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ELECTRON MICROSCOPY OF SYNAPTIC STRUCTURE OF *OCTOPUS* BRAIN

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ABSTRACT

The well known type of synapse between a presynaptic process containing vesicles and a "clear" postsynaptic process can be commonly observed in the various lobes of the brain of *Octopus*. The presynaptic vesicles are aggregated near regions of the synaptic membranes which show specialisation and asymmetric "thickening" indicating functional polarisation, and here chemical transmission is presumed to take place. In addition, in the *vertical* lobe a very interesting serial arrangement of synaptic contacts occurs. Presynaptic bags, formed from varicosities of fibres from the superior frontal lobe, contact the trunks of amacrine cells in the manner just described. The trunks, however, although apparently postsynaptic are themselves packed with synaptic vesicles. The trunks, in turn, make "presynaptic" contacts with clear spinous processes of other neurons of yet undetermined origin. Typical polarised membrane specialisations occur at the contact regions. The trunk vesicles aggregated closest to the contact regions have a shell of particles round their walls. At present, there is no way of telling whether the membrane conductance to the various ions is differently affected at either of the transmission sites, and, if an inhibitory mechanism is involved, whether it is of the presynaptic or postsynaptic variety.

INTRODUCTION

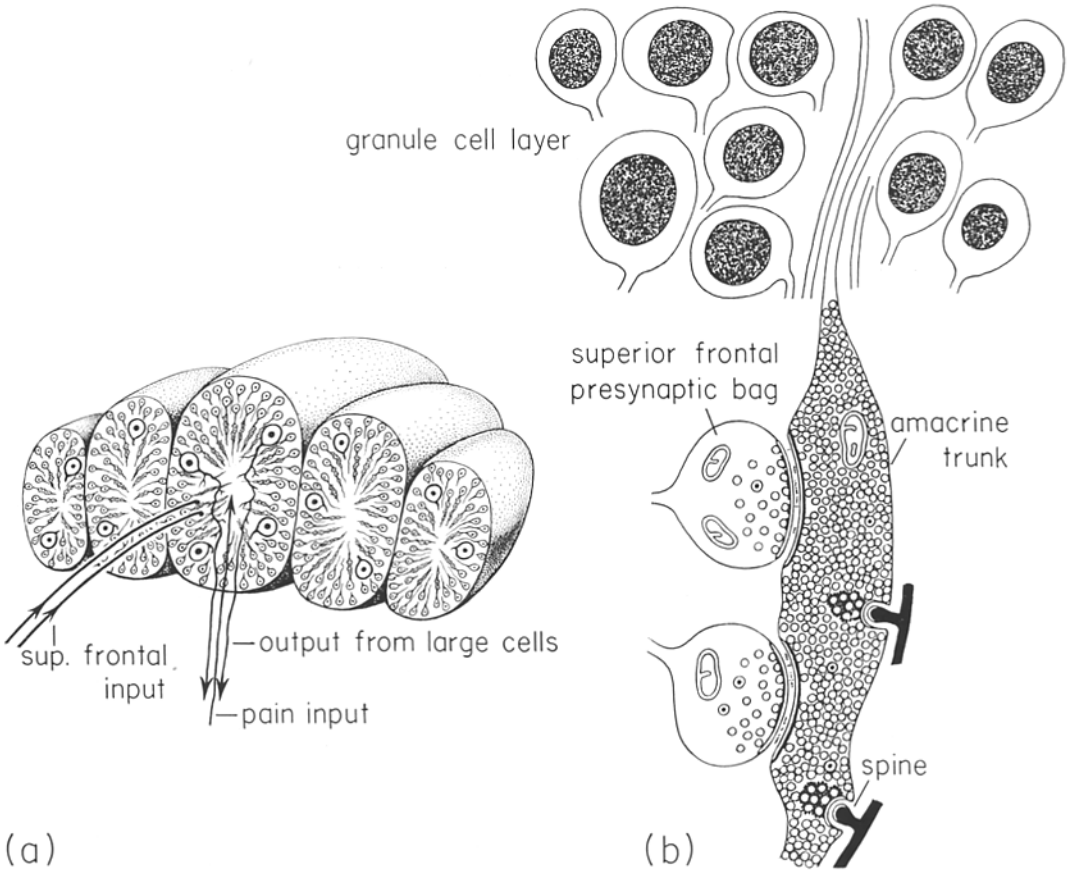
To understand the organisation of the central nervous system revealed by the electron microscope it is essential to develop rigid criteria (*a*) for the recognition of synaptic contacts and (*b*) for determining the direction of transmission so that pre- and postsynaptic components can be distinguished. Such criteria have become fairly well established for vertebrates, but much less is known about the fine structural organisation of synapses in the invertebrate central nervous system. With a few exceptions (13, 14), previous observations have been concentrated on the synaptic vesicles. Membrane thickenings and other specialisations, which might provide clues to the direction of transmission, have received less attention (3, 22, 15, 7).

The present work is an account of observations on the brain of *Octopus*, in which various lobes, the vertical, superior frontal, inferior frontal, subfrontal and pedal lobes, have been studied with the electron microscope. Most attention is given to certain serial synapses in the vertical lobe which may be the basis for the action of the lobe in suppressing attack (23). Apparently, the only other electron microscope study of *Octopus* brain so far is that on the optic lobes (4).

METHODS

Small pieces of the various lobes of *Octopus* were immersed in 1 per cent osmium tetroxide in saline at 3–4°C and buffered at pH 7.4. They were further sliced in the fixative with a dissecting microscope. Fixation was continued for 3 hours, followed by dehydration in ethanol, and staining for 3 hours in 1 per cent phosphotungstic acid in absolute ethanol. The material was embedded in Araldite for sectioning (11), and examined with a Siemens electron microscope.

Published April 1, 1964



Legends to Figures

a, vesicle-containing profile
amt, amacrine cell trunks
b, vesicle-containing profile
cp, clear profile
dv, dense-centred vesicle
dwv, dense-walled vesicles
gc, granule cells
ldv, large dense-centered vesicle
m, mitochondria
mt, synaptic membrane thickening
s, spine
sfb, presynaptic bags (varicosities) of superior frontal fibres
sp, shell of particles round vesicle
sv, synaptic vesicles
x, chemotactile fibres
y, visual fibres

FIGURE 1 Vertical lobe. (*a*) Diagram showing the five segments that constitute the vertical lobe of the *Octopus*. The anterior portion has been removed by a transverse cut. (*b*) Diagram derived from electron microscopy of part of one of the segments showing the superior frontal presynaptic bags contacting a vesicular amacrine trunk, which in turn makes contact with spines of other neurons.

OBSERVATIONS

1. *Vertical Lobe*

This lobe consists of five parallel tubular segments, lying in a longitudinal axis at the top of the brain (Fig. 1(*a*)). Its structure seen in Golgi and silver preparations has recently been described (23). In transverse sections the neuronal cell bodies can be seen to form a marginal zone and these send their trunks into the medulla, in which the intrinsic and extrinsic fibres form complicated synaptic relationships. Many of the

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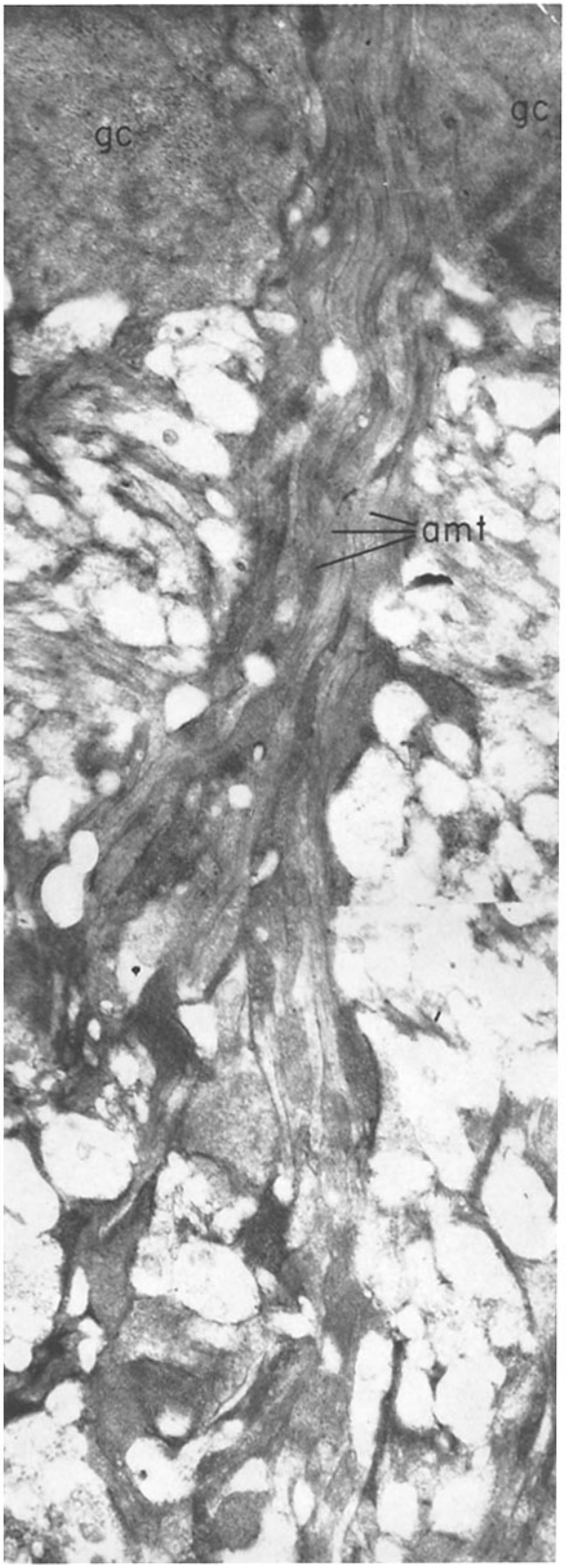

FIGURE 2 Vertical lobe. A group of trunks (*amt*) of the amacrine nerve cells emerging from the granule cell layer. The dense appearance results from tight packing of vesicle. × 5,500.

smaller granule cells are considered to be amacrine cells (23). Their trunks radiate inwards into the medulla; they have no fibres leaving the lobe and no processes that can be specifically identified as axons. The larger perikarya lie along the inner border of the marginal zone and it is these that send efferent fibres out of the lobe after making synapsis in the medulla (23).

The organelles of the cell bodies seen by electron microscopy resemble closely those of the optic lobes (4). The medulla under the electron microscope appears as a highly complex mass of interweaving fibres and synapses. This account is chiefly concerned with the outer zone of the medulla in which groups of trunks of the amacrine cells can be seen radiating inwards (Fig. 1(*a*)) and crossing at right angles the bundles of fibres arriving from the superior frontal lobe (23).

One of the amacrine trunks is shown diagrammatically in Fig. 1(*b*). The low power electron micrograph (Fig. 2) shows two of the innermost granule cell bodies (*gc*) and a group of amacrine trunks (*amt*), which originate from more superficial perikarya. These trunks increase in diameter as they emerge from the granule cell layer and they have a very dense appearance in contrast to the surrounding neuronal and glial profiles of this outer region of the medulla. This very dense appearance is the result of dense packing of synaptic vesicles within the amacrine trunks.

These dark trunks (*amt*) can be seen, at higher magnification, running from top to bottom in Fig. 3. The trunks are contacted on all sides by large presynaptic bags (*sfb*) (compare Fig. 1) whose cytoplasm, in contrast, contains fewer and less packed synaptic vesicles so that the bags appear relatively pale in comparison with the amacrine trunks. The bags are, in all probability, the "endings" of the fibres from the superior frontal lobe, from which the fibres can easily be followed, in Golgi preparations, into this outer zone of the medulla (Fig. 13). The varicosities along the length of these fibres as seen in the preparations, clearly correspond to the bags, which are, therefore, "*en passant*" contacts with the amacrine trunks. Further confirmation is provided from more recent work which shows that the bags degenerate as a result of section of the superior frontal fibres.

The superior frontal presynaptic bags (*sfb*) are shown at still higher magnifications in Figs. 4 and 5. Mitochondria and two sorts of synaptic vesicles are present, some with clear centres and others (*dv*) containing a dense granule. A special membrane "thickening" (*mt*) occurs at the region of apposition with the dark profile, which can be identified as a section of an amacrine trunk. The "thickening" will be described below in detail.

The superior frontal fibres form an important afferent supply to the vertical lobe, so the amacrine trunks are postsynaptic to the superior frontal fibres. However, the trunks, as already mentioned, are themselves packed with vesicles. A further special feature of the profiles of the trunks is groups of dense-walled vesicles (Fig. 4, *dwv*). Sections cut in other planes reveal that these special vesicles are not, in fact, lying in the centre of a mass of simple vesicles, but are aggregated against a further synaptic "thickening," which occurs at the apposition region between an amacrine trunk and a pale profile that is probably a neuronal dendritic spine, although the possibility that it is a glial profile cannot be entirely excluded. Fig. 5 shows the complete system in a favourable plane of section. The pale vesicle-containing superior frontal bag (sfb_1) contacts a dark amacrine profile (*amt*), which contains two groups of dense-walled vesicles (*dwv*). These are aggregated at membrane "thickenings" (mt_1 and mt_2) at contact points with two spines (*s*) having clear cytoplasm and no vesicles (compare Fig. 1(*b*)). A similar arrangement is shown in Fig. 6. Here the "thickening" (*mt*) between superior frontal fibre and amacrine trunk is sectioned obliquely, but the dense-walled vesicles can be clearly seen aggregated against a pale profile with no vesicles (*s*). The dense vesicles are surrounded by a mass of simple vesicles. In other sections (Fig. 7) the superior frontal contacting bag does not appear in the plane of section, only the amacrine profile with its special group of dense-walled vesicles (*dwv*) contacting the spine (*s*).

Hence we have here a serial arrangement of

FIGURE 3 Vertical lobe. A group of amacrine trunks (*amt*) containing tightly packed vesicles. Their concavities house the presynaptic bags (*sfb*) of the superior frontal fibres. × 16,000.

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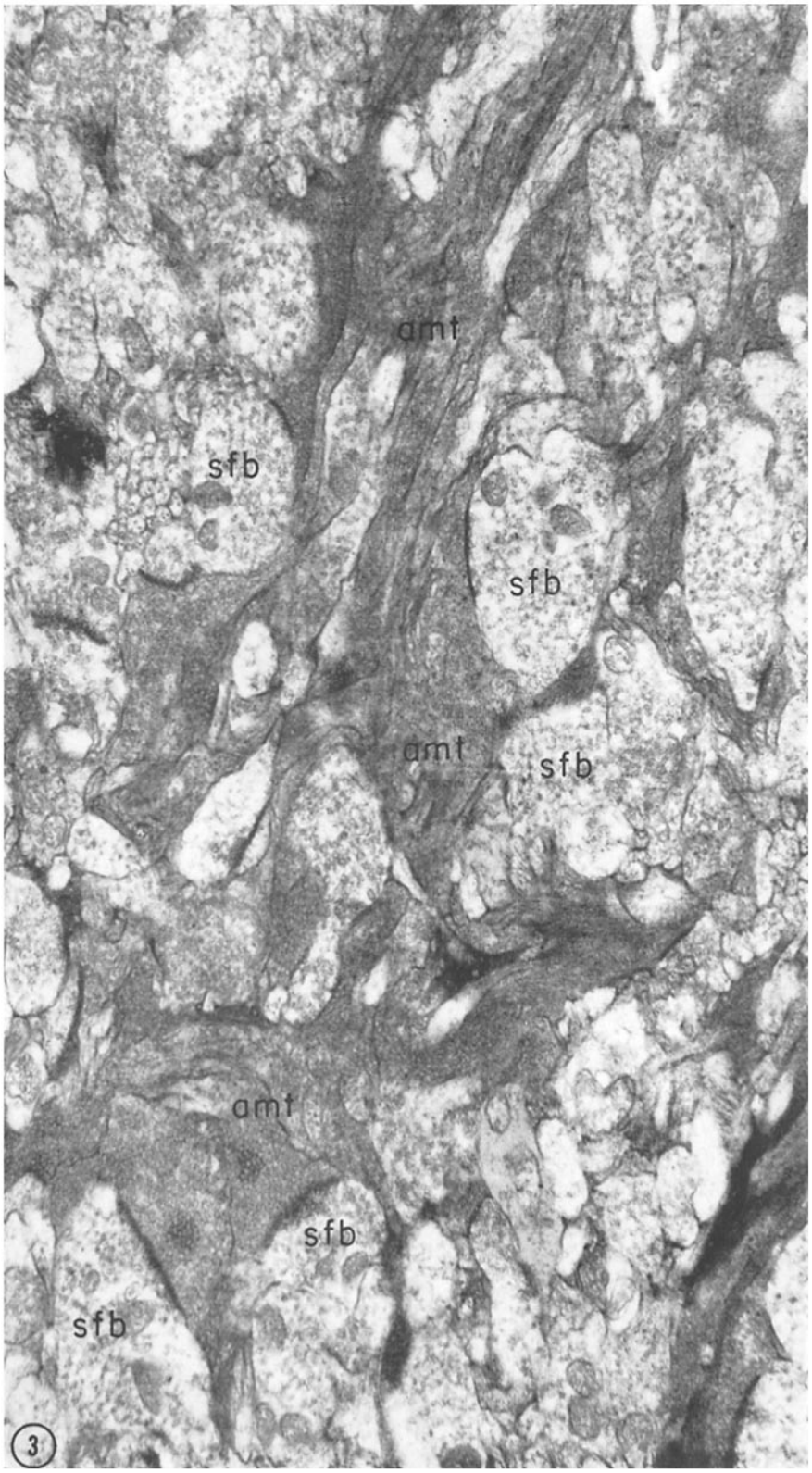

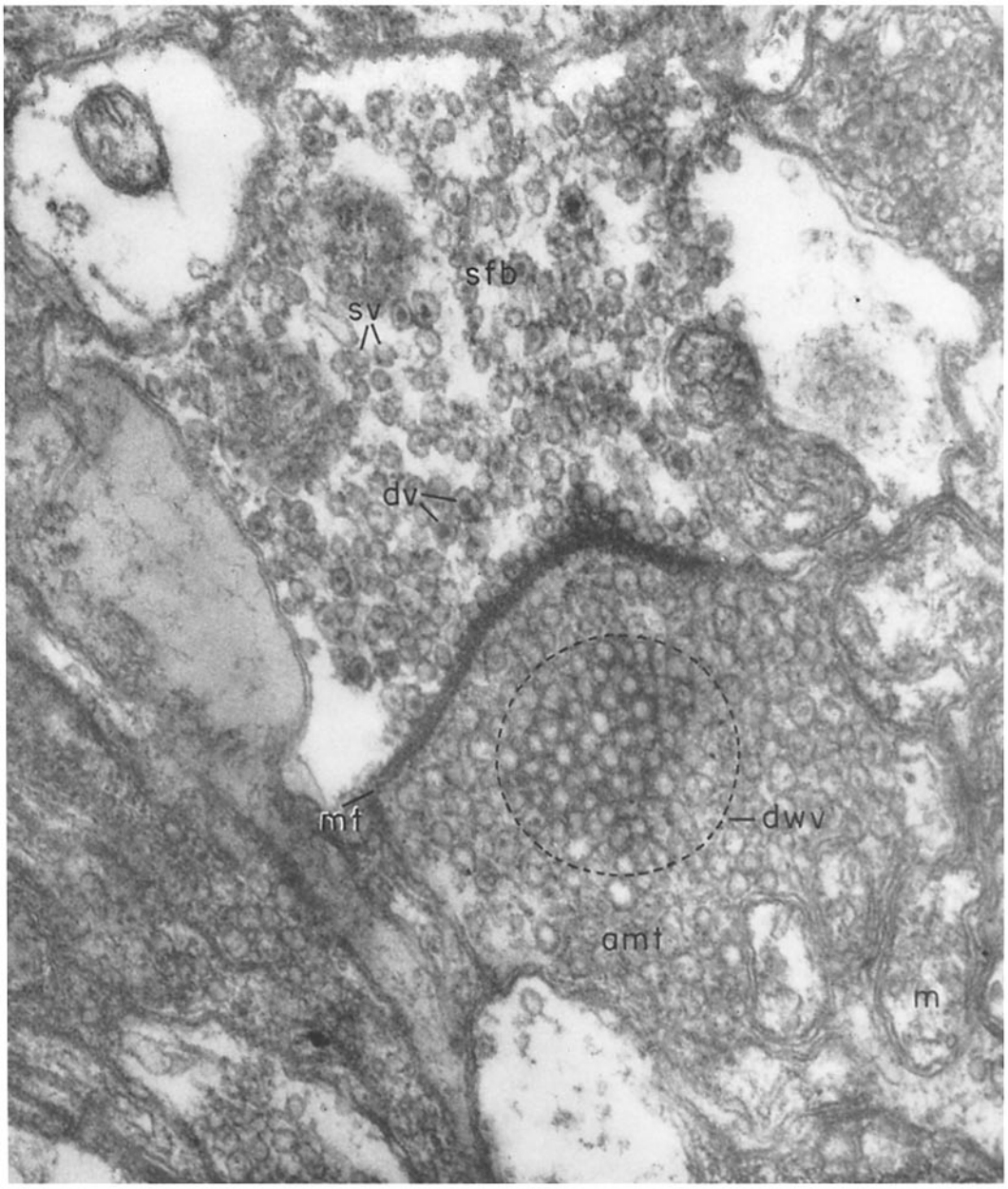

FIGURE 4 Vertical lobe. Superior frontal bag (*sfb*) contacting an amacrine trunk (*amt*). × 70,000.

synaptic contacts, that is, superior frontal fibres making "en passant" contacts with amacrine trunks, which, in turn, contact profiles with clear cytoplasm containing only a few fine granules or tubules. The cells of origin that give rise to the fibres of which these profiles are presumed to be fine spinous collaterals have not yet been certainly identified, but are possibly branches of the main efferent cells of the lobe (23).

The "thickenings" at the synaptic contacts show a complex asymmetrical structure, presumably indicative of functional polarisation.

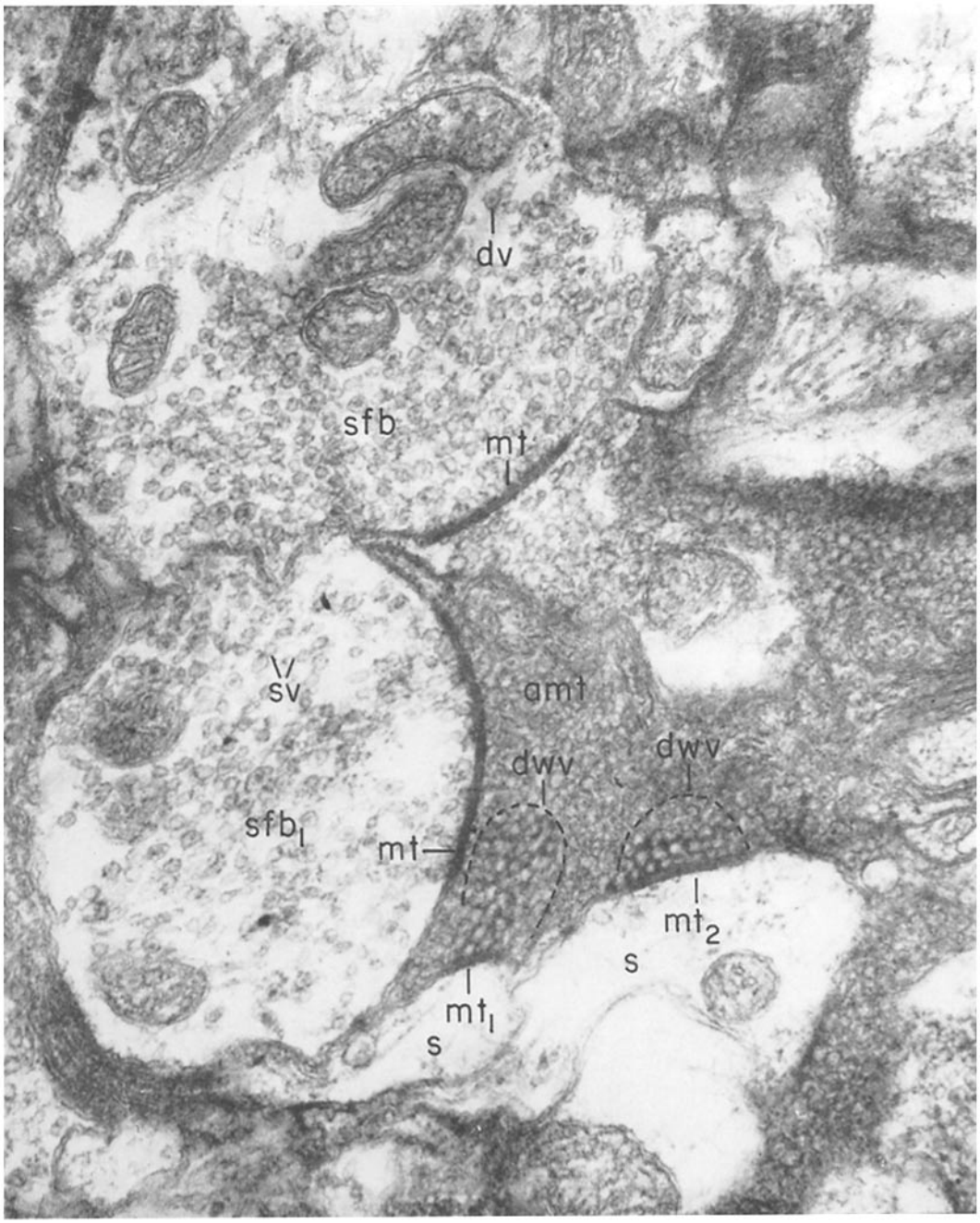

FIGURE 5 Vertical lobe. Two superior frontal bags (*sfb*) contacting amacrine trunks, one of which (*amt*) in turn contacts two spinous profiles (*s*). × 37,000.

A contact "thickening" between a superior frontal bag and an amacrine cell is shown at high magnification in Fig. 8 and in a region further enlarged as an inset. The vesicles (sv_1) form aggregations along the presumed presynaptic membrane and they have associated dense material (*1*) at the contact points. The presynaptic membrane itself (*2*) has a triple structure consisting

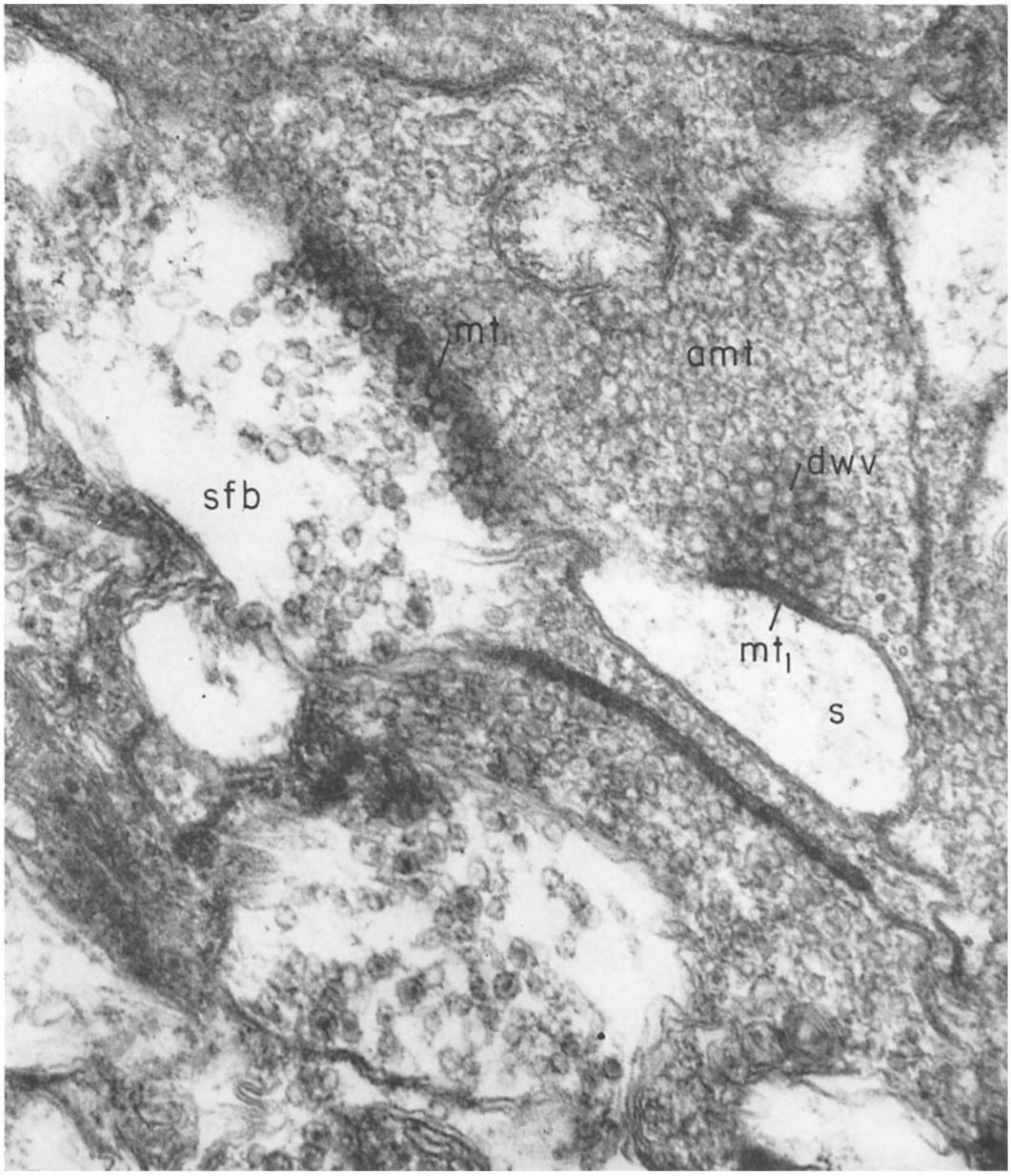

FIGURE 6 Vertical lobe. Superior frontal bag (*sfb*) contacting an amacrine trunk (*amt*). The membrane contact region is cut obliquely (*mt*). The trunk in turn contacts a spinous profile (*s*). × 51,000.

of two dense lines (50 A apart from centre to centre) with a clear zone between. The cleft (*3*) is about 200 A across and contains dense bars or particles that run either across the cleft or, in other regions, parallel with it. The postsynaptic membrane (*4*) also has a triple structure and, in addition, along its cytoplasmic surface a dense material (*5*) forms a continuous layer that is absent from the cytoplasmic surface of the presynaptic membrane. Non-specialised regions of apposed surface membranes (*6*) do not show the triple structure so clearly, have a cleft about

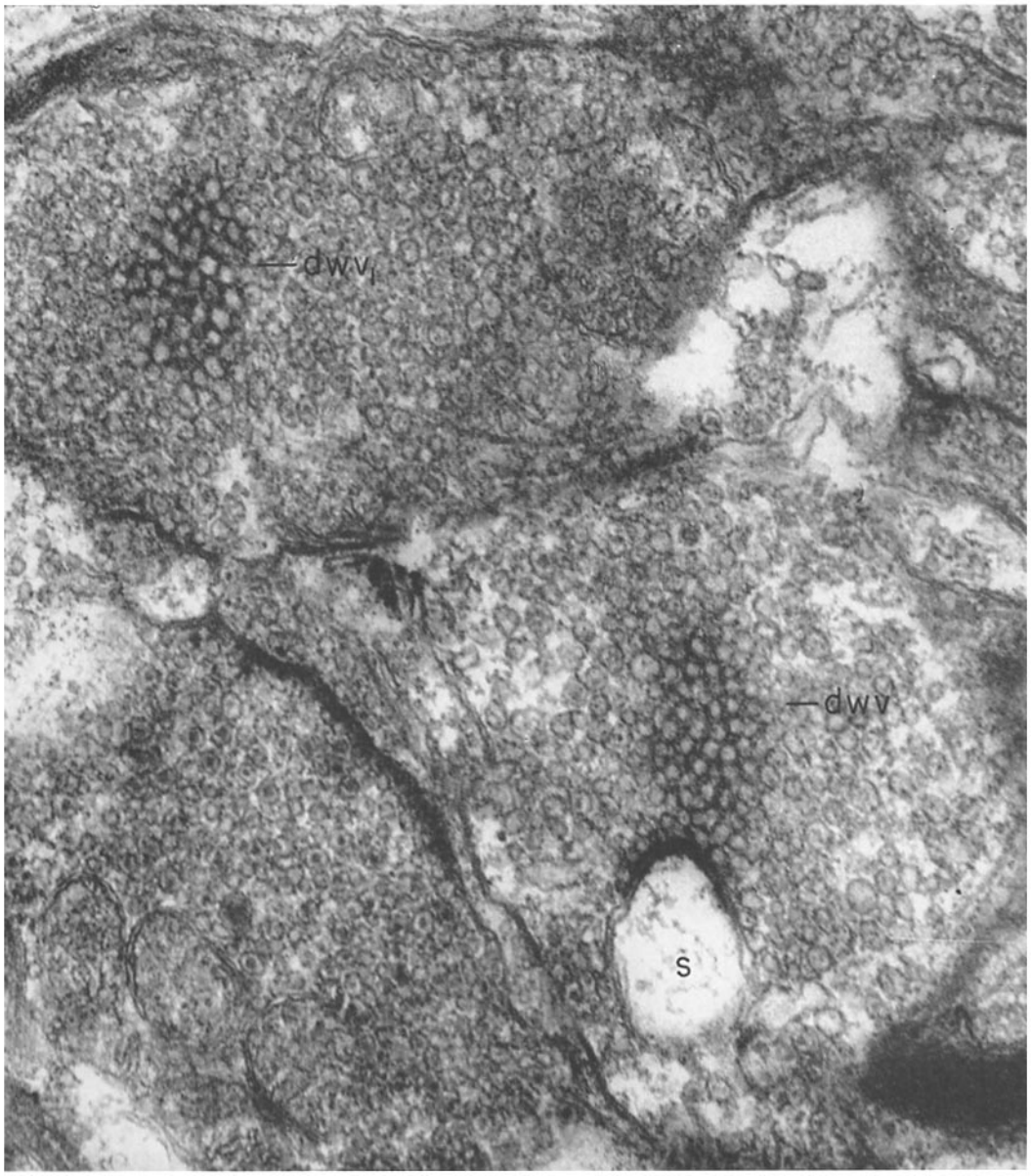

FIGURE 7 Vertical lobe. Two presumed profiles of sections of amacrine trunks. The synaptic vesicles surround an accumulation of dense-walled vesicles (*dwv*). ×46,000.

100 to 120 A across, and have no vesicle aggregations and no cleft material or other specialisations.

The structure of the amacrine dense-walled vesicles that form aggregations against the membrane "thickenings" at the contacts between amacrine trunks and spines is shown in Fig. 9 (enlarged from Fig. 4). These vesicles seem to have a shell of smaller particles (*sp*) around their walls, which gives them the dense appearance that marks them out from the surrounding mass of closely packed vesicles.

At present, it cannot be decided whether the over-all dark appearance resulting from the close packing of vesicles is a rigid criterion for identifying amacrine trunks under all conditions, although this seems to be the rule in sections ex-

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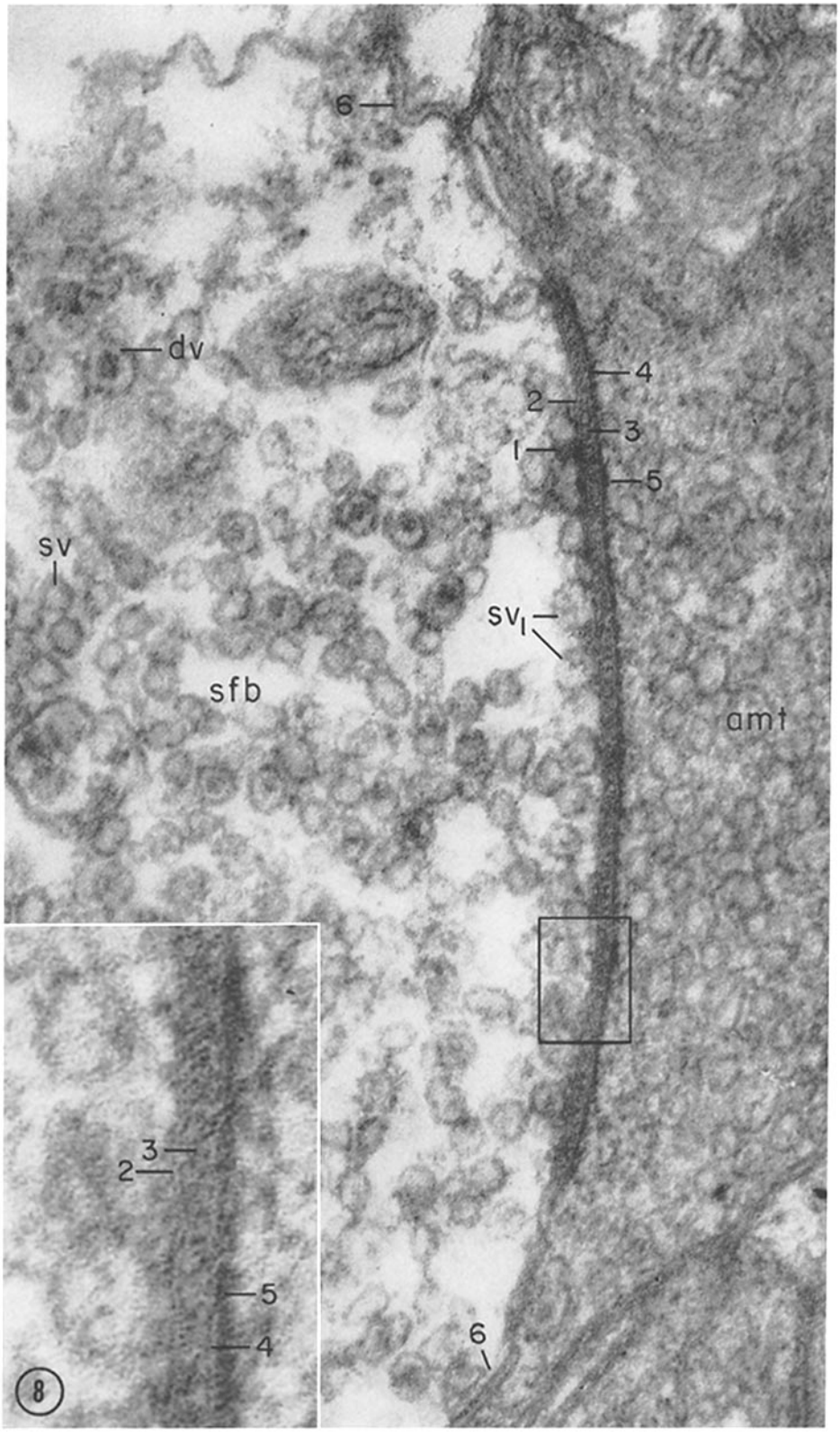

FIGURE 8 Vertical lobe. Details of membrane specialization at a superior frontal-amacrine contact, × 110,000. *Inset:* enlargement of part of the synaptic membranes. ×270,000.

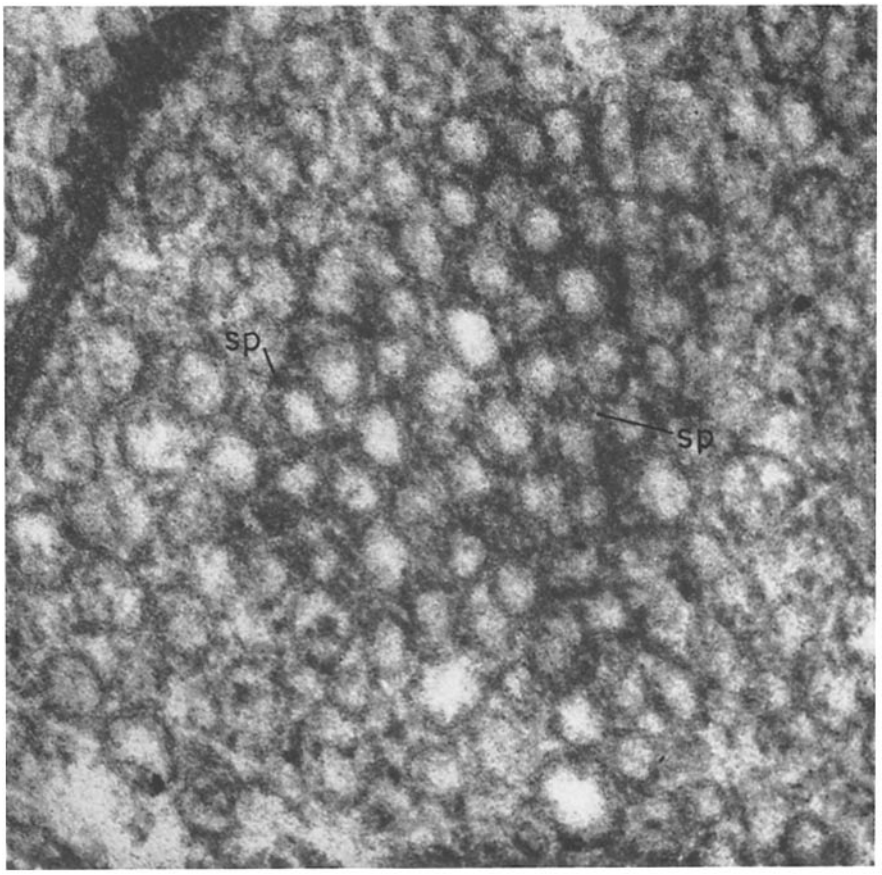

FIGURE 9 Vertical lobe. Group of dense-walled vesicles in amacrine trunk. Enlarged from Fig. 4. × 170,000.

amined so far. However, two clear processes containing well spaced synaptic vesicles can often be observed in presumed synaptic contact, as seen in Fig. 10, *a* and *b*, *a* being presynaptic to *b* since the cleft is polarized exactly as in Fig. 8. These could be two presynaptic bags of the superior frontal fibres or a completely different set of neuronal contacts. Conversely, membrane thickenings sometimes occur between two dark processes, presumably amacrine trunks.

2. *Superior Frontal Lobe*

The special feature of the vertical lobe described above is the presence of amacrine cells. The vertical lobe and the superior frontal lobe form a dual system controlling decisions to attack prey, especially where delayed rewards are involved (23). Light microscopy shows no amacrine cells in the superior frontal lobe. Electron microscopy of sections of this lobe shows no dark amacrinal cell trunks or serial synaptic arrangements such as those described above.

The synapses observed in the superior frontal lobe have all been of the single two-element type. Fig. 11 shows several presynaptic bags, containing both clear- and dense-centred vesicles, contacting a non-vesicular postsynaptic process (*ct*). These postsynaptic processes often take the form of spines (Fig. 12, *s*). They are presumably the tips of the dendritic collaterals seen in Golgi preparations of the trunks of the cells of the superior frontal lobe as they pass through the neuropil (Fig. 13). The presynaptic fibres are presumably the endings of the visual and chemotactile fibres that are known to enter the lobe, as shown diagrammatically in Fig. 13.

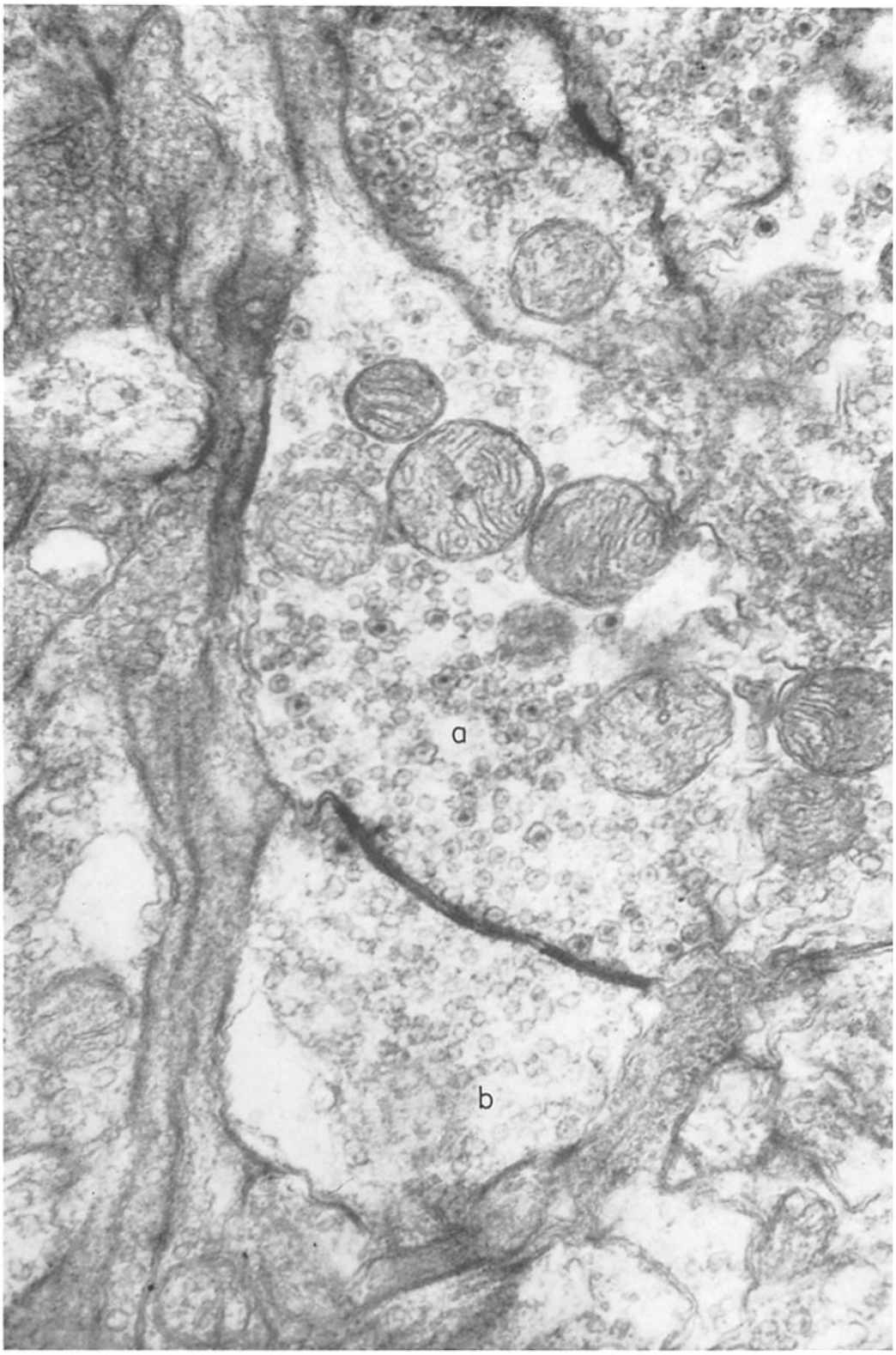

FIGURE 10 Vertical lobe. Two "clear" synaptic processes (*a*) and (*b*) with membrane specialisations at region of apposition. × 53,000.

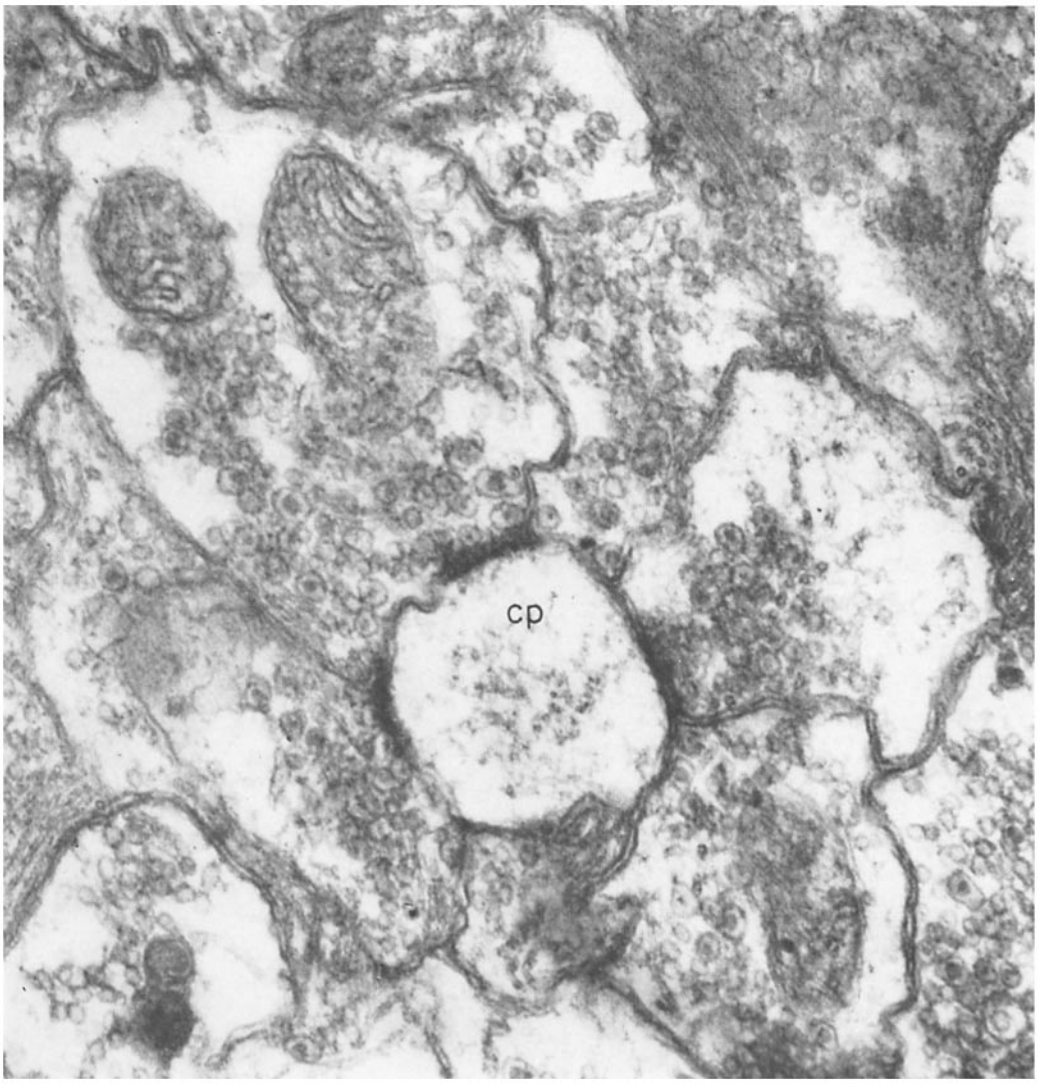

FIGURE 11 Superior frontal lobe. Clear process (*cp*) contacted by several presynaptic bags. × 30,000.

DISCUSSION

Synaptic Vesicles and Membrane Specialisations

In all the lobes of *Octopus* brain examined so far, synaptic contacts were observed resembling in essentials those of the vertebrate central nervous system fixed and stained in the same way. This general pattern of organisation is modified in the special serial synaptic arrangements of the vertical lobe. In the ordinary (non-serial) synapses, mitochondria and "clear" synaptic vesicles about 500 A in diameter characterise the presynaptic bag. The clear vesicles are mixed with others of similar size but having a dense core, similar to those of presynaptic bags in certain peripheral and central synapses of mammals, which are considered by some authors to be specific of adrenergic endings (18, 12, 21, 9). A third, larger type of dense-centred vesicle, 1000 to 1500 A in diameter (Fig. 14, from the vertical lobe),

is also a common feature within presumed presynaptic bags of *Octopus* brain. Three similar types of vesicles have been described in synapses of the gastropods by Gerschenfeld (7). The large dense vesicles are also common in the annelid central nervous system and are considered by some workers to be of a neurosecretory nature (20, 2). The neurosecretory vesicles of the vertebrate neurohypophysis are similar (18, 1).

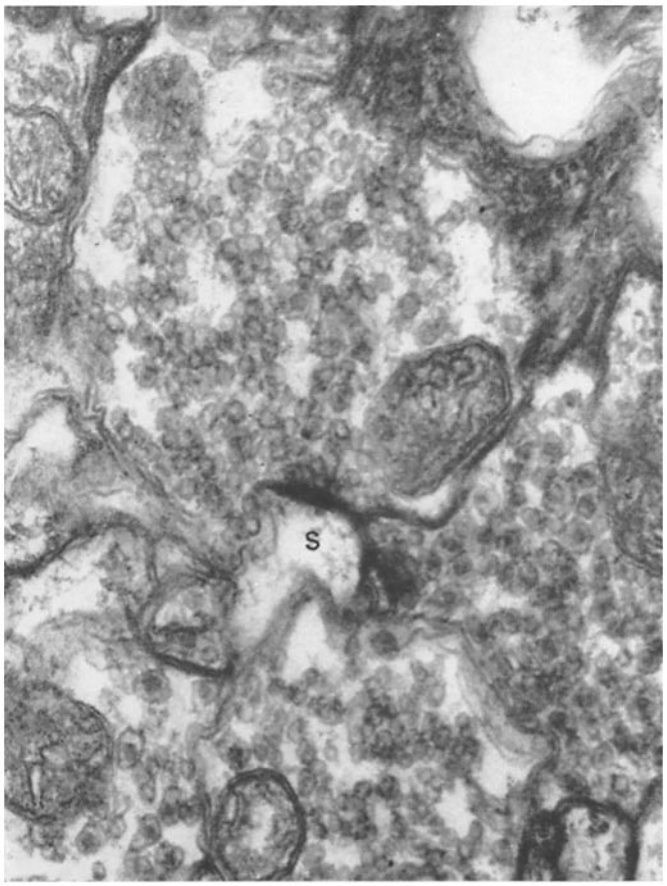

FIGURE 12 Superior frontal lobe. Spinous process (*s*) contacted by presynaptic bag. × 28,000.

As in vertebrates, the synaptic vesicles in *Octopus* form aggregates against the cytoplasmic surface of the presynaptic membrane, and dense material occurs at the regions of contact. The system of hexagonally arranged dense projections on the presynaptic membrane of vertebrates (9–11) has not been clearly observed in *Octopus* so far.

A striking feature of both pre- and postsynaptic membranes in osmium tetroxide–fixed *Octopus* brain is their triple structure, though this is less clearly seen in the non-specialised regions of the neuronal membranes. The structure resembles the unit membrane of Robertson (19), revealed in lipoprotein membranes after fixation with potassium permanganate. The sporadic occurrence of a triple structure in membranes after osmium tetroxide fixation has been reported from time to time and is not understood at present. In vertebrates, in contrast, the synaptic membranes are very dense and do not usually show a triple structure after osmium tetroxide fixation (8, 10, 11).

In both the vertebrates (especially type I synapses) (8) and *Octopus*, extracellular cleft material is present, and in both there is a layer of dense material along the cytoplasmic surface of the postsynaptic membrane. The material is rather less conspicuous in *Octopus*. The postsynaptic process in this type of contact may take

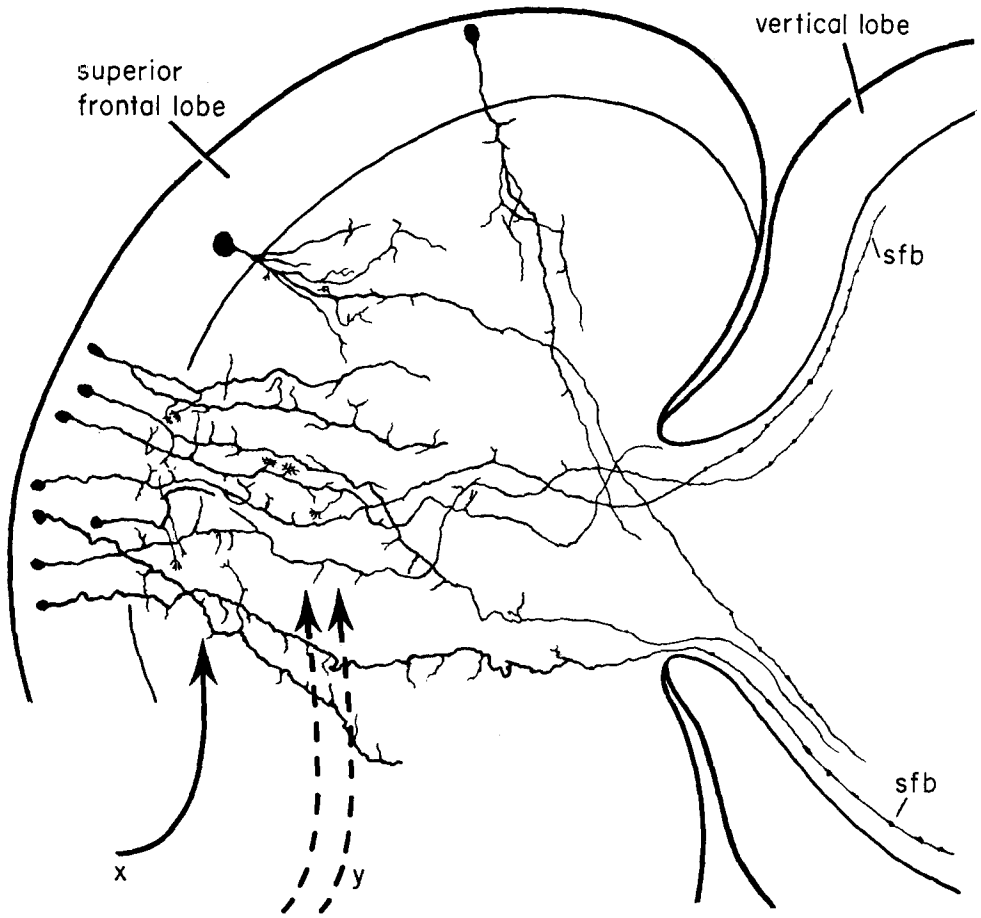

FIGURE 13 Superior frontal lobe. Diagram made from Golgi preparations. The input (chemotactile fibres *x*, and visual fibres *y*) is presumably directed to the numerous dendritic collaterals. The superior frontal cells send their fibres to the vertical lobe and become varicose. These are thought to be the presynaptic bags that contact the amacrine trunks.

the form of a "clear" neuronal trunk or its spinous process, without vesicles but with a few granules or tubules. Hence in *Octopus*, as in vertebrates, the presynaptic aggregations of vesicles and postsynaptic "thickening" make the contact asymmetrical and presumably functionally polarised.

Serial Synapses

A striking feature of the vertical lobe in *Octopus* is the arrangements of the synapses in a serial manner. The presynaptic bags are probably formed from fibres from the superior frontal lobe and these bags contact the trunks of the amacrine cells which can, therefore, be designated postsynaptic. However, the amacrine trunks, of which there are some thirty million in the vertical lobe, are remarkable in that they are also packed with synaptic vesicles, and, if our criteria are valid, the trunks are, in addition, presynaptic to the small non-vesicular postsynaptic profiles (presumably spines) that are invaginated into them. The vesicles nearest the presumed transmission point to the spine are each surrounded by a conspicuous shell of dense material. They are thus labelled and their detailed study may help to decide whether, in fact, synaptic vesicles do move to the synaptic cleft to discharge the transmitter there.

Thus, if we are to rely on our criteria for recognising synapses, there is a vast system of serial

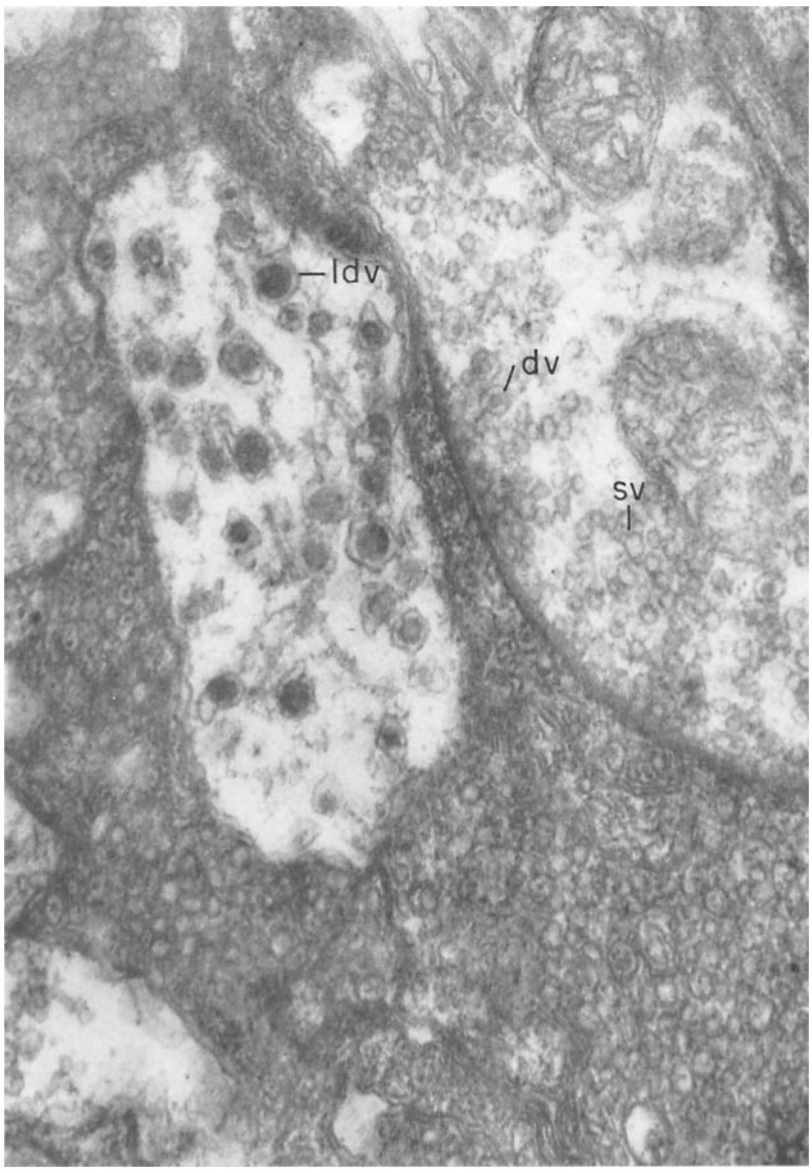

FIGURE 14 Vertical lobe. Profile containing large vesicles with dense interiors (*ldv*). Synaptic vesicles (*sv*) and vesicles with dense granules (*dv*) occur in a nearby presynaptic bag. × 56,000.

synaptic contacts in the vertical lobe. The amacrine trunks are both pre- and postsynaptic and so cannot be designated either dendrite or axon. The contacts parallel morphologically the serial synapses of vertebrates, which are thought to be responsible for presynaptic inhibition (16, 17, 9–11, 5, 6), but, as yet, there is no physiological evidence to support this view in *Octopus* and much more still needs to be learned about the connections in the vertical and superior frontal lobes. The integrated function of these two lobes has been considered in detail elsewhere (23).

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