THE SMALL PYRAMIDAL NEURON OF THE RAT CEREBRAL CORTEX

The Axon Hillock and Initial Segment

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ABSTRACT

The axon of the pyramidal neuron in the cerebral cortex arises either directly from the perikaryon or as a branch from a basal dendrite. When it arises from the perikaryon, an axon hillock is present. The hillock is a region in which there is a transition between the cytological features of the perikaryon and those of the initial segment of the axon. Thus, in the hillock there is a diminution in the number of ribosomes and a beginning of the fasciculation of microtubules that characterize the initial segment. Not all of the microtubules entering the hillock from the perikaryon continue into the initial segment. Distally, the axon hillock ends where the dense undercoating of the plasma membrane of the initial segment commences. Dense material also appears in the extracellular space surrounding the initial segment. The initial segment of the pyramidal cell axon contains a cisternal organelle consisting of stacks of flattened cisternae alternating with plates of dense granular material. These cisternal organelles resemble the spine apparatuses that occur in the dendritic spines of this same neuron. Axo-axonal synapses are formed between the initial segment and surrounding axon terminals. The axon terminals contain clear synaptic vesicles and, at the synaptic junctions, both synaptic complexes and *puncta adhaerentia* are present.

The axon originates from the neuronal perikaryon at the *axon hillock.* In light microscope preparations stained with basic dyes, the axon hillock is characterized by an absence of basophilic substance from its cytoplasm, and this is the only cytological feature that distinguishes it from the remainder of the perikaryon. In the same type of preparation, the axon emerging from the hillock is also devoid of Nissl substance, so that generally it is apparent only as a faintly outlined and unstained strand with smooth contours. These features distinguish the axon from the dendrites, which, in addition to containing Nissl substance, have irregular contours.

When an emergent axon acquires a myelin sheath, the sheath does not commence immediately. Instead there is a length of axon that remains bare. This is the *initial segment of the axon,* and it is a region of the neuron that has special properties. It is here that the action potential probably originates, and the membrane of the initial segment is considered to have a lower threshold to excitation than the membrane of dendrites (Eccles, 1964).

Although numerous electron microscope studies of central nervous tissue have been carried out in the past few years, there has been some difficulty in identifying both the axon hillock and the initial segment of the axon. Consequently, only a few descriptions of this portion of the neuron have been

given. Most of the available descriptions are fragmentary, and others are open to question with respect to the positive identification of the components being described. Recently, criteria for the identification of the initial segment of the axon have, however, been given by Palay, Sotelo, Peters, and Orkand (1968), in an account that refers to the initial segments of axons from a number of different neurons. The axon hillock has been even more neglected, and no complete descriptions of this portion of the neuron appear to be available in the literature.

The purpose of the present paper is to describe the morphology of the axon hillock and initial segment of the axon of one specific type of neuron, namely, the small pyramidal neurons of layers II and III of the rat cortex. In addition to further information about these structures, an account will be given of the axo-axonal synapses that occur on the initial segment of the axon.

MATERIALS AND METHODS

Material was taken from the parietal cortex of the rat after perfusion of young animals through the vascular tree. In the earlier studies, the brain was fixed with a 1% solution of osmium tetroxide in acetate-veronal buffer (pH 7.3–7.5) containing 5.4 mg of calcium chloride per ml (Palay, McGee-Russell, Gordon, and Grillo, 1962). More recent specimens were fixed by a perfusion with two aldehyde mixtures, and small pieces of tissue were postfixed with osmium tetroxide after they had been removed from the brain. The solutions of aldehydes were those given by Reese and Karnovsky (1967). The first aldehyde mixture, modified from Karnovsky's formula (1965), consisted of 1% paraformaldehyde and 1.25% glutaraldehyde in 0.067 M cacodylate buffer to which calcium chloride was added. After an initial fixation with this solution, the perfusion was continued with a stronger aldehyde solution containing 4% paraformaldehyde and 5% glutaraldehyde in the same buffer. The brain was left in the skull overnight, and the next day pieces of cortex were removed, washed in buffer, and postfixed in 1% osmium tetroxide in 0.1 M cacodylate buffer.

Blocks were embedded in Araldite, and thin sections of the parietal cortex were stained with uranyl acetate and lead citrate (Venable and Coggeshall, 1965) before examination in either an RCA 3F or AEI 6B electron microscope.

The light microscope study of the parietal cortex was performed by using the Golgi method described by Ramón-Moliner (1961).

DESCRIPTION

As shown by Cajal (1911) and other neuroanatomists, the axon of the pyramidal neuron most commonly takes origin from the center of the base of the conical perikaryon. We have encountered this situation in both Golgi (Fig. 1 *A*) and electron microscope (Figs. 2 and 3) preparations of layers II and III of the rat cerebral cortex. In some neurons, however, the axon arises more laterally. Then both the axon and a basal dendrite may emerge from the same site on the cell surface (Figs. 1 *B* and 4), or the axon may arise as a branch of a large basal dendrite, emerging a short distance away from the perikaryon (Figs. 1 *C*, 1 *D*, 5 and 6). In Golgi preparations, the axons are readily identified as thin processes of relatively constant diameter that have smooth contours. In contrast, dendrites have irregular contours and, although their primary stems may have few or even no thorns arising from their surfaces, these short lateral projections become abundant as soon as a primary dendrite divides into its secondary branches.

In most Golgi preparations of adult tissue, the axons of pyramidal neurons extend for only a short distance before the staining stops. This short stained segment of the axon, between the site of origin and the place at which the axon is no longer visible, is the initial segment. The failure of the remainder of the axon to stain in Golgi preparations is usually attributed to an inability of the chemicals to penetrate the myelin sheath.

As described by Palay, Sotelo, Peters, and Orkand (1968), in electron micrographs the initial segment of the axon is characterized by three special features. These are (*1*) a layer of dense granular material that lies beneath the plasma membrane to form an undercoating, (*2*) the presence of fascicles of microtubules within the cytoplasm, and (*3*) scattered clusters of ribosomes.

In the simplest example, when the initial segment of the axon arises directly from the perikaryon of the pyramidal neuron (Figs. 2 and 3) so that a well defined axon hillock (*ah*) is present, the 100-A-thick undercoating (*d*) of the axolemma begins (arrows) where the hillock narrows to assume the dimensions of the emerging axon. The beginning of the undercoating, which is separated from the axolemma by a clear interval of between 50 and 100 A (Figs. 3, 8, and 9), is considered to define the proximal boundary of the initial segment (*is*) of the axon. In those examples in which

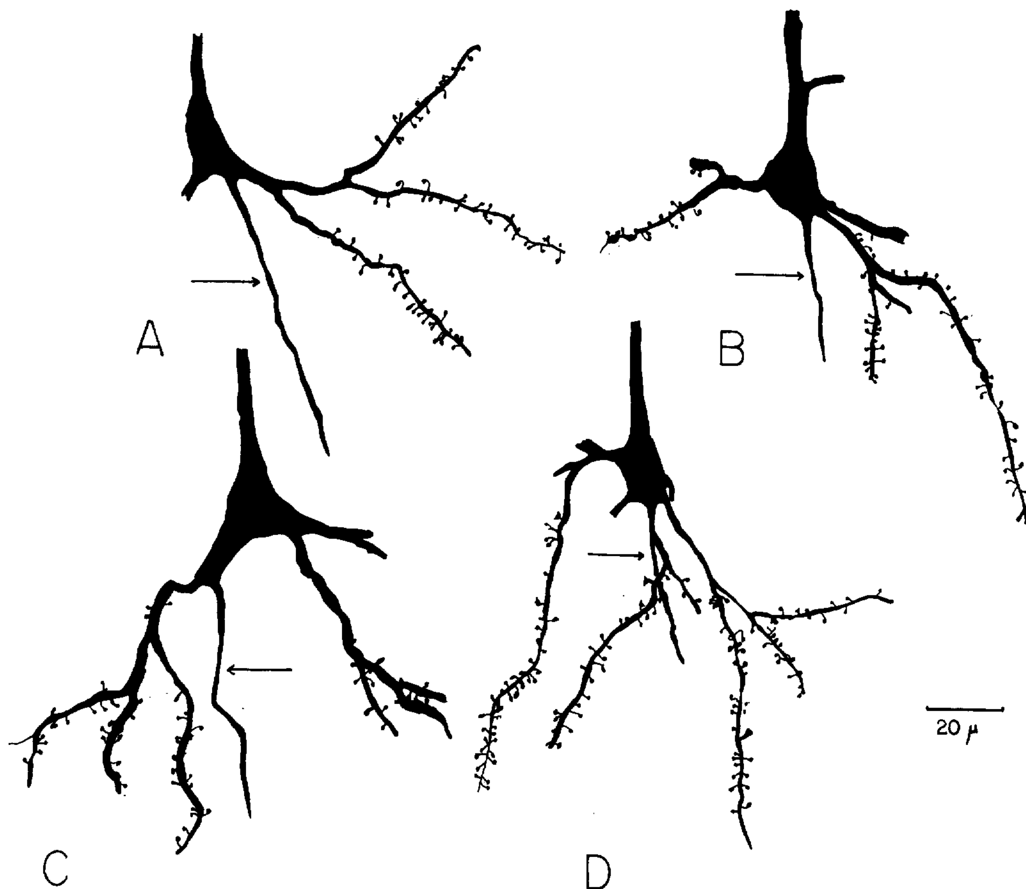
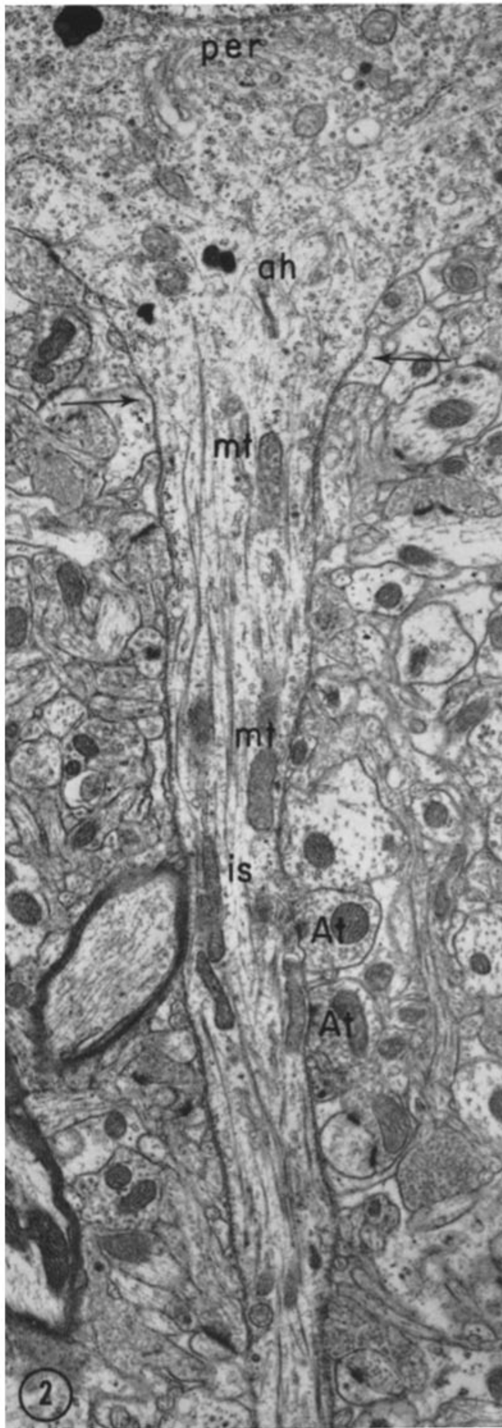


FIGURE 1 Drawings from Golgi preparations of the rat parietal cortex, to show the sites of origin of axons (arrows) of different pyramidal neurons. For further explanation, see text.

the axon initial segment takes origin from a basal dendrite (Figs. 4–6), the undercoating begins (arrows) almost exactly at the site at which the basal dendrite bifurcates into the initial segment of the axon and a secondary dendrite.

The undercoating persists beneath the plasma membrane of the initial segment as far as the site where the axon acquires its myelin sheath. There are, however, certain sites at which the undercoating appears to be deficient. These are where the plasma membrane takes part in the formation of a synaptic junction (Figs. 8 and 9) and where flattened cisternae lie in close proximity to the membrane (Figs. 9 and 11).

Granular material (Figs. 8 and 9; *x*) may also be present in the extracellular space surrounding the initial segment of the axon. Such material does not seem to occur in the extracellular space in other parts of the neuropil, except where plasma membranes are involved in the formation of a synaptic or adhesive junction. The extracellular space between the initial segment and the surrounding neuronal and neuroglial components has somewhat larger dimensions than that occurring elsewhere, and the granular material within the space may be either homogeneous in appearance, or condensed to form a line. It is probable that this extracellular dense material contributes to the apparent thickness of the plasma membrane of the initial segment observed in low power electron micrographs (Figs. 2 and 5). Because of the diffuse appearance of the extracellular material, one cannot ascertain exactly where it commences. But since the material is not present around the axon hillock (Fig. 7), it probably be-



gins to cover the initial segment at almost the same place as the undercoating.

Where the axon arises directly from the perikaryon, the fasciculation of the microtubulse (Figs. 2 and 3, *mt*) commences within the axon hillock (Figs. 2 and 3, *ah*), where aggregates of three or four microtubules are common. As these small aggregates pass down towards the initial segment, more microtubules are added to each one. Consequently, where the axon begins, as indicated by the presence of the undercoating, the number of microtubules in most fascicles has increased. Some fascicles within the initial segment of the axon still contain only three or four microtubules, but the majority contain between six and 12. In most initial segments five to seven such fascicles are present. In transverse sections (Figs. 8 and 9), it is most common for the microtubules (*mt*) of a fascicle to form a row in which each microtubule is separated from its neighbors by a distance of about 120 A. The profiles may form an almost straight line to give an appearance reminiscent of a string of beads, but in other instances they are staggered with respect to each other, giving a zig-zagged appearance. Less commonly, the microtubules in the fascicles are not aligned, but clustered into apparently random groupings, in which a regular spacing is still maintained between neighboring microtubules Bridging adjacent microtubules are thin whisps o. material. These bridges are most obvious in transverse sections of the initial segment (Figs. 8 and 9) and hillock (Fig. 7) of the axon, but they may also be apparent in longitudinal sections, when they have the appearance of rungs of a ladder (Fig. 3; *cb*).

When an axon arises from the perikaryon, the microtubules that form the fascicles within the axon hillock follow a relatively straight and parallel course (Fig. 3). Thus, in transverse sections of the axon hillock (Fig. 7), the microtubules in the

FIGURE 2 Longitudinal section through an initial segment (*is*) and axon hillock (*ah*) of an axon that arises from the perikaryon (*per*) of a pyramidal neuron. The fasciculation of the microtubules (*mt*) begins in the axon hillock, before the undercoating of the plasma membrane of the initial segment commences (arrows). Two axon terminals (*At*) are synapsing with the initial segment. Aldehyde perfusion. × 12,500.

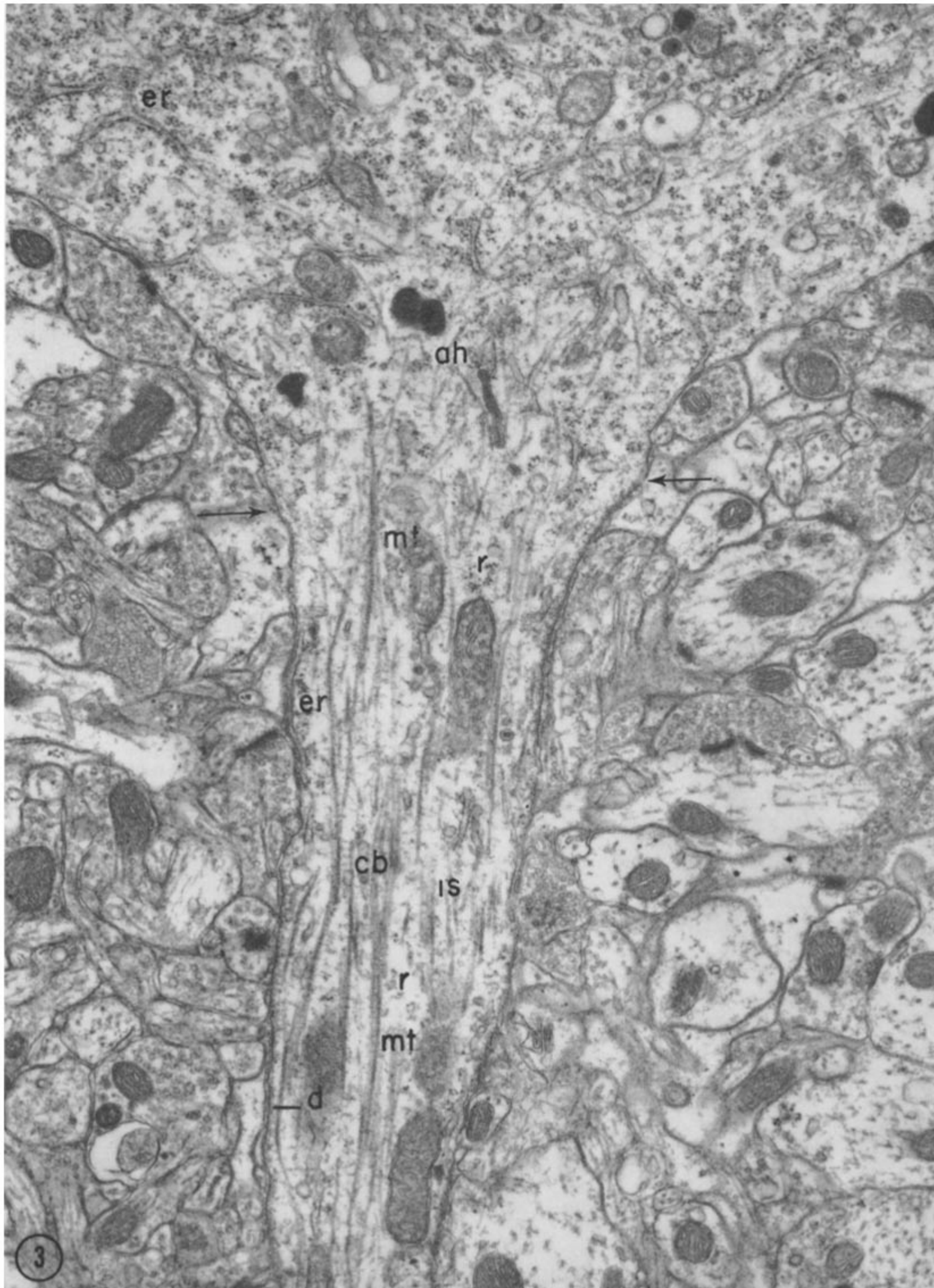


FIGURE 3 Enlargement of Fig. 2 to show details of the morphology of the axon hillock (*ah*) and the initial axon segment (*is*). The beginning of the undercoating (*d*) of the initial segment is indicated by arrows. Microtubules collect into fascicles (*mt*) in the axon hillock. In the fascicles they project straight into the initial segment and are linked together by cross-bridges (*cb*). Ribosomes, both free (*r*) and attached to the cisternae of endoplasmic reticulum (*er*), occur in the initial segment which also contains mitochondria and vesicles of smooth endoplasmic reticulum. Aldehyde perfusion. × 26,000.

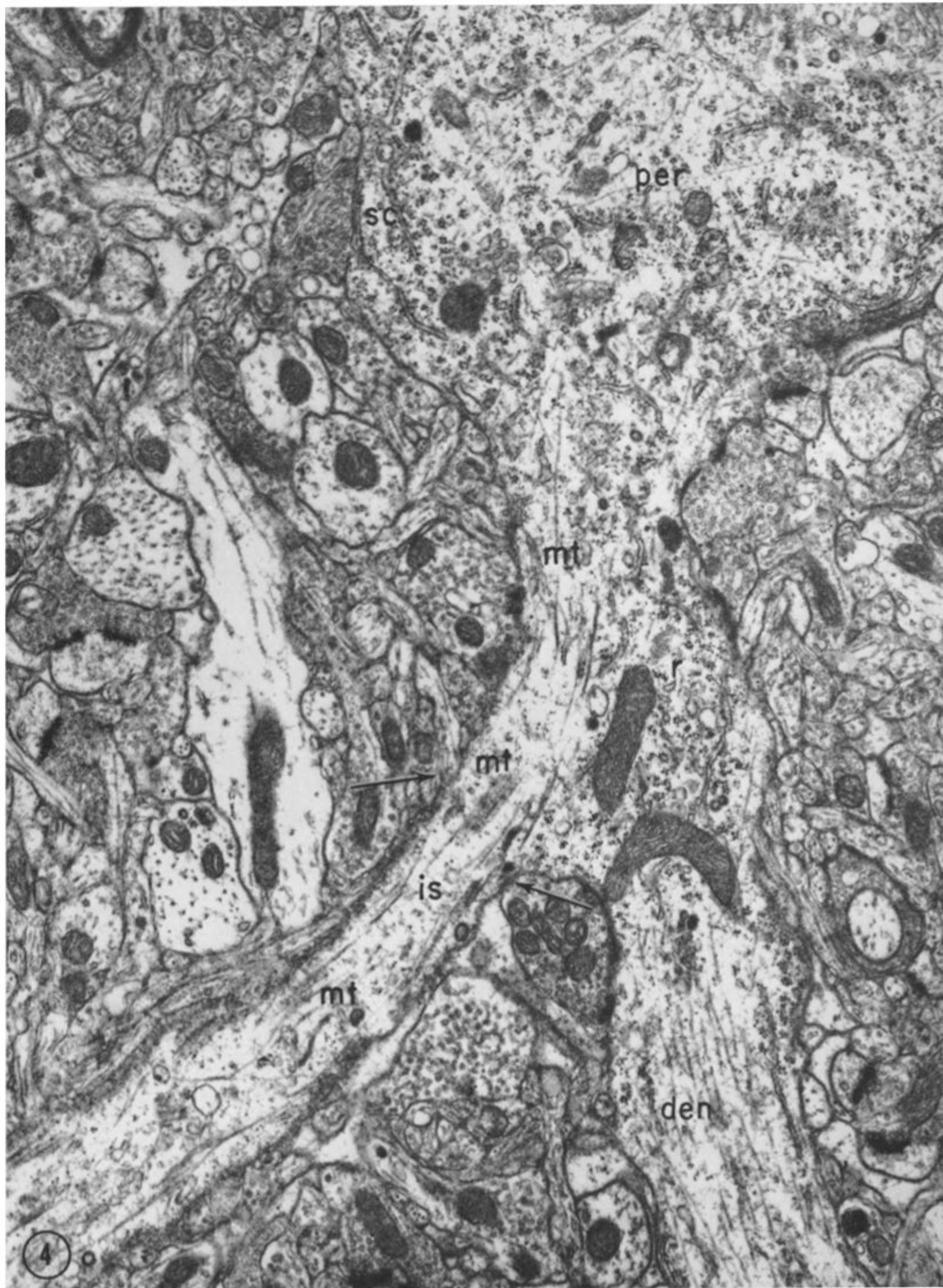
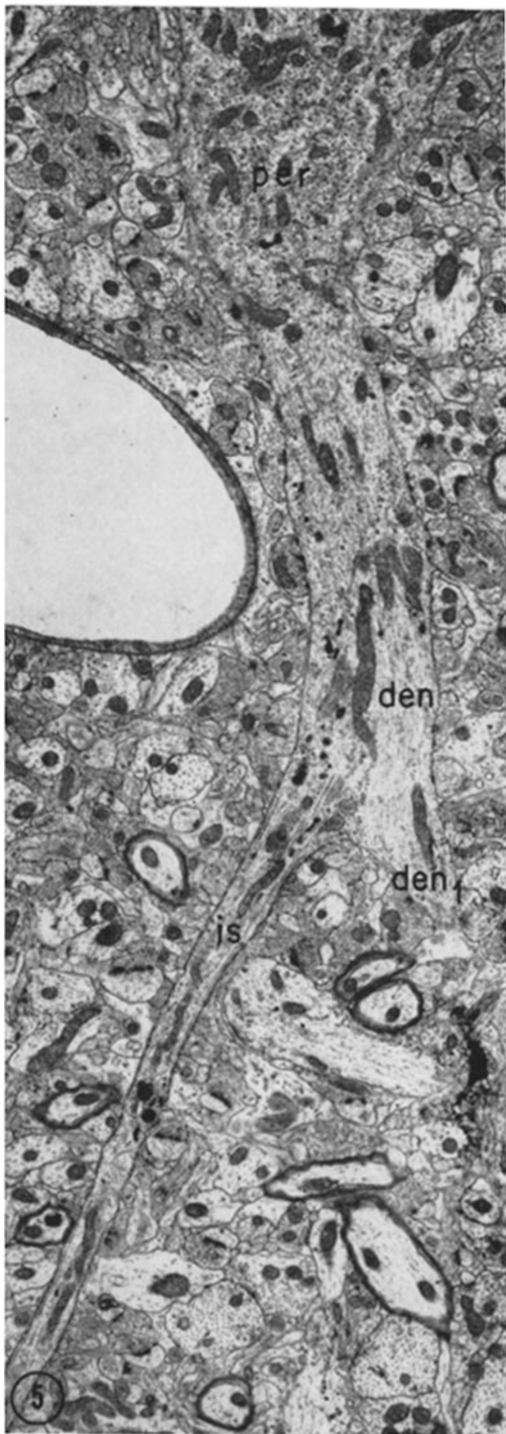


FIGURE 4 Longitudinal section through the basal portion of the perikaryon (*per*) of a pyramidal neuron that shows subsurface cisternae (*sc*). Arising from the perikaryon is a short common stem that bifurcates into a dendrite (*den*) and an axon initial segment (*is*) which soon acquires an undercoating (arrows). The fasciculation of the microtubules (*mt*) begins in the left side of the common stem. Aldehyde perfusion. × 18,000.



fascicles (*mt*) are nearly always seen in perfect cross-section. This makes them more obvious than the single microtubules (Fig. 7, *t*) lying nearby. The dimensions of both the individual and fasciculated microtubules appear to be the same. Both have an external diameter of about 220 A and in transverse sections have a dark wall, 60 A thick, that appears to be formed from globular elements (see Pease, 1963; Ledbetter and Porter, 1963; Peters and Vaughn, 1967). This dark wall surrounds a lighter center in the middle of which a dark dot, 30–40 A in diameter, is often visible (Figs. 7 and 9). The dark dot appears to represent a central filament, for in longitudinally oriented microtubules the interval between two dark lines representing the outer wall of the microtubules sometimes contains a third, more indefinite dark line passing down its center. Echandía, Piezzi, and Rodríguez (1968) suggest that the central element is not a filament but a series of granules.

One point indicated by an examination of transverse sections is that not all of the microtubules funneling into the axon hillock from the perikaryon contribute to the formation of the fascicles within the initial segment. Counts of total numbers of microtubules were made in profiles of axon hillocks and initial segments encountered in sections cut parallel to the surface of the cortex and passing through the lower border of layer II of the cortex, where probably the only neurons that provide these profiles are the small pyramids of layer II. In 20 initial segments in which complete counts were possible, the total number of microtubules varied between 22 and 50 and the axon diameters varied between 0.6 and 1.2 μ. One exception was an axon 0.3 μ in diameter which contained only nine microtubules. In five axon hillocks that allowed absolute counts of microtubules, both single ones and those associated with fascicles, 108 (Fig. 7), 84, 80, 70, and 67 microtubules were present. These numbers are higher than those for microtubules present in any profiles of initial segments encountered at the same level of the cortex. Since longitudinal sections give no indica-

FIGURE 5 Longitudinal section through a large basal dendrite (*den*) arising from the perikaryon of a pyramidal neuron (*per*). The basal dendrite bifurcates to form a secondary dendrite (*den*$_1$) and a thin axon initial segment (*is*), whose membrane appears thickened. Aldehyde perfusion. × 7,500.

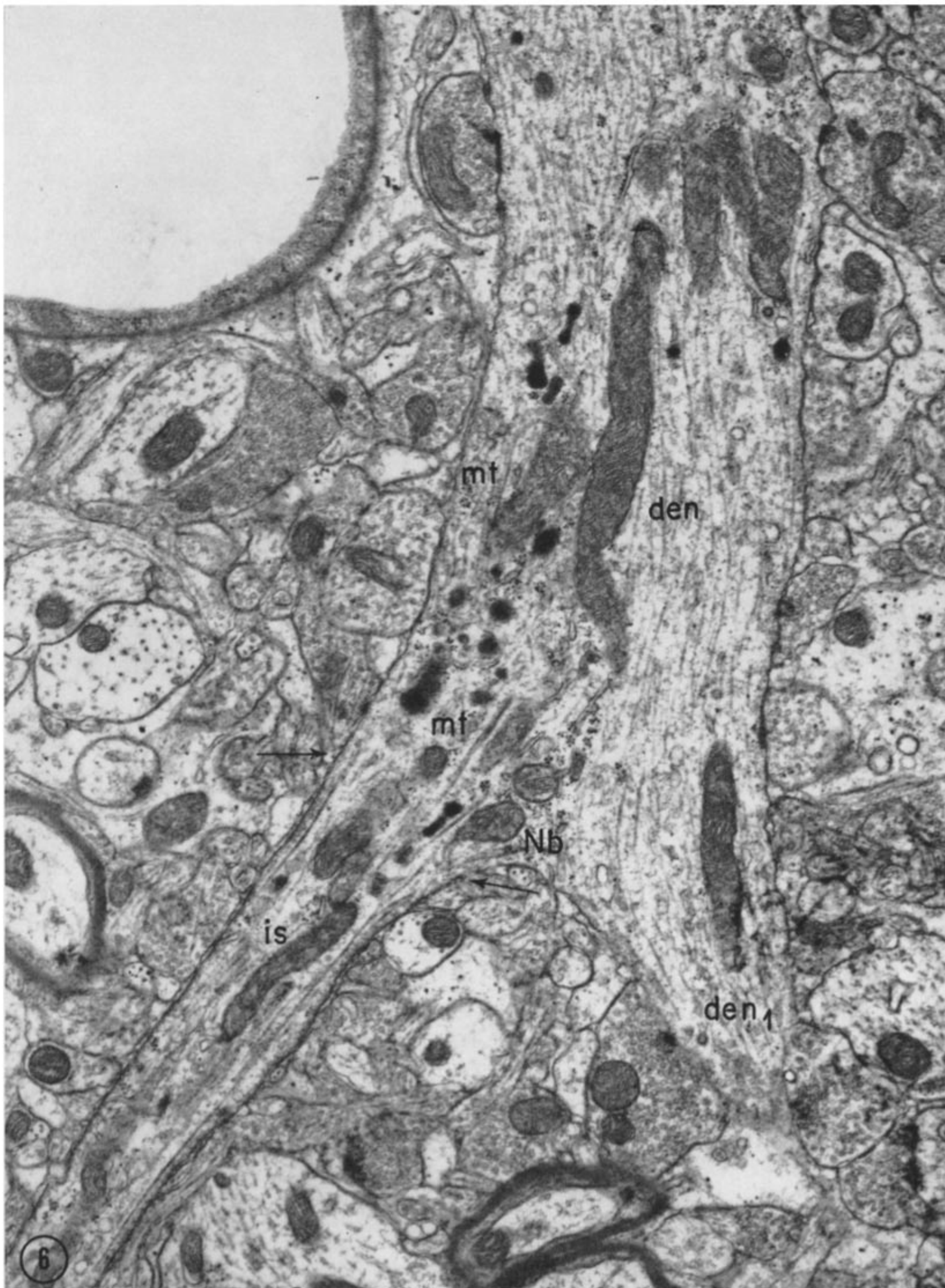


FIGURE 6 Enlargement of Fig. 5, to show the bifurcation of the large basal dendrite (*den*) into the secondary dendrite (*den*$_1$) and the axon initial segment (*is*). The undercoating of the initial segment begins (arrows) just beyond the bifurcation, but the fasciculation of the microtubules (*mt*) starts in the basal dendrite. Note the dark vesicles in the base of the axon, and the small Nissl body (*Nb*) in the angle of bifurcation. Aldehyde perfusion. × 22,000.

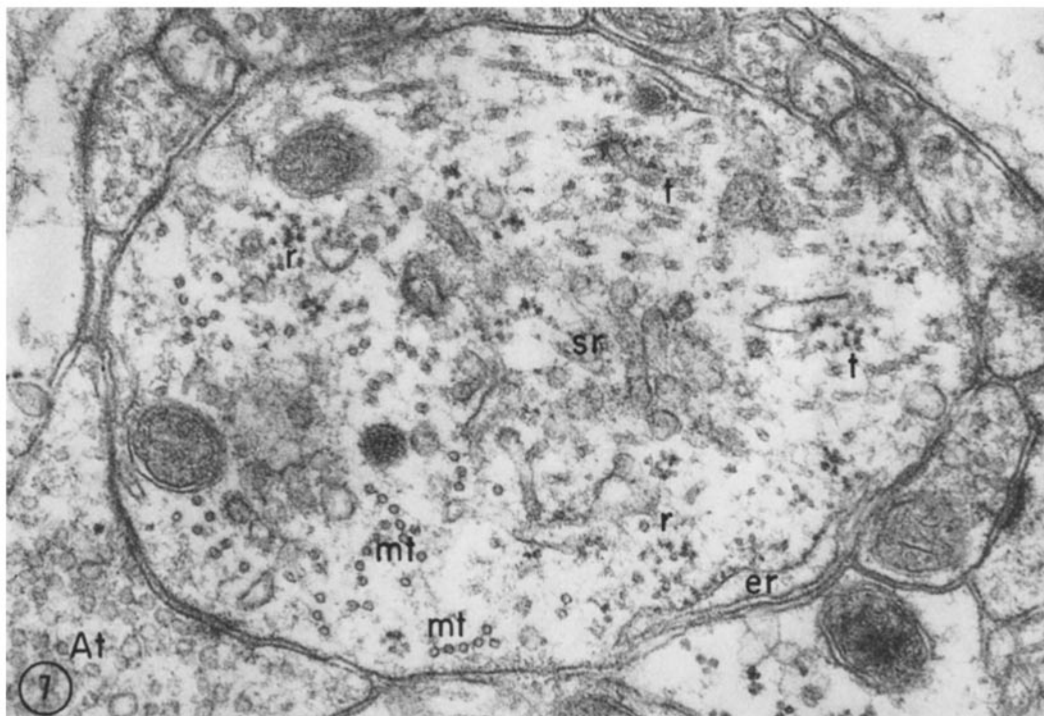


FIGURE 7 Transverse section of the axon hillock. This part of the neuron shows the beginning of the fasciculation of some microtubules (*mt*). Other, obliquely sectioned microtubules (*t*) are also present in the cytoplasm that contains ribosomes (*r*), a cistern of granular endoplasmic reticulum (*er*), and a number of smooth-surfaced cisternae (*sr*). Note the bouton (*At*) forming a synapse with the axon hillock. Aldehyde perfusion. × 58,000.

tion that microtubules loop into the axon hillock and then pass back into the perikaryon, it must be be presumed that at least some of the microtubules terminate in the distal portion of the axon hillock.

When the axon arises directly from the neuronal perikaryon, the axon hillock contains the beginnings of the fasciculation of some of the microtubules. It is this feature that allows transverse sections through the hillock (Fig. 7) to be distinguished from those through the base of a dendrite, in which the distribution of the microtubules is more homogeneous. Another feature of the axon hillock is the diminution of the number of ribosomes (Figs. 3 and 7) both in the form of free clusters (*r*) and attached to the outer surfaces of cisternae of the endoplasmic reticulum (*er*). Ribosomes continue into the initial segment as far as the site at which the axon acquires its myelin sheath, although the number of ribosomes are few and insufficient to produce a significant basophilia in light microscope preparations. The ribosomes that are present within the initial segment are generally in the form of small clusters. Only very occasionally are small cisternae of granular endoplasmic reticulum encountered in the initial segment (Fig. 3, *er*).

Other organelles commonly present in the axon hillock and initial segment (Figs. 2 and 3) are mitochondria, multivesicular bodies, neurofilaments, and various vesicles with both clear and dark content. These organelles become aligned in a parallel manner as they funnel into the axon from the perikaryon. The only other change that seems to occur is a slight increase in the number of multivesicular bodies within the axon hillock as compared with other portions of the neuron, but even this increase is not dramatic.

Apparently then, the axon hillock is essentially a portion of the neuron in which there is a transition between the cytological features of the peri-

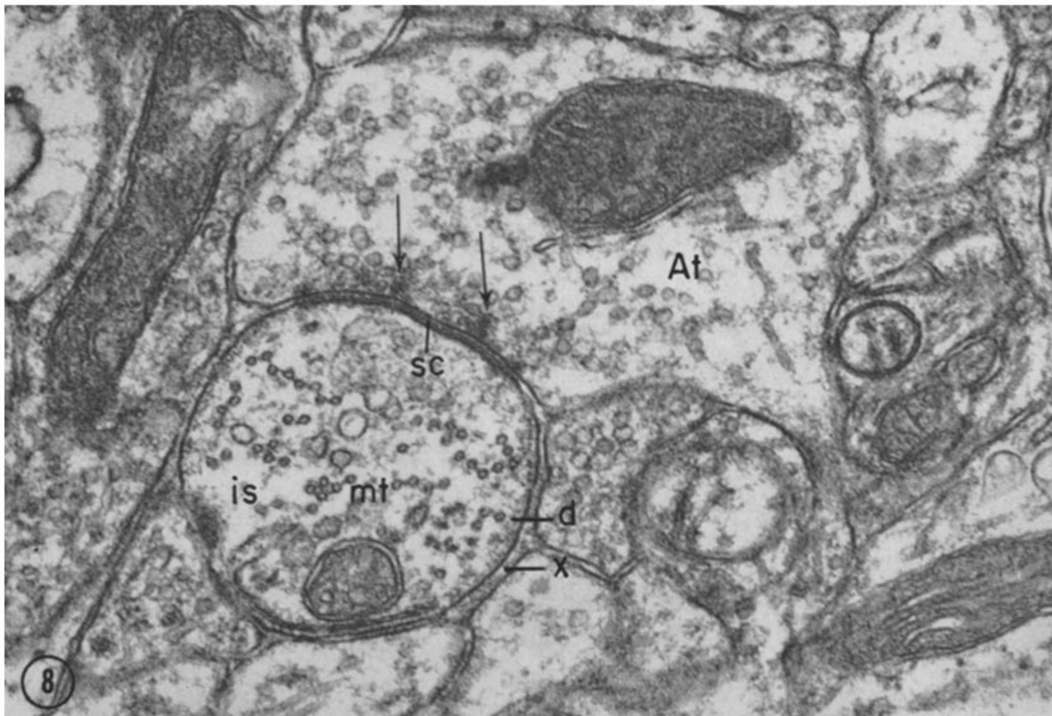


FIGURE 8 Transverse section of the initial segment (*is*) to show the fascicles of microtubules (*mt*) and the undercoating (*d*) of the plasma membrane. Synapsing with the initial segment is a large bouton (*At*). On the postsynaptic side of the junction (*sc*) is a thin layer of dense material and presynaptically there are dark patches (arrows). Dense material (*x*) is present in some parts of the extracellular space around the initial segment. Aldehyde perfusion. × 55,000.

karyon and those of the initial segment of the axon. Its distal limits are defined by the beginning of the undercoating of the initial segment, but toward the perikaryon there is no definite boundary. Thus, the axon hillock of the pyramidal cell is not a clearly delimited portion of the neuron. When both the initial segment of the axon and a dendrite arise from the same site on the perikaryon (Fig. 4), the short common stem has features of both types of process. On the side from which the axon (*is*) arises there is a diminution in the number of ribosomes (*r*) and a beginning of the fasciculation of the microtubules that pass into the axon. Nissl substance is retained on the side of the common stem from which the dendrite (*den*) arises, and this continues into the base of the dendrite. Moreover, on this side of the common stem, the microtubules remain separate. Consequently, there is no portion of the neuron that can be regarded as being an axon hillock.

In the situation in which both the initial segment of the axon and a dendrite arise from a common stem some distance from the perikaryon (Figs. 5 and 6), the common stem has the morphological characteristics of a dendrite (*den*). The only apparent difference between this common stem and any other basal dendrite is that a fasciculation of some of the microtubules (*mt*) that pass into the initial segment (*is*) begins a short distance prior to the origin of the axon. It should also be added that a small Nissl body (*Nb*) is often present in the angle of bifurcation of the common stem (*den*) into the axon (*is*) and the secondary dendrite (den_1). This location is also a favored position for a small Nissl body when a dendrite branches to form two smaller ones.

In all parts of the pyramidal neuron, subsurface cisternae of the type described by Rosenbluth (1962) are encountered. These are flattened cisternae that are closely applied to the inner aspect

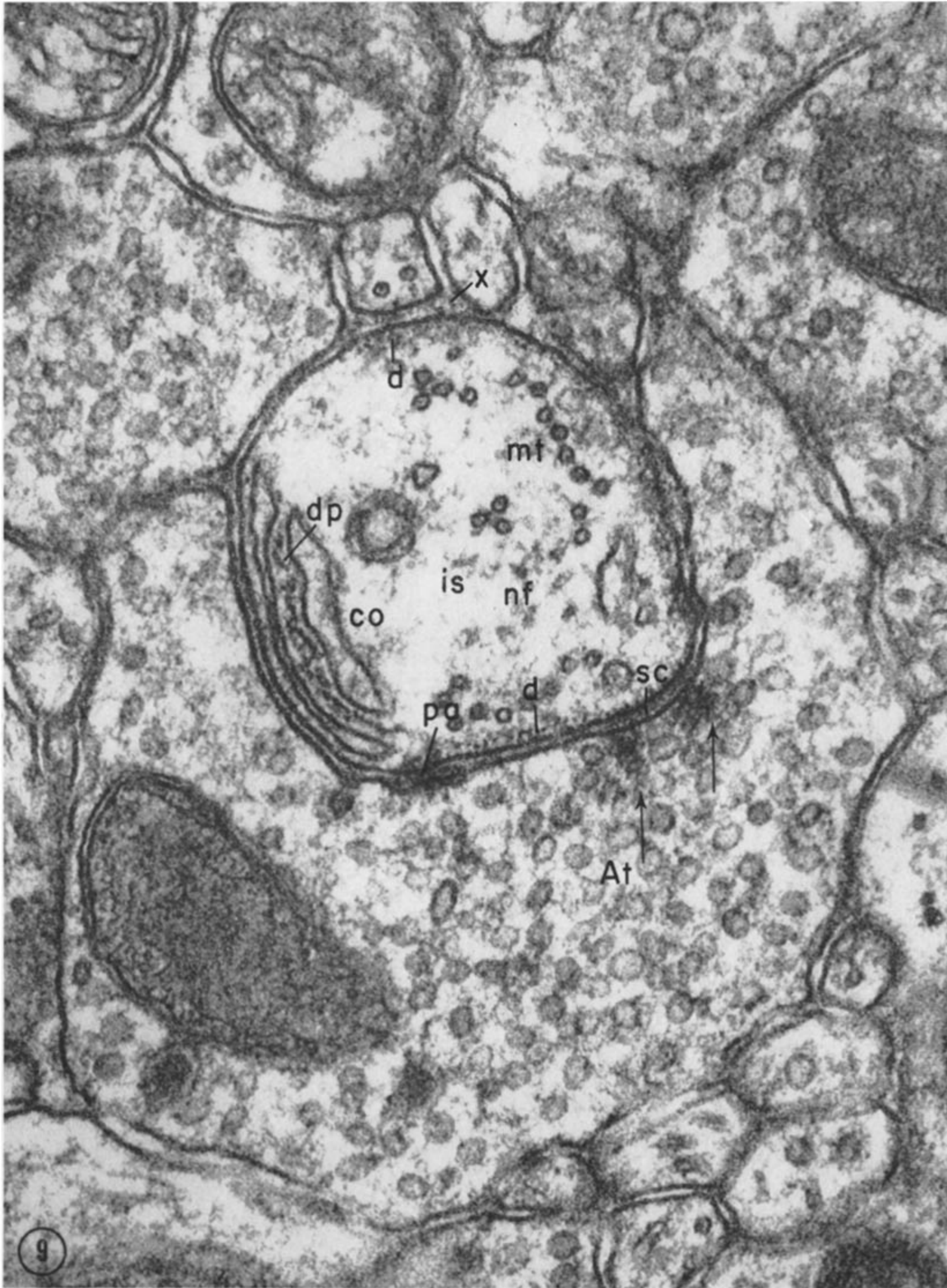


FIGURE 9 Transverse section of an initial segment (*is*) partially surrounded by a large axon terminal (*At*). In the initial segment are neurofilaments (*nf*), fascicles of microtubules (*mt*), and a cisternal organelle (*co*) in which the flattened cisternae are separated by an interval containing dense material (*dp*). At the synaptic junction is a synaptic complex (*sc*) with associated vesicles and dense patches (arrows) in the axon terminal. The other junctional specialization is a *punctum adhaerens* (*pa*). In the initial segment, the undercoating is absent beneath both the synaptic complex and the *punctum*. Note the granular material (*x*) in the extracellular space around the initial segment. Aldehyde perfusion. × 88,000.

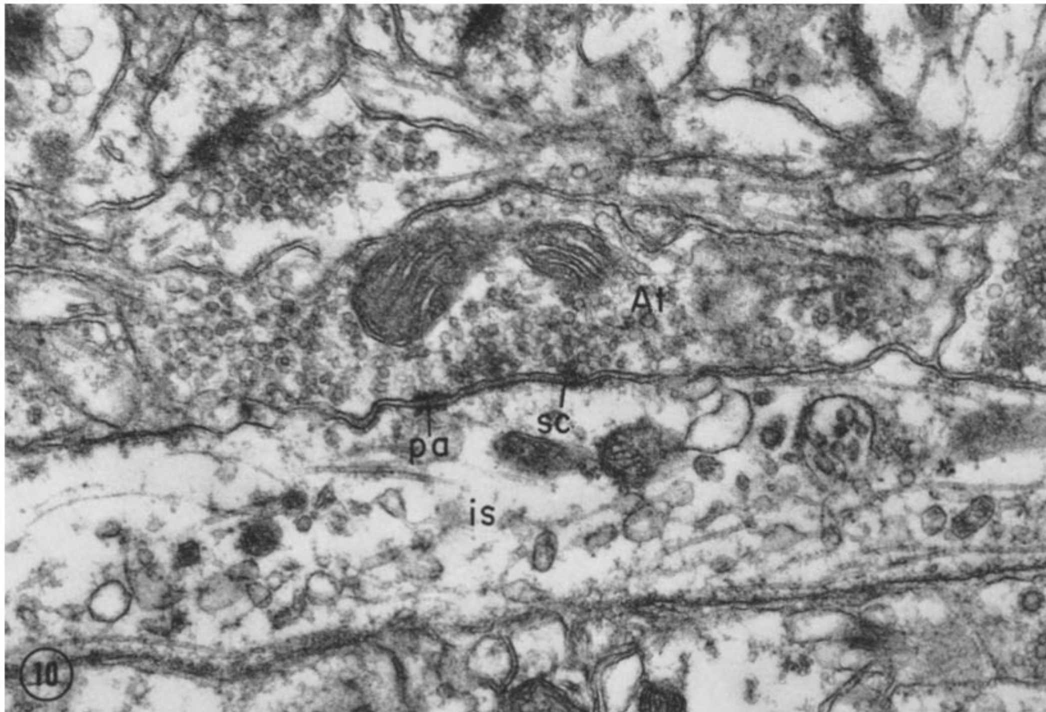


FIGURE 10 Longitudinal section of an initial segment (*is*) with a large bouton (*At*) attached to its surface. At the synaptic junction there are two specialized zones. One (*sc*) is a synaptic complex which has an asymmetric distribution of dense material and an accumulation of synaptic vesicles. The other is a *punctum adhaerens* (*pa*) which has no associated vesicles and a symmetrical distribution of dense material. OsO_4 perfusion. × 48,000.

of the neuronal plasma membrane. Sometimes a single cistern is present (Fig. 4; *sc*), but it is more common for additional cisternae to be aligned beneath the outer one so that a stack is formed. In such a stack, no other organelles intervene between adjacent cisternae which are often separated by a distance of only 100 A. Commonly, the cisternae are dilated at their ends, and continuities may be encountered between these cisternae and components of the smooth and granular endoplasmic reticulum. Sometimes, ribosomes stud the deep surface of the innermost cistern. This type of subsurface cistern is relatively common in the region of the perikaryon near the axon and may also be present in the proximal portion of the initial segment.

Within the initial segment another type of cisternal organelle is often present (Figs. 9 and 11; *co*) but is not prolific, for no more than two have ever been encountered in any given section; and in the majority of profiles of initial segment the organelle is absent. This organelle consists of one or more flattened cisternae that have no associated ribosomes. The cisternae are usually arranged parallel to the axolemma and are elongated in the direction of the length of the axon. When two or more cisternae are present, the ones situated nearer to the center of the axon become progressively smaller than those lying above (Fig. 11). The form of the packing of these cisternae differs from that of the subsurface cisternae present in the perikaryon and dendrites, for adjacent cisternae are separated by wider intervals, about 400 A, that contain a dense granular layer (*dp*). In sections, each dense layer extends for only the distance of the cistern lying below it and has a width of about 200 A. At high resolution, it is apparent that the dense layer consists of two dense plates separated by an interval of about 50 A. This figure is only approximate, since the two plates do not

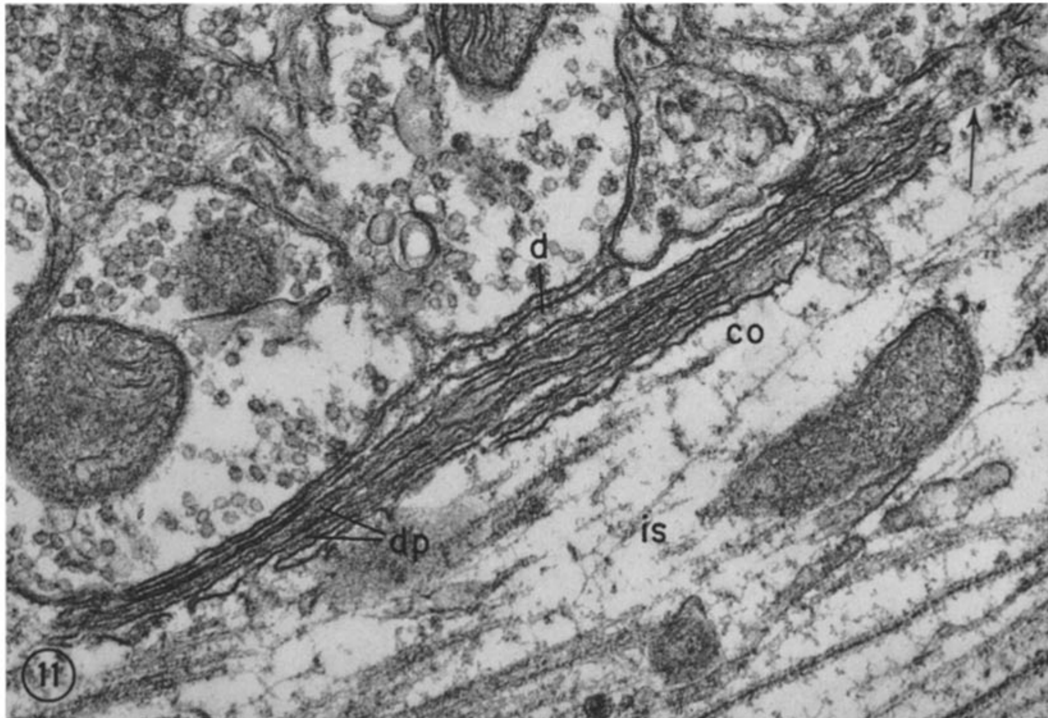


FIGURE 11 Longitudinal section of an initial segment (*is*) containing a large cisternal organelle (*co*). This organelle consists of flattened cisternae that alternate with dense plaques (*dp*). Where the outermost cistern lies close to the plasma membrane, the undercoating (*d*) is absent. At their ends some of the cisternae are in continuity (arrow) with the smooth endoplasmic reticulum. Aldehyde perfusion. × 57,000.

have well defined boundaries. When a cistern comes into close proximity to the inner face of the plasma membrane of the initial segment, the undercoating of the membrane may be absent (Fig. 9). The cisternae of these stacks in the initial segment are not dilated at their ends. In some instances the ends of the cisternae appear to be in continuity with the tubular system of smooth endoplasmic reticulum that occurs throughout the initial segment (Fig. 11; arrow), but no continuities with the granular endoplasmic reticulum have been encountered.

Boutons containing synaptic vesicles often surround the initial segment of the pyramidal neuron (Figs. 2, 8, 9, and 10; (*At*), and it is common for them to form synapses with the initial segment. At the site of junction of the pre- and postsynaptic membranes there are areas in which the distance between the two membranes is increased to about 200A. At these sites (Figs. 8–10; *sc*), the postsynaptic membrane has a thin layer of dense material, the postsynaptic thickening, attached to its cytoplasmic surface, and in the postsynaptic axon there are accumulations of synaptic vesicles. Areas of the synaptic junction with these features are the "synaptic complexes" (Palay, 1956) or "active zones" (Couteaux, 1961). At the synaptic complexes, there are also patches of dense filamentous material (Figs., 8 and 9; arrows) that extend from the postsynaptic membrane into the cytoplasm. As indicated by Palay et al. (1968), those portions of the plasma membrane of the initial segment that are occupied in the formation of synaptic complexes do not possess the undercoating that is present elsewhere. This is well illustrated in Fig. 9, where the undercoating (*d*) stops short of the synaptic complex (*sc*). Whether the undercoating and the thickening of the postsynaptic membrane at a complex are in continuity is uncertain. Sometimes, the two appear to be contiguous, but in other examples there seems to be a gap between them. In our tissue fixed by perfusion with either

osmium tetroxide or aldehyde mixtures, the synaptic vesicles are generally round and have clear contents (Figs. 8–10).

The "active zones" or "synaptic complexes" that occur at the synaptic junctions are generally considered to be sites of chemical transmission. In addition to these patches, however, there are areas of the synaptic junction in which the cytoplasmic faces of the pre- and postsynaptic membranes have a symmetrical disposition of electron-opaque material. These structures (Figs. 9 and 10; *pa*) have no synaptic vesicles intimately associated with them and are considered to represent *puncta adhaerentia* (Palay, 1967). Since *puncta adhaerentia* occur between other components of the central nervous system, both neuronal and neuroglial, in places in which no synaptic vesicles are present, they are considered to represent simple points of adhesion, and not synaptic transmission. *Puncta* interspersed with synaptic complexes have been described at other synaptic junctions (Peters and Palay, 1966).

Synapses essentially similar in form to those on the initial segment are also present on the axon hillock (Fig. 7).

DISCUSSION

Apart from a recent report by Palay, Sotelo, Peters, and Orkand (1968) describing the characteristic features of the cytoplasm of the initial segment of axons from a number of different neurons, only a few brief reports of this portion of the neuron have appeared in the literature. Palay (1962) first described the rather peculiar arrangement of the microtubules in an account of the initial segment of the axon from the Purkinje cell in the rat cerebellum, and a further mention of their bundling was made by Robertson, Bodenheimer, and Stage (1963), who studied the initial segment of the axon of the Mauthner cell of the goldfish. Kohno (1964) has also examined the arrangement of the microtubules in the initial segment of the Purkinje neuron in the frog. He gives the center-to-center spacing between adjacent microtubules as about 450 A, which is some 100 A greater than we have observed in the initial segment of the pyramidal neuron.

As shown in the present account and that of Palay et al. (1968), the fasciculation of microtubules begins prior to their entry into the axon initial segment, whose boundaries are defined by the limits of the undercoating. Thus, when an axon emerges directly from the perikaryon, the bundling commences in the axon hillock. An examination of transverse sections of the axon hillock and the initial segment has shown, however, that not all of the microtubules pass into the initial segment. It seems probable that the majority of microtubules that do not take part in the formation of the fascicles terminate before they reach the initial segment. This is suggested by the fact that no transverse sections of initial segments have so far been encountered that contain as many microtubules as transverse sections through the axon hillock, but no structure that could be identified as the end of a microtubule has been observed.

At present the significance of the fasciculation of the microtubules in the initial segment is unknown, although Palay et al. (1968) have speculated that these microtubules may be contractile.

In addition to the undercoating described previously (Palay et al., 1968), it has now been observed that the plasma membrane of the initial segment is surrounded by an extracellular space containing dense material. It is also apparent that this extracellular space is slightly larger than the one present elsewhere in the neuropil. An undercoating of the plasma membrane of the axon also occurs at the node of Ranvier (Elfvin, 1961; Andres, 1965; Peters, 1966) and, since both the node and the initial segment of the axon have similar electrical properties, it is reasonable to postulate that the undercoating is related to the production of an electrical signal. The dense material on the outside of the membrane may be related to this same function, and preliminary observations suggest that the node is also surrounded by a somewhat enlarged extracellular space containing dense material.

An unusual feature of the axon initial segment of the pyramidal neuron is the stacks of cisternae that occur beneath the plasma membrane. Such cisternae were not seen in the initial segments of other neurons examined by Palay et al. (1968). In appearance, the stacks of cisternae closely resemble the spine apparatus described by Gray (1959). In the spine apparatus, which occurs in the larger dendritic spines or thorns in the cerebral cortex, the adjacent sacs or cisternae are also separated by intervals of 300–500 A that contain a layer of dense material.

It is perhaps significant that, although the spine apparatus is often present within the dendritic spines of pyramidal neurons of the cerebral cortex

(see Gray and Guillery, 1966), including the hippocampal cortex (Hamlyn, 1962; Westrum and Blackstad, 1962), a similar but modified organelle has been only encountered infrequently in other neurons. Therefore, on the basis of the available evidence, there is a strong indication that this type of cisternal organelle, consisting of flattened cisternae separated by dense plates, is peculiar to pyramidal neurons of the cerebral cortex. From the location of the organelle, one can speculate that in some way the organelle is concerned with the synaptic transmission or excitability of these two specific portions of the pyramidal neuron, the initial segment of the axon and the dendritic spines, for it has not been seen in other parts of this same neuron. Although the organelle is frequently encountered in dendritic spines, it is most common in the larger ones. Small spines very often do not contain the organelle, but the largest spines may have two separate ones. In the initial segment of the axon there is no apparent relation between the location of the organelle and the site of attachment of the axo-axonic synapses. Since both are frequently encountered in sections of the initial segment of the pyramidal neuron, examples do occur in which the cisternal organelle lies beneath a part of the plasma membrane that is forming a synaptic junction with a bouton.

Axon-axonal synapses in the cerebral cortex of the rat have been previously described by Westrum (1966). Westrum was unable to decide whether the postsynaptic component was the preterminal unmyelinated portion of an afferent axon or the initial segment of a neuron situated within the cortex. Since Westrum found ribosomes within the cytoplasm of the postsynaptic axon, the component probably was an initial segment.

Our observations on the initial segment of the pyramidal cell axon indicate that synapses on this portion of the neuron are quite common. The synapses show no unusual features, and the form of the junction is very similar to that of synapses on both the axon hillock and the perikaryon. The origin of the axons terminating on the initial segments of the pyramidal cells is, so far as we are aware, unknown, and there are no Golgi studies relevant to this point. The location of these synapses places them in a very strategic position to effect inhibition of the pyramidal neuron. Inhibition of pyramidal neurons has been demonstrated by a number of workers (see Eccles, 1966), although the data mainly pertain to the Betz cells and the hippocampal pyramidal cells of the cat (see Andersen, 1966). However, both Eccles and Andersen believe that the inhibitory synapses are located on the soma of the pyramidal neuron.

If these axo-axonic synapses are inhibitory, then it is interesting that in our aldehyde-fixed material the boutons contain essentially spherical vesicles. Uchizono (1965), who studied the cerebellum, concluded that the presence of ellipsoidal vesicles could be equated with a bouton having an inhibitory function and that spherical vesicles occur in excitatory boutons. A similar conclusion has been drawn by Bodian (1966) who studied the anterior-horn cells of the spinal cord.

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