a given time; the former we have some knowledge of, the latter is a matter for speculation. It is probable that these parameters determine

the versatility of the structural relationship and its particular role in any site or at any time. The anatomical factors are the position of contacts in relation to the dendritic trees of the cells involved and the situation of axonal inputs on each of the dendrites, specifically those in close proximity to the dendro-dendritic synapses; a special case of the latter is whether or not the two reciprocally connected dendrites receive afferent input from the same process, or type of process, or not. For instance, the olfactory nerve terminals synapse on to both the periglomerular cell and the mitral/tufted cell dendrites taking part in the reciprocal synapses, and it is often the same olfactory nerve terminal that synapses on to each dendrite, in close proximity to the reciprocal synapse; on the other hand, the granule cell gemmules receive centrifugal and other afferent terminals that do not make any synaptic contact with the mitral/tufted cells, either in the immediate vicinity of the reciprocal synapse or at a distance. Functional factors determining the physiological role of this synapse include the type of synapse (excitatory, inhibitory) made by each component and its afferent connections; the functional nature of the synapse may be deduced from its morphological appearance (see below) and/or the action of the axons of the respective cells at other junctions, or it may be demonstrated physiologically. The extent to which the synapse is isolated from, or in contact with, the parent process is clearly of importance in determining both the influence that the ongoing activity in the dendrite as a whole has on the synaptic interaction and the effect of the dendro-dendritic input, and its associated

synapses, on dendritic activity as a whole. That this is likely to vary from cell to cell, and even within a cell, is indicated by the fact that some of the profiles are gemmules (granule and periglomerular cells) and others are dendritic shafts (mitral, tufted, and to a lesser extent granule and periglomerular cells). What the biophysical significance of the gemmules is as opposed to the dendritic shafts, is however still uncertain and largely speculative. The suggestions of Diamond et al. (1970) concerning spine function as a subunit of dendritic activity and integration are particularly interesting in this respect; one of their suggestions is that the events in the spine are largely unaffected by the activity in the dendrite. That this cannot be an absolute phenomenon is indicated by the granule cells of the bulb; these cells may be activated from the contralateral anterior olfactory by the fibres passing in the anterior commissure which terminate upon the deep dendrites of these cells (Yamamoto, Yamamoto & Iwama, 1963; Price & Powell, 1970c; Leveteau, Daval & MacLeod, 1972). Their effects are seen as an initial inhibition of mitral cells, mediated by the granule to mitral component of the reciprocal synapse (since this is the only efferent synapse from these cells); thus the activity is carried from the deep dendrites, down the peripheral processes and into the gemmules (there is no direct contact on to the gemmules from these fibres). It is thus clear that much has to be learnt from systems such as the bulb concerning spine and gemmule function as a specialised part of the dendritic tree; nevertheless, the concept of spines as subunits of the dendritic tree is an important one and is likely to be a fruitful

line of investigation.

One is now in a position to consider the functional capacity of the reciprocal dendro-dendritic synaptic arrangement (see also Rall et al., 1966; Rall & Shepherd, 1968; Shepherd, 1971); it is immediately obvious that this structure together with its afferent connections, its distribution, and that of other profiles allows of both temporal and spatial adjustment of a given signal. The spatial effects arise from the widespread interactions of different cells within the two basic types involved in a given reciprocal synapse (relay and primary interneuron); local interaction, within a glomerulus or within the field of a single granule cell (which is of the same order of lateral dimensions as a glomerulus) will predominate, but the dendro-dendritic input to the periglomerular cell will probably contribute to the axon spike propagation, which extends even further laterally to affect other relay cells. It may be useful to distinguish these two modes of spread, assuming that the glomerulus is the basic unit of integrative function in the bulb, as local integration and wider spatial interaction; this implies that the interneuronal connections in a given system are involved on the one hand in coding the afferent information and on the other hand are relating this to other afferent information arising parallel in the same system. The temporal aspects of reciprocal synaptic function arise largely from the simultaneous input to the two arrangements at glomerular level, or by the feedback of mitral/tufted cell collaterals on to the interneurons and ultimately back on to the relay neurons via the reciprocal synapse. In the former case,

this may be a reinforcement of periglomerular cell excitation by the excitatory mitral/tufted to periglomerular component, or a cut-off in the mitral/tufted dendritic activity by the periglomerular to mitral/tufted component. Thus the spatial (inhibitory) influence is reinforced, and the relay cell activity signal is sharpened, enhancing both spatial and temporal contrast. Also there is a possibility of self-inhibition after dendritic activation by the mitral-granule-mitral (or equivalent) route of the reciprocal synapses, in which the synapse is acting as a unit; this gives rise to the "rhythmical" activity to which the system is well suited (Rall & Shepherd, 1968). Here is an example of a local reciprocal feedback system on to which may be superimposed a feed-forward one-way system (periglomerular) or a feedback one-way system (granule and periglomerular). This highlights the point that the elements of the reciprocal synapse may act initially as a simple one-way system of transmission from one cell to another, mediating a specific influence, or it may act as a reciprocal unit, modifying the ongoing activity in one or both constituent processes. Clearly the former will merge to a greater or lesser extent, with time, into the latter, but conceptually it may be useful to realise that the reciprocal synapse is not only a reciprocal system, but also a combination of two one-way interactions between the same processes of the same two cells.

On a wider scale, two further functions emerge: the possibility of a fine reciprocal control at a local level of the present input (olfactory nerves), the immediately preceding successful input (relay cell collaterals) and the spatially adjacent input (relay cell collaterals,

periglomerular cell axons and short-axon cell axons), as well as the centrifugal afferent influences, including activity in the contralateral bulb. In addition the systems allow, potentially, the selective adjustment of input-output functions at this relay level, both by intrinsic and extrinsic (cerebral) mechanisms.

Although dendro-dendritic synapses including reciprocal synapses have been recently demonstrated in sites other than the retina and bulb (Ralston, 1968, Ralston & Herman, 1969; Lund, 1969; Guillery, 1969; Famlighetti, 1970; Wong, 1970; Harding, 1971; Sloper, 1971), they are clearly not present in the profusion exhibited in these two peripheral sensory relay sites. This leads one to enquire what particular functional aspect is so ideally suited to neuronal interactions of this type. In a very general way, it may be suggested that the peripheral siting of these synaptic specialisations on sensory pathways reflects the complexity of the neuronal interactions that are necessary in the transformation of information derived from those neuronal cells that are specifically involved in the transduction of the stimulus from the environmental modality to the neural one. Thus these reciprocal interactions, with their tremendous capacity for spatial and temporal contrast, and for local and selective types of action, could be seen to represent the tremendous task of sorting information into the spatial, temporal and qualitative parameters relevant to neuronal processing of information; information that is derived from receptor cells that are, of necessity, modified to suit to the modality of the stimulus. However, such generalisations must be

put in the context of sensory pathways other than olfactory and visual, and although the available evidence strongly suggests similar degrees of complexity in certain of these, dendro-dendritic or reciprocal synapses have yet to be described, with the exception of the somatic sensory (Ralston, 1968). It may be that axo-axonic synapses or other interneuron connections serve a different but analogous function in some of these systems. Unfortunately, so little is known of the physiological input in the olfactory nerves, and of the kinds of discrimination that are achieved at the ascending levels of the olfactory pathway that it is impossible to draw functional parallels between visual and olfactory pathways that could account for their similarity in this aspect of neuronal interaction.

Synaptic Patterns

Closely related to the question of dendro-dendritic synapses is that of synaptic patterns, in which several synaptic contacts are observed in close association with each other on a dendritic process or processes with a regularity suggestive of some physiological specificity. In the bulb, the commonest patterns are those relating to the reciprocal synapses that have already been discussed, and those relating to dendritic spines of interneurons. Again the hypothesis of Diamond et al. (1970) is central to the interpretation of these arrangements, for they certainly appear morphologically, as circumscribed subunits of the dendritic tree. The relation of these subunits to local dendritic output has been considered above, but the significance of both gemmules and

spines with these patterns to the whole dendritic tree may be briefly considered. Diamond et al. have suggested that the appendages are relatively isolated, so that local interactions in them will be to a greater or lesser degree independent of the activity in the parent dendritic shaft. This attractive notion of local integration in sub-units widens the field of intra-dendritic integration, despite the consideration above of the possibly exceptional granule cell gemmules; it would be consistent with the morphological diversity and apparently specific localisation of synapses, not to mention their numerical magnitude, that have struck most electron microscopists of the central nervous system. However, it would be dangerous to place too much emphasis on an extrapolation from the special case of the Mauthner cell interactions in the goldfish to the mammalian central nervous system, without some at least circumstantial evidence in favour of such an extension; clearly the morphological attractiveness of the concept is insufficient and so it is only possible to emphasize the relationship between the two sets of findings. Intra-dendritic integration, although important in conventional Class A dendrites, is of crucial significance in Class B dendrites where a single cell may be transmitting many different types of information that are derived from small subdivisions of its total input.

Physiological evidence (Spencer & Kandel, 1961; Rall et al., 1967; Llinás & Nicholson, 1971) suggests that it is the points of branching in dendrites at which convergence and possible dendritic spike initiation occurs; certainly these sites would be, teleologically, the "best" sites

for intra-dendritic integration of some sort. The morphological evidence from the bulb is in support of such a concept by two pieces of circumstantial evidence. Both of these relate to the part of the relay cell primary dendrite just deep to the glomerular arborisation. This is the only site where the periglomerular cell axons contact the relay cells, and it is also the site at which glial lamellae (or myelin in the monkey) are seen to wrap around the dendritic segment in a characteristic manner. The first observation suggests that this may be a similar site, functionally, to the initial segment on which similar isolated, inhibitory synapses are found, and it is self-evident that it would be a powerful site for inhibition, since all relay cell excitation is distal to this site. The second observation, although less easy to interpret, could be taken to indicate either spike initiation at this site or some insulating function that is crucial at this branch point; alternatively it could represent a point at which some form of glial activity is transmitted to the dendrite, relating to the olfactory nerve activity, around which the same glia are also wrapped, as discussed in a previous chapter (Chapter 3; Miller & Dowling, 1970).

Synaptic Structure and Function

As to the synaptic specialisations themselves, the various electron microscopic observations on the olfactory bulb, together with information available from physiological studies, has provided some evidence on the relationship between the morphology and the functional nature of synapses. Attempts to distinguish between excitatory and inhibitory synapses on

morphological grounds stem from the early work by Gray (1959); he described two types of synapse on grounds of situation and characteristics of the membrane thickenings but stated that no functional differences between the two could be suggested. Eccles (1964) pointed out the strong indications at that time that Type I was excitatory and Type II inhibitory. Uchizono (1965,1967), using aldehyde-fixed material of the cerebellum, showed that two types of presynaptic vesicles existed - spherical and flattened - each being present in different terminals, and he likewise suggested that these were related to excitation and inhibition respectively. Colonnier (1968) drew these studies together to some extent by relating the spherical type of vesicles to marked post-synaptic thickenings (a feature of Gray's Type I) and the flattened type of vesicles to a minimal post-synaptic thickening (a feature of Type II); these are termed asymmetrical and symmetrical thickenings respectively. Despite the somewhat tentative nature of his exposition, as much due to the fact that he was not using serial sections to determine the true nature of the few equivocal thickenings, this correlation has proved a reliable one, which, given the limitations and variability of the material, has been most strongly borne out by the evidence. Again, the correlations made by Eccles and Uchizono were applied to this situation, spherical vesicles and asymmetrical thickening being excitatory and flattened vesicles and symmetrical thickening inhibitory. Price and Powell (1970b) were able to divide the terminals in the olfactory bulb with symmetrical thickenings into two groups - those with large and those with small flattened vesicles -

using statistical methods; both were considered to be inhibitory, but to be derived from different cells and presumably act by means of different transmitters. Bodian (1970) showed that the use of cacodylate buffer would cause the flattening of a group of large, otherwise spherical, vesicles in terminals which were associated with symmetrical thickenings, and this has been confirmed in the caudate nucleus by Kemp and Powell (1971b); other vesicles are unaffected. Valdivia (1971) has shown that flattening of vesicles is dependent upon the osmolarity of the buffer solution used. Dennison (1971) has studied flattened vesicles in various situations, including the olfactory bulb, with stereoscopic electron microscopy, and has elegantly demonstrated that although some vesicles in terminals defined as flattened in type appear spherical, they are in fact flattened in the axis of the electron beam; she has shown that vesicles of the "flattened" type may be either a disc or a cylinder depending on site and species (cf. Hirata, 1964). Thus, over the course of a decade, clear criteria have emerged for distinguishing between various morphological types of synaptic specialization. It is tempting to assume that the morphological variation in vesicle shape in some way reflects the identity of the transmitter and that the nature of the post-synaptic thickening reflects the transmitter influence at that site, be it depolarising or hyperpolarising. Matus and Dennison (1971) have shown specific uptake of labelled glycine into terminals containing flattened vesicles; this promises to be a useful method in correlating structure and function. The enthusiasm for a morphological approach to synaptic function appears now to have provided

a correlation of substance, and the olfactory bulb has provided a situation for testing many of its tenets.

Firstly, the correlation between the vesicle type and the thickening type has been entirely borne out in every case, and defined by serial sections when equivocal. Secondly, the vesicle type and thickening type related to the synapses from a given cell type are consistent within the individuals and the group of that type, be the synapse from an axon terminal, a dendrite or even a cell soma. Finally, where physiological evidence is available on the functional type of the synapse this is in complete agreement with the prediction from morphological typing (see Table II).

Although this seems a clear-cut issue, instanceswhere these three important correlations fail may yet emerge; if it is borne in mind that these may represent instances of unusual transmitters and/or post-synaptic effects it may be found unnecessary to discard what is potentially a most useful system for at least postulating functional activity from morphological appearances. Naturally, such suggestions must remain speculative unless backed up by physiological or biophysical evidence; if the correlation becomes widely accepted, then it may serve as a means of predicting and confirming observations from electrophysiology. It should perhaps be stated explicitly that there is no a priori reason why, for example, excitatory transmitters should respond to aldehyde fixation methods by remaining spherical, although it is possible to imagine that post-synaptic effects may be determined by common structures that are revealed morphologically by heavy metal

staining; thus in equivocal situations it is probable that more reliance should be placed on the nature of the post-synaptic thickening.

Table II. A correlation of synaptic morphology with function.

Process	Morphology	Physiology	Source
Olfactory Nerves.	SV/AT	Excitatory.	Shepherd (1963a, 1971). Yamamoto et al. (1963).
Mitral cell.	SV/AT	Excitatory.	Rall et al. (1966); Rall & Shepherd (1968); Biedenbach & Stevens (1969); Nicoll (1970b).
Tufted cell.	SV/AT	Excitatory.	Shepherd (1971).
Periglomerular cell.	LFV/ST	Inhibitory.	Shepherd (1971).
Granule cell.	LFV/ST	Inhibitory.	Shepherd (1963b); Yamamoto et al. (1963); Rall et al. (1966); Rall & Shepherd (1968); Nicoll (1969).
Deep Short-axon cell.	SFV/ST	Inhibitory.	Yamamoto et al. (1963); Callens (1965).
Centrifugal.	SV/AT	Excitatory.	Dennis & Kerr (1968).
Anterior Commissure.	SV/AT	Excitatory.	Leveteau, Daval & MacLeod (1972).*

SV/AT = Spherical vesicles and asymmetrical membrane thickening.
LFV/ST = Large flattened vesicles and symmetrical membrane thickening.
SFV/ST = Small flattened vesicles and symmetrical membrane thickening.

* By extension.

ORGANISATIONAL PRINCIPLES

The olfactory bulb has long been regarded as a primitive part of the

brain and the assumption has thus arisen that it is also simple. The findings summarised above make it clear that, if this site is simple relative to the neuronal organisation to be found elsewhere in the brain, areas such as the neocortex must be almost prohibitively complex for contemporary study. While the question of the relative simplicity of the bulb is at present a teleological assumption, there underlies its apparent complexity a basically simple design comprising a limited number of neurons connected in a variety of ways. Although the exact transformation occurring in the bulb is still obscure, the pattern of organisation suggests that it may be common to many other sites, and be the basis for more complex systems. Indeed the most complex system so far analysed in detail - the cerebellum - shows a similar underlying organisation with certain elements in greater prominence, others less so, still others superimposed and, apparently, other features missed out. These latter are presumably those elements of neuronal organisation that are most specific to the bulb, sensory relays or to certain modes of transformation. There is little doubt that the bulb and cerebellum, insofar as we understand their function, are widely different. In the ensuing paragraphs certain features that have been seen in the bulb and elsewhere are brought out with a view to "isolating" certain of the common principles in neuronal organisation.

Basic Neuron Set

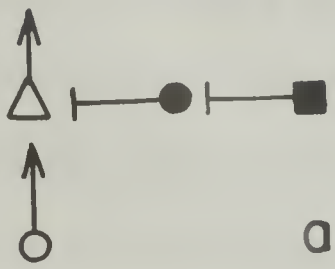
A striking feature is the main through pathway of excitatory neurons (olfactory nerves to relay neurons), with two systems of interneurons

acting on this pathway, each system consisting of two inhibitory interneurons acting in series (Fig. 205). In terms of the relay neurons these represent pathways for inhibition and disinhibition, and are an interesting model for integration. There is, at present, no evidence of a secondary interneuron in the retina; but the system of two inhibitory neurons in series acting on a through excitatory pathway may be discerned in the cerebellar cortex and nuclei, if one considers the climbing fibre input to the cerebellar nuclear cells and Purkinje cells, the inhibitory basket and Purkinje neurons acting on the cerebellar nuclear cells (Eccles, Ito & Szentagothai, 1967). The function of such a basic system must depend largely on the way in which it is connected to afferent, intrinsic and efferent collateral pathways, and here the individuality of a given nucleus will reveal itself.

Common features of intrinsic axonal projections

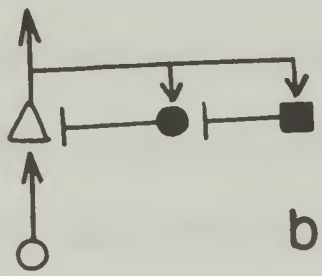
Perhaps the most interesting common principle of organisation of axonal projections is that each one, whether extrinsic, relay or interneuron, acts upon two (or rarely, three) different cell types in the bulb, which are linked in series (Fig. 205). The numerical proportions of these multiple projections vary, and although this may not be used as an absolute index of efficacy, their net effect may vary accordingly. A simple interpretation of these systems is that, in the steady state, each projection exerts an effect by means of the proximal connection, but also provides a means of cancelling it, after a short period of synaptic time; this is true in all the situations found in the bulb,

Fig. 205 Schematic diagrams to illustrate the various modes of interrelationship of intrinsic connections in the olfactory bulb, particularly the double projections of each axonal system. a. Basic neuron set: afferent input, relay cell, primary and secondary interneurons. b. Relay cell collaterals. c. periglomerular cell axons. d. short-axon cell axons. e. centrifugal pathways (including anterior olfactory nucleus and anterior commissure). Note that these figures indicate type connections and not those of individual neurons.

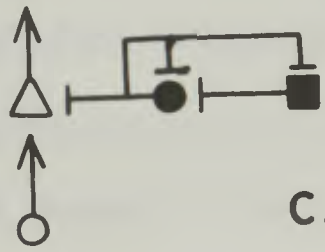


a.

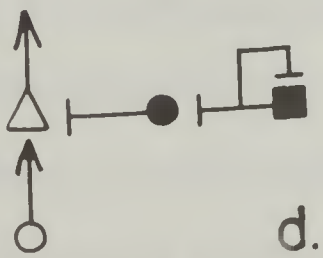
△ Relay cell
● Primary interneuron
■ Secondary interneuron
○ Olfactory receptor
⊗ Centrifugal pathways
↑ "Excitory" terminal
⊤ "Inhibitory" terminal



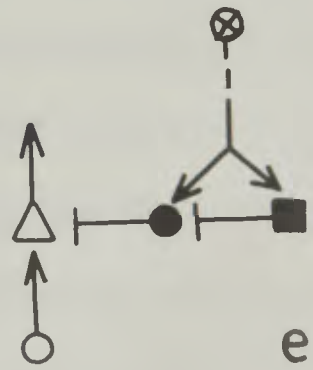
b.



c.



d.



e.

where at least one of the neurons is inhibitory. It is obvious that the success of this simple operation will be modified or prevented by other activity in the neurons and their inputs, but it is significant that every afferent system, whether extrinsic or intrinsic, is potentially self-limiting by virtue of anatomical connections; this kind of biological control is evidently not restricted to the nervous system. In addition, under certain circumstances, this type of projection may give rise to specific, laterally distributed, effects with characteristic time courses as a result of the output span of the post-synaptic neurons. It would be interesting to discover whether this double projection is in fact common to all or most projection pathways, short or long, within the central nervous system; its absence could be characterised by certain well-defined properties of the relevant nucleus. It should be noted, in parenthesis, that dendro-dendritic interactions do not show the same arrangements; however, the reciprocality of dendro-dendritic synapses in the bulb represent a system that has exactly similar functional properties, as discussed in the previous section. It would be interesting to discover whether sites having non-reciprocal dendro-dendritic synapses show some analogous system for self-limitation of activity. It is important to appreciate that this "self"-limiting system refers to cell types, not individual neurons; while there are clear instances where individual feedback is indicated, they cannot be demonstrated easily by current techniques. Nevertheless, the distribution of axons suggests that these interactions, if not acting back on the same cell, do not <u>locally</u>, having a similar effect in terms of neuronal trans-

formation. It may be a feature of the principal input to any nucleus that this projects to an excitatory neuron and an inhibitory interneuron that acts upon it, whereas modulating or collateral influences act solely via a double projection to two series-connected inhibitory interneurons, one of which acts on the relay neuron.

Why two sets of relay cells?

In addition to the serial duplication of axonal contacts, two subsystems of neurons operating in parallel may be distinguished in the olfactory bulb. The first of these is the tufted and mitral system of relay neurons, and the second, the deep and superficial sets of interneurons; these two parallel subsystems are in part related to one another but there are very definite regions of overlap, indicating that they may represent a common organisational principle, rather than the components of a single parallel system.

The tufted and mitral cells are, as has been emphasised already, essentially similar in general morphology, types of profile, types of synaptic interrelation and in central projection; they do however differ in size, situation, extent of dendritic and collateral spread, and the relative proportion and situation of afferent input. It is only as a result of recent studies that the fundamental similarity of these cells in terms of central projections has emerged (Lehman & Mentinck, 1969; Nicoll, 1970a). The minor variations in their characteristics, especially of distribution, lead one to suspect some division of function between them. The more limited glomerular arborisation of tufted cells could

be considered (if there is some degree of intra-glomerular localisation and specialisation of input function) to be more sensitive to "local" increases in olfactory nerve activity, either in terms of odour specificity or of intensity as represented by the proportion of fibres active. This is so in the sense that activity in a limited number of olfactory nerves could stimulate a greater proportion of the total possible excitatory input in the smaller tufted cells. Additionally, they are less subject to decrement in their short primary dendrites than the mitral cells and to the inhibitory influences from the granule cells (and thus central influences) acting in the external plexiform layer. Thus it is suggested that a lower threshold of activity in the olfactory receptor axons, in terms of the number of active fibres, could give rise to successful activation of tufted cells as opposed to the mitral cells; this would also have a gradation within the tufted cells, the external tufted being the most sensitive and the internal tufted being hardly more so than the mitral cells. Similar reasoning could be used to suggest that the tufted cells are more odour specific, if there is intraglomerular variation in odour quality representation; the necessary evidence for this is lacking however. The problem still remains as to why the tufted cells (which are about three times as common as mitral cells - Allison (1953)) are scattered through the external plexiform layer and the mitral cells are a more uniform population, forming a distinct layer. On the varying intensity hypothesis above, it could be considered that they represent a maximum level of intensity (neglecting the modulating influence of the interneurons). This could be

related to function as follows: The tufted cells pick up odour stimuli at low intensity (as in quiet breathing) and to some extent monitor the intensity; then sniffing occurs, increasing the number of neurons carrying information so as to define the odour properties more precisely. The hypothesis of odour specificity in the tufted cells is not in conflict with the intensity hypothesis as may at first sight appear: the increased activity in the olfactory nerves will not mask the quality detection by an increase in "noise" since they are receiving few additional synaptic terminals to those involved in the primary response; there are simply more tufted and mitral cells activated. The quality detection hypothesis is however dependent on the relationship between the neuroanatomical disposition of the olfactory nerves in the glomeruli and their odour specificity, a crucial problem as yet unsolved.

The collateral output of the relay cells in the bulb are interesting in regard to these hypotheses in that the organisation of the bulb, parallel to the surface, is in the form of these collateral and interneuronal axon influences. Mitral collaterals extend up to 25 glomerular widths (1 G.W. = the average lateral dimensions of a glomerulus) in either direction, while the more superficial tufted cells extend 10-12 G.W both superficially and deeply. This indicates that the maximum width of the surround interactions will vary according to the type of relay cell activated, since the relay collaterals are the widest intrabulbar projections. This activity will clearly modify the responses of adjacent glomeruli and relay cells to subsequent input, whatever the

relationship of this second stimulus to the original one, in terms of odour specifity. The available work certainly suggests that the glomeruli are not arranged spatially into groups receiving similar inputs in terms of odour specificity (Leveteau & MacLeod, 1966,1969), so irrespective of whether the tufted-mitral diversity is intensity-sensitive or specificity-sensitive, both the intensity and the quality of subsequent stimuli will be modified by immediately preceding stimuli.

Why two sets of interneurons?

The second of the parallel systems are the superficial and deep sets of interneurons; each of these groups consists of a primary interneuron, acting directly upon the relay neurons, and a secondary interneuron that acts only indirectly, by means of the primary interneuron. The characteristics of the input organisation to these two series-connected cells has already been considered. The question arises as to why the granule and deep short-axon cells are a quite separate group from the periglomerular and superficial short-axon cells, being completely separated by the deep border of the glomerular layer. As has been considered extensively in previous chapters, these two sets of interneurons are basically very similar, with corresponding synaptic characteristics, types of input, and general morphology, with one significant exception; that the periglomerular cells have axons and Class B dendrites, while granule cells have Class A dendrites and Class B dendrites, but no axon. Insofar as the features of the deep short-axon cells are known (Price & Powell, 1970d), they are very similar to those

of their more easily studied superficial counterparts. Thus the primary variant in the two interneuronal sets is the mode of its synaptic outflow - either axo-dendritic and dendro-dendritic or solely dendro-dendritic. Unfortunately since the relative effects of such different synapses are not yet fully understood, it is not possible to ascribe any fine significance to this difference, although it is likely to be important. One can simply observe that the superficial system, with the additional axo-dendritic output, will tend to have more immediate widespread actions on relay cells; the deep system, although it may act through the secondary dendrites of mitral cells to approximately the same distance as the periglomerular axons, will do so with a very different time course, if this pathway is of importance. It is interesting to note that all influences from other nerve nuclei act via interneurons, and principally those of the deep set (granule and deep short-axon).

The superficial group is distinguished by the olfactory nerve input to the periglomerular cells and minimal central feedback (a small centrifugal input to both interneurons), and the deep group by its input from several central pathways. This represents a further division of function between the two groups. The final distinction concerns the output, which feeds variously in both systems on to the relay neurons and bears some, but not a complete, relationship to the parallel system described for these neurons. Both dendro-dendritic synapses and axo-dendritic synapses from periglomerular cells feed on to the two groups of relay cells in the glomerular layer, and presumably in proportions corresponding with

the extent of the glomerular arborisations of the cells; however, the proportion of granule cell input to the relay cells varies according to the site of the cell body and the extent of the secondary dendrites (which appear to be closely related characteristics, the deeper cells having the more extensive secondary dendrites). Thus, for example, the mitral cells have maximal granule cell input, whereas the most superficial external tufted cells, which lie in the glomerular layer, receive none, the remaining tufted cell groups lying between these two extremes. Thus while the degree of feed-forward control (derived from the olfactory nerves and acting through periglomerular cells) is equivalent for both relay cell types, the more superficial cells are less subject to central influences, reciprocal effects from the opposite bulb and collateral feedback (acting through the granule cells). This relates to the speculation above that the deeper relay cells are "recruited" after the early low-threshold response of the more superficial tufted cells: in order for such a recruitment to occur, the level of inhibitory tone exerted on these deeper neurons would need to be reduced (in addition to peripheral changes such as sniffing). Such an effect could be mediated by a reduction of activity in the centrifugal pathway. This involves the assumption that the latter pathway is exerting a tonic influence on the granule cell to relay cell system; such an influence has not yet been demonstrated. If there were such a system, it would of course also have some effect on the periglomerular cell tone with similar effects, although these would probably be quantitatively less significant.

A further extension of this concept, where more direct knowledge is obtainable, is the observation of Leveteau, Daval and MacLeod (1972) that there is a reciprocal inhibition between the two bulbs after a unilateral olfactory stimulus, and that this is mediated by the anterior olfactory nucleus on the side of the stimulation. This nucleus feeds on to the deep dendrites of the granule cells of the opposite bulb and these cells synapse via the gemmules on their peripheral processes on to the relay cells. Thus the inhibition, recorded by these workers in the glomeruli, seems most likely to represent the "antidromic" conduction of the granule cell inhibition of the relay cells in their distal dendritic trees; the granule cells are activated by the excitatory terminals of the contralateral anterior olfactory nucleus. Clearly this effect will be exerted principally on the mitral and deeper tufted cells by virtue of their extensive granule cell connections, and the most superficial tufted cells will only be indirectly affected (by serial arrangements acting via the periglomerular cells), if at all. These theoretical applications of the anatomical network in the olfactory bulb give some indication of the degree of specialisation of cellular roles that has emerged. It is interesting to speculate that the granule and periglomerular cells were, phylogenetically speaking, a single cell, with "conventional" Class A dendrites and an axon. The increasing specialisation of function in the bulb led to the separation of the two interneuronal sets, necessitating the derivation of two primary interneurons - one bearing an axon and the other bearing Class A dendrites; in addition each derived a specialised intermediate process to complement its remaining

process and to retain some relationship between the two cells (via the relay cells) - this is the Class B dendrite. This, perhaps unsophisticated, idea may have at least some conceptual value.

It is interesting to note at this juncture the convergence of massive numbers of olfactory nerves into the glomeruli and the immediate divergence of two relay cell systems from the glomeruli; such a combination of convergence and divergence, seems to represent the "efficient" use of a single input to derive two forms of information. The extent to which they differ and the significance of this have been discussed, but require direct study. Divergent systems may also be seen in the cochlear nuclei and the spinal cord, and the different types of retinal bipolar cells suggest a similar differentiation there; needless to say, the detailed significance of this central duplication of pathways may differ from system to system, but may be a common principle in sensory transformation.

THE BULB AS A CORTICAL STRUCTURE

Reference has repeatedly been made to the characteristic lamination of the olfactory bulb - notably to its advantages for the identifications of profiles and its implications in the organisation of the various component neurons and axonal projections. The concept of lamination immediately allies this structure to the cerebral cortex, and it has indeed been suggested (Shepherd, 1963b) that the bulb represents a model for the more complex arrangement within the lamination of the cortex. It is difficult to assess the relevance of such a concept in the absence

of more detailed knowledge of cortical ultrastructure than is presently available. Clearly the fact that the neo-cortex receives and projects by the deep white matter, while the bulb receives its primary afferent input superficially and projects via the deep white matter, makes it possible that at least some of the basis for lamination is different in the two sites. Nevertheless, as further knowledge on the cellular subdivisions of the cortex becomes available, it may be possible to relate the two sets of pyramidal cells with the mitral and tufted cells of the bulb, as well as the interneurons of the two sites. It is of interest to note that the cerebral cortex, cerebellum, retina and olfactory bulbs, all of which show characteristic lamination, are outgrowths from the rostral neural tube, and may thus reflect some developmental similarity or specialisation.

Aside from the "horizontal" lamination, or as a function of it, the vertical disposition of the mitral and granule cells and their relationship to the superficial glomerular specialisations is of interest; this predominantly vertical arrangement and the very fact of the glomerular subdivisions of the primary olfactory nerve input, on which the output function of the relay cells must largely depend, are temptingly reminiscent of the functional "columns" described for the cerebral cortex (Powell & Mountcastle, 1959; Hubel & Weisel, 1962). Whether this analogy will serve any purpose in adding to our understanding of either site is uncertain; it is interesting to note that the seemingly random disposition of cortical columns with respect to functional specificity with a broad representation of the periphery is paralleled by a similarly

random "map" of odour specificities fed into the glomeruli (Levoteau & MacLeod, 1969), which may overlie a broad spatial representation of the olfactory epithelium (Le Gros Clark, 1951; Le Gros Clark, 1957; Adrian, 1950).

Conclusion

Systematic application of electron microscopy to the olfactory bulb has made it possible to construct a circuit diagram of the anatomical connections between its various component neurons and processes; although some gaps still remain, the knowledge derived in this way provides a means of predicting certain aspects of function and should facilitate physiological study. In view of the relative accessibility and simplicity of the bulb, it may serve as a model for sensory relay function in other pathways; it is also possible that detailed knowledge of the neuronal connections within the bulb will allow more accurate study of olfactory information processing at the periphery. While the connections within the olfactory bulb appear intricate, they have an underlying simplicity of design which may give some clue to the basis of neuronal coding in more complex parts of the central nervous system.

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ABSTRACT

A study of the olfactory bulb in certain mammals by light and electron microscopy of normal and experimental material. The neurons of the glomerular layer of the rat olfactory bulb have been studied using Nissl staining and Golgi-Kopsch impregnation in light microscopy to define the size, shape, and morphological features of individual cell somata, dendrites and axons; these have been correlated with electron microscopic material in which fine structural characteristics were also noted for each cell type, particularly synaptic specialisations. Three neuron types are described: the external tufted and periglomerular cells of classical microscopy, and additional, superficial short-axon cells; a description of the glomerular arborisations of the mitral and deep tufted cells is also included. The tufted and mitral cells show large, non-spiny glomerular dendritic arborisations, having terminal varicosities, the external tufted cells being more limited in their branching than the deeper cells. External tufted cells have large somata and abundant cytoplasm containing stacks of Nissl material; their main dendrites are characterised by pale cytoplasm and a regular array of neurotubules. Reciprocal dendro-dendritic and somato-dendritic synapses are commonly found, the tufted/mitral cells containing spherical vesicles and contacting by means of asymmetrical membrane thickenings; the other profile involved is a gemmule containing large flattened vesicles and associated with a symmetrical thickening. The periglomerular cells are smaller, with a spiny glomerular arborisation, as well as some other dendrites; all the dendrites of these cells tend to be of irregular

outline. They have a dark nucleus and very little somatic cytoplasm; somatic and dendritic appendages are common and often contain large flattened vesicles. Synapses orientated from the dendritic shaft or gemmule also show such vesicles, invariably associated with symmetrical thickenings. The superficial short-axon cells are characterised by the entirely periglomerular distribution of their dendrites, which are varicose and rarely branch. Of intermediate soma dimensions, but containing dispersed Nissl material, these cells and their stem dendrites show no synaptic specialisations directed from them.

Features of axon initial segments, axo-somatic and axo-dendritic synapses are also described for each cell, as well as some unusual glial relationships. Reasons are adduced for relating the superficial short-axon cell to the axon terminal type containing <u>small</u> flattened vesicles, as well as for considering that the external tufted and periglomerular cells show the same synaptic specialisations at their axon terminals as at their dendritic and somatic synapses.

The neuropil of the glomerular layer (consisting of glomeruli and periglomerular regions) of the rat olfactory bulb was studied with the electron microscope with a view to elucidating the type of processes involved - dendrites, appendages and axons - their cellular identity, and the synaptic relationships they establish. The problems encountered in defining these are considered and criteria based on neuron types and from examination of serial sections are put forward. The <u>glomeruli</u> are large structures containing many thousands of processes and are the sole site of termination of the olfactory receptor axons. The terminals

of the latter are characteristically electron-dense, allowing identification in normal material; they run through the glomeruli making many synapses by means of spherical vesicles and asymmetrical thickenings on to all types of dendritic profile. The glomerular dendritic arborisations of mitral and tufted cells, which are indistinguishable from each other, start as large, fairly regular, pale profiles but become increasingly varicose as they branch and diminish in size. They regularly show groups of spherical vesicles, often in association with asymmetrical synaptic thickenings directed from the dendrite; these are typically associated with return reciprocal synapses of the symmetrical type from profiles containing large flattened vesicles. These latter profiles are those of the dendrites and gemmules of periglomerular cells; the dendrites are of irregular outline and give rise to many appendages, mostly gemmules making synaptic contact with mitral or tufted cell dendrites. A small number of pale axon terminals containing either small or large flattened vesicles, derived from short-axon and periglomerular cells respectively, synapse with symmetrical thickenings on to the periglomerular cell dendritic processes. Close associations of particular types of axo-dendritic and dendro-dendritic synapses on interconnecting processes, termed synaptic patterns, are described and their significance considered. The nature of the glomerular interactions is discussed and then placed in the context of other, smaller glomeruli in the central nervous system; certain common principles of glomeruli are suggested.

The <u>periglomerular region</u> of the olfactory bulb, apart from

containing the somata and stem dendrites of the cells contributing to the glomeruli, is the sole region of distribution of the periglomerular cell thin dendrites and the short-axon cell dendrites. It is also the major site of termination of all axons to the glomerular layer except the olfactory axons - i.e. tufted cell collaterals, periglomerular cell and short-axon cell axons and centrifugal fibres. Its characteristic neuropil has been studied with the electron microscope to define the cells of origin of the types of neuronal process and their synaptic relationships. Three types of axon terminals have been found: those with spherical, large flattened and small flattened vesicles, which are deduced to derive from tufted cell collaterals or centrifugal fibres, periglomerular cell and short axon-cell axons respectively; those with spherical vesicles are consistently associated with asymmetrical membrane thickenings and those with either type of flattened vesicles with symmetrical thickenings. The thin periglomerular cell dendrites are very irregular and often have a somewhat dense cytoplasm, rich in ribosomes; they may become extended into very attenuated glia-like sheets that surround the mitral or tufted cell stem dendrites, from which they may receive synaptic contacts. Such dendrites also receive some synapses from all three types of axon in the periglomerular region. The short-axon cell dendrites are thick and varicose and show no sign of synaptic specialisation orientated from them; they have few spines but receive many asymmetrical type synapses on their shafts. Both axon terminal types synapsing with symmetrical thickenings are also found on the shafts. The evidence

obtained from the study of normal material is summarised and the various cellular roles considered. In the light of observations on the olfactory bulb, it is suggested that dendrites may be divided into two major classes: those that only receive synapses (Class A) and those that make synaptic contacts as well as receiving them (Class B). After a summary of the findings from normal material and a brief description of an unusual feature of the olfactory bulb in the monkey, a group of experimental studies on this site in the rat and rabbit are described.

The degeneration of axon terminals in the glomerular layer of the rat olfactory bulb has been studied, concentrating particularly on the sequence of degeneration in the olfactory nerve terminals and the long-term events in the degeneration process in several terminal types. Olfactory nerve terminal degeneration is divided into five stages, representing parts of the sequential changes taking place in the terminal after fibre section. The main features in the sequence are as follows: I. Swelling of the terminal and its vesicles. II. Initial shrinkage of the terminal, while vesicles remain swollen and some are distorted. III. Further shrinkage and darkening of the terminal, distortion of many of the vesicles and some mitochondrial swelling. IV. Extreme shrinkage of the terminal and loss of cytoplasmic detail; synaptic contact still intact or partially separated. V. Disappearance of the presynaptic terminal and persistence of the post-synaptic thickening.

The validity of the observation of the persistence of post-synaptic

membrane thickenings is considered and evidence adduced in its favour, both qualitative and quantitative. Characteristics of the newly apposed profiles are described, including cisternae and alveolate vesicles; the extra-cellular material of the synaptic cleft is considered in the light of its association with persisting thickenings. The relevance of these associated structures is discussed in terms of function and development. Spontaneous degeneration of olfactory nerves and the degeneration of the vomeronasal nerves in the accessory olfactory bulb are described, as well as Stage V in the degeneration of other terminal types in the glomeruli, following various lesions; persistence of post-synaptic thickenings after the degeneration of terminals showing symmetrical membrane thickenings is included. Observations on the persistence of post-synaptic thickenings in various sites in the central and peripheral nervous systems are reviewed in the context of the present study; the problems arising out of the morphological identification of apposition or reinnervation are discussed.

A study of the transneuronal degenerative changes in the cells of the olfactory bulb of the rabbit and rat following removal of the olfactory mucosa has also been made. The principal features of this type of change are cell shrinkage, a concentration of cytoplasmic and nuclear constituents, an alteration in the organisation of nucleic acid material, and a swelling of membrane-bound systems; some elements of cellular organisation appear unchanged, including synaptic specialisations. The changes are described for each part of the neuron - soma, dendrite, axon and terminal - as well as the variants found in different cells.

The degree of morphological alteration appears to be related to the extent of denervation for any individual cell and does not seem to be sequential. These changes are related to findings on altered impregnation properties of the same cells with the Golgi technique and are discussed in the context of the functional alteration produced by deafferentation. A new interpretation of some 'dark neurons' is also suggested on the basis of these findings.

The termination of the centrifugal fibres running in the lateral olfactory tract to the glomerular layer of the rat olfactory bulb has been determined with the electron microscope; this has been done with material perfused at various times after section of the lateral olfactory tract, as well as after a combination of this lesion with the long-term degeneration of olfactory nerves. The axon terminals are sparse at the glomerular level, but undergo typical degenerative changes; they are distributed solely in the periglomerular region and intermediate zone. The most common post-synaptic profiles are the processes of periglomerular cells, but a few centrifugal fibres terminate on short-axon, tufted and mitral cell dendrites. Evidence is produced to suggest that the anterior olfactory nucleus does not project as far as the glomerular layer. The findings are discussed in relation to previous studies with normal material and silver degeneration methods on similar experimental material; the functional implications of the centrifugal pathways in the bulb are briefly discussed.

Lesions have been made in the superficial layers of the dorsal surface of the rat olfactory bulb; the resulting axonal degeneration

has been studied with light and electron microscopy, in order to determine the mode and extent of interconnection of the intrinsic interneurons of the glomerular layer. Although the lesions necessarily involve extrinsic pathways, the axonal distribution of each of the intrinsic neurons may be deduced from this evidence and a knowledge of normal and experimental material. Tufted cell collaterals extend for 6-12 glomeruli, periglomerular cell axons for 4-5 glomeruli and superficial short-axon cell axons for 2-3 glomeruli; these figures correspond well with observations of Golgi-impregnated material. The experimental approach to the study of intrinsic connections and its applicability to other sites is considered, and the results are discussed from the point of view of neural coding and odour specificity.

In the last chapter concerned with results from this study, mention is made of the technique of combining Golgi-impregnation for light microscopy and electron microscopy of the same cell; its practical application is considered and examples are given from the study of a middle tufted cell by this technique. Finally, the general discussion brings together the results on the organisation of the olfactory bulb, particularly the glomerular layer. The discussion centres around two principal issues arising from these studies: the structure and function of individual neurons and their specialisations on the one hand, and the principles of neuronal organisation on the other; where possible, these are related to findings and function in other parts of the central nervous system, as well as to the function of the olfactory bulb in the olfactory pathway.