THE AXON HILLOCK AND

THE INITIAL SEGMENT

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ABSTRACT

Axon hillocks and initial segments have been recognized and studied in electron micrographs
of a wide variety of neurons. In all multipolar neurons the fine structure of the initial seg-
ment has the same pattern, whether or not the axon is ensheathed in myelin. The internal
structure of the initial segment is characterized by three special features: (a) a dense layer of
finely granular material undercoating the plasma membrane, (b) scattered clusters of ribo-
somes, and (c) fascicles of microtubules. A similar undercoating occurs beneath the plasma
membrane of myelinated axons at nodes of Ranvier. The ribosomes are not organized into
Nissl bodies and are too sparsely distributed to produce basophilia. They vanish at the end
of the initial segment. Fascicles of microtubules occur only in the axon hillock and initial
segment and nowhere else in the neuron. Therefore, they are the principal identifying mark.
Some speculations are presented on the relation between these special structural features and
the special function of the initial segment.

For more than 10 yr, since the introduction of
techniques for intracellular recording of electrical
potentials in nerve cells, neurophysiologists have
referred to a specialized region at the beginning
of the axon as the site where the action potential
originates. This region, somewhere between the
axon hillock and the beginning of the myelin
sheath, has been designated *the initial segment of
the axon*. Its membrane is thought to have special
properties, most notably a lower threshold to ex-
citation than the membrane of the dendrites and
the perikaryon (2, 12). In the light microscope
the axon hillock, from which the axon originates,
displays only one cytoplasmic feature that dis-
tinguishes it from the rest of the perikaryon: the
absence of basophilic material, i.e., the Nissl
substance. This characteristic pallor of both the
axon hillock and the process issuing from it has
long been regarded as an identifying mark that
permits the origin of the axon to be recognized

even in preparations where continuity with the
myelinated part of the axon is not evident. During
the past several years, in electron micrographic
studies of different types of neurons, we have
encountered numerous axon hillocks and initial
segments. In several cases we have been able to
observe the initial segment in its entirety, from its
origin to the first segment of myelin. This paper
describes the characteristic features that permit the
initial segment to be recognized in longitudinal
and transverse sections, in continuity with its
parent cell and nerve fiber, or in isolation.

MATERIALS AND METHODS

Specimens were taken from various parts of the
nervous system of adult rats after initial fixation *in
situ* by perfusion through the vascular tree (17).
Cerebral cortex, cerebellar cortex, spinal cord, and
brain stem were examined. The material for this
study was collected over a period of 6 yr, and a variety

of fixing solutions were used. The earlier tissues were fixed in a 1% solution of osmium tetroxide in acetate-Veronal buffer (pH 7.35–7.4) containing 5.4 mg of calcium chloride per milliliter. Later specimens were fixed in a mixture of aldehydes and postfixed in osmium tetroxide. The aldehyde mixture, modified from Karnovsky's recipe (11), consisted of 1% paraformaldehyde and 1.25% glutaraldehyde in either 0.067 M sodium cacodylate or 0.12 M phosphate buffer at pH 7.4. To each milliliter of fixative in cacodylate, 5 mg of $CaCl_2$ were added, and to that in phosphate 2 mg of $CaCl_2$ were added. Postfixation was carried out in 2% osmium tetroxide dissolved in the appropriate buffer with the same amounts of $CaCl_2$. In order to maintain approximately the same osmolality, we added 7 g of glucose to each 100 ml of solution. Blocks were embedded in either Araldite or Epon. Thin sections were double-stained with uranyl acetate and lead citrate (26).

OBSERVATIONS

In the present study we have recognized well over 100 different examples of the initial segment in either transverse or longitudinal sections, and in five cases we have followed the entire length of the initial segment from the perikaryon to onset of the myelin sheath in a composite of electron micrographs. The neuronal types whose initial segments we have documented with electron micrographs include the following: (a) Purkinje cells, basket cells, and granule cells in the cerebellar cortex; (b) pyramidal cells in the cerebral cortex; (c) Deiters cells and smaller cells in the lateral vestibular nucleus; and (d) motor neurons in the anterior horn of the spinal cord. Some observations on initial segments of Deiters cells (23) and Purkinje cells (16) were reported earlier along with illustrations that may also be used to supplement the present paper. In all cases, whether the axon is myelinated or not, the fine structure of the initial segment is essentially the same.

The initial segment of the axon is unlike any other known process of the nerve cell, and in certain respects it is unlike any other part of the axon itself. In the idealized nerve cell it arises from the summit of a conical projection on the surface of the perikaryon, the axon hillock (Fig. 1). The surface of the axon hillock is bounded by a plasma membrane with the usual trilaminate structure, but near the apex of the hillock (Fig. 2) a thin, dark layer of finely granular material appears just beneath the membrane. In low-power electron micrographs this granular material gives the membrane a dense appearance, so that on super-

ficial examination the plasmalemma seems to be reduplicated or thickened. But the undercoating is not really a part of the limiting membrane; instead, it is a thin layer of powdery densities, about 100 A thick, separated from the surface membrane by a clear interval of about 30 A (Figs. 3 and 5). As its margins are not distinct, these measurements cannot be precise. The undercoating extends from the narrow beginning of the axon to the onset of the myelin sheath, where it ceases as abruptly as it began. In unmyelinated axons the undercoating extends for a variable distance, 4–5 μ. Where the axon originates from a dendrite, the undercoating has the same sudden onset and is accompanied by other changes (see below) in the internal structure of the process that signal the beginning of an axon. The initial segment resembles the node of Ranvier (1, 4, 18) in having this undercoating, and the undercoating is, therefore, by itself not sufficient for identification of the initial segment.

There is, however, a second characteristic involving the internal structure that makes the initial segment instantly recognizable, and this is the arrangement of microtubules within it. In the axon hillock (Fig. 2) microtubules collect into bundles of three to five or more, which funnel into the initial segment and run parallel with one another throughout its length. Large initial segments have five or six such fascicles, while small ones may have only one. The number of microtubules in a bundle varies considerably but is probably characteristic of the type of neuron. In the Deiters cells of the lateral vestibular nucleus (Fig. 5) and in the motor neurons of the spinal cord, the number of microtubules included in a fascicle is usually three to five, and in the pyramidal cells of the cerebral cortex (Fig. 6) the number can reach 22. In the Purkinje cell of the frog, Kohno (13) found from 6 to 25 microtubules in a bundle, but in the rat the maximum number we have found in this type of neuron is only 10–12.

In longitudinal sections the fasciculated microtubules in the initial segment often appear darker than the single microtubules in the rest of the nerve cell and its processes. This appearance is due only partly to overlapping of the microtubules in a bundle within the thickness of the section. In addition, each microtubule is surrounded by a cloud of fine fibrillar material that contributes to the general density of the fascicle. In transverse sections it can be seen that the microtubules are
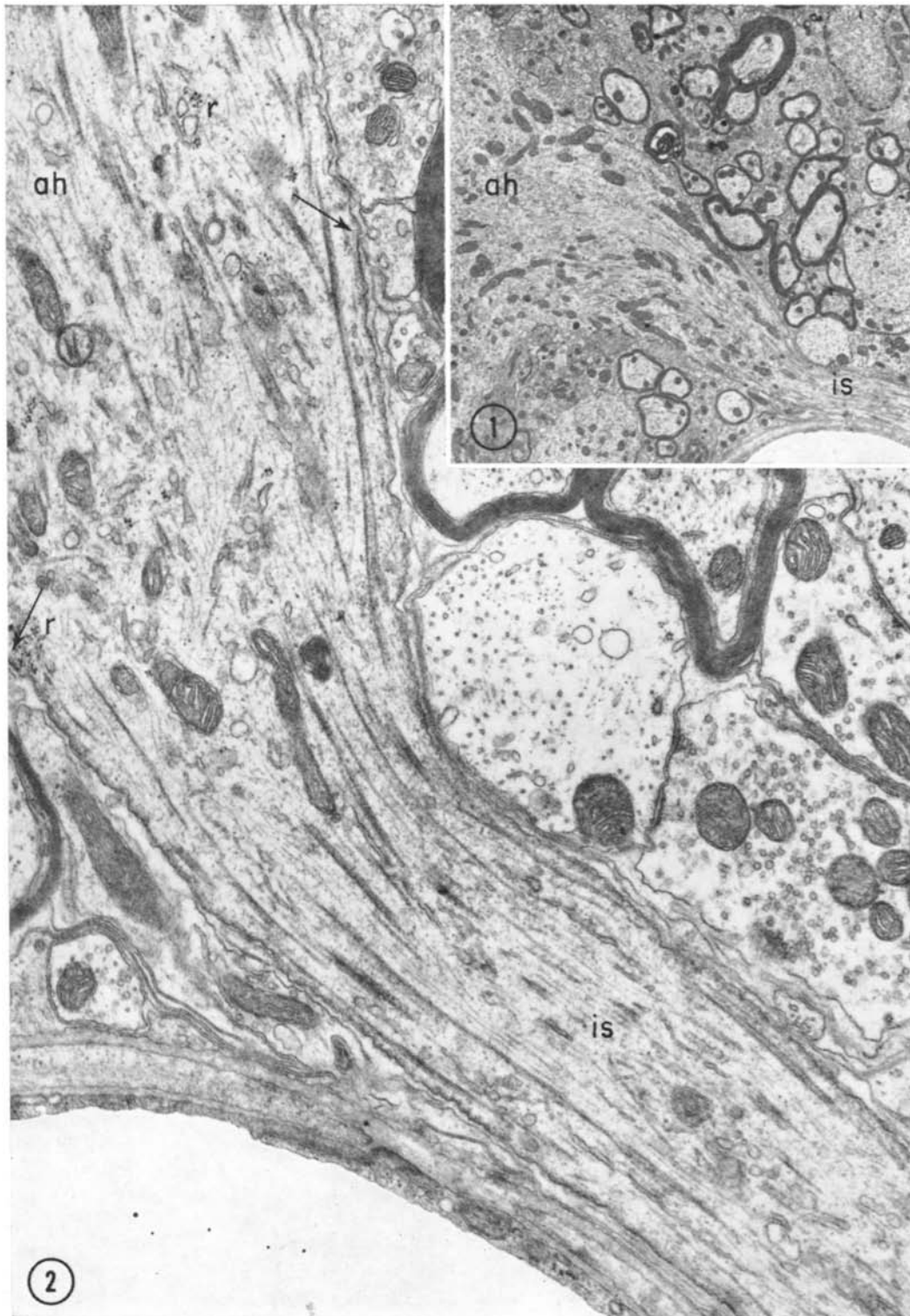
FIGURES 1 and 2   Lateral vestibular nucleus, rat. The axon of a Deiters cell emerges from the axon hillock (ah) and quickly narrows to become the initial segment (is). Mitochondria, neurofilaments, microtubules, and agranular endoplasmic reticulum all assume a linear and parallel orientation. Ribosomes (r) occur in clusters both in the hillock and in the initial segment. The onset (arrows) of the dense undercoating beneath the plasmalemma marks the beginning of the initial segment. Fig. 1, × 4000; Fig. 2, × 27,000.
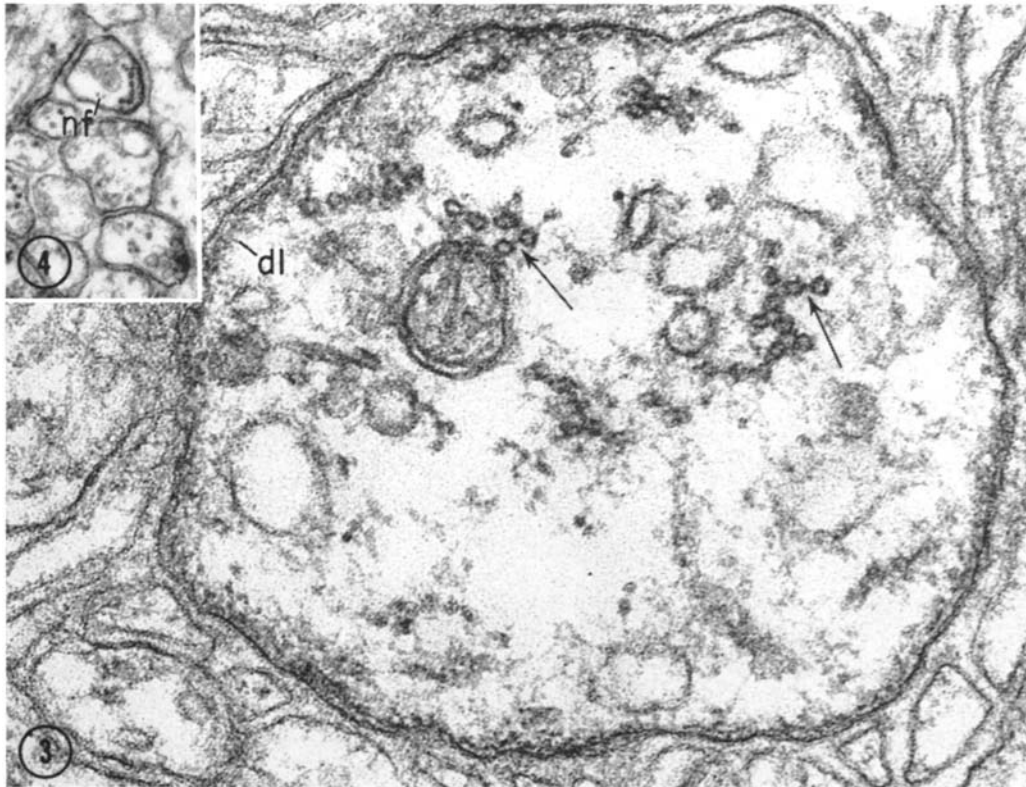
FIGURE 3 Cerebellar cortex, rat. Transverse section of the initial segment of a Purkinje cell, showing the dense granular layer (*dl*) underlying the axolemma, and the fascicles of microtubules. Slender cross-bars uniting neighboring microtubules in a fascicle are indicated by arrows. × 104,000.

FIGURE 4 Cerebellar cortex, rat. Transverse section of the initial segment of a granule cell. This axon is accompanied by other granule cell axons already beyond their initial segments. The initial segment contains a fascicle of five microtubules as well as four neurofilaments (*nf*) and a larger vesicle. The axolemma at this magnification appears thicker and denser than in the other axons in the field. × 31,000.

arrayed close together in a curving and sometimes branching line (Figs. 3, 4, and 6). Single or isolated microtubules are rarely encountered in the initial segment. Favorably oriented transverse sections show that the microtubules within the fascicles are bound together by thin, dark cross-bars or arms (Figs. 3 and 6).

The bundling of the microtubules ceases abruptly at the beginning of the myelin sheath. Whether they continue down the axons as isolated microtubules or are replaced by new tubules beginning in this region could not be determined from the sections that we examined.

Although the axon hillock and the beginning of the axon fail to stain with basic dyes, clusters and rosettes of ribosomes do occur in the axon

hillock and, in diminishing quantities, throughout the length of the initial segment. Apparently they are not numerous or concentrated enough to produce a basophilia that is recognizable in the light microscope. The ribosomes are usually, but not always, associated with a tubule or two of the endoplasmic reticulum. At the beginning of the myelin sheath they disappear while the endoplasmic reticulum continues in its agranular form throughout the axon.

Other cytoplasmic components of the axon, the neurofilaments, the mitochondria, multivesicular bodies, and various vesicles, all pass into the axon from the axon hillock without undergoing any distinctive change in their appearance or aggregation. Near the apex of the axon hillock the mito-
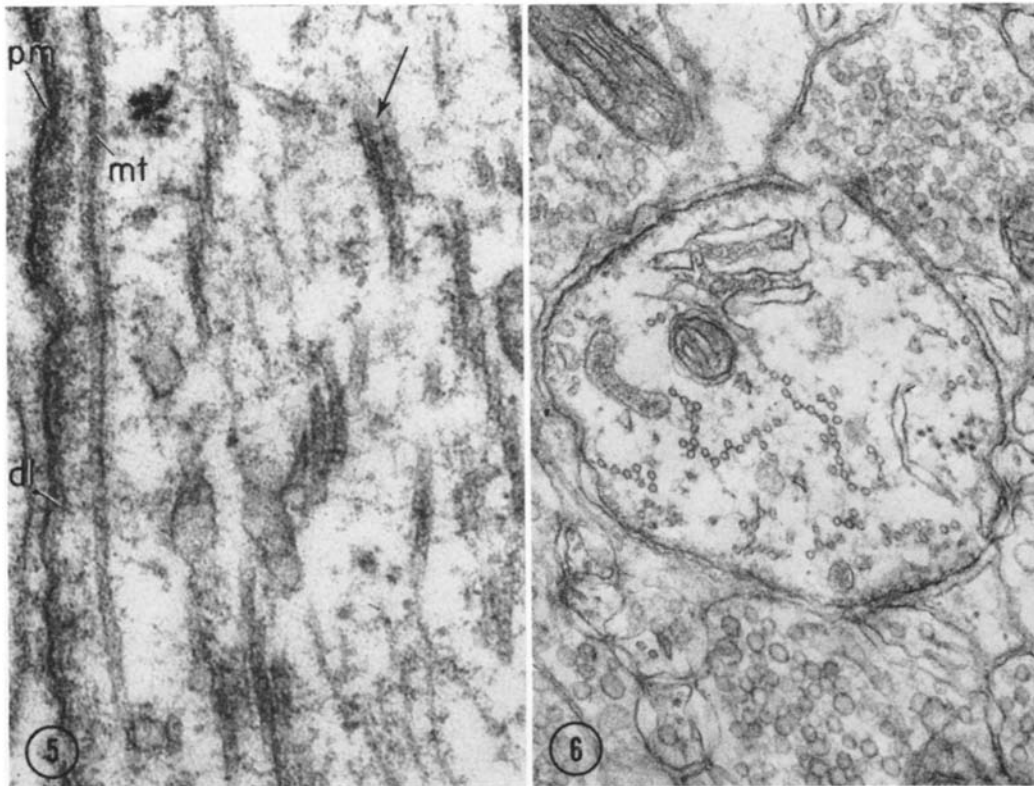
FIGURE 5 Lateral vestibular nucleus, rat. Initial segment of the axon of a Deiters cell. A dense granular layer (*dl*) underlies the plasma membrane (*pm*) of the axon. This undercoating varies in thickness and density from place to place. Bundles of microtubules (*mt*) are shown in the axoplasm, and cross-bars between them are indicated by an arrow. X 83,000.

FIGURE 6 Cerebral cortex, rat. Transverse section through the initial segment of the axon of a pyramidal cell. Fascicles of microtubules are large, and cross-bars between microtubules are shown. X 50,000.

chondria, neurofilaments, microtubules, and endoplasmic reticulum all assume a remarkably parallel orientation as they funnel into the narrow initial segment.

It is common to find synaptic boutons attached to the surface of the perikaryon at the axon hillock, but they are unusual on the surface of most initial segments. For example, in sections through the initial segments of some 60 different Purkinje cells only one synapsing bouton was found. In contrast, nearly every section through the initial segment of the cerebral pyramidal cell shows an attached bouton (Fig. 7). No examples have yet been encountered of initial segments studded with boutons like the dendrites and perikaryon of certain cells.

In a few cases in which the apposition between

an axon terminal and the initial segment was caught in a favorable plane of section, it was possible to see that the typical undercoating of finely granular material was interrupted at such sites and that the surface of the axon reverted to the appearance it normally has in the inter-nodal segments. Only at the location of the "synaptic complex" or "active zone" was there a deviation from the normal, here resulting from the aggregation of fine filamentous material that formed the postsynaptic density. The typical undercoating of the initial segment resumed beyond the margin of the apposing terminal. If a neuroglial process was inserted between the terminal and the axon, breaking the apposition, the typical undercoating reappeared beneath it. In our material the number of cases in which we
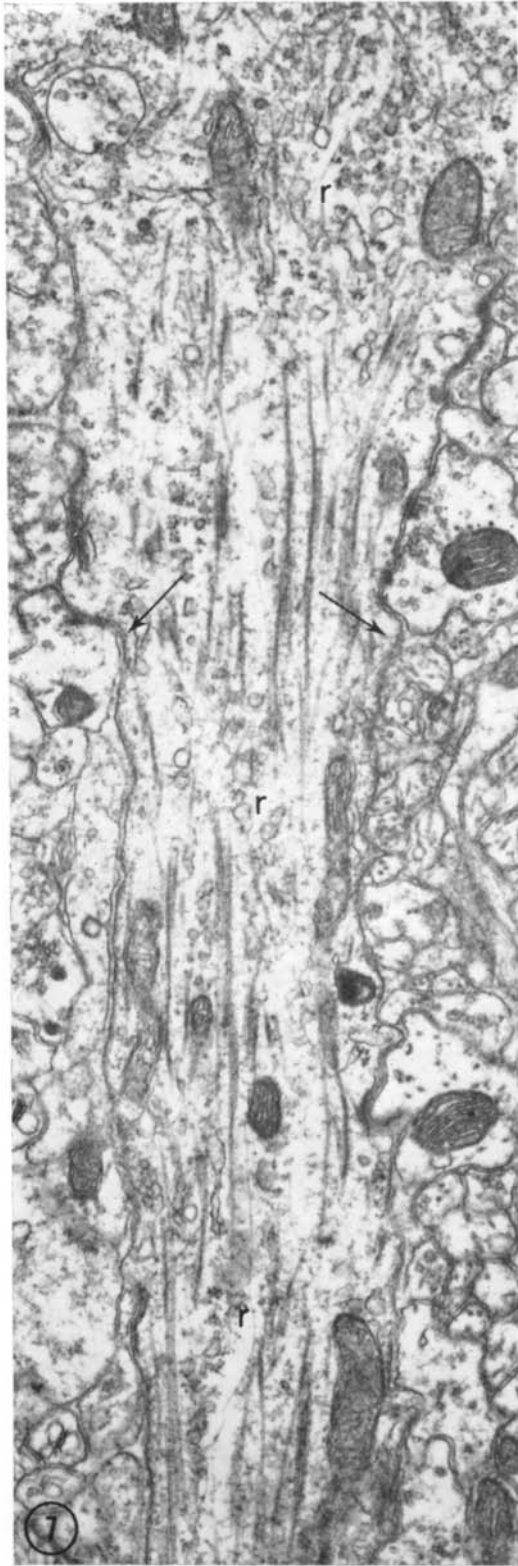
could clearly follow both the pre- and postsynaptic membranes was too small to allow us to generalize this description with assurance.

DISCUSSION

Identification of the Initial Segment

Because each neuron has only one axon, the axon hillock and the initial segment have been difficult to find in sections examined with the electron microscope. Only a few reports have appeared in which they have been mentioned. Laatsch and Cowan (14) point out that they could identify the initial segment of granule cells in the dentate gyrus only when continuous with the perikaryon. In a study of the prepyriform cortex Westrum (27) was unable to distinguish with certainty between preterminal unmyelinated portions of afferent axons and the initial segments of indigenous pyramidal cells. In both of these reports the authors noted the occurrence of a few ribosomes in the axoplasm.

Robertson et al. (20) published two electron micrographs of the initial segment of the Mauthner cell showing sheaves of microtubules associated with a finely granular axoplasmic material, but they did not study this part of the cell closely. A recent report (10) on the axon hillock and initial segment of neurons isolated from the lateral vestibular nucleus of the ox seems to be a case of mistaken identification.

The initial segment of the Purkinje cell axon has proved rather easy to find. Herndon (9) published a micrograph showing the main stem dendrite leaving from the apical pole of the peri-karyon and the slender axon leaving from the base. But the segment of axon was too short and the magnification of the micrograph was too small to permit one to analyze the structure of the initial segment. The peculiar disposition of microtubules in the initial segment of the Purkinje cell axon in the rat was described by Palay (16)

FIGURE 7 Cerebral cortex, rat. Longitudinal section through the initial segment of the axon of a pyramidal cell. At the top of the figure the axon leaves the axon hillock and the initial segment begins at a level indicated by the arrows, where the characteristic undercoating appears beneath the plasma membrane. Microtubules collect into fascicles near the beginning of the initial segment. Ribosomes (r) occur at various levels. A small terminal synapses on the axon at the tip of the hillock. × 27,000.

and in the frog was studied in some detail by Kohno (13). Hámori and Szentágothai (8) refer to the initial segment of the Purkinje cell as a "pre-axon" because its fine structure differs characteristically from the "true axon." But neither their description nor their electron micrographs are clear enough to provide reliable criteria for recognizing the initial segment, and indeed in one of their figures they included a profile of the Purkinje cell initial segment in a group of axonal profiles labeled "basket axons." Eccles et al. (3) published an additional micrograph of an initial segment of the Purkinje cell (cat) that displays some of the features discussed in the present paper, but they direct attention only to the ribosomes and the fasciculated microtubules, which had already been pointed out by Palay (16) and Kohno (13).

The whole constellation of characteristics belonging to the initial segment was not recognized until the present observations were made on a variety of neuronal types. The initial segment can be defined morphologically as that portion of the axon beginning close to its origin from the cell body or dendrite and characterized by three axoplasmic constituents: (a) dense granular material underlying the surface membrane, (b) fascicles of microtubules, and (c) scattered clusters of ribosomes. The structure of the initial segment is the same whether the axon is myelinated or not.

The Initial Segment and the Node of Ranvier

Clearly, the dense granular layer beneath the surface membrane of the initial segment resembles that found at the node of Ranvier (1, 4, 18). As these two regions of the axon are thought to have similar electrical properties, it is tempting to suggest that this undercoating represents a surface specialization in some way related to the production of the electrical signal. This suggestion is strengthened by the observation that the undercoating is apparently attenuated or absent beneath the axonal surface in contact with synaptic terminals. At these sites it gives way to the special and different, patchy postsynaptic densities. The undercoating, therefore, might be considered as a structural modification of the cell surface whose extent is congruent with the extent of specific electrical properties. It would be interesting to search for a similar undercoating beneath the axolemma in the preterminal stretches of sensory nerves. Another place of interest in this connection would be the branching apical dendrites of hippocampal pyramidal cells, where Spencer and Kandel (24) have shown that a booster potential originates.

In its other constituents the initial segment does not resemble the node of Ranvier, and therefore it becomes possible to distinguish even isolated profiles of the initial segment from the node, as well as from other unmyelinated axons or parts of axons. The microtubules are evenly dispersed across the node, not fasciculated as in the initial segment. If the initial segment is small, it may contain only one cluster of three or four microtubules, but the pattern is distinctive. In unmyelinated axons beyond their initial segment the microtubules appear as individuals, and an undercoating is absent. Furthermore, unmyelinated axons and nodes of Ranvier contain no ribosomes.

The appearance of ribosomes in the initial segment and axon hillock is surprising in view of the absence of basophilia in these parts of the cell. This finding demonstrates anew that there is no structural barrier to prevent ribosomes from passing into the axon. Nevertheless, it should be emphasized that the ribosomes in the axon hillock and initial segment are usually not attached to the endoplasmic reticulum and are not organized into Nissl bodies. Instead, they are sparsely disseminated in clusters and small polysomes. Perhaps the cause of the sharp reduction of basophilia and in the amount of ribosomes in the axon hillock should be sought in a local concentration of enzymes that disrupt or degrade the ribosomes.

All of the neuronal types that have a morphologically distinct initial segment are multipolar cells within the central nervous system. The origin of the axon has also been seen in ganglion cells of the dorsal roots and the acoustic and vestibular nerves of rats, and in ganglion cells of the lumbar sympathetic chain of the frog, but it does not resemble the initial segments described in this paper. These cells are bipolar or unipolar. In these sensory neurons, and perhaps in the amphibian autonomic cells, the axonal action potential originates in regions distant from the perikaryon

A SPECULATION: Although microtubules are found throughout the cytoplasm of the neuron, fasciculated microtubules are a special structural characteristic of the initial segment; they occur nowhere else in the neuron. Could they play a role in the specific function of the initial segment? At present this question can be answered only with

speculation, but it may be worth while to mention two possibilities. Many investigators have suggested that microtubules are contractile (5, 19, 22, 25). In particular, evidence is gradually mounting to the effect that those microtubules that are bound together by cross-bars are involved in contractility (6, 7, 15, 21). If we assume that the fascicles in the initial segment are contractile elements, then two apparently disparate functions of this part of the axon could be explained. First, the microtubules might provide the motive force for protoplasmic streaming away from the perikaryon into the axon. Second, and more specifically, the regulated contraction of the microtubules could change the shape of the initial segment and thus alter the configuration of the plasmalemma in this region, and consequently its permeability, with a resultant change in excitability. This suggestion links the contractility of the fascicles directly with the mechanism for electrical excitability at the initial segment. It should be pointed out that the initial segment is narrower than the axon extending beyond it and, of course, much narrower than the axon hillock. Only a very small shortening would be required to stretch the plasmalemma. The suggestion also implies that

contractions of the microtubules can be coupled with and regulated by potential changes occurring across the cell membrane as a result of synaptic activity. The funneling of current into the initial segment by the rapid narrowing of the axon hillock might be a sufficient condition to effect this coupling. Further discussion of these speculations seems premature at this time.

Note Added in Proof: The following paper has recently come to our attention: S. Conradi. 1966. Ultrastructural specialization of the initial axon segment of cat lumbar motoneurons. *Acta Soc. Med. Upsalien.* 71:281. The author points out that both the axon hillock and the initial segment contain ribosomes. He also recognized the undercoating beneath the plasma membrane of the initial segment and the large number of microtubules, but not their fasciculation.

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