General morphology of neurons and neuroglia

SANFORD L. PALAY
VICTORIA CHAN-PALAY

Departments of Anatomy and Neurobiology, Harvard Medical School, Boston, Massachusetts

CHAPTER CONTENTS

Les neurones sont des appareils générateurs et conducteurs de l'onde nerveuse. Voilà le fait fondamental.

Ramón y Cajal [(151), vol. I, p. 53]

THIS CHAPTER describes the form and internal structure of the cells composing the nervous system. These cells are divisible into two great classes: *1*) the specific parenchymal elements, the nerve cells, or neurons, and *2*) the supporting elements, the neuroglial cells. Both classes will be considered here. But as will be seen below, these cells are so highly diversified in shape, size, fine structure, and function that to define a typical cell in either class is to describe a chimera or will o' the wisp. Nerve cells, especially, deserve to be analyzed according to their specific characteristics, but they are manifestly too numerous to be considered individually even if some useful principle could be illuminated by doing so. Nevertheless nerve cells possess many features in common and it is to these qualities that the general discussions of this chapter must be confined.

It is important to note that the nervous system derives from the outermost cellular layer of the embryo, the ectoderm, and that it retains into maturity the morphological characteristics of an epithelium. That is, it consists almost entirely of cells. Intercellular space makes up only a very small proportion of its volume. Like some other epithelial organs it is invaded by blood vessels and connective tissue, but unlike these organs, the central nervous system contains relatively little connective tissue and the parenchyma is always separated from the blood vessels and perivascular connective tissue by an unbroken basal lamina, a delicate layer of fine collagen filaments embedded in a glycoprotein matrix.

In most epithelia the cells have a distinct polarity imposed by the fact that on one side they rest upon connective tissue while on the other they present a free surface. In the central nervous system a vestige of this primordial orientation is represented by the arrangement of the ependymal cells lining the ventricular cavities, and in a few places in which the wall of the brain is unusually thin, such as in the floor of the third ventricle, the original epithelial order can still be seen in the ependymal cells that stretch from the ventricular surface to the basal lamina on the external surface of the brain. But in most parts of the nervous system, the parenchymal cells are not oriented with respect to the primitive landmarks; instead they are oriented with respect to one another. This difference in orientation expresses a very important characteristic of the cells of the nervous system. In most epithelia the cells are independent morphological, trophic, and functional units. Although they are connected together by various junctional devices, their activities usually relate to exchanges across the surface which they cover and involve laterally at most only a few of their immediate neighbors. In contrast, the cells of the nervous system, although also independent morphological and trophic units with similar junctional devices, are profoundly interdependent functionally; and their func-

This work was supported by Public Health Service Grants NS03659 and NS10536, Training Grant NS05591 from the National Institute of Neurological and Communicative Diseases and Stroke, and an award from the William F. Milton Fund of Harvard University.

tional relations can extend for long distances through complex circuits of interconnections. That these connections are precisely ordered—not only grossly relating one part of the nervous system with another but also precisely disposed with respect to the geometry of each cell—indicates how far-reaching the transformation of the original embryonic epithelium has been.

NEURONAL SHAPE AS A SIGN OF FUNCTION

Nerve cells are notable for the variety of form and the extraordinary range of size that they display. No other cell type, not even the versatile macrophage, can compete with the nerve cell for sheer complexity of form. This very intricacy, the source of much difficulty for morphologists and biochemists, is intimately bound up with the function of the nerve cell. Indeed, as Ramón y Cajal puts it, "the form of the cell, insofar as it is the expression of its relations (with other cells), is consequently one of its most important attributes" [(151), vol I, p. 53].

It is the pursuit of these relations that rationalizes the effort of neuroanatomists to describe and classify nerve cells in all parts of the nervous system of different animals—attempts that entail a progressively more minute examination of the form, size, and disposition of the cells, the arborization patterns of their processes, their terminal ramifications and finest appendages, their internal fine structure, their ontogenesis, and their evolution. Such studies proceed on the premise that there exists for the nerve cell a fundamental, coherent, even ideal, form that is discernible in histological preparations despite the seemingly endless permutations displayed in nature. That ideal comprises a nucleated cell body that gives rise to two kinds of cytoplasmic processes, termed dendrites and axons. Each cell can have many dendrites, but should have only one axon. While the great majority of nerve cells in vertebrates conform to this paradigm, most nerve cells in invertebrates do not, since dendrites and axons in these animals are usually difficult to distinguish by their form alone. In vertebrates also there are nerve cells lacking a single characteristic process that can be called an axon, and there are even some well-known types in which the identification of proper dendrites is a subject of much disagreement. But these very deviations from the norm substantiate its existence in the minds of neuroanatomists at least, for they are seen as exceptions, as curiosities, that will probably be explained by a detailed functional analysis of the particular cells involved. We shall revert to this theme later when the significance of the synaptic junction has been considered.

The three major parts of the nerve cell, perikaryon or cell body, dendrites, and axon, have been regarded since the early studies of Ramón y Cajal as standard equipment of fully developed nerve cells, and, also following Cajal, the specifically neural aspects of neuronal function have been traditionally parceled out among them. For the nerve cell not only produces the nerve impulse; it also conducts that impulse over long or short distances, transmits it to other cells—other nerve, muscle, or gland cells—and is itself capable of being excited or inhibited by direct stimulation or by the activity of other nerve cells. It must possess the apparatus for generating, receiving, conducting, and transmitting the nerve impulse. To some extent these essential and characteristic functions are lodged in different parts of the nerve cell. In the first place, it must be indicated that the cell body, the seat of the nucleus, is specialized as the metabolic and synthetic center of the cell. It provides the great majority of the proteins used in the processes of the cell since the dendrites have very little and the axons have none of the apparatus necessary for protein synthesis. In addition, the dendrites and cell body appear to be specialized for receiving impulses, while the axon hillock and initial segment of the axon are specialized for generating the action potential, which is conducted by the axon to its terminals. These in turn are specialized for impulse transmission. The nerve cell therefore displays a dynamic polarity, first recognized by Ramón y Cajal (150), which is reflected in its complex form, its multiple dendrites funnelling information toward the single axon, the conducting and transmitting component.

In its original form the "law of dynamic polarization" referred to the streaming of nervous energy through the dendrites and cell body into the axon, whence it was led away to be transferred to the dendrites or cell body of another cell. As an indicator of the *direction* of information flow through the nerve cell this law still holds true in a general way, especially for those nerve cells that project their axons to distant points. It does not deal with the microanatomy and microphysiology underlying the polarity. Actually, it is the asymmetry of the synapse that results in the apparent functional polarity of the nerve cell. On closer view, it will be seen that in many nerve cells the traditional morphological subdivision into dendrites, cell body, and axon does not correspond to the expected restrictions of function, and, indeed, in many instances even the accepted morphological distinctions and subdivisions cannot be upheld at the level of fine structure. For these reasons the law of dynamic polarization must be understood as a propaedeutic principle, which applies particularly to the idealized nerve cell, modeled after such large cells as the motor neuron, but which should not be conceived of as a constraint either on the flexibility of the living nerve cell or on our ability to comprehend its mode of construction and operation.

The idea that the form of the nerve cell expresses its function extends, however, to a more recondite

principle than the simple law of dynamic polarization, which is, after all, an oversimplification. This concept is that the form of the nerve cell necessarily reflects its role in the nervous system. It cannot be otherwise, given the specific and orderly way in which nerve cells are connected together. Consider the dorsal root ganglion cell, which by way of one peripherally directed process and another centrally directed process connects the spinal cord with the surface of the body or with muscles and joints. The role of this cell is to transduce mechanical, thermal, or noxious stimuli into trains of nerve impulses and to distribute them to certain other nerve cells without loss of topographic information. The long afferent fiber with its secondary ascending and descending branches and their terminal tufts represents precisely this role of distributing primary sense data. In contrast a large pyramidal cell in the cerebral cortex (Fig. 1), with its apical and lateral dendrites, seems designed to collect information from the different sources impinging upon it in a regular spatial sequence and then to project its integrated response out of the cortex to other areas by way of a long axon with its own characteristic branching pattern. The double conical fields of the dendrites of such cells express the specificity of their connections in the different layers of the cortex. Similarly, the Purkinje cell in the cerebellar cortex is a prodigious integrator of hundreds of thousands of inputs received by way of its elaborate, fanlike dendritic tree, spread out to catch the greatest possible number of afferents. Its axon also projects out of the cortex, giving off recurrent collaterals to neighboring cells and terminating in leashes of thin, varicose fibers on the dendrites of nerve cells in the central nuclei.

Many small nerve cells display other patterns, for example, the basket cell in the cerebellar cortex, which provides inhibitory control over the Purkinje cells. Its dendrites radiate out through the cortex, receiving widespread inputs, fewer than those the Purkinje cell receives. Its axon extends laterally through a relatively narrow compass, sending out descending collaterals around successive Purkinje cell bodies and their initial axon segments as well as ascending collaterals that climb up the larger Purkinje cell dendrites. The role of the basket cell is defined by these connections, and its shape is their inevitable counterpart. A great deal of predictive value can therefore be attached to a knowledge of the shapes of nerve cells, even before the sign of their action has been ascertained by physiological means. When this knowledge has been united with a thorough analysis of their connections, the organization of the nervous system will be accessible for functional comprehension. We are far from this goal in all parts of the vertebrate nervous system, except perhaps in the cerebellar cortex.

In view of the extendedness of most nerve cells it is not surprising to find that parts of a single cell may lie in different parts of the nervous system. To take the dorsal root ganglion cell as an example again, the perikaryon and half or more of the axon lie in the

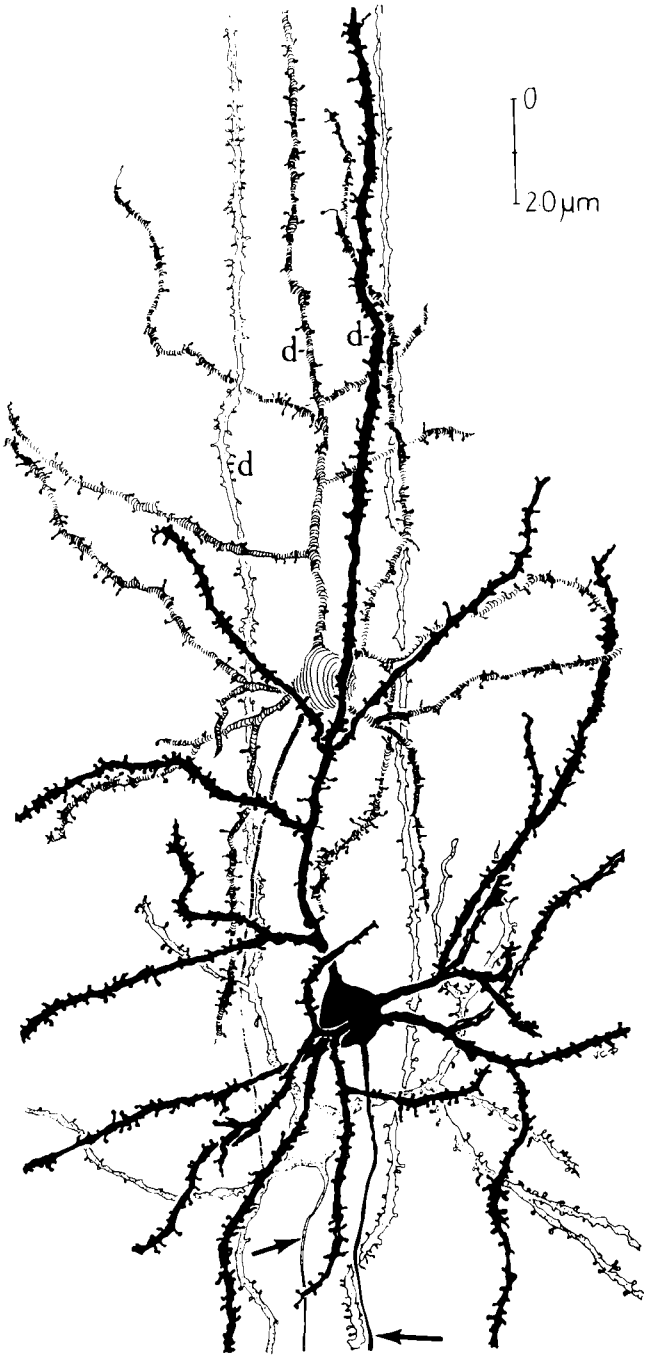


FIG. 1. Camera lucida drawing of 3 small pyramidal cells in layer 3 of cerebral cortex (area 17) of adult male *Macaca mulatta*. Cells give rise to apical and basal dendrites (*d*) of varying caliber, which display numerous spiny appendages of diverse shapes and sizes. In their ascent toward the pial surface the apical dendrites associate with those of other pyramidal cells in the same and deeper layers of cortex to form fascicles. Notice that cell bodies are also vertically aligned. A thin axon (*arrows*) springs from each cell body and descends to deeper layers. Rapid Golgi preparation.

peripheral nervous system, while the rest of the axonal tree lies in the spinal cord and the medulla oblongata. Or we may take the motor neuron with its perikaryon and dendrites in the central nervous system while its axon lies almost completely in the peripheral nervous system. On a smaller scale there is the giant pyramidal cell with its perikaryon and dendrites in the cerebral cortex and its axon coursing through the cerebral white matter, the internal capsule, peduncles, and pyramids into the spinal cord. Or in the reverse direction, the large cells of Clarke's column with their perikarya and dendrites in the spinal gray and their axons coursing through the white matter of the spinal cord up into the medulla and the cerebellar cortex. Many large cells in the reticular formation display a similar expansiveness. Less extreme examples are given by the granule cells of the cerebellar cortex with their cell bodies and dendrites in one layer, while their axons extend into and ramify in another layer. In all of these examples the dendrites and cell bodies occupy a relatively restricted territory from which the axons lead away to arborize at a greater or lesser distance.

Although this is a common pattern—and it is hard to conceive of a nervous system without such long or short through lines—there are also large numbers of cells, like some of the stellate cells in both cerebral and cerebellar cortices and in the reticular formation, whose axons arborize in the same field as their dendrites. It will be evident from the considerations set forth above that a cell with its dendrites and axon arborizing in the same circumscribed territory will have a different pattern of connections from that of a cell with its dendrites and axon deployed in widely separated fields. Therefore, the functional roles of these two kinds of cells must be different. The first cell, receiving much the same inputs as do the cells with which its axon articulates, should have a local, modulating, regulatory influence; whereas the second cell, receiving different inputs from those of the cells on which its axon terminates, should have a more critical role, performing more or less complex integrations and conveying them to distant points for further processing.

This quality of extensiveness is the basis for a simple classification of nerve cells first proposed by Golgi (53). He divided nerve cells into two categories: type I, those whose axons project out of the immediate surround of the cell body to distant sites, and type II, those whose axons arborize in the vicinity of the cell body, within a circumscribed field. This classification has proved useful as a quick, abbreviated description. To these minimal qualifications other characteristics can be added. The axons of type I cells are usually large and myelinated, while those of type II cells are usually slender and unmyelinated. This is consistent with the usually small size of type II cells. Size alone is, however, not a reliable criterion, for many type II cells are large enough to be confused with type I cells in the same region (e.g., in the cerebellar cortex, Golgi cells and Purkinje cells). The minimal requirements of this classification demand that the pattern of the axonal arborization of the cell in question be known. Since Golgi's classification does not take into account the varieties of dendritic pattern, it does not lend itself to rational fractionation. Other classifications, such as that of Ramón-Moliner (147), based upon dendritic pattern alone, also lack the coherence that a description of the whole cell would require. It must be admitted that the enormous diversity of form has proved an insuperable impediment to the construction of a satisfactory scheme for the classification of nerve cells.

SIZES OF NEURONS

Nerve cells, even within a single organism, vary greatly in size. In vertebrates the smallest cells, represented by the granule cells of the cerebellar cortex, are only about 5 or 6 μm in diameter, measured across the widest part of the perikaryon. Similar small cells are found in great numbers throughout the central nervous system. Especially well-known places in addition to the cerebellar cortex are the retina, the olfactory bulb, the cerebral cortex, and the substantia gelatinosa of the spinal cord. Large cells, like the dorsal root ganglion cells, the ventral horn motor neurons, and Betz cells in the motor cortex are much more widely known, because of their spectacular size. They may be 25–100 μm in diameter, depending upon the cell and the species in which it occurs. Other examples of large cells are the Deiters cells in the lateral vestibular nucleus, the Purkinje cells in the cerebellar cortex, the Meynert cells in the visual cortex, and the gigantic cells in the reticular formation. Such cells are often cited in textbooks as typical examples of the ideal nerve cell; they are, however, highly atypical, since they represent only a small percentage of the total neuronal population in vertebrates. Enormous nerve cells are found in some aquatic vertebrates, for example the Mauthner cells in the medulla of fishes and amphibians, the Müller cells in cyclostomes, and the gigantic neurosecretory cells in the spinal cord of skates and rays. In many invertebrates, certain nerve cells can be immense and increase in size with age. For example, in the visceral ganglion of *Aplysia*, cells 400–1,000 μm in diameter occur, along with small and middle-sized cells like those in vertebrates (16, 23, 158).

But it must be confessed that these examples give a false idea of the true size of the nerve cell. For most neurons, the perikaryon, where the measurements are easily made, represents only a small proportion of the whole cell. Some famous calculations were given by Heidenhain (61) in order to emphasize this fact. He selected a large dorsal root ganglion cell, 120 μm across, having an axon 12 μm across. Assuming the

cell body to be spherical, he calculated that its volume would be 864,000 μm^3, while its axon, if 1 m long, would have a volume of 108,000,000 μm^3. The ratio between these two volumes being 1:125, it can be seen that the perikaryon contains only 0.8% of the neuronal protoplasm and that the greatest part of it lies in the axon. The collaterals and terminal subdivisions of the axon have been neglected in the calculation. If they had been considered, the proportion of the cell occupied by the perikaryon would be still further diminished. Even if a smaller axon is chosen, for example an axon 6.4 μm in diameter, its volume would amount to 32,000,000 μm^3 or 35 times the volume of the cell body. In this example the perikaryon would occupy about 2.6% of the total neuronal volume, excluding the terminal axonal ramifications. Similar calculations for other large cells, such as the Betz cell in the mammalian motor cortex or the Mauthner cell in the teleost medulla, would give comparable results, thus demonstrating that even in very large neurons the perikaryon occupies a relatively small proportion of the whole cell. At the opposite extreme, among the smallest cells, the proportionate volumes occupied by the axon and the cell body are more in balance. For example, in a cerebellar granule cell, which has a cell body 6 μm in diameter and an axon 0.2 μm in diameter and 6 mm long, the volume of the cell body is about 100 μm^3, while the volume of the axon is about 180 μm^3 or only 1.8 times that of the perikaryon.

An even more impressive differential emerges when the surface areas of the perikaryon and axon are compared. In the first example given above, the dorsal root ganglion cell, considered as a sphere 120 μm in diameter, has a surface area of 43,200 μm^2. If the axon is 1 m long and 12 μm in diameter, it has a surface area of 36,000,000 μm^2, or 837 times the area of the cell body. If the axon is 6.4 μm across, as in the second example, its surface area would be 19,000,000 μm^2 or 441 times the surface area of the cell body. In the granule cell with a cell body 6 μm in diameter, its surface area is 108 μm^2, while the axon has a surface area about 3,800 μm^2, or more than 35 times that of the cell body. Since the cell types chosen for these calculations have either no dendrites or very short ones, the dendrites have been neglected. In many cell types, however, the dendrites expand the cell surface enormously while increasing the volume of the cell comparatively little. For example, in the giant cells of the reticular formation in the cat, Mannen (102, 103) found that the surface area of the dendritic tree was five times that of the soma. Similarly, Mungai (113), measuring pyramidal neurons in the somatosensory cortex of the cat, found that the cell body occupied only 4% of the total surface of soma and dendrites, and the small appendages of the dendrites, the spines, alone took up 43% of this total area.

The vast expansion of the neuronal surface achieved by extending axons and dendrites from the cell body underscores the importance of the cell surface for the specific function of the nerve cell. It is by way of its surface that a nerve cell connects with other cells and it is also by way of this surface that it carries on the physicochemical exchanges that underlie its integrative activity. The form of this surface (reflected in the shape of the cell), its extent, its apportionment among soma, axon, terminals, dendrites, and dendritic appendages—all the variations that permit the morphologist to recognize nerve cell types or, in some cases, even individual nerve cells—are the structural concomitants of the function of the cell in its context.

CYTOLOGY OF NEURONS

Soma or Cell Body

Structural compartmentation of functions is more explicit in nerve cells than in most other cells. Although the soma constitutes only a small proportion of the volume and surface of the nerve cell, it is charged with the task of maintaining and directing the metabolism of the entire cell. That the electrophysiological functions of the soma can be subsidiary is suggested by the existence of sensory root ganglion cells and certain other neuronal types in vertebrates and numerous ganglion cells in invertebrates that have no synaptic junctions on their somatic surfaces. These, however, must be contrasted with vertebrate motor neurons and certain cells of the reticular formation whose somatic surfaces are covered with thousands of synapsing terminals. Although generalization is hazardous, it should be pointed out that depending upon the geometry of the cell, the soma may not be invaded by the action potentials generated in the axon. Even in such cases, the soma should not be denied an important role in neural affairs on account of this apparent deficiency in electrical behavior. Lasting modifications in the nerve cell, such as those that underlie learning, memory, reasoning, and other activities of a high order, must be coupled with the responses of the internal machinery of the individual cells involved. The soma is first of all a metabolic and synthesizing center. It not only contains the nucleus of the neuron but it also concentrates within it borders nearly all of the machinery for protein synthesis. Therefore, it is to be expected that the cell body should exhibit a distinctive internal structure that is not shared by the processes of the neuron. And on this account it is the soma of the nerve cell, containing all of the organelles of typical metazoan cells, that most resembles other cells, particularly those with abundant protein-synthesizing machinery (Figs. 2 and 3).

NUCLEUS. The nucleus of nerve cells is characteristically large, filling most of the soma in all but the largest cells. For example, in cerebellar granule cells and in cerebral stellate cells, the nucleus barely

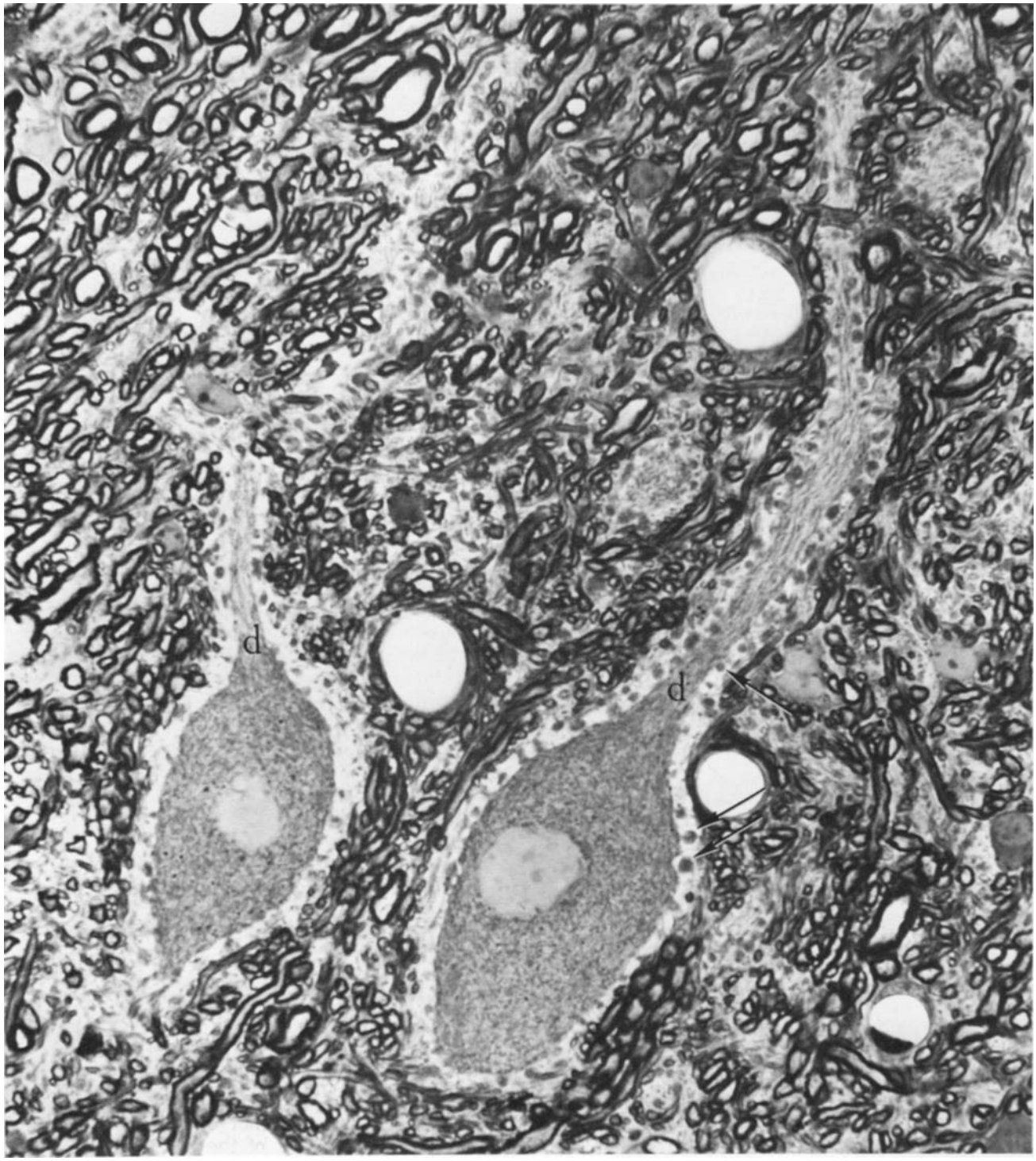


FIG. 2. Two large nerve cells in dentate nucleus of cerebellum of adult male *Macaca mulatta*. These nerve cells, immersed in field of heavily myelinated nerve fibers, emit heavy dendrites (*d*). Both cell body and dendrites are covered with spherical *boutons terminaux* (*arrows*). Within cells a dense population of granules and long mitochondria can be seen. Epon, 1.5-μm section, toluidine blue. × 1,300.

leaves space for a thin shell of cytoplasm. In the much larger Purkinje cells or pyramidal cells, as in motor neurons, the cytoplasm is more ample and the nucleus is also larger than it is in smaller cells.

The neuronal nucleus is usually described as vesicular, because it appears pale in preparations for the light microscope that have been stained with basic dyes. The nuclear membrane is sharply delineated

and the deeply staining nucleolus forms the most salient feature within it. On close inspection one or two lightly basophilic densities can be discerned, often attached to the nucleolus. Marginated condensations attached to the nuclear envelope can also be seen sometimes. This pallor apparently reflects the extended state of the chromatin and the dilution of the nucleoprotein in the nerve cell nucleus. For despite its large size, it contains the normal diploid complement of DNA in most instances.

Exceptions to this general rule have been reported for a variety of neurons in the vertebrate central nervous system: mature Purkinje cells, Betz cells, hippocampal pyramidal cells, and some large ventral horn cells. These neurons were said to possess the tetraploid amount of DNA (65, 66, 84, 105, 116, 161). All of them are large cells with large nuclei. In certain invertebrates, such as *Aplysia,* where even larger cells are known, an astounding polyploidy of over 75,000 has been reported according to one method (24) and of over 200,000 according to another method (87). More modest increases occur in other invertebrates; for example, Swift (182) has shown that certain insect neurons display a ploidy of 16. The significance of polyploidy in neurons is unknown. Cellular or nuclear size is not related to ploidy in any consistent fashion as it is in liver cells (66). Furthermore, many large neurons in vertebrates have the normal diploid complement of DNA, for example, the Mauthner cell in amphibians (11) and the Deiters giant cells in mammals (161). Recently the validity of the microspectrophotometric assessments of ploidy in neurons has been criticized on technical grounds (101) and has even been denied by contradictory evidence from other analytical methods. In autoradiographic studies in young mice and rats, Mareš and co-workers (105, 106) and Manuelidis & Manuelidis (104) reported that Purkinje cells fail to take up tritiated thymidine during the period of supposedly increasing ploidy. Furthermore, Cohen et al. (25) failed to find the expected heightened concentration of DNA per cell in Purkinje cells separated in bulk from mature rat cerebella. The discrepancy between these results and the earlier microspectrophotometric estimates on which claims of tetraploidy were based have been attributed to inadequate correction for nonspecific light loss suffered by the test beam as it passes through the cytoplasm surrounding the Purkinje cell nucleus (45, 46, 101). In a careful reassessment of the DNA content of Purkinje cells and large anterior horn cells, Fujita and his co-workers (45, 46) have confirmed the criticisms by Mann & Yates (101) of the procedures used in establishing tetraploidy. By taking into account nonspecific light loss in microspectrophotometry of sections and smears they were able to show that there is no significant deviation from the diploid amount of DNA in the nerve cells of either adult or developing man or rat (45, 46).

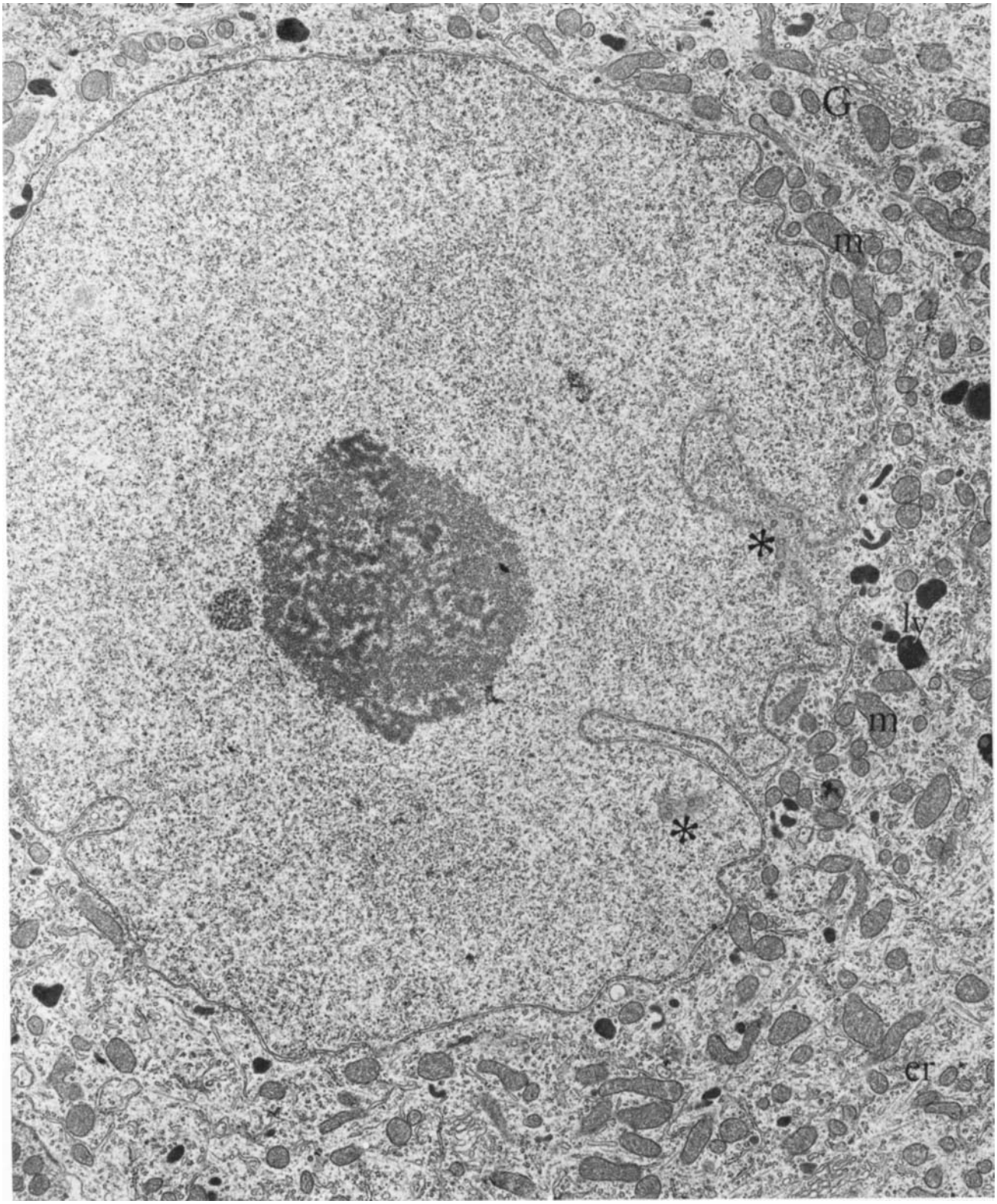
The nuclei of all nerve cell types do not, however, appear pale. The best example of the opposite appearance is given by the nucleus of the granule cell, which possesses a clock-face pattern of chromatin, similar to that of the lymphocyte or the plasma cell. The large amount of condensed chromatin in the nuclei of such nerve cells suggests that most of the chromosomal material is not so actively involved in transcription as it is in most other neuronal types (93). The nucleoli of such cells are also much less obvious in light-microscopic preparations, and the presence of nucleoli has even been denied (151).

The differences between neuronal nuclei are particularly well shown in electron micrographs. In most cells the chromatin is thinly dispersed as fine twisted threads more or less uniformly distributed over the nucleus. Small condensations usually appear just within the nuclear envelope and, as mentioned, in the neighborhood of the nucleolus, which is conspicuous because of its density and size. In the nuclei of cerebellar granule cells, however, the chromatin meshwork is condensed into large blocks of tightly coiled strands that fill most of the nucleus. In this dark field the nucleolus is hardly noticeable.

The nuclear envelope (Fig. 3), as in other cells, consists of a continuous more or less spherical cisterna of the endoplasmic reticulum (125, 131). On its inner, nucleoplasmic surface it is coated with a fine filamentous lamina, while on its outer surface it bears a small number of spiral or linear arrays of ribosomes. In some cell types the nuclear envelope is characteristically folded or creased, and the associated invagination of the cytoplasm is filled with ribosomes in polysomal aggregates. The envelope is perforated by pores, which are arranged in irregular or hexagonal arrays and which are filled with a highly organized fibrillogranular material known as the pore complex (42). The peripheral condensations of chromatin within the nucleus usually display clearings opposite the pores, as in other cell types.

In addition to the usual chromatin and nucleolus, various inclusions within the nucleus have been described. One of the most interesting inclusions is a circular or arcuate array of filaments 5–7 nm in diameter. The filaments are laid out in overlapping lamellae, within any one of which the filaments run parallel with one another and at 60° to the axis of the filaments in neighboring sheets (17, 39, 165). These inclusions correspond to curved, rodlike structures described in 1895 by Roncoroni (157) and termed *bâtonnets intranucléaires* by Ramón y Cajal (151). The most careful study of these inclusions has been made by Seïte and his colleagues (164–166), who have adduced experimental evidence strongly suggesting that in the nuclei of sympathetic neurons these inclusions become more numerous with increased neural activity.

It should be emphasized that the nerve cell is incapable of undergoing cell division. When nerve cells in the mature animal are severely injured or die, no


G
m
*
ly
m
*
cr

replacements are available. Mitosis in the nerve cell line ceases very early in the life history of the neuroblast. Once differentiation in the direction of a neuron has begun, mitotic potential is suppressed or lost, and the cell cannot revert to a more plastic state in which mitosis may be initiated. This characteristic has provided a valuable tool for the elucidation of development and the tracing of cell migrations during maturation of the central nervous system. Cells can be marked by the uptake of tritiated thymidine during the last mitotic cycle before differentiation. They then retain their label for the remainder of their lives and can be identified by autoradiography (170). In appropriately timed sequences of developing animals the regular succession of nerve cells in the layers of the cerebral or cerebellar cortices can be followed and the migration of neuroblasts from their sites of origin to their definitive stations can be traced by means of the radioactive label introduced at the time when DNA was last synthesized in the ventricular zone (ependyma) (5, 10, 110, 183).

The stage in ontogeny when the last mitotic cycle of a particular nerve cell precursor occurs varies considerably according to location in the central nervous system and cell type. In most mammals, all mitotic activity in neuronal precursors generally ceases at the time of birth or soon afterward. However, in certain areas some cell types continue to proliferate for weeks or months after birth. Two well-known examples of cells undergoing postnatal divisions are the granule cells in the cerebeller cortex (44, 110) and in the dentate gyrus (4). The time of birth is not magically related to the mitotic index of the central nervous system of the fetus but is related to complex endocrine interactions. The irrelevance of the time of birth is indicated by the wide range in maturity of the central nervous system at birth in the mammalian series, for example, as in the opossum compared with the guinea pig.

PERIKARYON. Strictly speaking, the term *perikaryon* signifies the cytoplasm surrounding the nucleus of the neuron and excluding that in the processes. Often the term is used interchangeably with the words cell body or soma, but it does not include the nucleus of the cell. The cytoplasm of the nerve cell contains all of the organelles usually seen in epithelial cells (Fig. 3). It is convenient to start a description of the cytoplasm with the large cells, in which it is voluminous and elaborate. In such cells the cytoplasm is permeated by a coarse network of flocculent basophilic material called the Nissl substance, which corresponds to the ergastoplasm of gland cells and is the protein-synthesizing apparatus of the cytoplasm (33). It consists of the granular endoplasmic reticulum and associated free polyribosomes (125).

As may be seen in Figure 4, motor neurons and other large neurons display large masses of Nissl substance, which have a highly ordered fine structure. The cisternae of the endoplasmic reticulum are arranged in imbricated arrays, seven or eight deep, with numerous anastomoses among them. The attached ribosomes suspended in the cytoplasmic matrix between the cisternae are almost all clustered into small rosettes of six to eight members. In smaller nerve cells and in some of the larger ones, such as the Deiters cell in the lateral vestibular nucleus, the Nissl bodies are all small and exhibit much less orderly fine structure. Nevertheless, the elements are the same, often only a meandering tubule or folded cisterna of the endoplasmic reticulum accompanied by a swarm of ribosomes in rosettes. Such small and irregular Nissl bodies also occur in large cells among the more orderly examples (Fig. 4). When the cytoplasm is ample, as in Purkinje cells and motor cells in general, the Nissl substance is arranged in two concentric zones around the nucleus. The second zone lies more peripherally, although usually avoiding the region immediately beneath the plasmalemma. Small strands of Nissl substance extend across the intervening clear zone. The smaller nerve cells seem to lack this dual lamination since they do not have space for the outer layer.

Typically the Golgi apparatus occupies the clear zone between the two basophilic zones. This organelle encloses the nucleus of the nerve cell in a cagelike reticulum. With the electron microscope it can be seen that it consists of tightly packed, parallel, fenestrated cisternae and a host of associated small vesicles and vacuoles. It is extensively developed in nerve

FIG. 3. Perikaryon of large cell in dentate nucleus of cerebellum of adult male *Macaca mulatta*. Folded nuclear envelope encloses a dilute and fairly homogeneous karyoplasm, in which the large dense nucleolus is conspicuous. Beside the nucleolus is a fragment of condensed heterochromatin, the nucleolar satellite or Barr body. Section passes obliquely through nuclear envelope at *asterisks*, disclosing arrays of pores. In the perikaryon, cytoplasm is crowded with organelles, among the most prominent of which are mitochondria (*m*), lysosomes (*ly*), Golgi complexes (*G*), and the granular endoplasmic reticulum (*er*). Neurofilaments and microtubules can be seen here and there. Notice that mitochondrial profiles occur in groups, indicating that each cluster represents a single mitochondrion of complex configuration. × 11,000.

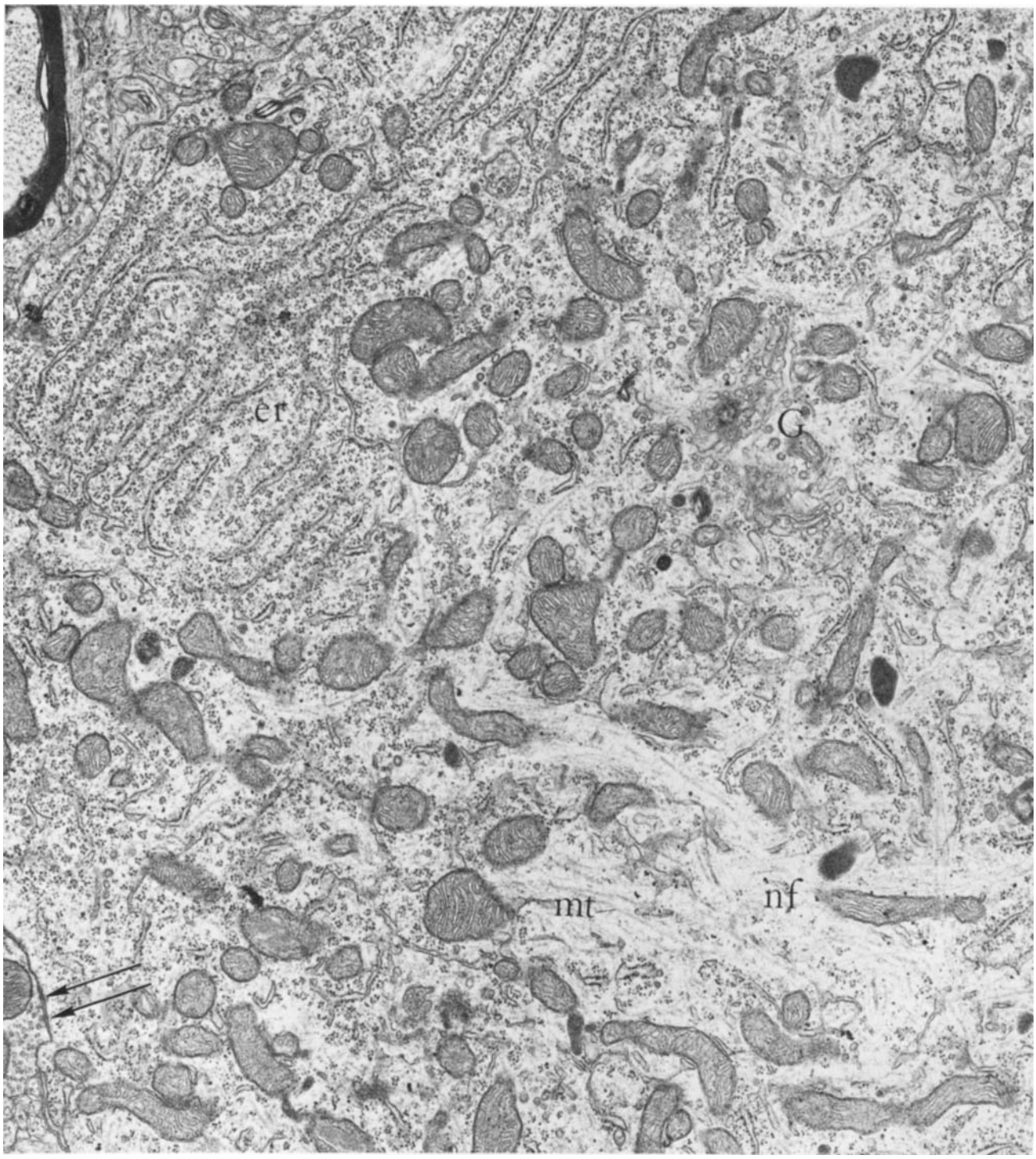


FIG. 4. Cytoplasm of a Meynert cell in cerebral cortex of adult male *Macaca mulatta*. Well-developed Nissl body lies in left upper corner of field, displaying its constituent cisternae of endoplasmic reticulum (*er*) and both attached and free ribosomes in polysomal array. Clustering of mitochondrial profiles is well shown and the fragment of Golgi apparatus (*G*) included in the field exhibits typical fenestrated cisternae. An open space to the right of center contains microtubules (*mt*) and neurofilaments (*nf*) aligned in *Plasmastrasse*. Axosomatic synapse with a terminal containing flattened vesicles occurs at lower left (*arrows*). Area 17. × 13,000.

cells. Only one cisterna on the outer, or forming, face impregnates with osmium or silver in the usual methods for demonstrating the Golgi apparatus at the light microscope level. Examination of thick sections with the high-voltage electron microscope shows that the disconnected stacks of cisternae usually seen in

thin sections form a continuous three-dimensional network lying in the intermediate circumnuclear zone (38, 146). Furthermore, this method confirms the observations in thin sections that the stacked cisternae of the Golgi apparatus are fenestrated in a regular pattern, or, to put it another way, they are composed of a regular lattice of continuous tubular elements (146). The deeper face, the so-called maturing face, of the Golgi apparatus contains thiamine pyrophosphatase and glycoprotein (117, 145, 185). In nerve cells in which the Golgi apparatus is elaborate, as in the motor neurons or the Purkinje cell, it is not easy to determine which face is maturing and which is forming without applying histochemical tests. In many small neuronal types, however, the Golgi apparatus is restricted to a single locus, for example, within a depression in the nucleus or at the base of a large dendrite. In such cases it is possible to speak of a cytocenter or centrosphere similar to that found in undifferentiated cells in embryonic tissues. Such a centrosphere comprises a cup-shaped array of Golgi complexes centered on a pair of centrioles and their associated microtubules.

Another characteristic feature of the neuronal perikaryon, and probably the only organelle that is specifically neural, is the neurofilaments, which course singly or in bundles through the cytoplasm and into the processes (Fig. 4). These threadlike structures consist of a single acid protein with a molecular weight of about 50,000 (28, 200). Amino acid analyses indicate that it is quite distinct from the protein found in microtubules and actin (28, 70). The protein occurs in globular subunits assembled into a coiled thread about 10 nm in diameter. The substructure has been described as consisting of tetramers stacked one on top of the other but staggered so that a helix is generated about an empty core measuring 3.5 nm in diameter (199). The neurofilaments usually run in the open spaces among the Nissl bodies and the Golgi complexes—the *Plasmastrassen* of Andres (3).

At the level of the light microscope, certain silver stains reveal a network of fine fibrillae permeating the cytoplasm of the cell body and its processes. These neurofibrillae have long been considered characteristic structures of nerve cells. By comparing nerve cells stained with these silver methods with material stained with basic dyes, it becomes evident that the pattern of the basophilic Nissl substance is complementary to the pattern of the argyrophilic neurofibrillae. The latter run to a large degree in the open spaces among the Nissl bodies, in other words, in the *Plasmastrassen*. Correlated observations of this kind suggested that the neurofibrillae are bundles of neurofilaments stained with silver (125), and Gray & Guillery (58) have given substance to this suggestion by their observations of annular fascicles of neurofilaments in electron micrographs of nerve endings that display ringlike neurofibrillae in silver preparations at the light microscopic level.

Neurofilaments, however, are not the only structures in the *Plasmastrassen*. As may be seen in Figures 3 and 4, various other organelles are also found there: microtubules, mitochondria, multivesicular bodies, assorted vesicles, lysosomes, and pigment granules. All of these organelles are found in other cell types and do not differ importantly from their congeners in such cells. The mitochondria deserve a special word because they vary widely in shape and size from one neuron to another and even within the same neuron. Elongated, bacillary shapes and subspherical, granular shapes occur side by side in the same cell (Fig. 3); giants and dwarfs are often neighbors. Furthermore complicated digitate or labyrinthine forms are quite common, and these yield highly varied profiles in thin sections. Generalizations concerning typical neuronal mitochondria are hazardous. Nevertheless, the fine structure of the mitochondria in nerve cells displays two peculiarities. Longitudinal, shelflike cristae are common in long mitochondria, although they are not the exclusive type, and the dense matrix granules that accumulate calcium phosphate (apatite) as a concomitant of oxidative phosphorylation are much less common in neuronal mitochondria than they are in other cells, such as renal tubular cells, liver cells, or striated muscle cells (88, 125, 131). The matrix of neuronal mitochondria is generally denser than that of other cells, but this is a quality that is quite sensitive to preparatory procedures.

It should be noted that in living cells the smaller organelles, especially the mitochondria, vesicles, and granules, appear to be in continuous motion, gliding, jumping, or slithering along the *Plasmastrassen*, like vehicles in crowded city streets (140). The mitochondria seem to be confined to narrow channels, in which they move forward or backward with slow, vermiform movements or with sudden jerks. They also undergo continuous changes in shape and size, varying from a single, long strand to a row of granules, or coalescing to form a large sphere, or coiling about into circles or cup-shaped and looped forms. Cinematographic records of nerve cells maintained in culture chambers show that the nerve cell is far from static internally and make one skeptical of attempts to quantitate mitochondria by counting them in either electron micrographs or subcellular fractions. The nucleus also appears to be rotating within its envelope. In view of what can be seen in these living cells, there is no reason to suppose that the Nissl bodies, Golgi apparatus, microtubules, and neurofilaments are completely quiescent. Studies with radioactive labeling [e.g., (85, 86)] indicate that proteins produced in the cell body are transported into the processes (see the chapter by Grafstein in this *Handbook*). The mechanism for this flow is apparently dependent upon the microtubules and perhaps other longitudinally oriented structures (7).

Dendrites

Most nerve cells give off one or several tapering

processes that branch repeatedly at acute angles to the parent stem (Fig. 1). These processes, the dendrites, begin as smooth, funnel-shaped extrusions of the cell body (Fig. 2) and become narrower with each successive subdivision. They can be relatively straight or gnarled and highly contorted, they can be long or short, they can branch profusely or sparsely. As has already been indicated, the form of the dendritic tree is directly related to the role of the cell in the organization of the nervous system, for it defines the field from which the cell receives its afferent input. Therefore, the pattern of the dendritic tree is characteristic of the cell type in much the same fashion as the branching pattern of a tree trunk is characteristic of the species.

Different cell types also differ widely in the extent to which their dendrites are furnished with secondary appendages. These are given the generic name spines or thorns, although their form ranges from long, thin filipodia to short, sessile knobs. A common form is a rounded bulb tethered to the shaft of the dendrite by a thin stalk. Some neurons, most dramatically exemplified by the Purkinje cells and the cerebral pyramidal cells, possess large numbers of spines; others, for example, cerebellar basket cells and spinal cord motor neurons, have relatively few spines. After their discovery by Ramón y Cajal (149) there was considerable disagreement concerning the nature of these appendages, which are beautifully displayed by the Golgi method but are invisible in reduced silver preparations. Opinions varied from the false idea that they are axonal terminals to the correct idea that they are miniscule branches of the dendrites, and there was a strong suspicion that they were inconsequential artifacts. As late as 1957 Fox & Barnard (41) still felt it necessary to justify their study of the spines on Purkinje cell dendrites with the explanation that they *believed* spines to be integral parts of the dendrites. In 1959 Gray (55) demonstrated conclusively by means of electron microscopy that spines are projections from the shafts of dendrites and are the sites of axodendritic synapses. Synapses also occur, however, on the shafts of dendrites. The differences between these two sites and the types of synapses on them will be discussed after the fine structure of dendrites and synaptic terminals has been described.

As is suggested by optical microscopy of sections stained with basic dyes, dendrites usually begin as simple elongations of the perikaryon (Fig. 2). The cytoplasm with all of its organelles flows out into each process. After a short distance, however, the cytoplasm is no longer stainable, and except for triangular clumps of Nissl substance lodged at the branch points, the dendrite becomes a clear, colorless track that with successive subdivisions is soon lost to view in the weakly staining neuropil. In reduced silver preparations it is often possible to trace the dendrites for longer distances by following the neurofibrillae as they course into the secondary and tertiary branches, but in many cell types they, too, disappear quickly into the confusing neuropil.

The internal structure of dendrites and their subdivisions can best be brought out in electron micrographs. In the proximal portions of the major trunks the Nissl substance, Golgi apparatus, mitochondria, microtubules, and neurofilaments are all drawn outward, rapidly assuming a more or less linear orientation parallel with the longitudinal axis of the dendrite. The Golgi apparatus does not continue much beyond the first subdivision, and the Nissl substance becomes progressively less massive until it is represented by only an occasional curved tubule of the endoplasmic reticulum in the midst of clustering ribosomes. In the more distal branches the Nissl substance disappears altogether, unless a few polysomes should still be designated by that name. The progressive dilution of the Nissl substance with distance from the perikaryon accounts for the clarity of the dendrites in ordinary histological preparations.

The other organelles remain in place, all longitudinally arranged and becoming the typical components of the dendrite just a short distance from its origin. These organelles are the microtubules, mitochondria, and the agranular endoplasmic reticulum. The last appears in the form of long tubules exhibiting irregular dilatations and branches, but running through the dendrite from its origin to its terminal branches and sending offshoots even into the spines. In some cells, for example, Purkinje cells, the reticular arrangement of the tubules is exaggerated so that the dendrites appear to be crisscrossed by a system of anastomosing, membrane-limited channels. In the larger dendrites the reticulum also approaches close to the inner side of the surface membrane to form subsurface cisternae as it does in the perikaryon. In the Purkinje cell dendrites these subsurface cisternae are specialized parts of the more general hypolemmal cisterna that is so highly developed in this cell type (124). The mitochondria elongate into extended flexuous forms, lined up in the peripheral cytoplasm of the larger dendrites. As the subdivisions produce narrower and narrower branches, the mitochondria appear to become more concentrated, so that in the finest branches there seems to be little room for anything else. Mitochondria do not usually enter the dendritic spines.

From the cell body onwards the microtubules are conspicuous components of dendrites. In the large trunks they run longitudinally, uniformly spaced over the cross section of the dendrites, and deviating in their course only to avoid mitochondria and other organelles. In some instances they pass through fenestrae in cisternae of the endoplasmic reticulum that lie athwart their path. At branch points a contingent swerves out of the main stream to enter the branch, while the rest continue on their way. Microtubules do not, however, enter into dendritic spines. Neurofila-

ments also occur in dendrites, especially the larger ones, but they are not so prominent as the microtubules. Usually they appear as individuals or parallel groups of two or three. In the large dendrites of spinal motor neurons (201), Betz cells (72), and Meynert cells in the cerebral cortex (20), large numbers of neurofilaments occur. Like the microtubules, neurofilaments do not branch but are distributed to the successive subdivisions of a dendrite in proportion to the caliber of the subdivision. The smaller dendrites generally contain few or no neurofilaments.

Although dendritic spines have a variety of shapes as mentioned above, they display a monotonous internal structure (131). The usual dendritic organelles, except for the endoplasmic reticulum, do not enter into the appendage. It is filled with a fine, fluffy network of microfilaments, and beneath the synaptic junction a heavy plaque of such filaments adheres to the inner side of the surface membrane. One or several tubules extend from the hypolemmal cisterna of the endoplasmic reticulum through the stem of the spine into its head where they expand into flattened cisternae. In the spines of pyramidal cells in the cerebral cortex these miniature cisternae are more elaborate than elsewhere and are recognized as a special organelle, the spine apparatus (55, 58). Two and sometimes three, apparently independent, dilated cisternae, close together or with a dense, thin plaque between them, constitute this organelle. Peters & Kaiserman-Abramof (129) have shown that the cisternae of the spine apparatus are continuous with the endoplasmic reticulum of the dendritic shaft through connecting tubules that lie in the stem of the spine. The spine apparatus, therefore, is only an extension of that ubiquitous organelle, the endoplasmic reticulum. In specimens with inadequate fixation or in certain pathological conditions such as anoxia, the spine apparatus can be retracted into the stalk of the dendrite. Further notes about dendrites and dendritic spines are included in the subsection *Synapse*.

Axon

In general each nerve cell possesses only a single axon, but examples of multiple axons are not difficult to find among sensory root (32, 61, 162) and sympathetic ganglion cells (180) and among the smaller neurons of the central nervous system, such as the Golgi cells in the cerebellar cortex (124). Usually the separate roots unite into a single axon not far from their origins. Typically the axon arises from a conical protuberance, the axon hillock, which bulges from the perikaryon. Frequently, however, the axon springs from one of the major dendrites by way of a less conspicuous projection.

At the light microscope level, in material stained with basic dyes, the axon hillock can be distinguished from the tapering dendrites by means of its striking pallor. The Nissl bodies rather abruptly diminish along the curving base of the axon hillock, and they do not intrude into the axon itself, at least so far as the light microscope can reveal. The axon emerges from the apex of the hillock as a smooth, relatively straight thread, which tends to become progressively more slender until it reaches the first segment of its myelin sheath or the first branch point. At this point the axon suddenly enlarges to its definitive caliber, which is maintained for long distances despite branching until the preterminal subdivisions are reached. Characteristically the branches, or collaterals, of axons are given off at nearly right angles to the parent stem.

The tenuous stretch of axon between the apex of the hillock and the point where it enlarges (at the beginning of the myelin sheath) is known as the initial segment. It was given this name by neurophysiologists when they discovered by means of intracellular recordings that the action potential originates in this part of the neuron (34, 48). Although the narrow caliber of the initial segment and the absence of Nissl substance had been recognized by morphologists since the last decades of the nineteenth century, these characteristics gave no clue to the nature of the functional specialization in this region. Electron microscopy has added still further particulars, but their coordination with the function of this part of the cell continues to be a subject of speculation (126). With the electron microscope it can be seen that the pallor of this region in light-microscopic preparations is due to the marked reduction in the granular endoplasmic reticulum. However, small cisternae and associated polysomal arrays are still scattered throughout it. Apparently the aggregates are not large enough to produce an appreciable basophilia at the light microscope level of examination. Clusters of ribosomes also occur in the initial segment for variable distances, in some nerve cells, down into the first myelinated segment (173, 203). The microtubules of the axon hillock veer toward its summit, and at the beginning of the axon they become bundled together into several parallel fascicles that run longitudinally through the initial segment (26, 27, 76, 121, 126, 131, 132). The number of microtubules in a fascicle varies from 3 or 4 to 20 or more. The microtubules seem to be held together by struts or crossbars at irregular intervals; they do not usually touch one another (124, 126). These fascicles of microtubules occur only in the axon initial segment and not in any other part of the neuron.

A second characteristic of the initial segment appears beneath the plasmalemma. It is a dense undercoating having a tripartite construction (18). Just beneath the surface membrane is a layer of discrete granules, 7.5 nm in diameter and spaced 9.5 nm apart in the circumferential direction and 7.5 nm apart in the longitudinal direction. These granules are seated upon a dense lamina about 7.5 nm thick, and under this is a helicoidal ridge composed of extremely fine interwoven filaments. A similar undercoating occurs

beneath the axonal plasmalemma of the node of Ranvier (131). Unmyelinated axons display the same initial segment features as myelinated axons (126). Since, however, the end of the initial segment is not demarcated in the former by the onset of a myelin sheath, the termination of the undercoating and the dispersal of the fascicles of microtubules are sufficient to distinguish it from the remainder of the axon. So far no information is available concerning the role of these morphological features of the initial segment in the generation of the action potential [but see (126) for a discussion of some speculations].

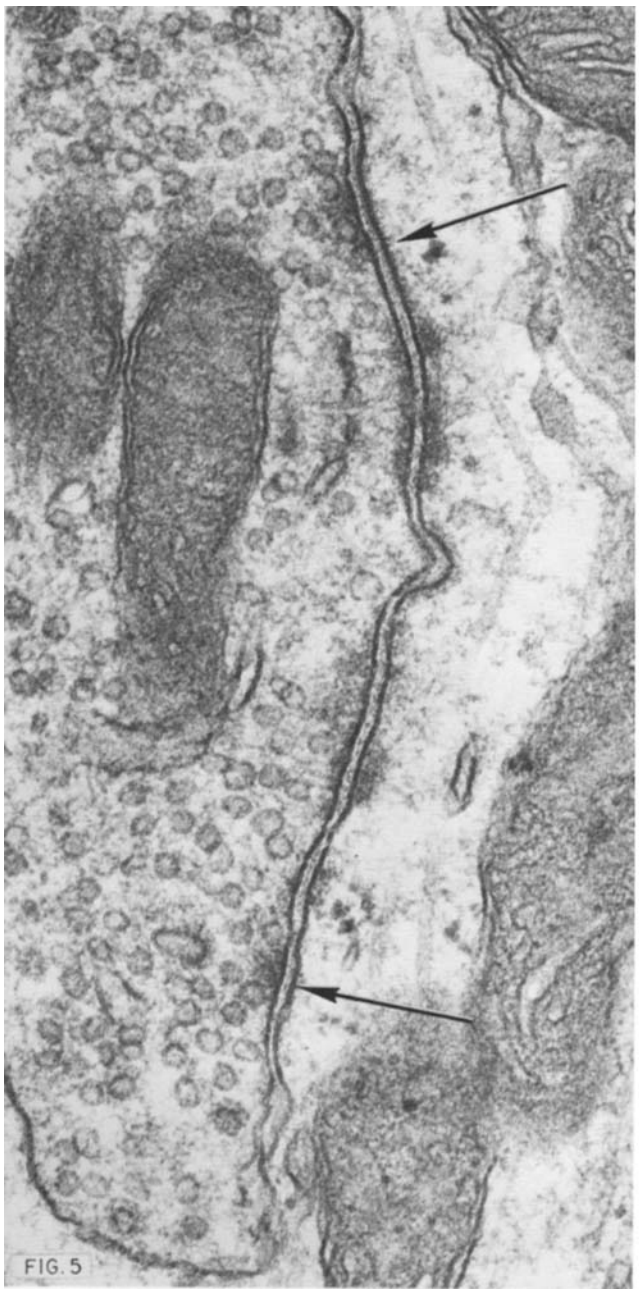
It will be noted that although Nissl substance, endoplasmic reticulum, mitochondria, microtubules, and neurofilaments enter into the initial segment, the Golgi apparatus does not. As has just been described, the Nissl substance rapidly diminishes in amount and a short distance beyond the initial segment it is not to be found. That leaves the definitive axon with a diminished complement of organelles, all of them arranged linearly, parallel to the longitudinal axis of the process. The mitochondria can be extraordinarily long and flexuous, as they are in dendrites; in large axons they are scattered over the cross section, whereas in small axons a single organelle can fill almost the entire cross section. There appears to be a balance between the numbers of microtubules and neurofilaments. Both are found in all kinds of axons. In the large, heavily myelinated axons neurofilaments are very conspicuous and are regularly dispersed across the section. The filaments are never bundled together. Interspersed among them are the microtubules, also spaced at regular but larger intervals, and in much smaller numbers. The mitochondria and the more vagrant tubules of the endoplasmic reticulum perturb the regularity of these arrays. In the small axons, especially in thin, unmyelinated fibers, neurofilaments are few and the microtubules are more conspicuous. A cross section of a small axon, 0.1–0.2 μm in diameter, can contain only three or four microtubules and one or two neurofilaments, accompanied by a single tubule of the endoplasmic reticulum or a very slender mitochondrion. The small, unmyelinated fibers resemble very closely the preterminal subdivisions of axons of any original caliber. They are characterized by periodic swellings or varicosities containing mitochondria and vesicles, which are the presynaptic components of synaptic terminals.

Synapse

Nerve fibers usually end by ramifying into a number of fine preterminal filaments, which terminate in small swellings applied to the surface of another nerve cell. These swellings are termed *boutons terminaux* in order to emphasize their location at the actual termination of the nerve fiber. Perhaps equally or more common are *boutons en passant,* swellings in the course of a fiber that are applied to the surfaces of dendrites or perikarya and have the same internal structure as *boutons terminaux*. Many nerve fibers arborize in a kind of terminal plexus of fine, unmyelinated fibers that are nothing more than a chain of *boutons en passant*. In contrast there are a few examples of nerve fibers that terminate in a single massive ending, for instance, the end bulbs and calyces of Held in the ventral cochlear nucleus and the nucleus of the trapezoid body, respectively. In this chapter all of these variations will be referred to as terminals even though some are not located at the actual ends of fibers. The enormous variety of form shown by nerve endings has been described in classic works by Ramón y Cajal (151, 152). For the present purpose it is only necessary to point out that the form of the nerve ending reflects to a significant degree the nature of the interaction between the afferent fiber and the postsynaptic element or elements. Since consideration of the nerve ending brings us to the interface between nerve cells, an appreciation of its form and structure is essential for the ultimate understanding of the functional organization of the nervous system.

The junction of any nerve ending and another element comprises three fundamental units: *1*) the presynaptic nerve terminal; *2*) the postsynaptic member, i.e., the dendrite, perikaryon, or other entity; and *3*) the synaptic cleft between them. Each of these has its own morphological peculiarities and variations, which must be closely related to the function of each particular synapse, although our information is incomplete at present. We shall first consider the standard chemical synapse, then take up variations on this type, and finally examine deviations from it.

PRESYNAPTIC ENDING. The presynaptic terminal displays four characteristic features: mitochondria, synaptic vesicles, presynaptic densities, and intramembranous specializations. Nerve terminals were early recognized because of the dense accumulation of mitochondria within them (62). In electron micrographs one to several mitochondrial profiles usually occur in the terminal (119, 120, 131). They are located in the proximal part of the terminal swelling away from the synaptic surface, and they are usually small rods or spherules. Synaptic vesicles are small membrane-limited vacuoles 20–60 nm in diameter (Fig. 5). They can be either round and spherical or ellipsoidal, flattened, and elongated (12, 187, 188). Terminals contain either one or the other type, not both, but it must be remembered that the degree of flattening depends upon the fixative mixture used (189). Furthermore, flattened vesicles often appear perfectly round when seen *en face*. Although the vesicles can fill up most of the space in an ending left free by the mitochondria, they often congregate especially close against the internal face of the presynaptic membrane. In adrenergic nerve endings, especially when the tissue has been fixed in permanganate solutions, the synaptic vesicles contain a small dense core, apparently the remnant of a protein that binds catecholamines.



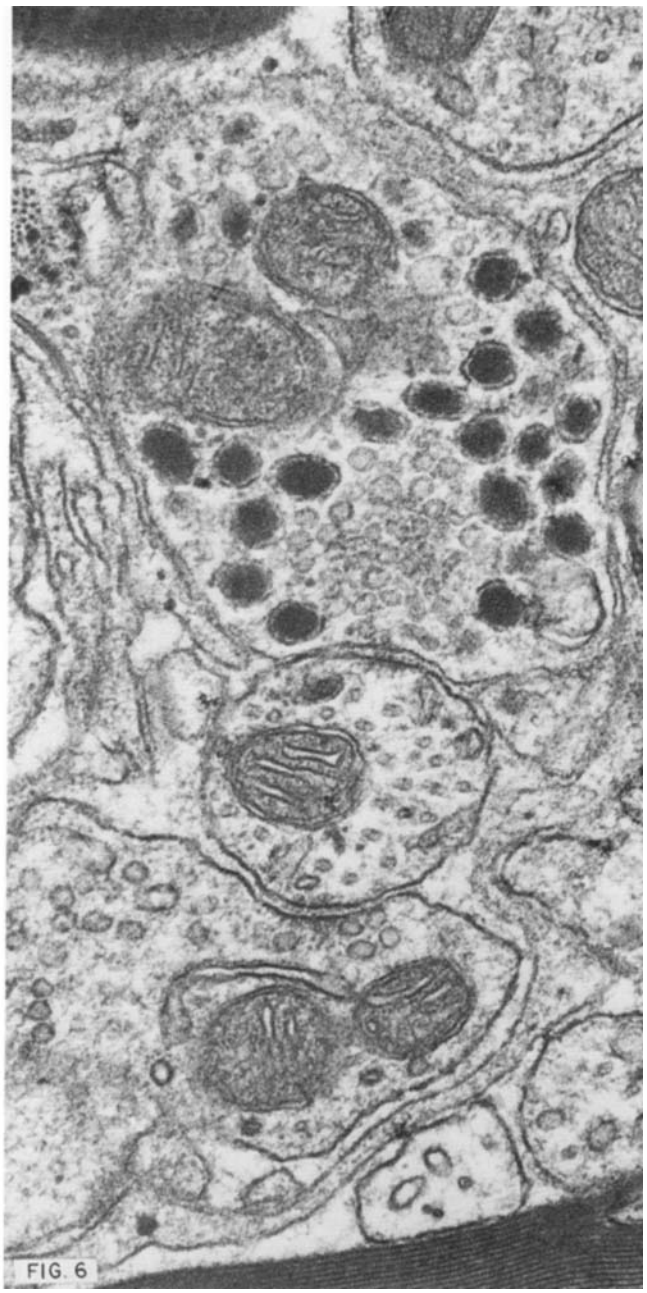


FIG. 5. Axodendritic synapse of Purkinje cell axon terminal in dentate nucleus of cerebellum of *Macaca mulatta*. Synaptic cleft is clearly shown. Patches of dense filamentous material adhere to both pre- and postsynaptic membranes. In two places (*arrows*), where synaptic vesicles approach surface of terminal, adherent densities are more prominent on presynaptic than on postsynaptic side. Synaptic cleft contains ill-defined densities lying in a plane roughly bisecting the cleft. Synaptic vesicles are pleomorphic with high proportion of ellipsoidal or flattened outlines. × 74,000.

FIG. 6. Catecholamine-containing terminal synapsing on small dendrite in dentate nucleus of cerebellum of *Macaca mulatta*. Terminal contains 2 kinds of synaptic vesicles, one small and clear centered, the other about 120 nm in diameter and filled with a dense core. Synaptic junction is characterized by some widening of cleft and modest asymmetry of dense adherent plaques. Small dendrite is nearly filled with microtubules and a single mitochondrion in transverse section. × 77,000.

More widely distributed are large vesicles 80–120 nm in diameter that contain dense cores even without the special permanganate fixation (Fig. 6). The nature of these large, dense-core vesicles is uncertain at present, but possibly they also contain a biogenic catecholamine-binding material (51, 60).

Recently a new variety of dense core has come to light. In neural tissues that have been preserved in

fixative solutions containing a relatively high concentration of calcium ions, a small dense spot appears within the synaptic vesicles, even in cholinergic nerve endings (13). The spot is often triangular or amygdaloid, fails to fill the whole vesicle, and is usually eccentrically situated within it. Careful examination shows that it is attached to the inner face of the vesicular membrane (138). Under similar conditions of fixation rows of dense dots or patches of linear density appear along the surface membranes of unmyelinated axons in the squid (67). The densities in all of these cases are apparently due to a high concentration of calcium ions attached to a carrier protein.

Attached to the inner surface of the presynaptic membrane are shaggy densities that appear to form a triangular or hexagonal grid, in the meshes of which the synaptic vesicles are supposed to be trapped (59, 135). This grid is best brought out with phosphotungstic acid stains. The extent of this adherent plaque varies a great deal in different types of synapse but is characteristic of the cell type from which the presynaptic terminal originates [see (124) for examples]. It usually does not cover the entire plasmalemmal surface involved in the junctional interface, and it matches only approximately the extent of the postsynaptic adherent densities on the other side of the synaptic cleft.

The intramembranous specializations have been known only a short time, and probably further details will become available in the next few years. By means of freeze-fracturing techniques, the distribution of particulate components in the plasmalemma has been examined. In this technique the plasmalemma is split down the middle so that a pair of reciprocally smooth or granular surfaces is exposed (14). One surface, the A face, represents the inner leaflet of the plasmalemma as seen from outside the cell, while the other, the complementary surface or the B face represents the outer leaflet of the plasmalemma as seen from inside the cell. The technique thus gives an otherwise inaccessible view of the internal architecture of the plasmalemma. The A face is strewn with 8–10-nm spherical particles, while the B face is relatively smooth. At the synaptic interface the A face of the presynaptic side is marked by rounded pits and is free of particles except around the mouths of the pits. The B face is distinguished by corresponding protuberances and associated clusters of particles. The significance of these patterns is under investigation, but it is most likely that the A face pits are the mouths of synaptic vesicles fused with the plasmalemma, while the B face protuberances represent the invaginated plasmalemma of these points, where the vesicles are releasing their contents into the synaptic cleft [(82, 83, 124, 134, 181); see also the chapter by Heuser and Reese in this *Handbook*].

POSTSYNAPTIC MEMBRANE. The postsynaptic membrane also has a characteristic intramembranous structure. In freeze-fractured preparations (Fig. 7) the A face of this membrane is relatively free of particles, whereas the B face displays a dense collection of them (2, 124). Attached to the cytoplasmic surface of this membrane are collections of filaments, which vary in different kinds of synapses from a just barely detectable, furry layer to a thick, shaggy layer, 20 or 30 nm deep (55, 119, 120). Secondary densifications beneath this layer have also been found in sympathetic ganglion cells (184) and in several centers in the brain, for example, the habenular nucleus, the subcommissural organ, and the lateral cerebellar nucleus (1, 19, 111).

SYNAPTIC CLEFT. The synaptic cleft is an extension of the thin interstitial space that separates all cellular elements in the central nervous system. At synaptic junctions it can become deeper or shallower or remain the same as elsewhere. The space is crossed by slender filaments and often appears to contain a thin, dense plate midway between the pre- and postsynaptic surfaces.

Of all the components of the synapse, the synaptic cleft is the most critical. It is into this gap that the transmitter is discharged by the presynaptic terminal. The transmitter must cross the synaptic cleft in order to affect the postsynatic membrane and must diffuse through it in order to be dispersed or to be attacked by destructive enzymes. From this gap the transmitter or its degradation products can also be taken up by the presynaptic terminal for reincorporation into synaptic vesicles. Therefore the dimensions and shape of the cleft might be expected to have an important influence on the dynamics of synaptic action. Unfortunately almost nothing is known about this subject. Kuno and his co-workers (80, 81) have shown that in the column of Clarke large nerve endings, having a large synaptic interface, display a greater effectiveness per impulse than small nerve endings. Similarly the well-known high efficiency of the climbing fiber in the cerebellar cortex has been correlated with its extensive synaptic interface with the Purkinje cell dendrites (35). In a recent study on the effects of stimulating afferent fibers to the superior cervical ganglion in the cat, Pysh & Wiley (141) demonstrated that the coaptation between the synaptic terminals and the dendrites of ganglion cells undergoes a considerable expansion that is reversible after the stimulation has ceased. Interestingly, however, no change in the dimensions of the so-called active zone (marked by adherent pre- and postsynaptic densities) is discernible in the published illustrations, and the authors did not comment on this puzzling stability.

From the morphological point of view it is convenient to classify synaptic junctions according to the characteristics of the cleft (55). Two types are recognized: *1*) those in which the cleft is widened by an elevation of the presynaptic surface membrane away from the postsynaptic membrane, and *2*) those in which the cleft retains the dimensions of the usual

FIG. 7. Dendrite and spines of a Purkinje cell of rat cerebellar cortex. Replica obtained by freeze-fracture technique provides three-dimensional view of dendritic spines. Dendrite stem passing across upper left corner of field gives off 4 spines (S_1–S_4) that reach out to synapse with parallel fibers (*pf*) coursing diagonally across the field. A fifth spine (S_5) appears in fracture plane, but its connection with dendritic stem is not shown. Dendrite and its spines are coated with neuroglial processes (*ng*). S_1 and S_4 are intact, presenting unbroken A faces along their entire length. S_2 is broken so that only the B face of its stem remains, whereas the head is complete. At the extremity of S_2 is a rounded, flattened area that is almost clear of A face particles. S_3 is represented entirely by its B face, which bears a collection of particles near its tip. Varicosity in pf_3 underlying S_3 signals presence of a synaptic junction between these 2 structures. Similarly, B face of S_5 displays a cluster of particles at site of its synaptic junction with pf_5. Widened synaptic cleft is clearly shown. × 47,000. [From Palay & Chan-Palay (124).]

interstitial space or is even slightly narrowed. The first type of junction is frequently associated with an asymmetry of the densities adherent to the inner surfaces of the apposed membranes, such that the postsynaptic density is much thicker and more compact than the presynaptic. This type of synaptic junction, designated by Gray (55) as type 1, is particularly well expressed in axodendritic synapses, especially those on dendritic spines. Furthermore this junction is invariably associated with spherical synaptic vesicles in the axonal terminal. Since such junctions, characterized by a widened cleft, asymmetric densities, and spherical synaptic vesicles, have been correlated in the cerebellar cortex with excitatory synaptic action [(187, 188); for review see (124)], it is presumed that such characteristics, wherever found, always signify an excitatory synapse.

The second type of junction, in which the cleft is narrow, is associated with symmetrical pre- and postsynaptic densities such that both have about the same thickness but neither is conspicuous. This junction was designated by Gray as type 2. It is usually found on the shafts of dendrites and on the perikarya of nerve cells. Also it is frequently associated with flattened or pleomorphic synaptic vesicles in the axonal terminal. This type of synapse, characterized by a narrow cleft, symmetrical densities, and flattened synaptic vesicles, has been correlated in the cerebellar cortex with inhibitory action, and it is widely presumed that these characteristics indicate an inhibitory synapse wherever they occur.

Thus there are two clearly different constellations of morphological features that correlate with two physiological functions of opposite sign. It must be remembered that this correspondence between morphological and physiological parameters is still not established in a wide variety of examples. The correlation has been successful in the cerebellar cortex, where physiological analysis on a cellular basis has been more intensively pursued than elsewhere [see (35)], and where nearly all the possible synapses have been characterized morphologically (124). Extension of the correlation to other regions depends on data that are not yet available. Even in the cerebellar cortex, however, there are certain difficulties with this classification. Gray's type 1 seems relatively clear and consistent from preparation to preparation and from region to region of the central nervous system. But many intermediates exist between types 1 and 2—junctions with widened clefts and thin, symmetrical densities; others with narrow clefts and asymmetric densities. These cannot be easily classified as belonging to either type and therefore leave the correlation with physiology somewhat ambiguous.

SYNAPTIC VESICLES. Almost all of the data bearing on the significance of the synaptic vesicles derive from experiments on the neuromuscular junction and the biochemistry of subcellular fractions from brain. These data are discussed in later chapters in this *Handbook*. For the present purpose, we need only record the prevailing hypothesis that the vesicles contain neurotransmitter substances probably bound to a carrier protein, that the vesicles release their content into the synaptic cleft by fusing with the presynaptic cell membrane, and that this fusion is regulated by the action potential and the local concentration of calcium ions.

The shape of the synaptic vesicles has been the subject of much interest and speculation. When first discovered, synaptic vesicles were always described as round or spherical membrane-limited cavities with a clear content. This is how they appeared in tissues fixed with osmium tetroxide. With the introduction of aldehyde fixation in the later 1950's the formerly uniformly spherical synaptic vesicles divided into two populations, as already noted, spherical and flattened. Now the shape of the synaptic vesicles could be added to the list of morphological features required for identifying the nerve endings of particular cells; for example, in the cerebellar cortex climbing fibers and parallel fibers contain only spherical synaptic vesicles, whereas basket fibers and Purkinje cell recurrent collaterals contain flattened vesicles. Since electrophysiological experiments showed that climbing and parallel fibers exert an excitatory action on the postsynaptic neuron, while basket fibers and Purkinje cell collaterals have an inhibitory action, it was an easy step to propose that spherical synaptic vesicles signify excitation and flattened vesicles signify inhibition wherever they are found (187). This correlation seems to be correct in the cerebellar cortex, but it is difficult to justify this extension from the particular to the general on logical grounds alone, without a longer list of correlated examples in many different regions of the vertebrate nervous system.

Excitation and inhibition are responses of the *postsynaptic* element. Why should they be correlated with a morphological difference in the *presynaptic* member of a synapse? The underlying assumptions are that excitation or inhibition is the consistent response to a particular transmitter and that the shape of the vesicle in some indirect way reflects the physicochemical properties of the transmitter and its specific carrier protein. If these assumptions prove to be correct, the vertebrate nervous system will have gained a simplifying consistency, because in invertebrates examples have already been collected of opposite actions produced by the same transmitter at different sites or under different conditions of stimulation (74). Even two branches of the same fiber may have different actions on the postsynaptic member (73). Furthermore the flattening of the vesicles seems to be an effect produced by fixation with aldehydes in association with subsequent treatments of the tissue (189). Flattened vesicles have not yet been seen in freeze-fractured tissue, even after aldehyde fixation.

Therefore it would be sensible at present to withhold judgement concerning the general correlation of vesicle size and shape with physiological action until many more systems have been examined in detail by physiological and fine structural means. In the meantime the tentative prediction of function on the basis of morphology could be used as a guide to experimental design.

DENDRITIC SPINE. Before leaving the subject of the axodendritic synapse, it would be desirable to emphasize certain aspects of the postsynaptic member. We have already noted that the dendritic tree vastly increases the surface area of the perikaryon, and in dendrites richly furnished with spines, like the pyramidal and Purkinje cells, these appendages expand the surface of the dendrite still more. Fox & Barnard (41) provided measurements indicating that dendritic spines of the Purkinje cell occupy 42.3% of the total surface area of the smaller dendritic branches. Since the dendrite is conventionally regarded as the receptive part of the neuron, the expansion of the surface should coincide with a concomitant increase in the extent of the surface occupied by synapses. This presumption, however, is not supported by the evidence. The neuron does not put all this increased surface to use as receptive sites for synapses. Peter & Kaiserman-Abramof (129) call attention to the fact that in the cerebral cortex the surface of pyramidal cell apical dendrites is far from covered by synapses. The rest is covered by neuroglial processes. In most synapses on spines the axon contacts only the head of the spine, not the stem. Therefore it is necessary to look for other implications in the existence of dendritic spines. Increasing the surface available for contact is evidently not an important one, nor is the necessity of reaching out from the dendritic shaft to select the appropriate axon from the welter of afferents passing by, as suggested by Peters & Kaiserman-Abramof (129), although such an argument might point to an accessory advantage.

The role of the spine is indicated by its shape: a rounded or clavate head attached to the dendritic shaft by a thin stem—a broad surface for presenting receptor sites to a prospective transmitter and a tenuous connector with high electrical resistance. The spine appears to be a device for electrically isolating a bit of the postsynaptic dendrite from the potential changes in the dendritic shaft that result from synaptic activity elsewhere along its length and perhaps from antidromic invasion of current from the axon hillock (31, 96). This isolation is achieved at the cost of a considerable attenuation in the strength of the signal generated in the spine, because of the high longitudinal resistance of its stem. Therefore it seems that the spines are constructed for the transmission of information from a large number of similar inputs in which the individual signal counts for very little so far as the postsynaptic cell is concerned. The advantages of isolation can be preserved while circumventing the disadvantages by clustering the spines into groups and arranging for them to synapse with a single varicosity or fiber in succession, as in the synapse between the climbing fiber and the Purkinje-cell dendrite (21, 124). Both the single spine and the complex cluster of spines appear to be old devices phylogenetically, for they appear in species from all vertebrate classes.

OTHER VARIETIES OF SYNAPSE. The previous sections have described what may be considered more or less standard, traditional, even classic synapses. The use of electron microscopy has not only increased the amount of detail that can be seen in these junctions but has also disclosed completely new and unexpected types of synapse, which greatly enlarge the repertoire of operations that neurons in a complex nervous system can utilize. These new varieties also greatly complicate the analysis of the central nervous system for morphologists and neurophysiologists alike.

Among the first new junctions to be discovered is the axoaxonal synapse, which has been found in the spinal cord, the retina, the lateral geniculate body, the cerebellar and cerebral cortices, and other places. There are two varieties of this synapse: *1*) one in which the boutons of an axon synapse upon the terminals of another axon, which, in turn, ends upon a dendrite—the so-called serial synapse, and *2*) one in which the boutons of an axon terminate upon the axon initial segment of another cell. In both cases it is thought that the effect of the axoaxonal synapse is inhibitory, in keeping with the flattened vesicles and type 2 characteristics of the junction. The first variety—the serial synapse—is supposed to be the basis for presynaptic inhibition (56).

Synapses between somata and between soma and dendrites have also been described (91, 148, 167, 198). Their existence indicates that the neuronal soma can have certain functional properties traditionally reserved for the axon. In some instances, the soma-somatic junctions are reciprocal, that is, synaptic vesicles occur in alternating clusters on either side of the junction. Such forms suggest an unanticipated subtlety in the interaction of neighboring neurons.

An even more anomalous kind of synapse was described by Rall et al. (143), who discovered dendrodendritic junctions between mitral cells and granule cells in the olfactory bulb (137, 167). Similar synapses were later recognized in the glomeruli of the medial and lateral geniculate bodies (36, 37, 90–92, 112, 128, 142, 144, 198) and in the superior colliculus (99, 100, 190), and no doubt they will turn up elsewhere. The dendrodendritic synapses in the olfactory bulb are reciprocal (the mitral-to-granule junction being Gray's type 1 and the granule-to-mitral junction being type 2), whereas those in the thalamic nuclei are one-way synapses in a serial arrangement with axons. The functional implications of these synapses are not yet

clear, but that they make possible very complex and delicate interactions between nerve cells is inevitable.

In all of these unconventional junctions the synaptic cleft has been preserved. Although the partners in the synapse may be somata, axons, or dendrites, they all display the fundamental morphological features that have been accepted as signs of a chemical synapse: synaptic vesicles, synaptic cleft, and densities adherent to one or both membranes. An entirely different order of synaptic junction is also known, which involves electrical coupling of the members and the almost total obliteration of the synaptic cleft. Electrical transmission was shown to be a reality in the crayfish giant fiber synapse in 1959 (50), and shortly thereafter attempts were made to demonstrate the structural characteristics of that junction. Although a narrowed cleft was found at first, it was not until advanced techniques for the examination of the plasmalemma were applied that the junction could be characterized. Meanwhile electrical coupling between cells was shown to be a widespread phenomenon, for example, between cardiac muscle cells, smooth muscle cells, epithelial cells, embryonic cells, neuroglial cells, and nerve cells in many species. In all instances examined [see (49) for an early compilation], electrical coupling is correlated with the existence of specialized intercellular junctions of various types: gap junctions, occluding junctions, or septate desmosomes (8, 9). Usually one of these varieties occurs in company with one or both of the other two. Current opinion tends to the belief that the gap junction is most likely to be responsible for the electrical coupling. In the crayfish giant fiber synapse (127), the Mauthner cell club ending synapse (15, 114, 155, 156, 202), and the tegmental and spinal electromotor cells in various teleosts (8, 9), electrical coupling is correlated with the presence of gap junctions between the synaptic members. Such junctions are characterized by a reduction in the depth of the synaptic cleft to 2 nm or less, and within the cleft a hexagonal array of densities spaced about 9 nm apart can be seen. This array can be outlined by lanthanum salts penetrating extracellularly into the plane of the junction (15, 154). Currently the best way to characterize the gap junction is by the freeze-fracturing technique. In such preparations small patches of particles are found in close-packed hexagonal arrays on the A face of the synaptic membrane, while a reciprocal array of tiny pits appears in the B face of the apposed cell membrane (54, 109). Since the two plasmalemmas are tightly adherent to each other at these sites and their substructures are in perfect register with each other, they are often found together in freeze-fractured preparations. The minute pits in the B faces are thought to be the mouths of pores capable of conducting ions from one cell to another.

In the mammalian nervous system, gap junctions have been found between neurons in only a few sites so far: the lateral vestibular nucleus (157, 176, 177), the inferior olive (95, 175), the somatosensory and motor areas of the cerebral cortex (172), the olfactory glomeruli (137), and the molecular layer of the cerebellar cortex (174). In the lateral vestibular nucleus large boutonlike terminals form intermittent gap junctions with the perikarya of Deiters cells. Electrophysiological studies indicate that the Deiters cells are electrically coupled, but it is not clear how these synapses are related to that coupling (78). In the inferior olive the principal cells are coupled by means of gap junctions betwen their dendrites, and these electrotonic junctions are associated with conventional chemical axon terminals of unknown origin that synapse on one or both dendrites of the pair. The speculation has been offered (95) that the operation of the chemical synapse might uncouple the members of the gap junction by altering the permeability of the postsynaptic dendritic membranes. This possibility has been demonstrated in the motor system of a mollusc (179). In the mesencephalic nucleus of the fifth cranial nerve close apposition of the nerve cells is associated with the formation of tight junctions between the somata (15, 68). Baker & Llinas (6) have provided evidence that these cells are also electrotonically coupled.

It is noteworthy, but usually ignored, that in all of the examples of interneuronal gap junctions in vertebrates, the presynaptic terminal also contains synaptic vesicles, which aggregate in clusters along the synaptic interface but avoid the vicinity of the gap junctions. The spots at which they aggregate exhibit all of the features of chemical synaptic junctions—vesicles, widened synaptic cleft, and asymmetric pre- and postsynaptic membrane densities. Thus the junctional interface possesses the morphological signs of both electrotonic and chemical synapses. For this reason they have been termed mixed synapses (131). Although these mixed synapses are known to occur in a wide variety of animals, in only two sites has evidence for both electrotonic coupling and chemical transmission been found when they were tested physiologically: in the chick ciliary ganglion (15, 107) and in several synapses in the spinal cord of the lamprey (159, 160). In all other tested examples where morphology indicates the coexistence of electrical and chemical transmission only electrical transmission has been demonstrated (8, 9). In invertebrates, although the dual mode of transmission has been shown in a number of instances, morphological correlates are thus far lacking. The value of having both kinds of transmission mechanisms at the same synaptic interface is not obvious [but see (177) for proposals regarding the significance of such junctions].

Lastly, electron microscopy has brought to light the existence within the central nervous system of synapses *à distance*, synapses in which the presynaptic terminal fails to contact the postsynaptic partner. The model for this type of synapse is the neuromuscu-

lar junction in smooth or cardiac muscle, just as the model for the conventional chemical synapse is the neuromuscular junction in skeletal muscle. In most types of smooth muscle the nerve fibers fail to articulate closely with the muscle cells. Instead they merely accompany the muscle fibers, ramifying in the interstitial spaces, escaping here and there from their Schwann cell sheaths, but generally remaining within their own basal lamina and not penetrating beneath that of the muscle cells. The nerve fibers develop *terminaisons en passant*, varicosities in the course of the fibers, which contain mitochondria and numerous synaptic vesicles, but the familiar specializations of the presynaptic plasmalemma and adjacent cytoplasm are lacking. Similarly the muscle fibers exhibit no sign of the postsynaptic specializations that mark the neuromuscular junction of skeletal muscle. Apparently the transmitter agents are discharged indiscriminately from the varicosities into the intercellular space.

The best-known example of this type of junction in the central nervous system is the pinceau of basket cell axons surrounding the initial segment of the Purkinje cell axon in the cerebellar cortex (124, 151). Here the terminal collaterals of the basket cell axon descend around the perikaryon of the Purkinje cell, with which they effect conventional type 2 synapses, and then continue down to the Purkinje cell axon. When they reach below the cell body, they ramify into a dense plexus, which encapsulates the initial segment in a tangle of unmyelinated nerve fibers. Although a number of basket cells contribute axons to this plexus, only one of them forms a chemical synapse directly on the initial segment of the Purkinje cell (124). The rest terminate freely within the plexus in dozens of small digitiform or grapelike endings filled with synaptic vesicles. The initial segment of the Purkinje cell axon is completely encased in neuroglial cell processes, which separate it from the axonal plexus but allow access to the one or two terminals that contact it. A similar fiber plexus composes the axon cap of the Mauthner cell axon in teleosts (114, 156), but this complex synapse has not been so thoroughly investigated as the pinceau. The several small terminal branches of the basket fiber plexus are joined together by special junctions that have been variously regarded as septate desmosomes, attachment plaques, or ambiguous densities (43, 52, 124, 174). Their nature is still under investigation. Although the pinceau is believe to be an inhibitory synapse, since that is the effect of basket cell activation upon the Purkinje cell, its mode of operation is still obscure.

Neuron Doctrine

In view of this long list of unanticipated types of interneuronal connections, what becomes of the traditional, fundamental principle of the nervous system known as the neuron doctrine? The neuron doctrine simply states that the nervous system is composed of independent cells termed neurons. By independent it is meant that each cell has its own nucleus and its own boundary or plasmalemma and that the cells communicate with one another by means of contact. They are *contiguous*, not *continuous*. Put in another way with a slight shift in emphasis, the neuron doctrine declares that the nerve cell is the independent trophic and functional unit of the nervous system. A summary of observations by Ramón y Cajal (150) and other writers, the doctrine was framed in this way from the beginning of its history as a counter to the prevailing and recurrent notion that the nervous system consisted of a continuous reticulum in which the perikarya of nerve cells were simple nodes or intersections. This latter idea, known as reticularism or the reticular theory, was held by large numbers of scientists during the nineteenth century, and it tenaciously survived, despite its inherently unpractical chaos, well into the first half of the twentieth century. It was conclusively disproved by the electron-microscopic demonstration of the synaptic cleft (119, 120).

The concept of the synapse is the heart of the neuron doctrine. When Sherrington (40) introduced this term in 1897, he required a word for the point at which a nerve impulse is transmitted from one nerve cell to another. He clearly accepted the proposal of Ramón y Cajal that the system is disjunctive and recognized that the points of functional contact or transmission are connections, or links, between independent elements. Subsequent physiological and biochemical investigation has shown that for a large part of the nervous system these independent units are connected by way of chemical transmitters, which, released from a nerve cell in some close dependence upon its integrated activity, cross the interstitial space and set off an appropriate response in another cell. This journey takes a small amount of time, included in the period known as the synaptic delay. Thus far, the physiological and biochemical data are in accord with the anatomic description and its conceptualization. It will be noticed that the synaptic cleft is critical for this generalized description of the operation of the synapse. The electrochemical perturbation of a nerve cell is confined to that cell by the electrical properties of its cell membrane and the low resistance of the interstitial fluid, so that it is not transmitted to a neighboring cell without the intermediary of specific transmitter molecules and specific release sites in the first cell and specific receptor sites in the neighboring cell. This description seems to be applicable to most synapses in vertebrates and invertebrates.

As we have seen, however, there are places where the synaptic cleft is all but eliminated in the formation of a gap junction and through these junctions there is electrical continuity between the mem-

bers of the synapse. Does this discovery invalidate the neuron doctrine? The partners in these junctions still have their independent existence, their nuclei, and their separate surface membranes. If in a few small spots their surface membranes come into register so that small molecules or ions can pass between them, their essential individuality is not compromised. The gap junctions are specializations, differentiations for a specific function (e.g., coordinating the rhythmic discharges of cooperating cells as in the inferior olive or in the command center of the electric organ in mormyrids), not an indiscriminate confluence of cell processes.

The newly recognized varieties of synaptic junctions have, however, set in motion an attempt to rephrase the neuron doctrine, to reform it in a more flexible and inclusive mode (169). The impetus for this revision derives from a misapprehension of the doctrine's rigidity, perhaps as a result of overzealous teaching in the past, as is implied in the English word doctrine. It might have been better if the English-speaking supporters of this concept had taken their cue from the German *die Neuronlehre*, the neuron theory, and used a less intransigent word. It is, after all, only the cell theory applied specifically to the nervous system. In particular, the new dendrodendritic and axoaxonal synapses have been felt as a challenge to the neuron doctrine. Such synapses demonstrate that the separation of functions within the neuron is neither so sharp nor so inflexible as had been thought and that therefore a considerable confusion may arise by trying to maintain the old morphological distinctions in describing the nervous system. In these new synapses dendrites appear to be acting like axons and axons like dendrites. There is consequently a loss of confidence in the definitions and interpretations that have been constructed by generations of investigators.

In coping with this problem it is necessary to review briefly the origins of these definitions. Dendrites and axons were first clearly distinguished by Deiters (29) on the basis of their form and branching patterns. This distinction is primarily a morphological one and remains so for both optical and electron microscopy. When they cannot be distinguished, as in some tissue culture preparations and in invertebrate ganglia, authors have resorted to the ambiguous term neurite (which sometimes is synonymous with axon but usually is equivocal) in describing the processes of nerve cells. The axon was early recognized as carrying the neural currents away from the nerve cell body, since the axons of motor cells make up the ventral root going out to muscle, but the role of the dendrites was for a long time obscure and their function in a reticular nervous system did not seem very specific. In fact, Golgi for a time maintained that they were merely nutritional appendages. It was Ramón y Cajal (150) who had the insight to realize that the dendrites have a specific role in each cell. His concentrated study of nerve cells in all parts of the nervous system, especially in the cerebellar and cerebral cortices, led him to the proposition that the nerve cell is polarized: the dendrites receive incoming impulses and direct them to the cell body, while the axon conducts them away from the cell body and transmits them to the dendrites of the next cell in line. Shortly afterward Ramón y Cajal realized that this statement was too restrictive, and he enlarged it to include the cell body in the receptive apparatus of the neuron. Thus the law of dynamic polarization (150) became the principle that the dendrites and the cell body receive afferent inputs and conduct them to the axon, which in turn conducts them away from its origin and transmits them to the receptive parts of the next cell. Ramoń y Cajal had an idea of convergence, summation, and integration, but of course he did not have the present concept of synaptic potentials and action potentials. For him the nervous system operated in terms of action and silence; inhibition as now understood was still unknown. Consequently it does not seem appropriate to expect that the law of dynamic polarization should be applicable today to all known nervous interactions. The remarkable aspect is that such an intuitive statement should be as close to general applicability as it is. For Ramón y Cajal it was a necessary explanation of the two different kinds of processes extended by the nerve cell, and it complemented the neuron doctrine, which his observations supported. But this law was not a necessary part of the doctrine. It does not require the independence of neurons; it suggests how they might work if they are independent.

The newly recognized types of synapse may be seen as exceptions to the general law of dynamic polarization. The rule would have to be modified to include them, if it is important to enunciate such rules. Conceptually, however, it only needs to be remembered that the synapse is a specialized region in the surface membrane and subjacent cytoplasm of the neurons involved. The asymmetry and polarity of this specialization and its mode of action can be viewed as the result of local structural and chemical differentiation. Receptive and conducting or transmitting parts of neurons are commonly intermingled in invertebrates. In these animals dendrites are frequently branches of axons, and the morphological difference between them is rather ambiguous. In vertebrates the most highly differentiated nerve cells, such as motor horn cells, have their receptive and conducting or transmitting functions widely separated in different parts of the neuron, but in many other cells—and the examples may increase with further study—receptive and conducting or transmitting functions can be to some extent intermingled. There are even examples of dendrites, formerly thought to be capable only of graded potentials and electrotonic conduction, capable now of generating action potentials (47, 97, 98, 178). And there is now an example of a dendrite in the

olfactory bulb that is myelinated (136), although only lightly, to put beside the troublesome and anomalous peripheral process of the sensory root ganglion cell, which many had regarded as a dendrite with axonal structure and functions. If this line of argument is pursued, the neuron becomes a mosaic with its different functional mechanisms and their congruent structures distributed in a characteristic way for a particular cell type. Dendritic and axonal morphology in general correspond to the functions of reception and conduction or transmission, respectively, but they may be intermingled in special regions, and there the fine details of the local structure should correspond to the local functional mechanisms. According to this view, a nerve cell is a mosaic of functions and appropriately correlated structures. The distribution of these functions and structures in any one cell is integral with the role of that cell in the circuitry of the nervous system.

NEUROGLIA

The interstitial or supporting cells of the central nervous system are divided into three types: astrocytes, oligodendrocytes, and microglia. Their counterpart in the peripheral nervous system is a single cell type, the Schwann cell. Abnormal neuroglial cells and pathological reactions will not be considered in this chapter. Astrocytes, oligodendrocytes, and Schwann cells are all descendants from ectodermal derivatives in the embryo, those in the central nervous system coming from the walls of the neural tube and the Schwann cells originating from the neural crest. The ancestry of the microglia is still obscure; some investigators believe that they are mesenchymal invaders into the central nervous system (77), and others believe that at least some microglial cells originate from undifferentiated glioblasts surviving in the mature animal (193). Perhaps both ideas are correct to some degree. In the following paragraphs the structure of the neuroglial cells will be described and their relationships to nerve cells will be analyzed.

Astrocytes

Astrocytes are small, rounded cells with large numbers of radiating processes. They are found in all parts of the central nervous system, including the white and gray matter, in the olfactory and optic nerves, and in the cranial and spinal nerve roots until they leave the dura. Two kinds of astrocytes are recognized, fibrous and protoplasmic. Fibrous astrocytes contain heavy fibers that pass through the cell body into the processes. They are found principally in the white matter and in the nerves, but they are also seen in the parts of the gray matter that contain large numbers of myelinated fibers. Protoplasmic astrocytes also contain fibers, but they are more tenuous and the processes of these cells are also more delicate. They occur principally in the gray matter, and they are often satellites to the large nerve cell bodies. Both types of astrocyte send out processes that end in palisades on the walls of blood vessels and beneath the pial surface of the brain and spinal cord. Together with the basal lamina coating the central nervous system, the palisaded end feet of the astrocytes compose the external limiting membrane or the glia limitans externa.

In light microscope preparations stained with the usual histological dyes, astrocytes appear as pale bean-shaped nuclei surrounded by lightly basophilic cytoplasm. These perikarya are about 9 or 10 μm in diameter and are not always easily distinguishable from those of small neurons. However, the cytoplasm of the astrocyte is usually so attenuated and so pale that it is not possible to discern the outline of the perikaryon, whereas that of the neuronal perikaryon, containing a more basophilic cytoplasm, can often be made out. A prominent nucleolus in a clear vesicular nucleus usually identifies a neuron, but even this sign may fail if the nucleolus is obscured for some reason. In regions populated with small cells, like the substantia gelatinosa of the spinal cord or the granular layers of the cerebral cortex, it may be quite impossible to distinguish the two cell types in preparations stained with basic dyes. Although special procedures are available that stain the astrocytes more or less selectively, Golgi preparations provide the best means of visualizing their true shapes and the marvelous delicacy of their processes (Fig. 8). These preparations correlate faithfully with the electron microscope appearance (131).

In electron micrographs the two kinds of astrocyte are readily differentiated. Both contain bundles composed of fine filaments about 7.5 nm in diameter (199). The fibrous astrocyte is nearly full of them whereas the protoplasmic astrocyte has a few thin, delicate bundles. In both, the cytoplasm is loosely textured with meandering granular endoplasmic reticulum and a few rather large mitochondria. The Golgi apparatus consists of small assemblages of appressed cisternae, usually at one pole of the nucleus or in its hilus. In the spaces between the long bundles of filaments the cytoplasm is peppered with solitary glycogen granules and numerous ribosomes in clusters and spiral or polysomal arrays. The nucleus bears the usual fenestrated envelope, and the nuclear chromatin is fairly uniformly distributed with some margination (Fig. 9). The nucleolus, which is usually not distinguished with the light microscope, is clear enough in electron micrographs but has no special features.

In electron micrographs the distinctions between astrocytes and neurons are usually quite clear, and the two cell types are easily identified (Fig. 9). In contrast to the smooth, regular outline of the neuronal perikaryon, that of the astrocyte is typically

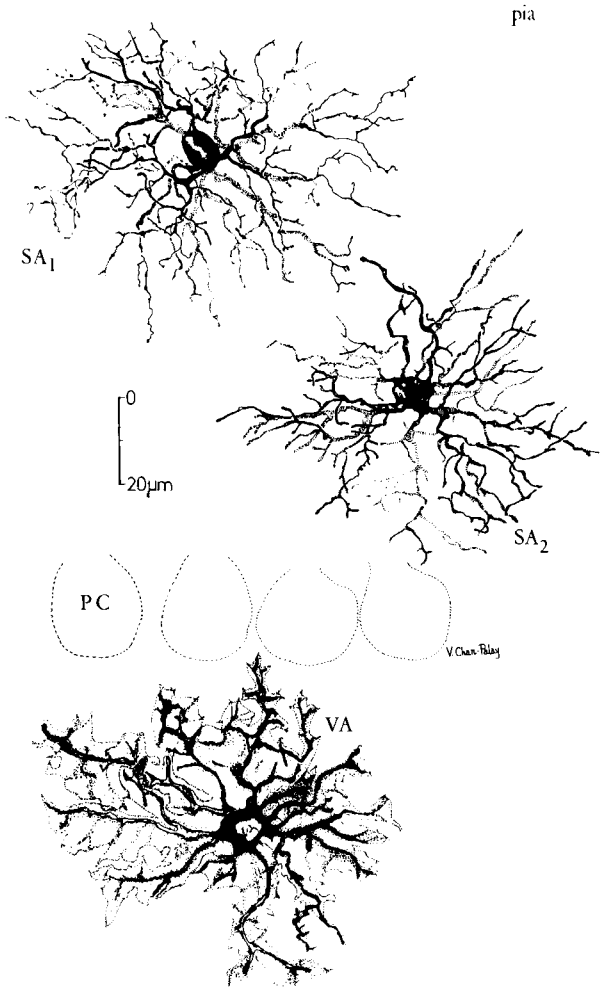


FIG. 8. Camera lucida drawing of protoplasmic astrocytes in cerebellar cortex of adult rat. Two cells in molecular layer (SA_1 and SA_2) are examples of smooth protoplasmic astrocytes, the radiating branching processes of which lack elaborate appendages. Cell (*VA*) below Purkinje cells (*PC*) is a velate astrocyte displaying its velamentous processes, which spread out in all directions among nerve cells and fibers of gray matter. Golgi-Kopsch preparation; *pia*, pial surface of brain. [From Palay & Chan-Palay (124).]

irregular, as this cell emits numerous fine processes that lose themselves in the surrounding neuropil. The shape of the perikaryon is consequently not delineated by its immediate neighbors in the way that the neuronal perikaryon is. And the lack of axonal terminals synapsing on the surface of the astrocyte underscores this difference. The astrocyte nucleus is remarkable for the homogeneous, rather tight disposition of its chromatin in contrast to the dilute chromatin of the neuronal nucleus. Finally, the pattern of the cytoplasmic organelles and inclusions is distinctly different from that of the neurons. The loose arrangement of the sparse granular endoplasmic reticulum, the scattered glycogen granules, and the bundles of neuroglial filaments are unlike the components of neuronal cytoplasm.

The most remarkable quality of the astrocyte is the exuberant pleomorphism of its processes. The usual pictures in textbooks do not do it justice. They are ordinarily drawings of fibrous astrocytes with the fine details left out. It is true, however, that the processes of fibrous astrocytes are fundamentally cylindrical extrusions with broadened tips; their lateral offshoots and pseudopodia are rather restrained in comparison to those of protoplasmic astrocytes. This type emits large numbers of flowing velamentous processes, consisting often of little more than reduplicated plasmalemma, which insinuate themselves between the cellular constituents of the neuropil and enclose whole neurons in multilaminate sheaths (122, 130, 196, 197). In Golgi preparations these attenuated sheets can be seen scrolling about dendrites, subdividing clusters of perikarya, and forming the leaflike walls of compartments in the neuropil (22, 123, 124). In electron micrographs the thin sheets can be seen extending everywhere into crevices and along axons, dendrites, and perikarya. They surround thorns and enwrap synaptic pairs. The processes often contain no organelles except for a few glycogen granules, but sometimes they thicken into ridges and ribbons of triangular profile in transverse section. These thickenings are occupied by thin bundles of neuroglial filaments or bits of endoplasmic reticulum and mitochondria. The images seen in well-impregnated Golgi preparations and in conventional electron micrographs are consonant with the cinematographic images of living astrocytes growing in tissue culture chambers (139, 140). Under these conditions these cells exhibit highly active surfaces, emitting flowing membranes, streamers, and irregular veils which undulate, fold and unfold, retract and flow out again in continuous motion. It is not known whether the surface of the astrocyte is so active in situ as it is in vitro, but that it puts out such velamentous folds is evident enough in both Golgi preparations and electron micrographs (22, 124). Both high-voltage electron microscopy of Golgi preparations and freeze-fracture preparations of briefly fixed tissues confirm this interpretation of the images. In order to emphasize the laminar processes of these astrocytes, we have given them the name *velate astrocytes*, and we reserve the term *smooth protoplasmic astrocytes* for the small number that seem to have much less elaborate extrusions (Fig. 8).

The role of these cells is discussed in detail in the chapter by Orkand in this *Handbook*. Only a few comments need to be entered here. Although indifferent space-filling and supportive or nutritional functions have been suggested, and may to some extent be correct, the distribution of the velamentous processes in the neuropil indicates a more specific and

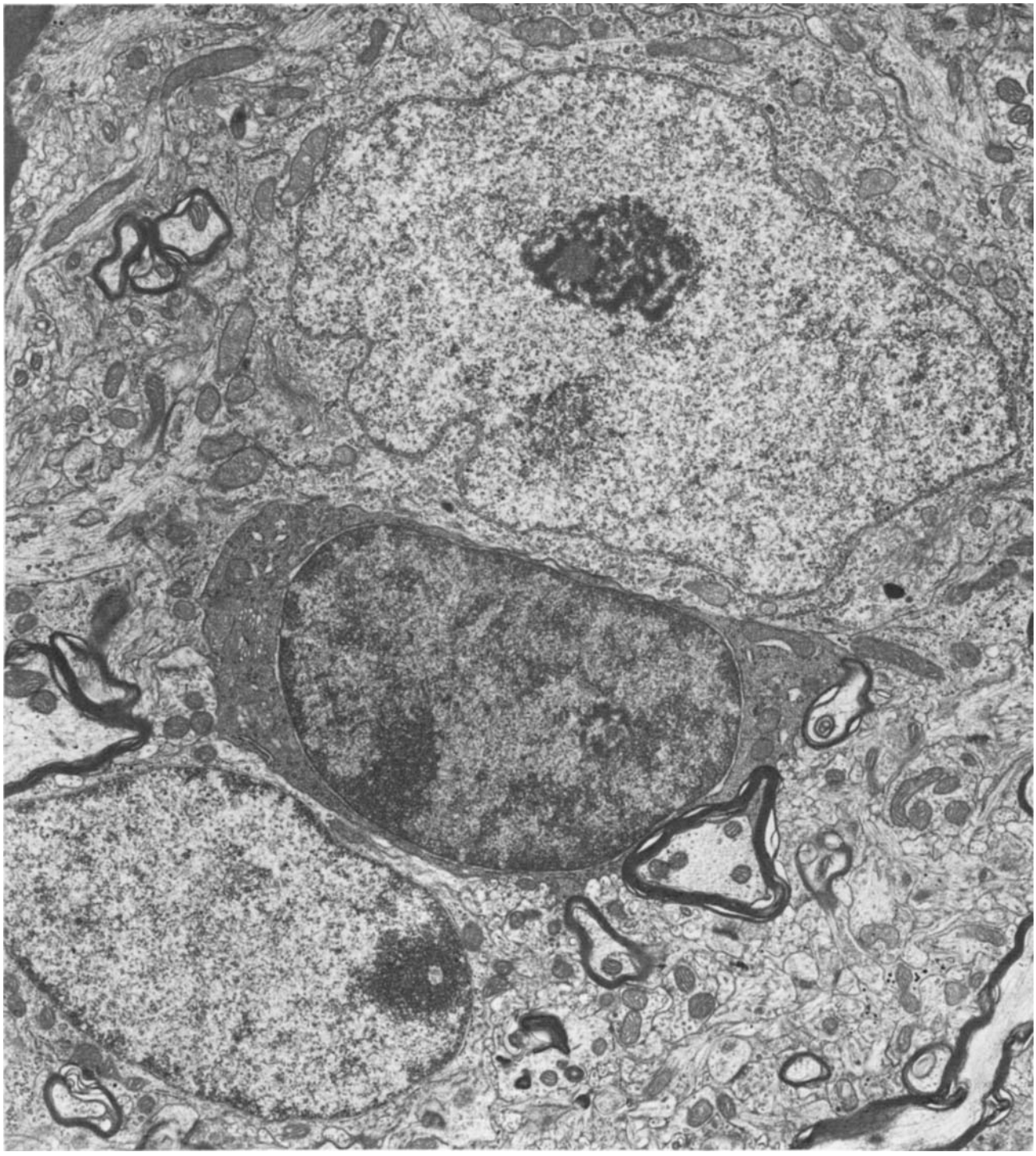


FIG. 9. Three cells in layer 4 of cerebral cortex of adult male *Macaca mulatta*. Upper half of field is occupied by a small neuron (stellate cell), which sends off a dendrite to left and its axon to upper margin of picture. Nucleus is relatively large and cytoplasm is limited to a narrow rim about it. Below the neuron is a rounded, dark cell with a distinct outline and dense nucleus. This is an oligodendrocyte with characteristic cytological features: dense nucleus and cytoplasm, small fragmented profiles of endoplasmic reticulum, small Golgi apparatus, numerous microtubules. In left corner of field is a protoplasmic astrocyte with its typically irregular outline and light nucleus and cytoplasm. Visual cortex, area 17. × 10,000.

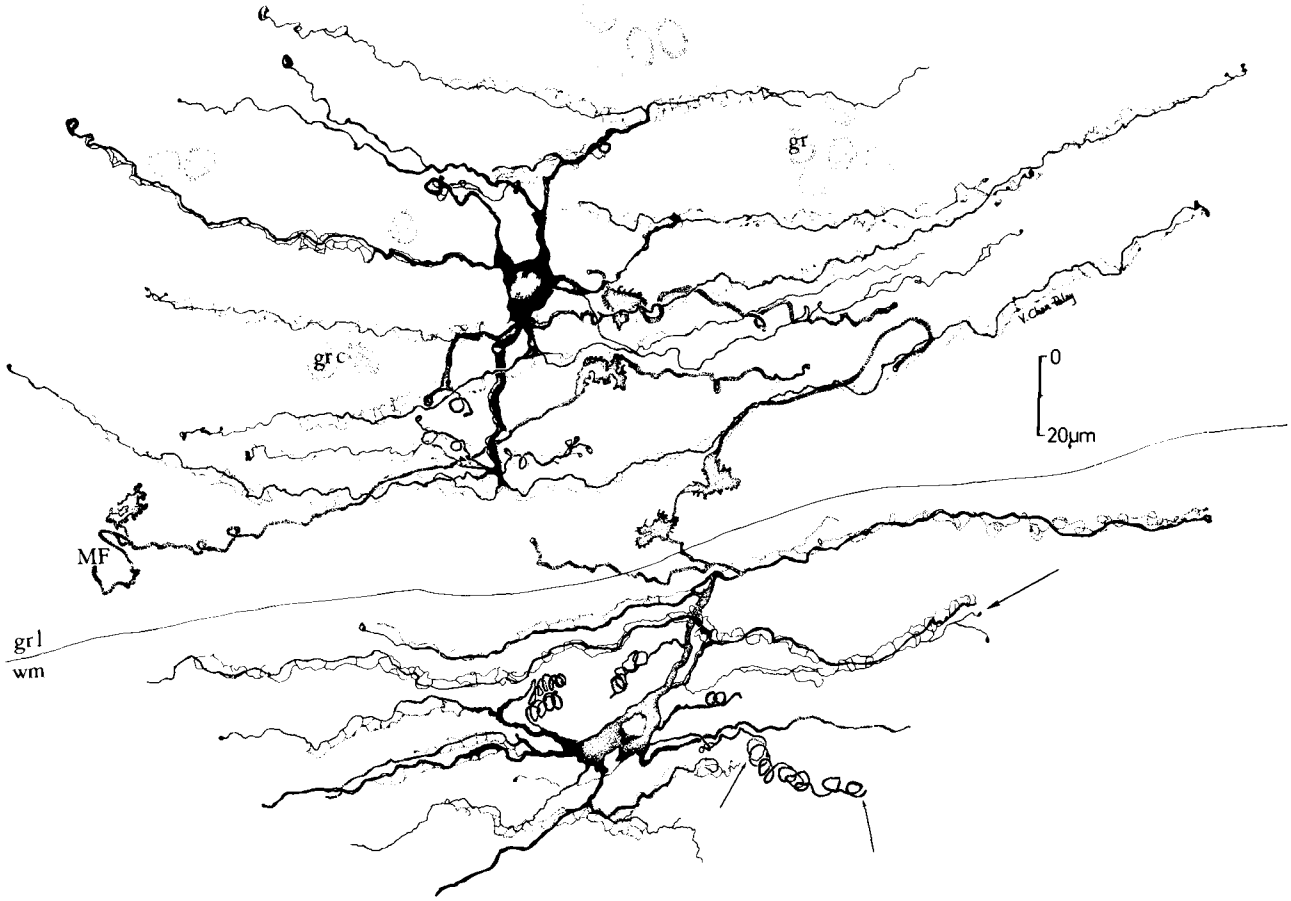


FIG. 10. Camera lucida drawing of oligodendrocytes in white matter and granular layer of cerebellum of adult rat. Cell in lower half of picture lies entirely within white matter. Here myelinated fibers are plaited in regular criss-crossing groups. Long, coiled processes extending from oligodendrocyte are continuous cytoplasmic ribbons in myelin sheaths. These processes serve axons that run in either longitudinal (*heavy arrow*) or transverse (*fine arrows*) directions. Cell in upper half of field lies in granular layer, and nearly all of its processes run approximately longitudinally, although some run transversely. They follow trajectory of myelinated Purkinje axons and mossy fibers. Limits of white matter (*wm*) and granular layer (*grl*) are indicated by a line. Several mossy fibers (*MF*) with their elaborate synaptic varicosities (rosettes) are also shown, along with cell bodies of a few granule cells (*gr c*). Golgi-Kopsch preparation. [From Palay & Chan-Palay (124).]

discriminate function. In most regions of the central nervous system these astrocytic processes ensheath receptive surfaces, particularly dendrites and perikarya; they tend to enclose groups of terminals around dendrites or paired axonal endings and dendritic thorns. They appear to insulate the postsynaptic membrane from nonspecific synaptic influences, for example, from diffusing transmitter, or from ionic imbalance in the interstitial space (122). The latter possibility has received some support from studies on neuroglial responses to alterations in their environment associated with prolonged neural activation (79). Neuroglial cells seem to be particularly avid imbibers of potassium ions from the interstitial space. They may therefore have a role in maintaining the homeostasis of the interstitial fluid upon which continued neural activity depends (30, 63, 118, 131). There is also histochemical and cytochemical evidence that at least some neuroglial cells take up and destroy neurotransmitters (64, 71, 115).

The distribution of the velamentous processes also suggests that these astrocytes may have an important role in organizing the neuropil and even clusters of cells at a local level, so that fibers and cells having similar topographical or functional relations are held together in the same compartment. With such an idea astrocytes are likened to the cement in a mosaic pattern or the leading in a stained glass window—its pattern creates organization in the effective elements of the structure (22).

Oligodendrocytes

The oligodendrocytes are small rounded cells with dark nuclei and dark cytoplasm. They are found both in the white matter and in the cranial nerves and spinal roots. In well-fixed material stained with basic dyes, the cytoplasm is a thin, dark layer surrounding an oval or bean-shaped nucleus, but in poorly fixed material the cells are often grossly swollen and the

cytoplasm appears colorless and vacuous. In silver-stained material (Fig. 10) these cells are small, about 6 or 8 μm across, and they have only a few radiating, branching processes in contrast to the multitudinous bristles of the astrocyte. The appearance of these cells in the electron microscope is distinctive (Fig. 9). The nucleus is round or kidney-shaped and very dense. The chromatin is concentrated in blocks that are almost continuous with one another, and the nucleolus, although recognizable, is difficult to discern. The perinuclear cisterna within the nuclear envelope stands out starkly, separating the dense karyoplasm from the thin shell of cytoplasm. The cytoplasm contains the usual organelles, but they are peculiarly composed. The mitochondria are sparse, plump, short rods and the granular endoplasmic reticulum is represented by a few bent and branching cisternae. All of these organelles are immersed in a dark matrix filled with moderately dense granules, among which several clusters stand out as polysomal arrays of ribosomes. The nature of the background granules is obscure. Furthermore the cytoplasm is permeated by large numbers of microtubules coursing in all directions and out into the processes. These processes are generally cylindrical without laminate expansions (except where they produce myelin), and they are packed with longitudinal microtubules in a dark matrix. Since the concentration of microtubules is noticeably higher than in any other type of neural or neuroglial process, the profiles of oligodendroglial processes can be easily recognized even when separated by sectioning from their cell of origin.

The oligodendrocyte is responsible for forming and maintaining the myelin sheath surrounding medullated axons in the central nervous system. This sheath consists of cell membrane helically wrapped around the axon in numerous turns. A single oligodendrocyte can generate as many as 40 or more myelin sheaths, as can be demonstrated in Golgi preparations (Fig. 10), in which the cytoplasmic continuity between the cell body and the spirally wound processes can be seen (69). Each segment of myelin sheath is produced by a separate process. At the points where successive segments of myelin touch along the course of a nerve fiber, the myelin is interrupted for variable distances. In the central nervous system such interruptions (the nodes of Ranvier) tend to be rather long; they may stretch for several micrometers. Further discussion of the myelin sheath is deferred to the subsection *Schwann Cell*.

Microglia

Microglia, as indicated earlier, may be a composite group of cells including not only elements native to the central nervous system but also invading mesenchymal cells that originate from the blood or from perivascular components. In any case they are not numerous in the normal nervous system of an animal that has not been exposed to infection, trauma, and other disease. They appear in large numbers at the sites of lesions, hemorrhages, tumors, and other conditions destructive of nervous tissue. The cells in these lesions rapidly take up fragments of debris and enclose them in phagocytic vacuoles. Depending upon the amount of destruction and the rate at which it proceeds, these cells digest the debris locally or, laden with their booty, carry it off to the blood vessels, around which they congregate. Active or recently active microglial cells are therefore labeled by their burden of inclusions and thus can be identified in electron micrographs as well as in light microscope preparations. These cells have dense, elongated nuclei and a cytoplasm that is intermediate in density between that of the astrocyte and the oligodendrocyte. The granular endoplasmic reticulum is loosely arranged as simple, meandering cisternae with scattered polyribosomal arrays. The mitochondria are small spheres or rods and are not distinctive. The Golgi apparatus is small and confined to one pole of the cell or to the hof indenting the nucleus. The inclusions are heterogeneous, varying from large masses of dense, laminated material to small, finely granular lysosomes. Droplets of lipid are also seen. Such cells are difficult to find in the brains of healthy, uninjured animals such as the laboratory rat but appear with increased frequency in animals that suffer from or have suffered from various illnesses such as pneumonitis. They also increase in numbers with increasing age, even without a history of disease (191). In street animals, apparently healthy but of unknown history, cells of this type are frequent in the perivascular tissues. In experimental studies in which blood cells were labeled with radioactive thymidine in advance of inflicting a small brain lesion (75, 77), nearly all of the phagocytic microglia around the lesions were shown to originate from the bloodstream.

In the brains of healthy animals, however, there is a small population of cells that resemble these microglia except, of course, for the phagocytic vacuoles. They have the dark, oval nuclei and the strung out endoplasmic reticulum in a light cytoplasmic matrix that characterize the activated microglia. Vaughn & Peters (193) proposed that these are undifferentiated neuroglial precursors, capable of migrating to a scene of injury and phagocytizing debris. Vaughn and his co-workers (170, 192) found that these cells increased in number in optic nerve undergoing degeneration after enucleation but that they were not labeled by prior injections of tritiated thymidine. They concluded that this "third neuroglial cell" is a native of the nervous system and is not hematogenous. The two views concerning the origin of the phagocytic cells are not irreconcilable. Cells of local origin as well as hematogenous elements can participate in the response to injury, the relative numbers of each depending upon such factors as severity of vascular damage, chemical attractants, amount and concentration of dying tissue, and rate of development of the lesion. In this connection it should also be remembered that astrocytes, especially protoplasmic astrocytes, are capable of phagocytosis. In lesions involv-

ing diffuse degeneration of nerve terminals, as after a remote tract section, they may be the only neuroglial elements in the neuropil that respond. Their activity is so efficient and discreet that they can remove dying terminals and digest them in a few hours, leaving the site of the lesion swept clean as if nothing had happened. Finally, it is the astrocytes that reconstitute the breach in the glia limitans externa by marshalling fresh palisades of end feet at the new surface.

Schwann Cell

The Schwann cell in the peripheral nervous system corresponds to all three neuroglial cells in the central nervous system. Like the astrocytes it takes its shape from its location and function. It sends out sheetlike cytoplasmic streamers and encapsulates nerve cells and fibers. Like the oligodendrocyte it produces myelin. Like the microglial cell it can become a phagocyte in time of need. But there are certain differences. Schwann cells deal with each axon individually. When a Schwann cell ensheathes a bundle of axons, as in an unmyelinated nerve, each axon runs in a separate tunnel or furrow in the surface of the cell. Astrocytic processes accompany axons and run in planes between them, like connective tissue fascia. A single Schwann cell generates the myelin sheath for only one axon, while oligodendrocytes may service dozens. The nodes of Ranvier in peripheral myelin sheaths are usually narrow, abrupt separations between segments, whereas they are long interruptions in the central myelin sheath. Finally, the Schwann cell retains a good deal more cytoplasm in the membranes wrapped into the myelin sheath, so that not only is there cytoplasm in the outermost and innermost layers of the myelin, but also there are ribbons of cytoplasm incarcerated in the windings of membrane composing the myelin. These residual ribbons of cytoplasm give rise to the funnel-shaped Schmidt-Lanterman incisures that are characteristic of peripheral myelin but are usually absent in the central nervous system.

The Schwann cell has a fairly compact, busy cytoplasm (131). The granular endoplasmic reticulum is often collected into imbricated aggregates, which if seen in a nerve cell would be called Nissl substance. Polyribosomes are numerous. The mitochondria are plump rods with many transverse cristae, and the Golgi apparatus is well developed. The cell surface exhibits many pits and coated vesicles. The nucleus is large with dense, blocky chromatin masses.

Probably the most impressive evidence of the activity of the Schwann cell is the formation of the myelin sheath. As the nerve fibers grow out from the spinal cord or the dorsal root ganglia, the precursors of the Schwann cell follow them, undergo cell divisions, and take up serial positions on the nerve. In the beginning any individual Schwann cell is associated with numerous axons, and this is the condition that with some refinement prevails in unmyelinated nerves. The first step in the formation of myelin is the establishment of a 1:1 ratio between a Schwann cell and a length of an axon, the isolation of a single axon within the territory of a single Schwann cell (108, 194, 195). Each axon undergoing myelination is encapsulated by a succession of single Schwann cells forming a segmented chain. Myelination can begin in any of these segments, not necessarily in neighboring segments or in more proximal segments first. The axon sinks deep into a furrow in the satellite cell, which gradually forms a tunnel-like sleeve around it. In this process the lips of the furrow come together, overlap, and seal together in several longitudinal tight junctions. The membrane complex so formed is known as the mesaxon, which elongates irregularly as the overlapping folds continue to grow. A spiral sheet of cytoplasm bound by the plasmalemma is gradually wound around the axon becoming more regular with advancing age. At varying points in its expanse the cytoplasm is resorbed or disappears, and the plasmalemma is allowed to condense upon itself forming the major dense line, as it appears in thin sections examined in the electron microscope. The residual interstitial space also condenses as the spiral matures, and where the outer face of the plasmalemma compacts upon itself in successive gyres, it gives rise to the minor or the intraperiod dense line. However, with special techniques this dense line is always separable into two thin lines, corresponding to the outer leaflet of the plasmalemma of successive gyres bounding the interstitial space between (153). The interval between dense lines, the major period, varies according to species of animal and importantly according to the technique of preparation. In a beautiful study of myelin using water-soluble embedding materials Peterson & Pease (133) have demonstrated that the period of about 18 nm shown in the X-ray diffraction analyses of fresh nerve, can be preserved in sectioned material and seen in the electron microscope.

In the process of elaborating this myelin sheath a Schwann cell expands the extent of its cell membrane some 100 times while the axon increases only 5 times in circumference (194). Thin ribbons of cytoplasm remain in the sheath at its ends (the edges of the original cytoplasmic sleeve) and laced throughout it. Because these ribbons are also spirally wound around the axon, they result in the formation of cone-shaped interruptions in the continuity of the myelin, which are known from light microscopy as the Schmidt-Lanterman incisures or clefts. These ribbons of cytoplasm, continuous with the main perinuclear cytoplasmic mass, may provide routes for metabolic exchanges and renewal of structural components that go on within the myelin sheath, which otherwise appears almost crystalline in organization and stability.

Successive Schwann cells in the series along an

axon are separated from each other by thin interruptions, which leave the axonal surface bare except for the continuous basal lamina investing the entire nervous system. Since the myelin sheath is an integral part of the Schwann cell, it is also interrupted at the junctions between successive Schwann cells. These interruptions are known as nodes of Ranvier. The axonal plasmalemma between segments of myelin displays an undercoating similar to that found in the initial segment. In the paranodal region, where adjacent myelin segments terminate, the edges of the successive laminae of the myelin sheath touch the surface of the axon at a greater and greater distance from the midpoint of the segment. This is a simple consequence of the fact that the sheath is a spiral wrapping of a single sheet, which grows progressively larger with each successive turn. Since a ribbon of cytoplasm runs along this edge, it appears in longitudinal sections of a nerve as a series of membrane loops finishing off each successive lamina and containing a few microtubules and occasionally a mitochondrion. These loops are attached to the surface of the axon by a series of circumferential linear septate junctions that tend to seal off the myelin sheath from the interstitial space that surrounds the axon at the node of Ranvier (94, 163). These details of the structure of the myelin sheath correlate quite well with its electrical properties, and the morphological differences between the axonal membrane at the nodes and beneath the sheath are consonant with the requirements of saltatory conduction. Further discussion of these problems will be found in the chapters by Hille and by Orkand in this *Handbook*.

REFERENCES

1. AKERT, K., K. PFENNINGER, AND C. SANDRI. The fine structure of synapses in the subfornical organ of the cat. *Z. Zellforsch. Mikroskop. Anat.* 81: 537–556, 1967.
2. AKERT, K., K. PFENNINGER, C. SANDRI, AND M. MOOR. Freeze etching and cytochemistry of vesicles and membrane complexes in synapses of the central nervous system. In: *Structure and Function of Synapses*, edited by G. D. Pappas and D. P. Purpura. New York: Raven Press, 1972, p. 67–86.
3. ANDRES, K. H. Untersuchungen über den Feinbau von Spinalganglien. *Z. Zellforsch. Mikroskop. Anat.* 55: 1–48, 1961.
4. ANGEVINE, J. B., JR. Time of neuron origin in the hippocampal region. An autoradiographic study in the mouse. *Exptl. Neurol. Suppl.* 2: 1–70, 1965.
5. ANGEVINE, J. B., JR., AND R. L. SIDMAN. Autoradiographic study of cell migration during histogenesis of cerebral cortex in the mouse. *Nature* 192: 766–768, 1961.
6. BAKER, R., AND R. LLINÁS. Electrotonic coupling between neurones in the rat mesencephalic nucleus. *J. Physiol. London* 212: 45–63, 1971.
7. BARONDES, S. H. Axoplasmic transport: a report of an NRP work session. *Neurosci. Res. Progr. Bull.* 5: 307–419, 1967.
8. BENNETT, M. V. L., G. D. PAPPAS, E. ALJURE, AND Y. NAKAJIMA. Physiology and ultrastructure of electrotonic junctions. II. Spinal and medullary electromotor nuclei in mormyrid fish. *J. Neurophysiol.* 30: 180–208, 1967.
9. BENNETT, M. V. L., G. D. PAPPAS, M. GIMÉNEZ, AND Y. NAKAJIMA. Physiology and ultrastructure of electrotonic junctions. IV. Medullary electromotor nuclei in gymnotid fish. *J. Neurophysiol.* 30: 236–300, 1967.
10. BERRY, M., AND A. W. ROGERS. The migration of neuroblasts in the developing cerebral cortex. *J. Anat.* 99: 691–709, 1965.
11. BILLINGS, S. M., AND F. J. SWARTZ. DNA content of Mauthner cell nuclei in *Xenopus laevis*: a spectrophotometric study. *Z. Anat. Entwicklungs.* 129: 14–23, 1969.
12. BODIAN, D. Electron microscopy: two major synaptic types on spinal motoneurons. *Science* 151: 1093–1094, 1966.
13. BOHAN, T. B., A. F. BOYNE, P. S. GUTH, Y. NARAYANAN, AND T. H. WILLIAMS. Electron-dense particle in cholinergic synaptic vesicles. *Nature* 244: 32, 1973.
14. BRANTON, D. Fracture faces of frozen membranes. *Proc. Natl. Acad. Sci. US* 55: 1048–1056, 1966.
15. BRIGHTMAN, M. W., AND T. S. REESE. Junctions between intimately apposed cell membranes in the vertebrate brain. *J. Cell Biol.* 40: 648–677, 1969.
16. BULLOCK, T. H. On the anatomy of the giant neurons of the visceral ganglion of *Aplysia*. In: *Nervous Inhibition*, edited by E. Florey. New York: Pergamon, 1961, p. 233–240.
17. CHANDLER, R. L., AND R. WILLIS. An intranuclear fibrillar lattice in neurons. *J. Cell Sci.* 1: 283–286, 1966.
18. CHAN-PALAY, V. The tripartite structure of the undercoat in initial segments of Purkinje cell axons. *Z. Anat. Entwicklungs.* 139: 1–10, 1972.
19. CHAN-PALAY, V. On the identification of the afferent axon terminals in the nucleus lateralis of the cerebellum. An electron microscope study. *Z. Anat. Entwicklungs.* 142: 149–186, 1973.
20. CHAN-PALAY, V., S. M. BILLINGS-GAGLIARDI, AND S. L. PALAY. Meynert cells in the primate visual cortex. *J. Neurocytol.* 3: 631–658, 1974.
21. CHAN-PALAY, V., AND S. L. PALAY. Interrelations of basket cell axons and climbing fibers in the cerebellar cortex of the rat. *Z. Anat. Entwicklungs.* 132: 191–227, 1970.
22. CHAN-PALAY, V., AND S. L. PALAY. The form of velate astrocytes in the cerebellar cortex of monkey and rat: high voltage electron microscopy of rapid Golgi preparations. *Z. Anat. Entwicklungs.* 138: 1–19, 1972.
23. COGGESHALL, R. E. A light and electron microscope study of the abdominal ganglion of *Aplysia californica*. *J. Neurophysiol.* 30: 1263–1287, 1967.
24. COGGESHALL, R. E., B. A. YAKSTA, AND F. J. SWARTZ. A cytophotometric analysis of the DNA in the nucleus of the giant cell, R-2, in *Aplysia*. *Chromosoma* 32: 205–212, 1970.
25. COHEN, J., V. MAREŠ, AND Z. LODIN. DNA content of purified preparations of mouse Purkinje neurons isolated by a velocity sedimentation technique. *J. Neurochem.* 20: 651–657, 1973.
26. CONRADI, S. Ultrastructural specialization of the initial axon segment of cat lumbar motoneurons. Preliminary observations. *Acta Soc. Med. Upsalien.* 71: 281–284, 1966.
27. CONRADI, S. Observations on the ultrastructure of the axon hillock and initial axon segment of lumbosacral motoneurons in the cat. *Acta Physiol. Scand. Suppl.* 332: 65–84, 1969.
28. DAVISON, P. F., AND B. WINSLOW. The protein subunit of calf brain neurofilaments. *J. Neurobiol.* 5: 119–133, 1974.
29. DEITERS, O. *Untersuchungen über Gehirn und Rückenmark*. Braunschweig: Vieweg, 1865.
30. DENNIS, M. J., AND H. M. GERSCHENFELD. Some physiological properties of identified mammalian neuroglial cells. *J. Physiol. London* 203: 211–222, 1969.
31. DIAMOND, J., E. G. GRAY, AND G. M. YASARGIL. The function of the dendritic spine: an hypothesis. In: *Excitatory Synaptic Mechanisms*, edited by P. Andersen and J. K. S. Jansen. Oslo: Universitetsforlaget, 1970, p. 213–222.
32. DOGIEL, A. S. *Der Bau der Spinalganglien des Menschen und der Säugetiere*. Jena: Fischer, 1908.
33. DROZ, B. Synthèse et transfert des protéines cellulaires dans

les neurones ganglionnaires. Étude radioautographique quantitative en microscopie électronique. *J. Microscopie* 6: 201–228, 1967.
34. ECCLES, J. C. *The Physiology of Nerve Cells*. Baltimore: Johns Hopkins Press, 1957.
35. ECCLES, J. C., M. ITO, AND J. SZENTÁGOTHAI. *The Cerebellum as a Neuronal Machine*. New York: Springer, 1967.
36. FAMIGLIETTI, E. V., JR. Dendro-dendritic synapses in the lateral geniculate nucleus of the cat. *Brain Res.* 20: 181–191, 1970.
37. FAMIGLIETTI, E. V., JR., AND A. PETERS. The synaptic glomerulus and the intrinsic neuron in the dorsal lateral geniculate nucleus of the cat. *J. Comp. Neurol.* 144: 285–334, 1972.
38. FAVARD, P., AND N. CARASSO. The preparation and observation of thick biological sections in the high voltage electron microscope. *J. Microscopy* 97: 59–81, 1973.
39. FELDMAN, M. L., AND A. PETERS. Intranuclear rods and sheets in rat cochlear nucleus. *J. Neurocytol.* 1: 109–127, 1972.
40. FOSTER, M., AND C. S. SHERRINGTON. *A Text Book of Physiology, Part III: The Central Nervous System* (7th ed.). London: Macmillan, 1897.
41. FOX, C. A., AND J. W. BARNARD. A quantitative study of the Purkinje cell dendritic branchlets and their relationship to afferent fibres. *J. Anat.* 91: 299–313, 1957.
42. FRANKE, W. W. Structure, biochemistry, and functions of the nuclear envelope. *Intern. Rev. Cytol. Suppl.* 4: 71–236, 1974.
43. FRIEND, D. S., AND N. B. GILULA. A distinctive cell contact in the rat adrenal cortex. *J. Cell Biol.* 53: 148–163, 1972.
44. FUJITA, S. Quantitative analysis of cell proliferation and differentiation in the cortex of the postnatal cerebellum. *J. Cell Biol.* 32: 277–287, 1967.
45. FUJITA, S. DNA constancy in neurons of the human cerebellum and spinal cord as revealed by Feulgen cytophotometry and cytofluorometry. *J. Comp. Neurol.* 155: 195–202, 1974.
46. FUJITA, S., T. HATTORI, M. FUKUDA, AND T. KITAMURA. DNA contents in Purkinje cells and inner granule neurons in the developing rat cerebellum. *Develop. Growth Differentiation* 16: 205–211, 1974.
47. FUJITA, Y. Activity of dendrites of single Purkinje cells and its relationship to so-called inactivation response in rabbit cerebellum. *J. Neurophysiol.* 31: 131–141, 1968.
48. FUORTES, M. G. F., K. FRANK, AND M. C. BECKER. Steps in the production of motoneuron spikes. *J. Gen. Physiol.* 40: 735–752, 1957.
49. FURSHPAN, E. J. "Electrical transmission" at an excitatory synapse in a vertebrate brain. *Science* 144: 878–880, 1964.
50. FURSHPAN, E. J., AND D. D. POTTER. Transmission at the giant motor synapses of the crayfish. *J. Physiol. London* 145: 289–325, 1959.
51. GEFFEN, L. B., AND B. G. LIVETT. Synaptic vesicles in sympathetic neurons. *Physiol. Rev.* 51: 98–157, 1971.
52. GOBEL, S. Axo-axonic septate junctions in the basket formations of the cat cerebellar cortex. *J. Cell Biol.* 51: 328–333, 1971.
53. GOLGI, C. Sulla fina anatomia degli organi centrali del sistema nervoso. I. Note preliminari sulla struttura, morfologia e vicendevoli rapporti delle cellule gangliari. *Riv. Sper. Freniat. Med. Leg. Alienazioni Ment.* 8: 165–195, 1882.
54. GOODENOUGH, D. A., AND J.-P. REVEL. A fine structural analysis of intercellular junctions in the mouse liver. *J. Cell Biol.* 45: 272–290, 1970.
55. GRAY, E. G. Axo-somatic and axo-dendritic synapses of the cerebral cortex: an electron microscope study. *J. Anat.* 93: 420–433, 1959.
56. GRAY, E. G. A morphological basis for pre-synaptic inhibition? *Nature* 193: 82–83, 1962.
57. GRAY, E. G., AND R. W. GUILLERY. The basis for silver staining of synapses of the mammalian spinal cord: a light and electron microscope study. *J. Physiol. London* 157: 581–588, 1961.
58. GRAY, E. G., AND R. W. GUILLERY. A note on the dendritic spine apparatus. *J. Anat.* 97: 389–392, 1963.
59. GRAY, E. G., AND R. A. WILLIS. On synaptic vesicles, complex vesicles, and dense projections. *Brain Res.* 24: 149–168, 1970.
60. GRILLO, M. A. Electron microscopy of sympathetic tissues. *Pharmacol. Rev.* 18: 387–399, 1966.
61. HEIDENHAIN, M. *Plasma und Zelle*. Jena: Fischer, vol. 2, 1911.
62. HELD, H. Beiträge zur Structur der Nervenzellen und ihrer Fortsätze. Part II. *Arch Anat. Physiol. Anat. Abt.* 204–293, 1897. Part III. *Arch. Anat. Physiol. Anat. Abt. Suppl.* 273–312, 1897.
63. HENN, F. A., H. HALJAMAE, AND A. HAMBERGER. Glial cell function: active control of extracellular K^+ concentration. *Brain Res.* 43: 437–443, 1972.
64. HENN, F. A., AND A. HAMBERGER. Glial cell function. Uptake of transmitter substances. *Proc. Natl. Acad. Sci. US* 68: 2686–2690, 1971.
65. HERMAN, C. J., AND L. W. LAPHAM. DNA content of neurons in the cat hippocampus. *Science* 160: 537, 1968.
66. HERMAN, C. J., AND L. W. LAPHAM. Neuronal polyploidy and nuclear volumes in the cat central nervous system. *Brain Res.* 15: 35–48, 1969.
67. HILLMAN, D. E., AND R. LLINÁS. Calcium-containing electron-dense structures in the axons of the squid giant synapse. *J. Cell Biol.* 61: 146–155, 1974.
68. HINRICHSEN, C. F. L., AND L. M. H. LARRAMENDI. Synapses and cluster formation of the mouse mesencephalic fifth nucleus. *Brain Res.* 7: 296–299, 1968.
69. HORTEGA, P. DEL RÍO. Tercerea aportación al conocimiento morfológico e interpretación functional de la oligodendroglía. *Mem. Real Soc. Esp. Hist. Nat. Madrid* 14: 5–122, 1928.
70. HUNEEUS, F. C., AND P. F. DAVISON. Fibrillar proteins of the squid axoplasm. I. Neurofilament protein. *J. Mol. Biol.* 52: 415–428, 1970.
71. HYDE, J. C., AND N. ROBINSON. Localization of sites of GABA catabolism in the rat retina. *Nature* 248: 432–433, 1974.
72. KAISERMAN-ABRAMOF, I. R., AND A. PETERS. Some aspects of the morphology of Betz cells in the cerebral cortex of the cat. *Brain Res.* 43: 527–546, 1972.
73. KANDEL, E. R., W. T. FRAZIER, AND R. E. COGGESHALL. Opposite synaptic actions mediated by different branches of an identifiable interneuron in *Aplysia*. *Science* 155: 346–349, 1967.
74. KANDEL, E. R., AND D. GARDNER. The synaptic actions mediated by the different branches of a single neuron. *Res. Publ. Assoc. Res. Nervous Mental Disease* 50: 91–146, 1972.
75. KITAMURA, T., H. HATTORI, AND S. FUJITA. Autoradiographic studies on histogenesis of brain macrophages in the mouse. *J. Neuropathol. Exptl. Neurol.* 31: 502–518, 1972.
76. KOHNO, K. Neurotubules contained within the dendrite and axon of Purkinje cell of frog. *Bull. Tokyo Med. Dent. Univ.* 11: 411–442, 1964.
77. KONIGSMARK, B. W., AND R. L. SIDMAN. Origin of brain macrophages in the mouse. *J. Neuropathol. Exptl. Neurol.* 22: 643–676, 1963.
78. KORN, H., C. SOTELO, AND F. CREPEL. Electrotonic coupling between neurons in the rat lateral vestibular nucleus. *Exptl. Brain Res.* 16: 255–275, 1973.
79. KUFFLER, S. W., AND J. G. NICHOLLS. The physiology of neuroglial cells. *Ergeb. Physiol. Biol. Chem. Exptl. Pharmakol.* 57: 1–90, 1966.
80. KUNO, M., AND J. T. MIYAHARA. Factors responsible for multiple discharge of neurons in Clarke's column. *J. Neurophysiol.* 31: 624–638, 1968.
81. KUNO, M., E. MUÑOZ-MARTINEZ, AND M. RANDIC. Synaptic action on Clarke's column neurones in relation to afferent terminal size. *J. Physiol. London* 228: 343–360, 1973.
82. LANDIS, D. M. D., AND T. S. REESE. Differences in membrane structure between excitatory and inhibitory synapses

in the cerebellar cortex. *J. Comp. Neurol.* 155: 93–126, 1974.
83. LANDIS, D. M. D., T. S. REESE, AND E. RAVIOLA. Differences in membrane structure between excitatory and inhibitory components of the reciprocal synapse in the olfactory bulb. *J. Comp. Neurol.* 155: 67–92, 1974.
84. LAPHAM, L. W. Tetraploid DNA content of Purkinje neurons of human cerebellar cortex. *Science* 159: 310–312, 1968.
85. LASEK, R. Axoplasmic transport in cat dorsal root ganglion cells: as studied with [^{3}H]-*l*-leucine. *Brain Res.* 7: 360–377, 1968.
86. LASEK, R. Axoplasmic transport of labeled proteins in rat ventral motoneurons. *Exptl. Neurol.* 21: 41–51, 1968.
87. LASEK, R., AND W. J. DOWER. *Aplysia californica*: analysis of nuclear DNA in individual nuclei of giant neurons. *Science* 172: 278–280, 1971.
88. LEHNINGER, A. L. Cell organelles: the mitochondrion. In: *The Neurosciences*, edited by G. C. Quarton, T. Melnechuk, and F. O. Schmitt. New York: Rockefeller Univ. Press, 1967, p. 91–100.
89. LENTZ, R. D., AND L. W. LAPHAM. Postnatal development of tetraploid DNA content in rat Purkinje cells: a quantitative cytochemical study. *J. Neuropathol. Exptl. Neurol.* 29: 43–56, 1970.
90. LEVAY, S. On the neurons and synapses of the lateral geniculate nucleus of the monkey, and the effects of eye enucleation. *Z. Zellforsch. Mikroskop. Anat.* 113: 396–419, 1971.
91. LIEBERMAN, A. R. Neurons with presynaptic perikarya and presynaptic dendrites in the rat lateral geniculate nucleus. *Brain Res.* 59: 35–59, 1973.
92. LIEBERMAN, A. R., AND K. E. WEBSTER. Presynaptic dendrites and a distinctive class of synaptic vesicle in the rat dorsal lateral geniculate nucleus. *Brain Res.* 42: 169–200, 1972.
93. LITTAU, V. C., V. G. ALLFREY, J. H. FRENSTER, AND A. E. MIRSKY. Active and inactive regions of nuclear chromatin as revealed by electron microscope autoradiography. *Proc. Natl. Acad. Sci. US* 52: 93–100, 1964.
94. LIVINGSTON, R. B., K. PFENNINGER, H. MOOR, AND K. AKERT. Specialized paranodal and interparanodal glial-axonal junctions in the peripheral and central nervous system: a freeze-etching study. *Brain Res.* 58: 1–24, 1973.
95. LLINÁS, R., R. BAKER, AND C. SOTELO. Electrotonic coupling between neurons in cat inferior olive. *Brain Res.* 67: 560–571, 1974.
96. LLINÁS, R., AND D. E. HILLMAN. Physiological and morphological organization of the cerebellar circuits in various vertebrates. In: *Neurobiology of Cerebellar Evolution and Development*, edited by R. Llinás. Chicago: AMA-ERF Institute for Biomedical Research, 1969, p. 43–73.
97. LLINÁS, R., AND C. NICHOLSON. Electrophysiological analysis of alligator cerebellar cortex: a study on dendritic spikes. In: *Neurobiology of Cerebellar Evolution and Development*, edited by R. Llinás. Chicago: AMA-ERF Institute for Biomedical Research, 1969, p. 431–464.
98. LLINÁS, R., AND C. NICHOLSON. Electrophysiological properties of dendrites and somata in alligator Purkinje cells. *J. Neurophysiol.* 34: 532–551, 1971.
99. LUND, R. D. Synaptic patterns in the superficial layers of the superior colliculus of the rat. *J. Comp. Neurol.* 135: 179–208, 1969.
100. LUND, R. D. Synaptic patterns in the superficial layers of the superior colliculus of the monkey *Macaca mulatta*. *Exptl. Brain Res.* 15: 194–211, 1972.
101. MANN, D. M. A., AND P. O. YATES. Polypoidy in the human nervous system. Part 1. The DNA content of neurones and glia of the cerebellum. *J. Neurol. Sci.* 18: 183–196, 1973.
102. MANNEN, H. Arborisations dendritiques. Étide topographique et quantitative dans le noyau vestibulaire du chat. *Arch. Ital. Biol.* 103: 197–219, 1965.
103. MANNEN, H. Contribution to the quantitative study of the nervous tissue. A new method for measurement of the volume and surface area of neurons. *J. Comp. Neurol.* 126: 75–89, 1966.
104. MANUELIDIS, L., AND E. E. MANUELIDIS. On the DNA content of cerebellar Purkinje cells *in vivo* and *in vitro*. *Exptl. Neurol.* 43: 192–206, 1974.
105. MAREŠ, V., Z. LODIN, AND J. ŠÁCHA. A cytochemical and autoradiographic study of nuclear DNA in mouse Purkinje cells. *Brain Res.* 53: 273–289, 1973.
106. MAREŠ, V., B. SCHULTZE, AND W. MAURER. Stability of DNA in Purkinje cell nuclei of the mouse. An autoradiographic study. *J. Cell Biol.* 63: 665–674, 1974.
107. MARTIN, A. R., AND G. PILAR. Dual mode of synaptic transmission in the avian ciliary ganglion. *J. Physiol. London* 168: 443–463, 1963.
108. MARTIN, J. R., AND H. DE F. WEBSTER. Mitotic Schwann cells in developing nerve: their changes in shape, fine structure, and axonal relationships. *Develop. Biol.* 32: 417–431, 1973.
109. MCNUTT, N. S., AND R. S. WEINSTEIN. The ultrastructure of the nexus. A correlated thin-section and freeze-cleave study. *J. Cell Biol.* 47: 666–688, 1970.
110. MIALE, I., AND R. L. SIDMAN. An autoradiographic analysis of histogenesis in the mouse cerebellum. *Exptl. Neurol.* 4: 277–296, 1961.
111. MILHAUD, M., AND G. D. PAPPAS. Postsynaptic bodies in the habenula and interpeduncular nuclei of the cat. *J. Cell Biol.* 30: 437–441, 1966.
112. MOREST, D. K. Dendrodendritic synapses of cells that have axons: the fine structure of the Golgi type II cell in the medial geniculate body of the cat. *Z. Anat. Entwicklungs.* 133: 216–246, 1971.
113. MUNGAI, J. M. Dendritic patterns in the somatic sensory cortex of the cat. *J. Anat.* 101: 403–418, 1967.
114. NAKAJIMA, Y. Fine structure of the synaptic endings on the Mauthner cell of the goldfish. *J. Comp. Neurol.* 156: 375–402, 1974.
115. NEAL, M. J., AND L. L. IVERSEN. Autoradiographic localization of ^{3}H-Gaba in rat retina. *Nature New Biol.* 235: 217–218, 1972.
116. NOVÁKOVÁ, V., W. SANDRITTER, AND G. SCHLUETER. DNA content in rat central nervous system. *Exptl. Cell Res.* 60: 454–456, 1970.
117. NOVIKOFF, A. B. Enzyme localization and ultrastructure of neurons. In: *The Neuron*, edited by H. Hydén. Amsterdam: Elsevier, 1967, p. 255–318.
118. ORKAND, R. K., J. G. NICHOLLS, AND S. W. KUFFLER. Effect of nerve impulses on the membrane potential of glial cells in the central nervous system of amphibia. *J. Neurophysiol.* 29: 788–806, 1966.
119. PALAY, S. L. Synapses in the central nervous system. *J. Biophys. Biochem. Cytol.* 2, Suppl. 193–202, 1956.
120. PALAY, S. L. The morphology of synapses in the central nervous system. *Exptl. Cell Res. Suppl.* 5: 275–293, 1958.
121. PALAY, S. L. The structural basis for neural action. In: *Brain Function. Vol. 2. RNA and Brain Function; Memory and Learning*, edited by M. A. B. Brazier. Los Angeles: Univ. of California Press, 1964, p. 69–108.
122. PALAY, S. L. The role of neuroglia in the organization of the central nervous system. In: *Nerve as a Tissue*, edited by K. Rodahl and B. Issekutz, Jr. New York: Hoeber-Harper, 1966, p. 3–10.
123. PALAY, S. L., AND V. CHAN-PALAY. High voltage electron microscopy of the central nervous system in Golgi preparations. *J. Microscopy* 97: 41–47, 1973.
124. PALAY, S. L., AND V. CHAN-PALAY. *Cerebellar Cortex, Cytology and Organization*. Berlin: Springer Verlag, 1974.
125. PALAY, S. L., AND G. E. PALADE. The fine structure of neurons. *J. Biophys. Biochem. Cytol.* 1: 69–88, 1955.
126. PALAY, S. L., C. SOTELO, A. PETERS, AND P. M. ORKAND. The axon hillock and the initial segment. *J. Cell Biol.* 38: 193–201, 1968.
127. PAPPAS, G. D., Y. ASADA, AND M. V. L. BENNETT. Morphological correlates of increased coupling resistance at an electrotonic synapse. *J. Cell Biol.* 49: 173–188, 1971.

128. PASIK, P., T. PASIK, J. HÁMORI, AND J. SZENTÁGOTHAI. Golgi type II interneurons in the neuronal circuit of the monkey lateral geniculate nucleus. *Exptl. Brain Res.* 17: 18–34, 1973.
129. PETERS, A., AND I. R. KAISERMAN-ABRAMOF. The small pyramidal neuron of the rat cerebral cortex. The perikaryon, dendrites and spines. *Am. J. Anat.* 127: 321–355, 1970.
130. PETERS, A., AND S. L. PALAY. An electron microscope study of the distribution and patterns of astroglial processes in the central nervous system. *J. Anat.* 99: 419, 1965.
131. PETERS, A., S. L. PALAY, AND H. DE F. WEBSTER. *The Fine Structure of the Nervous System. The Neurons and Supporting Cells.* Philadelphia: Saunders, 1976.
132. PETERS, A., C. C. PROSKAUER, AND I. R. KAISERMAN-ABRAMOF. The small pyramidal neuron of the rat cerebral cortex. The axon hillock and initial segment. *J. Cell Biol.* 39: 604–619, 1968.
133. PETERSON, R. G., AND D. C. PEASE. Myelin embedded in polymerized glutaraldehyde-urea. *J. Ultrastruct. Res.* 41: 115–132, 1972.
134. PFENNINGER, K., K. AKERT, H. MOOR, AND C. SANDRI. The fine structure of freeze-fractured presynaptic membranes. *J. Neurocytol.* 1: 129–149, 1972.
135. PFENNINGER, K., C. SANDRI, K. AKERT, AND C. H. ENGSTER. Contribution to the problem of structural organization of the presynaptic area. *Brain Res.* 12: 10–18, 1969.
136. PINCHING, A. J. Myelinated dendritic segments in the monkey olfactory bulb. *Brain Res.* 29: 133–138, 1971.
137. PINCHING, A. J., AND T. P. S. POWELL. The neuropil of the glomeruli of the olfactory bulb. *J. Cell Sci.* 9: 347–377, 1971.
138. POLITOFF, A. L., S. ROSE, AND G. D. PAPPAS. Calcium binding sites in synaptic vesicles of the frog sartorius neuromuscular junction. *J. Cell Biol.* 61: 818–823, 1974.
139. POMERAT, C. M. Dynamic neurogliology. *Texas Rept. Biol. Med.* 10: 885–913, 1952.
140. POMERAT, C. M., W. J. HENDELMAN, C. W. RAIBORN, AND J. F. MASSEY. Dynamic activities of nervous tissues *in vitro*. In: *The Neuron*, edited by H. Hydén. Amsterdam: Elsevier, 1967, p. 119–178.
141. PYSH, J. J., AND R. G. WILEY. Synaptic vesicle depletion and recovery in cat sympathetic ganglia electrically stimulated *in vivo*. Evidence for transmitter secretion by exocytosis. *J. Cell Biol.* 60: 365–374, 1974.
142. RAFOLS, J. A., AND F. VALVERDE. The structure of the dorsal lateral geniculate nucleus in the mouse. A Golgi and electron microscopic study. *J. Comp. Neurol.* 150: 303–332, 1973.
143. RALL, W., G. M. SHEPHERD, T. S. REESE, AND M. W. BRIGHTMAN. Dendrodendritic synaptic pathway for inhibition in the olfactory bulb. *Exptl. Neurol.* 14: 44–56, 1966.
144. RALSTON, H. J. Presynaptic dendrites: evidence for their existence and a proposal for their mechanism. *Nature* 230: 585–587, 1970.
145. RAMBOURG, A. Detection des glycoprotéines en microscopie électronique: coloration de la surface cellulaire et de l'appareil de Golgi par un mélange acide chromique-phosphotungstique. *Compt. Rend.* 265: 1426–1428, 1967.
146. RAMBOURG, A., A. MARRAUD, AND M. CHRETIEN. Tridimensional structure of the forming face of the Golgi apparatus as seen in the high voltage electron microscope after osmium impregnation of the small nerve cells in the semilunar ganglion of the trigeminal nerve. *J. Microscopy* 97: 49–57, 1973.
147. RAMÓN-MOLINER, E. The morphology of dendrites. In: *The Structure and Function of Nervous Tissue*, edited by G. H. Bourne. New York: Academic, vol. 1, 1968, p. 205–267.
148. RAMÓN-MOLINER, E. Presynaptic perikarya in olfactory bulb of guinea pig. *Brain Res.* 63: 351–356, 1973.
149. RAMÓN Y CAJAL, S. Sur la structure de l'écorce cérébrale de quelques mammifères. *Cellule* 7: 125–176, 1891.
150. RAMÓN Y CAJAL, S. Croonian Lecture. La fine structure des centres nerveux. *Proc. Roy. Soc. London* 55: 444–468, 1894.
151. RAMÓN Y CAJAL, S. *Histologie du Système Nerveux de l'Homme et des Vertébrés*, translated by L. Azoulay. Paris: Maloine, vols. 1 and 2, 1909 and 1911.
152. RAMÓN Y CAJAL, S. Les preuves objectives de l'unité anatomique des cellules nerveuses. *Trabajos Lab. Invest. Biol. Madrid* 29: 1–137, 1934. [Translated into English by M. U. Purkiss and C. A. FOX. *Neuron Theory or Reticular Theory? Objective Evidence of the Anatomical Unity of Nerve Cells.* Madrid: Consejo Superior de Investigaciones Cientificas, 1954.]
153. REVEL, J.-P., AND D. W. HAMILTON. The double nature of the intermediate dense line in peripheral nerve myelin. *Anat. Record* 163: 7–16, 1969.
154. REVEL, J.-P., AND M. J. KARNOVSKY. Hexagonal array of subunits in intercellular junctions of the mouse heart and liver. *J. Cell Biol.* 33: C7–C12, 1967.
155. ROBERTSON, J. D. The occurrence of a subunit in the unit membranes of club endings in Mauthner cell synapses in goldfish brains. *J. Cell Biol.* 19: 201–221, 1963.
156. ROBERTSON, J. D., T. S. BODENHEIMER, AND D. E. STAGE. The ultrastructure of Mauthner cell synapses and nodes in goldfish brains. *J. Cell Biol.* 19: 159–199, 1963.
157. RONCORONI, L. Su un nuovo reperto nel nucleo delle cellule nervose. *Arch. Psichiat.* 16: 447–450, 1895.
158. ROSENBLUTH, J. The visceral ganglion of *Aplysia californica*. *Z. Zellforsch. Mikroskop. Anat.* 60: 213–236, 1963.
159. ROVAINEN, C. M. Synaptic interactions of identified nerve cells in the spinal cord of the sea lamprey. *J. Comp. Neurol.* 154: 189–206, 1974.
160. ROVAINEN, C. M. Synaptic interaction of reticulospinal neurons and nerve cells in the spinal cord of the sea lamprey. *J. Comp. Neurol.* 154: 207–223, 1974.
161. SANDRITTER, W., V. NOVÁKOVÁ, J. PILNY, AND G. KIEFER. Cytophotometrische Messungen des Nukleinsäure- und Proteingehaltes von Ganglienzellen der Ratte während der postnatalen Entwicklung und im Alter. *Z. Zellforsch. Mikroskop. Anat.* 80: 145–152, 1967.
162. SCHARFF, J.-H. Sensible Ganglien. In: *Handbuch der mikroskopischen Anatomie des Menschen*, edited by W. Bargmann. Berlin: Springer Verlag, IV/1, 3, 1958.
163. SCHNAPP, B., AND E. MUGNAINI. The myelin sheath: electron microscopic studies with thin sections and freeze-fracture. In: *Golgi Centennial Symposium: Perspectives in Neurobiology*, edited by M. Santini. New York: Raven Press, 1975, p. 209–233.
164. SEÏTE, R. Étude ultrastructurale de divers types d'inclusions nucléaires dans les neurones sympathiques du chat. *J. Ultrastruct. Res.* 30: 152–165, 1970.
165. SEÏTE, R., J. ESCAIG, AND S. COUINEAU. Microfilaments et microtubules nucléaires et organisation ultrastructurale des bâtonnets intranucléaires des neurones sympathiques. *J. Ultrastruct. Res.* 37: 449–478, 1971.
166. SEÏTE, R., N. MEI, AND S. COUINEAU. Modification quantitative des bâtonnets intranucléaries des neurones sympathiques sou l'influence de la stimulation électrique. *Brain Res.* 34: 277–290, 1971.
167. SÉTÁLÓ, G., AND G. SZÉKELY. The presence of membrane specializations indicative of somato-dendritic synaptic junctions in the optic tectum of the frog. *Exptl. Brain Res.* 4: 237–242, 1967.
168. SHEPHERD, G. M. Synaptic organization of the mammalian olfactory bulb. *Physiol. Rev.* 52: 864–917, 1972.
169. SHEPHERD, G. M. The neuron doctrine: a revision of functional concepts. *Yale J. Biol. Med.* 45: 584–599, 1972.
170. SIDMAN, R. L. Autoradiographic methods and principles for study of the nervous system with thymidine-H^3. In: *Contemporary Research Methods in Neuroanatomy*, edited by W. J. H. Nauta and S. O. E. Ebbeson. New York: Springer Verlag, 1970, p. 252–273.
171. SKOFF, R. P., AND J. E. VAUGHN. An autoradiographic study of cellular proliferation in degenerating optic nerve. *J. Comp. Neurol.* 141: 133–156, 1971.
172. SLOPER, J. J. Gap junctions between dendrites in the primate neocortex. *Brain Res.* 44: 641–646, 1972.

173. SOTELO, C. Ultrastructural aspects of the cerebellar cortex of the frog. In: *Neurobiology of Cerebellar Evolution and Development*, edited by R. Llinás. Chicago: AMA-ERF Institute for Biomedical Research, 1969, p. 327–367.
174. SOTELO, C., AND R. LLINÁS. Specialized membrane junctions between neurons in the vertebrate cerebellar cortex. *J. Cell Biol.* 53: 271–289, 1972.
175. SOTELO, C., R. LLINÁS, AND R. BAKER. Structural study of inferior olivary nucleus of the cat: morphological correlates of electrotonic coupling. *J. Neurophysiol.* 37: 541–559, 1974.
176. SOTELO, C., AND S. L. PALAY. Synapses avec des contacts étroits (tight junctions) dans le noyau vestibulaire latéral du rat. *J. Microscopie* 6: 83a, 1967.
177. SOTELO, C., AND S. L. PALAY. The fine structure of the lateral vestibular nucleus in the rat. II. Synaptic organization. *Brain Res.* 18: 93–115, 1970.
178. SPENCER, W. A., AND E. R. KANDEL. Electrophysiology of hippocampal neurons. IV. Fast prepotentials. *J. Neurophysiol.* 24: 272–285, 1961.
179. SPIRA, M. E., AND M. V. L. BENNETT. Synaptic control of electrotonic coupling between neurons. *Brain Res.* 37: 294–300, 1972.
180. STÖHR, P., JR. *Mikroskopische Anatomie des vegetativen Nervensystems*. In: *Handbuch der Mikroskopischen Anatomie des Menschen*, edited by W. Bargmann. Berlin: Springer Verlag, IV/1, 5, 1957.
181. STREIT, P., K. AKERT, C. SANDRI, R. B. LIVINGSTON, AND H. MOOR. Dynamic ultrastructure in presynaptic membranes at nerve terminals in the spinal cord of rats. Anesthetized and unanesthetized preparations compared. *Brain Res.* 48: 11–26, 1972.
182. SWIFT, H. Nucleic acids and cell morphology in dipteran salivary glands. In: *The Molecular Control of Cellular Activity*, edited by J. M. Allen. New York: McGraw-Hill, 1962, p. 73–125.
183. TABER-PIERCE, E. Histogenesis of the nuclei griseum pontis corporis pontobulbaris and reticularis tegmenti pontis (Bechterew) in the mouse. An autoradiographic study. *J. Comp. Neurol.* 126: 219–240, 1966.
184. TAXI, J. Contribution à l'étude des connexions des neurones moteurs du système nerveux autonome. *Ann. Sci. Nat. Zool.* 7: 413–674, 1965.
185. THIÉRY, J. P. Role de l'appareil de Golgi dans la synthèse des mucopolysaccharides. Étude cytochimique. I. Mise en évidence de mucopolysaccharides dans les vesicules de transition entre l'ergastoplasme et l'appareil de Golgi. *J. Microscopie* 8: 689–708, 1969.
186. TRACHTENBERG, M. C., AND D. A. POLLEN. Neuroglia: biophysical properties and physiological function. *Science* 167: 1248–1252, 1970.
187. UCHIZONO, K. Characteristics of excitatory and inhibitory synapses in the central nervous system of the cat. *Nature* 207: 642–643, 1965.
188. UCHIZONO, K. Synaptic organization of the mammalian cerebellum. In: *Neurobiology of Cerebellar Evolution and Development*, edited by R. Llinás. Chicago: AMA-ERF Institute for Biomedical Research, 1969, p. 549–581.
189. VALDIVIA, O. Methods of fixation and the morphology of synaptic vesicles. *J. Comp. Neurol.* 142: 257–277, 1971.
190. VALVERDE, F. The neuropil in superficial layers of the superior colliculus of the mouse: a correlated Golgi and electron microscopic study. *Z. Anat. Entwicklungs.* 142: 117–147, 1973.
191. VAUGHN, D. W., AND A. PETERS. Neuroglial cells in the cerebral cortex of rats from young adulthood to old age: an electron microscope study. *J. Neurocytol.* 3: 405–429, 1974.
192. VAUGHN, J. E., P. L. HINDS, AND R. P. SKOFF. Electron microscopic studies of Wallerian degeneration in rat optic nerves. I. The multipotential glia. *J. Comp. Neurol.* 140: 175–206, 1970.
193. VAUGHN, J. E., AND A. PETERS. A third neuroglial cell type. An electron microscope study. *J. Comp. Neurol.* 133: 269–288, 1968.
194. WEBSTER, H. DE F. The geometry of peripheral myelin sheaths during their formation and growth in cat sciatic nerves. *J. Cell Biol.* 48: 348–367, 1971.
195. WEBSTER, H. DE F., J. R. MARTIN, AND M. F. O'CONNELL. The relationships between interphase Schwann cells and axons before myelination: a quantitative electron microscopic study. *Develop. Biol.* 32: 401–416, 1973.
196. WOLFF, J. Elektronenmikroskopische Untersuchungen über Struktur und Gestalt von Astrozytenfortsätzen. *Z. Zellforsch. Mikroskop. Anat.* 66: 11–28, 1965.
197. WOLFF, J. Die Astroglia im Gewebsverband des Gehirns. *Acta Neuropathol. Suppl.* IV: 33–39, 1968.
198. WONG, M. T. T. Somato-dendritic and dendro-dendritic synapses in the squirrel monkey lateral geniculate nucleus. *Brain Res.* 20: 135–139, 1970.
199. WUERKER, R. B. Neurofilaments and glial filaments. *Tissue Cell* 2: 1–9, 1970.
200. WUERKER, R. B., AND J. B. KIRKPATRICK. Neuronal microtubules, neurofilaments, and microfilaments. *Intern. Rev. Cytol.* 33: 45–75, 1972.
201. WUERKER, R. B., AND S. L. PALAY. Neurofilaments and microtubules in anterior horn cells of the rat. *Tissue Cell* 1: 387–402, 1969.
202. ZAMPIGHI, G., AND J. D. ROBERTSON. Fine structure of the synaptic discs separated from the goldfish medulla oblongata. *J. Cell Biol.* 56: 92–105, 1973.
203. ZELENÁ, J. Ribosome-like particles in myelinated axons of the rat. *Brain Res.* 24: 359–363, 1970.