STRUCTURE AND FUNCTION OF SYNAPSES

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Preface

With the publication of this handsome volume the New York Society of Electron Microscopists enters upon a new phase of its activities, yet one thoroughly in keeping with its traditional aims—the acquisition and dissemination of knowledge arising from electron microscopy. The thirteen chapters elegantly display the integration of morphological, physiological, and biochemical techniques to produce a new synthesis of experimental data on the structure and function of synapses. The success of this endeavor amply demonstrates that it is through the melding of structural and functional approaches to biological problems that electron microscopy can make its greatest contributions.

In selecting the topic for this initial monograph, the Society followed the same criteria that it has used to choose the topics for its annual symposia. It determined to focus on an area of timely and fundamental importance. The rapid development of the neurosciences, as exemplified by the increasing number of workers and publications devoted to it, assures us that the subject selected fulfills our requirements.

The authors who have so ably contributed to this volume are all outstanding researchers in their own right who have added significantly to our understanding of neural and synaptic function through their research over the years. In writing their chapters for this monograph they have undertaken the review of background information and current findings within their respective fields of expertise. Through the support of the Society this impressive body of information was assembled without restrictions on the length of the texts and with the inclusion of eighty-seven high quality reproductions of electron micrograph plates.

It is hoped that this resulting volume will be of equal value to students and to experienced workers as an up-to-date text and reference source.

Michael H. Ross
President, New York Society of Electron Microscopists

Introduction

> "*If there exists any surface or separation at the nexus between neurone and neurone, much of what is characteristic of the conduction exhibited by the reflex-arc might be more easily explainable . . . It seems therefore likely that the nexus between neurone and neurone in the reflex-arc, at least in the spinal arc of the vertebrate, involves a surface of separation between neurone and neurone; and this as a transverse membrane across the conductor must be an important element in intercellular conduction. The characters distinguishing reflex-arc conduction from nerve-trunk conduction may therefore be largely due to intercellular barriers, delicate transverse membranes, in the former.*
>
> *In view, therefore, of the probable importance physiologically of this mode of nexus between neurone and neurone, it is convenient to have a term for it. The term introduced has been* synapse."
>
> —Charles S. Sherrington,
> *The Integrative Action of the Nervous System, 1906.*

The development of synaptology as a major neurobiological discipline may be traced to two conceptual formulations, the neuron doctrine and the hypothesis of the nexus. The former was derived from largely morphological considerations of the neuron as a structural and functional entity. Its most effective champion was Santiago Ramón y Cajal whose life-long contributions to neurohistology disclosed much of what is known today concerning the morphological heterogeneity of neurons and their hodological relations. The other seminal concept was formulated by Charles S. Sherrington to account for the characteristic differences between conduction in nerve trunks and reflex-arcs. According to Sherrington the most parsimonious explanation for these differences would best be met by assuming a "surface of separation" in that part of the reflex-arc which lies in gray matter, the "field of nexus between neurone and neurone." The quotation from Sherrington, cited above, contains the first reference to the concept of the synapse derived from physiological considerations. It is of interest that only once did Sherrington consider the alternative possibilities of a surface *or* separation, rather than a surface *of* separation at the nexus or junction between neurons. Electron microscopy has provided us with many additional characteristics of junctions between excitable cells. What has emerged from such studies, complemented by electrophysiological investigations, is the realization that these junctions may involve a true separation in some instances and a surface of separation, i.e., a transverse membrane with special properties, in other instances. Clearly, both types of junction are entitled to the designation of synapse in accordance with Sherrington's original usage of the term.

It is generally accepted that the nexus with a true separation (and other important features of the pre- and postsynaptic elements) constitutes a class of synapses in which signal transmission from one element to another is usually neurohumorally

mediated. The nexus exhibiting "intercalation of a transverse surface of separation or membrane into the conductor" (to use Sherrington's words) includes a class of synapses in which junctional transmission is effected by currents generated during the spike potential. The former are designated *chemical* synapses, the latter *electrotonic* synapses. The controversy as to whether synaptic transmission is chemical or electrical has long since waned following the unequivocal demonstrations of both modes of operation at different (and, rarely, the same) synapses. The issues to be joined are no longer semantic but, appropriately, biological. For the future course of neurobiology depends in no little measure upon the continuing orderly acquisition of knowledge concerning the morphological substrate of chemical and electrotonic synapses, their mode of operation and the manner in which they may be functionally and structurally modified. Information along these lines can be expected to be of fundamental importance in the analysis of the organization of the brain and the neural basis of normal and abnormal behaviors. It follows that without an adequate understanding of synaptic mechanisms, including identification of transmitter substances and their metabolic pathways, there can be no rational development of an effective program of pharmacotherapy for many chronic neurological and psychiatric disorders. To be sure, several quantal jumps in progress in neuropsychopharmacology have occurred in recent years which have had dramatic effects on the management of severe and debilitative disorders of brain function. The oft-cited beneficial effects of *l*-DOPA in the pharmacotherapy of parkinsonism is by now a classical example of this progress. But in many instances the treatment has proved to be accompanied by prohibitive complications which reflect in part incomplete knowledge of the site and mode of action of *l*-DOPA, its effects of synaptic processes and the actions of its many metabolic by-products on different neuronal organizations. This is not to say that clinical applications of possibly beneficial neuropharmacological agents should be deferred until their actions are satisfactorily defined at the level of the neuron and synapse. Still, there are valid reasons to adopt a cautious posture in the face of extravagant claims for neuro- and psychotropic drugs in view of the paucity of information on the nature of the synaptic substrate responsible for particular symptom complexes and present difficulties in defining adequate models for evaluating drug effectiveness. In a word, progress in neuropsychopharmacology will be measured to the extent that programs of drug research and development evolve from principles of synaptology. Elucidation of these principles cannot be regarded as a trivial concern, but a mission of central importance in the health sciences.

Research in synaptology has proceeded at an ever-increasing pace in the past few decades, catalyzed by technological developments that have permitted detailed visualization of the structural features of different types of junctions and by physiological and pharmacological studies of pre-and postsynaptic components. Analyses of transmitter synthesis, storage, release and re-uptake have been carried out at identifiable synapses with the aid of subcellular fractionation methods, radioautography and biochemical and histochemical techniques. Any adequate summary of the extant data on the synapse would doubtless fill several handbooks or exhaust the capabilities of any single worker in the field. The classical *Handbuch* approach has the dubious virtue of completeness, but may suffer the disadvantage of diluting basic fact with overwhelming details. Notable success has been achieved with the monograph approach, especially for conveying a particular point of view of the operation of synapses or for presenting a general survey of current trends in the field. The alternative to these approaches is the multi-authored text, of which the present volume is an example.

In this book the editors have attempted to meet the need for a survey of important new philosophical and operational approaches to synaptology which would at the same time provide the student with a critical appraisal of established concepts. The major objective has been to emphasize those aspects of the subject in which notable advances have resulted from the combination of several technological approaches to the study of synapses. In many instances attempts to answer specific questions have generated even more complex problems, which can be expected at this stage in the historical development of a field that has only recently felt the impact of the "new" molecular biology. The casual reader will find here no ready solution to the problem of how neurons recognize each other and thereby establish appropriate synapses. Nor will the search herein for the mystery of how behavior modifies synaptic organization be particularly rewarding. But it is not to be inferred from this that the material presented does not address these vital issues. For it goes without saying that solutions to these and other neurobiological problems are already prophesied by the very nature of the data currently at hand on the structure and function of synapses.

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Synaptic fine structure—morphological correlates of chemical and electrotonic transmission

George D. Pappas and Stephen G. Waxman

In the original use of the word synapse as "the surface of separation" between neurons (Sherrington, 1906) a functional polarization, indicating a one-way transmission of the signal from cell to cell, was implied. Even the earliest studies of synapses with the electron microscope demonstrated a structural basis of this functional asymmetry (Palay and Palade, 1955; De Robertis and Bennett, 1955). Studies of neuronal fine structure have shown conclusively that the Neuron Doctrine, espoused at the turn of the century by Ramón y Cajal (1909–1911) and others, is in fact correct, and that the nervous system is composed of discrete cellular elements and is not a syncytial net. Moreover, the early studies of nervous tissue with the electron microscope showed that neuropil, the structure of which had not previously been well characterized, is the major site of synaptic contact in the vertebrate nervous system (Wyckoff and Young, 1956). The high resolution provided by electron optics has allowed study of the synapse at a level which was not possible with the light microscope, and has allowed us to begin to approach meaningful correlations between structure and function. In the past decade it has become clear that there are two modes of synaptic transmission: chemical and electrotonic. These physiologically distinct mechanisms of synaptic transmission are characterized at the fine structural level by distinct morphological characteristics.

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I. THE CHEMICALLY TRANSMITTING SYNAPSE

Neuronal and glial cells and their processes are closely packed in the central nervous system, and as viewed by electron microscopy are separated by an extracellular space approximately 200 Å wide. The chemical synapse is no exception. A distinct synaptic cleft is present at most synapses and separates the pre- and postsynaptic membranes, providing a *morphological* correlate (cf. Figs. 1 and 2) for the physiological distinctiveness of pre- and postsynaptic elements (Eccles, 1964).

Following aldehyde fixation, material of intermediate density can be observed within the synaptic cleft, occasionally demonstrating an orderly structure (cf. Fig. 3). Early studies with the light microscope (Hess, 1953) suggested the presence of PAS-positive material (i.e., mucopolysaccharides) in neuropil. In more recent studies with the electron microscope the presence of mucopolysaccharide in the synaptic cleft could be demonstrated when silver methanamine was substituted for Schiff's reagent (Rambourg and LeBlond, 1964). The presence of basic protein in the synaptic cleft has been demonstrated with alcoholic phosphotungstic acid (Bloom and Aghajanian, 1966). Thus, synaptic gap substance contains carbohydrate and protein moieties similar to those found as the "extraneous coat" substance or "glycocalyx" in almost all cell types (Pappas and Purpura, 1966). It has been shown that PAS-positive extraneous coat substance binds the dye ruthenium red in *Amoeba* (Pappas, 1954) and in most other cells (Luft, 1966). The synaptic gap substance also binds ruthenium red (Bondareff, 1967). It has been suggested that surface glycoproteins may be concentrated at nerve endings (Barondes, 1969), as well as substances containing sialic acid

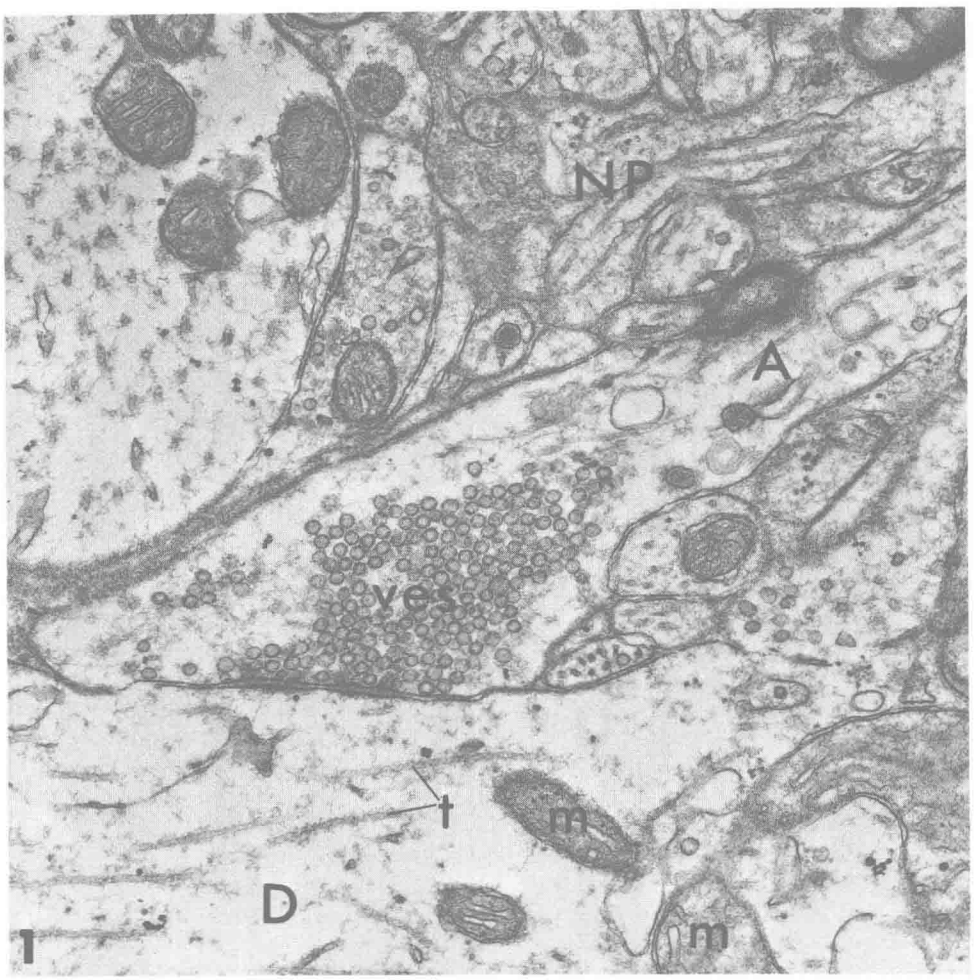


FIG. 1. An axodendritic synapse in the oculomotor nucleus of the cat. Numerous synaptic vesicles (*ves*) are clustered near the presynaptic membrane. There is a distinct synaptic cleft at this chemical synapse. The dendrite (*D*) contains mitochondria (*m*) and microtubules (*t*). Axonal and dendritic processes are seen above in the neuropil (*NP*). *A* = axon. ×36,000.

(Bondareff and Sjöstrand, 1969). The presence of polyanionic substances in the synaptic cleft may well bear on the movements of ions or transmitter molecules within the cleft. Both the pre- and postsynaptic membranes are capable of micropinocytotic uptake from the synaptic cleft (see below). Since it has been demonstrated that "extraneous coat" substance binds pinocytotic-stimulating agents (Brandt and Pappas, 1960), the synaptic gap substance may be important in micropinocytosis at this site. Another attractive hypothesis is that the synaptic gap substance, which could be highly specific in composition, plays a role in intercellular recognition and is implicated in the mechanism of specificity in synaptogenesis.

The physiological specialization of the synaptic membranes (see, e.g., Eccles, 1964) has not as yet been correlated with specific ultrastructural specializations of the apposed membranes themselves. Studies of the apposing pre- and postsynaptic membranes reveal the same unit membrane structure (Robertson, 1964) which characterizes other regions of the neuronal plasma membrane.

There is usually some dense material associated with the cytoplasmic surfaces of both the pre- and postsynaptic membranes (Fig. 4). The relative distribution of dense cytoplasmic material at the synapse varies. Synaptic profiles may appear, as a result of the distribution of dense material, as symmetric or asymmetric. This has been used as a basis for morphological classification of synapses (Gray, 1959). While distinct types can be seen, it may be that these represent the end points in a continuum rather than examples of two distinct populations (Peters et al., 1970). Akert and his co-workers (this volume), using specialized staining techniques, have visualized

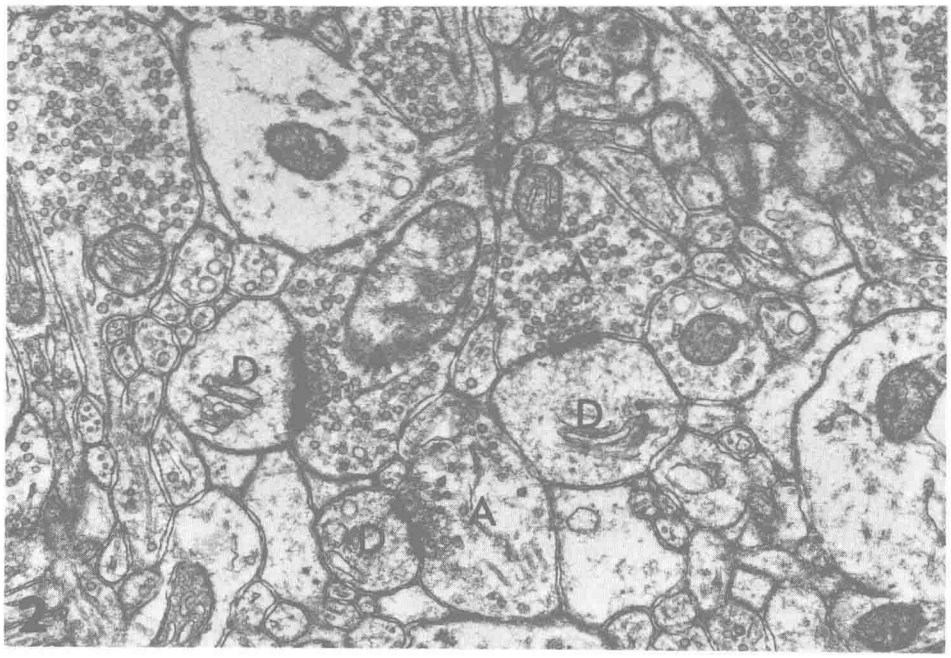


FIG. 2. This electron micrograph illustrates a region of neuropil from sensorimotor cortex of the cat. Numerous neuronal processes and some glial elements are present in this area. Three axodendritic synapses are present in the central part of the field. At each, an axonal process (*A*) contains presynaptic vesicles, which are clustered near the synaptic cleft. There is an accumulation of dense cytoplasmic material at the postsynaptic membranes. The dendritic spine apparatus is seen in two of the dendrites (see also Fig. 16). ×30,000.

a pattern of distribution of dense material in the presynaptic element which they characterize as a "grid."

A. Presynaptic specializations

Presynaptic processes may contain a number of structures. Glycogen particles are often present. Mitochondria are also observed, along with multivesicular bodies and tubular elements of endoplasmic reticulum.

Synaptic vesicles. The most outstanding aspect of the asymmetric appearance of the synaptic junction is the conspicuous presence of vesicles clustered close to the presynaptic membrane. It is at present generally accepted that transmitter is released in discrete quantal units (Del Castillo & Katz, 1954; Martin, 1955; Kuffler and Dudel, 1961). Numerous studies have implicated the presynaptic vesicles with the quantal release of transmitter substance. [It has been noted, however, that single transmitter molecules can have measurable effects on the postsynaptic membrane (Katz and Miledi, 1970)]. Fractionation studies isolating synaptosomes and further isolation of the presynaptic vesicles themselves have revealed a close association between these vesicles and transmitter substance (Whittaker, this volume). That the membranous profiles present in the presynaptic terminal are, in fact, discrete and membrane-bounded and are not part of a continuous tubular network is accepted by most investigators, although there is some evidence to the contrary. It is not unusual to find in micrographs the profiles of a tubular membranous reticulum in close proximity to synaptic vesicles (Fig. 5), a finding which could be interpreted as indicating the origin of vesicles from tubular reticulum near the synaptic site. Vesicle production can either take place at or near the synapse, or at some distant site in the axon or cell body (cf. Barondes, 1969). Vesicles have been observed within the axon and cell body and have been interpreted as synaptic vesicles in transit (Gray, 1970).

It has been suggested that pinocytosis at the presynaptic terminal (i.e., uptake of extracellular material into membrane-bounded vesicles) may be a part of the production of synaptic vesicles (Andres, 1964; Westrum, 1965). Birks (1960) and Brightman (1967) have demonstrated the uptake of extracellular marker substances into structures in the presynaptic process which resemble synaptic vesicles. Some invaginations at the presynaptic membrane are "coated" (Roth and Porter, 1964) and thus resemble typical micropinocytotic vesicles more than synaptic vesicles. Gray and Willis (1970) have discussed the presence of "complex vesicles" at synapses. They interpret these as vesicles in formation. Some investigators (de Robertis, 1964; Nagasawa et al., 1970)

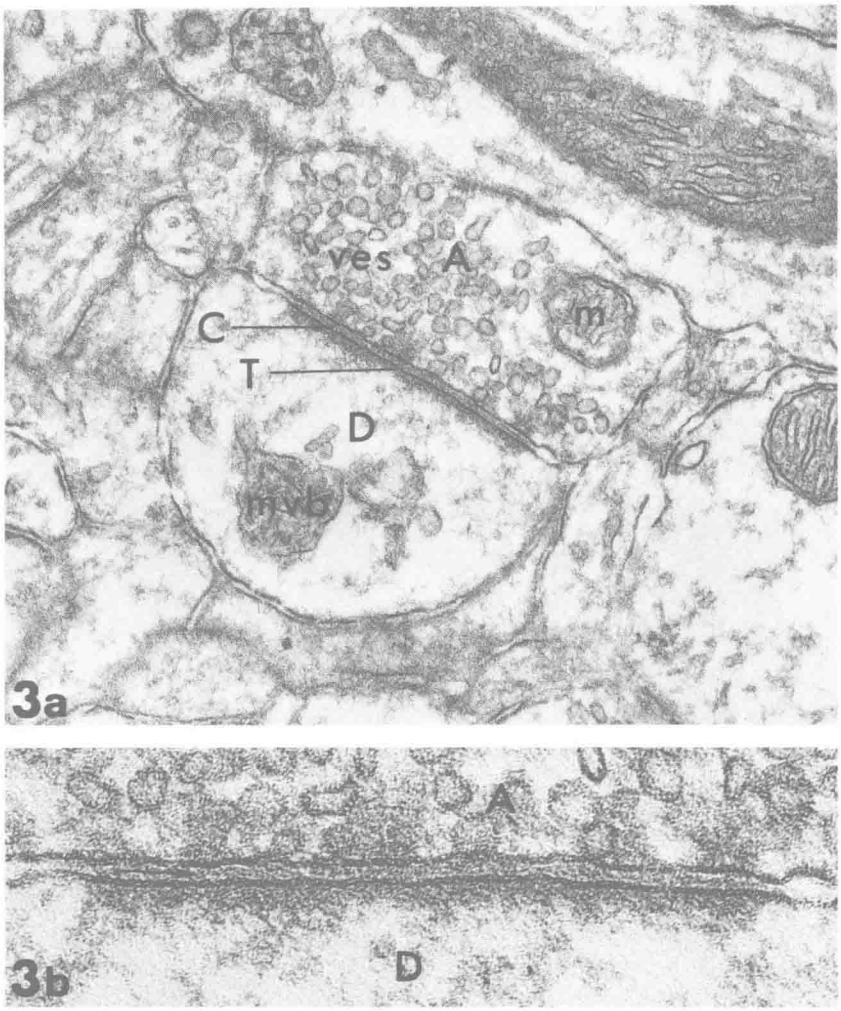


FIG. 3. Axodendritic synapse from the cerebral cortex of the kangaroo rat *Diplodomys merriami*. The presynaptic element contains vesicles (*ves*) and a mitochondrion (*m*). A multivesicular body (*mvb*) is present in the postsynaptic dendrite. Dense material is present in the synaptic cleft (*C*). There is dense cytoplasmic material associated with the postsynaptic membrane (*T*). Pre- and postsynaptic membranes are enlarged in Fig. 3b. A fine density (*arrow*) runs between the pre- and postsynaptic membranes, bisecting the synaptic cleft, which is approximately 200 Å wide. Micrograph courtesy of R. Melker. 3a, ×52,000; 3b, ×155,000.

have interpreted pit-like indentations of the presynaptic membranes as vesicles extruding their contents into the synaptic cleft.

There can be no doubt that presynaptic vesicles fall into several classes on the basis of size and content. At most synapses in the vertebrate CNS and at the motor endplate, the predominant type of vesicle is small (400–600 Å diameter) and electron-lucent. Occasionally in the CNS, scattered among these small clear vesicles are one or more larger (about 700–1000 Å diameter) vesicles with dense cores (Fig. 5). These larger vesicles are often located in that portion of the vesicle population farthest from the synaptic cleft.

The axon terminals of autonomic nerve fibers contain vesicles about 500 Å in diameter with dense cores. Wolfe et al. (1962) have shown by autoradiography that tritiated norepinephrine localizes in terminals containing small dense core vesicles. More recent autoradiographic studies by Budd and Salpeter (1969) show a similar localization, although the distributions of the dense core vesicle population and ^{3}H-labeled norepinephrine do not coincide perfectly. Hökfelt (1967) has shown that there are small vesicles (700 Å) in areas of the brain known to have a high content of biogenic amines, which display dense cores after fixation with potassium permanganate. Other workers (Bak et al., 1969)

have shown that depletion of norepinephrine and dopamine with oxypertine is accompanied by loss of dense core material. It is clear that there must be at least several classes of synaptic vesicles; morphological differentiation of these classes is not yet unequivocal. Vesicle shape may serve as an important indicator in this respect; this is discussed briefly below, and is taken up in detail in Dr. Bodian's contribution to this volume.

A Consideration of Synaptic Vesicle Localization. The problem of how vesicles come to be confined in distribution primarily to the synaptic area has received only minor consideration. Smith et al. (1970) have noted an association between microtubules and

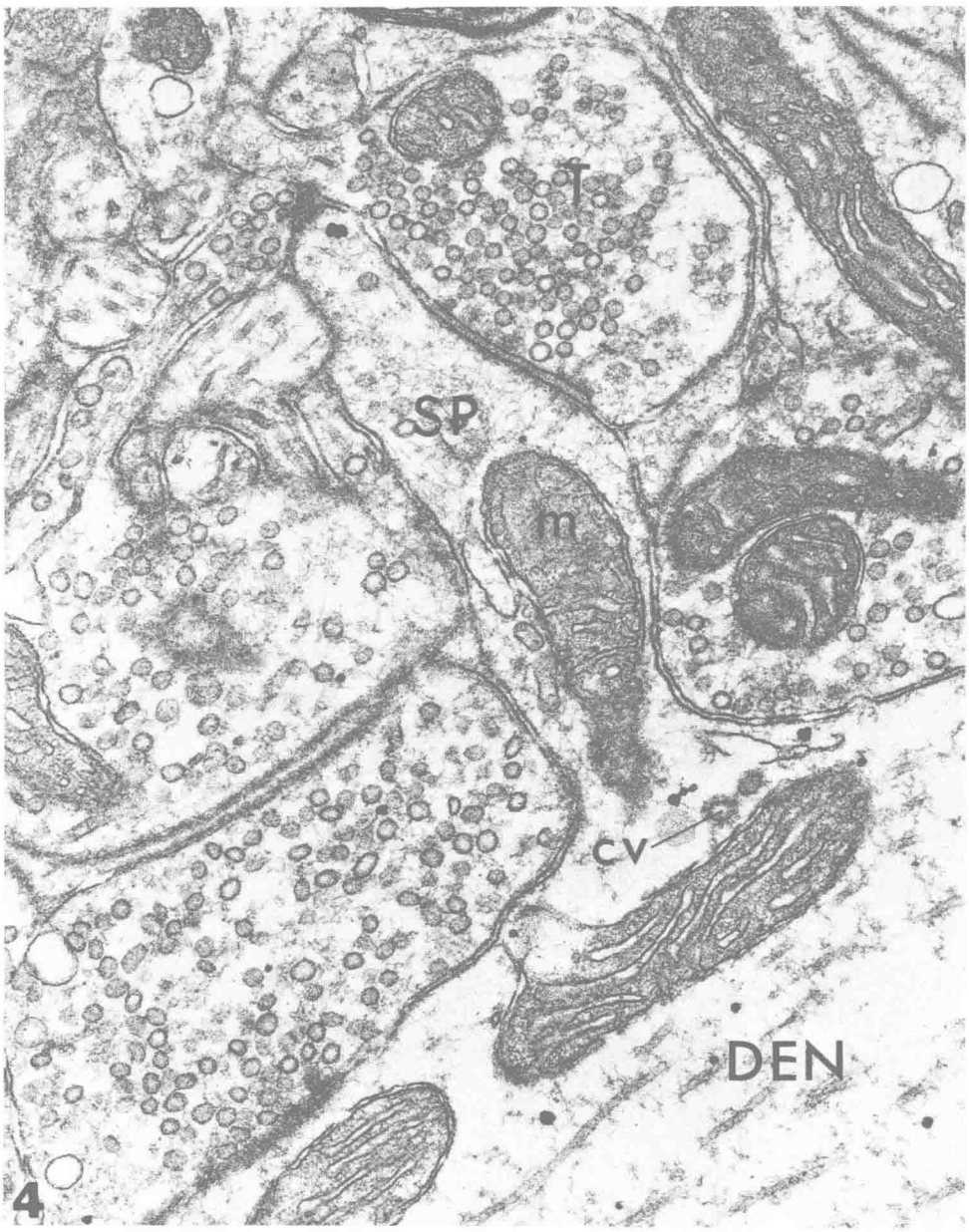


FIG. 4. A dendritic spine (*SP*) from the oculomotor nucleus of the cat. The spine contains a mitochondrion (*m*) and its continuity with a dendrite (*DEN*) can be seen in this section. A cytoplasmic vesicle with a radially striated coating (*cv*) is seen at the base of the spine. Axonal processes surround the spine; one of the processes (*T*) establishes synaptic contact with the spine. There is dense cytoplasmic material associated with both pre- and postsynaptic membranes, which are separated by a synaptic cleft about 200 Å wide. Dendritic spines may function so as to isolate certain synapses and allow for linear summation of synaptic potentials. ×52,000.

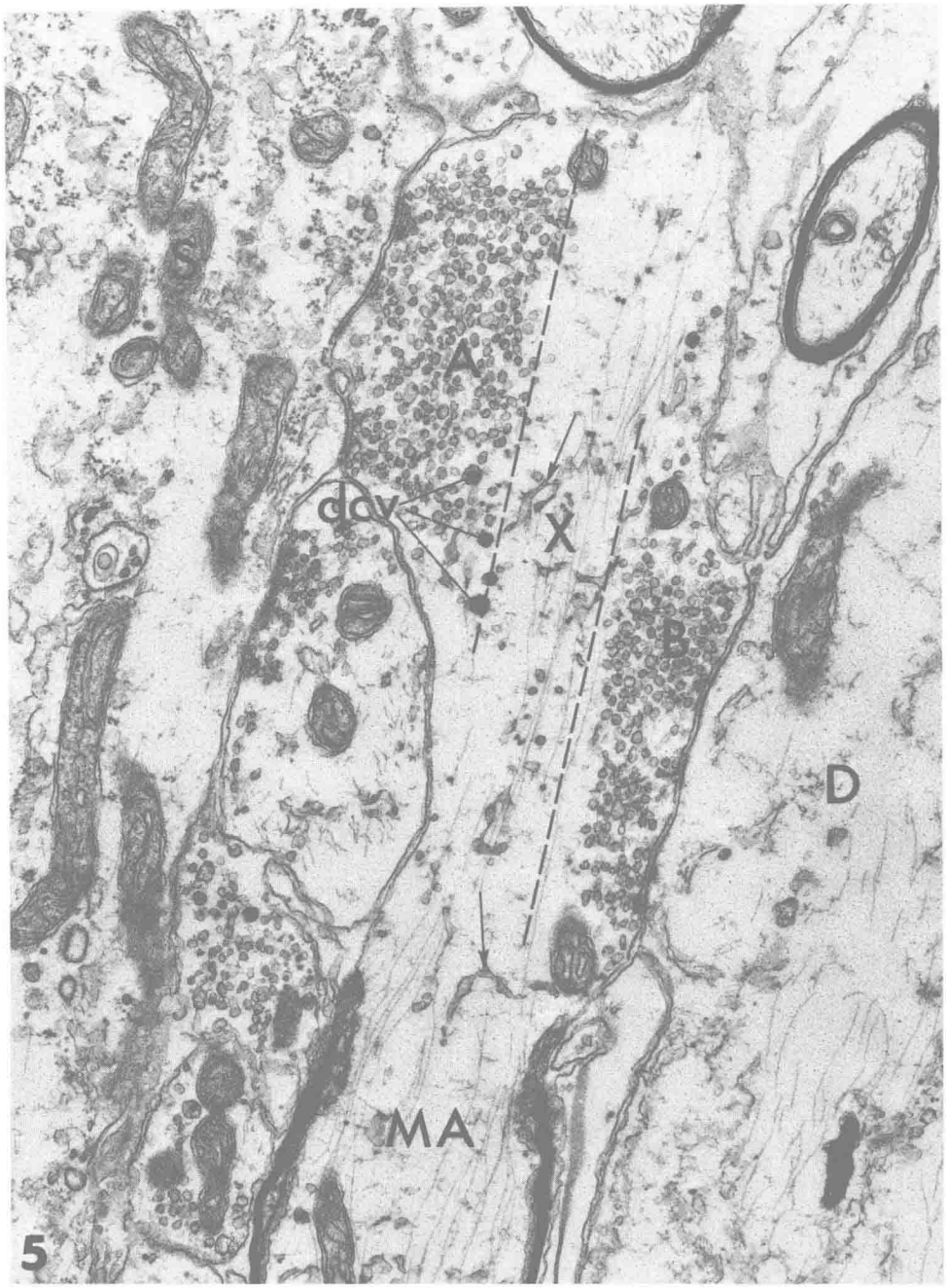


FIG. 5. A myelinated axon (*MA*) from the oculomotor nucleus of the spiny boxfish *Chilomycterus*. Synapses are established with a cell body (*S*) and a dendrite (*D*). The populations of vesicles at both synapses (*A*, *B*) are of uniform density, and both populations of vesicles exhibit discrete boundaries (*dotted lines*). The boundaries lie along an extension of the circumference of the axon cylinder, and their presence suggests that a barrier phenomenon is implicated in vesicle localization. The area marked *X*, in the central part of the axon, contains only a few vesicles, but longitudinally oriented filaments are present. Tubular elements of endoplasmic reticulum (*arrow*) are also present in the axon cylinder, and there are some dense core vesicles (*dcv*) in that part of the synaptic vesicle population farthest from the synaptic cleft. ×30,000.

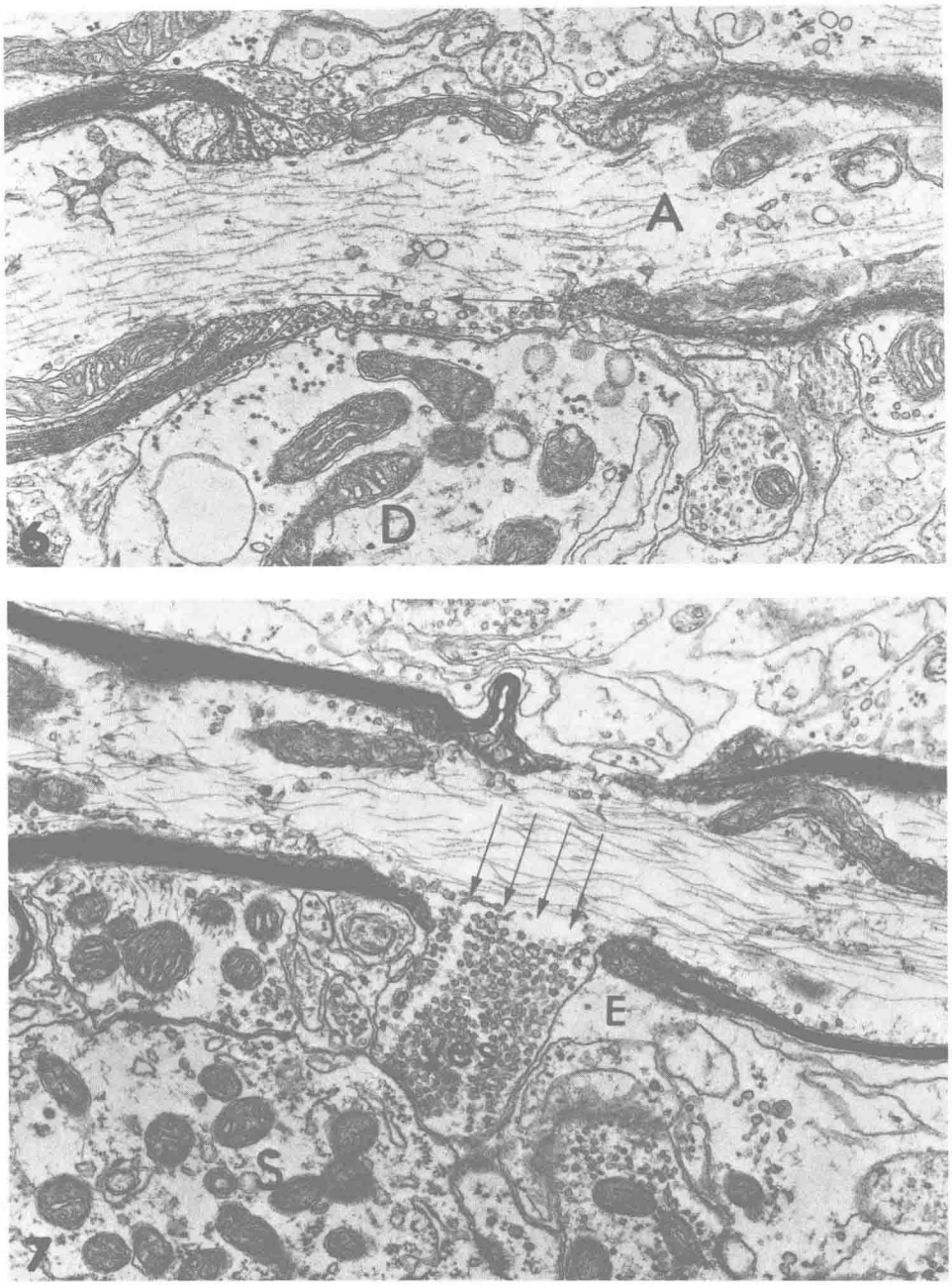
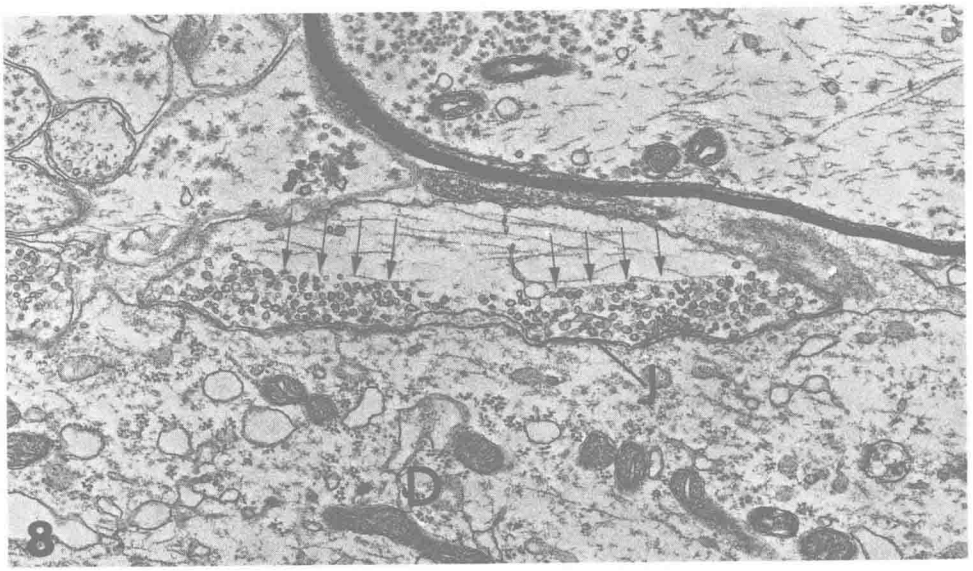
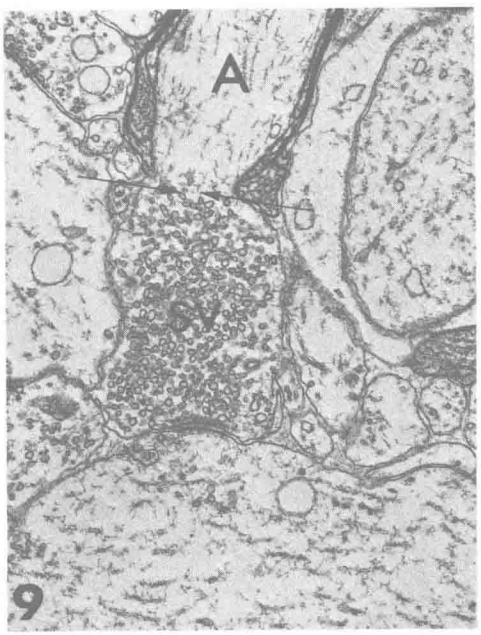


FIG. 6. A myelinated axon (*A*) establishes synaptic contact with a dendrite at a node of Ranvier. Presynaptic vesicles are present, but do not extend into the axon cylinder (*arrows*). ×24,000.

FIG. 7. At this nodal synapse from the oculomotor nucleus of the spiny boxfish, a protrusion of the axon at the node contains a dense population of presynaptic vesicles (*ves*). The postsynaptic element is a cell body (*S*). Note the apparent boundary to the vesicle population, along an extension of the circumference of the axon cylinder (*arrows*). A network of neurofilaments is present in the axon cylinder, but does not extend into the nodal protrusion. ×24,000.

"synaptic vesicles" in the vicinity of synapses in the lamprey *Petromyzon*. Robertson et al. (1963) have noted the sharp boundary to the vesicle population in club ending synapses on the lateral dendrite of the Mauthner cell in fish. Many other published micrographs include synaptic vesicle populations with discrete boundaries, but reference is rarely made to this feature of the aggregation of vesicles.





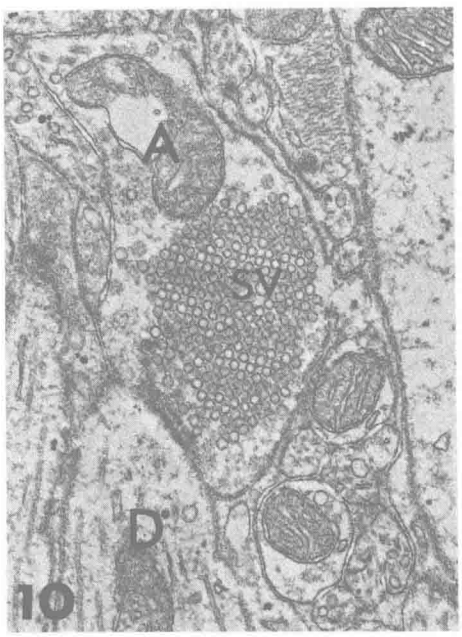


FIG. 8. An axodendritic synapse from the oculomotor nucleus of the frog. There is a close membrane apposition (*J*) at this electrotonic synapse. There is a distinct boundary to the population of vesicles (*arrows*). ×20,000.

FIG. 9. This axon terminal (*A*) from the frog oculomotor nucleus establishes a synapse with a large dendrite. A distinct synaptic cleft is present. Note the sharp boundary (*arrows*) to the accumulation of presynaptic vesicles (*SV*). ×21,000.

FIG. 10. The synaptic vesicles (*SV*) at this synapse from the feline oculomotor nucleus are closely packed and appear to form an orderly array. Such paracrystalline arrays of vesicles have been described at a number of sites in the normal and pathological nervous system. The significance of the strikingly regular arrangement of these vesicles is not yet clear. ×30,000.

The high frequency of occurrence of vesicles at synapses, and the low frequency of similar vesicles in nonsynaptic regions of the neuron, constitute the two end-points in a frequency distribution. The distribution of synaptic vesicles in the transition region can be distinctly non-random. At many synapses, the frequency distribution profile of the vesicle population, plotted against distance from the synaptic