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THE FINE STRUCTURE OF THE VERTICAL LOBE OF *OCTOPUS* BRAIN

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[Plates 73 to 92]

CONTENTS

Although much is known about the structural organization and connexions of the various lobes of the octopus brain from light microscopy, this is the first attempt at a detailed analysis of one of the lobes—the vertical lobe, with the electron microscope. The vertical lobe consists of five lobules. The median superior frontal (MSF) axons enter each lobule from the MSF lobe. The MSF axons contain both microtubules and neurofilaments. The varicosities of the MSF axons contain both agranular and dense-cored vesicles and synapse with trunks of the amacrine cells. These trunks run together in bundles termed amacrine tracts into the centres of the lobules. The amacrine trunks contain microtubules but no neurofilaments. The trunks contain large and small agranular synaptic vesicles and synapse with what are in all probability branches of the trunks of the large cells. These trunks contain microtubules but no neurofilaments. They run out through the bases of the lobules probably without forming synaptic contacts within the lobule. Fibres signalling 'pain' (nocifensor) enter the lobules from below. They can be recognized by their content of neurofilaments. Their terminals contain numerous very small synaptic vesicles and a few larger and dense-cored ones. These 'pain' fibres appear to synapse mostly with processes of the large cells.

J. Z. Young has shown that the vertical lobe is especially concerned with the integrative action of the visual system, linked with the chemo-tactile system. Electron microscopy supports Young's suggestion that the superior frontal and interconnected vertical lobe systems constitute a loop which could sustain a positive feed-back mechanism (MSF → amacrine → large cell → lateral superior frontal → MSF) while the 'pain' (nocifensor) input could exert a suppressor (inhibitory) effect on the loop by its action on the large cells.

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Introduction

Much is known of the organization of the octopus brain as seen by light microscopy (see Young 1964, 1970), but there have as yet been few electron microscopic (EM) studies, partly no doubt because the octopus brain is proving an extremely difficult tissue to fix. The only previous EM investigation of the vertical lobe (Gray & Young 1964) gave a brief account of the synaptic organization of the outer zone of the neuropil. In the present work an improved fixation technique has been used, enabling the vertical lobe to be described in more detail. In particular the amacrine cells and their trunks are described in detail, together with the synaptic contacts they receive from the incoming fibres that originate in the median superior frontal lobe. It is shown that the amacrine trunks synapse with processes that are probably dendritic branches of the large cells of the vertical lobe. Further details of these latter cells and their dendritic organization are given. Finally, a distinct group of nerve endings is described, which are thought to belong to the presumed 'pain' (nocifensor) axons that enter the neuropil of the vertical lobe from below (Young 1964, 1970).

Methods

Various aldehyde and osmium tetroxide fixatives at various tonicities were tried. The best fixative consisted of 80 ml of distilled water to which was added 0.398 g of sodium chloride, 0.014 g calcium chloride, 0.012 g potassium chloride, 10 ml of N/5 HCl, 10 ml of veronal-acetate buffer (prepared from 14.7 g sodium barbitone plus 9.7 g of sodium acetate $3H_2O$ dissolved in 500 ml) and in the total 100 ml, 1 g of osmium tetroxide was dissolved. This gave a final pH of 7.3. Satisfactory fixation could sometimes be achieved by cutting up the lobe into minute fragments in the fixative, but by this method knowledge of the orientation and region, which is essential to the study, is lost. The method finally adopted was to micro-inject the fixative into the lobe. For this purpose a fine glass pipette with a tip bore of about 80 μm, fitted with a rubber teat and broken to a point in the shape of a hypodermic needle, was used. A small piece of Plasticene, with a notch in it of sufficient size to accommodate and support the vertical lobe, was stuck into the inside of a 2 in (5 cm) block watch-glass and then the fixative was added.

An octopus was anaesthetized in 3 % urethane in sea water and the vertical lobe was removed and placed in the notch in the Plasticene immersed in the fixative. The lobe was repeatedly punctured and injected with fixative with the aid of a stereo dissecting microscope, for 10–15 min. If the tip became blocked a new, ready-filled pipette was used. After this time the lobe had become blackened and hardened and was then cut into four or five pieces of known orientation. These were placed in fresh fixative for 2 to 3 h and then rinsed in 15 % ethanol. Dehydration was continued with ethanol and at the 70 % stage the pieces were stained with 1 % uranyl acetate in the 70 % ethanol for 2 h. After complete dehydration with absolute ethanol, the pieces were placed for half an hour in 50/50 ethanol-epoxypropane, half an hour in epoxypropane, half an hour in 50/50 epoxypropane-Araldite mixture (without accelerator) and then in Araldite mixture alone (without accelerator), where the pieces were left for 3 to 5 days at 50 °C. Finally, they were embedded in final Araldite mixture with two or three changes (see Gray 1964). Sections were cut and stained for five minutes with lead citrate (Reynolds 1963).

Fixation was by no means always successful with this method, and it was necessary to avoid regions close to the penetration point of the micropipette. Nevertheless, when a satisfactorily-

fixed block was found, the whole cortex and medulla of one of the lobules of the lobe could be sectioned by hand (see Gray 1961) and known regions selected with precision for sectioning on the ultramicrotome.

Observations

Organization of the vertical lobe

Details of the light microscopy of normal and experimentally degenerated material are given in Young (1964, 1970). Briefly, the vertical lobe is the most dorsally situated lobe of the octopus brain (figures 1 to 5, plate 73). It is a medial structure and consists of five cylindrical lobules that lie antero-posteriorly. Each lobule has a cortex, 150 μm thick, consisting of the neuronal perikarya. The core or medulla of each lobule constitutes the neuropil and is about 300 to 400 μm across. Two distinct classes of neuronal perikarya are found in the cortex. The majority are small cells with trunks* running radially into the centre of the medulla. These are termed *amacrine cells* by Young, since the single trunk can be classified neither as a dendrite nor as an axon. The *large cells* form the small minority of the perikarya and these lie in the inner zones of the cortex, either singly or in clusters of up to six. Their trunks also run radially into the medulla, where they give off dendritic collaterals, which branch profusely in the more central regions of the medulla. The main trunks of these large cells continue as efferent axons and run out ventrally through channels between the clusters of amacrine perikarya in the ventral cortical zones of each lobule. Such channels may appropriately be termed *lobule hila*. The axons run down from the hila into the subvertical lobe. Afferent axons run up into the medulla of the lobules through these hila where (in silver preparations) they appear tortuous and tangled.

A second group of afferents to the vertical lobe are axons whose cell bodies are situated in the median superior frontal (MSF) lobe. These *MSF axons* stream into the medulla of the vertical lobules and they can be seen in Golgi preparations as fine varicose fibres. They are largely restricted to the outer zone of the medulla which is about 70 μm thick. Electron microscopy (Gray & Young 1964) confirms that they make cruciform connexions with the (radial) trunks of the amacrines, each varicosity containing the characteristic presynaptic vesicles (see below). The vertical lobe, then, has two afferent supplies, the MSF axons coming from in front and a second afferent supply from below, thought to be of 'pain' (nocifensor) *fibres*. The lobe has an efferent system—the axons of the large cells with their (intrinsic) dendritic system. The amacrine cells with their trunks are wholly intrinsic to the vertical lobe.

For electron microscopy numerous sections were cut in two planes normal to each other. By cutting accurately orientated transverse (i.e. with respect to the animal) sections of a lobule so that the plane of section runs parallel with the amacrine trunks and the large-cell trunks, both sets can be seen in section in their longitudinal aspects and can be followed for some distance through the neuropil and are often seen in continuity with their perikarya. In this plane the MSF fibres will appear in transverse section. Secondly, sections were cut horizontally (with respect to the animal) at intervals down through a lobule. Thus, the most dorsal sections will show amacrine perikarya in the cortex and cross-sections of their trunks. Deeper, horizontal sections will include these and sections of the large cells and cross-sections of their trunks lying near the inner margin of the cortex. Deeper still, the outer medulla will appear in the centre of the section with the amacrine trunks and large cell trunks seen in cross section, while the

* The term 'trunk' is used throughout to denote the parent or single process of a neuron, when such a process cannot be clearly classified as axon or dendrite.

inter-mingled MSF fibres will now appear, seen in their longitudinal aspect, and this zone will be flanked by cortex. Deeper still, horizontal sections will pass through the centre of the lobule showing the central zone of the neuropil flanked by amacrine and large cell trunks now seen in the longitudinal aspect and the MSF fibres will also be seen in their longitudinal aspect. This is a most revealing plane to study the cruciform connexions of the MSF fibres with amacrine trunks, for since both appear in longitudinal section they can both be followed for some distance with the EM. However, it is a difficult plane to hit accurately with the ultramicrotome.

In the neuropil of the centre of the medulla, the amacrine trunks become irregularly orientated, and the tangle of these with the dendritic branches of the large cells and the pain fibres makes it impossible to follow any of these components far in any plane of section, and so criteria have to be developed for recognizing their isolated profiles in EM sections. Such criteria include location, dimensions and the size, nature and presence or absence of, for example, vesicles, mitochondria, neurofilaments, microtubules, neuroglial fibrils and glycogen and ribosomal granules. The differences between two sorts of profile may be quite subtle and may be overlooked if fixation is less than adequate.

The amacrine cells

The closely packed perikarya (6 to 10 μm in diameter) of these small cells occupy most of the cortex of each lobule. Golgi preparations (Young 1964, 1970) show that they are unipolar cells with the single trunk orientated inwards to the medullary neuropil. In EM sections the nucleus (*amn*) occupies most of the perikaryon (figure 6, plate 74). The cytoplasm (figure 8, plate 75) contains small scattered units of granular endoplasmic reticulum and clusters of free ribosomes, mitochondria and scattered microtubules. One and rarely two Golgi units are seen. They usually lie away from the origin of the trunk. Frequently, a centriole occurs adjacent to the Golgi apparatus and the microtubules sometimes appear to be radiating from the centriole (figure 10, plate 76). The swarms of vesicles associated with the apparatus (figure 8) vary widely in diameter. Some groups range from 30 to 50 nm, others from 90 to 100 nm and some may be twice this size. A detailed analysis of the Golgi vesicles has been made elsewhere (Gray 1970*a*). The large lamellated bodies described by Dilly, Gray & Young (1963) in neurons of the optic lobe occur also in these amacrine cells (figures 6 and 10).

Each amacrine perikaryon has a single, inwardly directed trunk. The trunks, however, do not run down independently between the other amacrine cell bodies into the neuropil, but cascade together into bundles of up to 300 or more before running out from the cortex of the lobule. These bundles will be called *amacrine tracts*. Figure 9, plate 76, shows an amacrine trunk at its origin from the perikaryon, and it can be seen joining the left side of a tract already formed by the more superficial amacrine perikarya. Here the tract is sectioned longitudinally. A tract cut transversely is shown in figure 6, plate 74 at low magnification. It contains at this level in the cortex about 200 trunks (*amt*). Two trunks are seen joining it (arrow). This applies equally to the innermost amacrine perikarya, whose trunks (figure 11, plate 77 amt^1 & amt^2) do not run directly out into the neuropil, but first run to join an amacrine tract. The arrangement suggests that the amacrine trunks are grouped together into functional units.

The trunks of the amacrine cells grouped into a tract can be seen in longitudinal section in the cortical zone in figure 9. In their thinnest parts they are approximately 0.2 to 0.3 μm across and they have ovoid varicosities two or three times this diameter. The trunks contain microtubules that run out from the perikaryon, elongated mitochondria, elongated profiles of agranular

reticulum, clusters of vesicles of various dimensions (see below), which are concentrated in the varicosities, and groups of small granules thought to be ribosomes.

Sections of an amacrine tract cut the other way to reveal the trunks in cross section (e.g. figure 7, plate 75) show how the profiles of the individual trunks interdigitate with each other. Their surface membranes are directly apposed without intervening glial processes. A range of cross sectional diameters can be seen, the wider ones being cross-sections through the varicosities, and this shows that the varicosities are staggered. Part of a tract is seen at higher magnification in figure 12, plate 77 and shows the microtubules and other organelles of the amacrine trunks mentioned above, in cross-section. Neurofilaments are absent from these trunks. Glial folds (figure 7, arrow) with bundles of glial filaments (see Gray 1969*a*) partly encase the amacrine tracts (again suggesting that the trunks may be aggregated into functional units), but a glial fold never completely isolates an amacrine tract from the surrounding tissue for in the upper part of figures 7 and in figure 12 the surface membranes of marginal trunks can be seen to be in direct apposition with the membranes of the neighbouring amacrine perikarya. A similar arrangement is shown in figure 13, plate 78, in a section cut near the inner margin of the cortex. Here two processes of a dark glial cell (*gl*, see Gray 1969*a*) intrude between the amacrine trunks and so lie within the amacrine tract.

The large cells

These have their perikarya up to 100 μm in diameter situated singly or in groups of two, three or more in the inner margin of the cortex. Glial folds often partly enclose them. A single cell is shown in figure 14, plate 79, at very low magnification: the pale glial folds contain bundles of glial filaments. This cell is probably one of a group, but appears alone, because of the plane of section. The cytoplasm appears dense because of the large amounts of granular endoplasmic reticulum and free ribosomes.

Figure 16, plate 80, shows a section through parts of the perikarya of three adjacent large cells. In one the nucleolus appears in the nucleus in the plane of section. Golgi apparatuses are conspicuous, also mitochondria and parallel arrays of granular endoplasmic reticulum. At higher magnifications dense material can be seen within the Golgi lumina, suggesting that the dense-cored vesicles seen around the Golgi apparatus may be formed by budding from the Golgi membranes. In addition, the cytoplasm contains multivesicular bodies, coated vesicles, mitochondria, granular endoplasmic reticulum, free ribosomes, agranular reticulum and scattered microtubules. Centrioles have not yet been encountered in these cells.

The trunks of these large cells run out into the neuropil in groups, or sometimes singly. A cross-section through such a group of four trunks taken at the inner edge of the cortex is shown in figure 17, plate 81. The profiles of the trunks interdigitate and the trunks may be up to 5 μm across at this level. The two central profiles have their membranes directly apposed, while the others are separated from each other and from the perikaryon of the large cell (left) by thin glial lamellae. The trunks contain mitochondria and a conspicuous array of fairly regularly spaced microtubules, profiles of agranular reticulum of various dimensions and groups of smaller round and elongated vesicles (arrow). Groups of granules, probably ribosomes (*r*) also occur at this level. Neurofilaments are absent from these trunks as well as from the amacrine trunks.

In the outer zone of the neuropil the bundle of trunks of the large cells or isolated ones may be partly surrounded by glial lamellae. In figure 18, plate 81, a trunk (*lc*) is seen embedded in an amacrine tract (above).

Figure 15, plate 79, shows two adjacent trunks of large cells in longitudinal section.

Microtubules, granular and agranular endoplasmic reticulum are present and also agranular vesicles and two dense-cored vesicles can be seen. One of the mitochondria has granular or fibrous material (*fi*) in its matrix.

Synapses of the neuropil of the vertical lobe

(1) *The MSF-amacrine contacts*

The axons from the median superior frontal (MSF) lobe run in bundles in the outer zone of the neuropil medulla of the vertical lobe and there they meet the amacrine tracts running at right angles and make cruciform connections with them.

An MSF tract is seen in figure 19, plate 82 (left), cut longitudinally to reveal the varicose nature of the constituent MSF axons. An amacrine tract appears in cross-section since it meets the MSF tracts at right angles (see below). Part of another MSF tract lies to the right of the amacrine tract. The thin regions of the MSF axons are about 0.2 to 0.3 μm thick and the varicosities may be several times thicker. At higher magnification (figure 20, plate 83) the MSF axons can be seen to contain both microtubules and much finer threads, the neurofilaments. Mitochondria are present together with swarms of vesicles, many of which have dense cores (see below). These features can be used to recognize an MSF tract when cut the other way, i.e. in cross-section. Note how the varicosities are staggered (as in the amacrine trunks) so that small and large cross-sectional profiles are cut in the same plane.

Numerous two-component synaptic profiles can be seen in the outer neuropil zone (figure 22, plate 84), the MSF to amacrine contacts. Here the upper component contains numerous vesicles, both dense-cored and agranular, with diameters ranging from about 20 to 80 nm. The percentage of vesicles with dense cores in sectional profiles varies widely with a mean of about 30 %. The MSF component contacts a second varicose component, which contains numerous vesicles that are all agranular and form a bimodal population with peak diameters at about 30 and 90 nm. Granules ranging from 15 to 30 nm occur between the vesicles. Sometimes they appear inside the vesicles, but stereo-electron-microscopic studies (Gray & Willis 1970, in preparation) show that usually they lie above or below them within the thickness of the section. (This possibility has always to be taken into account when deciding whether a given vesicle is dense-cored or agranular, and on such occasions stereo-electron-microscopy using tilted sections is indispensable.) The larger granules are probably glycogen, but the smaller ones may be ribosomes. The granules are also present in the MSF component lying in the cytoplasm between the agranular and dense-cored vesicles.

Most of the region of contact (figure 22) shows membrane thickenings and cleft material and so we regard this region as the synaptic cleft where transmission takes place. The cleft shows asymmetry, there being slightly more dense material along what we shall recognize as the postsynaptic membrane (see below). Both the dense-cored and agranular vesicles of the presynaptic component can be seen crowded against the presynaptic membrane. These synaptic cleft asymmetrical specializations are more readily seen after phosphotungstic acid staining, and have been described in detail by Gray & Young (1964).

The presynaptic components with their percentage of dense-cored vesicles are readily identified as the varicose *en passant* contacts of the MSF fibres. The MSF tracts have axons with dense-cored and agranular vesicles scattered throughout their length (figures 19 and 20) and occasionally an axon of an MSF tract can be followed along in the plane of section and be seen to form a presynaptic dilatation followed by a narrowing as it continues along in the MSF

tract. Thus, the MSF axons have non-synaptic, slight dilatations and pronounced dilatations, the presynaptic varicosities.

The postsynaptic components are identified as the varicosities of the amacrine trunks that run down in the amacrine tracts to meet the MSF axons at right angles and make cruciform connexions with them. Occasionally it is possible to trace an amacrine trunk down from its perikaryon (figure 24, plate 85) in the plane of section with the EM. This is possible at the inner margin of the cortex where an amacrine cell body lies directly against an amacrine tract, so that its trunk joins the tract without having to curve for some distance (when it would be lost out of the plane of section). The characteristic agranular vesicles can be seen scattered along its length and are concentrated in the regions of pronounced varicosity. MSF contacts on the amacrine trunks are not seen in this plane of section.

Usually, the MSF presynaptic component is convex and fits into a concavity in the dilatation of the amacrine trunk (figures 19 and 21), but not invariably so (figure 22). An MSF axon may sometimes synapse not with the vesicle-filled varicose part of the amacrine trunk but with the narrow zone between the varicosities (figure 20, plate 83). This narrow zone, as in this illustration, may not contain vesicles. The zone is seen in cross-section as one of the outermost components of an amacrine tract, and for this reason is identified as an amacrine trunk.

One MSF presynaptic varicosity can sometimes be seen to synapse with two (presumably different) amacrine trunks (figure 23, plate 84). Only once has the converse been seen (figure 25, plate 86) where two MSF components contact one amacrine varicosity.

Gray & Young (1964) distinguished amacrine trunks from MSF axons and their presynaptic varicosities, partly on the basis of the very dark or dense appearance of the former after PTA staining. With the present method this criterion is less reliable for although it usually holds (e.g. figure 19) the reverse is sometimes the case; for example, figure 22 where the MSF component is the darker one. In figure 21, plate 83, the left MSF varicosity is paler than the amacrine component and the situation is reversed in the central lower synapse. The darkness or paleness of the component really seems to depend on the packing of the vesicles and the effect this has on the crowding together of the granular material between them. In figure 23, plate 84, MSF and amacrine components appear equally pale in the section. When, in sections, the synaptic components cannot be directly traced from their respective tracts the main criteria are: (*a*) the presence of dense-cored vesicles in the MSF (presynaptic) component; (*b*) the asymmetry of the cleft—the denser side being postsynaptic, i.e. amacrine; (*c*) the presence of the large agranular vesicles in the amacrine component; and (*d*) less reliably, which is convex and which concave.

In some micrographs where the MSF presynaptic varicosity appears dark the impression is gained that it has become shrunken, while the granulated vesicles in it have become swollen possibly by the osmotic transfer of water from the cytoplasm into the vesicle. This needs further investigation, however. Also, it is not known whether the varying densities (i.e. organelle packing) of the MSF and amacrine varicosities reflect different states of *in vivo* activity or are fixation artefacts.

(2) *The amacrine to large-cell synapses*

Although the MSF-amacrine synapses are mainly peripheral, observations on the central zone of the medulla show that a few such contacts also occur there, formed by the few MSF axons that curve inwards from the outer zone of the medulla to synapse on the more distal

(deeper) parts of the amacrine trunks. Under the electron microscope, in these distal parts they are very conspicuous. They occur in vast aggregations and this is to be expected since the amacrine trunks radiate inwards into this central zone, and since they retain their diameters they occupy more and more of an ever decreasing volume. Figure 27, plate 87 shows a field in the inner medullary zone packed with the extremities of the amacrine trunks cut here mainly obliquely. Their characteristic vesicles and microtubules can be seen as described above in outer regions of their trunks.

Here and there they synapse with pale processes that contain glycogen (or ribosomal?) granules and a few vesicles and microtubules (see also inset). Figure 26, plate 86, shows such a process at higher magnification. It is contacted by three amacrine trunks. These pale processes are thought to be (see Discussion) the fine twigs and branches of the trunks of the large cells as described by Young (1964, 1970) in Golgi preparations seen by light microscopy. Figure 28, plate 88, shows a larger branch or perhaps the main trunk of a large cell contacted by an amacrine varicosity. Since these side branches or twigs (i.e. the dendritic collaterals of Young 1964) twist and turn in all directions they are difficult to follow in the plane of section (*tw*, figure 29, plate 88).

The side branches are most common in the central zone of the medulla, but they also occur in the peripheral zone (figure 25, plate 86). Here the entire serial arrangement can be seen (Gray & Young 1964), MSF varicosities synapsing with an amacrine varicosity which in turn synapses with a microtubule-containing branchlet, which we assume is a dendritic collateral of a large cell. The trunks of the large cells, after giving off the collaterals, run down through the lobule and out through the lobule hila. This region has not yet been examined with the electron microscope.

So far there is no evidence that any of the branches given off from the main trunks of these large cells within the lobule neuropil can be regarded as axonal, i.e. they have not been seen in sections in continuity with presynaptic knobs. Nor do the trunks or branches themselves show the morphological features of presynaptic components; for although vesicles, including dense-cored ones (see figure 15, plate 79), can be seen occasionally in them, they have not been seen to form aggregates near 'thickened' contact regions. These vesicles are presumably destined for the presynaptic knobs that the trunks form outside the vertical lobe, probably in the sub-vertical, lateral superior frontal and inferior frontal systems.

(3) *The incoming 'pain' (nocifensor) axons and their synapses*

So far we have considered the MSF afferent inputs synapsing with the amacrine trunks, which in turn contact dendritic collaterals of the trunks of the large cells. These trunks run down and out through the lobule hila. Young (1964, 1970) has described relatively coarse

Description of plate 73

Figure 1. Sagittal section through the supra-oesophageal lobes of an octopus brain. Figure 1 to 5 Cajal silver.

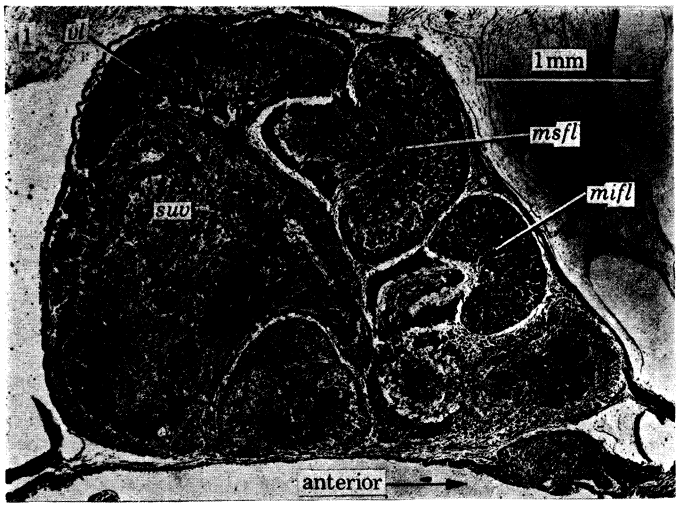
Figure 2. Longitudinal (parasagittal) section through one of the lobules of the vertical lobe.

Figure 3. Transverse section through a lobule of the vertical lobe (see figure 4).

Figure 4. Transverse section showing the five lobules that constitute the vertical lobe.

Figure 5. Transverse section through a lobule of the vertical lobe (see figure 4).

Note: The MSF fibres are cut longitudinally in figure 2, and appear as dots or short lines in figures 3 and 5, where they are cut transversely or obliquely. The trunks of the amacrines and large cells are not seen in these silver preparations—Golgi preparations are needed (see Discussion).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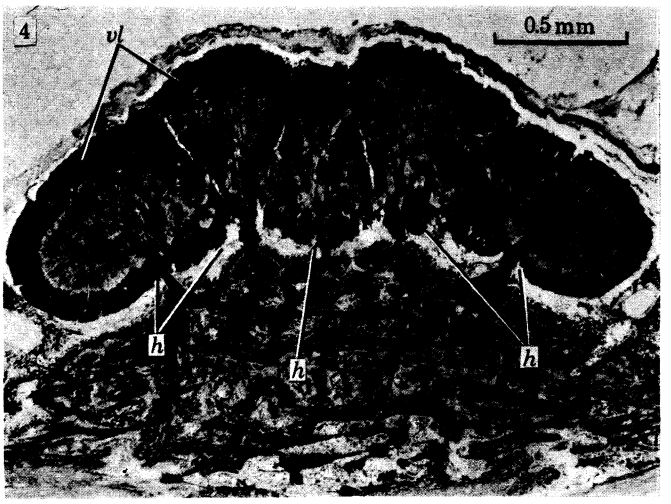
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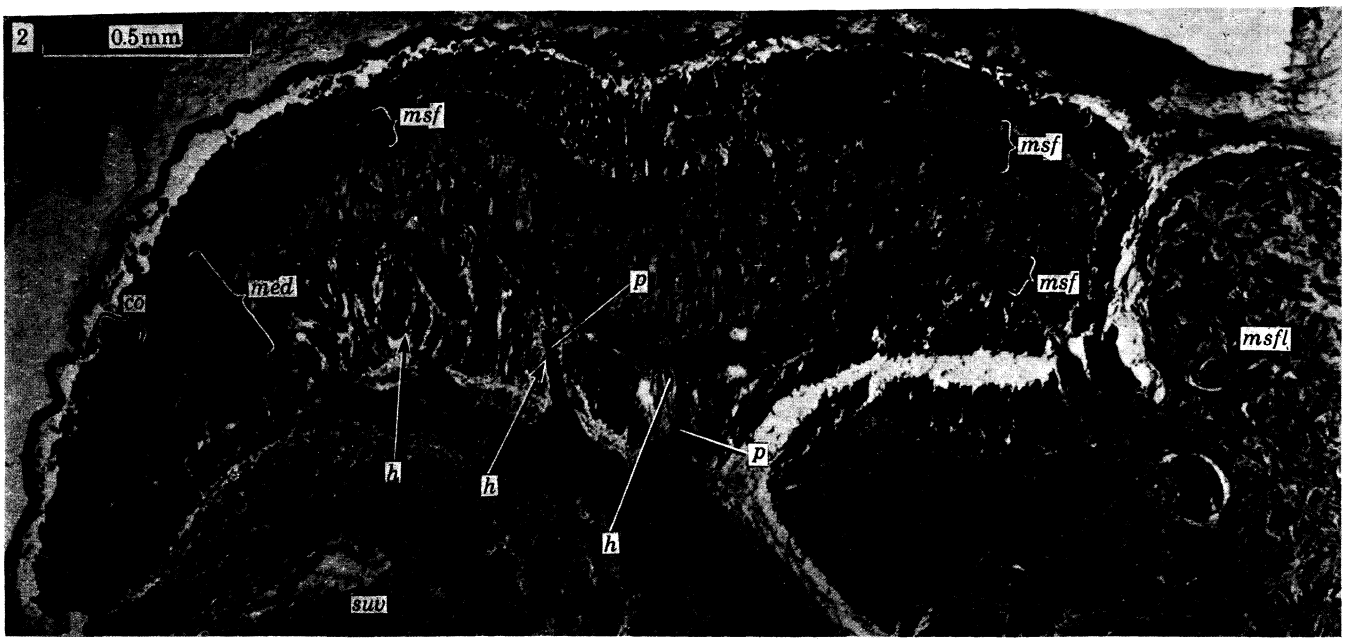
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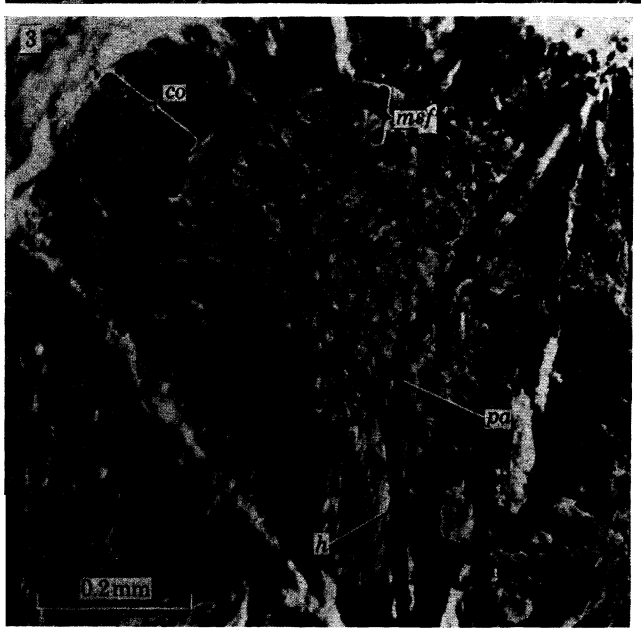
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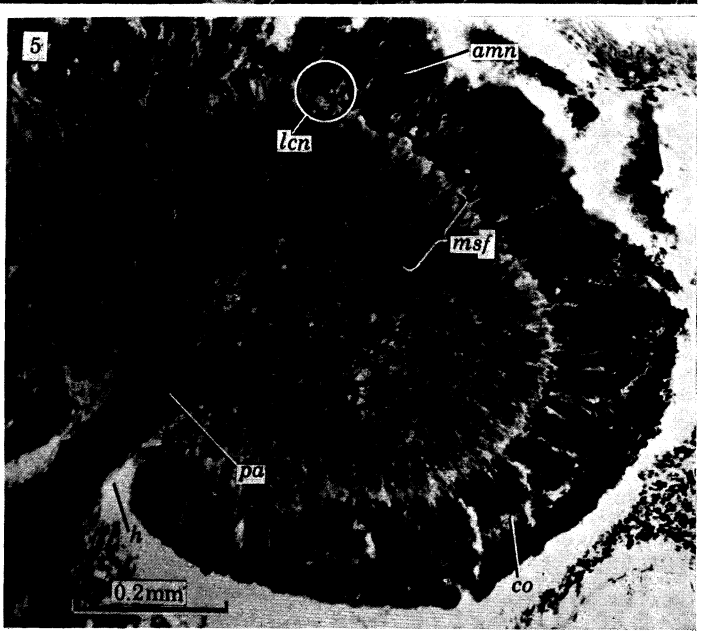
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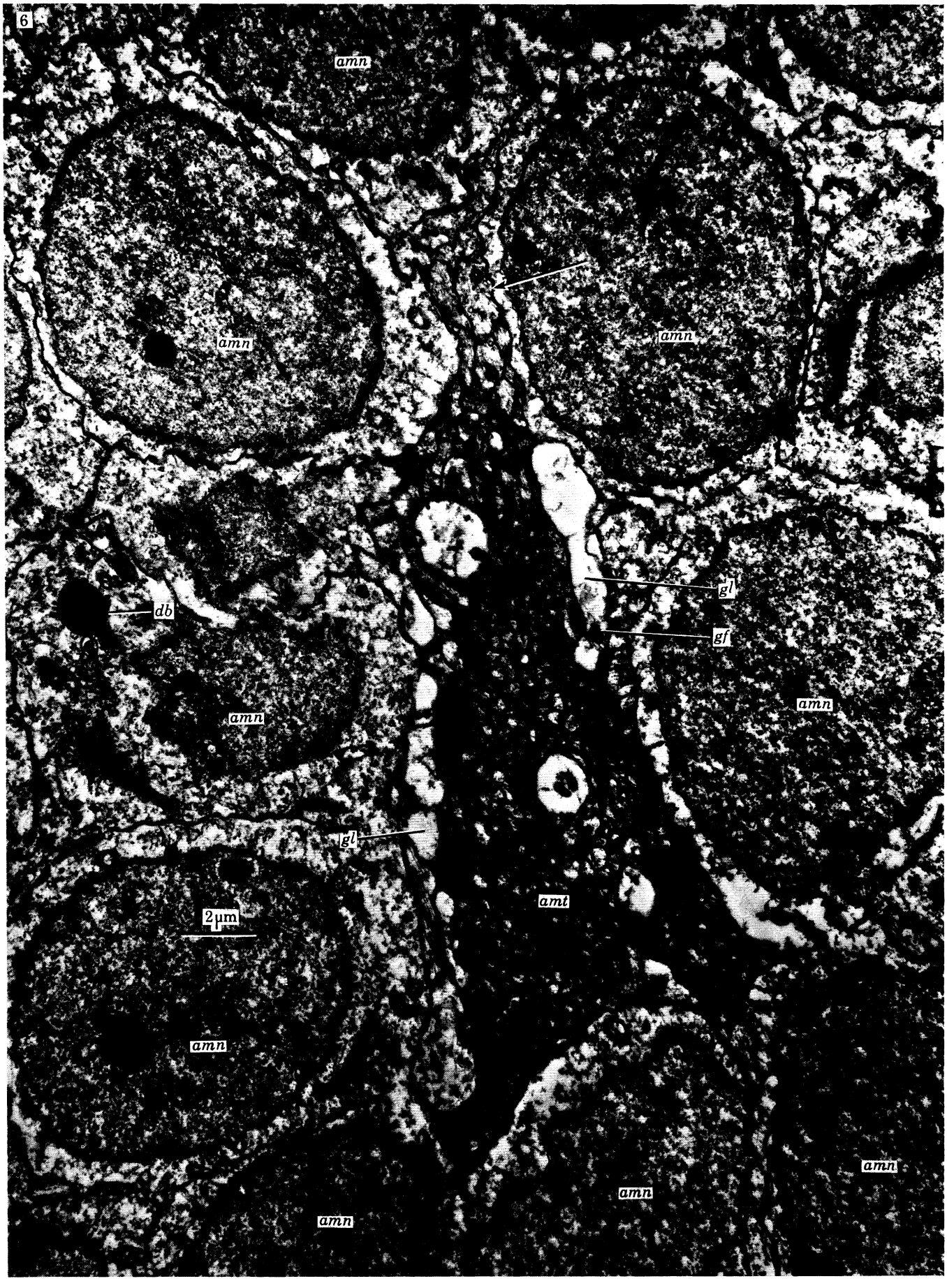
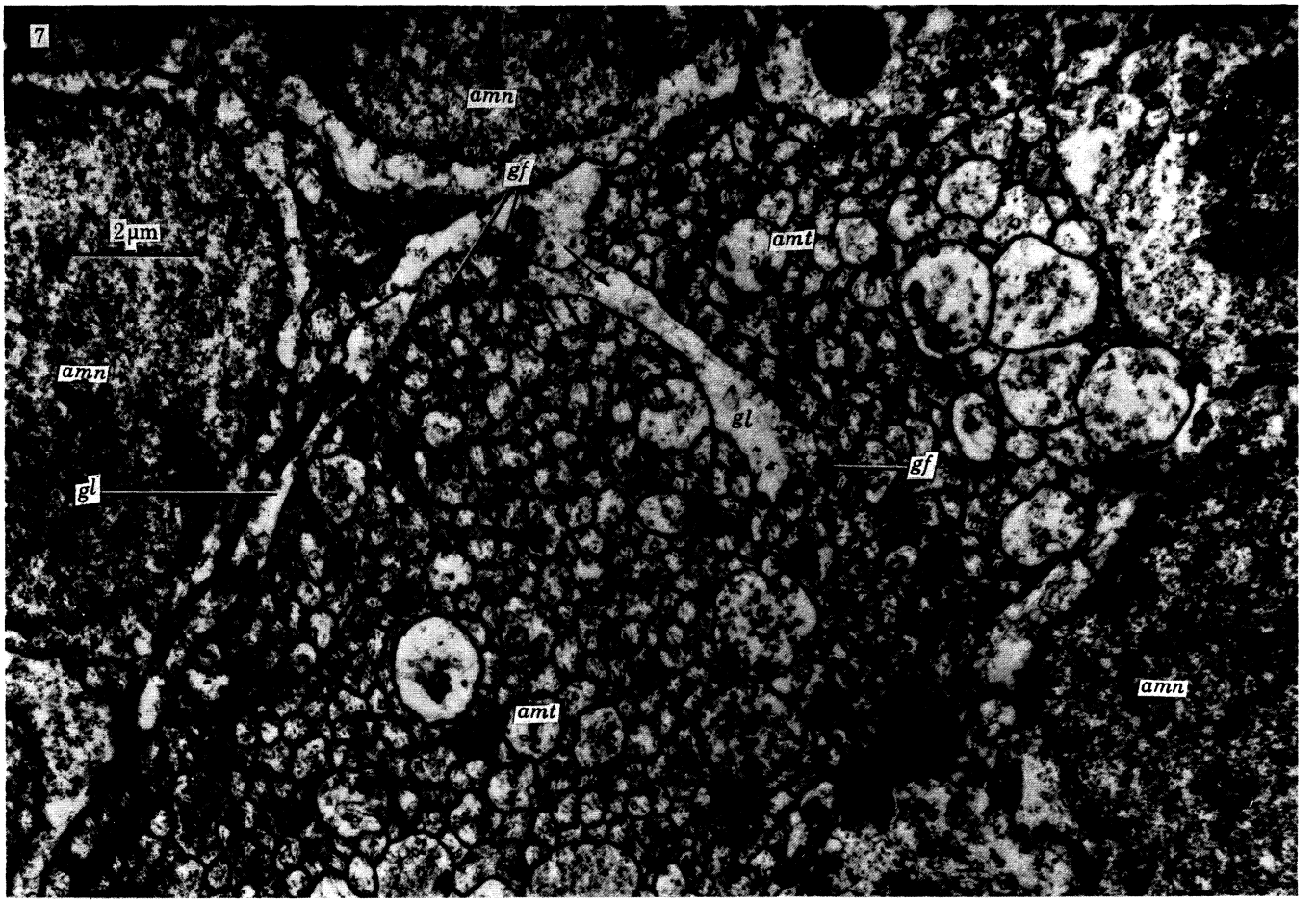


FIGURE 6. An amacrine tract (sectioned transversely) surrounded by amacrine perikarya. Cortex.



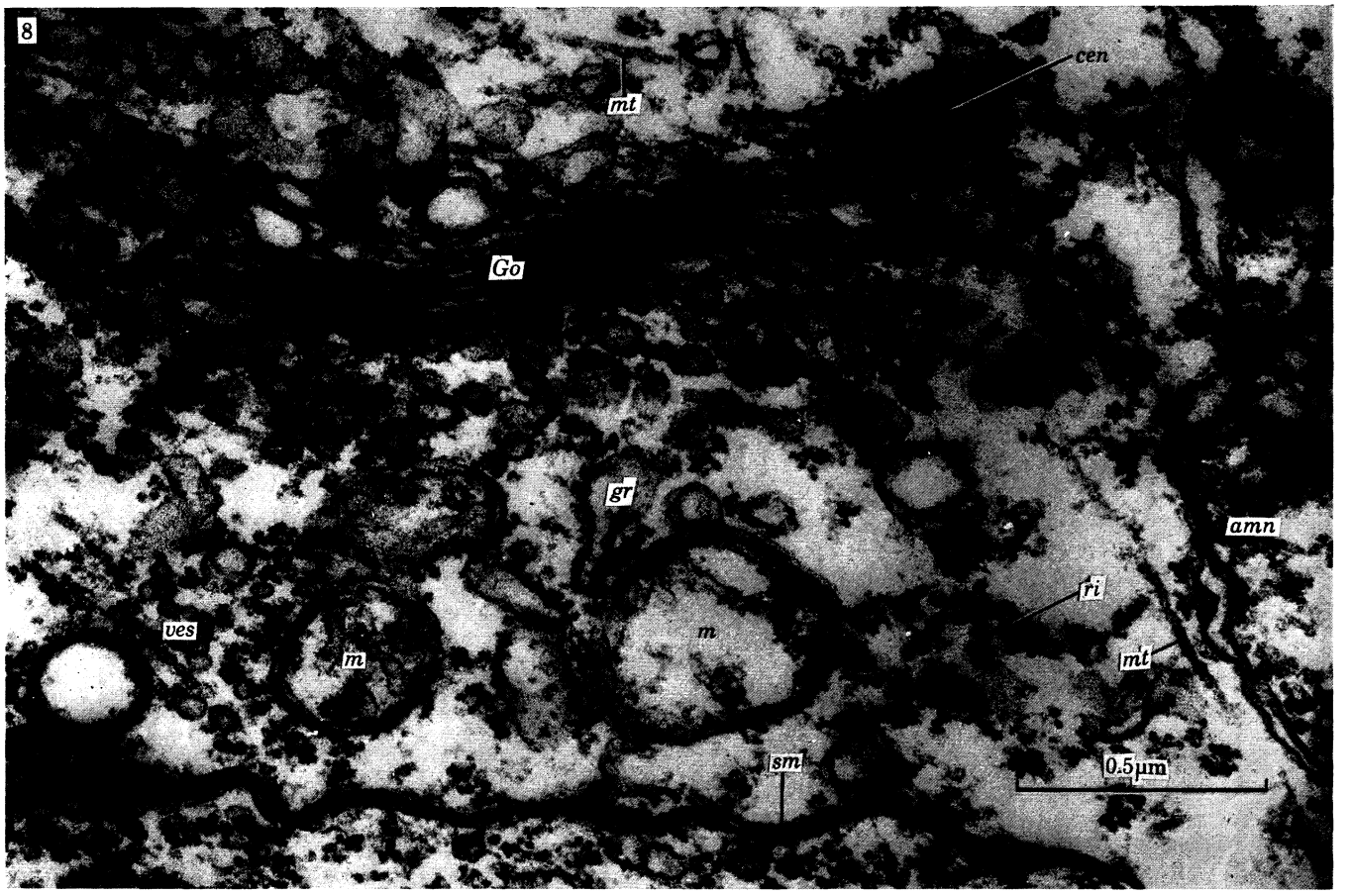


FIGURE 7. Amacrine tract (cut transversely) partly separated from the surrounding amacrine perikarya by a glial fold. Cortex.

FIGURE 8. Golgi zone of an amacrine perikaryon. Cortex.

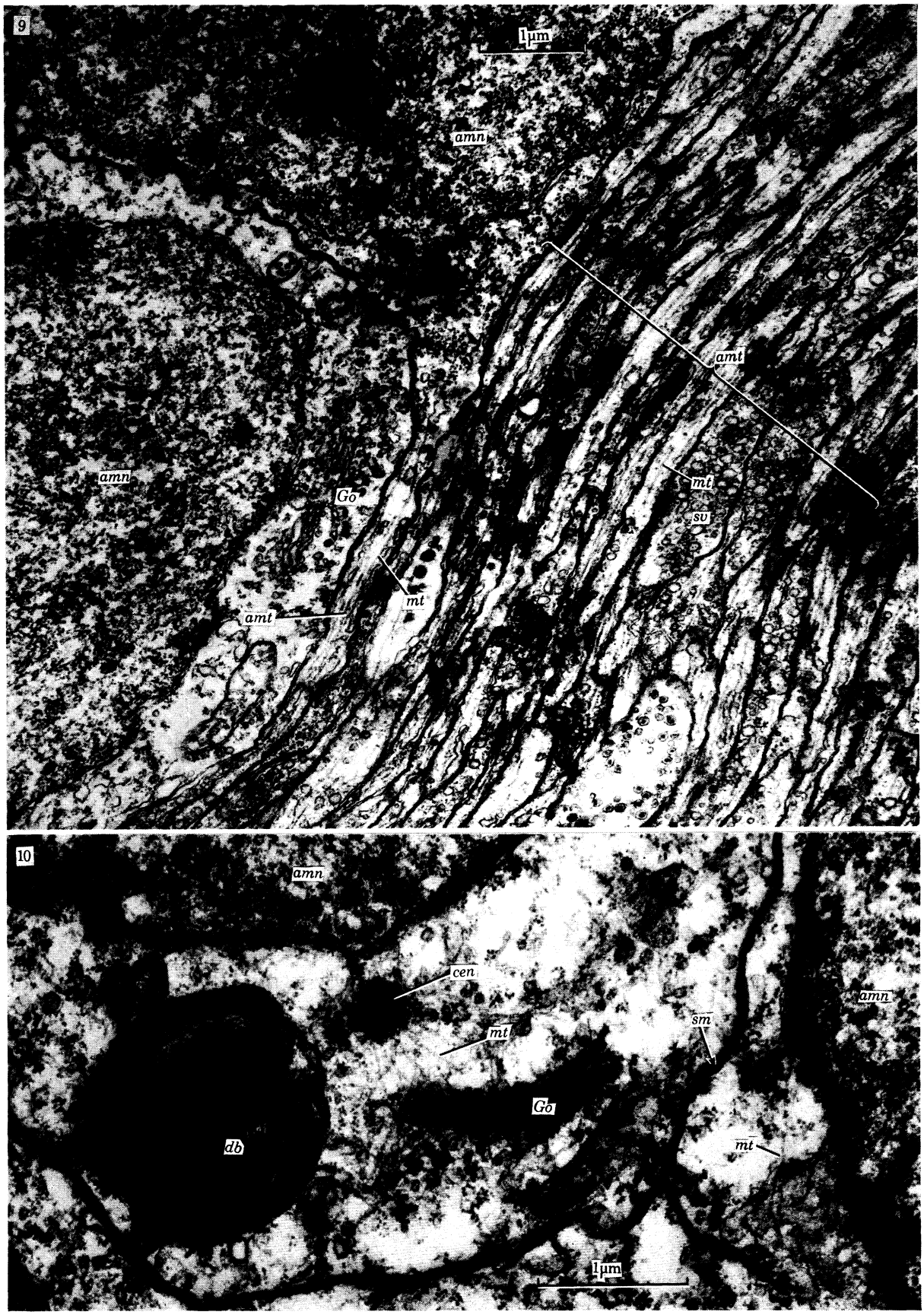
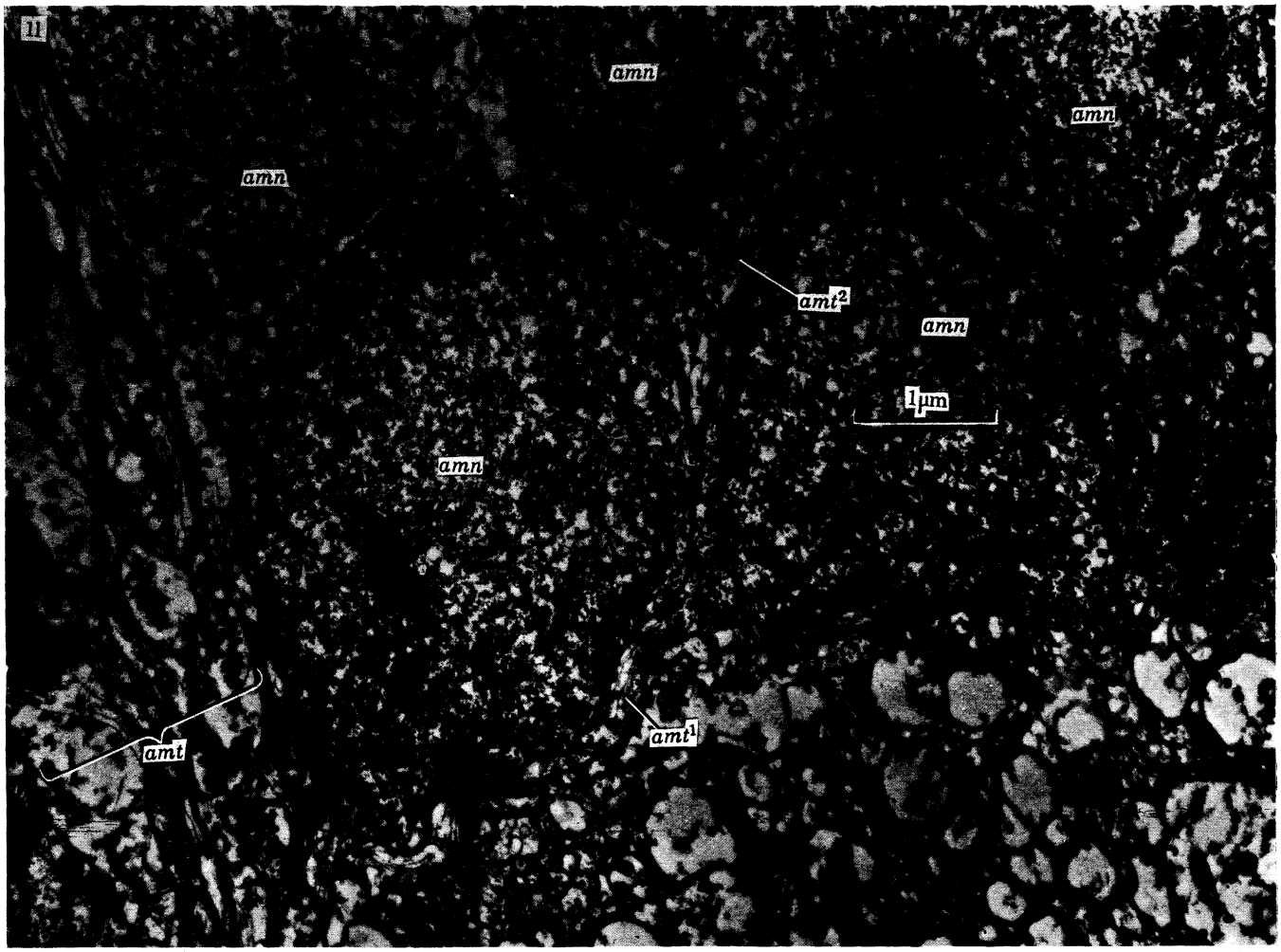


FIGURE 9. Two amacrine perikarya; the trunk of one can be seen joining (contributing to) an amacrine tract. Cortex.

FIGURE 10. An amacrine cell perikaryon with a dense body, centriole and Golgi apparatus. Cortex.



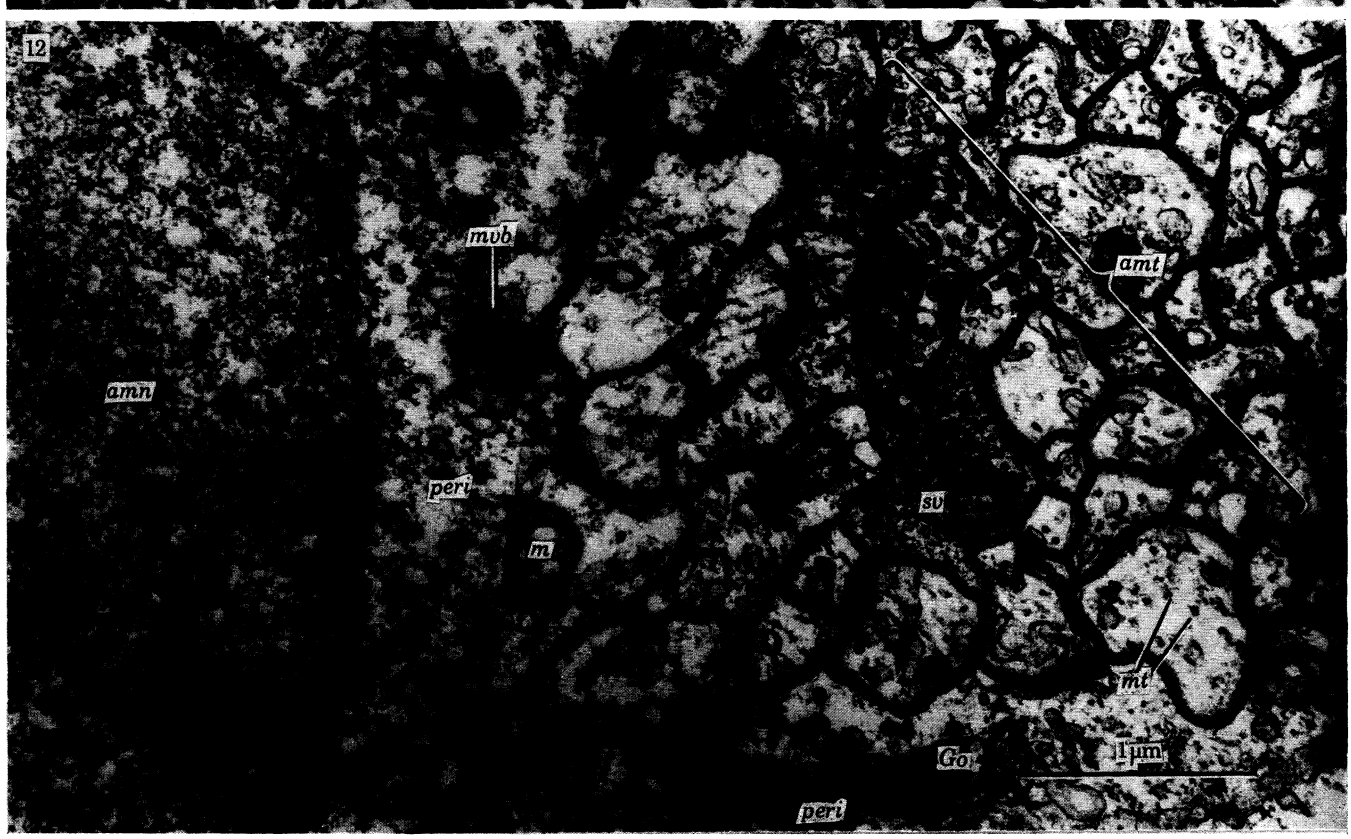


FIGURE 11. Two amacrine cells with their trunks joining an amacrine tract. Cortico-medullary junction.
FIGURE 12. Part of an amacrine cell and adjacent amacrine tract. Cortico-medullary junction.

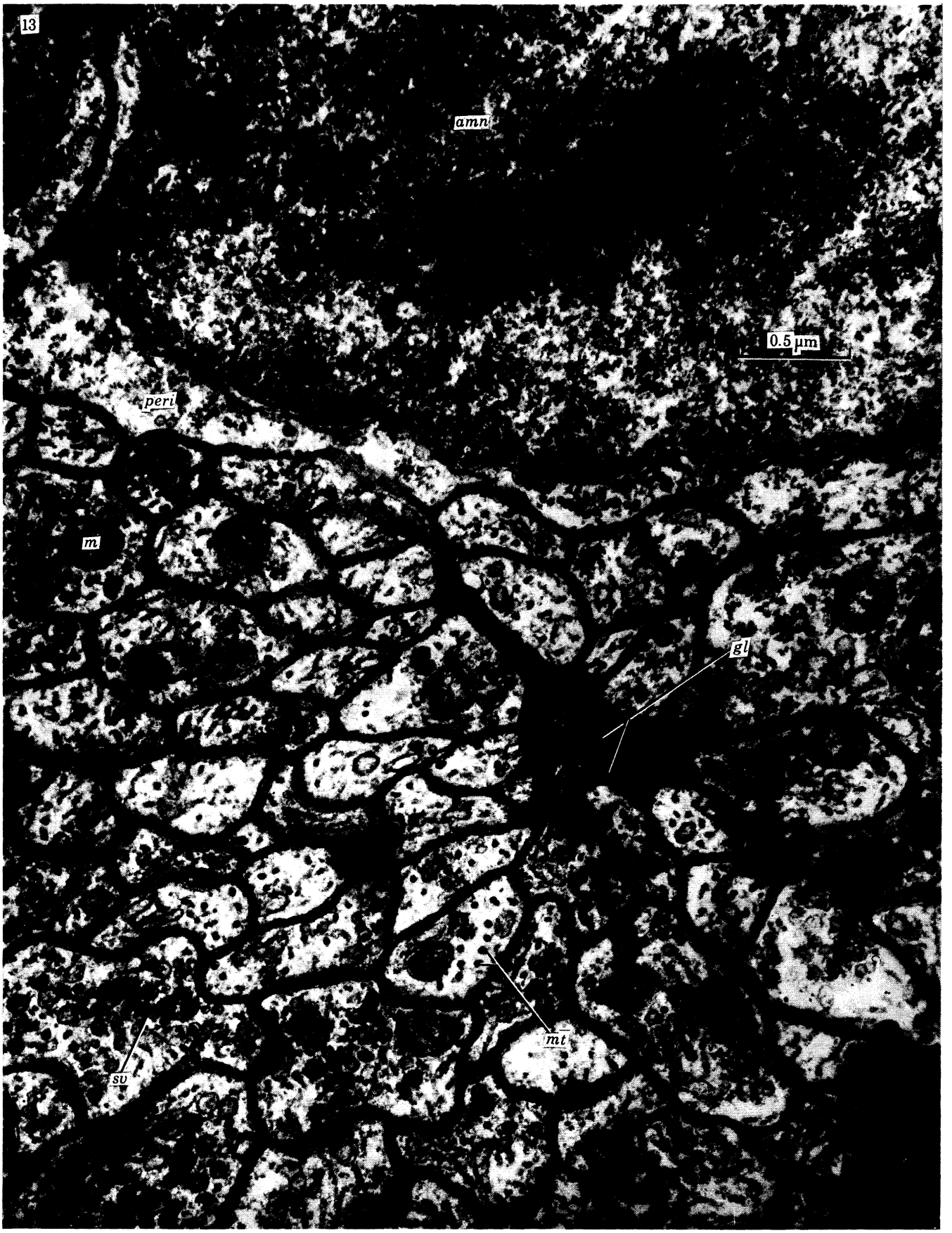
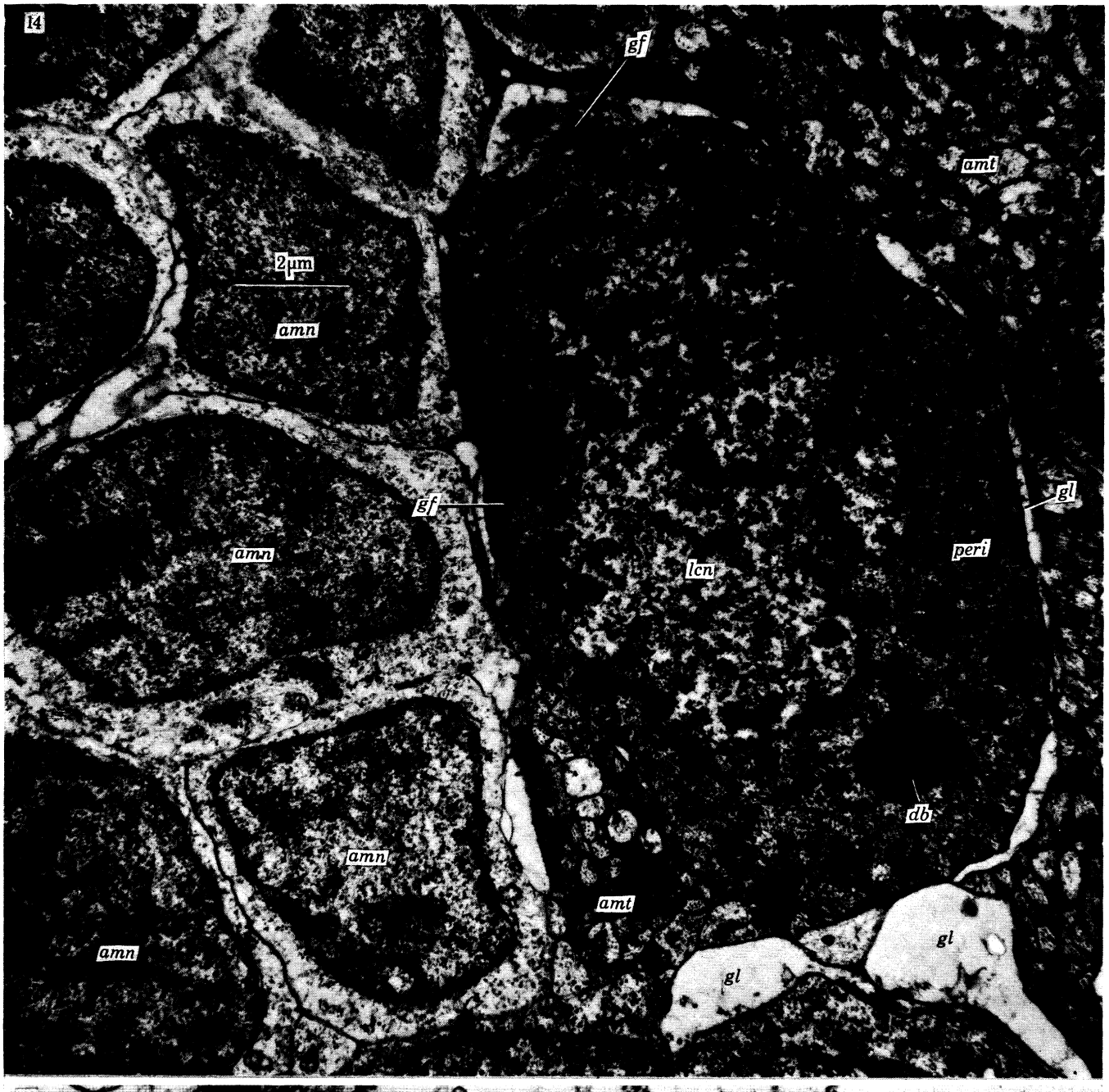


FIGURE 13. Amacrine tract (cut transversely). Two 'dark' glial processes intrude between the trunks. Outer medulla.



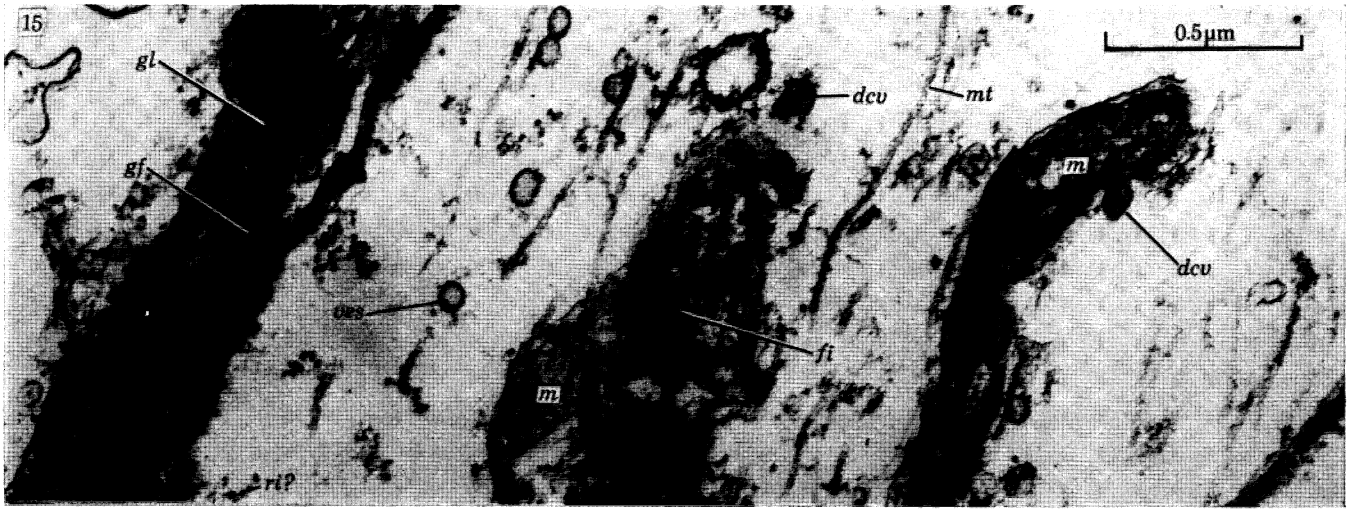


FIGURE 14. A large cell and numerous amacrine perikarya. Inner zone of cortex.

FIGURE 15. Part of the trunk of a large cell cut longitudinally. Outer medulla.

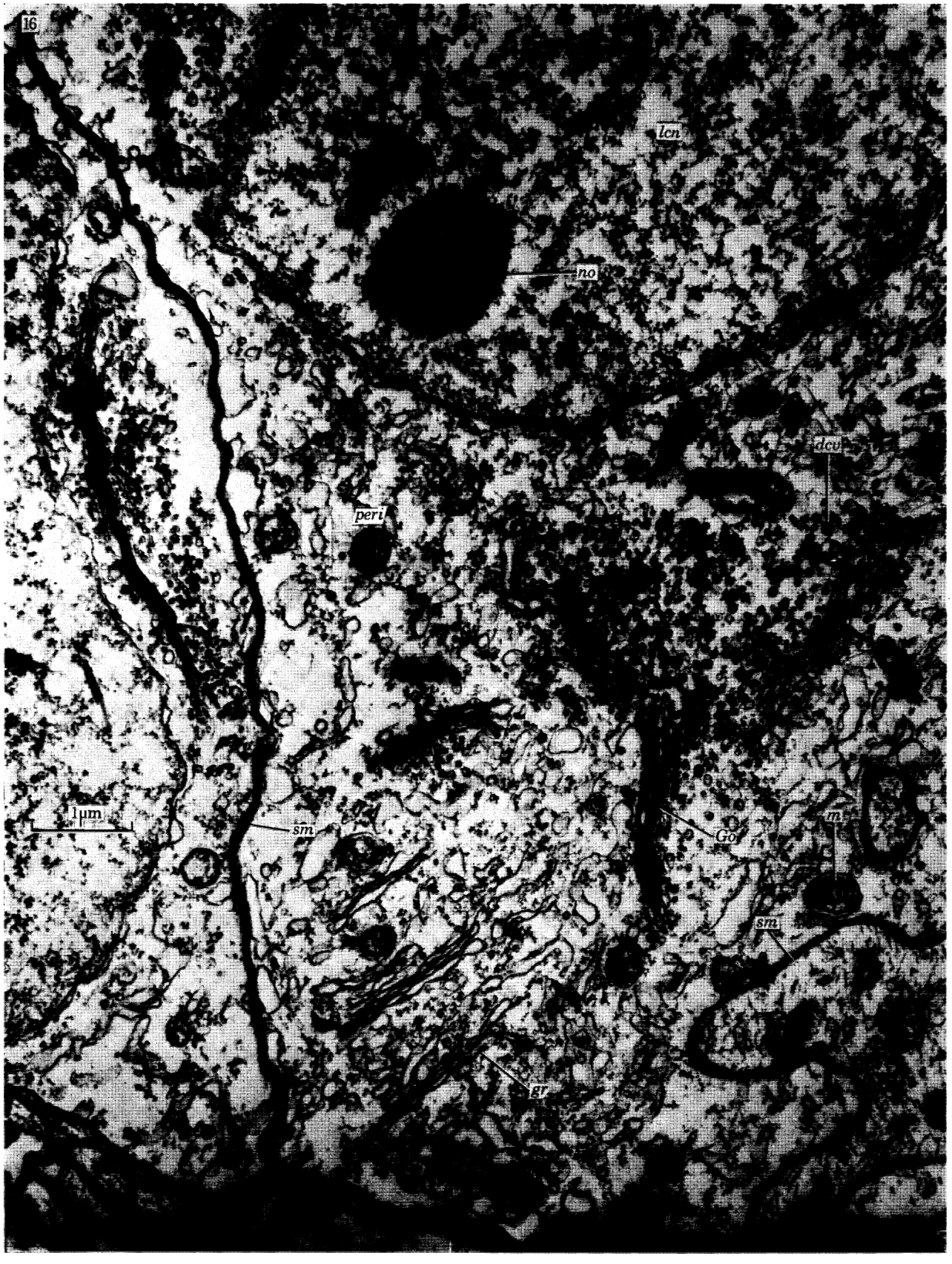
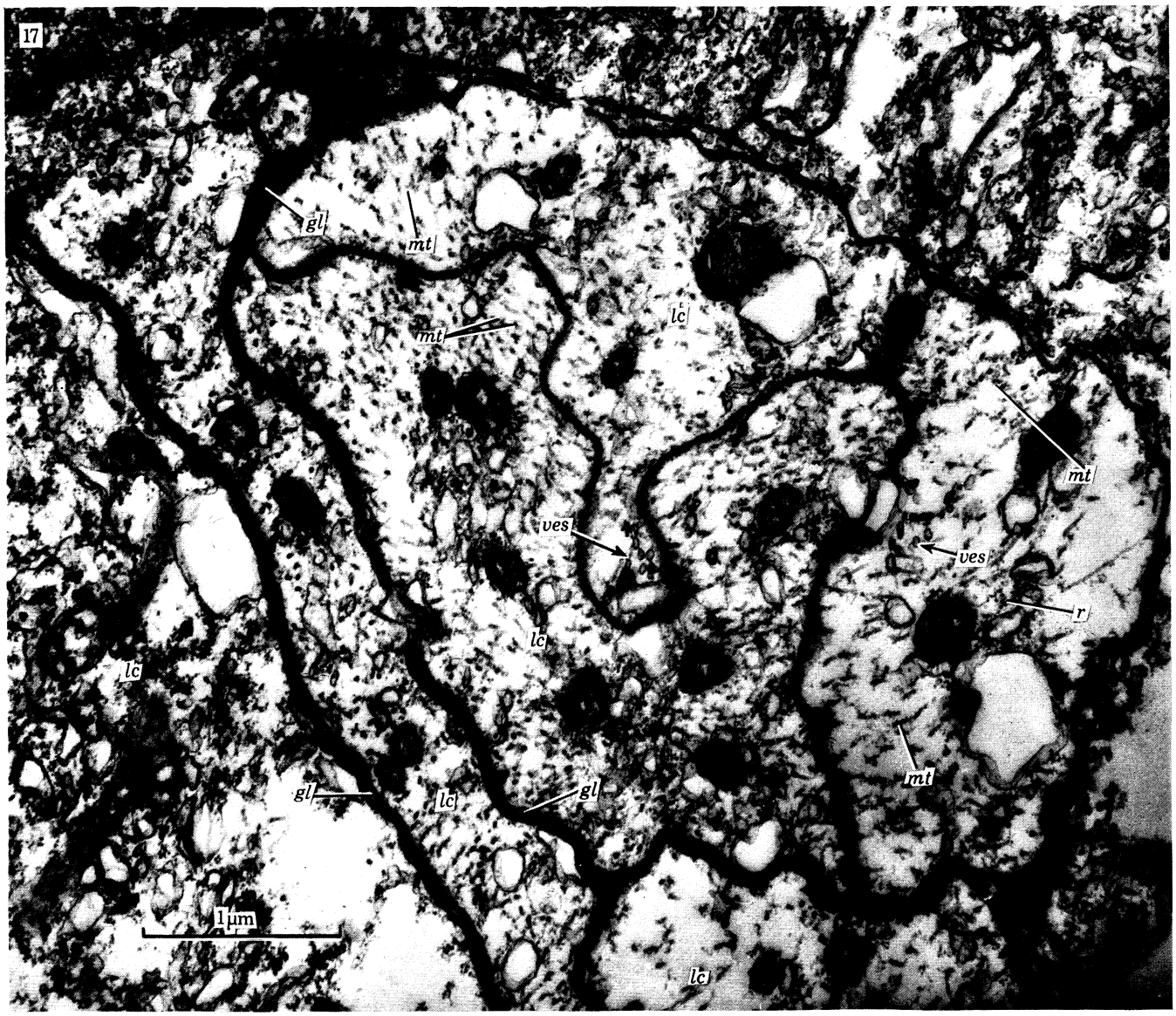


FIGURE 16. Parts of the perikarya of two adjacent large cells. Inner border of cortex.



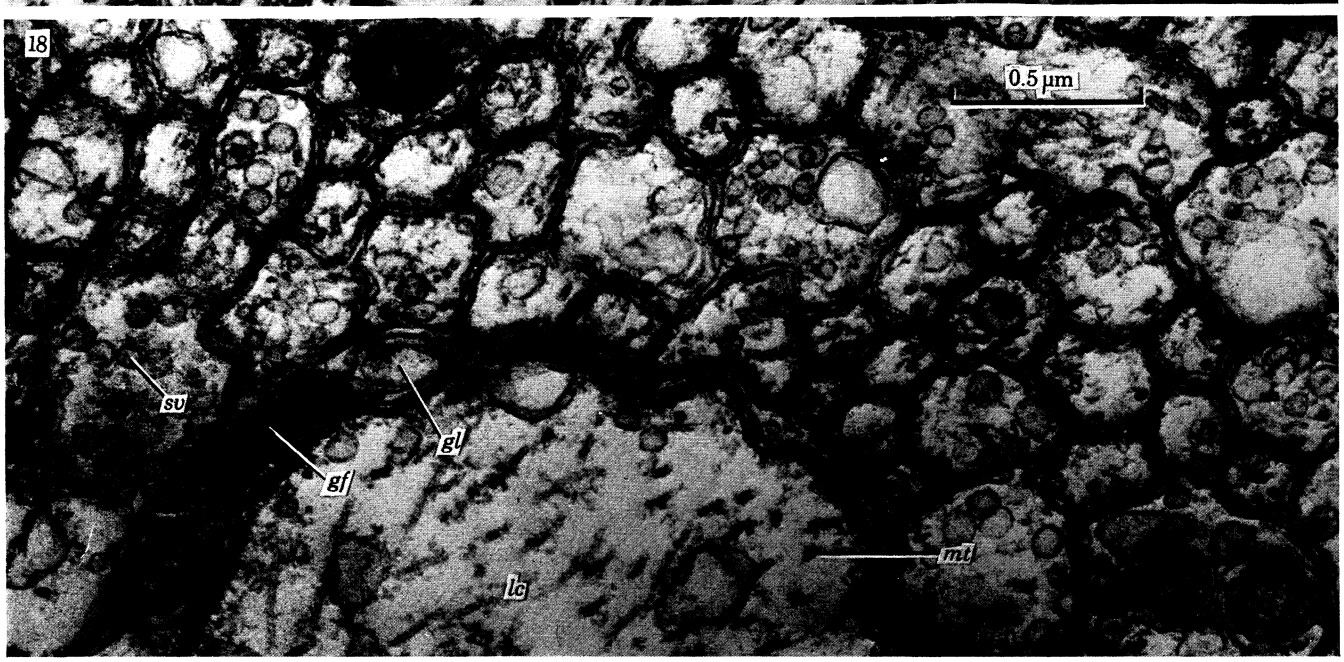


FIGURE 17 Trunks of large cells (cut transversely) showing interlocking profiles. Cortico-medullary zone.

FIGURE 18. Part of large cell trunk (cut transversely), separated from an adjacent amacrine tract by a glial fold. Outer medulla.

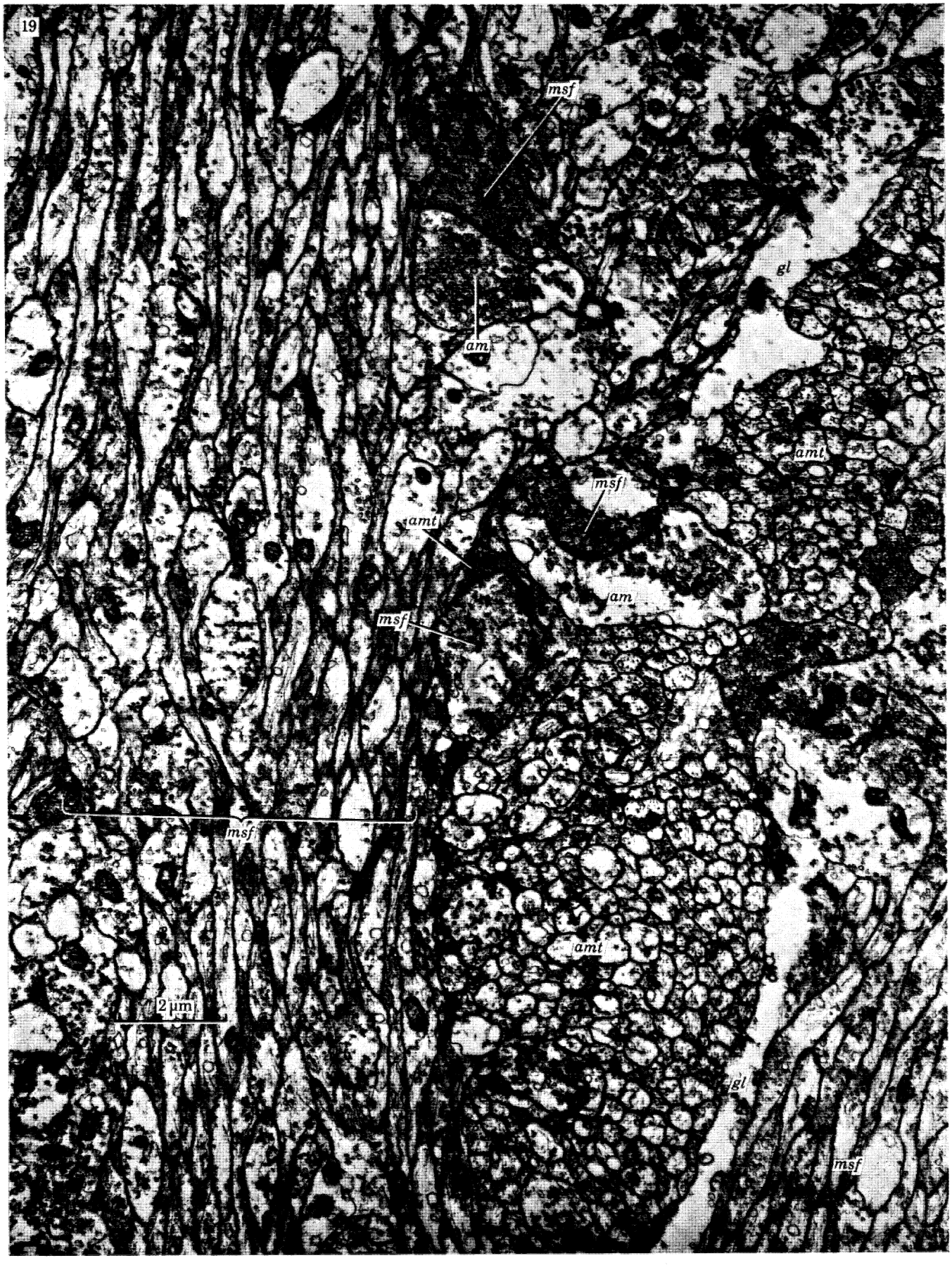


FIGURE 19. Bundles of MSF axons (cut longitudinally) and an amacrine tract (cut transversely). MSF-amacrine synapses occur as cruciform connexions. Outer medulla.

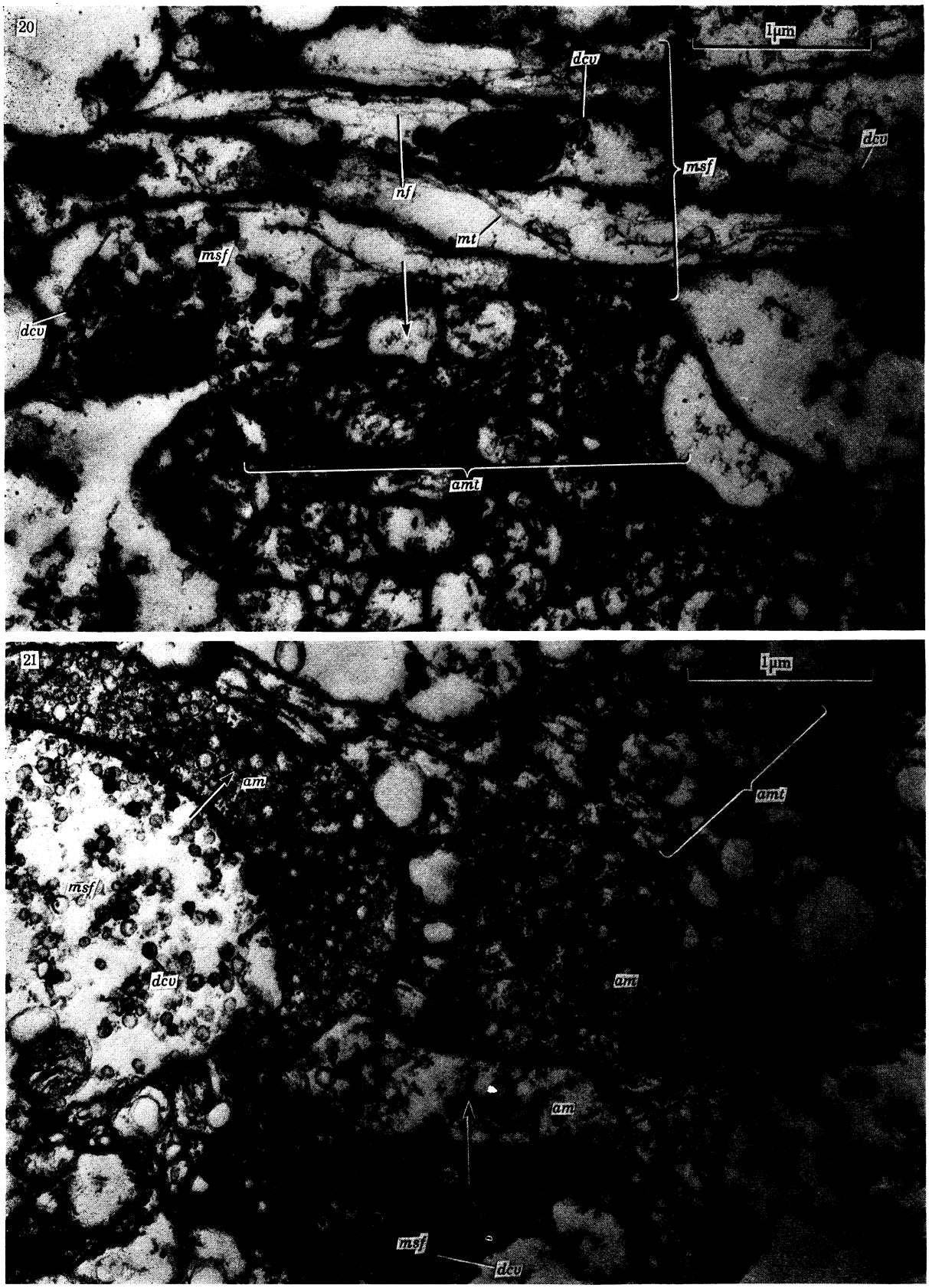


FIGURE 20. An MSF axon in synaptic contact with a profile thought to be an amacrine trunk since the profile forms part of an amacrine tract. Outer medulla.

FIGURE 21. Synapses between MSF varicosities and amacrine trunk varicosities. Left—the MSF varicosity is the pale one; centre—the amacrine trunk is the paler one. Medulla.

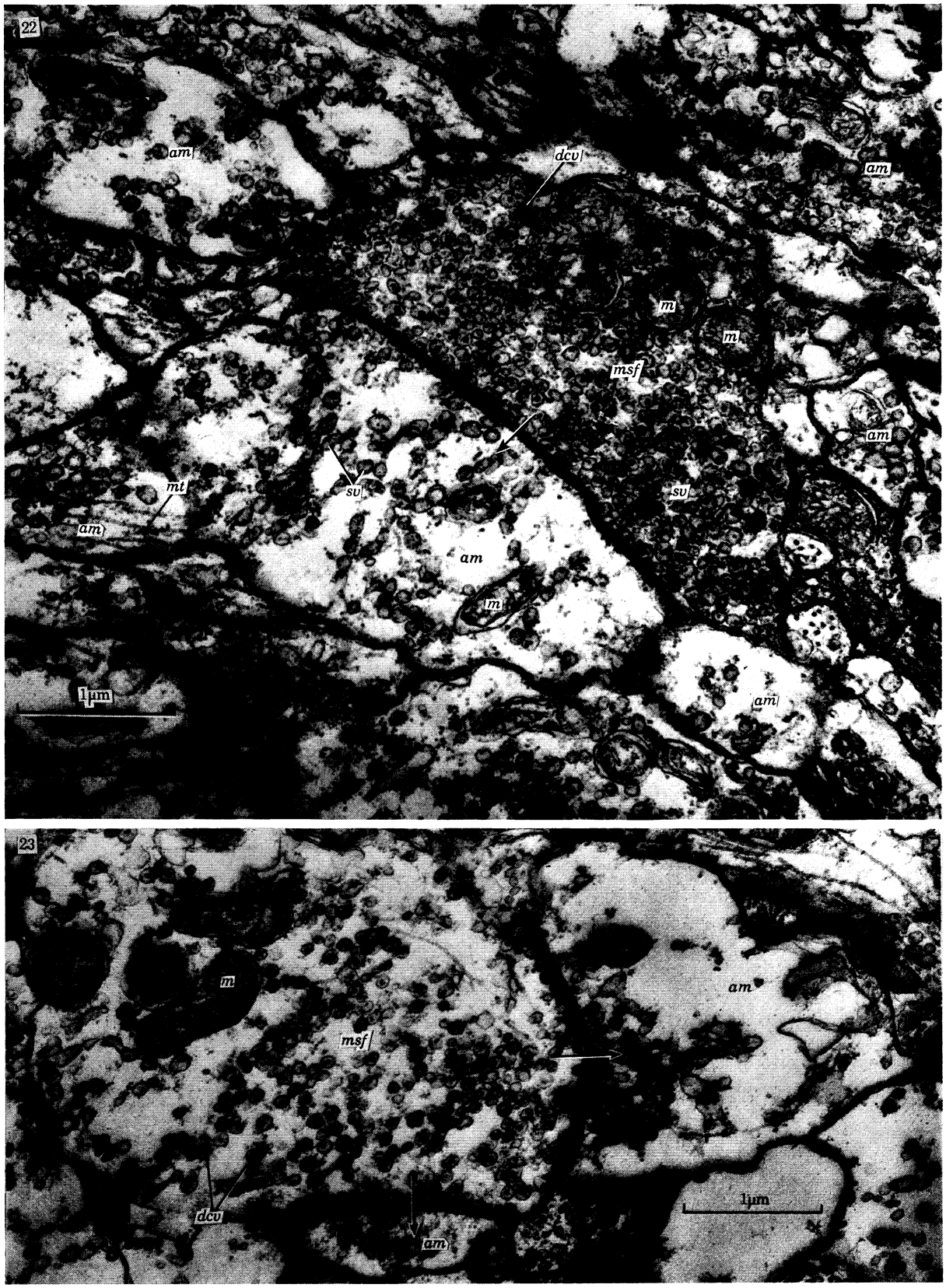


FIGURE 22. An MSF varicosity in synaptic contact with a varicosity of an amacrine trunk. Outer medulla. Arrows in this and other figures indicate the presumed direction of transmission across the synaptic cleft.

FIGURE 23. An MSF varicosity in synaptic contact with the varicosities of two amacrine trunks. Outer medulla.

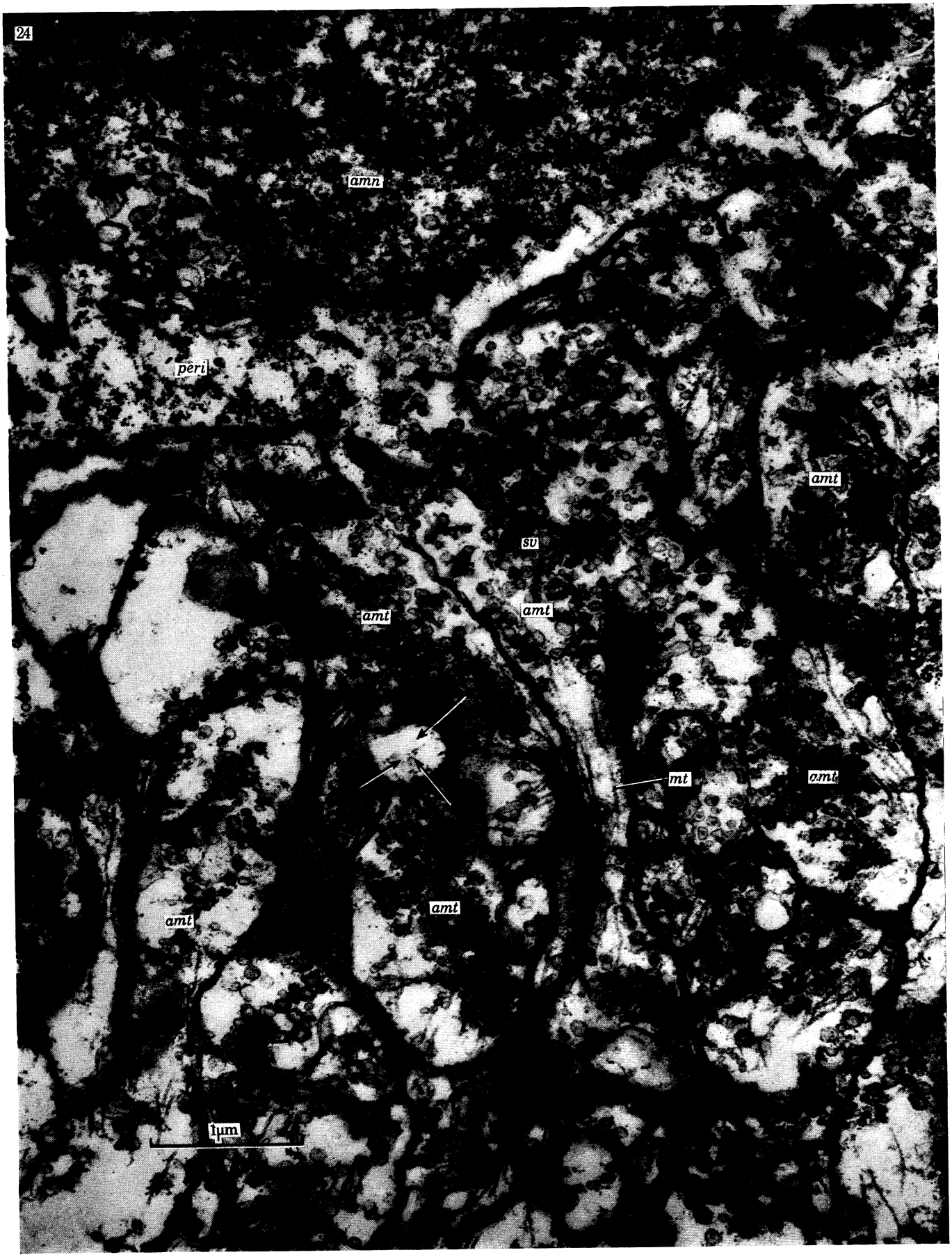
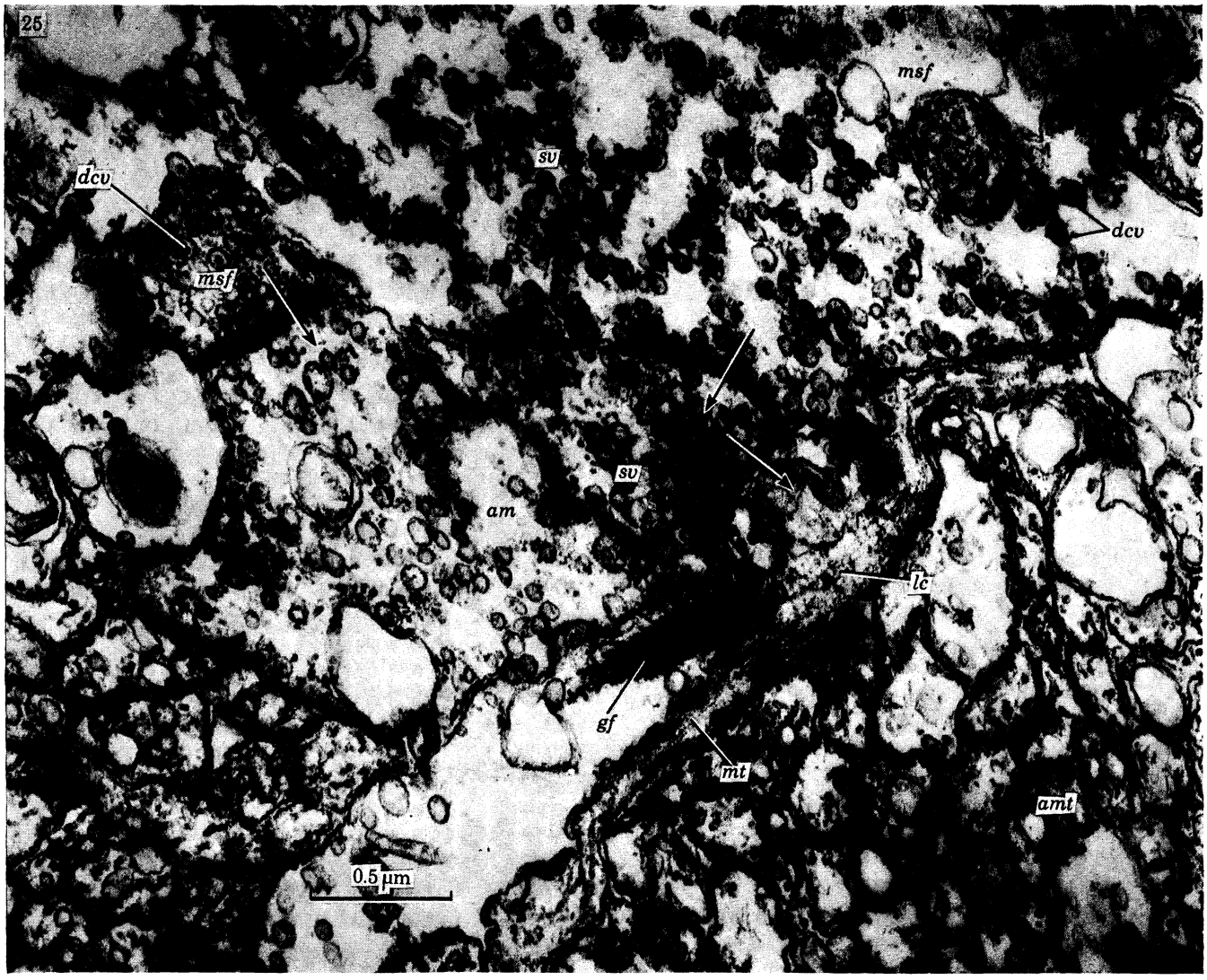


FIGURE 24. An amacrine cell in the inner cortical margin; its trunk extends into the medulla.



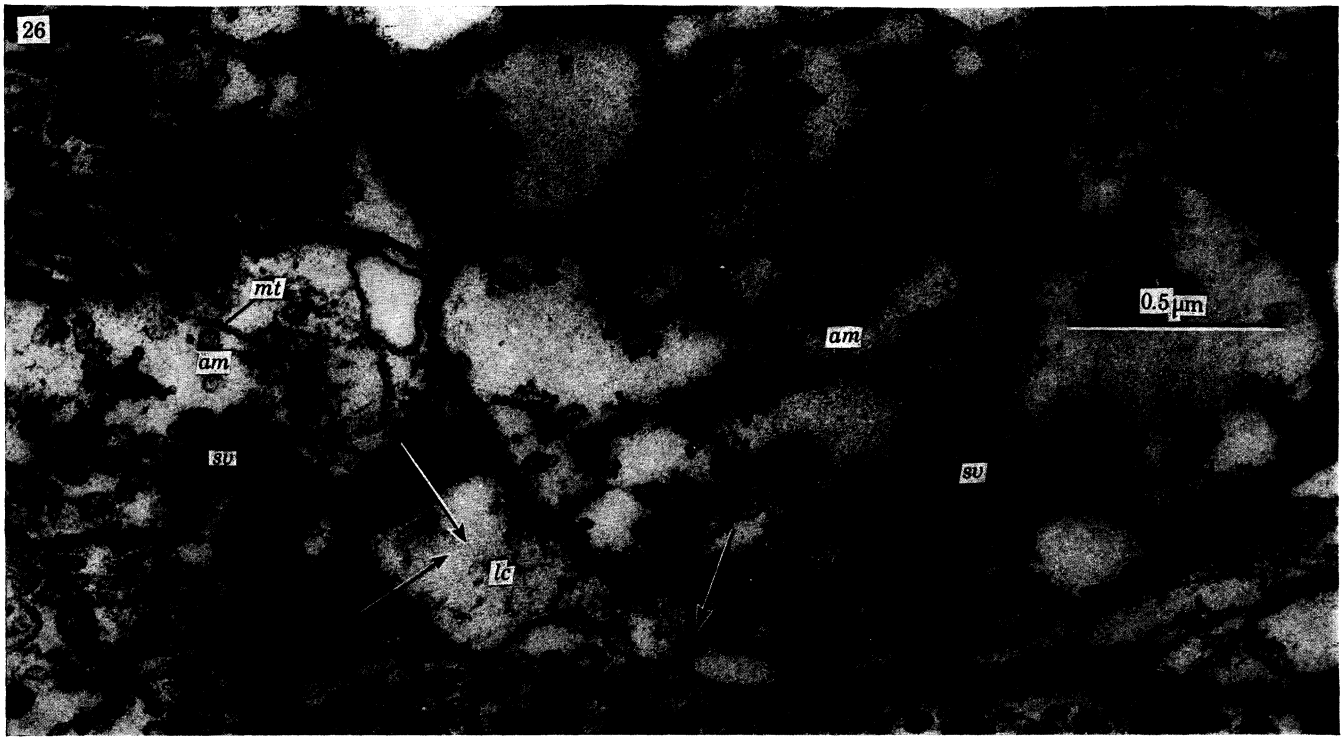


FIGURE 25. Two MSF varicosities synapsing with an amacrine varicosity, which in turn is synapsing with a collateral of a large cell. Outer medulla.

FIGURE 26. Three amacrine varicosities in synaptic contact with a pale process thought to be the collateral of a large cell.

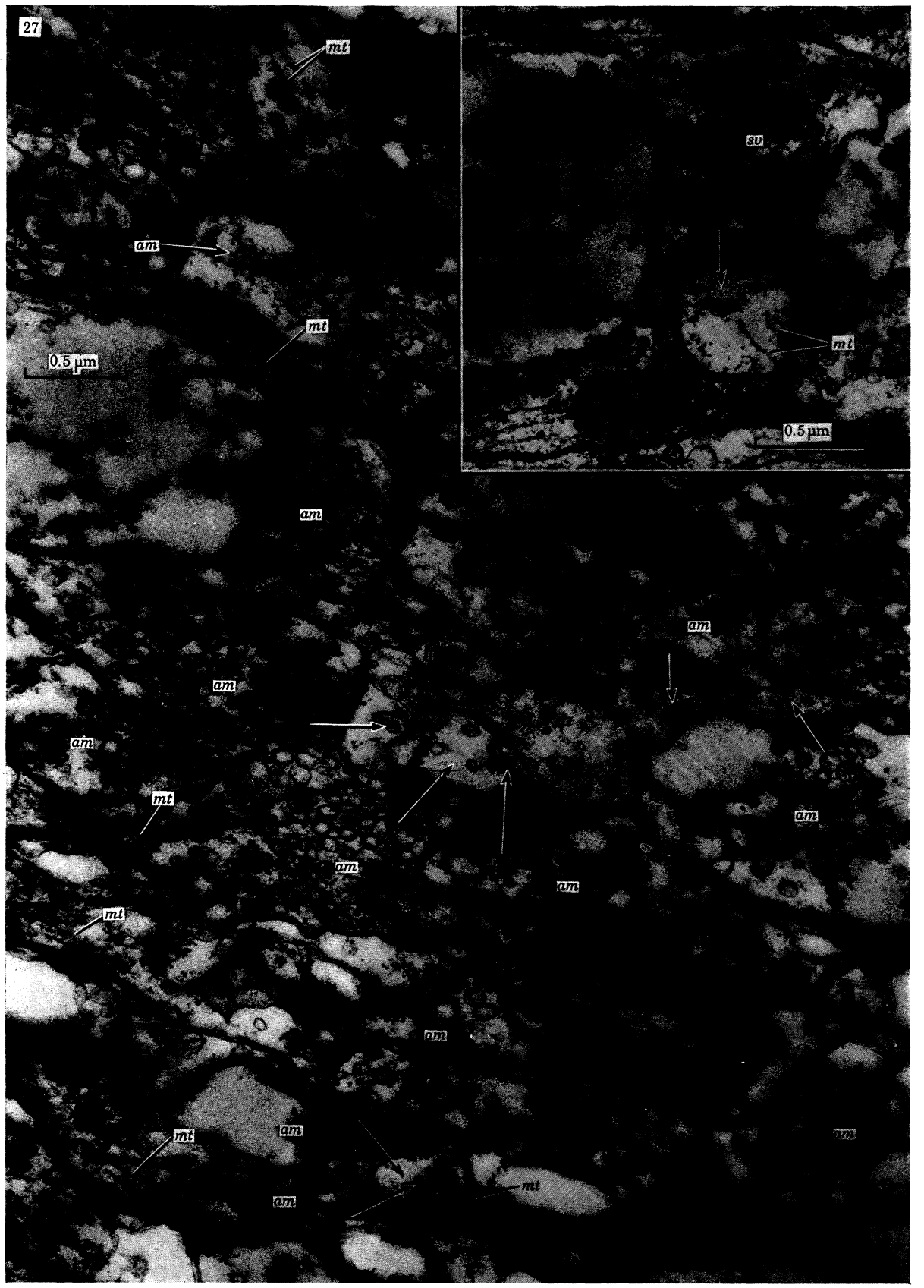


FIGURE 27. The central zone of the medulla contains numerous amacrine trunks synapsing with branches presumed to originate from trunks of large cells. Broken lines represent probable membranes, which are invisible because of tilt. Microtubules and small granules (ribosomes or glycogen?) present (but not invariably) in the pale postsynaptic component. (Inset) such a contact at higher magnification.

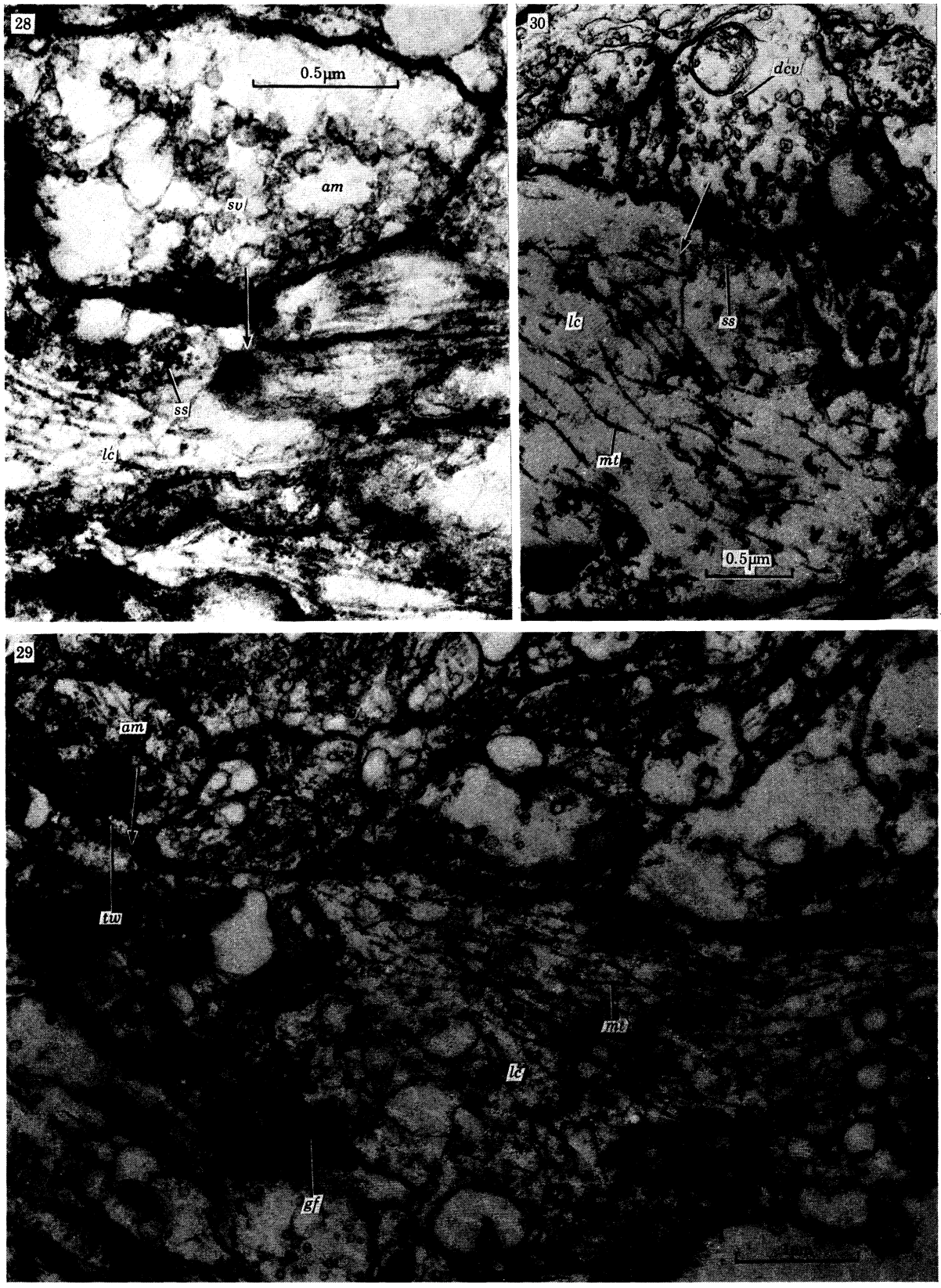


FIGURE 28. Amacrine varicosity contacting a process of a large cell. Central medulla.

FIGURE 29. Fine collateral of a process of a large cell contacted by an amacrine presynaptic varicosity. Central medulla.

FIGURE 30. A synaptic knob (amacrine or MSF?) contacting a process of a large cell. Central medulla.

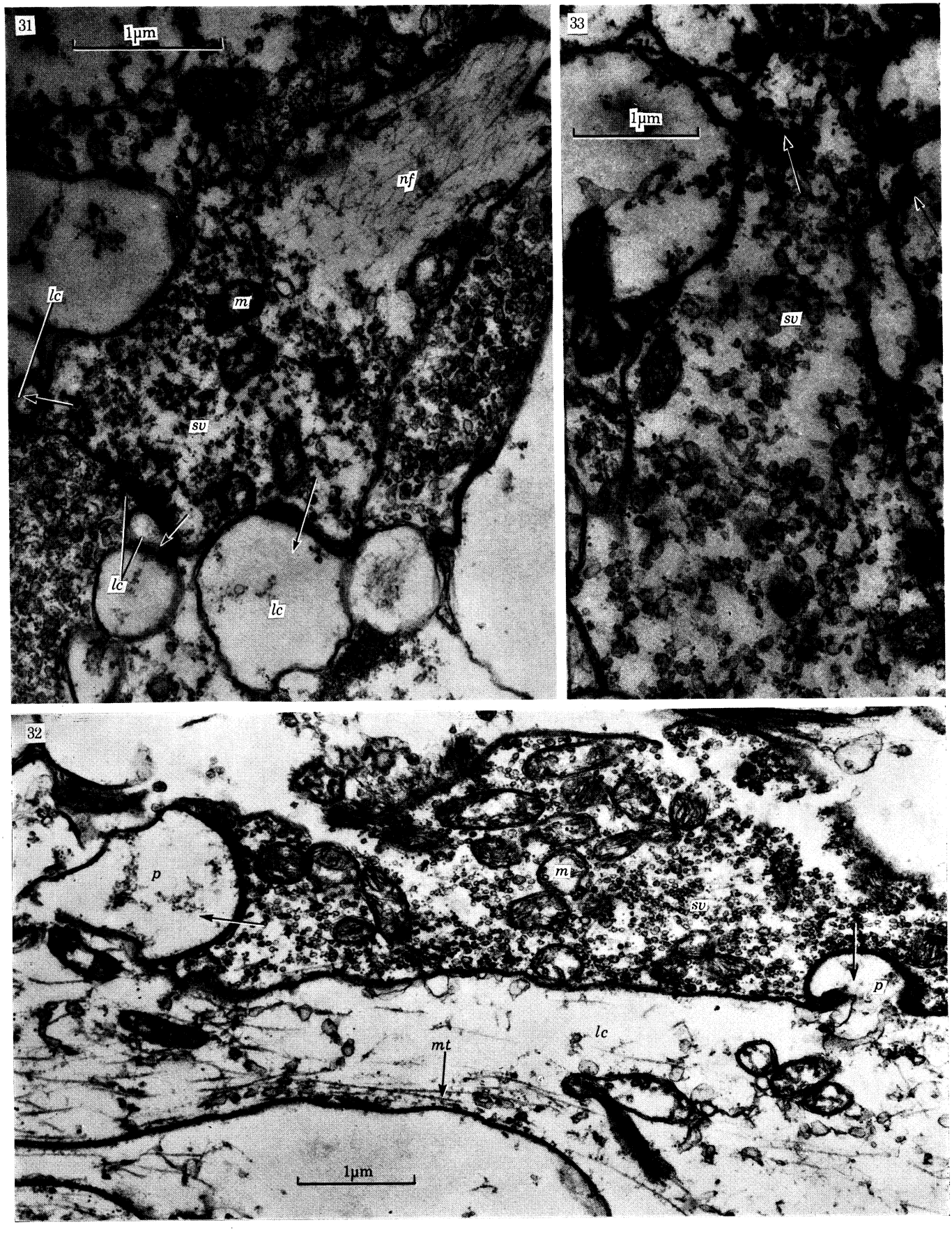


FIGURE 31. Presumed 'pain' ending contacting processes presumed to arise from trunks of large cells. Central medulla.

FIGURE 32. Presumed 'pain' ending contacting protruberance of process of large cells. Central medulla.

FIGURE 33. Presumed 'pain' endings contacting spinous processes (presumably of collaterals of large cells). Central medulla.

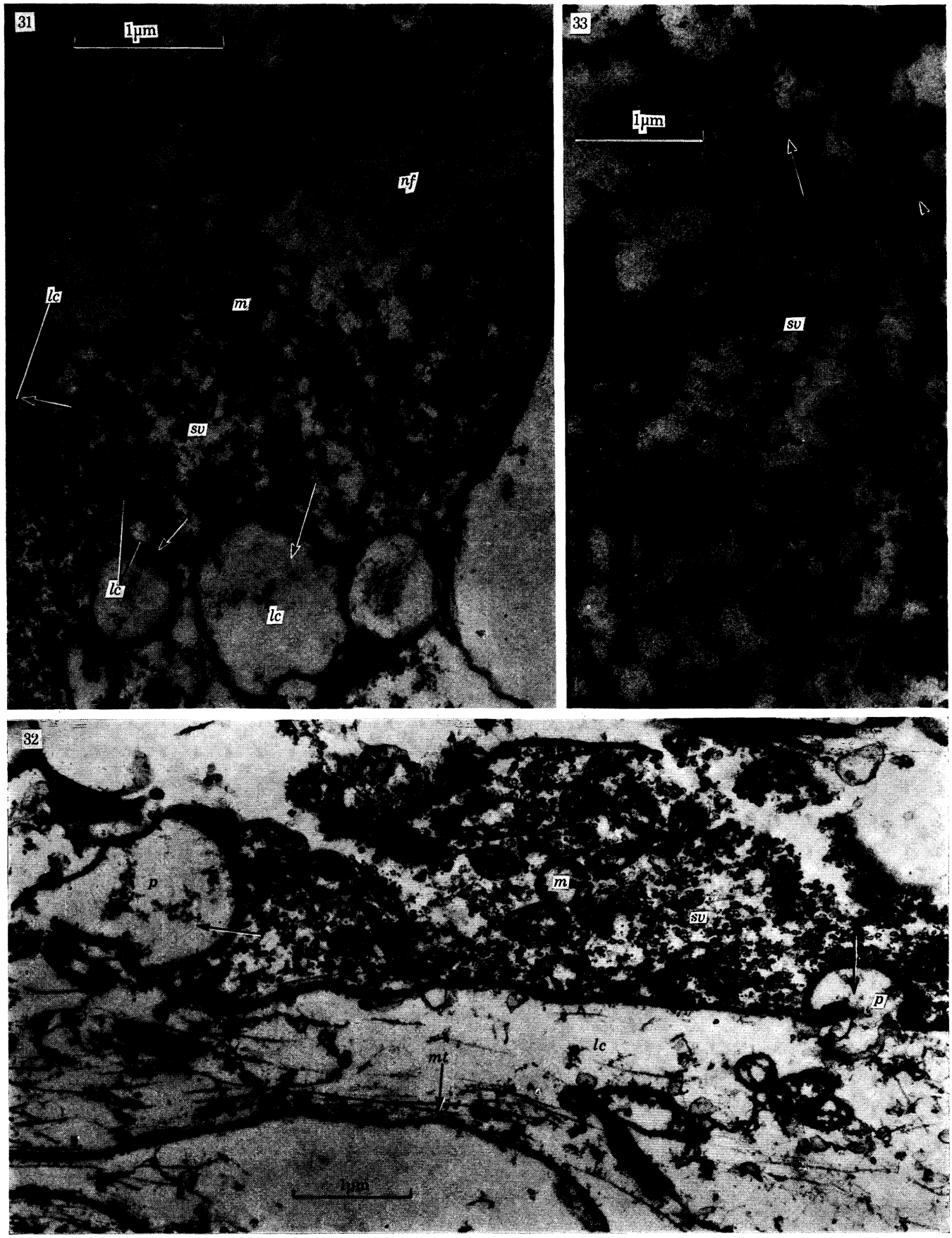
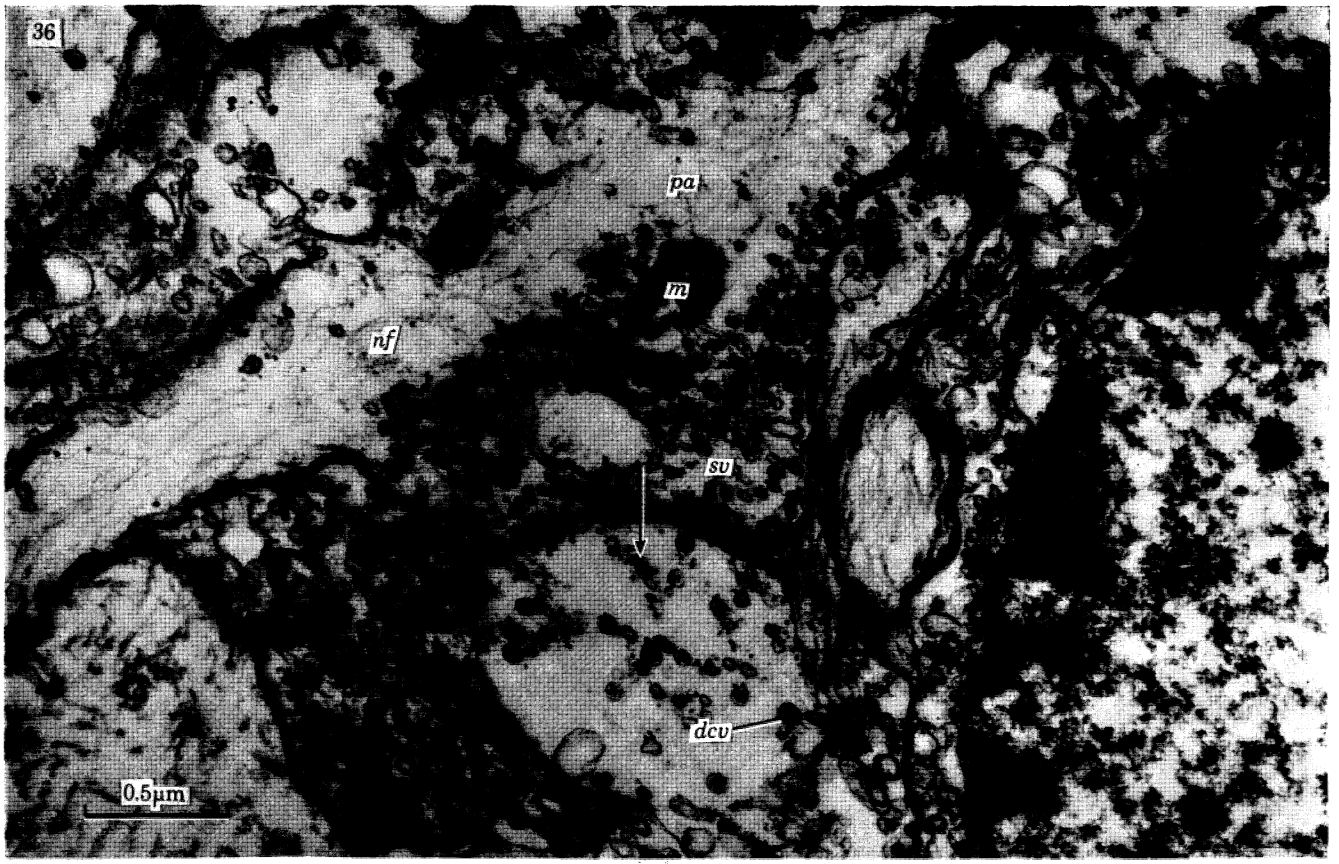


FIGURE 31. Presumed 'pain' ending contacting processes presumed to arise from trunks of large cells. Central medulla.

FIGURE 32. Presumed 'pain' ending contacting protruberance of process of large cells. Central medulla.

FIGURE 33. Presumed 'pain' endings contacting spinous processes (presumably of collaterals of large cells). Central medulla.



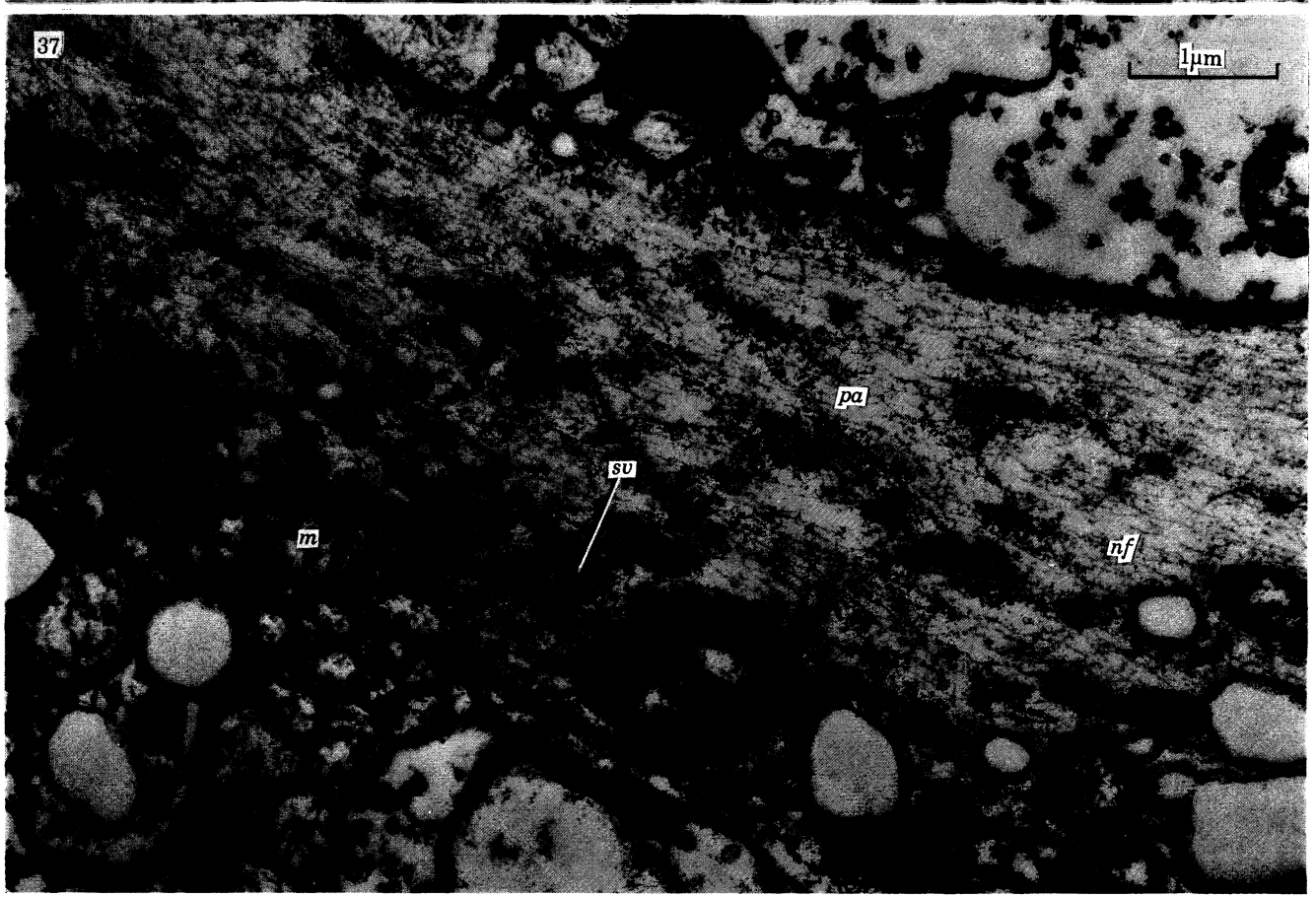


FIGURE 36. Presumed 'pain' axon making synaptic contact with a process containing dense-cored vesicles. Central medulla.

FIGURE 37. 'Pain' axon (aldehyde-fixed) containing small and flattened vesicles and neurofilaments. Central medulla.

FIGURE 38. An axo-somatic synapse on an amacrine cell. Cortex.

fibres (figures 1 to 5, plate 73) that can be seen in silver preparations, running into the lobule medulla up through the lobule hila. He suggested that these are 'pain' or nocifensor fibres. Electron microscopy shows a morphologically distinct set of synaptic knobs in the lobule medulla, which are in all probability the 'pain' axon terminals (see Discussion).

One such knob is shown lying in the central neuropil region of the medulla of a lobule (figure 31, plate 89). The preterminal region contains numerous neurofilaments that are quite distinct from neurotubules and, as we shall see, these neurofilaments serve as a valuable criterion for identification of the pain axons. It is the neurofilaments that account for their strong argyrophilia described by Young (see Discussion).

The synaptic vesicles in the knob (figure 31) have distinctive features that facilitate the identification of the knob even when in the plane of section it cannot be followed to the neurofilament-containing preterminal region of its axon. The majority of the synaptic vesicles are very small with diameters ranging from 15 to 30 nm. They are round, flattened or irregular in shape. A few of the vesicles are larger (50 or 60 nm) and these sometimes have small dense cores. In this illustration the knob is presynaptic to four pale profiles (*lc*).

In favourable planes of section these knobs can be seen to be ovoid and several micrometres long (e.g. figure 32, plate 89). They are thus much larger than the MSF and amacrine presynaptic dilatations (knobs) already encountered.

These pain fibre knobs are thought to synapse with the trunks or their collaterals of the large cells. Here (figure 32) the trunk is identified by its size and content of microtubules, and the synapses are formed on small bulbous protrusions of the trunk. The pale post-synaptic profiles seen in figure 31 are probably sections through thin dendritic collaterals of the large cell trunks. Sometimes the postsynaptic components take the form of fine spines that invaginate the presynaptic bag (figure 33, plate 89). The thin spine presumably originates from the trunk of a large cell. Sometimes two closely apposed presynaptic knobs thought to be 'pain' endings contact one postsynaptic process.

The identification of the presynaptic components in figures 32 and 33 relies on the criteria of size and appearance of the synaptic vesicles, for in these the neurofilamentous preterminal region they are assumed to have does not appear in the plane of section. In figure 31 we saw that the neurofilaments stop short where the synaptic vesicles appear. Other observations confirmed this view, where the preterminal axon could be followed for some distance in the plane of section. It contained the characteristic neurofilaments (*nf*) that stop short at the beginning of the synaptic bulb. In this zone a few microtubules can sometimes be seen running between the vesicles.

The distinction between neurofilaments in the 'pain' fibres and microtubules in trunks of the large cells is illustrated in figure 34, plate 90, where profiles of two 'pain' endings (with the characteristic vesicles) can be seen adjacent to profiles of a group of four trunks of large cells cut across.

Sometimes neurites can be seen with coarse filaments (figure 35, plate 90). Since these filaments appear solid they are regarded as neurofilaments not microtubules. Groups of the small polymorphic vesicles (*sv*) occur in the neurites, which are thought also to be 'pain' fibres.

Occasionally (figure 36, plate 91), structures identified as 'pain' knobs, because of their small vesicles and neurofilaments, are seen to be presynaptic to profiles which, judging by their content of plain and dense-cored vesicles, could be MSF synaptic varicosities. The latter we have seen to be presynaptic to amacrine knobs, so this might be a serial arrangement (see Discussion).

(4) *Anomalous contacts on amacrine cells*

Synaptic contacts between MSF fibres and amacrine trunks were described on p. 384. Here certain MSF-amacrine contacts are described separately because of their unusual nature. Several hundred amacrine cell bodies were examined in EM sections in the course of this study and on three occasions apparent axo-somatic contacts on amacrine perikarya were observed. They can be seen where an amacrine perikaryon borders directly on an amacrine tract (consisting, as we have seen, of the grouped trunks of the neighbouring and more superficial amacrines and joined by the trunk of this amacrine). Figure 38, plate 92, shows such an amacrine perikaryon with the proximal portion of its trunk (*amt*) joining an amacrine tract. The perikaryon is contacted by a knob that contains mostly dense-cored vesicles and so is apparently a terminal or *en passant* varicosity of an MSF axon. The vesicles show the characteristic aggregations against the contact region of the membranes, but unlike the MSF-amacrine contacts already described there is (*a*) no pronounced thickening and widening of the synaptic membranes, and (*b*) amacrine vesicles (*sv*) are aggregated against the amacrine side of the cleft. Thus this contact has some of the features of a two-way or reciprocal synapse. This and the fact that axo-somatic synapses are not usual features of *Octopus* (and invertebrates in general) neurons presents an intriguing situation.*

Further evidence that the presynaptic knobs are from MSF axons comes from examination of the amacrine tracts in the cortex, where varicose fibres apparently ascending between the down-coming amacrine trunks could be seen. The varicosities contained the dense-cored as well as plane vesicles and so it would appear that a small percentage of the MSF fibres instead of running anterio-posteriorly along the outer part of the lobule medulla turn at right angles up into the cortex within an amacrine tract. Contact regions could be seen with vesicles on the *amacrine side* aggregated against the surface membrane as in the axo-somatic contact (see Discussion).

Discussion

Figures 39 and 40 show the suggested basic circuitry within a lobule of the vertical lobe. The MSF inputs make *en passant* cruciform contacts with the radial trunks of the amacrine fibres (*amt*). These in turn synapse with the trunks and their dendritic collaterals of the large cells (*lc*). The 'pain' fibres (*pa*) ascend into the lobules from below and also synapse with the trunks and dendritic collaterals of the large cells. The collaterals of the large cells are apparently entirely receiving components within the lobules, for observations so far indicate that they do not appear to have any processes that are axon-like, and hence presynaptic, to any of the other neurites. The trunks are the only output and they continue as axons which presumably convey information signals down and out of the lobule and synapse in the subvertical and lateral superior frontal lobes and perhaps in the inferior frontal system (see Young 1964, 1970).

In Golgi preparations (Young 1964, 1970) the varicose MSF fibres correspond exactly with the EM picture, as do the amacrine trunks with which they make cruciform contacts. In the Golgi preparations, however, because of the selective staining of this method, only one or a few amacrine trunks are seen running down together into the neuropil. The EM shows in fact that they are bundled together into groups of two or three hundred or more, and so we term the bundles *amacrine tracts*. These might constitute functional units but there is no evidence for this at present. The trunks of the large cells run down singly or in bundles of two to six and we rely

* Nagy & Sakharov (1969, *Experientia* **25**, p. 258) described axosomatic synapses in gastropods.

on the Golgi preparation for a picture of the ramifying dendritic collaterals they give off (Young 1964, 1970). From this and from EM sections cut occasionally in suitable planes we deduce that the numerous fine clear or microtubule-containing processes seen in the EM to form the chief receptor surfaces for the amacrine and 'pain' fibre synapses are in fact dendritic collaterals of the large cells.

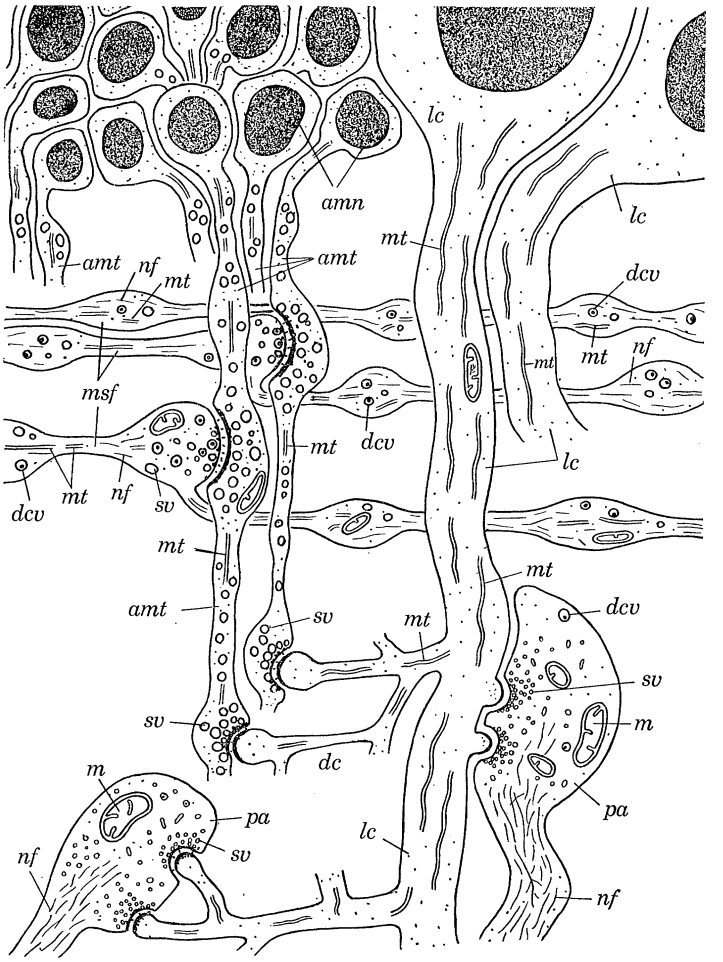


FIGURE 39. Diagram to show what is thought to be the basic circuitry of the vertical lobe (much simplified and not strictly to scale).

Turning now from the Golgi to the Cajal neurofibrillar silver preparations (Young 1964, 1970), we find a remarkable correlation between light and electron microscopy. There is good evidence in vertebrates and invertebrates that axons and dendrites containing the thin (8 to 10 nm) threadlike neurofilaments that run in bundles are silver-positive (see Gray & Guillery 1966), while axons and dendrites containing only microtubules are refractory to the silver stain. In the octopus there is already evidence for this. Dilly *et al.* (1963) described neurofilaments in the optic axons which are silver positive. The initial regions of these axons in the retina, however, only contain bundles of microtubules (see Gray 1970*b*) and cannot be seen in silver preparations. Also, Lund (1966, and personal communication) has shown a similar correlation in the stellate ganglion.

Now, returning to the vertical lobe, the MSF fibres can be seen as neurofibrillar threads in

silver preparations (figures 3 and 5, plate 73) and under the EM the MSF fibres are seen to contain neurofilaments (*nf*) and microtubules (*mt*) (figure 39). The amacrine trunks are invisible after Cajal silver staining and contain only microtubules when viewed with the EM. The trunks of large cells are invisible under the light microscope in silver-stained preparations and they contain only microtubules when viewed with the EM, though the region in which they lie in the silver preparations can easily be located since the silver-positive nuclei in their cell bodies can be seen. The pain fibres contain masses of neurofilaments and in the silver preparations visible by light microscopy they are heavily stained and can be seen streaming in through the lobule hila and forming dense tangles in the neuropil. Thus it may be possible to generalize from these observations and, when turning to study the other brain lobes with the electron microscope, predict that the silver-impregnated fibres seen under the light microscope will be the ones containing neurofilaments.

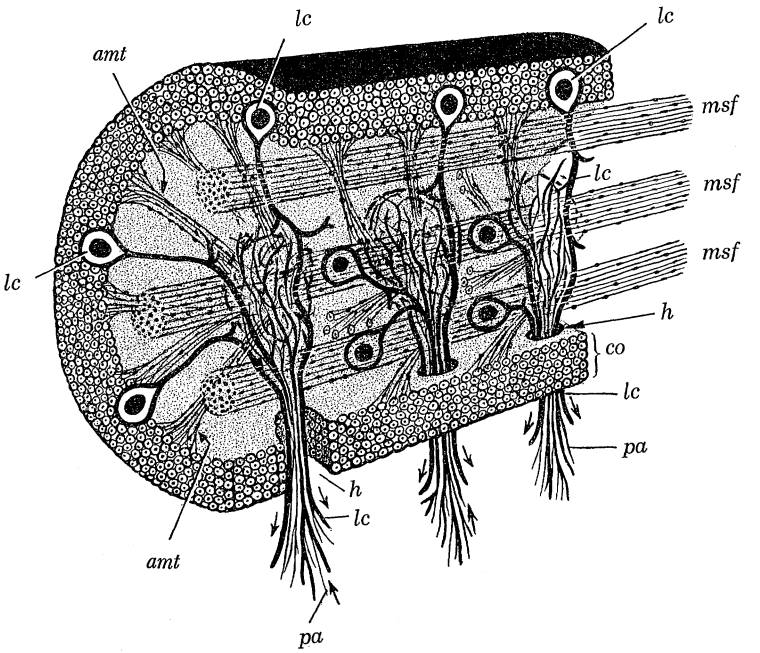


FIGURE 40. Diagram to give a three-dimensional representation of the circuitry in the vertical lobe. Part of a lobule is shown with a portion cut away longitudinally. (Much simplified and not strictly to scale.)

In the Cajal silver preparations (Young 1964) some of the 'pain' fibres can be seen extending up into the outer zones of the neuropil and sometimes into the cortex. These have not yet been identified with the electron microscope, possibly because of the sampling problem. Presumably they synapse with the more proximal regions of the large cell trunks or their collaterals in this zone. Possibly the silver-positive fibres extending to the cortex are in fact not pain fibres but descending amacrine trunks. These might occasionally become impregnated with silver, perhaps because rarely they contain neurofilaments, a feature perhaps missed because of EM sampling.

Turning now to the synapses, so far none have been encountered with tight junctions (nor for that matter have any been found at synapses in any of the other brain lobes of octopus so far examined). Tight junctions, where the synaptic cleft is obliterated by 'fusion' of the pre- and postsynaptic membranes, can be seen at synapses known to have an electrical mode of transmission (see Gray 1969*b* for discussion and references). The octopus synapses all show the characteristic vesicle aggregations apparently focused against a distinct synaptic cleft which has

thickenings. Thus it seems likely that all these synapses have a chemical mode of transmission with synaptic delays and the numerous other implications involved.

Very little is known at present about the types of transmitter substances involved in the different sorts of synapse in *Octopus*. The problem is at present under investigation and will be described elsewhere. Suffice it to say that of the three sorts of ending under consideration here, the MSF, amacrine and 'pain' knobs, the transmitter in the MSF knobs is possibly a monoamine since a proportion of the synaptic vesicles have dense cores (see Hökfelt 1968 and Gray 1970*a*). In the vertebrates aldehyde fixation reveals two categories of synaptic knob, ones with round vesicles and ones with flattened or polymorphic vesicles (Uchizono 1965; Gray 1969*b*) and there is strong evidence that the former might be chemically excitatory and the latter chemically inhibitory. An EM survey of the nervous systems of invertebrates after aldehyde fixation does not reveal two such clear-cut categories (Dennison 1970, in preparation), however, except in certain instances in the arthropods (Uchizono 1967, see Gray 1969*b*). In *Octopus* brain there are several varieties of synaptic vesicle related to the different sorts of endings. In the vertical lobe the 'pain' endings were shown above to contain numerous small and flattened vesicles and it may be more than a coincidence that Young (1964, 1970) suggests that these endings are inhibitory in function. The synaptic vesicles in the amacrine trunks are mostly spherical in appearance and so possibly excitatory. It is idle to speculate further at present, however. Our task at present is to identify the transmitters involved. Even then, though, this may not tell us which synapses are excitatory and which are inhibitory, for in another mollusc, *Aplysia* there is evidence that this might depend on the nature of the receptors in the postsynaptic membrane rather than the type of chemical transmitter released from the presynaptic component (see Tauc 1967).

The scheme of connexions proposed above will undoubtedly turn out to be an oversimplification in the light of future work. For example, no EM observations have been made on the commisural fibres described by Young (1964, 1970) running between adjacent lobules. Are they MSF fibres that perhaps in ontogeny took the 'wrong' turning as they entered the vertical lobe and later sorted themselves out by crossing back into the correct lobule, or are they an entirely different set of fibres yet to be encountered with the EM?

No doubt it is an over-simplification to call the fibres coming in from below simply 'pain' fibres (see Young 1970). They probably consist of a number of modalities and originate in various parts of the brain as well as the subvertical lobe.

Also, here only one category of amacrine neurons has been described. There may well be more than one variety (see Young 1970) perhaps with different sorts of connexions.

Other micrographs that at present do not fit the scheme are: (*a*) an isolated example (figure 30 plate 88) of an ending with granulated vesicles contacting what appears to be a trunk of a large cell. This ending could be a 'pain' knob, however, with an unusual preponderance of dense-cored vesicles. (The dense cores look smaller than those in MSF vesicles, and more like those found in 'pain' vesicles.) (*b*) An apparent 'pain' ending presynaptic to an MSF varicosity (figure 36, plate 91). This would be a serial arrangement since the MSF varicosities are themselves presynaptic to amacrines. More observations are needed to clarify this possibility.

The anomalous axo-somatic contacts described on the amacrine cell perikarya present a puzzle in that the *postsynaptic* (amacrine) membrane has an aggregation of vesicles against its surface, just as has the MSF presynaptic membrane. Since it is thought that such arrangements indicate that at these points synaptic vesicles are strategically situated to liberate their

transmitter into the cleft, then this synapse would appear to have a two-way action, i.e. MSF to amacrine feeding back to MSF. Also, in the neuropil aggregations of amacrine vesicles can be seen lying against the *postsynaptic* membrane. This needs much further investigation, but it should be mentioned that synapses with both pre- and postsynaptic vesicle-aggregations (where there is evidence for two-way chemical transmission) have been described in the giant fibre system of the squids (Martin 1969). Also, in the mammalian mitral cell-granule cell contacts of the olfactory bulb, reciprocal synapses have been described (Rall *et al.* 1965; Price 1969), and in the bipolar amacrine contacts of the retina (Dowling & Boycott 1966).

Finally, and of special interest, it should be mentioned that observations support Young's (see 1964, 1970) use of the term 'amacrine' for the small 'granule' neurons—some 25 000 000 of which are present in the vertical lobe compared with only 65 000 of the large cells. The EM confirms that the single trunks of these small cells can be regarded neither as axon nor dendrite since they fulfil the role of both. The surface of the trunk is receptive over some parts (postsynaptic to the MSF axons) and effective over others (presynaptic to the dendritic collaterals of the large cells). Even the perikaryal surface of the amacrine shows morphological features suggestive of both pre- and postsynaptic sites (mentioned above).

In the vertebrates, amacrine neurons have been described with the electron microscope in the retina (see Dowling & Boycott 1966), in the olfactory bulb (the granule cells, Rall *et al.* 1966; Price 1969) and more recently in the superior cervical ganglion (Matthews & Raisman 1969). The latter two types are probably inhibitory, but the function of the retinal amacrines is probably more complex.

Little is known of the function of the amacrines in the vertical lobe of the octopus. A single MSF axon probably makes a series of cruciform connexions with the amacrines throughout the longitudinal extent of the outer medulla of the lobule (Young 1970) and the related amacrines relay the influence into the centre of the medulla (in a manner not yet understood) to be picked up there by the dendritic collaterals of large cells. These collaterals branch in a complicated way possibly with specific orientations of their receptor fields (see Young 1962).

The incoming pain fibres are also related (i.e. presynaptic) to those collaterals of the large cells. The pain fibres certainly show evidence of orientation (figure 40). In cross-sections they can be seen to spread out in the transverse plane like tennis rackets (figures 3 and 5, plate 73) rather than as randomly arranged balls of fibres (compare their appearance when cut in a plane at right angles, i.e. longitudinally, figure 2, plate 73).

The role of the vertical lobe in the general integrative action of the octopus brain (particularly its visual system) has been considered in detail by Young (1964, 1970). Suffice it to say that the electron microscopy supports Young's suggestion that the superior frontal-vertical lobe constitutes a loop which could sustain a positive feed-back mechanism (MSF → to amacrine → large cell → lateral superior frontal → MSF), while the pain (nocifensor) input could exert a suppressor (inhibitory) effect on the loop by its action on the large cells. Whether the amacrine and pain fibres have different addresses on the large cells (different dendritic collaterals or perhaps just different patches of trunk or collateral surface membrane) remains to be seen.

I am indebted to Professor J. Z. Young, F.R.S., for his collaboration and guidance and for access to his material. Figures 1–5, plate 73, are taken from his material. Mr S. Waterman gave his skilled assistance with photography. Miss H. Pease and Mrs M. Chessum gave technical assistance and Mrs J. Astafiev drew the text figures.

KEY TO ABBREVIATIONS

am	amacrine cell varicosity or presynaptic component
amn	amacrine cell nucleus
amt	amacrine trunk or tract formed by bundle of amacrine trunks
cen	centriole
co	cortex of vertical lobe lobule
cv	coated vesicle
db	dense body
dc	dendritic collaterals of large cell
dcv	dense-cored vesicle
fi	fibrous material in mitochondrion
gf	glial fibrils
gl	glial cell or its process
Go	Golgi apparatus
gr	granular endoplasmic reticulum
h	hilum or hila of vertical lobe
lc	body or trunk of large cell
lcn	nucleus of large cell
m	mitochondrion
med	medulla of vertical lobe lobule
mifl	median inferior frontal lobule
msf	median superior frontal axon or its presynaptic varicosity or bundle of such axons
msfl	median superior frontal lobe
mt	microtubule
mvb	multi-vesicular body
nf	neurofilaments
no	nucleolus
p	protruberance of process of large cell
pa	axons or their presynaptic knobs thought to signal 'pain' (nocifensor)
peri	perikaryon of neuron
ri	ribosomes
ss	subsynaptic granules (ribosomes?)
suv	subvertical lobe
sv	synaptic vesicles
tw	side branches from trunks of large cells
ves	vesicles
vl	lobule of vertical lobe

N.B. Unlabelled arrows drawn across the synaptic cleft indicate presumed direction of transmission.

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1 *vl* 1 mm *msfl* *mifl* *suv* anterior

4 *vl* 0.5 mm *h* *h* *h*

2 0.5 mm *msf* *msf* *med* *co* *p* *msf* *msfl* *h* *h* *h* *p* *suv*

3 *co* *msf* *pa* *h* 0.2 mm

5 *amn* *lcn* *msf* *pa* *h* 0.2 mm *co*

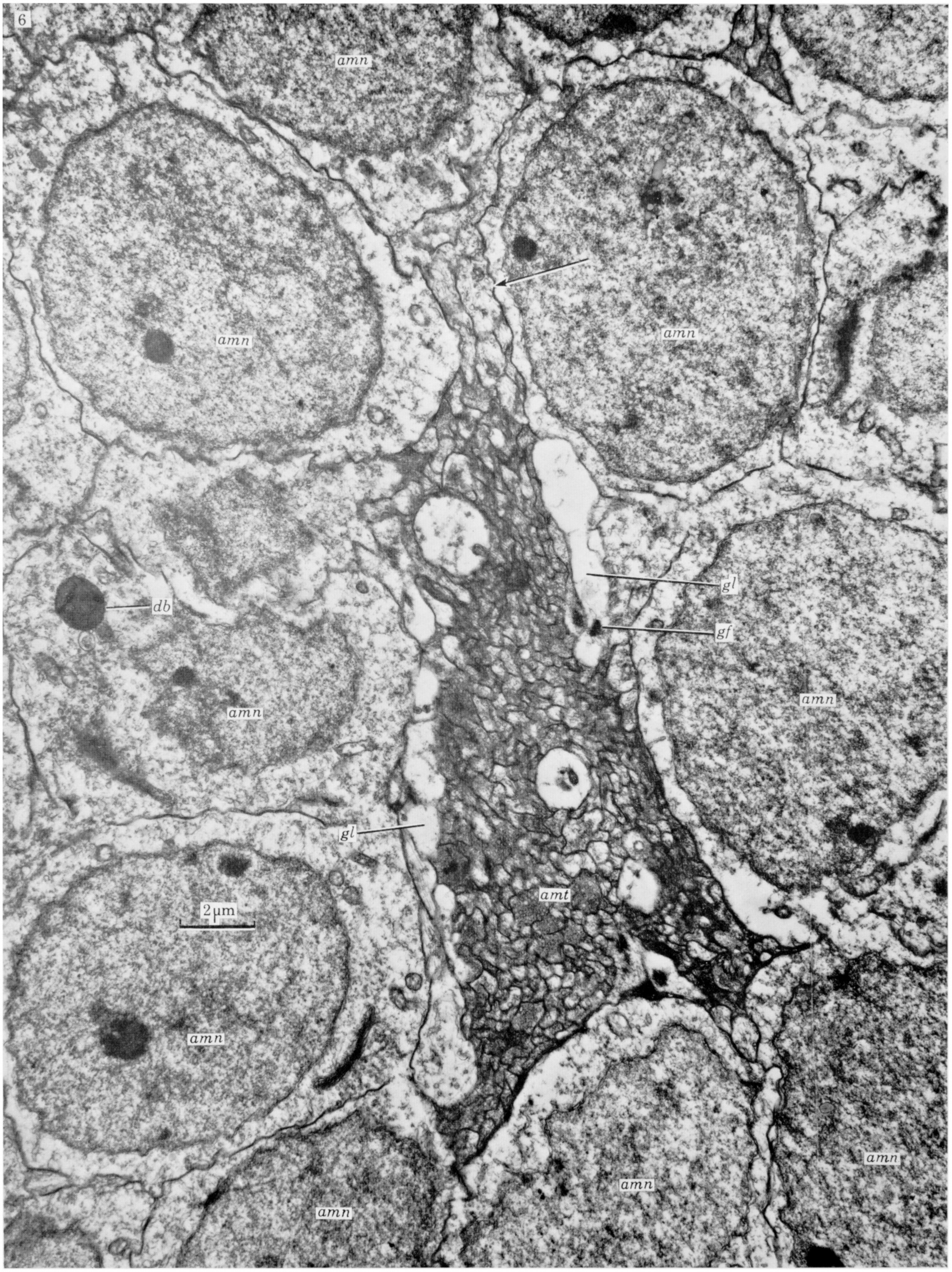
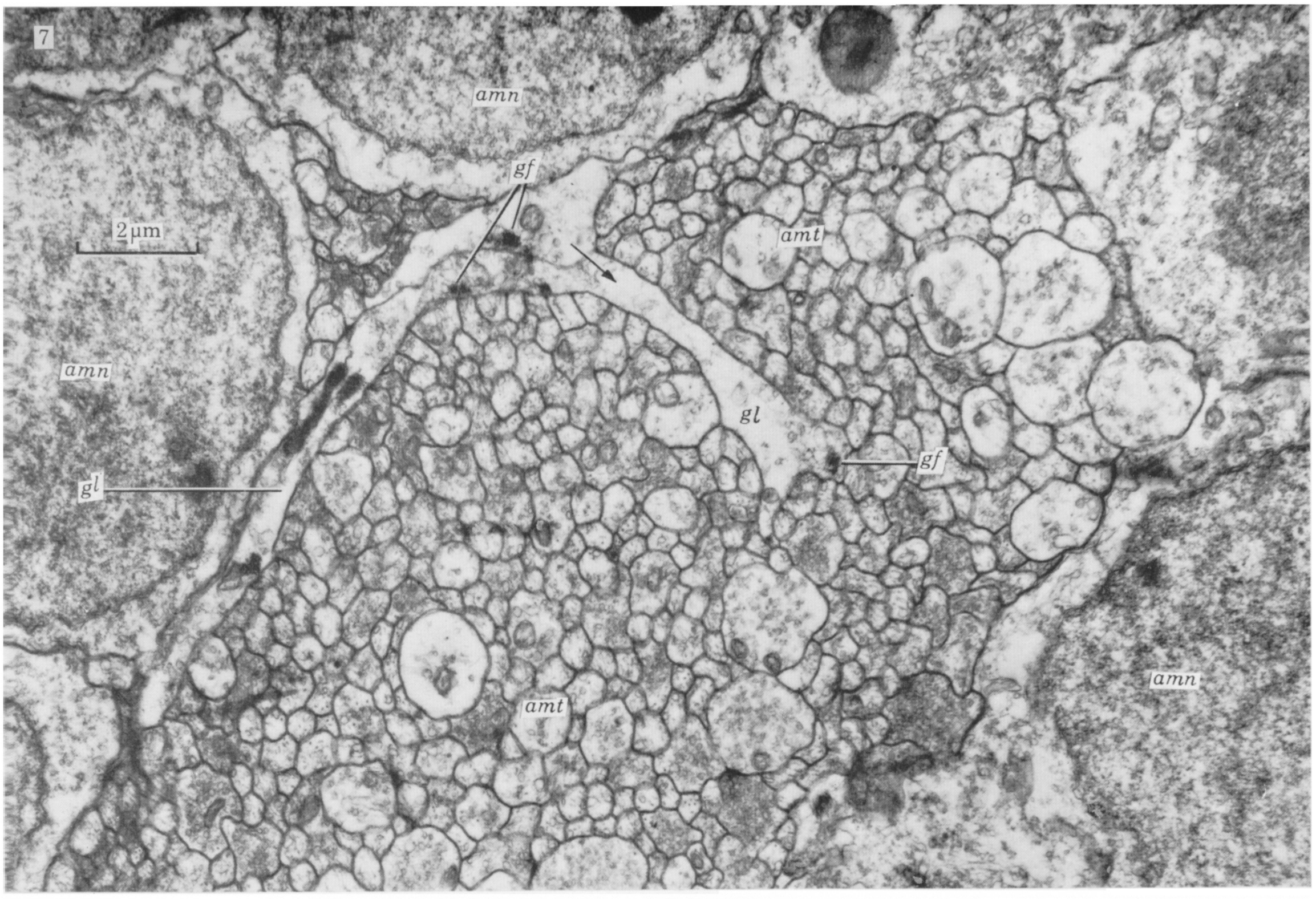


FIGURE 6. An amacrine tract (sectioned transversely) surrounded by amacrine perikarya. Cortex.



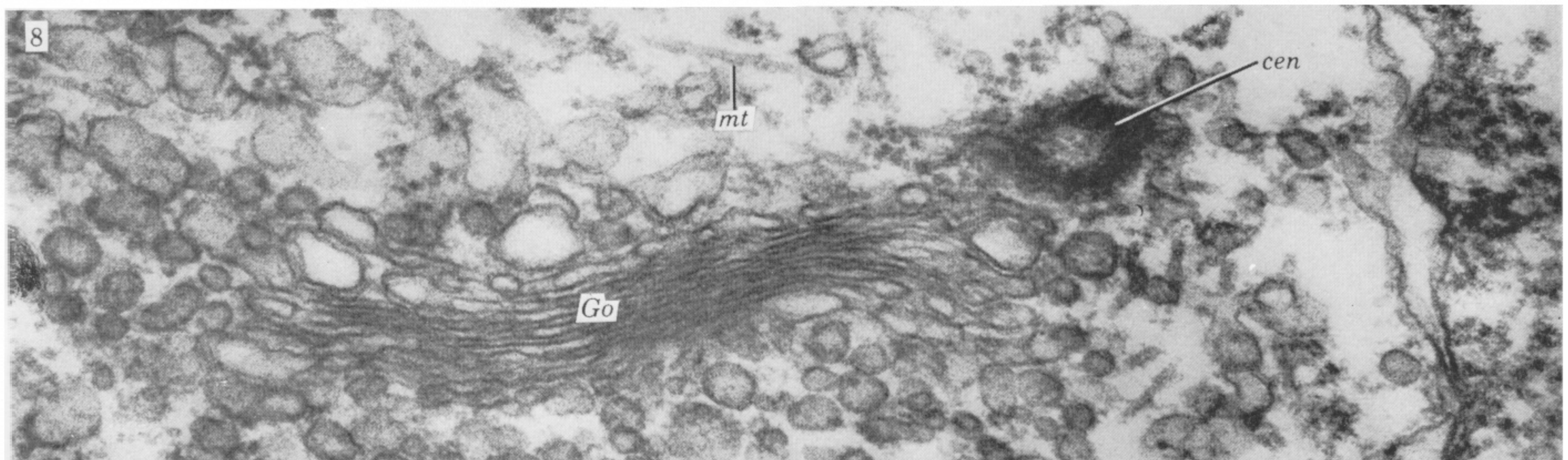
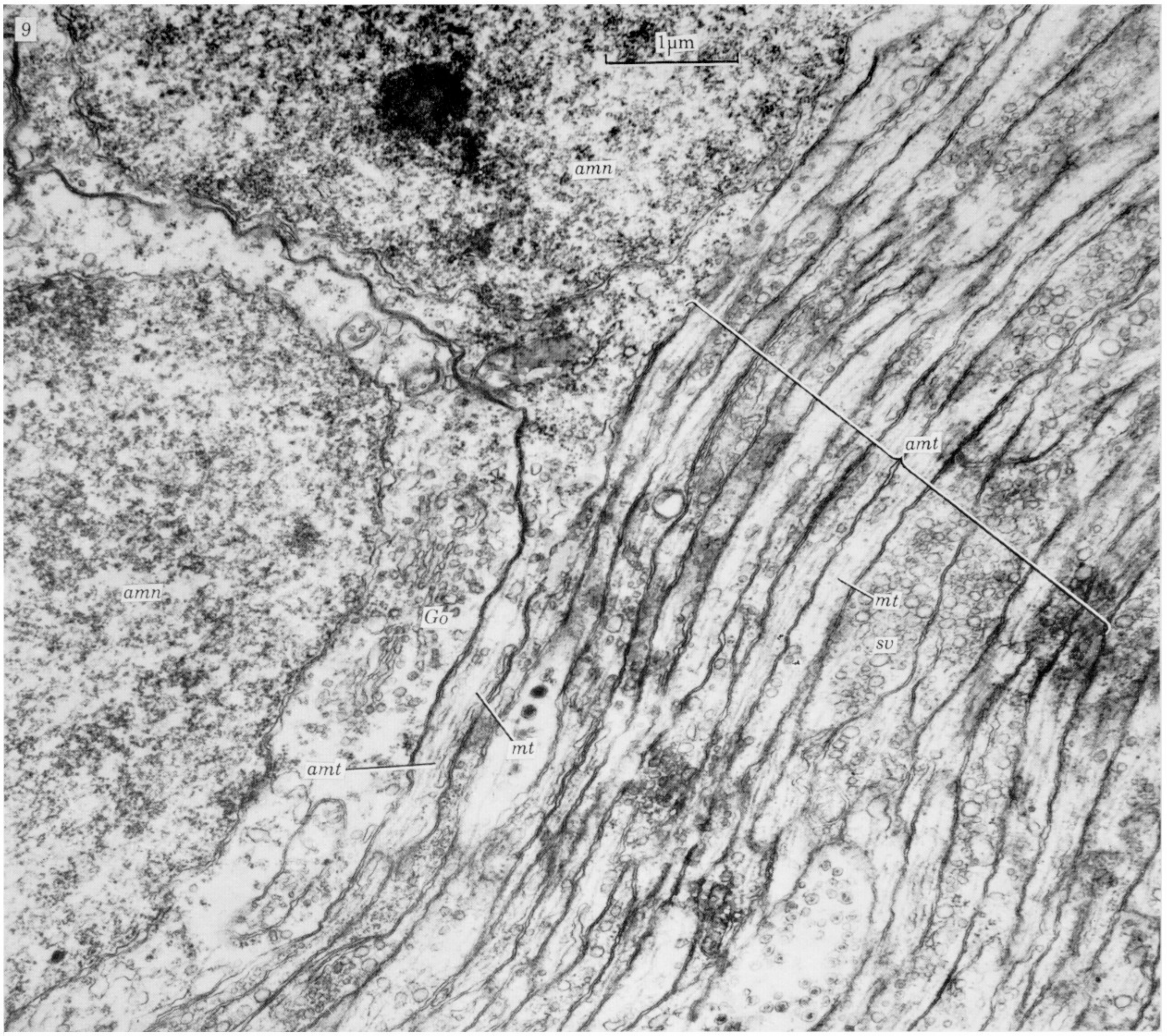


FIGURE 7. Amacrine tract (cut transversely) partly separated from the surrounding amacrine perikarya by a glial fold. Cortex.

FIGURE 8. Golgi zone of an amacrine perikaryon. Cortex.



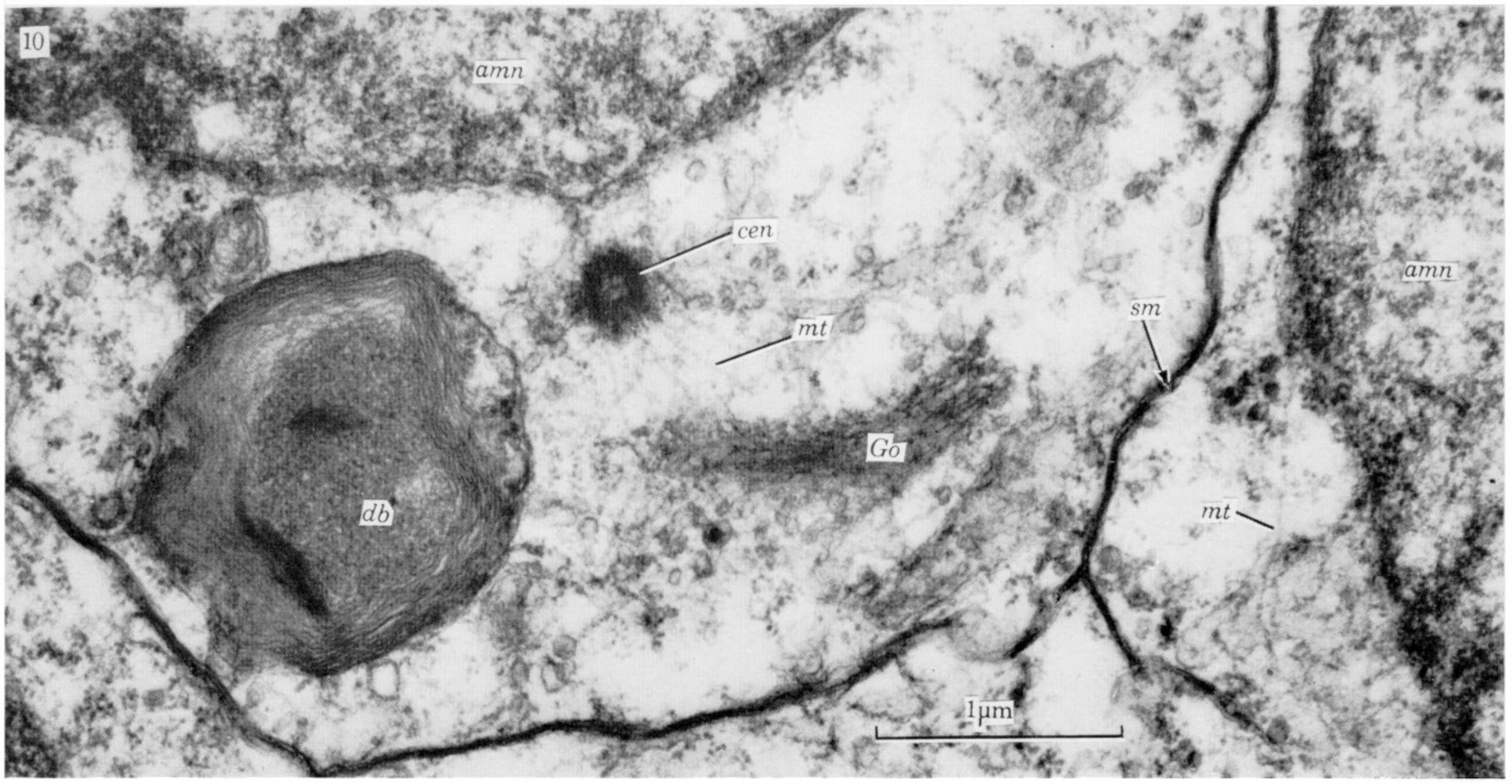


FIGURE 9. Two amacrine perikarya; the trunk of one can be seen joining (contributing to) an amacrine tract. Cortex.

FIGURE 10. An amacrine cell perikaryon with a dense body, centriole and Golgi apparatus. Cortex.

11

amn

amn

amn

amn

amn

amt²

1μm

amt

amt¹

12

mvb

amn

amt

peri

su

m

mt

Go

1μm

peri

FIGURE 11. Two amacrine cells with their trunks joining an amacrine tract. Cortico-medullary junction.

FIGURE 12. Part of an amacrine cell and adjacent amacrine tract. Cortico-medullary junction.

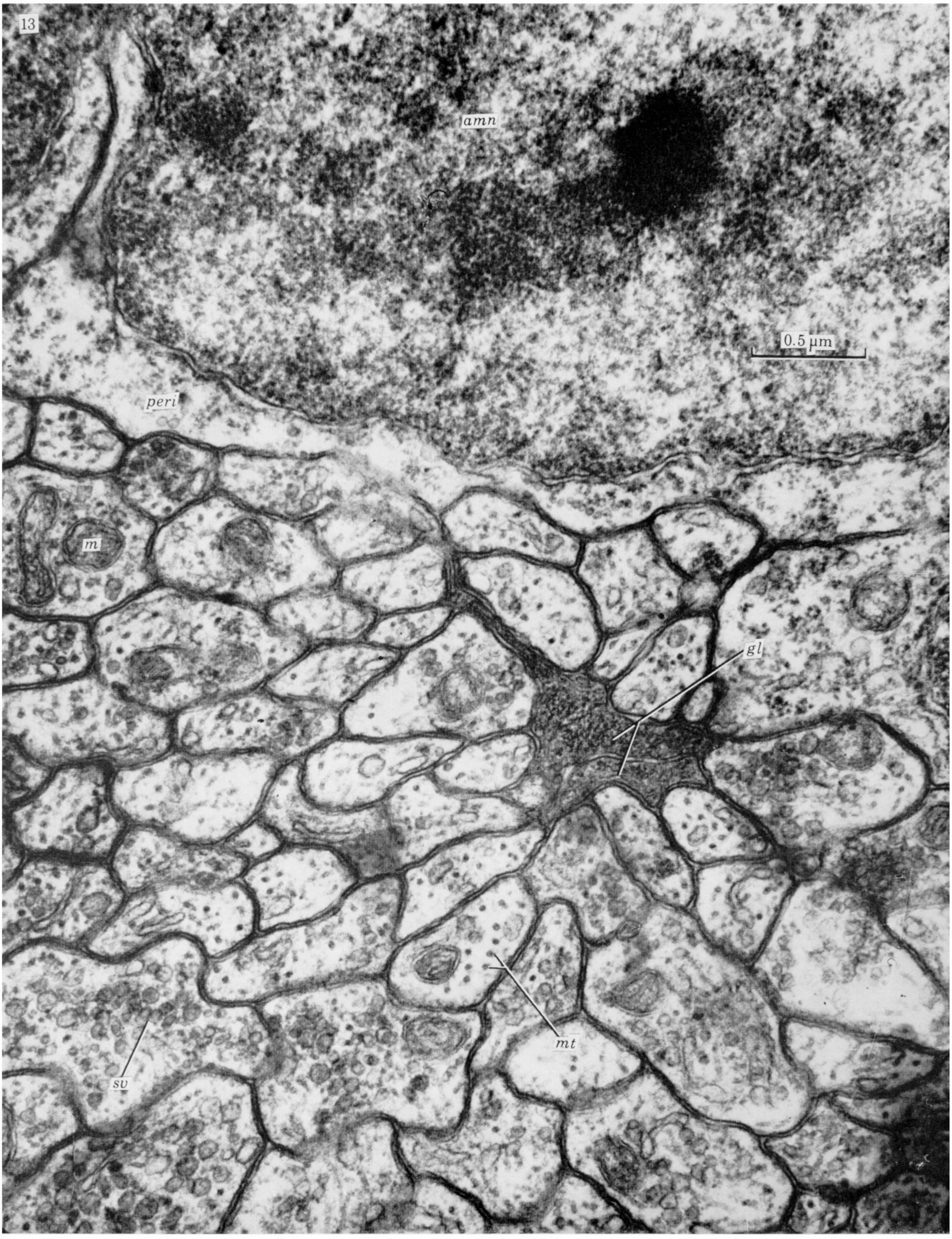
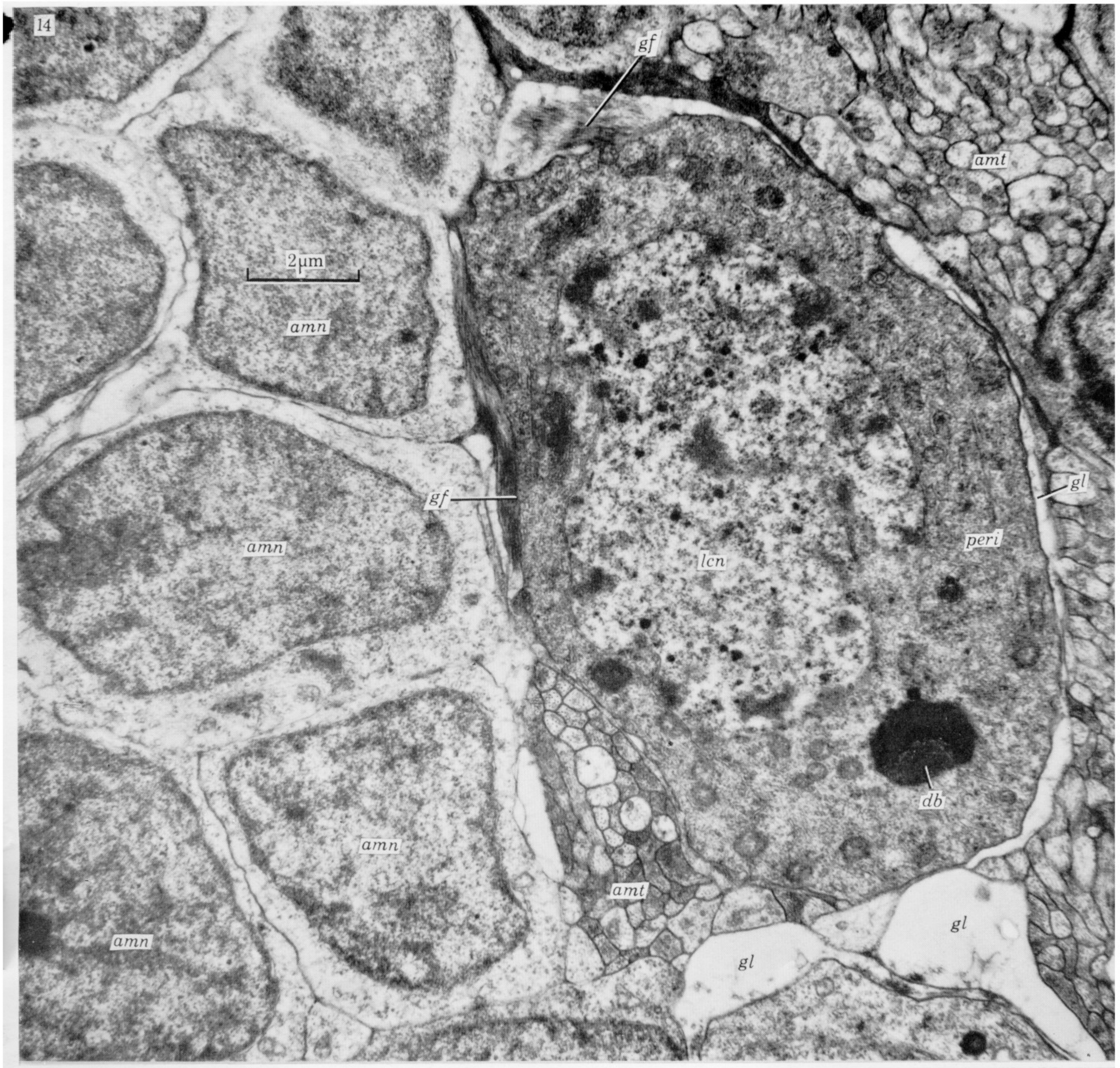


FIGURE 13. Amacrine tract (cut transversely). Two 'dark' glial processes intrude between the trunks. Outer medulla.



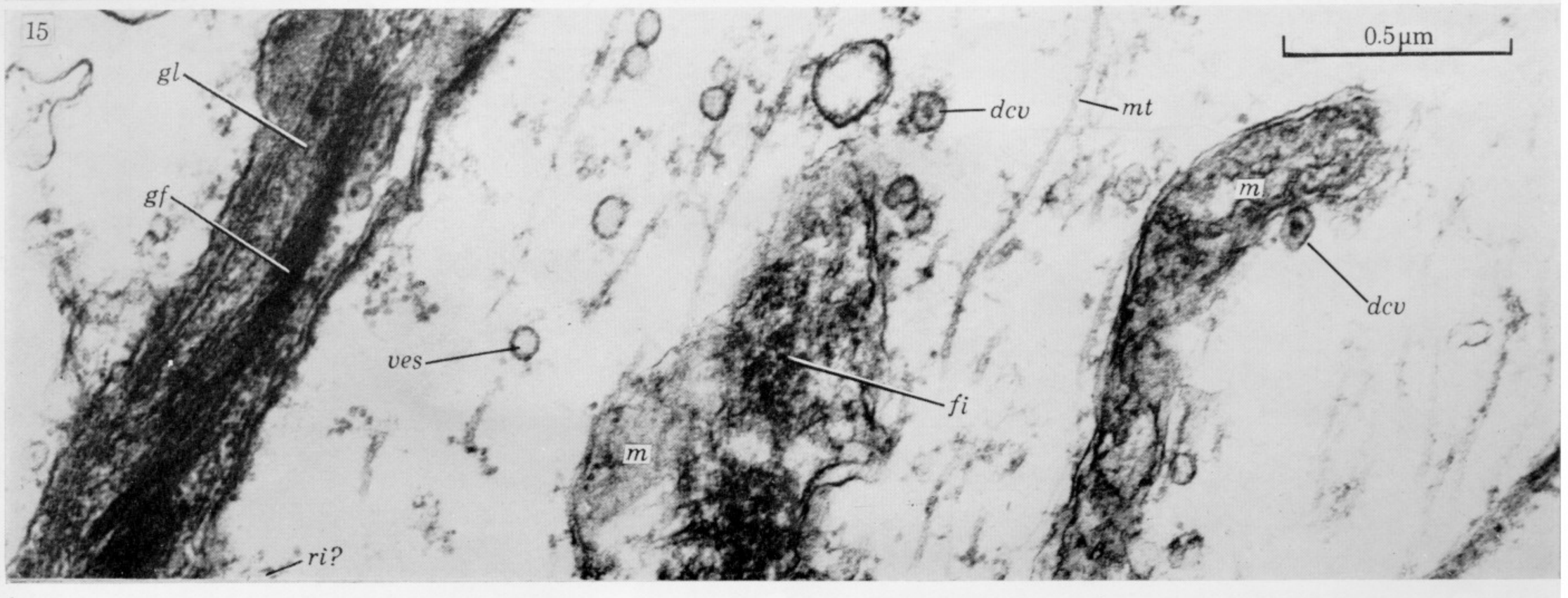


FIGURE 14. A large cell and numerous amacrine perikarya. Inner zone of cortex.

FIGURE 15. Part of the trunk of a large cell cut longitudinally. Outer medulla.

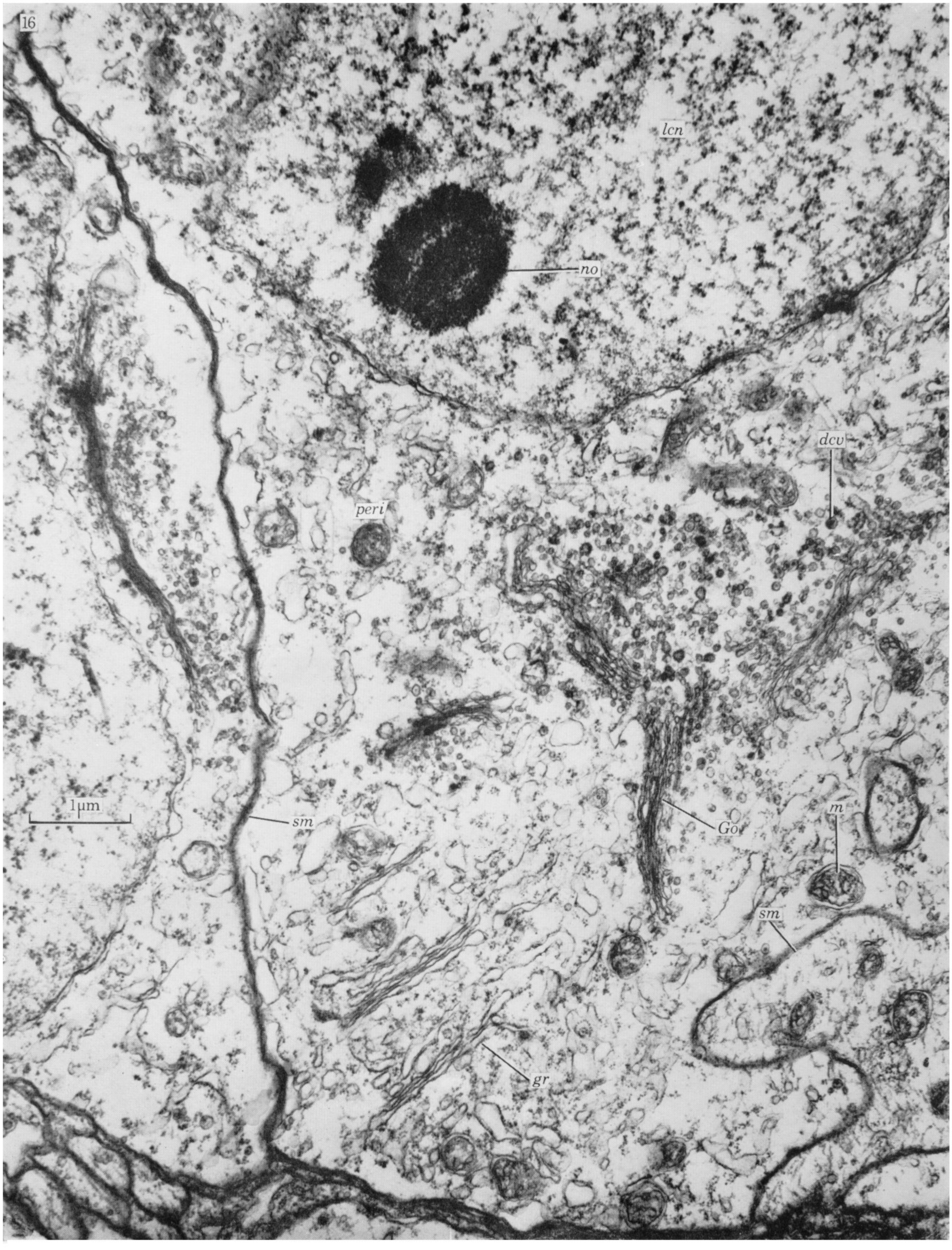


FIGURE 16. Parts of the perikarya of two adjacent large cells. Inner border of cortex.

17

gl
mt
mt
lc
mt
ves
ves
lc
r
lc
mt
gl
lc
gl
lc
1 μm

18

0.5 μm
su
gf
gl
mt
lc

FIGURE 17 Trunks of large cells (cut transversely) showing interlocking profiles. Cortico-medullary zone.

FIGURE 18. Part of large cell trunk (cut transversely), separated from an adjacent amacrine tract by a glial fold. Outer medulla.

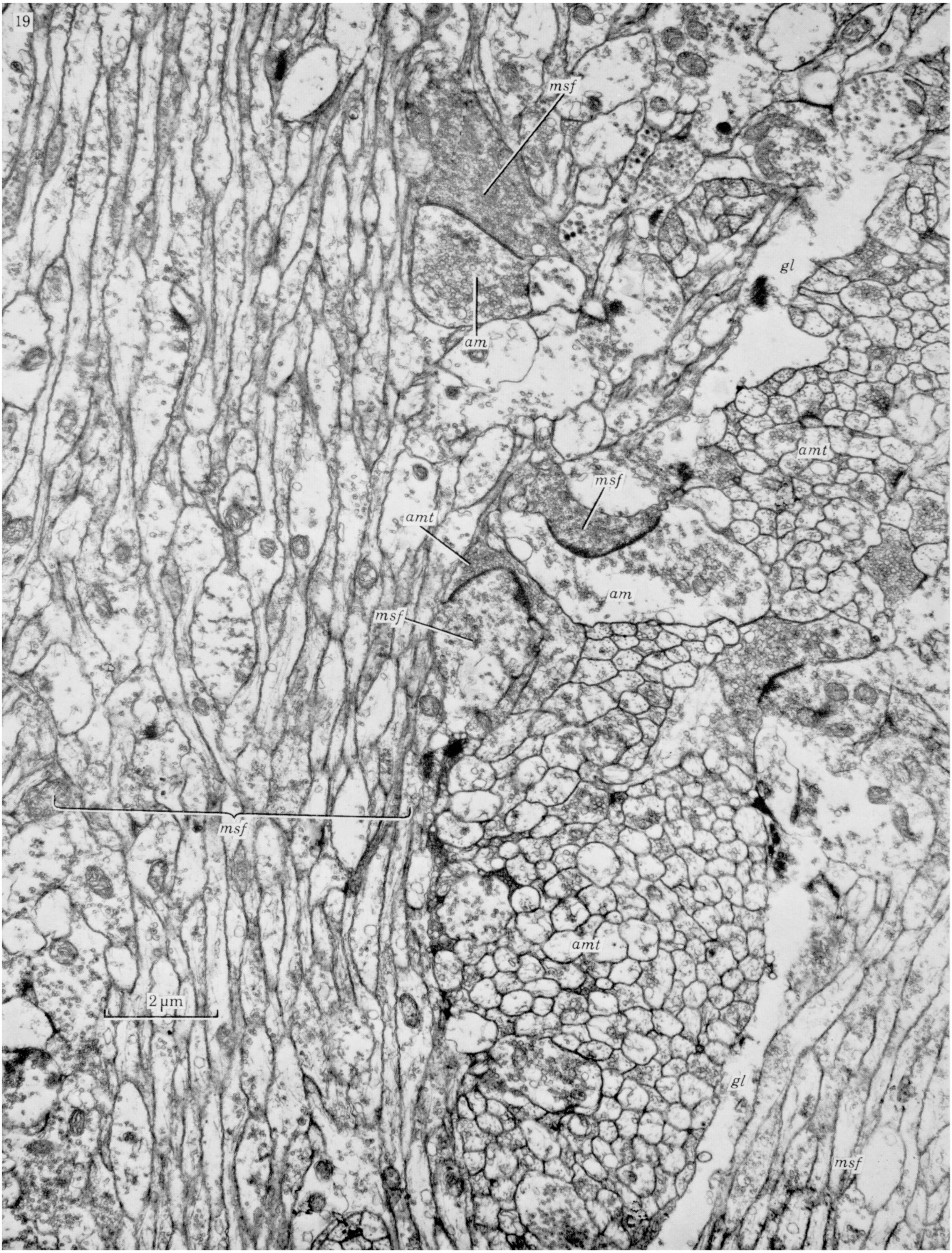
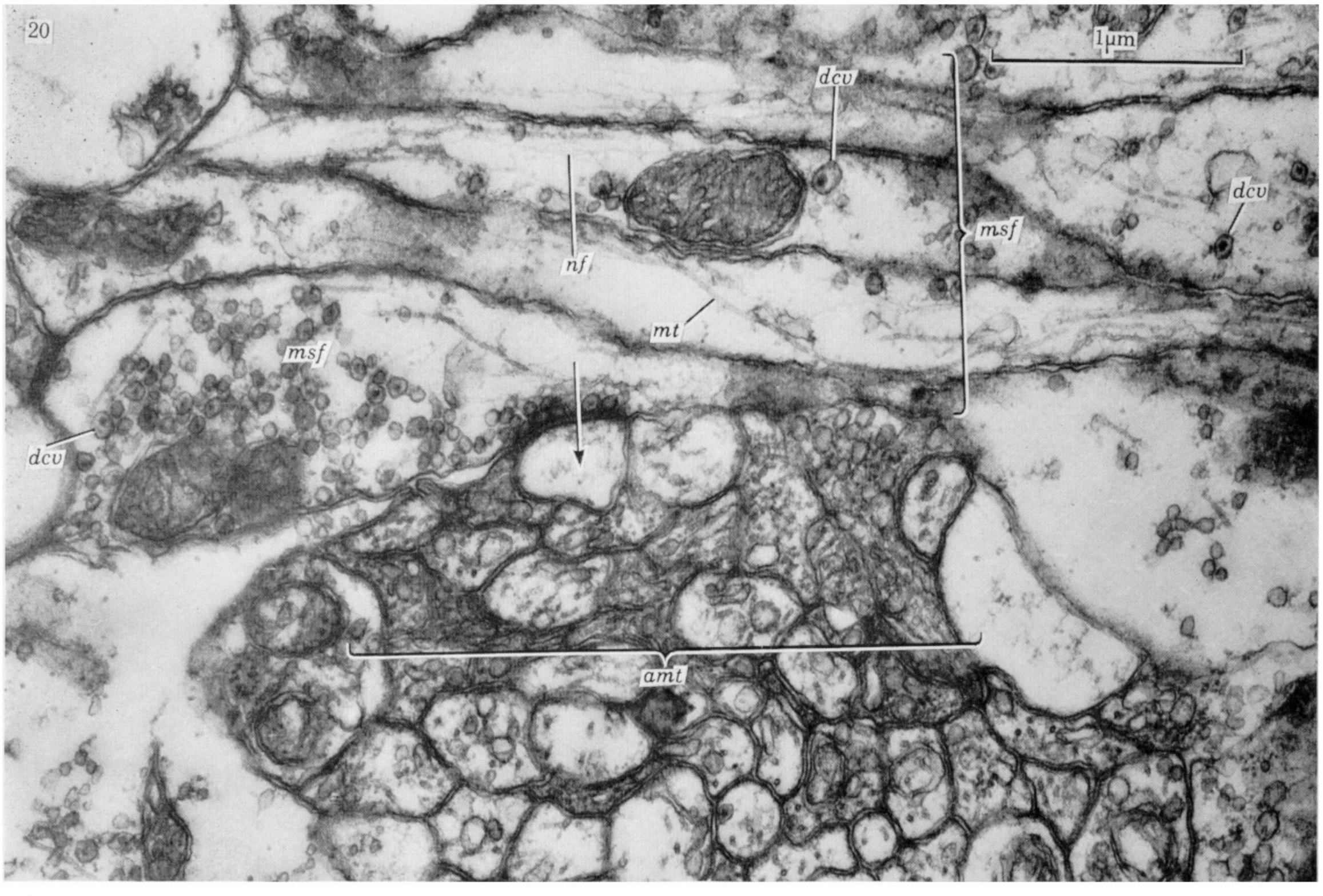


FIGURE 19. Bundles of MSF axons (cut longitudinally) and an amacrine tract (cut transversely). MSF-amacrine synapses occur as cruciform connexions. Outer medulla.



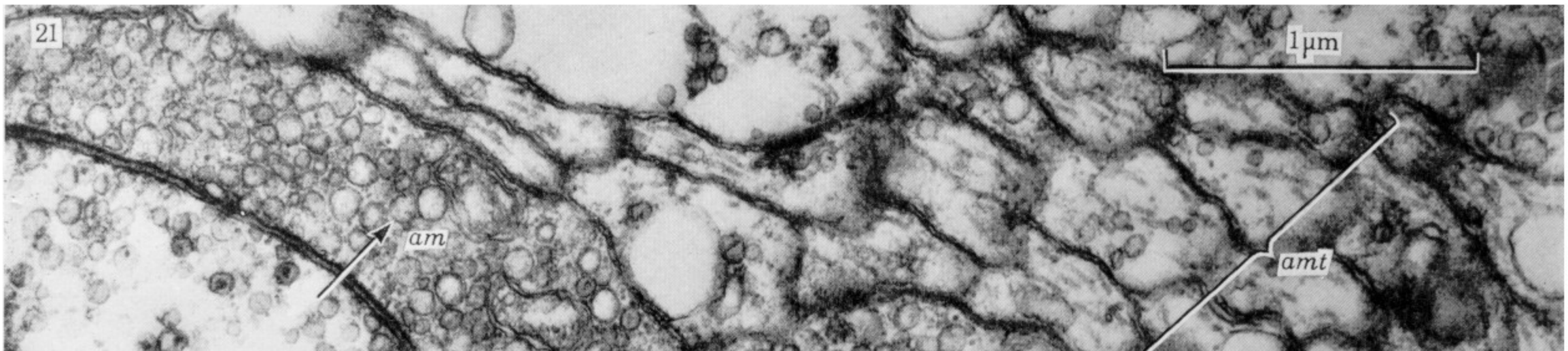


FIGURE 20. An MSF axon in synaptic contact with a profile thought to be an amacrine trunk since the profile forms part of an amacrine tract. Outer medulla.

FIGURE 21. Synapses between MSF varicosities and amacrine trunk varicosities. Left—the MSF varicosity is the pale one; centre—the amacrine trunk is the paler one. Medulla.

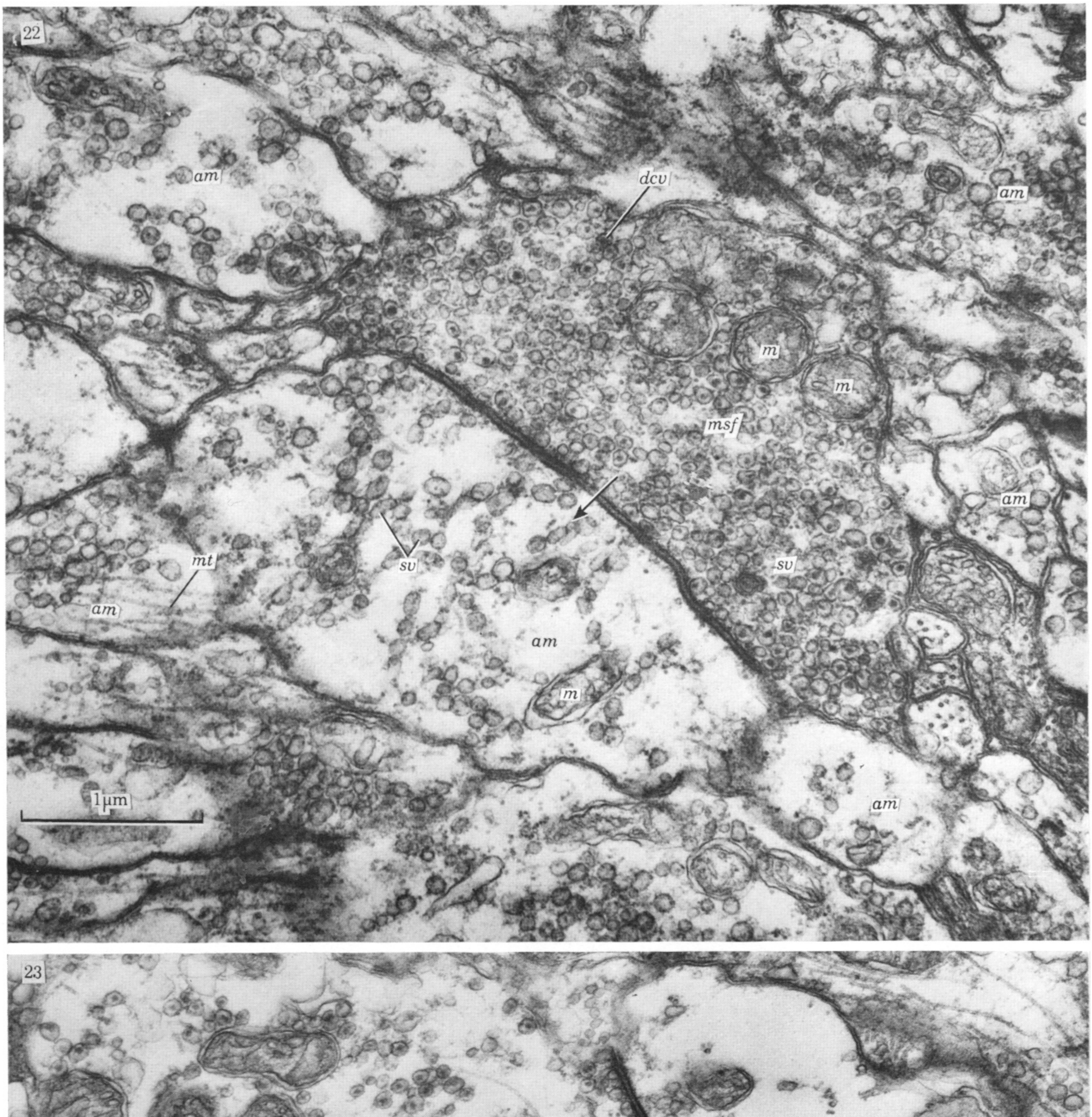


FIGURE 22. An MSF varicosity in synaptic contact with a varicosity of an amacrine trunk. Outer medulla. Arrows in this and other figures indicate the presumed direction of transmission across the synaptic cleft.

FIGURE 23. An MSF varicosity in synaptic contact with the varicosities of two amacrine trunks. Outer medulla.

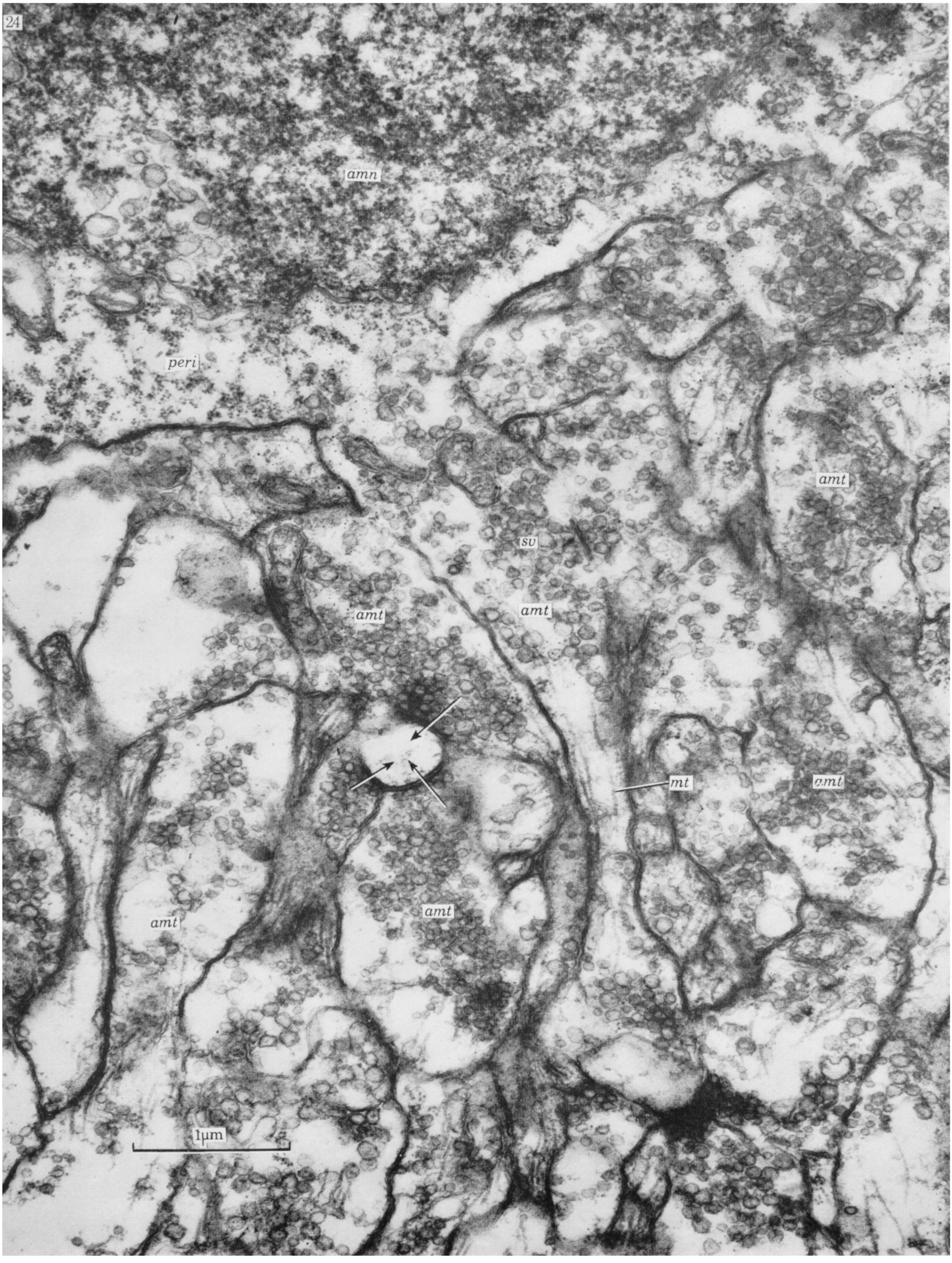
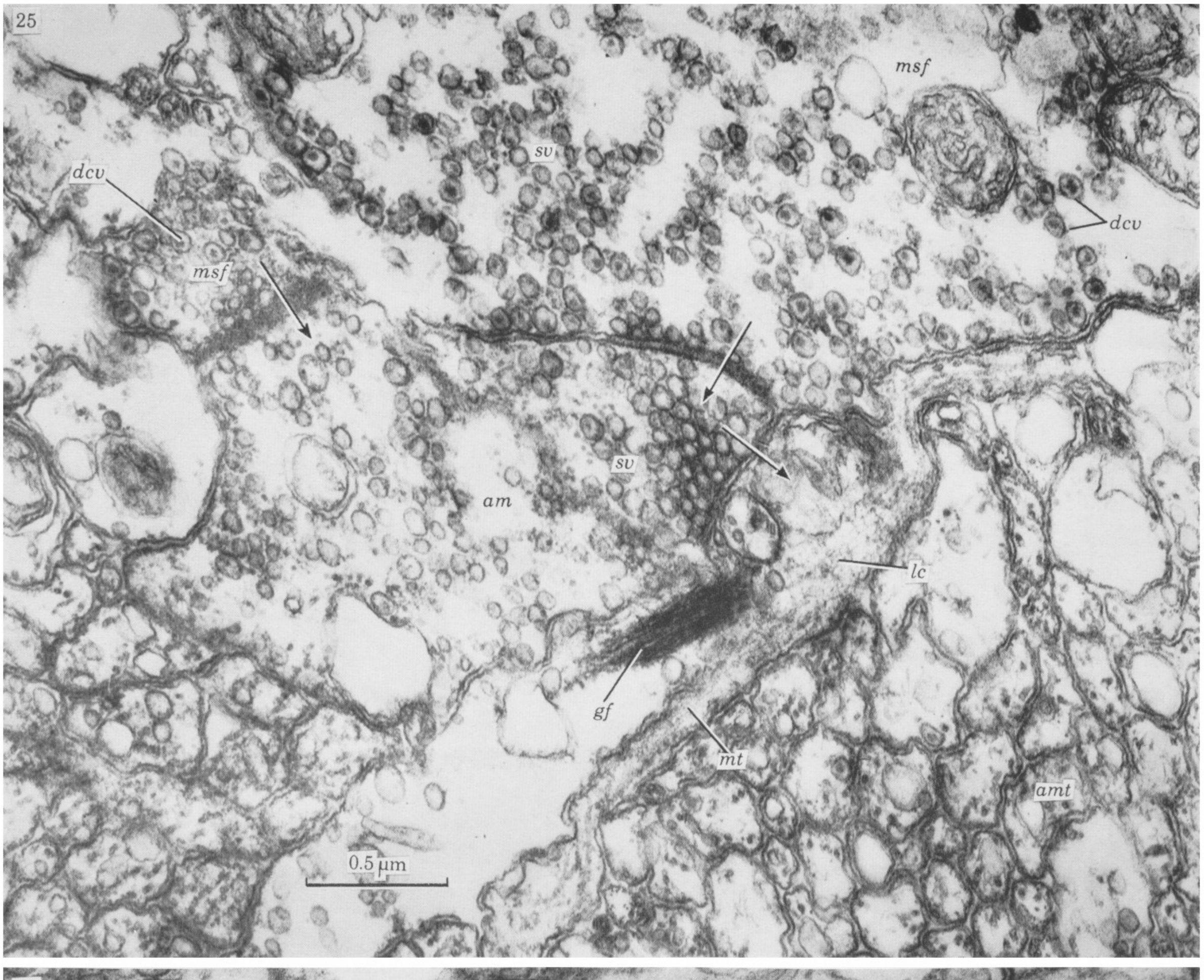


FIGURE 24. An amacrine cell in the inner cortical margin; its trunk extends into the medulla.



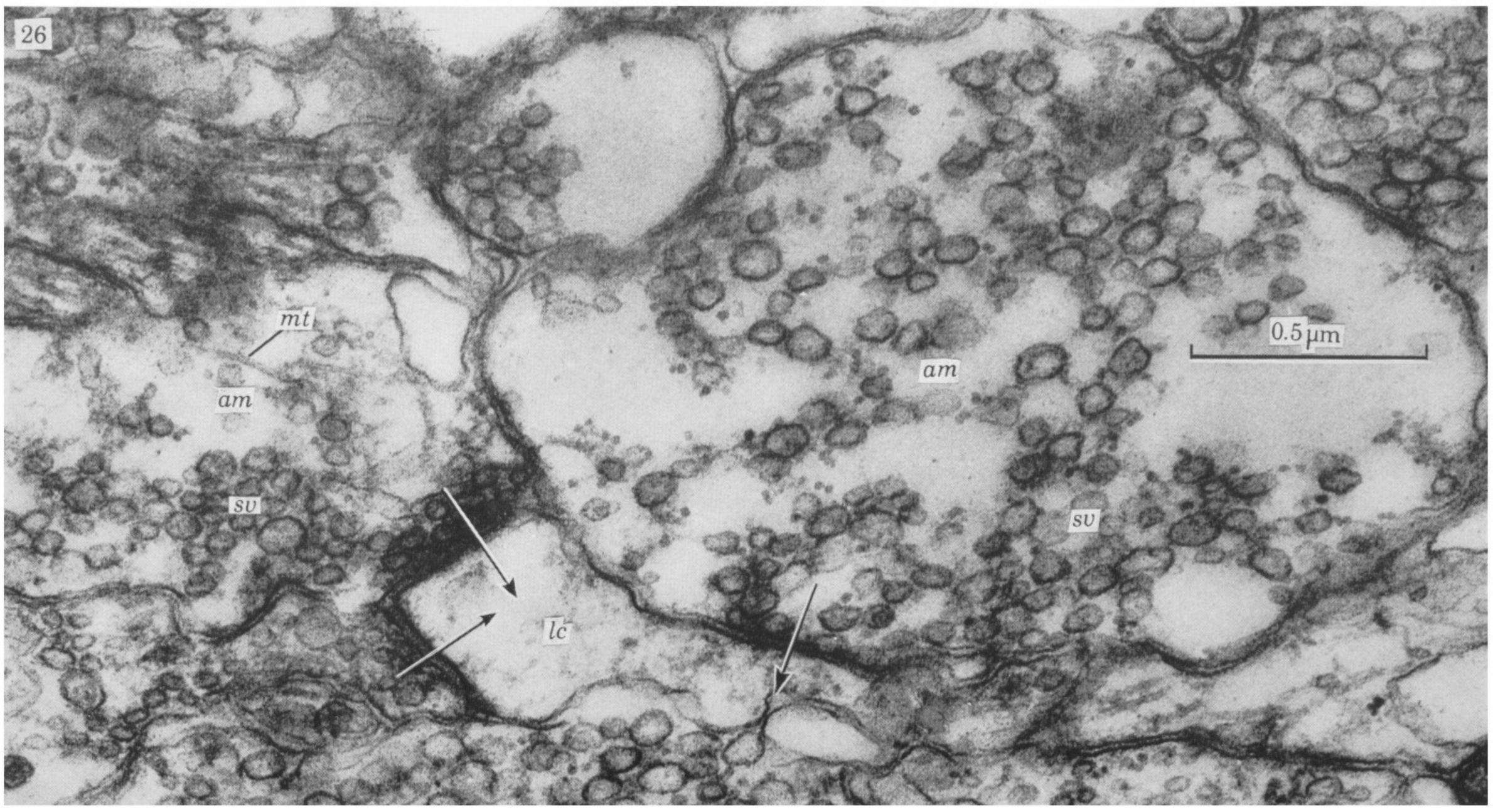


FIGURE 25. Two MSF varicosities synapsing with an amacrine varicosity, which in turn is synapsing with a collateral of a large cell. Outer medulla.

FIGURE 26. Three amacrine varicosities in synaptic contact with a pale process thought to be the collateral of a large cell.

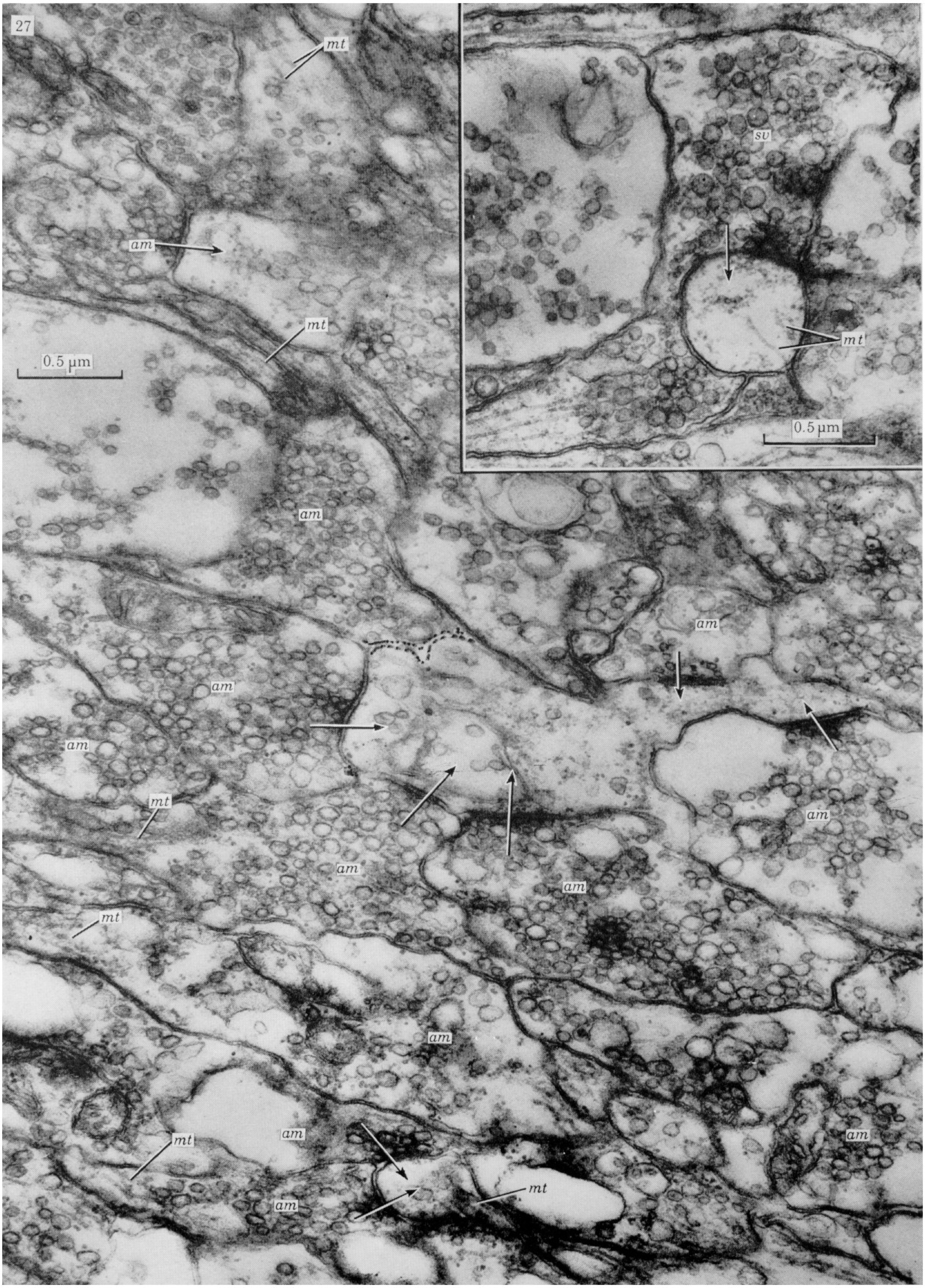
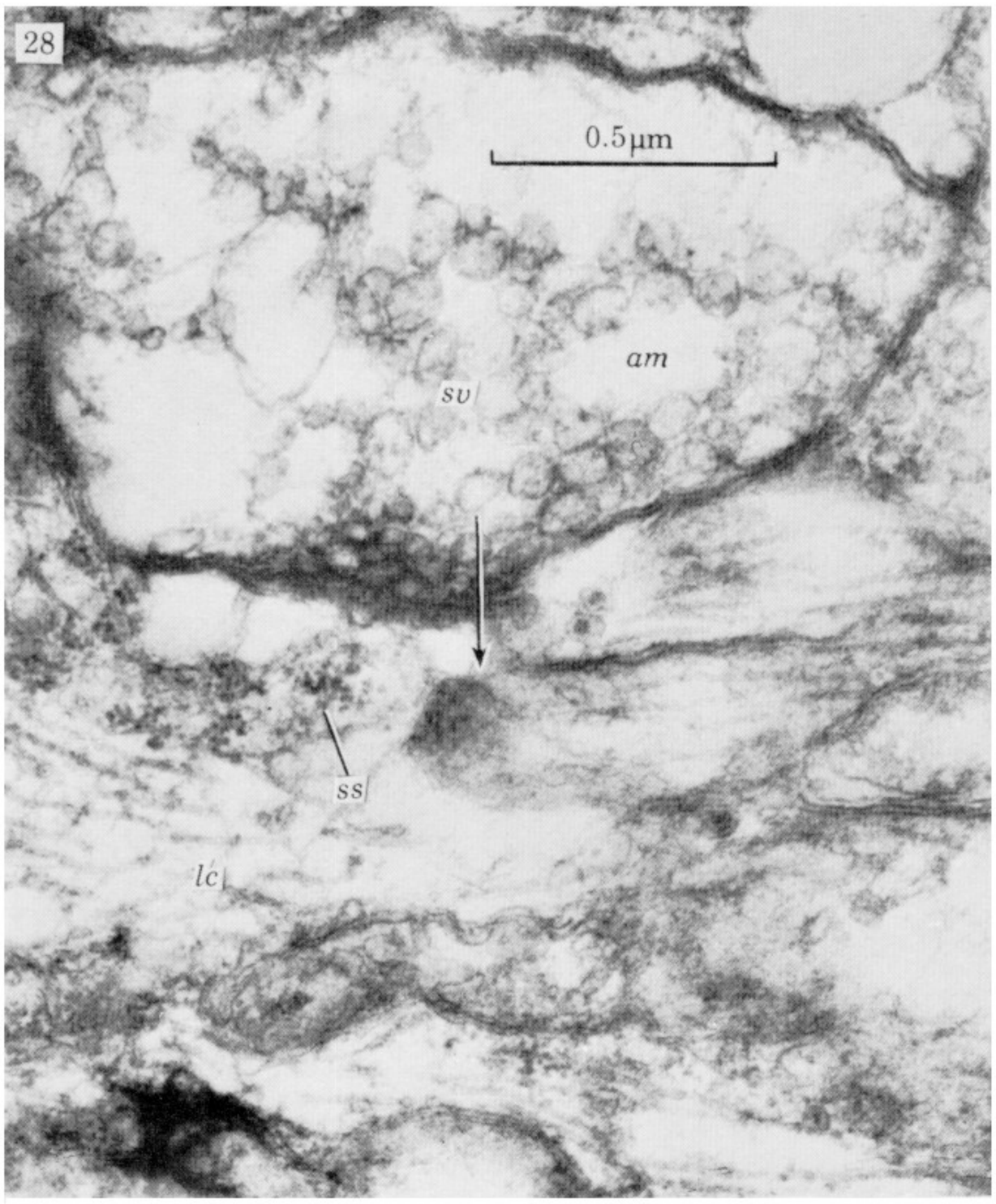
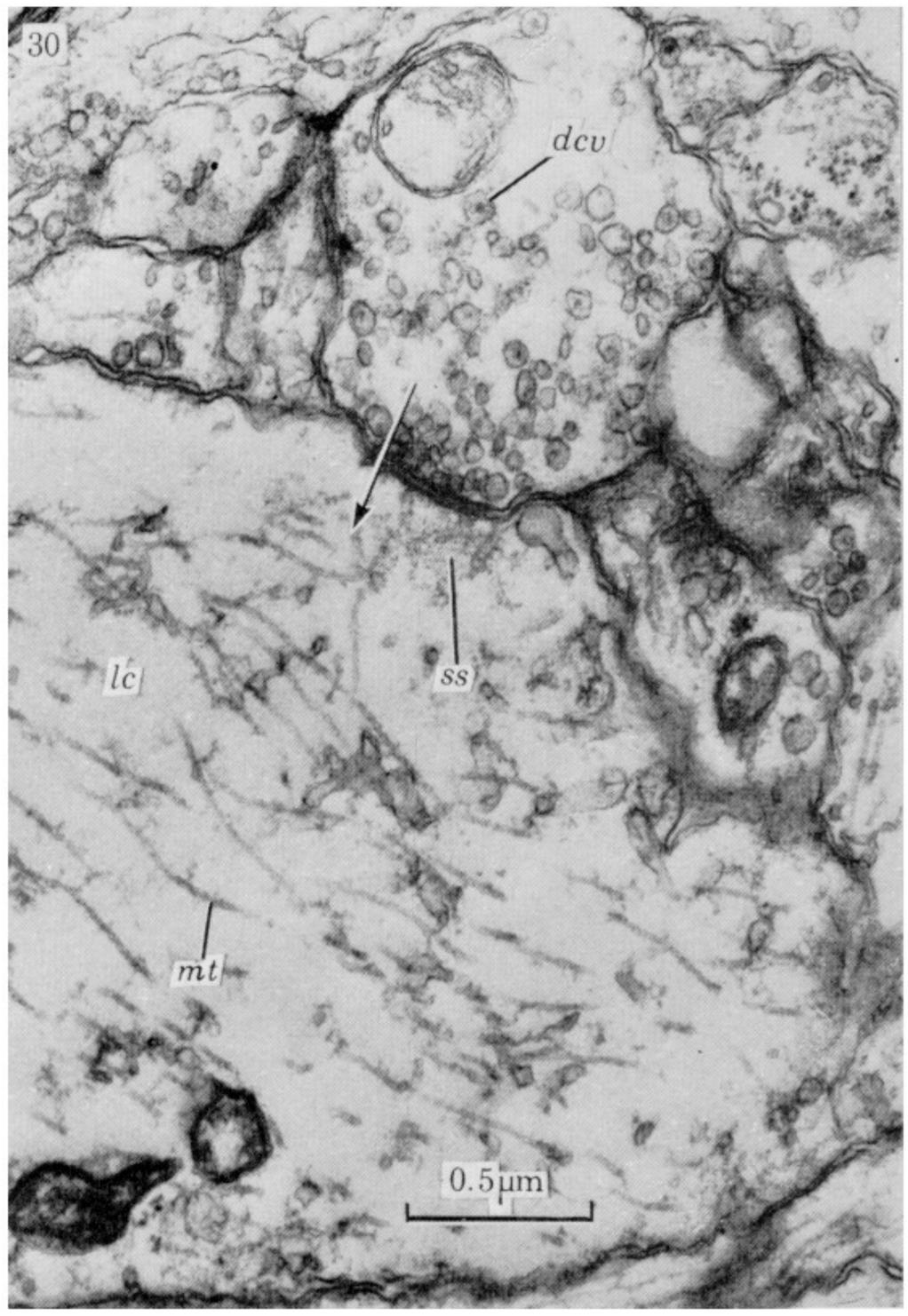


FIGURE 27. The central zone of the medulla contains numerous amacrine trunks synapsing with branches presumed to originate from trunks of large cells. Broken lines represent probable membranes, which are invisible because of tilt. Microtubules and small granules (ribosomes or glycogen?) present (but not invariably) in the pale postsynaptic component. (Inset) such a contact at higher magnification.





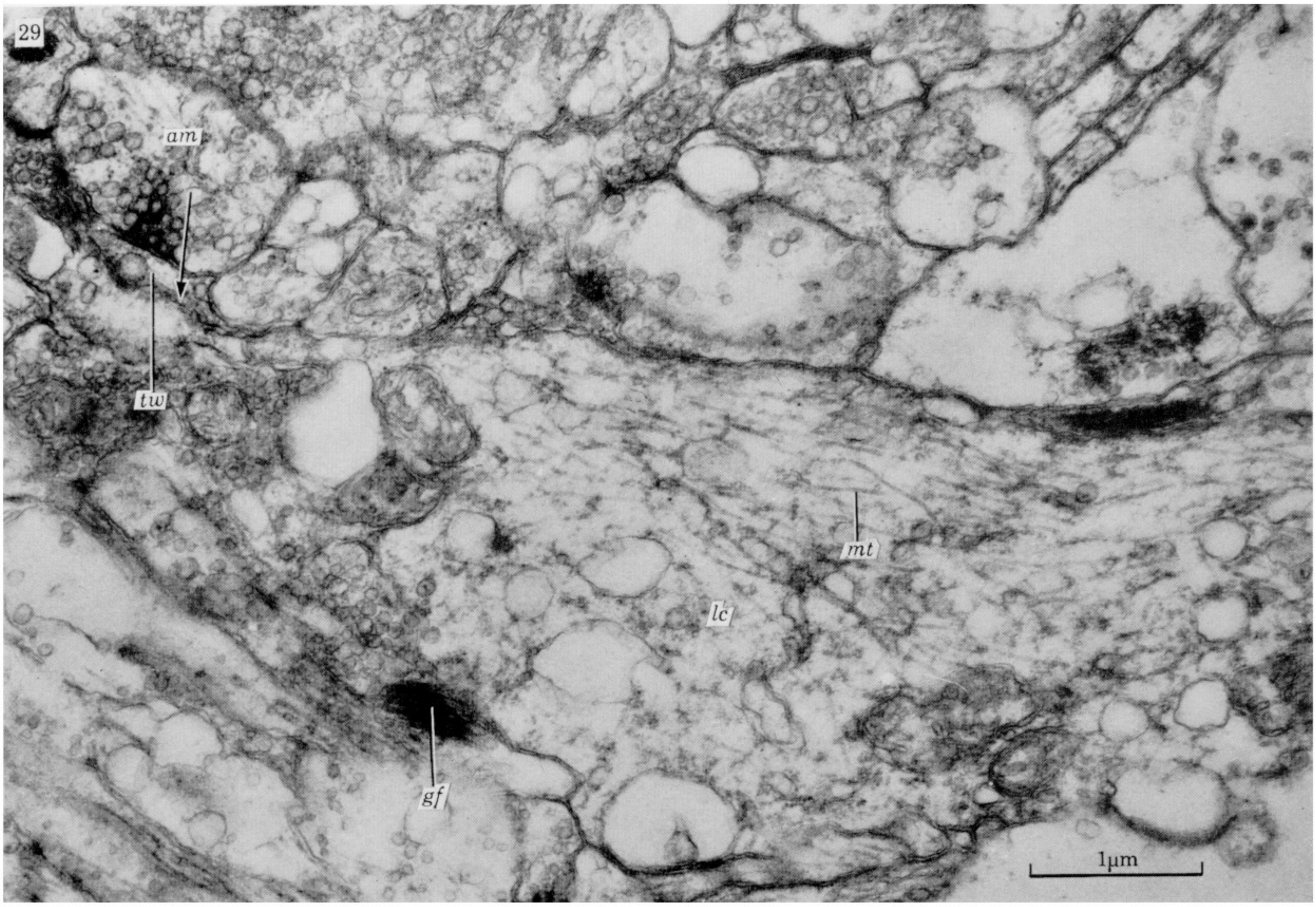
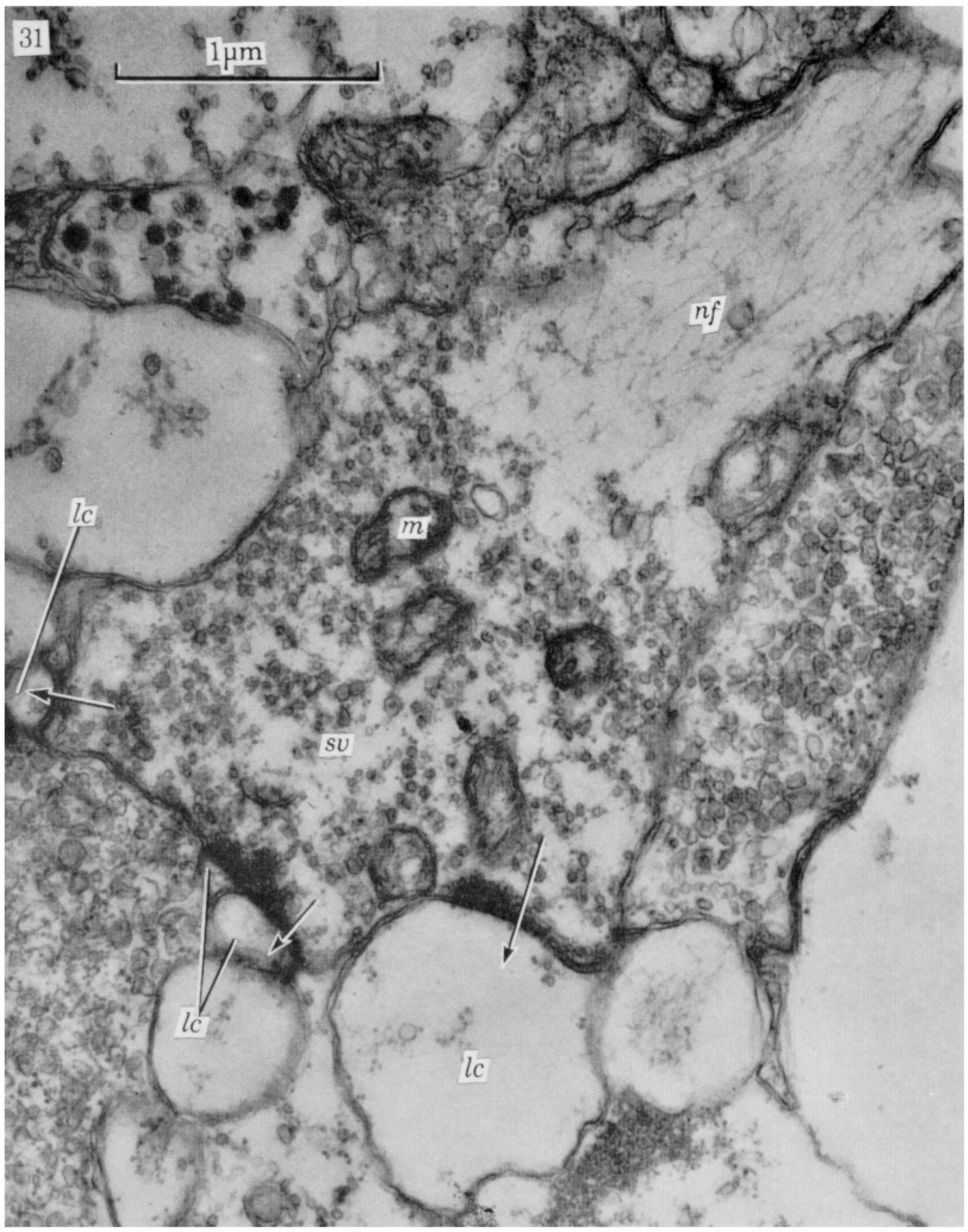


FIGURE 28. Amacrine varicosity contacting a process of a large cell. Central medulla.

FIGURE 29. Fine collateral of a process of a large cell contacted by an amacrine presynaptic varicosity. Central medulla.

FIGURE 30. A synaptic knob (amacrine or MSF?) contacting a process of a large cell. Central medulla.



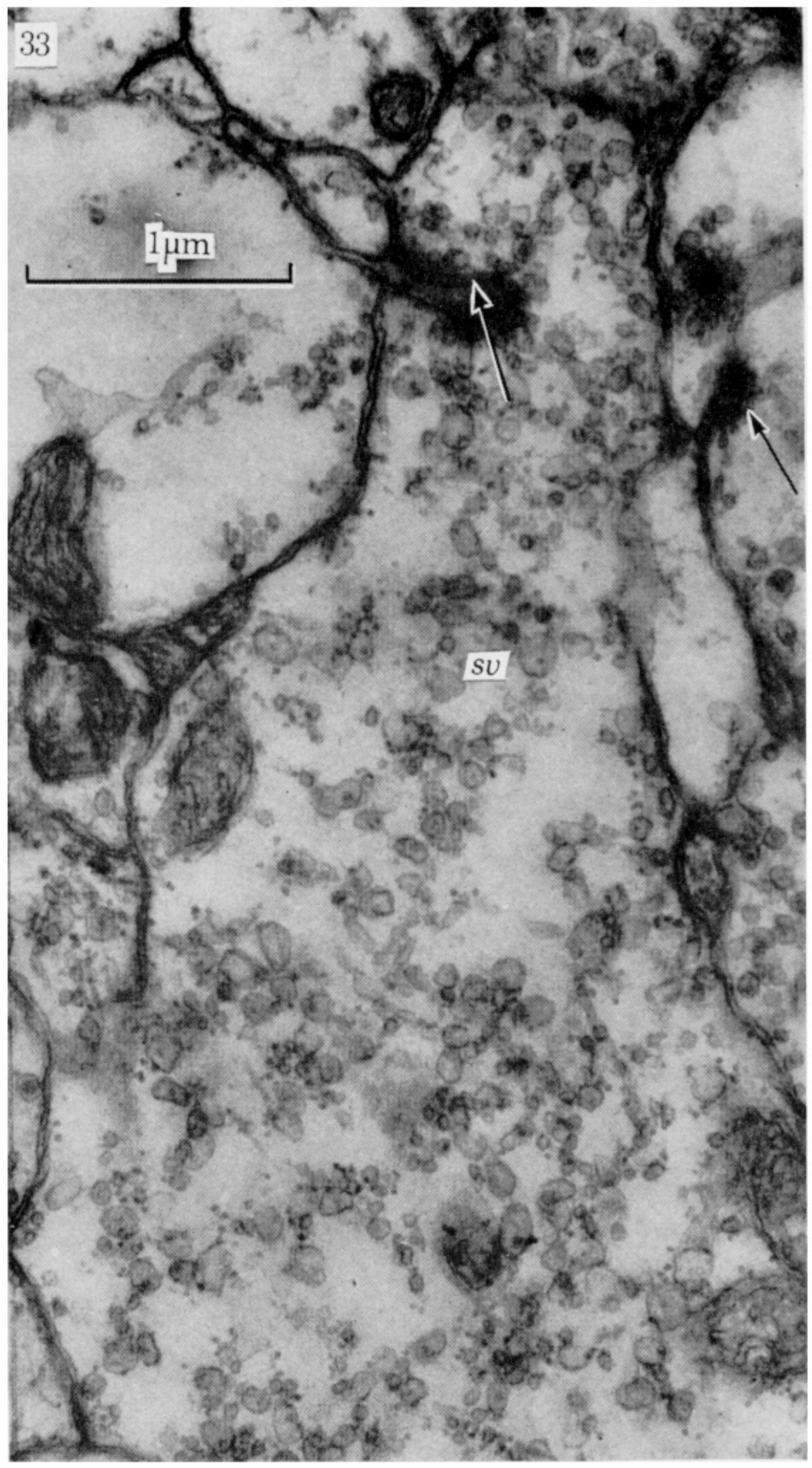
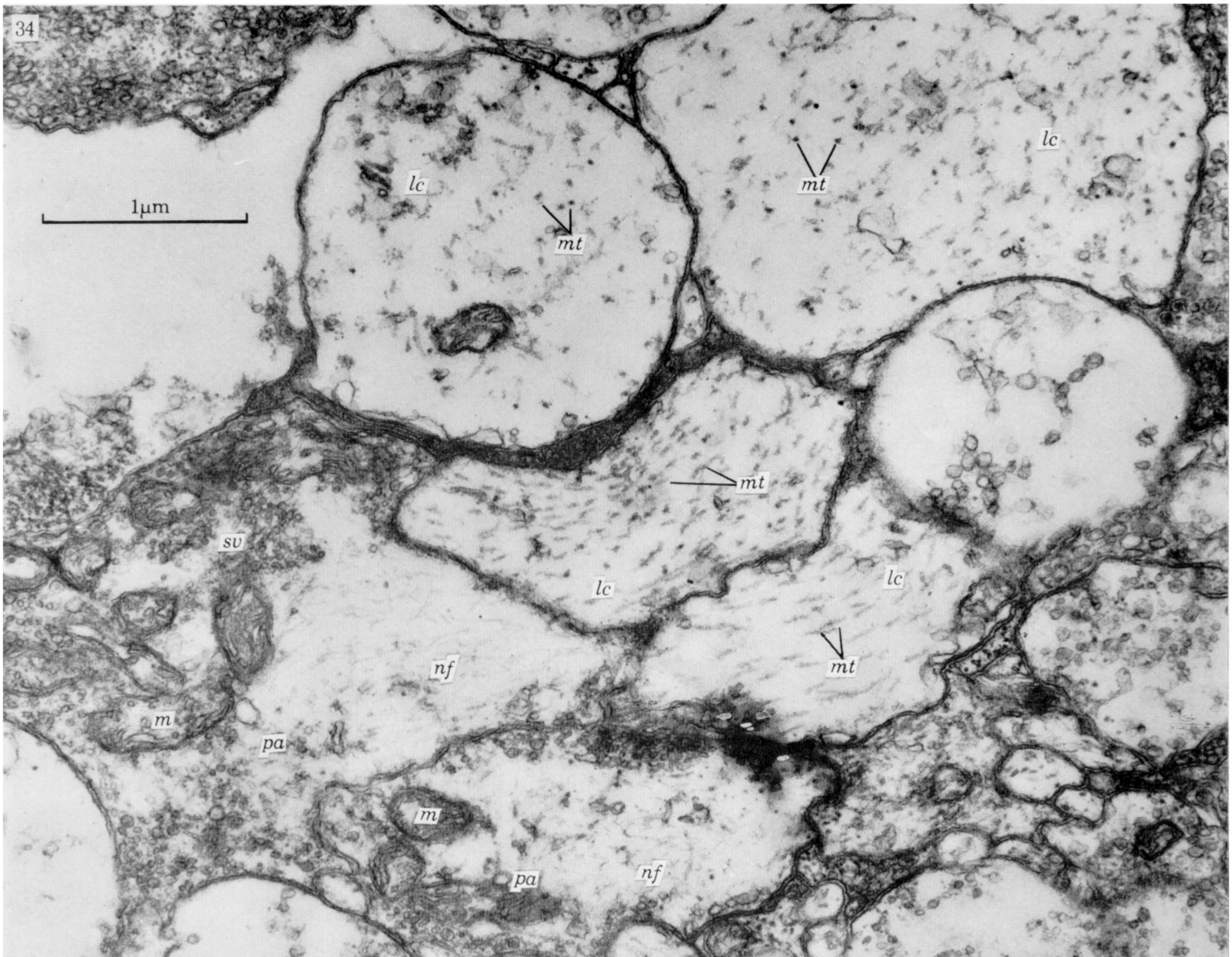


FIGURE 31. Presumed 'pain' ending contacting processes presumed to arise from trunks of large cells. Central medulla.

FIGURE 32. Presumed 'pain' ending contacting protruberance of process of large cells. Central medulla.

FIGURE 33. Presumed 'pain' endings contacting spinous processes (presumably of collaterals of large cells). Central medulla.



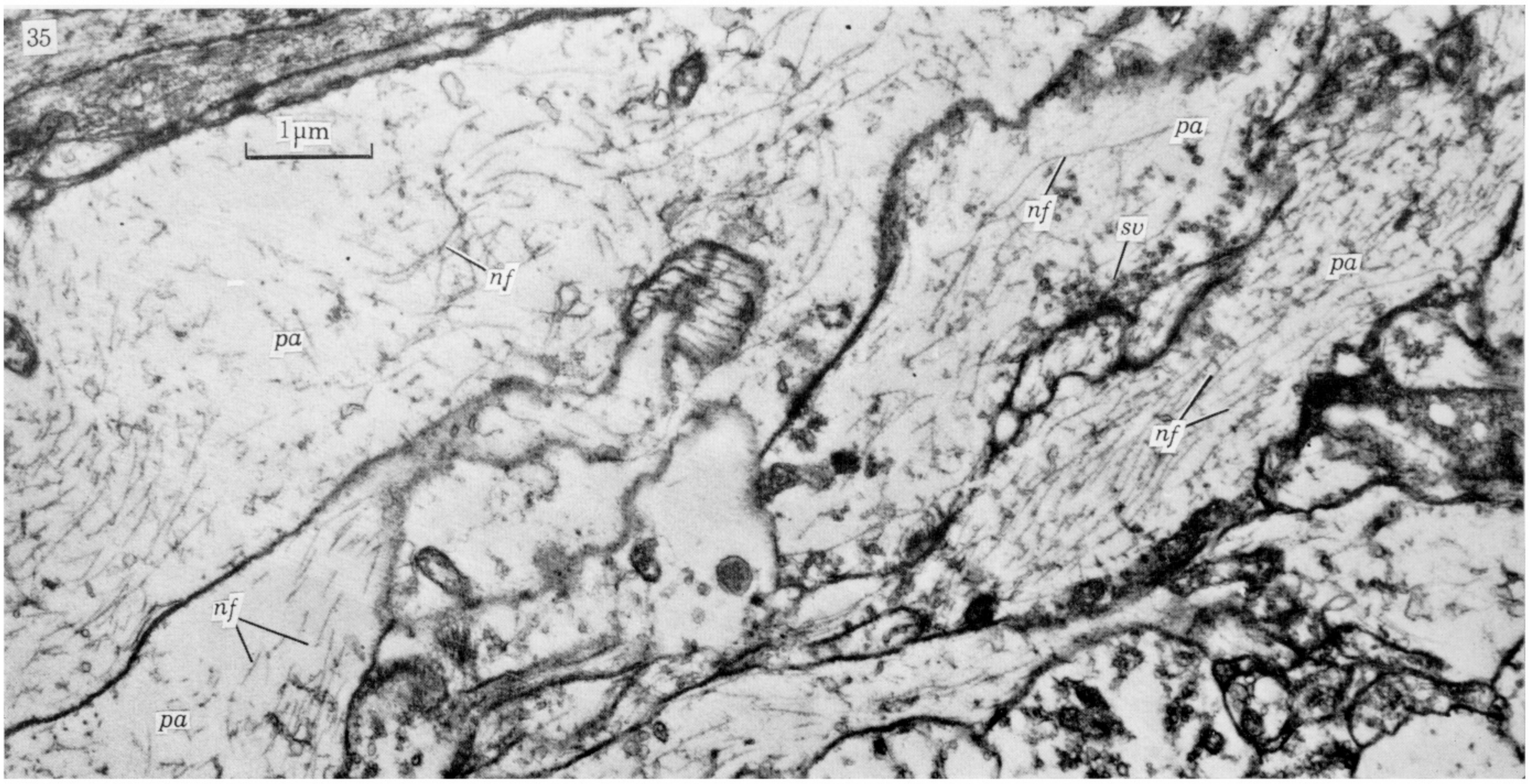
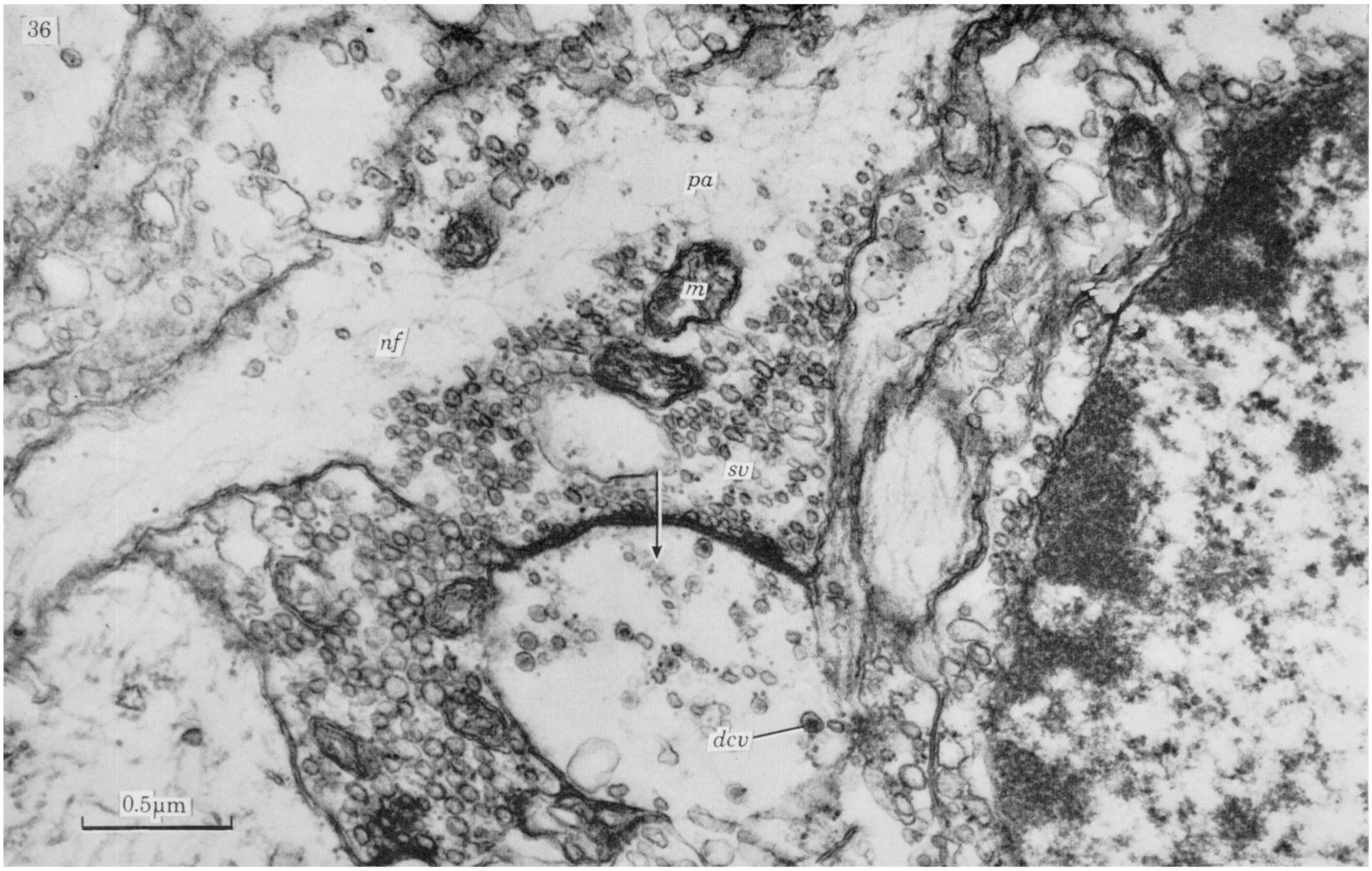


FIGURE 34. Four trunks of large cells (cut transversely). Compare the appearance of their microtubules with the neurofilaments in two neighbouring 'pain' endings. Central medulla.

FIGURE 35. Group of fibres (cut longitudinally). They contain coarse neurofilaments and are thought to be 'pain' axons. Central medulla.



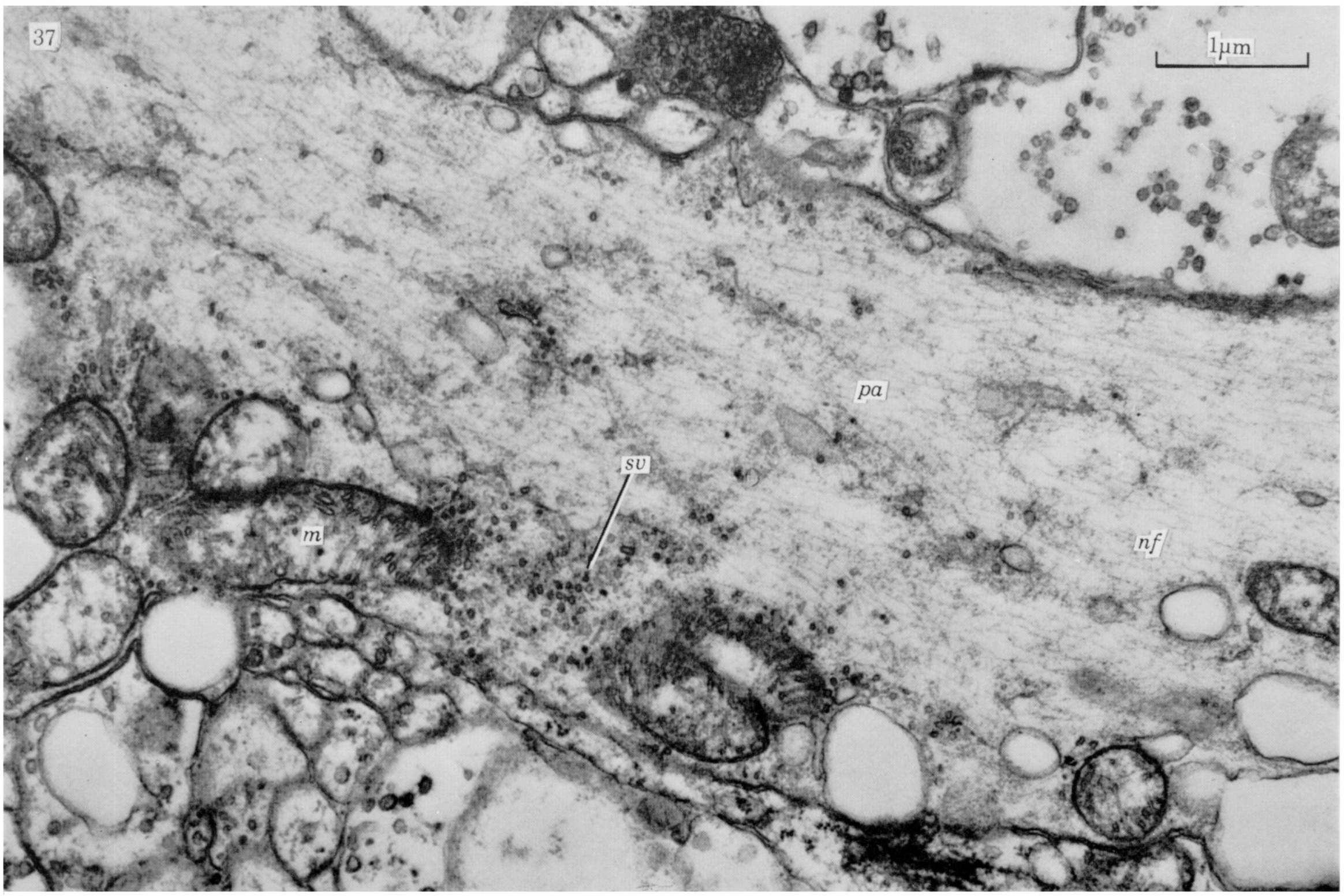


FIGURE 36. Presumed 'pain' axon making synaptic contact with a process containing dense-cored vesicles. Central medulla.

FIGURE 37. 'Pain' axon (aldehyde-fixed) containing small and flattened vesicles and neurofilaments. Central medulla.

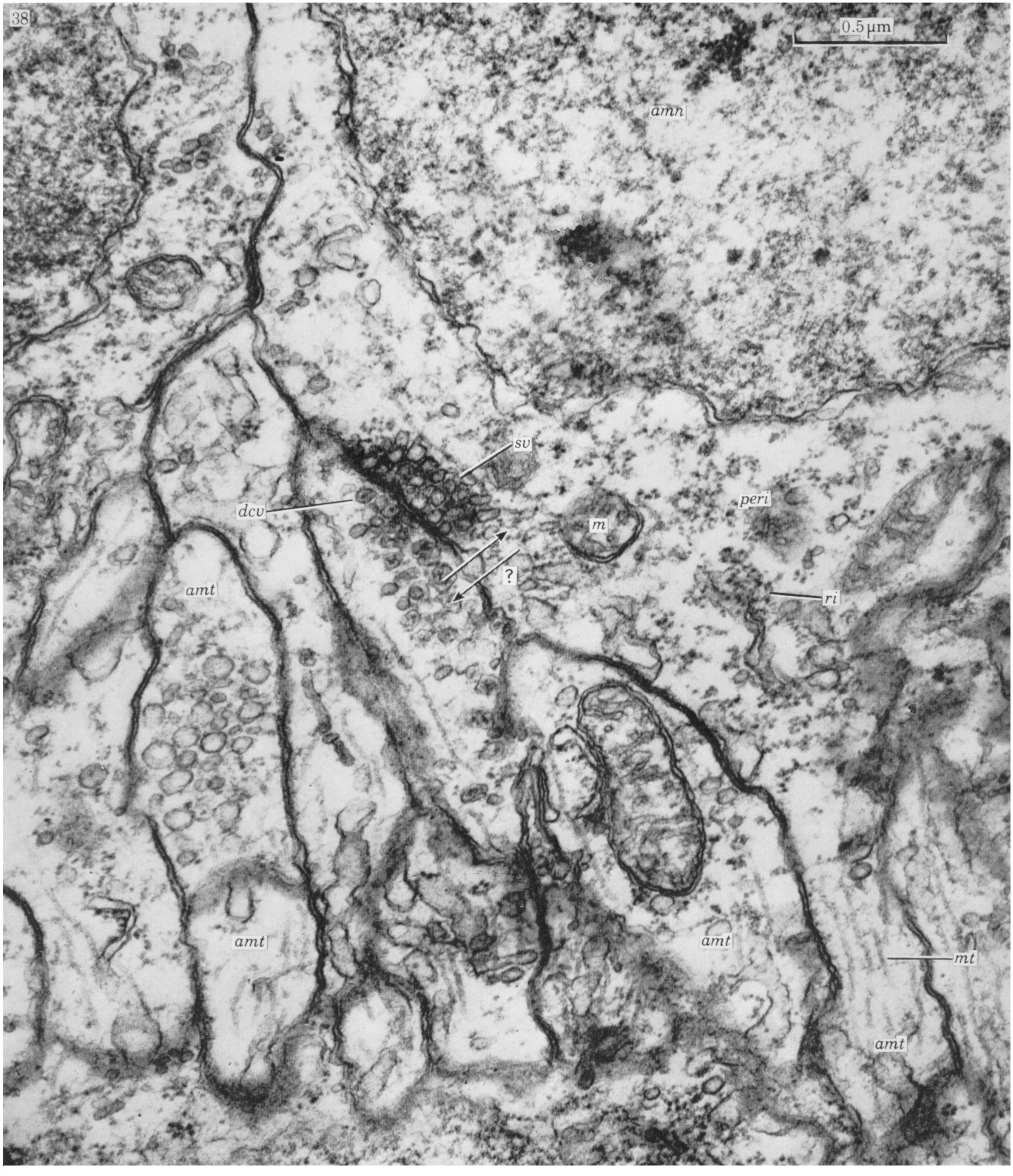


FIGURE 38. An axo-somatic synapse on an amacrine cell. Cortex.