J. Anat. (1974), **117**, 3, pp. 487–516
With 23 figures
Printed in Great Britain

Ultrastructure and three-dimensional organization of synaptic glomeruli in rat somatosensory thalamus

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(*Accepted* 7 *March* 1974)

INTRODUCTION

A number of sites in the mammalian central nervous system (C.N.S.) are characterized by complex synaptic arrangements in which several axonal and dendritic components, partially or totally ensheathed by glial cell processes, establish synaptic contacts of various kinds (see, for example, Szentágothai, 1970; Pinching & Powell, 1971*b*). There are considerable differences between such complexes in different sites and even in homologous nuclear regions of different species. But wherever they occur, and whatever the architecture of the participating components and the nature of the specialized contacts between them, such complexes are usually clearly different from the rest of the neuropil in that region. Thus, as Szentágothai (1962) has stated, they constitute recognizable tissue units. Many different names have been given to such regions (viz. synaptic islands, encapsulated synaptic zones, large synaptic complexes, complex interneuronal contacts, complex glomerular synapses, synaptic agglomerulations, synaptic nests and synaptic glomeruli). Of these, the most commonly used term, *synaptic glomeruli*, is particularly appropriate for circumscribed synaptic complexes with a well-defined glial capsule (Szentágothai, 1970).

Synaptic glomeruli are particularly, though not exclusively, associated with nuclear regions receiving a major sensory input, and have been described in the nuclei of the spinal cord and brainstem in which primary afferent fibres terminate (e.g. Wolff & Němeček, 1971; Réthelyi & Szentágothai, 1973; Coimbra, Sodré-Borges & Magalhães, 1974), and in the olfactory bulb (Pinching & Powell, 1971*b*; White, 1973). But it is in the thalamus, especially in the sensory relay nuclei, that synaptic glomeruli have attracted the most attention (see, for example, Jones & Powell, 1969; Ralston & Herman, 1969; Szentágothai, 1970; Famiglietti & Peters, 1972).

The complex interneuronal relationships within synaptic glomeruli are difficult, if not impossible, to analyse fully from non-serial sections, and the need for three-dimensional studies has often been stressed. In the present study, some results of which have been reported previously in an abbreviated form (Lieberman & Špaček, 1971), we have investigated the three-dimensional relationships between the neuronal

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and glial components of synaptic glomeruli in the rat somatosensory thalamus, employing reconstruction and quantitative techniques with electron micrographs of serial sections.

MATERIALS AND METHODS

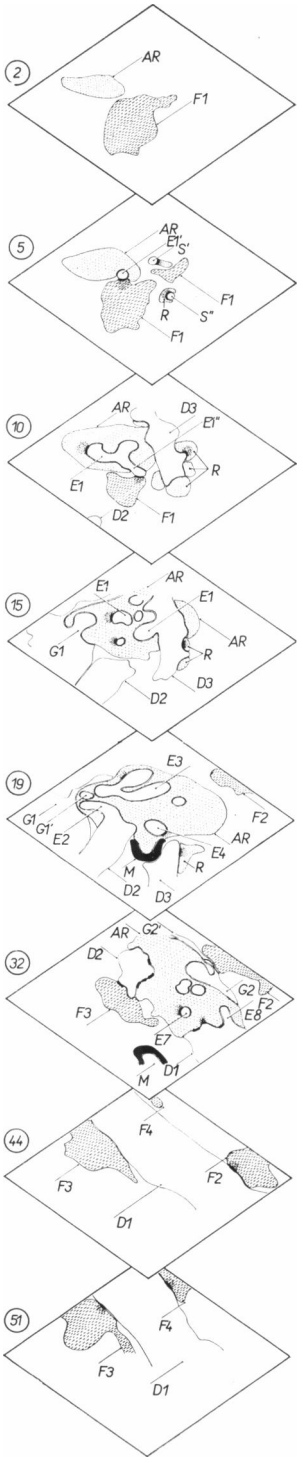
Young adult albino rats weighing about 200 g were used for both light and electron microscopy. A variety of Golgi impregnation techniques was used for light microscope studies and the light micrographs in Fig. 2 derive both from Golgi rapid and perfusion Kopsch preparations. Tissue for electron microscopy was prepared by perfusion of aldehyde solutions through the left cardiac ventricle of animals anaesthetized with ether. The reconstructions and electron micrographs shown here all derive from animals perfused with a high osmolarity, phosphate-buffered mixture of 4% paraformaldehyde and 0·5% glutaraldehyde at pH 7·3–7·4. Immediately following perfusion the brain was removed and thin (less than 1 mm) coronal slices were taken through the diencephalon at the level of the VB complex. Blocks of tissue removed under a dissecting microscope from the *pars externa* of this nucleus, and from the posterolateral complex *pars lateralis* (LP) (Webster & Lund, 1967) were placed in fresh fixative solution for about 1 hour and, if necessary, cut into smaller pieces. Cresyl violet-stained frozen sections of the coronal slices were used to confirm the accuracy of the sampling technique. After further fixation in ice-cold 2% osmium tetroxide in the same buffer for 1½–2 hours, tissue blocks were dehydrated in ethanol (or occasionally in acetone), stained for 1½ hours in 1% uranyl acetate in absolute ethanol (or occasionally acetone), passed through propylene oxide and embedded in Araldite.

Series of thin sections displaying pale gold interference colours, which would be about 100 nm thick according to Peachey (1958), were cut with glass knives on manual or automatic Porter–Blum microtomes. Ribbons were picked up on single aperture grids (LKB 4829 A-13; 1 mm × 2 mm aperture) bearing a Formvar film. After staining with lead citrate for 15 minutes at room temperature, the sections were supported by the evaporation on to their free surface of a thin film of carbon, and were studied and photographed in a Siemens Elmiskop IB electron microscope.

Fig. 1. Representative tracings of serial sections after transformation and tilting into a rectangular axonometric projection. The sections in this series (section numbers circled) were used to reconstruct the VB glomerulus shown in Figs. 15 and 16. For an explanation of the labelling, see Results, section B.

Key to labelling of micrographs. AR, axon terminal with spherical synaptic vesicles (lemniscal bouton); a, astrocyte process; D, dendrite; E, dendritic excrescence; F, axon terminal with cylindrical synaptic vesicles; f, neurofilaments; P, neuronal perikaryon; R, small axon terminal with spherical synaptic vesicles (in extraglomerular neuropil); r, ribosome cluster; →, synaptic contact; ▷, filamentous contact; O→, dendritic SER; ➔, axonal SER.

Fig. 2. Golgi impregnations of thalamocortical relay neurons in VB. (*a*) Dendritic excrescence clusters indicating the sites of synaptic glomeruli are arrowed. Finer non-clustered excrescences can be seen along some of the distal dendrites of this cell. Golgi-rapid, × 320. (*b*) Higher magnification of same cell showing one of the excrescence clusters (arrowed) at a different focal depth. × 1230. (*c*) Dendritic excrescences (arrowed) at the branch point of a large, smooth, stem dendrite. Golgi–Kopsch, × 1200. (*d*) The arrows indicate probable sites of synaptic glomeruli whose closely packed dendritic excrescences are not individually resolved, or only poorly so. Golgi-rapid, × 1230.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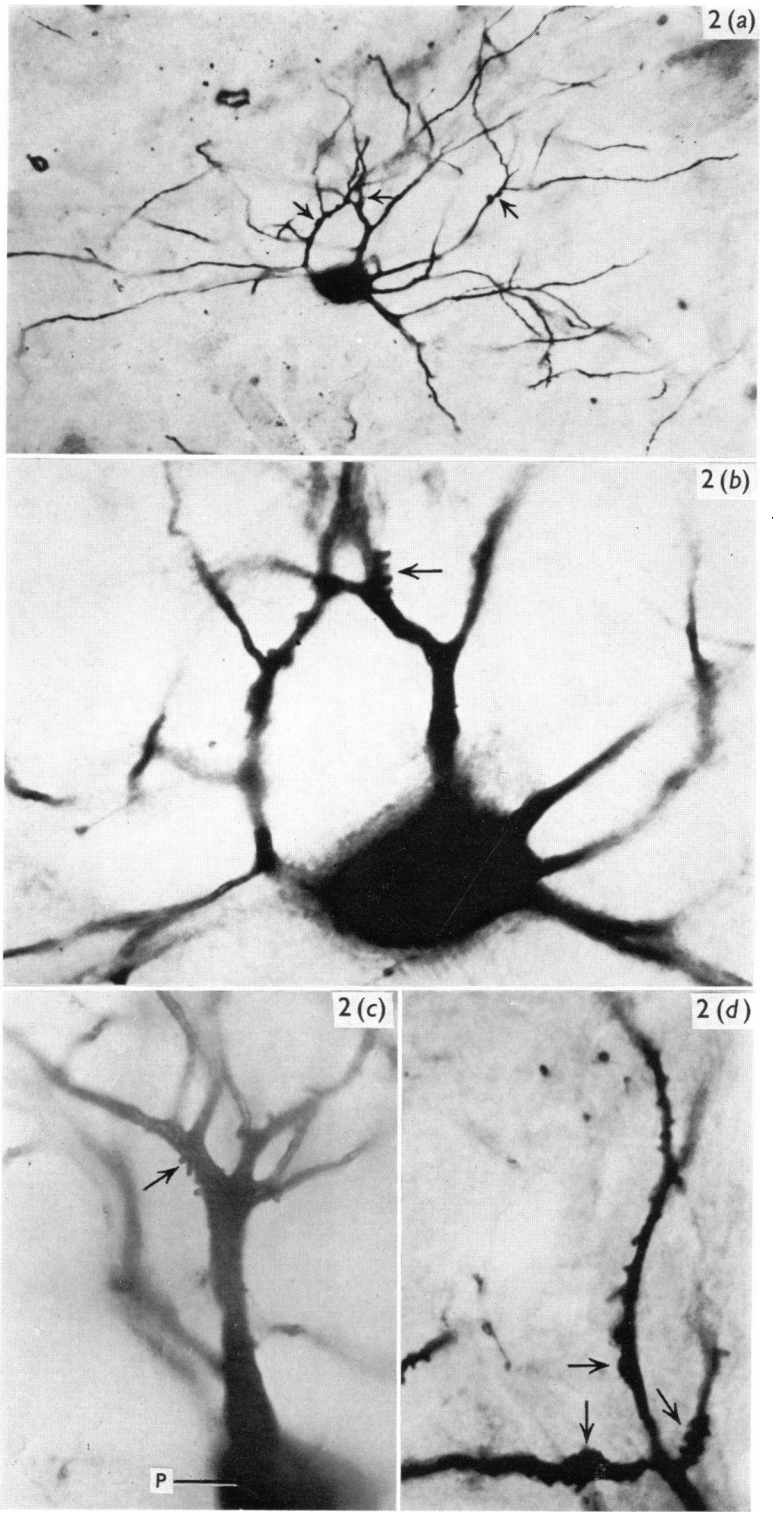
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2 (b)
2 (c)
P
2 (d)

Fig. 1.

Photographs for reconstruction purposes were taken at initial magnifications of ×12000 or ×18000. In none of the series from which reconstructions were made was a single section lost or obscured in such a way that a clear photographic record could not be produced. The series from which the graphic reconstructions in Figs. 15–17 and the drawings in Fig. 18 were made, and from which selected micrographs are shown in Figs. 11 and 12, comprised 60 sections. Reconstructions based on several shorter series of about 20 sections each, which only partially encompassed synaptic glomeruli, were used for comparative purposes, and the model in Fig. 20 was reconstructed from an unbroken series of 22 gold sections.

Reconstructions were based on every section in the series. Tracings of threefold photographic enlargements of the electron micrographs were transposed into oblique or rectangular axonometric projections (Fig. 1) and the aggregated profile outlines were joined and shaded to produce the graphic reconstructions. Solid reconstructions were made from outlines traced on to and cut from thin metal foil sheets, and subsequently soldered together at separations proportional to section thickness. The interstices of the model were filled with putty to produce a smooth surface contoured to the rims of the metal sheets.

Great care was taken to ensure as far as possible the correct mutual orientation of profiles in adjacent sections and to minimize distortion of the final reconstruction. Mitochondria appearing in several adjacent sections and not themselves subjects of a reconstruction attempt, or membranes cut perpendicularly in several adjacent sections, were used as reference points. Ensuring the correct orientation and superimposition of adjacent sections was a relatively minor problem because of the short vertical distance between adjacent levels in the reconstruction, and the fact that many clearly defined structures and relationships changed only slightly from section to section. Further details concerning the reconstruction techniques employed are to be found in Špaček & Lieberman (1974).

RESULTS

A. *General features of VB glomeruli*

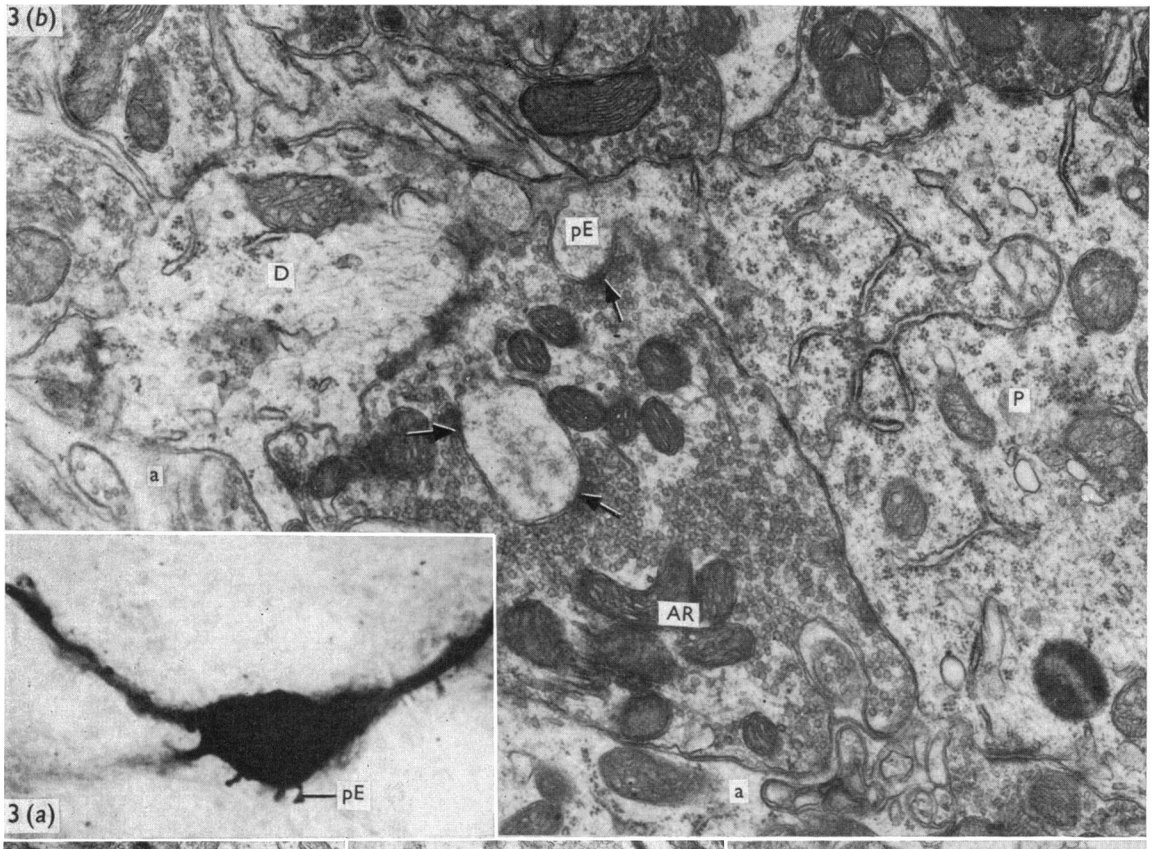
In the *pars externa* of VB, the synaptic glomeruli are ovoidal, spherical or hemispherical in shape and from 2 to 10 μm or more in diameter or long axis. There is clear evidence both from electron microscopy and from light microscopy of Golgi impregnations (e.g. Figs. 2*a–d*) that the glomeruli are associated primarily with the

Fig. 3. Golgi-rapid impregnation (Fig. 3*a*) and electron micrograph (Fig. 3*b*) showing perikaryal excrescences (pE). In Fig. 3(*b*) the perikaryal excrescence is clearly associated with a synaptic glomerulus. Fig. 3(*a*) ×1230; Fig. 3(*b*) ×19800.

Fig. 4. Part of a small glomerulus showing the AR terminal, dendritic excrescences and a loosely arranged multilamellate astrocytic sheath. ×16000.

Fig. 5. Part of a synaptic glomerulus showing a lemniscal bouton invaginated by a series of excrescences originating from a dendrite of large diameter. ×15000.

Fig. 6. Part of a large synaptic glomerulus sectioned parallel to the principal dendrite and perpendicular to the large number of dendritic excrescences invaginating the AR bouton (both profiles of which are part of a single large terminal). The asterisk indicates a glial protrusion into the AR bouton. ×12000.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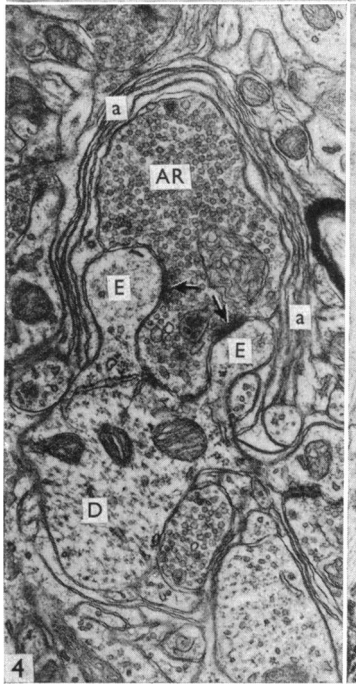
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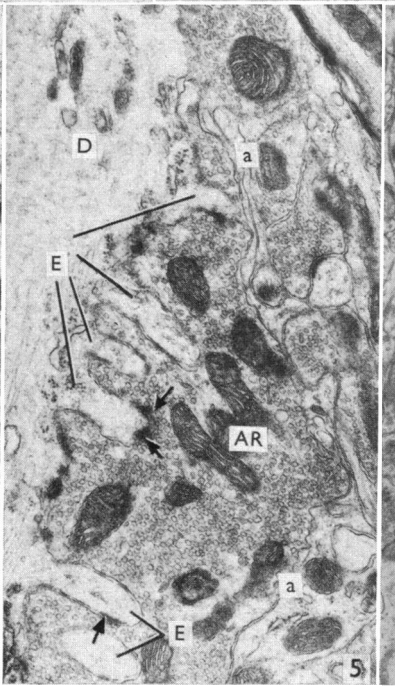
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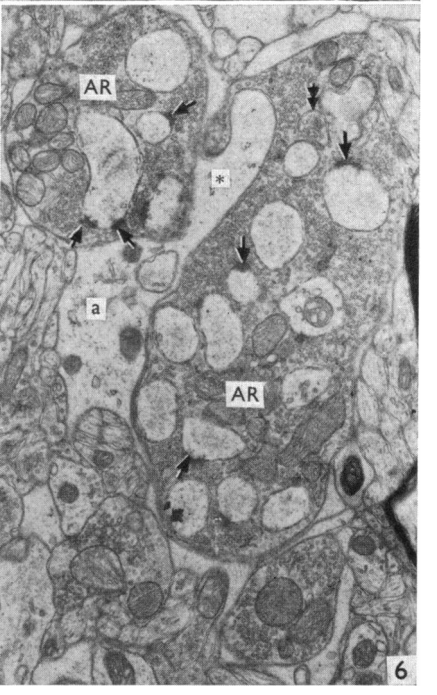
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proximal portions of the dendritic arbors of the thalamo-cortical relay (TCR) neurons, and even occasionally with cell bodies (Figs. 3*a* and *b*). They are characterized by the following principal features, not all of which will be seen in every section passing through a glomerulus.

Lemniscal axon terminal: AR bouton

The most conspicuous component of the glomerulus is a large axonal bag containing spherical vesicles with a diameter of approximately 50 nm. These boutons often occupy almost the entire cross-sectional area of glomeruli cut through their equatorial region and are referred to here as AR boutons (Figs. 3*b*, 4–6). Many AR boutons arise from myelinated preterminal axons of about 1 μm overall diameter (Fig. 11 and inset), but it is not yet clear what proportion are true terminals as opposed to boutons *en passant*. AR boutons undergo rapid degeneration and astrocytic engulfment after ablation of the contralateral dorsal column nuclei (Lieberman & Webster, unpublished). In addition to the synaptic vesicles, which are tightly packed in the boutons, there are large mitochondria distributed in irregular clumps, and often some approximately 100 nm diameter dense-cored vesicles, coated vesicles, multivesicular bodies, and electron-dense, lysosome-like bodies. AR boutons contain very few microtubules or neurofilaments although both are present in their preterminal axons.

In most AR boutons there is an extensive system of smooth-surfaced tubules, sacs and cisterns, probably components of the agranular endoplasmic reticulum (Figs. 7–9, wide arrows). The cisterns are commonly fenestrated, and are usually prominent close to and parallel with the boutonal plasmalemma in the vicinity of specialized, non-synaptic, axodendritic junctions (see below).

Membranous elements of quite a different kind are also present in AR boutons. These are unfenestrated flattened sacs with particularly prominent membranes, arranged as short, straight, narrow cisterns (commonly below and parallel to segments of unspecialized plasma membrane) or as curved or cup-shaped cisterns enclosing a group of vesicles whose profiles are commonly less regular than those of the main body of synaptic vesicles (Figs. 6, 11 at double-headed arrows). Occasionally one or both 'ends' of such profiles display a vesicular expansion and at such sites the membrane frequently appears to be coated with a material similar to that of coated vesicles.

Dendrites and dendritic excrescences

Invaginated into the AR boutons are protrusions originating in clusters from the surface of the dendrite (or dendrites) with which the AR bouton is in contact. These protrusions contain no distinct spine apparatus (although tubules of agranular endoplasmic reticulum may run into them from the dendritic shaft) and seldom have the characteristic shape of cortical spines: they are referred to here as excrescences. The excrescences have a clear counterpart in Golgi preparations (Figs. 2*a–d*, arrows), and while some arise directly from the surface of the dendrite (simple excrescences) others branch from a thick stem (compound or ramified excrescences). Although the excrescences are low in organelle content, the dendrites from which they arise are generally large and rich in organelles (Figs. 4, 5, 7). It is

sometimes apparent that mitochondrial concentration is greater in segments of dendrites from which excrescence clusters arise than elsewhere.

Commonly found in the dendrites at the site of a synaptic glomerulus is a peculiar organelle, the multitubular body (Lieberman, Špaček & Webster, 1971) often associated with smooth endoplasmic cisternae running below the dendritic plasma membrane in relation to the paramembranous densities of the non-synaptic membrane specializations established with the AR boutons (see below).

Specialized contacts between AR boutons and dendritic components

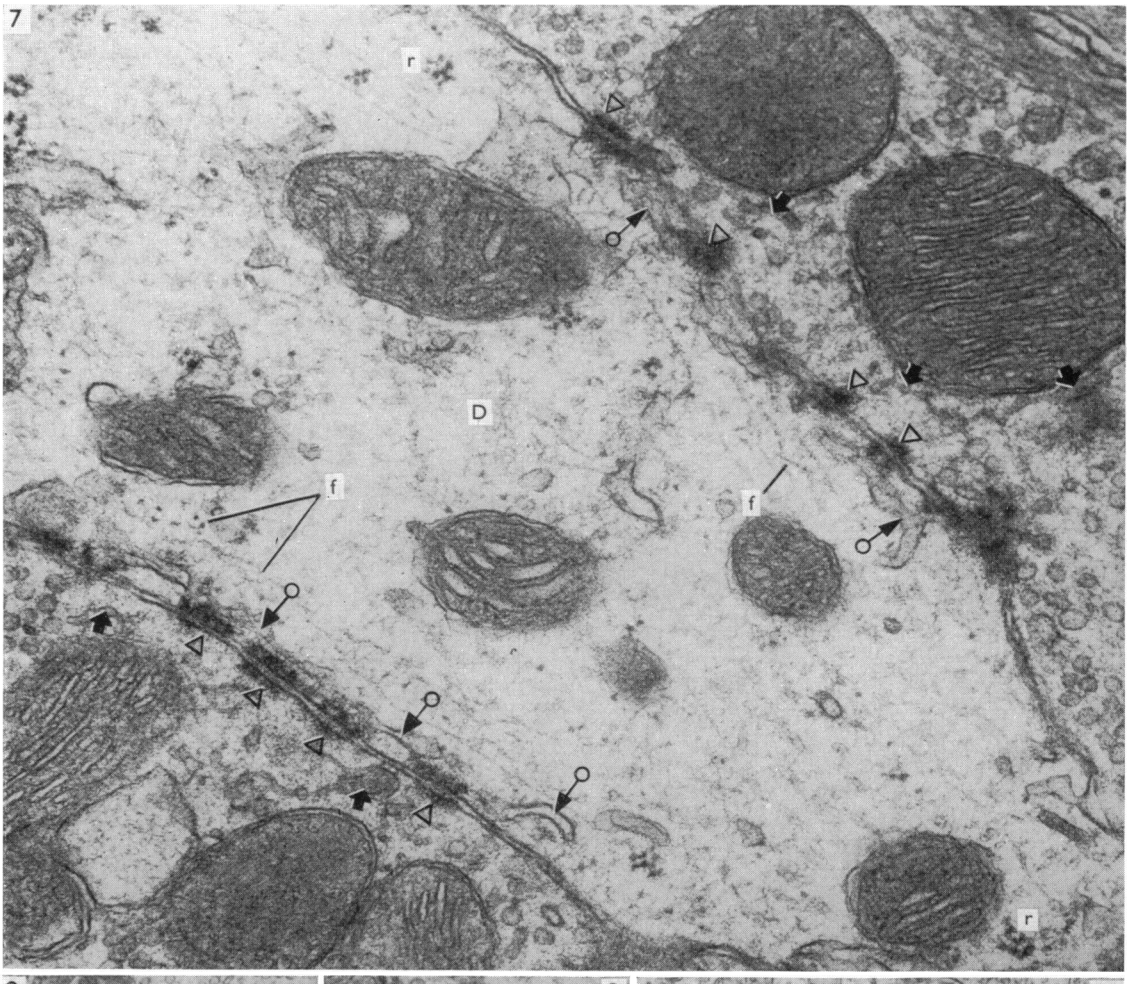
(i) *Synapses.* The excrescences receive at least one and generally a number of synaptic contacts with Gray type I morphology. The synaptic cleft, within which both radial sub-units and an intermembranous line or plate can sometimes be resolved, is slightly widened, while presynaptic dense projections associated with synaptic vesicle clusters, as well as a continuous postsynaptic density, are both prominent (Figs. 3*b*, 4, 6, 11, 21, arrows).

(ii) *Filamentous contacts.* The area of apposition between the smooth surface of the dendrite and the AR bouton rarely shows synaptic contacts, but is characterized instead by non-synaptic specializations, resembling the filamentous contacts of Colonnier & Guillery (1964) and the adhesion plaques of other authors (e.g. Jones & Powell, 1969). The filamentous contacts (Fig. 7, at arrow heads) consist of co-extensive stretches of apposed axonal and dendritic plasma membranes associated with electron-dense paramembranous material, on either side of a widened intercellular space containing an intermediate lamina of electron-dense material similar to that seen at synaptic contacts. Filamentous contacts are clearly asymmetrical, with the dense material extending more deeply into the dendritic than into the axonal cytoplasm (Fig. 7; see also Peters & Palay, 1966; Jones & Powell, 1969; Guillery, 1967). Furthermore, the paramembranous dense material is not homogeneous but comprises sub-units of slightly irregular size (Figs. 7, 9; cf. Gray & Guillery, 1966) not always symmetrically arranged on either side of the contact.

The filamentous contacts are not punctate or plaque-like as perpendicular sections suggest and many authors have concluded (e.g. Peters & Palay, 1966; Pappas, Cohen & Purpura, 1966). Three-dimensional reconstruction and single sections passing tangentially along an interface with filamentous contacts (Figs. 12, 13) show that the contacts between AR boutons and dendritic shafts seen in perpendicular sections constitute part of a reticulum, and that the entire interface is characterized by an extensive, interconnected, reticular filamentous contact.

On the dendritic side of the contact, 7–10 nm filaments are often prominent (f, Fig. 7; see Colonnier & Guillery, 1964, and Guillery, 1967). The filaments sometimes run in one or more directions parallel with the contacts and sometimes appear to insert into the paramembranous densities. In the dendritic cytoplasm there is also an elaborate system of tubules (and some narrow cisterns) of smooth-surfaced endoplasmic reticulum (SER) the complexity and extent of which can seldom be appreciated from single sections. Components of this system are indicated by ringed arrows in Figs. 7–9. In places the SER tubules are continuous with cisterns of granular ER and with multitubular bodies.

Commonly the fenestrated SER system of the AR boutons lies directly opposite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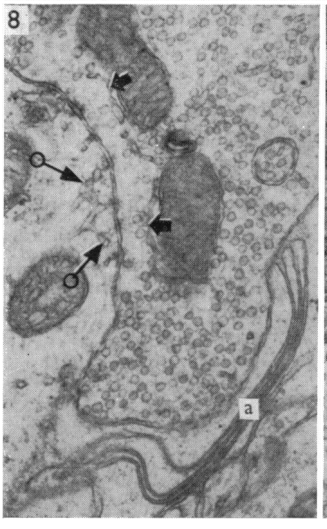
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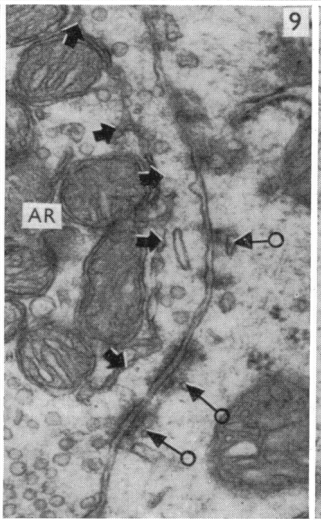
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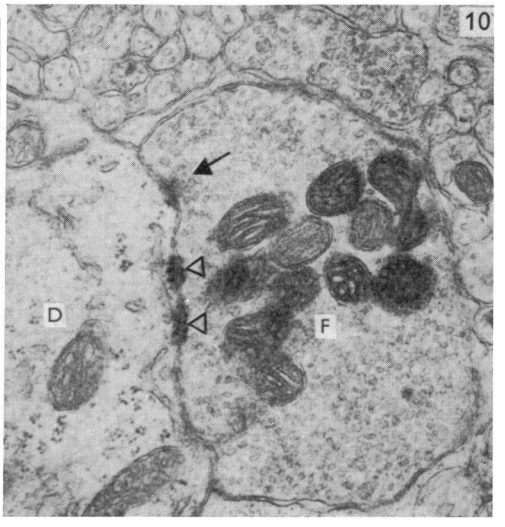
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the filamentous contact and the associated dendritic SER tubules, although the axonal SER always lies further from the contact zone than the dendritic SER (Figs. 7–9). Filamentous contacts differ in a number of respects from the apparently plaque-like non-synaptic specializations found at dendrite-to-dendrite, dendrite-to-soma, and soma-to-soma plasma membrane contacts in this tissue (e.g. Fig. 14). Such 'attachment plaques' are symmetrical, both with respect to the extent and homogeneity of the paramembranous dense material and to the associated SER elements which are generally situated equidistant from the two apposed plasma membranes.

Axon terminals containing flattened synaptic vesicles

Other axonal boutons, containing flattened synaptic vesicles (F-boutons) also participate in the glomeruli (F, Figs. 10, 11, 21). With the fixation and staining techniques used in this study the mitochondria of F-boutons display a particularly electron-dense matrix and closely packed cristae orientated along their long axis. These boutons do not degenerate after lesions of the dorsal column nuclei nor after ablation of the somatosensory cortex (unpublished studies of Lieberman, Webster, Winfield & Yiu). Some, at least, arise from myelinated axons, and many are clearly *en passant* rather than terminal boutons.

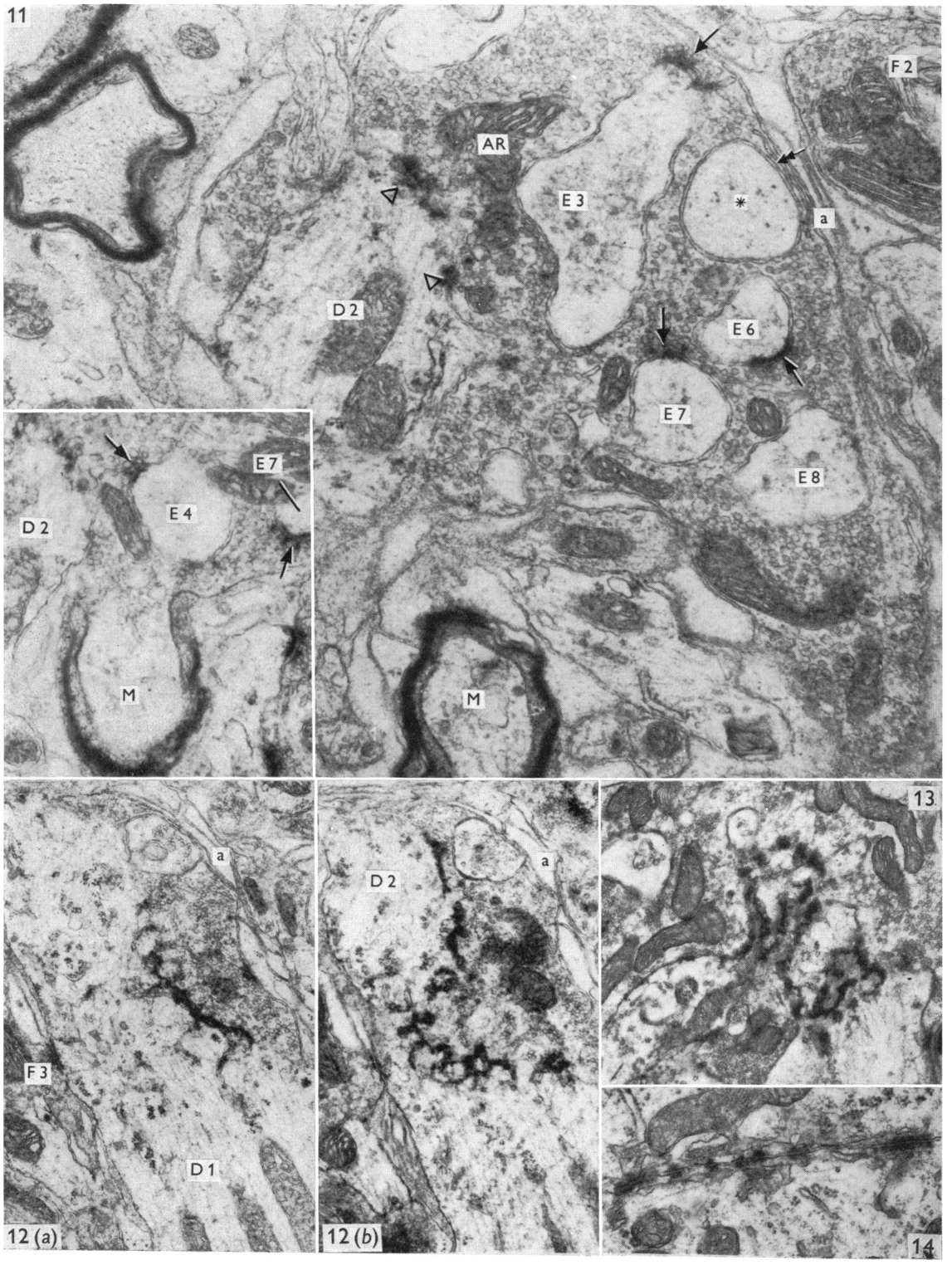
The F-boutons establish synaptic contacts (and also filamentous contacts) with the smooth surface of the glomerular dendrites (Fig. 10). While some boutons are within or partially within the glial capsule of the glomerulus, others lie technically outside the glomeruli, contacting the smooth surface of the dendrite opposite that generating the excrescences. F-boutons are not exclusively associated with glomeruli or juxta-glomerular dendrites: they also establish frequent synaptic contacts with dendrites and spine-like dendritic protrusions in the extraglomerular neuropil and with the perikarya and initial axon segments of the TCR cells. The synaptic specializations generally show only moderate widening of the cleft, have vesicle clusters associated with the presynaptic dense projections, and a post-synaptic density which though continuous, is far less marked than that of the AR bouton synapses. F-boutons are often of extremely irregular form and are quite large (1–4 μm). They are only very rarely invaginated by dendritic excrescences and they do not receive synaptic contacts from AR boutons or other presynaptic elements.

Fig. 7. Part of a large glomerulus showing filamentous contacts between the dendrite and the AR bouton (▷). Dendritic filaments, and axonal and dendritic SER elements are all indicated. × 51000.

Fig. 8. Part of a synaptic glomerulus with a partly multilamellate astrocytic sheath (at bottom). SER systems of the axon terminal and the dendrite on either side of a filamentous contact are indicated. × 21600.

Fig. 9. Part of a glomerulus showing the filamentous contact between an AR bouton and a dendritic shaft. The close relationship between mitochondria and the boutonal SER is apparent. Here, as in Fig. 8, the dendritic SER can be seen to lie closer to the filamentous contacts than does the axonal SER. × 34000.

Fig. 10. F-bouton contacting a large dendrite and establishing a synaptic contact and two filamentous contacts. × 25500.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a
12 (b)
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14

The final component of the glomerulus, the glial capsule surrounding the neural elements, is described in section C of the Results.

B. *Three-dimensional reconstructions of glomeruli*

One glomerulus was reconstructed graphically from a series of 60 consecutive sections encompassing the entire glomerulus. Graphic and solid reconstructions of parts of several other glomeruli were also prepared.

Completely reconstructed glomerulus

(i) *General description.* The glomerulus was of medium size (approximately $6 \times 4 \times 4$ μm excluding the glial capsule). It was situated at the branch point of a large dendrite about 2 μm in diameter, almost certainly a primary or stem dendrite of a TCR cell (D 1 and D 2, Figs. 11 and 12). The dendritic components of the glomerulus are reconstructed in Fig. 15(*b*) and in Fig. 16(*b*) the large axon terminal (AR bouton) of the glomerulus is also shown in a more complete reconstruction. In the interests of clarity the glial envelope and four axon terminals containing flattened synaptic vesicles (F-boutons) have been omitted from both reconstructions.

The arrangement of the glomerular components can best be understood by first referring to the orientation diagrams inset into Figs. 15(*a*) and 16(*a*) and to the labelled, key drawings of the reconstructions in Figs. 15(*a*) and 16(*a*).

(ii) *The AR bouton.* The single large AR bouton originated from a myelinated preterminal axon immediately adjacent to the glomerulus and expanded to cover an extensive surface area of the main dendrite D 1, its branch D 2 generated within the glomerulus, and a smaller dendrite D 3 about 0·7 μm in diameter and not apparently connected with either D 1 or D 2.

At its origin from the preterminal axon the AR bouton expanded into a large sac lying on the surface of D 1 and wrapped around D 2. It was indented by excrescences E 1–4 and then bent around dendrite D 3 to envelop excrescences E 5–E 10 and to lie upon the surface of D 1 very close to the preterminal axon (M, Fig. 16*a*).

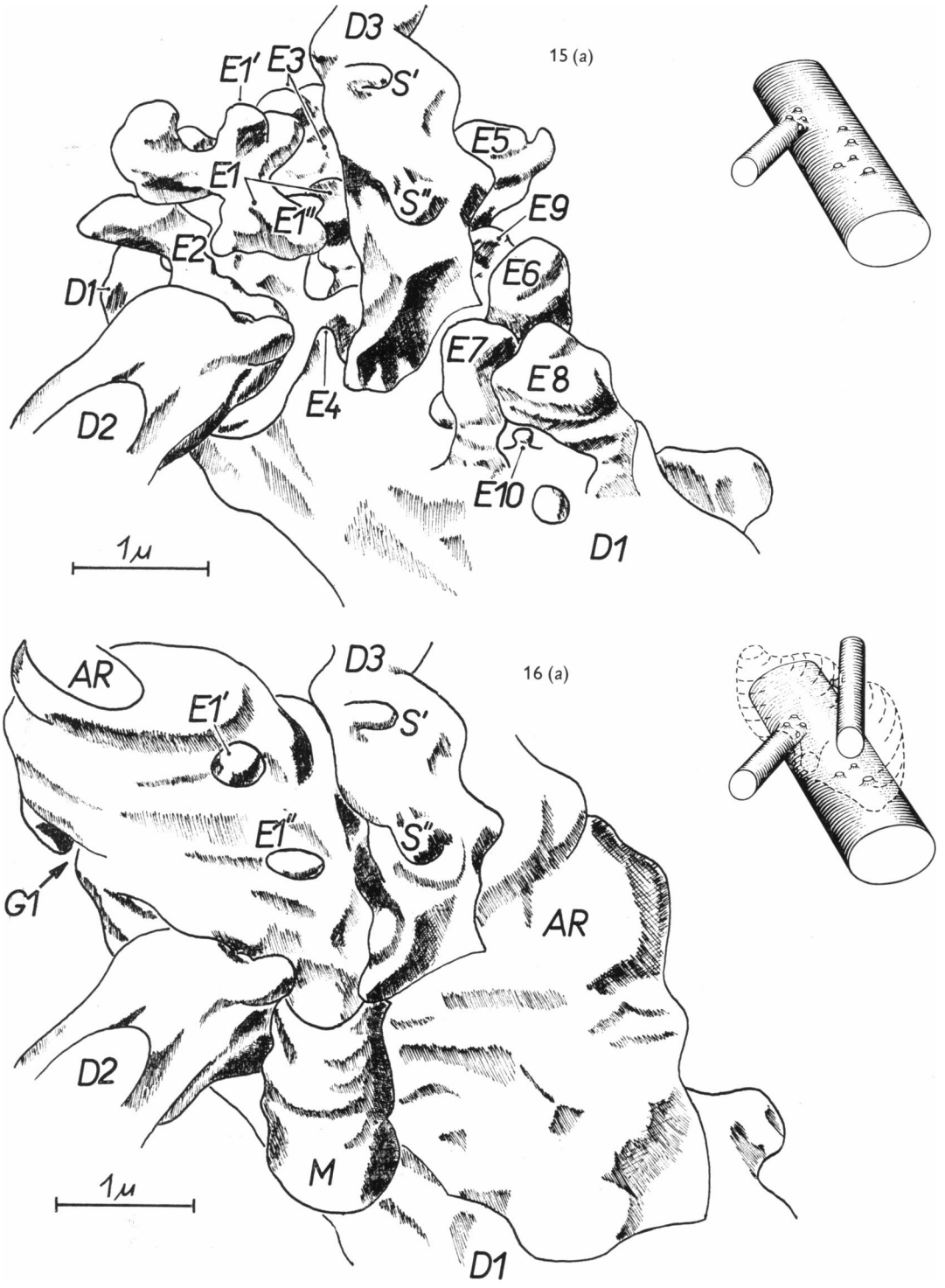
Almost the entire area of contact between the shafts of D 1 and D 2 and the AR bouton was characterized by a reticularly arranged filamentous contact (Figs. 11, 12).

Fig. 11. Section 27 of the series from which the reconstructions in Figs. 15 and 16 were made. The myelinated fibre (M) is the preterminal of the glomerular AR bouton and is shown at the termination of the myelin sheath in the inset (section 21). The labelling of the excrescences and dendrites corresponds to that in Figs. 15 (*a*) and 16 (*a*). The asterisk indicates a glial protrusion into the AR bouton (see G 2 in section 32 of Fig. 1 and Špaček & Lieberman, 1971). × 27600.

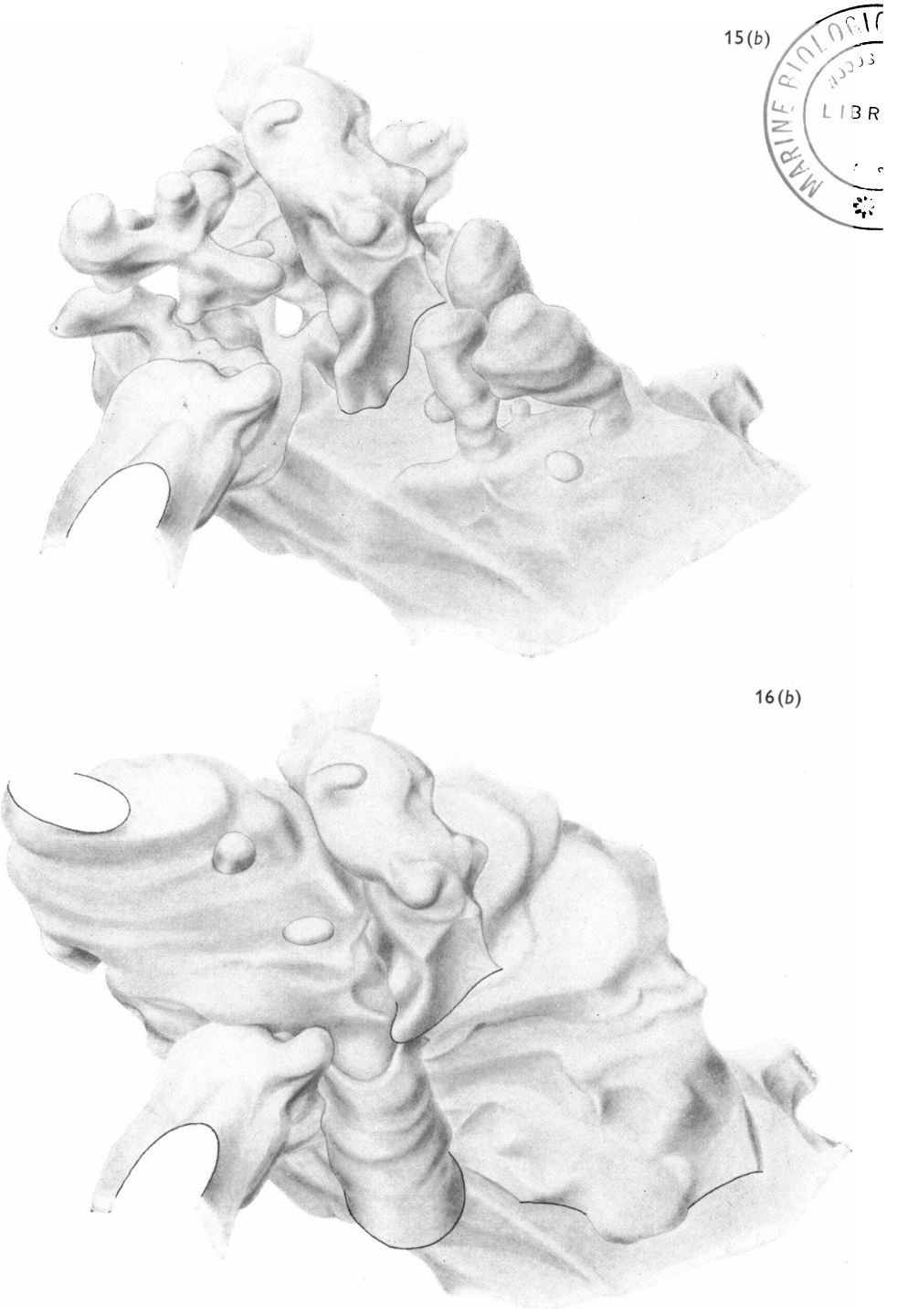
Fig. 12. Immediately serial sections (37 and 38) from the same series at the level of separation of D 2 and D 1. The reticular arrangement of the filamentous contact between the surface of the dendrites and the AR bouton is apparent. × 16800.

Fig. 13. Part of a large glomerulus showing an extensive area of apposition between an AR bouton and a dendritic shaft. Some portions of the interface are sectioned perpendicularly but in the centre of the micrograph tangential sectioning shows the reticularly arranged filamentous contact. × 10900.

Fig. 14. Symmetrical intercellular specializations between two large cell bodies. Differences between these contacts (symmetry of paramembranous dense material and SER cisterns) and filamentous contacts are apparent. × 26000.



Figs. 15 and 16. Orientation guides (insets), key drawings (Figs. 15*a*, 16*a*) and graphic reconstructions (Figs. 15*b*, 16*b*) of the dendritic components (Fig. 15) and of the dendritic components together with the lemniscal bouton and its preterminal axon (Fig. 16) of a synaptic glomerulus in VB.



Figs. 15(*b*) and 16(*b*). For legend see opposite.

Table 1. *Source, form, surface to volume ratio, number of synaptic contacts and percentage surface area specialized as postsynaptic membrane of excrescences E1–E10*

Excrescence number	Dendrite of origin	Form of excrescence	Number of synaptic contacts	Surface to volume ratio	Percentage synaptic surface area
E 1	D 3	Compound (5)	10	24	7·8
E 2	D 2	Simple (1)	2	21	7·0
E 3	D 2	Compound (3)	8	20	5·6
E 4	D 2	Compound (2)	7	22	9·6
E 5	D 2	Compound (2)	8	27	6·5
E 6	D 1	Simple (1)	1	27	5·7
E 7	D 1	Simple (1)	4	24	7·3
E 8	D 1	Simple (1)	3	20	5·2
E 9	D 1	Simple (1)	2	30	4·4
E 10	D 1	Simple (1)	1	36	25·0

Filamentous contacts were not however established with the dendritic excrescences or with the surface of D 3.

Summated planimeter readings were used to estimate the percentage volume of the AR bouton occupied by excrescences (13·4%), mitochondria (11·6%) and by glial protrusions (1·8%; see section C below).

A small segment of the bouton extended beyond the level of the reconstruction (see AR at top left of Figs. 16*a* and *b*) and this may have marked the origin of an interterminal axon leading from the reconstructed AR bouton to another or to a series of such boutons.

(iii) *Dendritic excrescences and their synaptic contacts with the AR bouton.* Six simple and four compound or ramified excrescences originated from D 1, D 2 and D 3. All were invaginated into the AR bouton and received from it a total of 44 distinct synaptic contacts, all of them circular or oval in shape. The compound excrescences displayed between two and five distinct branches or heads (figures in parentheses in column 3 of Table 1) and the excrescences received between 1 and 10 distinct synaptic contacts (column 4 of Table 1). The relative volumes of the excrescences (obtained by summating planimeter values for the areas of profiles in all sections containing them) and estimates of the surface areas of excrescences and of synaptic specializations (obtained by summating map reader – measured lengths of excrescence profiles and synaptic membranes) were determined, and the surface to volume ratios and the percentage surface area of each excrescence occupied by synaptic specializations were calculated (Table 1).

If excrescence E 10 is excluded,* the surface to volume ratios for the excrescences are rather constant and the percentage surface area specialized as synaptic membrane is of the same order for all the excrescences, viz. between 4·5 and 9·5%. The distribution of these synaptic contacts is shown in Fig. 17 (black stipple). It is worth emphasizing that although synaptic contacts between AR boutons and the

* Excrescence E 10 was an unusually small evagination of the surface membrane of D 1 (Fig. 15). Although it received a synaptic contact from the AR bouton and can thus be accepted as a differentiation comparable to the larger excrescences, it was clearly atypical. Moreover the error in estimating the relative volume and surface area of such a small evagination was probably very great.

dendritic shaft do occur in some glomeruli, they are relatively uncommon, and in the reconstructed glomerulus no synaptic contacts were established between the AR bouton and the surface of any of the three dendrites involved.

(iv) *The F-boutons.* Of the four F-boutons associated with the glomerulus, only two, F 1 and F 3, established synaptic contact within the astrocytic capsule of the glomerulus proper, and all four also established synaptic contact with nearby non-glomerular dendrites. However, since F-boutons are invariably found establishing synaptic contact with dendritic shafts immediately adjacent to excrescence clusters, and often partly within the periglomerular astrocytic capsule, they are legitimately considered as components of the glomerular complex.

Bouton F 1 established synaptic contacts with D 2 and D 3, the contacts with the latter lying within the glomerulus proper. Boutons F 2 and F 4 each made 3 synaptic contacts with D 1, and bouton F 3 established 4 synaptic contacts with D 1, two with D 2 and an adhaerens-like macula with both dendrites, all within the glomerulus proper. The synaptic areas of the F-boutons were circular or oval and generally 0·3–0·4 μm in diameter: some oval synaptic areas were up to 0·7 μm in long diameter.

Two of the contacts made by F 1 were particularly interesting, contributing to an unusual arrangement of synaptic contacts at the tips of two branches of the compound excrescence E 1. These excrescence sub-units (E 1′ and E 1″ in Figs. 1 and 15) received synaptic contacts from the AR bouton before protruding from within the latter to receive the F 1 synaptic contacts at their tips (see Fig. 1, levels 5 and 10 and Figs. 16 and 17).

In the largest of the F-boutons (F 3) mitochondria accounted for 40·9% of the volume of the terminal.

Two of the F-boutons (F 3 and F 4) showed similar, interesting relationships to axons of unknown origin and uncertain type, invaginating them and passing between them and the surface of the dendrites they contacted. In Fig. 18 are drawings from four sections of the series, illustrating the relationship between F 4, the invaginating axon (I) and the surface of D 1. The axon was about 0·1 μm in diameter and contained somewhat irregular but predominantly spherical synaptic vesicles (Fig. 18*a*). Within F 4 the axon enlarged to an approximately spherical dilatation of about 0·4 μm diameter containing vesicles and some irregular smooth surfaced cisterns (Fig. 18*b* and *c*) and subsequently narrowed to its original diameter (Fig. 18*d*). Neither this axon nor the slightly larger one (diameter approx. 0·3 μm), passing between D 2 and F 3 and invaginating the latter, established any synaptic contact with the dendritic shaft, and neither showed any synaptic relationship with the F-boutons. These were, then, simply axons 'de passage'.

Partially reconstructed glomeruli

Results obtained from graphic and solid reconstructions of parts of other glomeruli were in close agreement with those already described. In Figs. 20(*a*)–(*c*) photographs from different aspects of a model of the principal dendritic component and derived excrescences of part of a VB glomerulus are shown. The series of sections from which the reconstruction was made passed approximately perpendicular to the long axis of an elongate, presumably ovoid glomerulus with a circular to oval cross-sectional

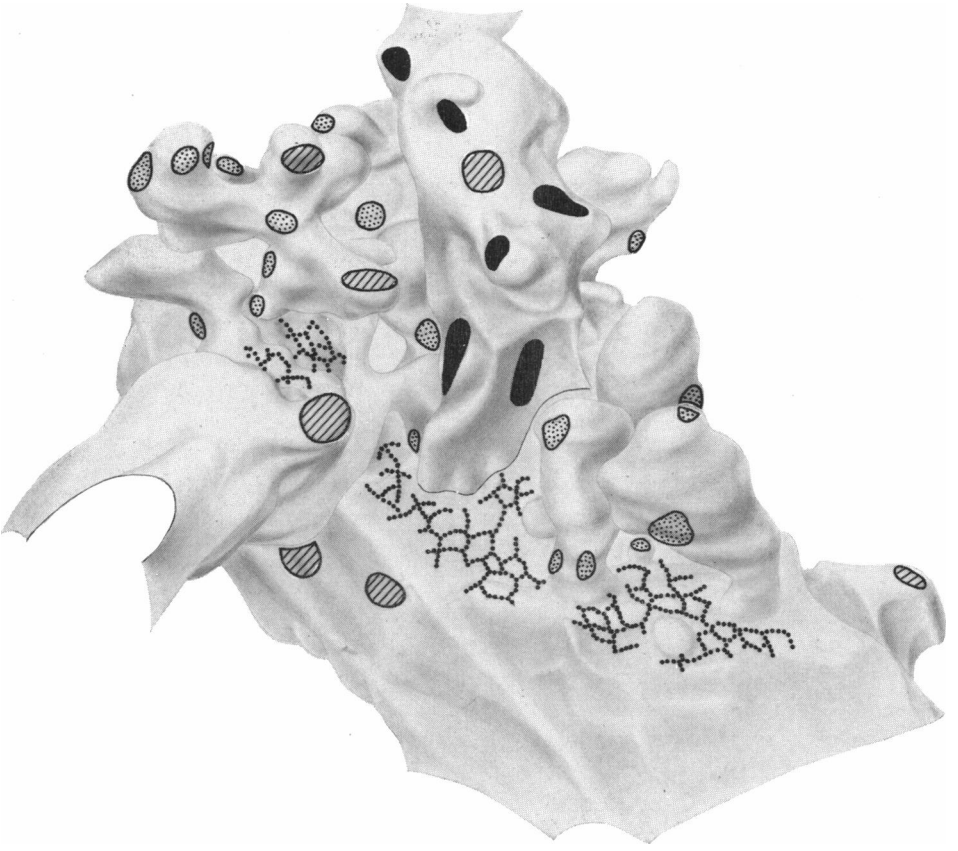


Fig. 17

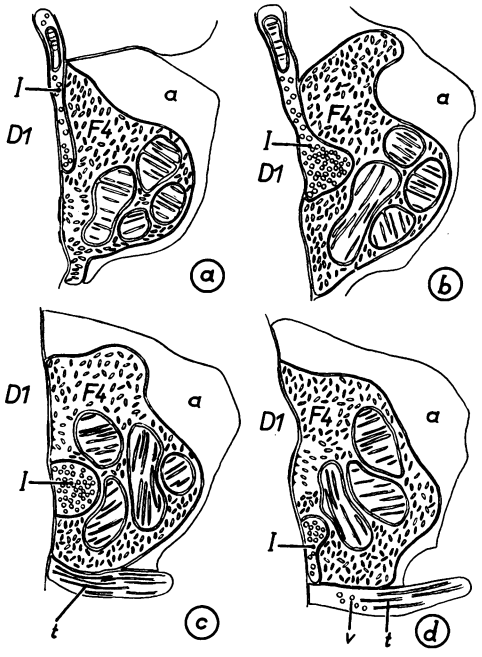
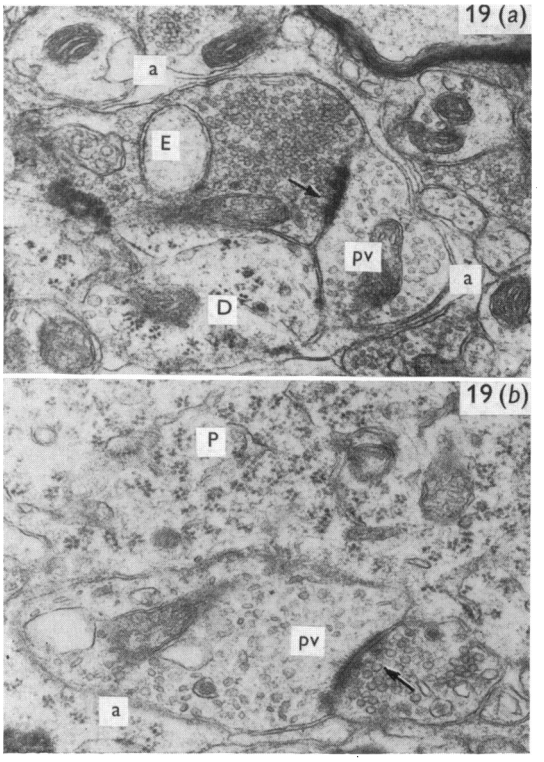


Fig. 18



profile and a diameter of 3–3½ μm. Of the 5 excrescences reconstructed (all of which originated from the single dendrite forming the 'backbone' of the glomerulus at this level) 4 were compound and 1 simple. The excrescences were closely packed together and extensively interdigitated, and received a total of 21 discrete synaptic contacts from the AR bouton into which they were invaginated. A striking feature of this glomerulus was the deep invagination of the dendrite by an outpushing of the AR bouton (see Fig. 20*c*). In Fig. 21(*a*)–(*f*) are representative pairs of micrographs of the series from which the reconstruction was made. The completely invaginated portion of the AR bouton is shown in Fig. 21(*a*) and (*b*) (where arrow heads indicate the filamentous contacts between the dendrite and the invaginated AR bouton). Two of the F-boutons associated with the glomerulus are indicated in Fig. 21(*b*), (*e*) and (*f*).

C. *The glial capsule*

Around the large axon terminal and the other components of the glomerulus is a glial sheath formed by the processes of astrocytes (a, Figs. 3*b*, 4–6, 8, 11). In glomeruli of all sizes, the glial capsule is commonly arranged in an irregular cup-like or goblet-like fashion around the AR terminal and its associated elements, with the open mouth of the sheath apposed to the surface of the main dendritic branch from which arise the invaginating excrescences. The sheath is also interrupted at the point of entry of the preterminal axon of the AR bouton and of other elements participating in the glomerulus. In some micrographs the glial sheath is constituted by a single astrocytic lamella of rather irregular width and appearance, conforming at one surface to the contour of the glomerular components and at the other to that of surrounding neuropil elements. Multilamellate sheaths are extremely common, and examples are shown in Figs. 4, 8 and 22. The sheath of the glomerulus reconstructed in Figs. 15 and 16 was in part multilamellate (at a in Figs. 11, 12), but extensive areas of the surface of the glomerulus were related to only a single, but rather wide astrocyte process.

The individual lamellae of multilamellate glial sheaths are usually extremely attenuated (some are less than 25 nm thick), and practically organelle-free. Furthermore, the extracellular space between individual lamellae is frequently narrower than the normal 15 to 20 nm gap characteristic of aldehyde perfusion-fixed material. Commonly, this narrowing of the extracellular space is sufficiently marked to produce the appearance of membrane fusion so that the sheath takes on something of the appearance of loose myelin (Figs. 8, 22), particularly after dehydration and

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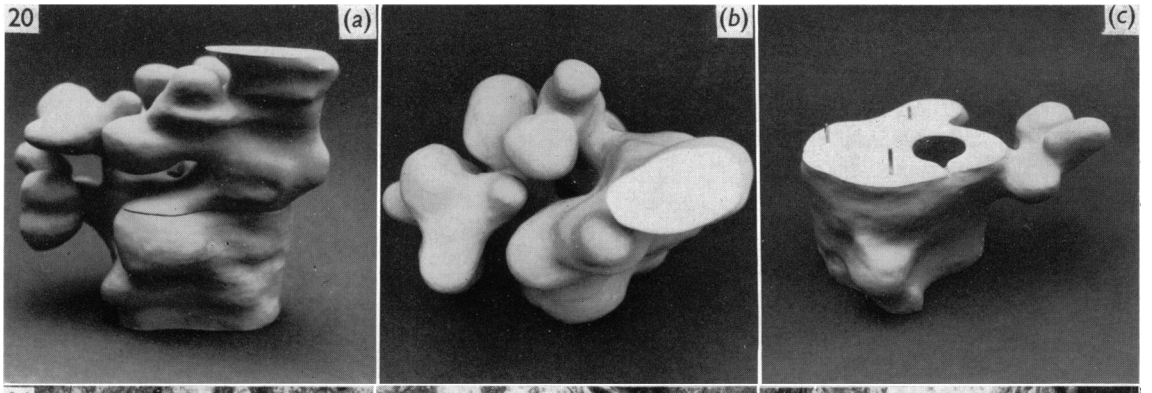
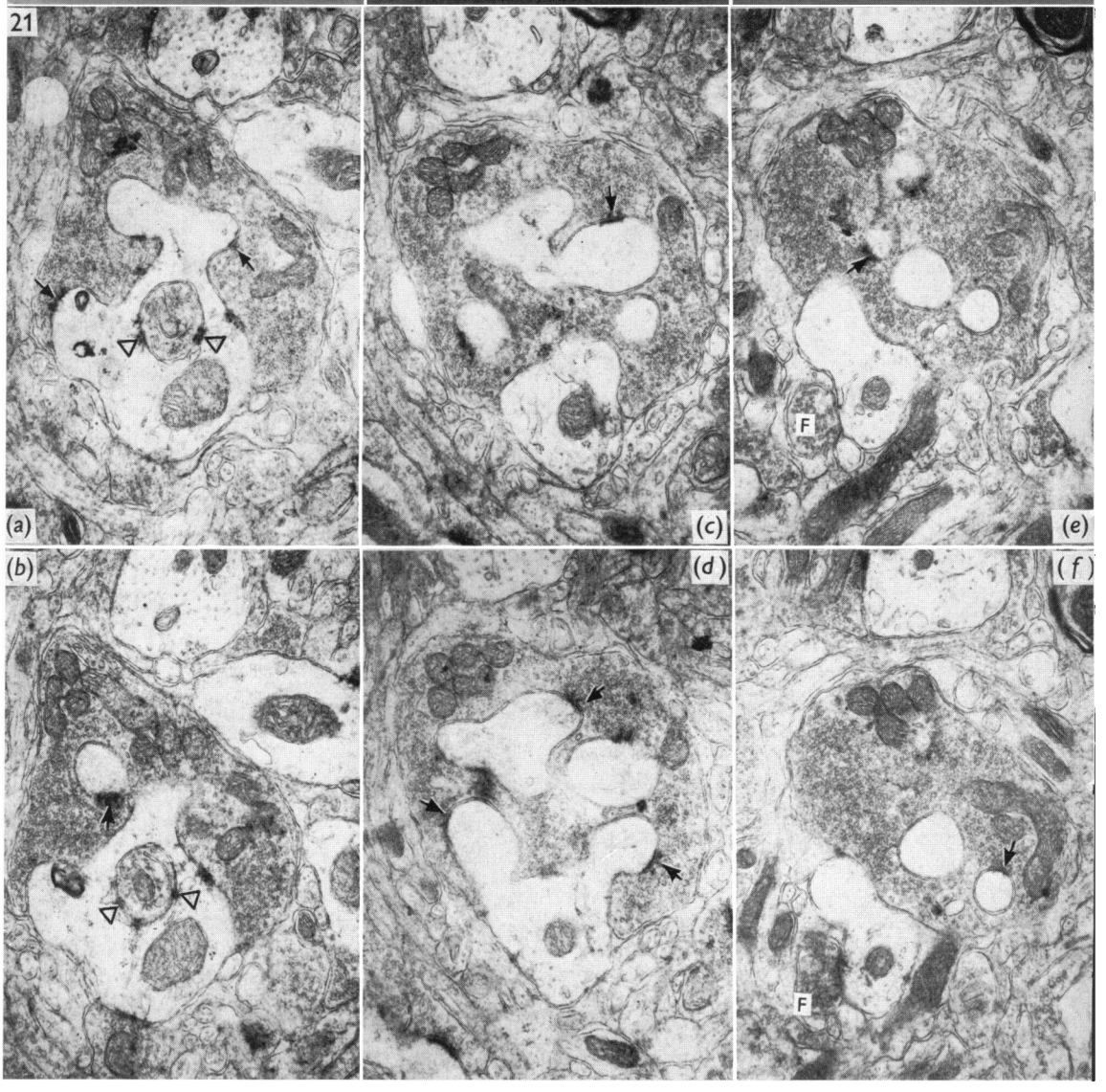
Fig. 17. Dendritic reconstruction as in Fig. 15(*b*), but with the filamentous contact and the synaptic contacts mapped to scale on the visible surfaces. ▦, synaptic contacts from the AR bouton; ▨, synaptic contacts from F-boutons; ■, synaptic contacts from small extraglomerular boutons containing spherical synaptic vesicles.

Fig. 18. Drawings of parts of four sections in a series showing the relationship between an F-terminal (F4), the dendritic shaft it contacts (D 1) and a small axon (I) passing between the two.

Fig. 19(*a*), (*b*). Sections from caudo-lateral portion of VB complex of two different animals showing rare bouton-bouton synapses between terminals containing spherical synaptic vesicles and pale profiles containing pleomorphic vesicles (pv). (*a*) ×27200; (*b*) ×26300.


20
(a)
(b)
(c)


21
(a)
(c)
F
(e)
(b)
(d)
(f)
F

block staining in acetone (see also Colonnier & Guillery, 1964; Karlsson, 1967). We have, however, never seen convincing evidence for a spiral arrangement of the laminae of multilamellate sheaths. Although Karlsson (1967) describes the multilamellate sheaths of synaptic glomeruli in rat LGd as spirally arranged, his illustration of a multilamellate sheath (Fig. 10 of Karlsson, 1967) shows no continuity between any of the seven astrocytic laminae that partially or completely surround the glomerulus.

Fig. 22 is a drawing based on a number of electron micrographs of serial sections extending approximately 1 μm through a medium-sized glomerulus in LP. Although the arrangement of the multitubular body (mtb) and of the dendritic excrescences (E) and their synapses with the AR bouton changed dramatically through the extent of the series, the glial sheath consisting of several individual concentric curved astrocytic laminae changed only slightly. In Fig. 22, three attenuated and more-or-less co-extensive laminae (L 1–3) are moulded closely to the contour of the AR bouton and are reflected from it on to the surface of the dendrite where the latter contacts the AR bouton. Another, short lamina (L 0) lies enclosed by L 1 at the left of the glomerulus. Outside L 1–3 is a fourth lamina (L 4), rather wider in places than the inner laminae, and becoming the innermost and only lamina after the termination of L 1–3 (at lower right). Both 'arms' of L 4 embracing the glomerulus are continuous with a stouter process applied to the basement lamina of an adjacent capillary (lumen at v). The termination of L 4 (bottom left) is only about 0·5 μm from the terminations of L 1–3 and is separated from them by a small F-bouton (F).

Periglomerular astrocytic laminae terminate in a most distinctive manner. At the termination or free edge of the lamina there is almost invariably an expansion, reminiscent of the terminal myelin loops found at nodes and heminodes in peripheral and central nerve fibres. Within the terminal expansion there is usually a profile of SER, and both terminal expansions of all four laminae in Fig. 22 contain an irregular, but more or less circular profile, which might be interpreted as vesicular but for the facts that such profiles are encountered with high frequency in sections passing through terminal expansions of periglomerular astrocyte laminae and that the dimensions of the profiles remain little changed when followed through several serial sections.

Thus our interpretation of multilamellate glomerular capsules is that they comprise separate flattened astrocytic processes, sometimes very extensive and cup-shaped, with a thickened free edge that is commonly occupied by a circumferential tubule or canaliculus of SER. Very similar features of periglomerular astrocyte processes have been recognized in LGd (personal observations). We are not aware of any previous description of these striking and consistent features of periglomerular

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Fig. 20(*a*)–(*c*). Photographs from different points of view of a model of a dendrite and the cluster of excrescences generated at the site of a synaptic glomerulus. The model photographed from the side in (*a*) and from above in (*b*), was built in two parts. In (*c*) (which is photographed from the side opposite to that in (*a*)) the model has been disarticulated to show the invagination of the dendrite by an outpushing of the AR bouton.

Fig. 21. Electron micrographs of pairs of immediately adjacent sections (*a*, *b*; *c*, *d*; *e*, *f*) at different levels in the series from which the model in Fig. 20 was reconstructed. × 12000.

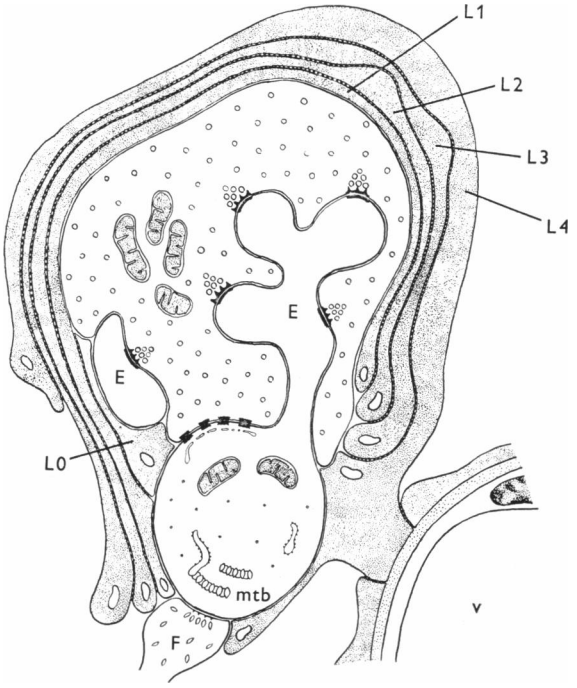

Fig. 22. Drawing based on tracings of serial sections of a synaptic glomerulus with a prominent multilamellate sheath in LP.

astrocytic processes. It is noteworthy that terminal loops of oligodendroglial cytoplasm at nodes or heminodes in this site also contain a somewhat similar tubule of SER (as well as one or more microtubules), spiralled around in the expanded free edge of the glial lamina (personal observations).

Periglomerular laminae have been traced to larger, typically astrocytic processes, and occasionally to astrocyte cell bodies but never to processes identifiable as oligodendroglial. Adjacent laminae in the multilamellate formations are probably derived from a single principal astrocytic process, although in some cases it appears that a single lamina may bend back on itself to constitute two adjacent laminae.

The innermost astrocytic laminae occasionally invaginate glomerular boutons in the form of irregular villiform or club-like protrusions; details and reconstructions of this relationship have been described elsewhere (Špaček & Lieberman, 1971).

DISCUSSION

The results obtained in this study confirm the value of three-dimensional reconstruction techniques in the analysis of C.N.S. tissue, particularly in regions of complex neuropil (see references in Špaček & Lieberman, 1974). Other studies in which effective use has been made of serial sections and reconstruction techniques in the

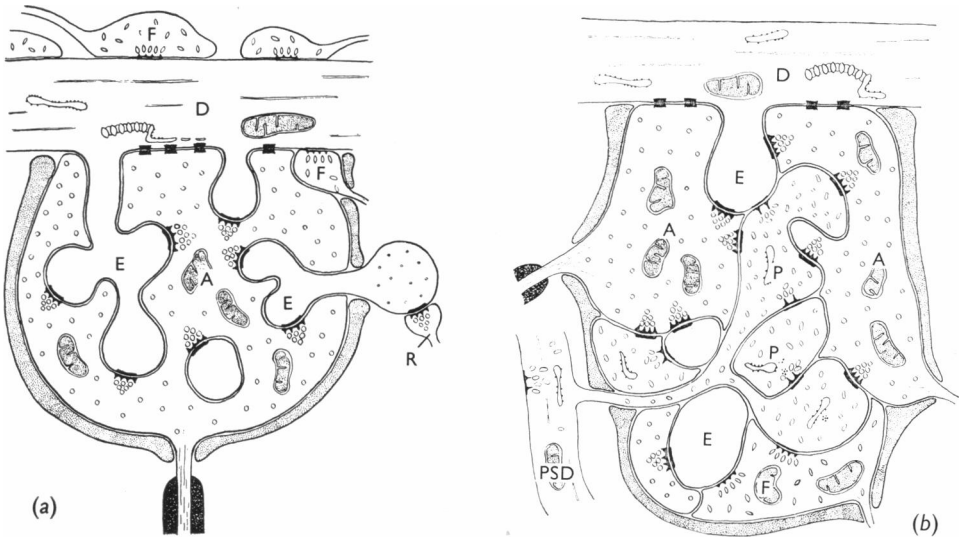

Fig. 23. Diagram summarizing the principal difference between glomerular organization in rat somatosensory thalamus (*a*) and in other mammalian sensory relay nuclei (*b*). (*b*) is based on studies of rat LGd by Lieberman & Webster (1974), cat LGd by Famiglietti & Peters (1972), cat medial geniculate nucleus by Morest (1971) and cat VB by Jones & Powell (1969) and Ralston & Herman (1969). See text for explanation of labelling.

analysis of synaptic glomeruli are those of Famiglietti & Peters (1972) and Harding (1973) on thalamic glomeruli and of Pinching & Powell (1971*b*) and White (1973) on olfactory glomeruli.

Basic organization of somatosensory glomeruli: comparisons with more complex glomeruli

Synaptic glomeruli of rat VB, though large by comparison with most simple synapses, and with an intricate geometrical arrangement of their constituent elements, are nevertheless rather simple. Only three classes of neural element appear to be involved and a relatively small number of each class participate in individual glomeruli.

A dendrite, or dendrites, of exclusively postsynaptic character which bears clusters of prominent excrescences constitutes the first component (D, E, Fig. 23*a*). The second component is a large lemniscal axon terminal, in contact with the dendrite(s), deeply invaginated by the excrescences, and making multiple synaptic contacts with the excrescences and filamentous contacts with the dendritic shaft (A, Fig. 23*a*). The final neural component comprises a small number of axon terminals of uncertain origin (see later) containing flattened synaptic vesicles and making synaptic contact with the dendritic shafts at the periphery of and immediately adjacent to the glomerulus (F, Fig. 23*a*). LP glomeruli are organized in a very similar way, but the origin of the AR bouton is less certain.

In the homologous nucleus of the cat thalamus, as well as in the LGd of all mammalian species so far studied, and in other thalamic relay nuclei of cat and monkey,

synaptic glomeruli are very much larger, and considerably more complex than in rat VB or LP (see for example, Jones & Powell, 1969, and Ralston & Herman, 1969, for cat VB; Guillery, 1971, for cat and monkey LGd; Lieberman & Webster, 1974, and Lieberman, 1973, for rat LGd; Jones & Powell, 1969, and Morest, 1971, for cat medial geniculate nucleus; Harding, 1973, for monkey ventrolateral nucleus). In the synaptic glomeruli of these nuclei there are at least four distinct classes of neural element (Fig. 23*b*). There are first of all specific afferent terminals (A), derived in some situations at least from more than a single axon (e.g. Famiglietti & Peters, 1972), relay cell dendrites (D) blunt protrusions, or somewhat smaller excrescences originating from the dendrites (E), flat-vesicle axon terminals (F) similar to the F-boutons of rat VB, and large numbers of boutons containing discoid synaptic vesicles (P) and derived from presynaptic dendrites (PSD, Fig. 23*b*; Famiglietti & Peters, 1972; Harding, 1973; Lieberman & Webster, 1974).

In rat VB the presynaptic dendrite-derived glomerular boutons do not appear to be present – at least not in significant numbers (see below). Thus the synapses between two (or more) vesicle-containing boutons in the more complex glomeruli, which were formerly characterized as axo-axonal synapses but which are in fact axo-dendritic synapses (A → P and F → P in Fig. 23*b*) or dendro-dendritic synapses (P → P in Fig. 23*b*) are absent in the rat VB. The functional implications of this deficit, and of the generally simple synaptic organization of rat VB by comparison with the markedly more complex cat VB (Jones & Powell, 1969; Ralston & Herman, 1969) are presumably considerable, but cannot at present be specified. For example, although feed-forward inhibition mediated by the axon terminals of lemniscally-activated intrinsic neurons may occur (see later), *rapid* feed-forward inhibition of the type involving suppression of excitatory postsynaptic potentials in TCR cells by inhibitory postsynaptic potentials occurring before the relay cell discharges (see Purpura, 1972) is unlikely to be a feature of the functional organization of rat VB. This prediction stems from the fact that triplet synapses, which consist of a presynaptic dendrite-derived bouton intercalated between a specific afferent terminal and a relay cell dendrite at the site of a monosynaptic connexion between the latter two elements (see Fig. 23*b*), and which are the probable morphological substrate for inhibition of this kind (Lieberman & Webster, 1974), are not present in rat VB.

Although it is certain that in the great majority of VB tissue blocks examined by electron microscopy no presynaptic dendrites or bouton to bouton synapses were seen, in some a sparse population of boutons containing pleomorphic vesicular profiles and lying in postsynaptic relation to boutons containing spherical synaptic vesicles was observed (Figs. 19*a*, *b*). These boutons were morphologically similar to the presynaptic dendrite-derived P-boutons of rat LGd. There may therefore be some, but certainly relatively few presynaptic dendrites in VB. Sections containing P-boutons were derived predominantly from tissue blocks of the most lateral zone of VB, and it is possible that special features of synaptic (and even cellular) organization exist in this region. For example, in anatomical studies Webster & Lund (1967) have demonstrated that in the lateral part of caudal VB, spino-thalamic input and possibly also input from the lateral cervical nucleus overlap with the lemniscal input. Also, in physiological studies, Davidson (1965) reported bilateral evoked responses

in this region of VB only and Emmers (1965) placed (the anterior) part of the second somatosensory receiving area (SII) in this area, with bilateral input and with overlapping spino-cervico-thalamic and spino-thalamic projections (Tomasulo & Emmers, 1970) constituting a zone of convergence similar to that demonstrated in the cat by Landgren, Nordwall & Wengström (1965).

The fact that lemniscal boutons in the glomeruli were never observed in postsynaptic relation to any other axon terminal implies that a presynaptic inhibitory pathway, such as that described by Andersen, Eccles & Sears (1964) on the basis of electrophysiological studies of cat VB, is unlikely to be a feature of the functional organization of rat VB.

Intrinsic neurons, double dendritic participation in VB glomeruli and the significance of F-boutons

In the last few years much interest has focussed on thalamic intrinsic neurons. There is now substantial evidence that such cells are the source of presynaptic dendrites in several thalamic nuclei (see Lieberman, 1973). It may therefore be reasonable to ask whether the absence or paucity of presynaptic dendrites and their glomerular boutons in rat VB should be taken to imply that intrinsic neurons are absent in this site. Cajal (1911) and, more recently, Scheibel & Scheibel (1966) have expressed the opinion that such neurons are very rare in, or absent from, rodent thalamic nuclei; but since there is in fact a distinctive population of intrinsic neurons in rodent LGd (Grossman, Lieberman & Webster, 1973; Lieberman, 1973), it is probable that a careful study with the Golgi methods and electron microscopy will also reveal a population of intrinsic neurons in rat VB, comparable with that found in cat VB and other thalamic relay nuclei.

The principal dendritic component of rat VB glomeruli (and in some glomeruli possibly the only dendritic component) certainly originates from a TCR cell. In the reconstructed glomerulus another dendrite (D 3), clearly different from the principal dendritic components (D 1 and its branch D 2), received synaptic contacts from the AR bouton. This dendrite was of small diameter, and in dramatic contrast to the other dendrites, received no filamentous contacts from the AR bouton. Its non-glomerular surface was also distinct, bearing irregularly spaced, simple, spine-like excrescences and received synaptic contacts from small axon terminals containing spherical synaptic vesicles (Fig. 17 and R in Fig. 23*a*) of the type that degenerate in large numbers after lesions of the S1 cerebral cortex.

Because of these distinctive features it is probable that the small dendrite was either a distal dendrite of a TCR cell or the dendrite of an intrinsic neuron. We have no way of knowing at present how extensive double dendritic participation of this type may be, but if in such cases one dendrite is of relay cell origin and the other of intrinsic neuron origin, the arrangement would constitute an appropriate anatomical substrate for feed-forward postsynaptic inhibition. This would involve simultaneous excitation of TCR and intrinsic neuron dendrites by lemniscal afferents and subsequent postsynaptic inhibition of TCR cells mediated by intrinsic neuron axon terminals, probably including some or all of the F-bouton population. Similarly, the F-boutons would be expected to mediate the feed-back (postsynaptic) inhibition of TCR cells initiated by TCR cell recurrent collaterals (Andersen *et al.* 1964). The fact

that F-boutons do not degenerate after ipsilateral SI cortical lesions or ablation of the contralateral dorsal column nuclei (Lieberman & Webster, unpublished) is in accord with this interpretation. It is possible, however, that some F-boutons (like some of the small non-glomerular boutons with spherical synaptic vesicles that do not degenerate after cortical ablation) originate in other regions of the C.N.S. such as for example, the brain stem reticular formation (see Scheibel & Scheibel, 1966; Bowsher, 1971).

The location, characteristics and significance of the glomerular synapse between the lemniscal afferent and the TCR cell

The proximal situation of the lemniscal axon terminals in relation to the dendritic tree of the TCR cells, the large size of the terminals and the large number of closely spaced synaptic contacts each terminal establishes with the relay cell dendritic excrescences, almost certainly combine to produce an extremely powerful excitatory effect on the relay cell. This has as its physiological expression the short latency and high degree of synaptic security of transmission through the VB complex following an afferent volley in the medial lemniscus (Eccles, 1966; Welker, 1973). Thus, it might be appropriate to view the relationship between the lemniscal terminal and the relay cell dendritic excrescences as an amplifying device by which a maximal postsynaptic effect can be generated with a minimal and highly specific lemniscal input. Because of the physiological evidence of specificity of transmission through VB, it is unlikely that the excitatory effect is much enhanced by the convergence of several topically related lemniscal axons upon a single relay cell (see also Welker, 1973). On the other hand, postsynaptic excitation of relay cells would be considerably enhanced if lemniscal fibres terminated in a series of *en passant* glomerular boutons on the dendrite(s) of a single cell. Such a relationship, resembling that between the cerebellar Purkyně cell and its powerfully excitatory climbing fibre, is hinted at often enough in Golgi material and electron micrographs (e.g. see Harding, 1973) but has yet to be convincingly demonstrated. Additionally (perhaps alternatively) preterminal branching of individual afferents to provide several glomerular axon terminals on dendrites of the same cell may occur, and recent electron microscopic observations of multiple myelinated branches originating at nodes of Ranvier of presumed lemniscal afferents in VB suggest this possibility (Lieberman, Webster & Špaček, 1972).

Among the most interesting observations to emerge from the present study, raising a variety of developmental and functional questions, were the following:

(1) The lemniscal afferents establish synaptic contacts preferentially (in some glomeruli exclusively) with the dendritic excrescences, whereas they make filamentous contacts only with the dendritic shafts.

(2) Excrescences of all sizes and degrees of complexity (except perhaps the smallest and most inconspicuous) maintain a fairly constant surface area to volume ratio and the combined surface area of the synaptic contacts each receives is closely related to the surface area of the excrescence, constituting in every case only about 5–10% of the total.

The significance of the filamentous contacts is unknown. In VB glomeruli they are highly differentiated structures, arranged in a reticulum exclusively upon the

surface of the dendritic shaft, and in association with dendritic and axonal systems of SER. Although many authors have concluded that contacts of this type are desmosome-like with purely mechanical or adhesive properties (e.g. Pappas *et al.* 1966; Peters & Palay, 1966) they in fact resemble desmosomes and other symmetrical adhaerens-like contacts only superficially. Others have viewed them as a class of synaptic junction (Karlsson, 1966), or as related in some way to the specificity of the connexions between certain nerve cells (Colonnier & Guillery, 1964; Gray & Guillery, 1966).

The reason the lemniscal input is concentrated on the dendritic excrescences and virtually excluded from other receptive surfaces is not known and probably will not be until the specific roles of various kinds of dendritic appendage are more completely understood. That dendritic spines comprise specialized receptive surfaces for specific (and usually excitatory) synaptic inputs to neurons is well documented, and the situation in the glomeruli of VB is a particularly striking example of this phenomenon. Furthermore, although the excrescences do not have the characteristic form of 'classical' cortical spines, and do not contain a spine apparatus, they may be comparable in functional significance to the spines of other types of neuron.

From the relationship found between the surface area of synaptic specializations on excrescences and of the excrescences themselves, it would appear that for this synapse at least, areas of the excrescence membrane in which transmitter receptor sites are situated (assuming that the latter are confined to the areas of postsynaptic specialization) are surrounded by rather large and relatively constant areas of non-synaptically specialized membrane. Presumably at this synapse, 5–10% of the total surface area represents the optimum proportion of specialized to non-specialized receptive membrane for the effect of transmitter release from the lemniscal terminal to be maximal. It would be of considerable interest to determine the extent to which this relationship holds for other spine-like neuronal appendages and for other types of postsynaptic membrane.

A general discussion on the principles of organization and significance of synaptic glomeruli in the C.N.S. is beyond the scope of this paper. Jones & Powell (1969), Szentágothai (1970), Pinching & Powell (1971*b*), Wolff & Němeček (1971), Famiglietti & Peters (1972) and Gobel (1974), among others, have contributed interesting discussions on these topics.

Relative volumes of mitochondria in glomerular axon terminals

The data obtained on the relative volume of mitochondria in the AR bouton and in the largest F-bouton of the reconstructed glomerulus is very limited. Nevertheless, the difference between the two terminals is very marked (11·6% in the AR bouton; 40·9% in the F-bouton). There are also obvious differences in the fine morphology of mitochondria in AR and in F-boutons. These observations suggest that the amount and type of mitochondria in different classes of axon terminal might be related to different metabolic needs (perhaps both qualitative and quantitative) in the terminals. It is of interest that in the only other study in which comparable data was obtained, Nafstad & Blackstad (1966) found that the relative area occupied by mitochondrial profiles was significantly greater in the flat-vesicle-containing Gray type II axo-somatic boutons on adult rat hippocampal cortical neurons (17%) than in the mossy

fibre terminals (11%) which (with spherical synaptic vesicles, invaginated dendritic excrescences and Gray type I contacts) closely resemble VB lemniscal terminals.

The glial capsule

Synaptic glomeruli in all parts of the C.N.S. are characterized by the absence of glial cell processes between the neural elements that synapse within them, and in most cases also, by a more or less complete investment of astrocyte processes around them. In rat somatosensory thalamus the periglomerular astrocytic sheath is particularly prominent. In nuclei with larger and more complex glomeruli an astrocytic capsule is often less obvious. This is due in large measure to the absence of a single, circumscribed hilus in such glomeruli. The many entering and exciting neural components necessitate frequent interruptions in the continuity of the capsule and are responsible for rendering the latter less conspicuous. Furthermore, it is not certain that the glomerular neuropil in such nuclei as the cat and monkey LGd constitutes glomeruli as discrete as those of rat VB. It may be rather that glomerular neuropil comprises an extensive, continuous reticulum (encapsulated synaptic zone) embedded in the non-glomerular (interstitial) neuropil (see Guillery, 1969, 1971). If such is the case, the glial–related surface of the encapsulated synaptic zones would be relatively limited in extent. Nevertheless it appears that, in all thalamic nuclei, glomeruli or areas of glomerular neuropil are separated from the extraglomerular neuropil by astrocytic laminae.

The functional significance of periglomerular glial sheaths is unknown. It has been suggested that astrocyte laminae enclosing certain synaptic complexes serve to segregate axon terminals of different origin, and perhaps also to insulate the activity of specific synaptic groupings from that of other neural elements (Palay, 1966; Chan-Palay & Palay, 1972). Clearly the astrocytic sheaths of glomeruli, particularly of large and complex glomeruli, do not segregate, but rather hold together axon terminals and other neural processes of different origins. The proposal that astrocyte processes insulate certain neuronal surfaces or synaptic groupings from others is still at the general conceptual level, representing little more than a re-statement of morphological observations: precisely what is insulated from what, how the insulation is effected, and why it should be necessary, are not clear. Multilamellate sheaths with extensive zones of close membrane apposition between contiguous laminae (resembling but not anatomically equivalent to loose myelin) are common in VB and LP and would presumably be particularly effective in 'insulating' the synaptic glomeruli.

Perhaps the glial capsule serves in part to limit the diffusion from the glomerular environs of certain extracellular molecules and ions. Repetitive discharge of TCR cells following single afferent volleys is a well-established phenomenon (Andersen *et al.* 1964; Eccles, 1966). It is also a feature of the response of TCR cells in rat VB (Waite, 1973), and may be related to a relatively slow breakdown of the large amount of transmitter released from the specific afferent terminals and retention of the transmitter close to the postsynaptic receptor sites (see also Pappas *et al.* 1966). Depolarization of the specific afferent terminal will result in a considerable increase in the local extracellular concentration of potassium ions (Baylor & Nicholls, 1969*a*; Vyklický, Syková, Kříž & Ujec, 1972) and the periglomerular sheath may be

responsible for maintaining a high extracellular concentration of potassium around the specific afferent terminal following this event. The high extracellular potassium concentration would be expected to reduce the excitability of the terminal, particularly following prolonged periods of activity in the latter (Baylor & Nicholls, 1969*b*; see also Pinching & Powell, 1971*a*), and thus might explain the presynaptic inhibition (presynaptic afferent depolarization) that has been described in the mammalian VB complex (Eccles, 1966) and in other sites in which the expected morphological basis for presynaptic inhibition has not so far been demonstrated (see also Vyklický *et al.* 1972).

We have considered elsewhere possible metabolic relationships between astrocyte processes and glomerular axon terminals, particularly in relation to large terminals invaginated by spine-like protrusions originating from periglomerular astrocyte processes ('microtrophospongium'; Špaček & Lieberman, 1971).

SUMMARY

This paper describes synaptic glomeruli in rat ventrobasal and posterolateral thalamic nuclei using light and electron microscopy and graphic and solid reconstructions from serial thin sections.

In each glomerulus a single large lemniscal bouton packed with spherical synaptic vesicles comes into multiple synaptic contact with simple and compound dendritic excrescences. The excrescences have a fairly constant surface to volume ratio and originate from proximal dendrites of thalamocortical relay cells (and possibly also from interneuron dendrites). Only 5–10% of the surface area of the excrescences is synaptically specialized. Each lemniscal bouton also establishes an extensive reticular asymmetric filamentous contact with the dendritic shaft. The filamentous contact is associated with a system of tubular SER in the dendrite, and often also with SER in the lemniscal bouton. Axon terminals with flattened synaptic vesicles and a relative mitochondrial volume considerably greater than that of lemniscal boutons, synapse on to dendritic shafts within and immediately adjacent to the glomeruli.

Glomeruli are separated from the extraglomerular neuropil by a prominent, often multilamellate sheath of astrocyte processes. The free edges (rims) of curved and cup-shaped periglomerular astrocyte processes are expanded and contain a circumferential canaliculus of SER. The astrocytic capsule may serve to limit diffusion from the glomerular environs of extracellular ions (especially K^+) and of transmitter released by intraglomerular terminals.

In a fully reconstructed glomerulus approximately 5 μm in diameter, two different dendrites gave rise to 10 excrescences which received a total of 44 discrete contacts from the lemniscal bouton. Similar findings were made in other partially reconstructed glomeruli. Glomerular synapses of this type may act as amplifiers to ensure transmission from a small number of lemniscal fibres to specific relay cells.

Rat somatosensory glomeruli are relatively small and simple and lack the bouton to bouton synapses characteristic of glomeruli in other thalamic nuclei. Only rarely, and principally in the caudo-lateral area of the ventrobasal complex, were boutons resembling those derived from presynaptic dendrites in other thalamic nuclei identified. Thus interneurons in rat somatosensory thalamus probably do not bear

presynaptic dendrites: their axons, however, may contribute to the population of terminals with flattened synaptic vesicles.

These studies were carried out in 1969 in the Anatomy Department, University College London, during the tenure of a British Council Bursary by J.Š. We thank the Wellcome Trust for travel grants (to A.R.L.), Dr K. E. Webster for help in the removal of tissue samples from identified thalamic nuclei, Mr J. Špaček, Snr., for making the reconstruction drawings, D. Gunn for technical assistance, and Professors E. G. Gray, T. A. Sears and J. Z. Young, F.R.S., for helpful discussions.

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