AXO-SOMATIC AND AXO-DENDRITIC SYNAPSES OF THE CEREBRAL CORTEX: AN ELECTRON MICROSCOPE STUDY

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1. INTRODUCTION

The method of electron microscopy is, of course, of extreme value for the study of the cytology and membrane relations of neurons and neuroglia. However, the study of the nervous system is to an important degree a study of the connexions of neuronal processes. This is at present an impossible task for the electron microscope (EM), not only because of the impracticability of serially sectioning a useful volume of tissue, but because the cell processes of grey matter twist and turn in all directions. This results in oblique sectioning, causing membranes to become invisible (see Williams & Kallman, 1955). Nevertheless, electron microscopy should prove invaluable in this connexion in assessing the accuracy of information obtained by the light microscopist, who can then continue with his silver, Nissl, Golgi, Weigert and other techniques with added confidence.

Various regions of the mammalian central nervous system have been examined with the EM, e.g. the spinal cord (Wyckoff & Young, 1956), the thalamus (Fernández-Morán, 1955) and the ventral cochlear nucleus (de Robertis, 1956) (see also Sjöstrand, 1956). Palade (1954) has studied the cerebellum and medulla and Palay (1956, 1958) has studied the cerebrum, cerebellum, medulla and neurohypophysis. Horstmann & Meves (1958) have studied the brains of fishes and reptiles. All have reported that the pre-synaptic process contains numerous synaptic vesicles and often mitochondria and the contact region between pre- and post-synaptic components shows localized density increases and thickenings. Synapses in the cerebral cortex in particular have received little attention. Palay (1956) mentioned, without illustrating, that cortical synapses are in general similar to those elsewhere in the central nervous system, except that the pre-synaptic processes are smaller and contain fewer mitochondria. Luse (1956) studied the cerebral cortex and other regions, but did not describe synapses in the cortex. Schultz, Maynard & Pease (1957) made a more detailed study of the cerebral cortex and described structures containing 'synaptic vesicles' and thickened contacting-membranes. From their observations (see Discussion) they doubted that these features could be considered specific criteria for the identification of synapses.

From observations given in this report it is suggested that the criteria are still valid. Structural differences between axo-somatic and certain axo-dendritic synapses are described. The latter type show marked densities associated with the thickened region of the post-synaptic membrane, an increased spacing where the synaptic membranes are thickened, and the occurrence of an extracellular band of material between those regions of the synaptic membranes. Finally, the spines of dendrites

are shown to be sites of synaptic contact (Gray, 1959*b*). The term 'synapse' has been used to label morphologically specialized contacts between neuronal processes. These are presumed to be regions of transmission, but the precise site of transmission within these contacts is unknown.

2. METHODS

The visual area of the occipital cortex of adult rats (Lashley, 1944) has been used throughout. No attempt has been made to study specific layers, but layer 1 has been given least attention. Three methods have been used:

(*a*) A rat was anaesthetized with ether and the skull roof and dura removed from the visual cortex. Vertical slices were obtained by using four razor blades held parallel and 0·5 mm. apart in a clasp. The slices were transferred to 1 % osmium tetroxide in Ringer's sloution, buffered at pH 7·4 with veronal acetate and maintained at about 5° C. Fixation was continued for 4 hr. with continuous agitation. After dehydration with ethanol, the slices were 'stained' with 1 % phosphotungstic acid in absolute alcohol for 3 hr., again with continuous agitation. Araldite embedding (Glauert & Glauert, 1958) was used and the sections were examined with a Siemens Elmiskop 1*b* electron microscope.

(*b*) As (*a*), using osmium tetroxide for fixation but omitting immersion in phosphotungstic acid. This showed no structural differences from method (*a*), but the material showed very poor contrast.

(*c*) As (*a*), but 0·6 % $KMnO_4$ (Luft, 1956) was used in place of 1 % osmium tetroxide but again the phosphotungstic acid was omitted. Observations made by this method are described in §5.

In the following sections, method (*a*) has been used, except where otherwise stated.

3. NEURONAL PERIKARYA, DENDRITES AND AXONS

Profiles of neuronal perikarya (Pl. 2, fig. 8; Pl. 3, fig. 11) are recognized by size, shape, the cisternae and granules of the endoplasmic reticulum, the shape and density of the nucleus and other features (see Palay & Palade, 1955; Luse, 1956; Schultz, Maynard & Pease, 1957).

Palay (1956) described tubular structures running longitudinally in dendrites of the central nervous system and regarded them as part of the endoplasmic reticulum. Schultz *et al.* (1957) observed structures which appeared to them as dots in cross-sections of dendrites of the cerebral cortex and named them neural filaments. They made no mention of the tubular nature of these structures. Palay's description is confirmed here (Gray, 1959*a*).

The dendrite tubules are about 200 Å in diameter and are shown in longitudinal section in Pl. 1, fig. 3 and Pl. 3, fig. 10. Often they run parallel, but in places they approach each other and possibly anastomose (Pl. 2, fig. 5). In cross-sections the tubules are seen as regularly spaced ring profiles (Pl. 1, fig. 1, Pl. 5, fig. 19). When the dendrite branches or changes direction in the plane of section, the tubules can be seen cut in all planes (Pl. 5, fig. 17). The group at (*a*) are normal to the plane of section, those at (*b*) are cut obliquely, and those at (*c*) run longitudinally. It can be seen that the rings are most dense; the oblique profiles (*b*) appear as two short, less dense lines and

the longitudinally orientated tubules (*c*) appear the least dense. The effect, of course, depends on differences in the amount of electron-scattering material lying in the axis of the beam (Williams & Kallman, 1955). The result is that when dendrites are sectioned obliquely or longitudinally their tubules may be almost or completely invisible (Pl. 1, fig. 4, *b*). This is especially obvious in the low contrast preparations obtained when phosphotungstic acid was omitted from the technique. Such processes can appear 'clear' or 'empty' and become indistinguishable from certain neuroglial processes.

Myelinated axons are easily recognizable and nodes are observed occasionally (Pl. 6, fig. 24). Tubules can sometimes be observed in the preterminal regions of small diameter unmyelinated axons (Palay, 1956) and it is sometimes impossible to distinguish them from small dendrites (see §7). Systems of tubules have not so far been observed in processes, which could be traced to neuroglial cell-bodies (see Luse, 1956; Schultz *et al.* 1957; Gray, 1959*c*).

4. SYNAPSES OF THE CEREBRAL CORTEX

General. In sections pre-synaptic processes (diameters up to 1·5 μ) contain numerous ring profiles 200–600 Å. in diameter (Pl. 1, figs. 1, 3; Pl. 2, figs. 5, 6)—the so-called synaptic vesicles. Mitochondria are sometimes present (Pl. 2, fig. 5; Pl. 4, figs. 12, 13). Examination of sections of 300 pre-synaptic processes showed that 47 % contained no mitochondrial profiles, 43 % contained one, 8 % contained two, and 1 % contained four, the maximum observed. Serial sections are, of course, necessary to determine the mitochondrial content in any given pre-synaptic process, but such figures can be legitimately used to make a comparison with populations of pre-synaptic processes in electron micrographs of other regions of the central nervous system, for in these processes mitochondria are said to occur frequently (Palay, 1956; Schultz *et al.* 1957).

The post-synaptic component can be perikaryon, dendrite trunk of dendrite spine (see below). Often the post-synaptic component appears in the neuropil as a round, conical or oval-shaped profile. Many of these processes might be oblique sections through spines (§6).

Regions of the apposed membranes of the pre- and post-synaptic processes show thickenings and increased densities as noted by previous workers (Pl. 1, figs. 1–4). The synaptic vesicles occur in the pre-synaptic process and appear in clusters near these thickenings (Palay, 1956; Schultz *et al.* 1957).

These thickened regions show special properties. Pl. 5, fig. 18, shows a part of the damaged cut edge of a slice of cortex. The cell processes have become widely separated in places and their membranes are ruptured. The thickened regions, however, remain firmly attached. Three such regions are shown, the denser and thicker membrane of the post-synaptic process (see below) is seen attached to a pre-synaptic process in each case.

One very obvious feature of cortical synapses is that in certain contacts with dendrite trunks or their spines a high proportion of the length over which the membranes are apposed shows a thickening and increased density. Also the thickening and density is much more pronounced in the post- than the pre-synaptic membrane (see below). Such synapses are designated type 1. In synapses on neuronal perikarya

(axo-somatic) and in certain of those on dendrite trunks, on the other hand, the percentage of the length of the apposed membranes showing increase in density and thickness is small. Also there is no marked difference between the thickening of the pre- and post-synaptic membranes. These are termed type 2.

Type 1 *synapses.* In Pl. 1, figs. 1 and 3, type 1 synapses are shown where the increase in thickness and density (arrow) is present over 90–100 % of the contact region between pre- and post-synaptic processes. In Pl. 1, fig. 1, the post-synaptic component is a dendrite seen in transverse section and in Pl. 1, fig. 3, a dendrite in oblique section.

The contact region is shown in detail in Pl. 1, fig. 2. The synaptic vesicles lie near the pre-synaptic membrane (*a*), which shows irregular densities along its cytoplasmic surface. The post-synaptic membrane (*c*) is situated about 300 Å. away. Material associated with its cytoplasmic surface makes the post-synaptic membrane appear thicker (up to 400 Å.) and often denser than the pre-synaptic membrane (see also Pl. 1, figs. 1, 3 and 4). In the clear zone between the pre- and post-synaptic membranes and strictly confined between their thickened regions, is situated a band of material (*b*, Pl. 1, fig. 2), showing variations in density along its length. This intermediate band is sometimes situated asymmetrically nearer the post- than the pre-synaptic membrane (Pl. 1, figs. 2, 3; Pl. 4, figs. 14, 15); otherwise it appears equidistant between the two, but never nearer the pre-synaptic membrane.

At the thickened regions the pre- and post-synaptic membranes lie parallel and about 300 Å apart, whereas in general in the cerebral cortex the cell processes lie only about 200 Å. apart (Pl. 1, figs. 1*a*, 3*a*, 4*a*; Pl. 4, fig. 13, *a*), although the distance increases where three processes meet or occasionally elsewhere, especially near the damaged edge of the slice of cortex (see also §5).

Where the pre- and post-synaptic membranes are not thickened along their entire length, the difference in spacing is especially clear (Pl. 4, figs. 14, 15). On the right the apposed membranes are unthickened and they lie about 200 Å. apart. Where the thickening commences the membranes move apart (arrows) so that the gap increases to about 300 Å. and contains the intermediate band. Occasionally the apposed synaptic membranes are thickened in two regions, in which case the gap in the region between closes to about 200 Å. and the intermediate band disappears.

As mentioned above, the percentage length of thickening of the apposed synaptic membranes is large. Measurements on 75 type 1 synapses showed that in 88 % the thickened regions occupied 70–100 % of the total length of apposition. The mean of the absolute length of thickening seen in sections was 0·46 μ. The longest thickened region so far observed measured 1·1 μ.

Type 2 *synapses.* Observations of a large number of perikaryal surfaces have shown that axo-somatic contacts are present, but since they appear so consistently different in certain respects from those described above, they have been classified as a second type. As in type 1, the pre-synaptic process sometimes contains mitochondria (Pl. 2, fig. 8). However, the thickened regions (arrows) occur over only a small proportion of the length of apposition between pre-synaptic and perikaryal membranes (see below), and at these points the distance between the membranes often shows no obvious increase; nor does the intermediate band appear clearly defined. Also, unlike type 1 synapses, the thickening associated with the post-synaptic

membrane is not obviously wider than that associated with the pre-synaptic membrane (compare Pl. 2, fig. 8, with Pl. 1, figs. 1–4). One apparent exception has been observed, however, where the membranes showed an obvious increase in distance apart at the thickened regions in an axo-somatic contact (Pl. 2, fig. 9, *a*) and the intermediate band (*b*) could be clearly observed, in this case situated very close to the post-synaptic (perikaryal) membrane. In other respects this contact could be classified as a type 2 synapse.

These features make type 2 synapses inconspicuous, especially at low magnifications. Pl. 3, fig. 11, shows a type 2 synapse (circle) with thickened membranes (*a*) and vesicles in the pre-synaptic process. Another type 2 synapse is shown on the base of the apical dendrite of the same neuron (Pl. 3, fig. 10, *a*) and the pre-synaptic process makes a second type 2 contact (*b*) on a small dendrite (*c*) (seen in cross-section, with tubules scarcely visible). Compare a type 1 synapse (circle, Pl. 3, fig. 10) with these type 2 synapses.

Type 2 synapses also occur on the trunks of the smaller dendrites (Pl. 2, fig. 5). Here a single thickened region is shown but more than one thickened region along the synaptic membranes can sometimes be seen.

As mentioned above, the percentage length of thickening of the apposed membranes in type 2 synapses is small. Measurements on 47 axo-somatic (type 2) synapses showed that the percentage length of thickening in 90 % of the cases did not exceed 40 % (compare type 1). The mean for absolute length in sections was 0·25 μ. The longest length of thickening so far observed measured 0·45 μ.

Clear processes (Pl. 3, figs. 10, 11, *d*) also make contacts with the neuron surface as described by other workers. Some can be identified as neuroglial since they can sometimes be traced to their cell-bodies. Other contacting processes (*e*) can be identified as axon sections, because of the clusters of characteristic vesicles (200–600 Å. in diameter). In these cases the plane of section might not have passed through the thickened regions of the membranes.

Other processes directly related to the neuron surface include myelinated axons, neuroglial cell-bodies, dendrites, cell-bodies of other neurons and possibly the basement membranes of blood vessels. These relationships will be considered in detail elsewhere.

5. THE CEREBRAL CORTEX FIXED WITH POTASSIUM PERMANGANATE

The uses and limitations of $KMnO_4$ have been described by Luft (1956). Its use for study of the cerebral cortex is limited in several respects. The membranes appear clearer than with osmium fixation, but in both types of preparation apparent discontinuities appear in the membranes. This seems due to oblique sectioning (Williams & Kallman, 1955) rather than poor preservation or 'staining', and is especially obvious in sections of grey matter, where the cell processes frequently change direction of orientation. Also if the lack of large extracellular spaces seen in osmium-fixed cortex is considered a true representation, then the spaces (Pl. 2, fig. 6, *a*) in $KMnO_4$-fixed material must be considered artefacts. Also after this method of fixation neural and glial processes can seldom be distinguished, for perikaryal inclusions (in particular the granules of the endoplasmic reticulum) and dendrite tubules are invisible. These tubules have been termed endoplasmic reticulum by

Palay (1956), although they do not appear in $KMnO_4$ preparations, whereas the endoplasmic reticulum of the cell-body does.

Pre-synaptic processes can be identified by their vesicles (Pl. 2, fig. 6). The apposed membranes (*b*) show little if any specialized regions of increased density in contrast to osmium preparations. Nor is a discrete intermediate band apparent in the synaptic cleft, although a vague density is sometimes seen.

The use of permanganate is important in the study of membranes at high magnifications (see Robertson, 1959), especially in the study of the 75 Å. unit membrane structure, which Robertson has shown to be a feature of cell membranes in general. Their presence is confirmed in cell processes of the cerebral cortex (Gray, 1959*b*). The contact regions are shown in Pl. 2, figs. 6, 7. Each membrane (*a*) has the unit configuration of two 20 Å. dense lines with a 35 Å. clear zone between. As in osmium preparations, the synaptic cleft is wider (250–300 Å.) than between the more closely apposed membranes (i.e. where shrinkage is presumed to be least) of non-synaptic regions (e.g. Pl. 2, fig. 6, *c*).

In osmium-fixed peripheral nerves, when the membranes between processes are closed in certain situations, a third dense line is observed between them. This complex has been termed an external compound membrane (Robertson, 1959). However, the over-all dimensions eliminate the possibility that the intermediate band, seen between the thickened synaptic membranes in osmium preparations, is the middle line of an external compound membrane. The intermediate band can, in fact, be identified as extracellular in position.

6. SYNAPTIC CONTACTS ON DENDRITE SPINES

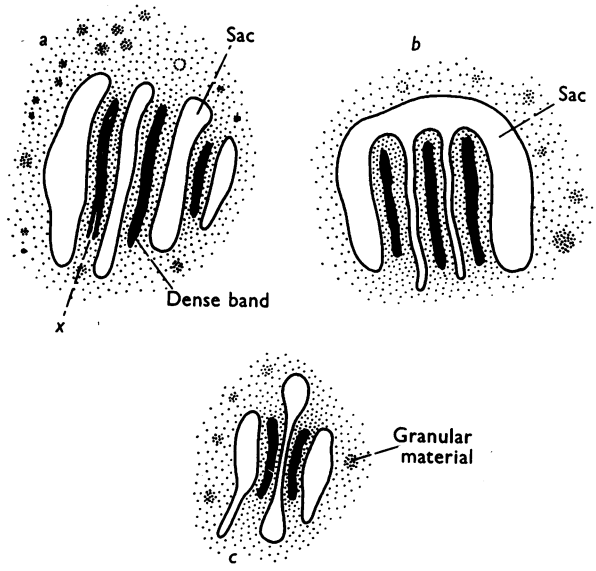
When stained by the Golgi or methylene-blue method for light microscopy, dendrites of the cerebral cortex and elsewhere are seen to have numerous spinous projections, whose function has been much disputed (see, for example, Cajal, 1911, 1954; Sholl, 1956; Fox & Bernard, 1957; Gray, 1959*b*). Here dendrite projections are described, which are seen to be sites of synaptic contact and are thought to correspond with the dendrite spines of light microscopy (Gray, 1959*b*).

Dendrite spines may be long (up to 2 μ) (Pl. 4, fig. 12), or short stumps (Pl. 4, fig. 16). At the distal end of the spine, which can be either flattened or rounded, a pre-synaptic process makes contact. This has always been identified as a type 1 synapse so far. The spine cytoplasm often contains a structure that does not occur in the dendrite trunk or perikaryon. This is termed a spine apparatus.

The *spine apparatus* (Pl. 4, figs. 12, 13 and 16; Text-fig. 1) consists of two, three or more membrane-bound spaces, here referred to as sacs, although they might represent continous channels. The sacs lie 300–500 Å. apart and only occasionally have they been seen to be connected (Text-fig. 1, *b*). Between each sac a dense band, 150–200 Å. wide, occurs, which occasionally appears as a double structure (*x*). This zone containing the band has dense cytoplasm and the zone inside the sacs appears clear in contrast. The spine cytoplasm surrounding the apparatus often contains discrete or clumped granules (diameter $\sim$100 Å.) and occasional ring profiles. Clear evidence that the dendrite tubules enter the spine has not yet been attained.

Often 'isolated' pairs of pre- and post-synaptic processes appear in sections of

the neuropil. In this case, when the post-synaptic process contains the apparatus (Pl. 4, fig. 13), it is presumed to be an oblique section through the distal region of a spine. Often part of the stalk of the spine is also seen, and, of course, in a small proportion of cases continuity with a dendrite is observed.



Text-fig. 1. Diagrams to illustrate the various arrangements seen in the spine apparatus of dendrites of the cerebral cortex. Sacs alternate with dense bands. The bands sometimes appear as double structures (*x*). The sacs may be continuous (*b*) or very narrow in certain regions (*c*). Granular material is also present in the spine cytoplasm.

7. A NOTE ON AXONS AND THEIR PRE-TERMINAL REGIONS

Certain perikaryal processes, thought to be cones of origin (to be described in detail elsewhere) are occasionally encountered in sections. They contain bundles of dense tubules, which run a remarkably straight course into the axon. The axon membrane appears straight and not indented by various contacting processes, in contrast to the dendrites. So far axons have only been followed a few microns into the initial segment, and the beginning of a myelin sheath has not been encountered.

Myelinated axons are, of course, common in the grey matter forming the cerebral cortex. Very occasionally a section shows the region of terminal myelin and the unmyelinated axon can be seen continuing to form a pre-synaptic process. This contains synaptic vesicles, which are aggregated in the region of the pre-synaptic membrane.

In Fig. 24 a node of Ranvier of the cerebral cortex is shown to illustrate the appearance of myelin, where it ends (at *x* and *y*). Pl. 6, fig. 20 shows an axon losing its myelin just as at the node. The unmyelinated axon expands and makes a synaptic contact at *x*. The post-synaptic process is identified as a section of a dendrite spine since it contains a spine apparatus (Pl. 6, fig. 21—continuation of the same region, serial section). The distance from terminal myelin to synaptic contact

is in this case 1·5 μ. Serial sections showed that the same process made another synaptic contact on what is presumed to be another dendrite spine (Pl. 6, figs. 20, 22, *y*) in this case only 0·5 μ from the myelin termination. In Pl. 6, fig. 23, a bouton is shown making a synaptic contact (type 2) on a dendrite immediately after the termination of the myelin.

On the other hand, unmyelinated axons can be followed for 4 or 5 μ before synapsing. An unmyelinated axon is shown in Pl. 5, fig. 19, running for 3 μ, containing a few tubules, and forming a bouton on a dendrite (seen in cross-section).

Such unmyelinated axons do not exceed 0·5 μ in diameter in longitudinal sections. Serial longitudinal or transverse sections are, of course, needed to determine their true diameters, but since longitudinal sections should occasionally pass through the widest regions, the diameters of unmyelinated axons are not likely to exceed this figure. Boutons usually appear rounded in profile and seldom exceed 1·5 μ in diameter in agreement with the figures given by Armstrong & Young (1957) for light-microscope preparations.

DISCUSSION

The initial problems are:

(1) What are synaptic contacts?

(2) Are they all alike in thin sections: (*a*) in different positions; (*b*) regardless of function, either at the moment of fixation or in the more remote past?

To answer these questions criteria for the identification of neuronal cell-bodies, the axons and their boutons, dendrites, and neuroglia seen in thin sections are required. Mainly satisfactory ones are available for the cell-bodies and axons. Some dendrites are readily identified by their tubules and other features and certain spinous processes can be seen attached to undoubted dendrites. Other fibres are seen that are probably dendrites but cannot rigidly be proved to be such. Boutons are identified by their vesicles. Criteria exist for some but not all glial processes (see Luse, 1956; Farquhar & Hartmann 1957; Schultz *et al.* 1957; Gray, 1959*c*).

Using these criteria we can be certain that the thickened membranes occur at synapses, because a process (containing vesicles) can be seen connected with a myelin-bearing axon and making contact with a tube-carrying dendrite or its spine (Pl. 6, figs. 20–23). When two apposed processes show the thickenings the clusters of vesicles are always present in one of the processes (the pre-synaptic one).

One cannot be certain, of course, that such a situation always represents a synapse, although this is considered probable. Doubts would arise if either the presumed pre- or post-synaptic process could be shown to be neuroglial. In the cortex undoubted neuroglial processes (seen in the section to be in continuity with their cell-bodies) have never been seen to have the thickened membrane-vesicle feature and, when the evidence of other workers (Introduction) is taken into account, there is little doubt that this feature is specific to the pre-synaptic processes of axons (see below).

Nor is there any evidence to indicate that the presumed post-synaptic component can be glial and not neuronal. Frequently the post-synaptic process appears clear or faintly granular and unless its origin is determined by serial sections, it cannot be decided whether it is a section of a dendrite trunk, spine or neuroglial process. Dendrites can sometimes appear clear if cut obliquely or longitudinally for in these situations the dendrite tubules lose contrast (§3) and may become invisible. This

effect is especially evident when phosphotungstic acid is omitted from the technique. A section through a dendrite spine, which misses the spine apparatus, is another situation where the post-synaptic process can appear clear.

Schultz *et al.*, (1957) have also observed 'synaptic' contacts on clear processes. However, these authors assumed that clear processes were specifically neuroglial and doubted that the features of the contacts (i.e. 'pre-synaptic' vesicles associated with thickened membranes) were valid criteria for identifying synapses. Since dendrites or their spines can appear clear in section, their doubts are not justified.

The author has found no evidence to show that neuroglia have synaptic contacts. Neuroglial cell-bodies and processes that can clearly be seen to originate from them, also the large clear end-feet on blood vessels, which are for several reasons likely to be glial processes (Luse, 1956; Schultz *et al.* 1957; Gray, 1959*c*) have been carefully examined and have not yet been found to occur as a post-synaptic component in a synapse.

Conversely, one cannot be certain that all synapses carry thickened membranes with associated pre-synaptic vesicles. Processes with vesicles but no thickened membrane regions are commonly observed. It could be argued (*a*) that the thickening is not in the plane of section, but would be observed in serial sections of the pre-synaptic process, or (*b*) that the thickenings are not permanent structures, but are produced during synaptic activity and then disappear. A study of the effects of degeneration of the pre-synaptic process would show whether or not the post-synaptic thickening is permanent. (*c*) They may be temporary structures, but when present, not related to synaptic activity.

In addition, there is the possibility that inter-neuronal contacts which show no specializations of their apposed membranes, may be sites of transfer of information. Occasionally a pair of boutons or dendrites, or a dendrite and a nerve cell-body, have membranes in apposition (200 Å. apart). It seems probable that there would be electrical interaction between these processes and if this can be shown to be more than chance interference, then such contacts can justifiably be termed synaptic.

Palay (1956) and others have suggested that the thickened regions might be the actual transmission points and/or adhesion regions analogous to the terminal bars of epidermal cells. Direct evidence from material damaged in preparation is given in this paper to support the latter view. If the situation in the central nervous system requires that synaptic contacts, once made, should remain fixed, then it is logical to suppose that adhesive and transmitting regions coincide.

The type 1 synapses described here show several morphological similarities to neuro-muscular junctions (Reger, 1957; Robertson, 1956, 1959). Pre-synaptic vesicles, of course, occur in both situations (Palay, 1956). In the synaptic gap a dense band is situated, which is perhaps homologous with the intermediate substance situated between the membranes of the neuro-muscular junction. There also, just as in the synapse, the post-synaptic membrane shows a greater thickening and density than the presynaptic membrane. Finally in both cases the apposed membranes are more widely separated than in non-synaptic regions. In both cases the intermediate layer may be cementing in function or perhaps also more directly concerned with the transmission mechanism. It is relevant that a band of extracellular material occurs between the apposed thickened membranes of the contact

zones of epidermal cells; here also the membranes are more widely separated (see Odland, 1958). However, in epidermal contacts, the two thickenings are of equal dimensions, the intermediate line is situated symmetrically in the centre of the cleft, tonofibrils are present and vesicles absent.

The occurrence of material producing a greater thickening in the post- than in the pre-synaptic membrane is not accounted for at present. This effect is still seen, although less clearly, when phosphotungstic acid is omitted from the technique (Methods (*b*)). The marked post-synaptic thickening described here in type 1 synapses is not a feature of synaptic contacts that have been described in other regions of the mammalian central nervous system (see Palay, 1956, 1958). The effect might be attributed to the use of phosphotungstic acid. However, in this case, one would expect the effect to be observed in all the post-synaptic thickenings, whereas in fact the post-synaptic thickening of axo-somatic and certain axo-dendritic synapses differs little from the pre-synaptic one. This is one of the reasons for considering axo-somatic contacts in a separate category (type 2). In fact it seems that the type 2 synapses correspond with those described by Palay (1956, 1958) in other regions of the central nervous system. They show, in common with type 2 and in addition to the feature just described, the occurrence of thickenings only over short distances of the apposed membranes, and only vague and irregular densities in the synaptic cleft (see Palay, 1956) and no clearly defined intermediate band. Perhaps type 1 synapses are a special feature of the cerebrum. However, other regions of the central nervous system must be examined by the phosphotungstic acid method before this conclusion can be reached.

At present there is no evidence to suggest that type 1 and type 2 synapses are functionally different. Even the morphological (i.e. three-dimensional) reality of these structures seen in thin sections must be carefully considered. For example it can be argued that the thickenings of the contact regions are oval or circular apposed plates (see Sjöstrand, 1956) and that around the margins the thickening of the post-synaptic plate decreases, the intermediate band becomes less defined and the membranes begin to close. Thus a section through the margin would produce a type 2 synapse and one through the centre a type 1 synapse. However, if this were so, then one would expect at least a proportion of axo-somatic synapses to appear in the type 1 category and a proportion of contacts on dendrite spines to appear in the type 2 category, but this has not been observed. Clearly serial sections are needed to decide this issue.

One wonders why so many of the synaptic contracts should be poised on spines away from the dendrite trunk (Gray, 1959*b*). Golgi preparations of the visual cortex show dendrites commonly covered with spinous processes. It is important to remember that in Golgi preparations only about one in seventy neurons with their dendrite ramifications is visible (Sholl, unpublished). The cortex is in fact packed with dendrites and their spines. At present it cannot be stated that all spines are sites of synaptic contact or that they all contain a spine apparatus. These features represent a new and intriguing situation for physiological investigation.

SUMMARY

1. The method found most suitable for the study of the cerebral cortex included fixation with osmium tetroxide, 'staining' with phosphotungstic acid and embedding in araldite.

2. Frequently, apposed membranes of two processes show thickened regions and clusters of vesicles near the thickened membrane of one of the processes. These are thought to be synaptic contacts, because:

(*a*) The vesicle-bearing (pre-synaptic) process can be seen to originate from a myelinated axon.

(*b*) The other (post-synaptic) process can be identified as a nerve cell-body, or a dendrite trunk or a dendrite spine.

(*c*) No such contacts have been observed on neuroglial cell-bodies or their processes.

(*d*) The thickened regions show special adhesive properties.

(*e*) Of the similarity with neuro-muscular junctions and correspondence with descriptions by other workers of synaptic contacts in other regions of the vertebrate nervous system.

3. Two categories of synaptic contacts are distinguished: (*a*) type 1 synapses, which occur on dendrite trunks and dendrite spines; (*b*) type 2 synapses, which occur on dendrite trunks and neuron cell-bodies.

4. In type 1 synapses a large percentage of the length of the apposed membranes shows increased thickness and density. The post-synaptic thickening is more pronounced than the pre-synaptic thickening. These thickened regions of the membranes lie farther apart than where the apposed membranes are unthickened and in the extracellular region between the thickened membranes an intermediate band of material can be seen.

5. In type 2 synapses the percentage length of thickening is small, the pre- and post-synaptic thickenings are of similar dimensions, the intermediate band is not clearly visible and the membrane spacings at these regions differ little from the non-thickened regions.

6. Dendrite spines are sites of synaptic contact (type 1). The spine cytoplasm contains a structure termed a spine apparatus which consists of a group of 'sacs' separated by bands of dense material.

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KEY TO PLATES

ax	axon (myelinated)	*post*	post-synaptic process
ci	cisternae of neuron endoplasmic reticulum	*pre*	pre-synaptic process
cyt	neuron cell-body cytoplasm	*s*	spine apparatus
den	dendrite	*sp*	dendrite spine
g	granules of the endoplasmic reticulum	*sv*	synaptic vesicles
m	mitochondria	*t*	dendrite tubules
my	myelin sheath	*tu*	axonal tubules
nuc	nucleus of neuron	*unmy*	unmyelinated axon

Other letters: see text and individual captions.

EXPLANATION OF PLATES

Visual cortex of adult rats. Figures 6 and 7 are of $KMnO_4$-fixed material. All others, OsO_4-fixed, phosphotungstic acid stained.

Plate 1

Fig. 1. Dendrite seen in transverse section containing tubules. It has a type 1 synapse. The pre-synaptic process contains vesicles. Arrow indicates thickened membranes at contact region. (*a*) Non-thickened regions of membranes (see text).

Fig. 2. Details of the thickened apposed membranes (*a* and *c*) of pre- and post-synaptic processes. A band of extracellular material (*b*) occurs in the synaptic cleft. The post-synaptic process is probably a dendrite spine.

Fig. 3. A type 1 synapse on a dendrite seen in longitudinal section. See fig. 1 for lettering.

Fig. 4. A group of synaptic contacts on a small dendrite. They are all type 1. Note how the vesicles of the pre-synaptic process are clustered near the thickened regions. The dendrite also has a spine synapse (bottom right). (*b*) Region of dendrite where tubules are not seen. (*a*) See text.

Plate 2

Fig. 5. A type 2 synapse on a small dendrite. Note apparent anastomoses of the dendrite tubules.

Fig. 6. A synaptic contact in material fixed with $KMnO_4$. The post-synaptic process is probably a dendrite spine. Part of the contact region is enlarged in fig. 7. (*a*) Extracellular spaces seen when this fixative is used. (*b*, *c*) See text.

Fig. 7. Enlarged portion of fig. 6. The triple structure of the pre- and post-synaptic membranes (*a*) can be seen ($KMnO_4$ fixation).

Fig. 8. Type 2 synapses on a neuron cell-body that contains the characteristic granules and cisternae of the endoplasmic reticulum. Arrows indicate thickenings of the synaptic membranes.

Fig. 9. Contact region of a synapse on a neuron perikaryon. Here the intermediate band (*b*) can be seen in the synaptic cleft: this is uncommon in an axo-somatic contact. (*a*) Region where distance apart of membranes increases.

Plate 3

Fig. 10. Base of an apical dendrite of a pyramidal neuron. Type 2 synapse at (*a*). Type 1 synapse (in circle) in the neuropil. (*d*) Presumed neuroglial process. (*e*) Axon terminals. (*b*, *c*) See text.

Fig. 11. Type 2 synapse (in circle) on neuron cell body. (*a*) Thickened region of synaptic membranes. (*d*) Presumed neuroglial process.

Plate 4

Fig. 12. Dendrite with spine. The spine contains a spine apparatus and has a type 1 synapse at its apex.

Fig. 13. Type 1 synapse. The isolated section of the post-synaptic process is identified as a dendrite spine profile since it contains a spine apparatus. (*a*) See text.

Figs. 14, 15. Type 1 synapses. The membranes of the apposed synaptic processes become wider (arrow) at the thickened regions and the intermediate band is seen in the cleft between the thickened regions.

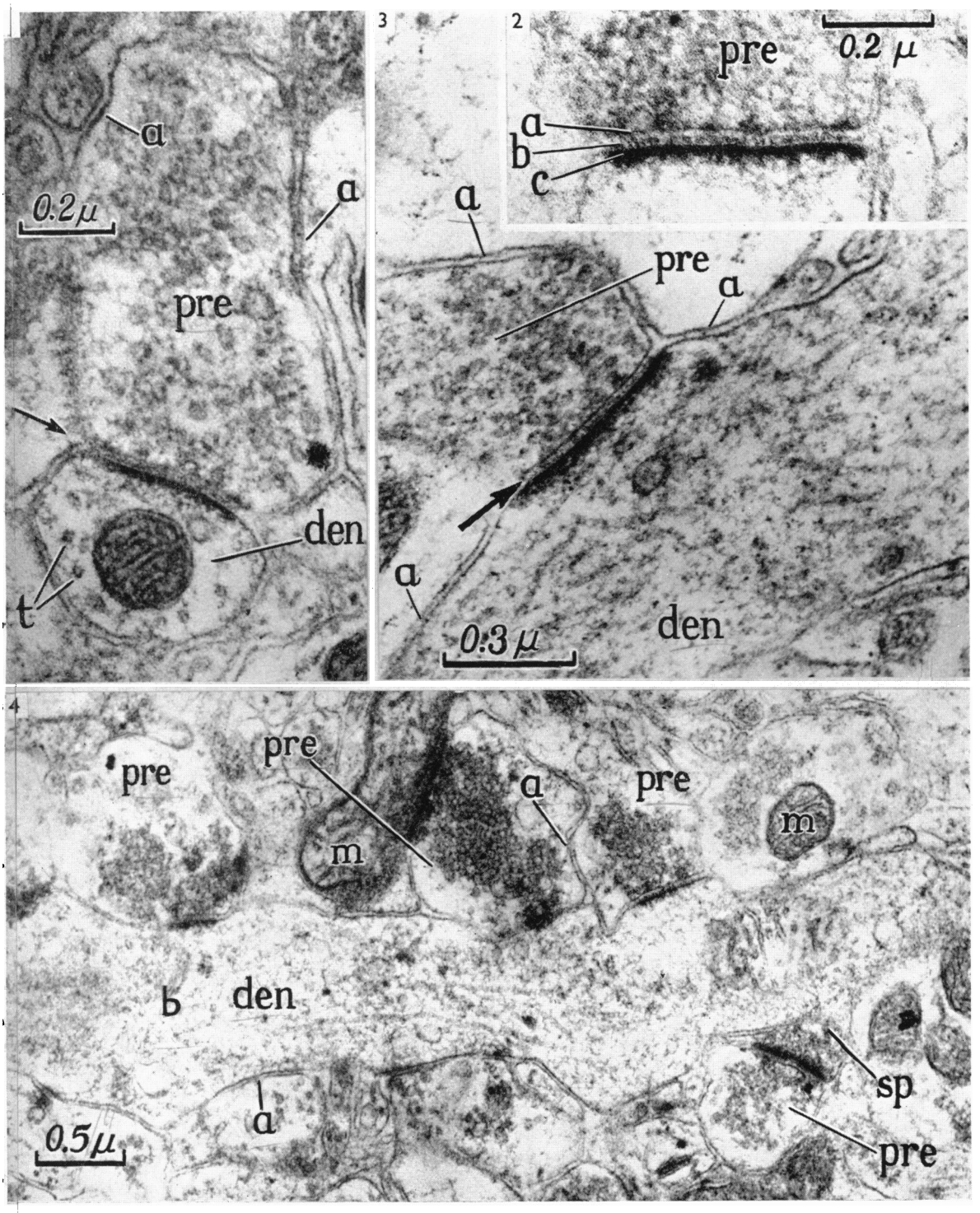
Fig. 16. Dendrite with two short spines each with a spine apparatus and a type 1 synaptic contact.

Plate 5

Fig. 17. Large dendrite with tubules orientated (*a*) normally, (*b*) obliquely, and (*c*) longitudinally to the plane of section. Different directions of orientation are related to branching or change of direction of the dendrite.

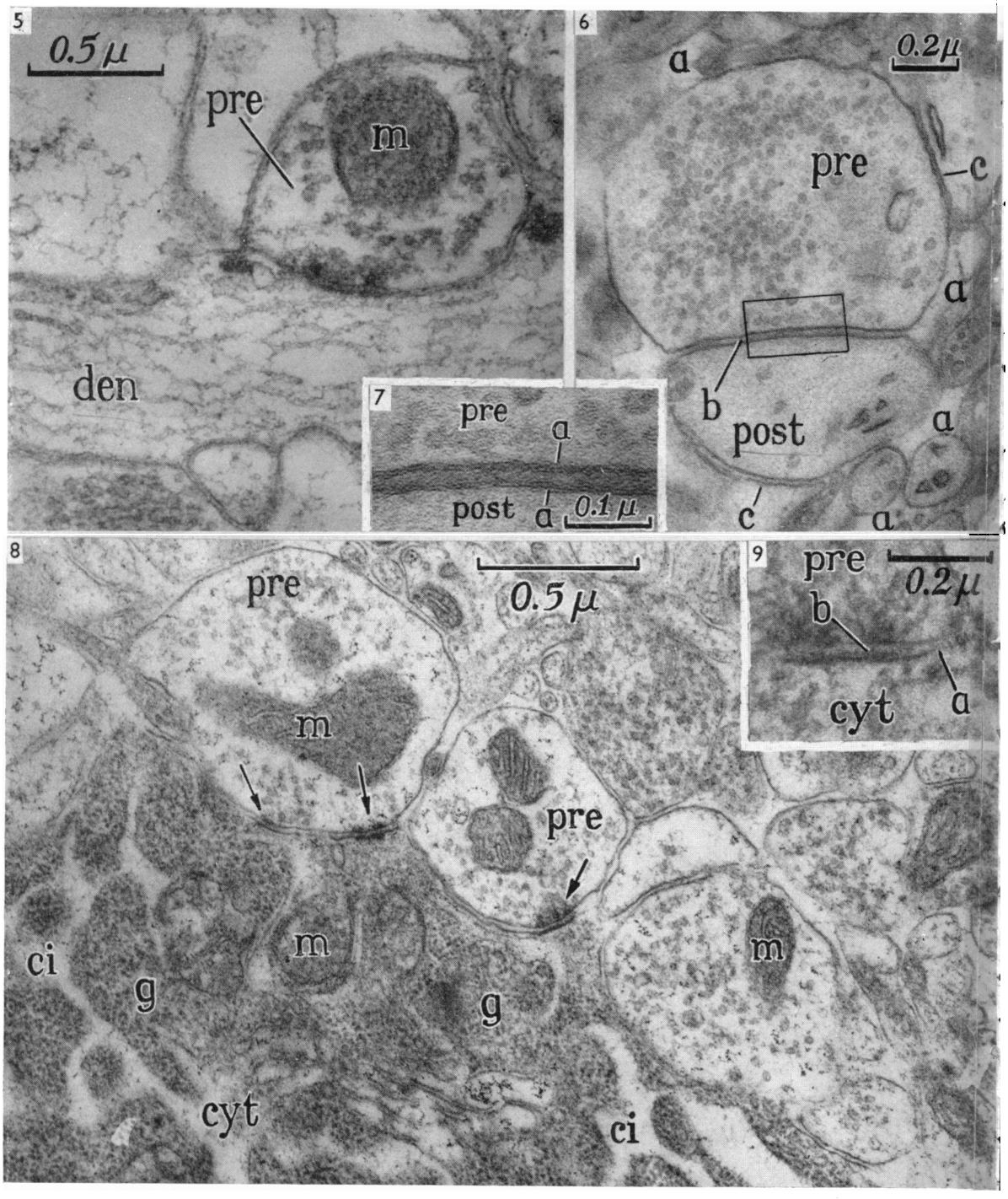
Fig. 18. Ruptured processes appear at the margins of cortical slices, where damage is caused during preparation. The thickened regions of the synaptic membranes remain firmly attached.

Fig. 19. Unmyelinated axon containing a few tubules in its pre-terminal region. It forms a bouton on a small dendrite containing the characteristic tubules (seen in cross-section).

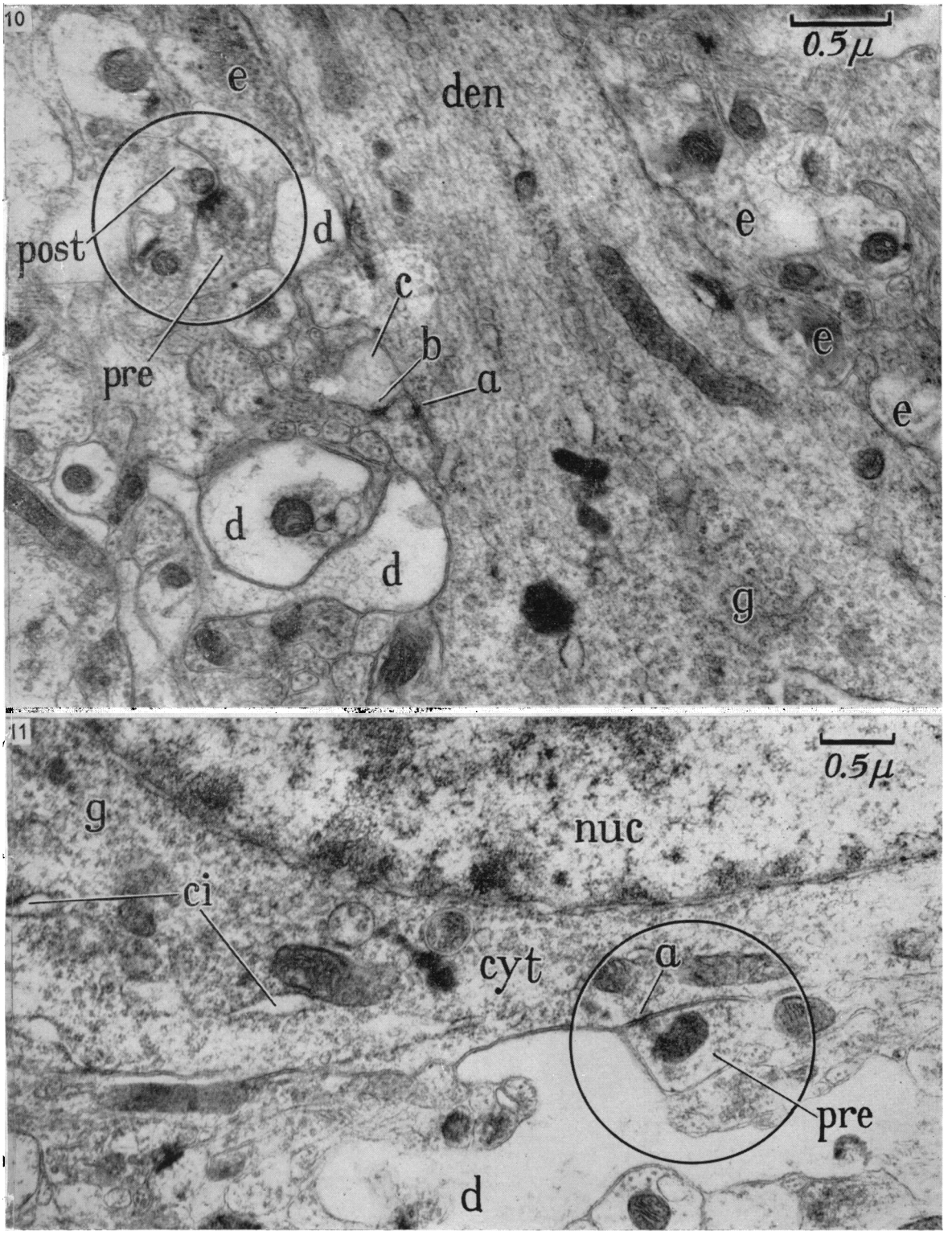


GRAY—Axo-somatic and axo-dendritic synapses of cerebral cortex

(*Facing p.* 432)



GRAY—Axo-somatic and axo-dendritic synapses of cerebral cortex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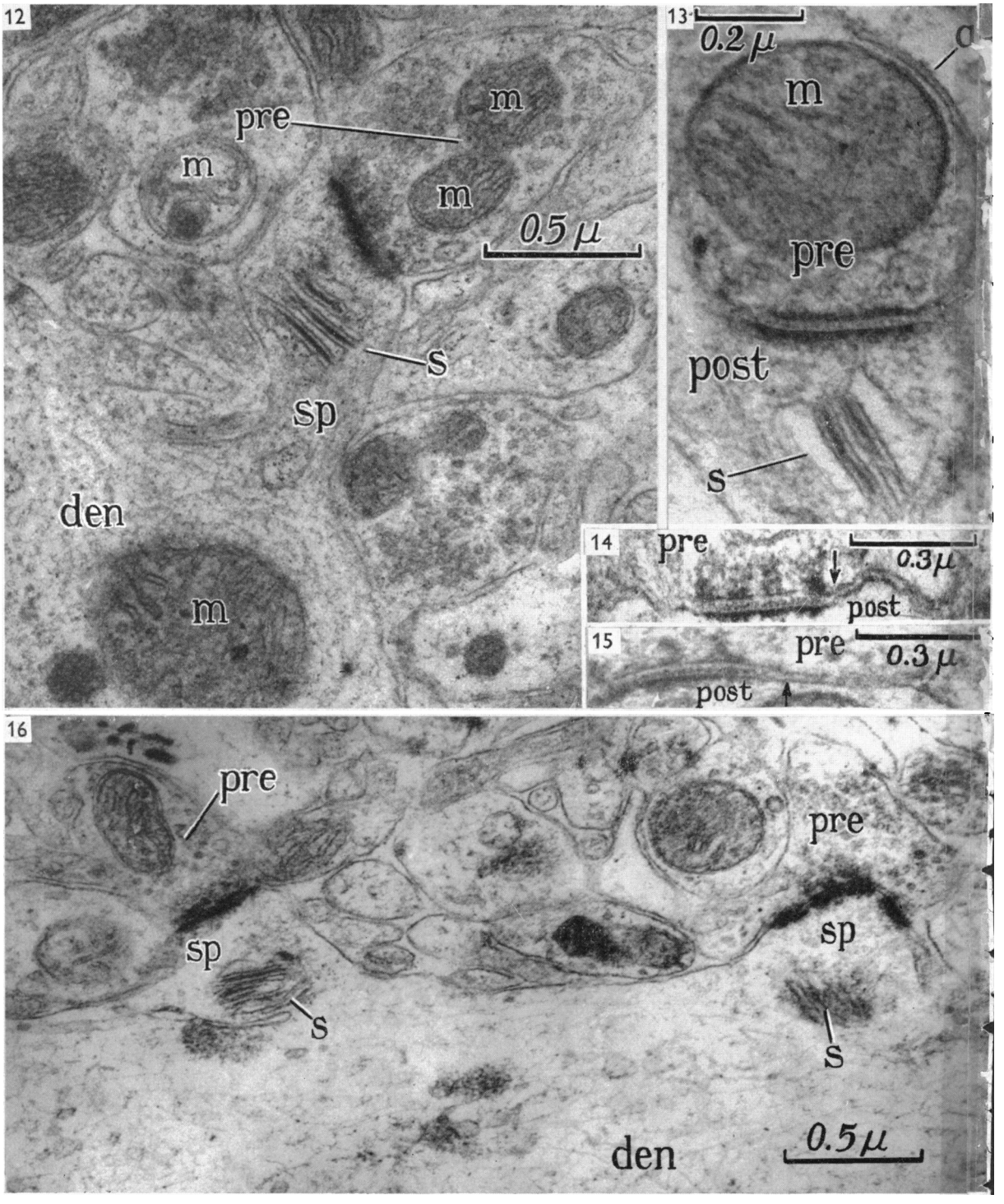
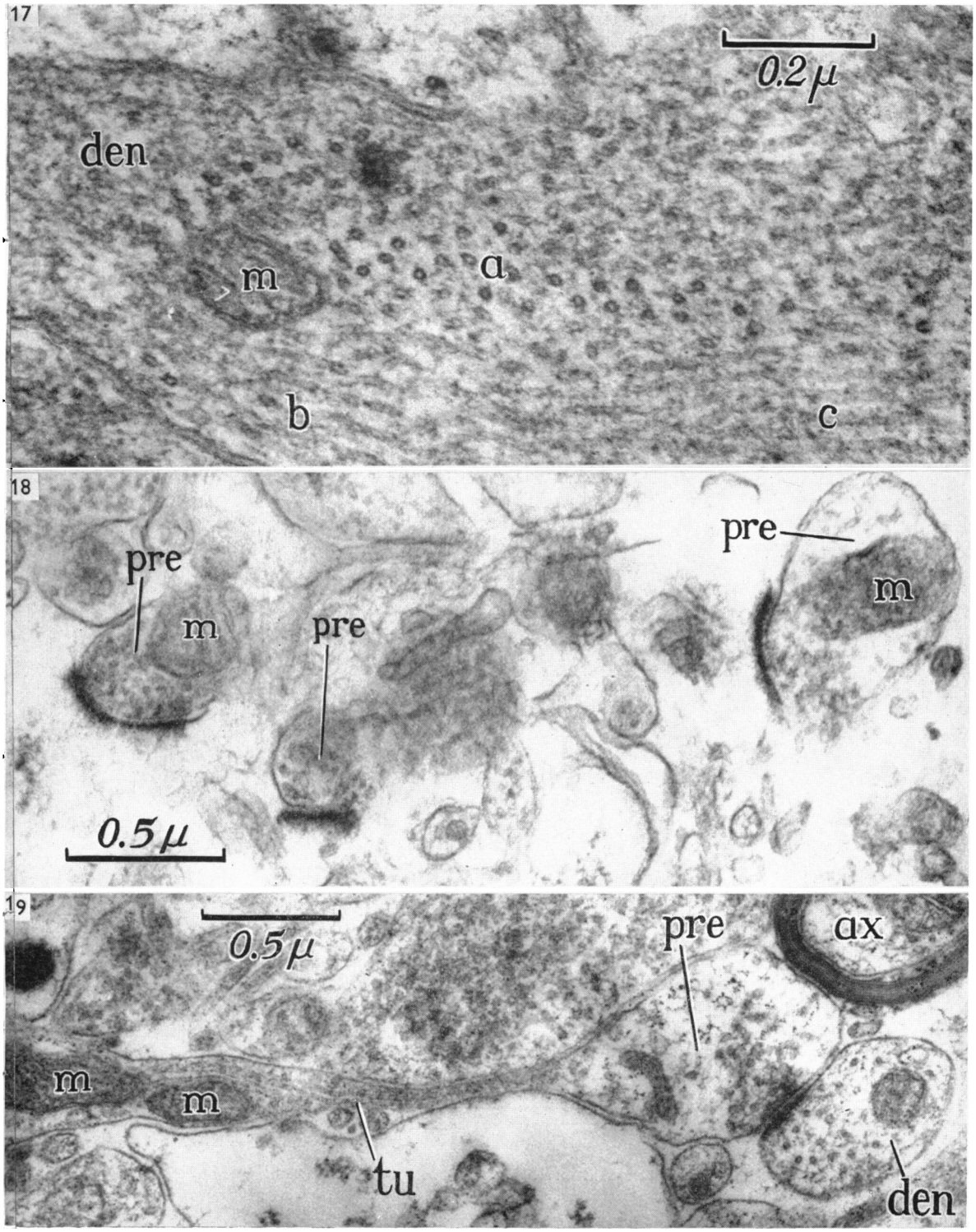
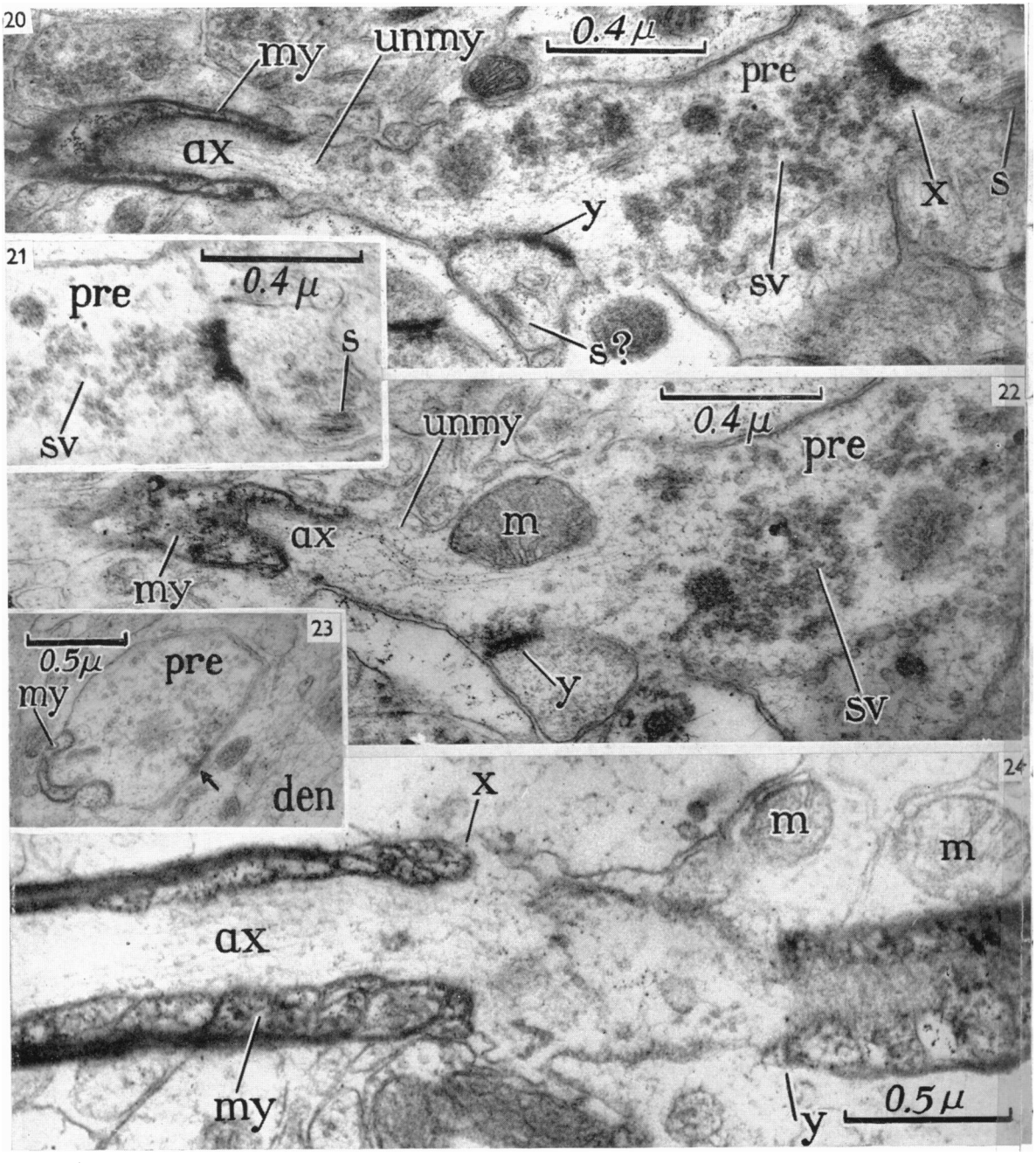


GRAY—Axo-somatic and axo-dendritic synapses of cerebral cortex



GRAY—Axo-somatic and axo-dendritic synapses of cerebral cortex



GRAY—Axo-somatic and axo-dendritic synapses of cerebral cortex

PLATE 6

Fig. 20. A pre-synaptic process seen originating from a myelinated axon. The process contains synaptic vesicles and makes synaptic contact at x with a dendrite spine—see figs. 21 and 22.

Fig. 21. Serial section of fig. 20. The post-synaptic process (of fig. 20) is shown to be a dendrite spine, since it contains a spine apparatus (s).

Fig. 22. Serial section of fig. 20. The pre-synaptic process makes a second synapse at y. The second post-synaptic process is also thought to be a dendrite spine, because of its shape and because it contains a vague structure (s?, fig. 20), which, however, cannot clearly be identified as a spine apparatus.

Fig. 23. A type 2 synapse on a dendrite trunk. The pre-synaptic process is seen emerging from a terminating myelin sheath. Arrow shows thickened contact region.

Fig. 24. A node of Ranvier of the cerebral cortex, included so that the terminating myelin configuration (x, y) can be compared with figs. 20, 22 and 23.