ULTRASTRUCTURE OF THE SPOON TYPE SYNAPTIC ENDINGS IN THE NUCLEUS VESTIBULARIS TANGENTIALIS OF THE CHICK

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ABSTRACT

The fine structure of the "spoon" type synaptic endings of the chick tangential nucleus was studied with the electron microscope. These endings often measure ~18 μ in length by ~3–4 μ in width. The axoplasm of the endings contains very few synaptic vesicles, a large number of neurofilaments oriented parallel to the long axis of the nerve fiber, and microtubules and numerous mitochondria. The synaptic membrane complex shows areas of localized occlusion of the synaptic cleft with the formation of an external compound membrane. It has not been decided whether these areas have a disc shape; their length measures between 0.04 and 0.47 μ. The five-layer pattern characteristic of an external compound membrane is shown in specimens fixed with formalin–OsO_4, glutaraldehyde–acrolein–OsO_4, and acrolein–$KMnO_4$ but it does not appear in the glutaraldehyde–OsO_4-fixed specimens. The over-all thickness of the external compound membrane varies depending upon the fixative used. The synaptic clefts in the regions between the external compound membrane discs are widened and measure ~300 A. A condensation of dense material occurs in pre- and postsynaptic cytoplasms all along the synaptic membrane complex. The morphological relationships described in the spoon endings are suggestive of electrical transmission.

INTRODUCTION

Cajal (4) in 1908 described the nucleus tangentialis in chick and fish brains as a component of the vestibular system (17). It lies superficially in the medulla at the level of entrance of the eighth nerve. One of its characteristic features is the presence of the unusual kinds of synapse now called "spoon" endings because of their peculiar shape. These are endings of large myelinated fibers of the eighth nerve which make synaptic contact with nerve cell perikarya and spread out in a shape that gives the impression, in silver preparations, of a microscopic spoon. The endings are often of the *en passant* type but also may be of the terminal variety. Their large size and shape are the main features that set them apart. Cajal described several varieties according to disposition, shape, and size. Some are thick and short, with the nerve fiber at an acute angle; others display a larger branch ending in a concave disc on one pole of the cell; still others are of the *en passant* type with the axon becoming remyelinated and running on to another cell.

We decided to investigate these endings because we wished to find out whether the vestibular endings in the vestibular nuclei of chicks and those in the Mauthner cell system in goldfish medulla might be analogous. In particular, the giant club endings on the lateral dendrites of Mauthner cells have been found to transmit electrically (11) and to have a characteristic morphological feature whereby the synaptic clefts are closed in disc-shaped regions referred to as "synaptic discs" (22, 23). We wondered whether similar synaptic discs might occur in the chick spoon endings since these are also vestibular endings of unusually large size. Our findings reveal that, indeed, the two types of endings are analogous and provide morphological evidence suggesting that the spoon endings may transmit impulses electrically.

MATERIALS AND METHODS

Chicks 1–10 days old were used throughout the study. The brains were fixed by a modification of a perfusion technique of Palay et al. (18) which is similar to the one used by Robertson et al. (22, 23). The chicks were anesthetized by intraperitoneal injection of pentobarbital sodium (0.075 mg per 10 g of body weight) and then perfused. A rigid cannula was made from a segment of a No. 18 hypodermic needle. The heart was rapidly exposed and the cannula was tied into the aorta. The cannula had been previously connected by a polyethylene tube (Clay-Adams, Inc., New York) to another No. 18 hypodermic needle attached to a 10 ml syringe filled with avian Ringer solution at room temperature. Different syringes containing the prefixing, buffer, or fixing solutions were used.

The most successful preparations were made with OsO_4 fixation preceded by fixation with formalin (22), distilled glutaraldehyde (31), or a combination of glutaraldehyde and acrolein according to Sandborn et al. (32). The concentration of the OsO_4 solution was 2.5% and that of the glutaraldehyde was 6.25%.

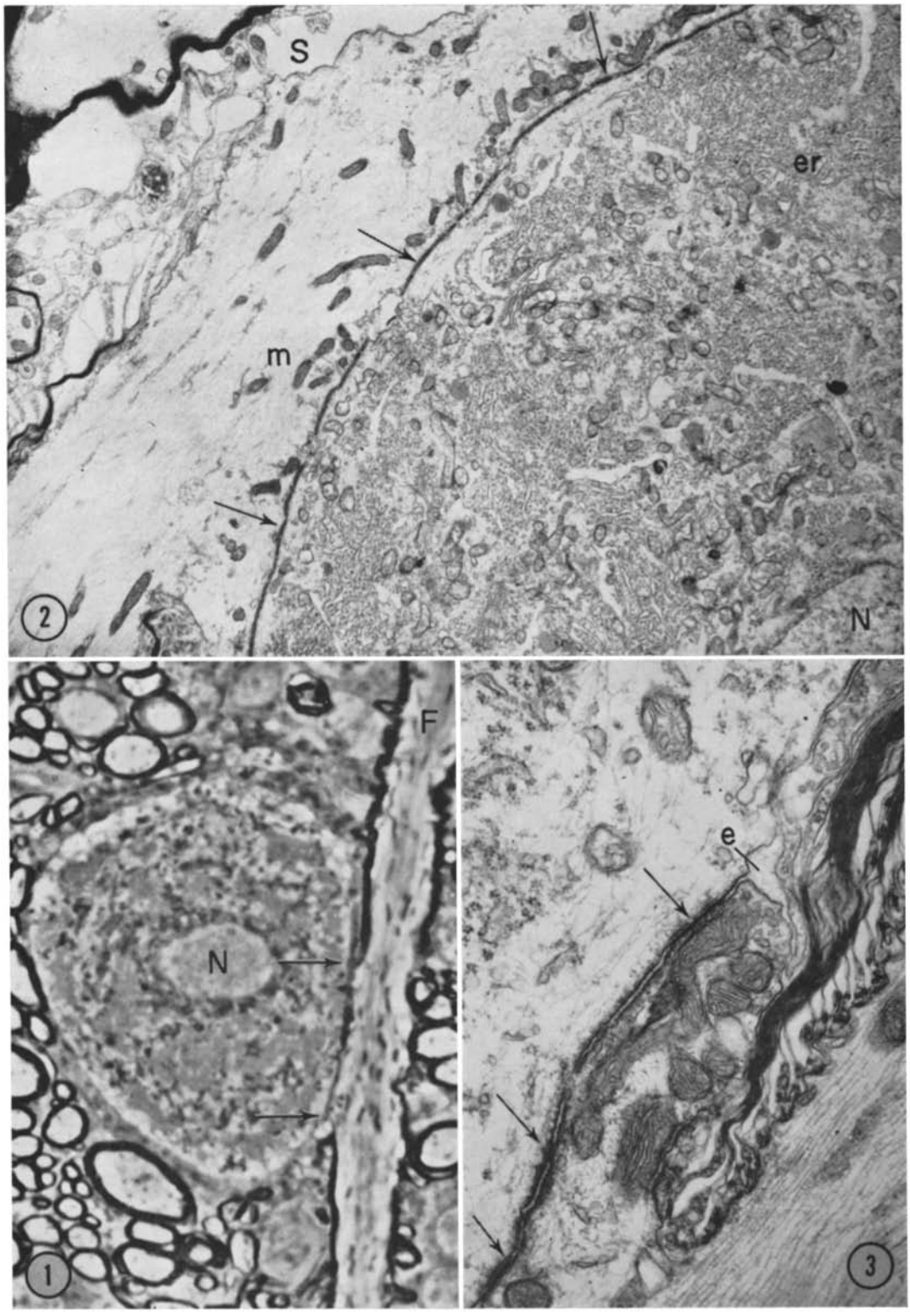
So far no consistently successful method has been devised for perfusing the appropriate region of these brains with permanganate fixatives. Some regions have been successfully fixed but the particular part of the medulla containing the vestibular endings has consistently failed to fix well.

The specimens were embedded in Araldite according to Robertson et al. (22), sectioned with an LKB Ultrotome with a diamond knife, and examined in a

FIGURE 1 Phase contrast light photomicrograph of 1-μ section of a nerve cell of the nucleus vestibularis tangentialis of the chick. It shows the nucleus (*N*), the cell body, and the large incoming myelinated vestibular fiber (*F*). The fiber approaches the cell body on one side and makes a synapse of *en passant* type (arrows). The fiber (*F*) loses its myelin sheath just before it makes contact with the perikaryon of the cell (upper arrow) and becomes remyelinated just beyond the contact region (lower arrow). Opposite the synaptic contact is a node of Ranvier. Acrolein–$KMnO_4$ fixation. × 1,980.

FIGURE 2 Low-power electron micrograph of a nerve cell of the nucleus vestibularis tangentialis in the chick showing a spoon type ending extending from the lower to the upper part of the micrograph (arrows). The nerve fiber is myelinated close to the terminal where the myelin sheath ends in a half-node configuration. The nerve ending contains numerous mitochondria (*m*) and abundant neurofilaments and microtubules. Note the apparent empty space (*S*) to the left of the ending. The cell body shows the nucleus (*N*) and abundant profiles of granular endoplasmic reticulum (*er*). Glutaraldehyde–OsO_4 fixation. × 7,600.

FIGURE 3 Section of an area of the spoon ending. A nerve cell body at the upper left contains clusters of ribosomes which suggest polysomal organization. A large myelinated fiber with abundant microtubules and neurofilaments extends across the field (lower right), its myelin sheath terminating in a nodal arrangement next to the spoon ending. A small collar of extracellular material (*e*) in a triangular enlargement is present between the synaptic terminal, nerve cell, and a glia cell which is related to the myelinated fiber. The small collar seems to contain some faint strands of matrix material, which suggests that it represents a real space. However, this is still uncertain. Note the ECM at the arrows. Here the synaptic clefts are closed, but in the intervening regions they are widened. The node to the lower right contains interesting circular bodies in the glial cytoplasm next to the axon but their nature is not yet understood. Glutaraldehyde–OsO_4 fixation. × 30,000.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Siemens Elmiskop Ib under instrumental conditions published elsewhere (23).

Light microscopy was done with a Zeiss Ultraphot with phase-contrast optics. Sections about 1 μ thick were cut with glass knives, mounted on glass slides and covered with immersion oil under a cover-slip before examination. Some sections were stained in 1.2% potassium permanganate.

OBSERVATIONS

Light Microscopy

In preliminary observations of Araldite sections by phase-contrast light microscopy we found some of the different types of synapses described by Cajal (4). In general the nerve cell body is globular or elongated and has a slightly eccentric nucleus. The nerve cells are often oval and taper at two poles. Usually the polar portions of the cell are free of closely apposed myelinated fibers and are covered by small globular profiles that we identified as *boutons terminaux* in electron micrographs. Sometimes, at one pole opposite the large incoming myelinated vestibular fibers, the axon could be observed running parallel to the incoming large myelinated fibers. The spoon synapses are easily identified by their size and shape. Synapsing nerve fibers approach the cell body on one side or one pole, depending upon their type. The fibers that have the spoon ending, either the terminal or *en passant* type, make synaptic contact on the lateral side of the cell body. The fiber loses its myelin sheath just before termination but this sheath reappears immediately in the *en passant* type of synapse (Fig. 1). The loss of myelin involves the entire perimeter of the presynaptic fiber, and it is clear that in the region of the synaptic contact a node is formed. The axon may synapse by terminating directly at this node, or may be remyelinated and extend for varying distances with the branch making a synapse *en passant*. The concave disclike synapse of the terminal type is observed at one pole of the cell and is very much like the club endings on the lateral dentrite of the Mauthner cell of the goldfish medulla. Like the club endings, the terminating fiber loses its myelin sheath a few microns from the postsynaptic neuron.

Electron Microscopy

Although the spoon type endings are the main subject of this report, the nerve cells of the tangential nucleus have some peculiarities that also deserve comment. These are large cells with a single large, round or oval, eccentric nucleus. Organelles are abundant, but their numbers vary in three clearly defined concentric zones. First, a light perinuclear region is demarcated. It contains mitochondria in moderate numbers and a few groups of free ribosomes. This region is surrounded by a second darker intermediate zone crowded with Nissl bodies and Golgi apparatus (21) (Fig. 2). Irregularly arranged mitochondria are scattered throughout this zone, but in some micrographs these appear to be more abundant farther toward the cell periphery. Intermingled with these elements are some moderately dark oval bodies of undetermined nature. A third thin peripheral zone of the cytoplasm is demarcated as a light region relatively free of organelles. It contains a few mitochondria, a few vesicles ~300–500 A in diameter, numerous neurofilaments ~50 A in diameter, and a few microtubules loosely associated in bundles irregularly arranged parallel to the cell membrane (Figs. 3–4).

The spoon ending in sections often measures ~18 μ in length by ~3–4 μ in width with the myelinated axon at one end (Figs. 2 and 3). At the ending the myelin sheath terminates usually in a symmetrical half-node configuration (9, 22, 24, 25, 34). The unmyelinated part of the ending is free of any satellite cell investment between the terminal myelin and the synaptic contact (Figs. 2 and 3), but sometimes apparent *boutons terminaux* occur on the terminal axon in this bare zone at the edge of the synaptic membrane complex (tip of spoon). More often around the naked axon terminal there appears to be a thin collar of extracellular material similar to that found in goldfish club endings (22). This material sometimes extends into the synaptic cleft (Fig. 3 *e*). However, we are not yet certain of the extent to which this space may represent artifact (35).

The axoplasm of the spoon ending contains very few vesicles. These measure ~300–700 A in diameter and are situated close to the synaptic membrane complex (SMC), but they do not have any special relationship to the disclike segments of SMC cleft occlusions. A large number of neurofilaments and microtubules are oriented roughly parallel to the plane of the SMC (Figs. 2–4). Because of the tangential approach of the terminative fiber this orientation also corresponds to the long axis of the axon. The neurofilaments are relatively more abundant than the microtubules. Their diameter is ~50 A, and they are closely packed with

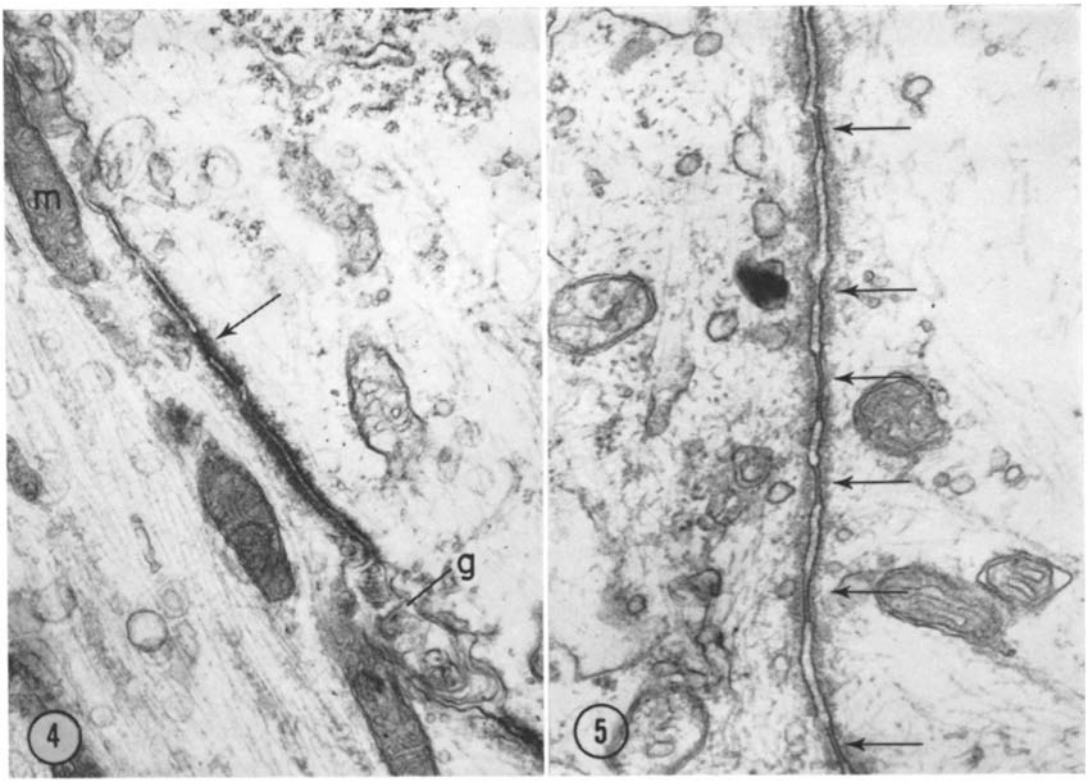

FIGURE 4 Portion of the synaptic membrane complex (SMC) of the spoon ending showing a synaptic disc (arrow). Notice interposition of a glial process with cytoplasm showing organelles and tubular profiles in cross-section (*g*). The terminal nerve fiber at the lower left shows abundant microtubules and neurofilaments and a few mitochondria (*m*) oriented parallel to the SMC. The cytoplasm of the nerve cell at the upper right shows bundles of loosely packed neurofilaments irregularly arranged parallel to the cell membrane. A few mitochondria and clusters of ribosomes are present in the cytoplasm. Glutaraldehyde–OsO_4 fixation. × 30,000.

FIGURE 5 Portion of a spoon type ending in the tangential nucleus showing synaptic ECM at the arrows. Note the dense, amorphous material in the cytoplasm to either side of the ECM and throughout the synaptic membrane complex. In between the discs, the synaptic cleft is widened. Neuronal cytoplasm lies to the left and terminal synaptic axoplasm to the right. Neurofilaments and microtubules are evident. Note the paucity of synaptic vesicles and mitochondria. Glutaraldehyde–OsO_4 fixation. × 48,000.

no sign of aggregation into bundles. A few microtubules ~200 A in diameter are distributed among the neurofilaments of the vestibular nerve fiber terminal. Mitochondria are increased in number near the SMC but are not abundant.

Along the ending the SMC appears in sections to contain many areas where the ~100–200 A gap between the presynaptic and postsynaptic unit membranes is occluded with the formation of external compound membranes (ECM) (27) (Figs. 2, 3, and 5). These areas appear as irregularly dispersed intermittent zones (Fig. 5) and measure 0.04–0.47 μ in length. They may or may not be disc-shaped like the corresponding structure in the Mauthner cell club endings. Our measurements in many electron micrographs obtained from specimens fixed with different fixatives lead us to believe that the length of the synaptic occlusions is not influenced by the fixative used.

The combined unit membrane pattern displaying five layers characteristic of the ECM does not generally appear in the glutaraldehyde–OsO_4-

fixed material but it is shown up in the material fixed in formalin–OsO_4, in glutaraldehyde–acrolein–OsO_4, or in acrolein–$KMnO_4$. The over-all thickness of these ECM differs depending upon the fixative. With the glutaraldehyde–OsO_4 fixative the ECM thickness is ~145–~155 A (Figs. 3 and 5). In the case of formalin–OsO_4 fixation the ECM measures ~140 A in thickness (Fig. 6), with glutaraldehyde–acrolein–OsO_4 fixation it is ~136–~163 A thick (Fig. 7), and with acrolein–permanganate it measures ~165 A (measured in one instance only) (Fig. 8). Microdensitometer traces of these areas in material fixed with each of the different fixatives are shown in insets of Figs. 6–8. The peak-to-peak measurement of the microdensitometer trace of formalin–OsO_4-fixed material (inset, Fig. 6) is ~100A, that of the glutaraldehyde–acrolein–OsO_4-fixed material (inset, Fig. 7) is ~105 A, and that of the acrolein–$KMnO_4$-fixed material (inset, Fig. 8) is ~140 A. The peak-to-

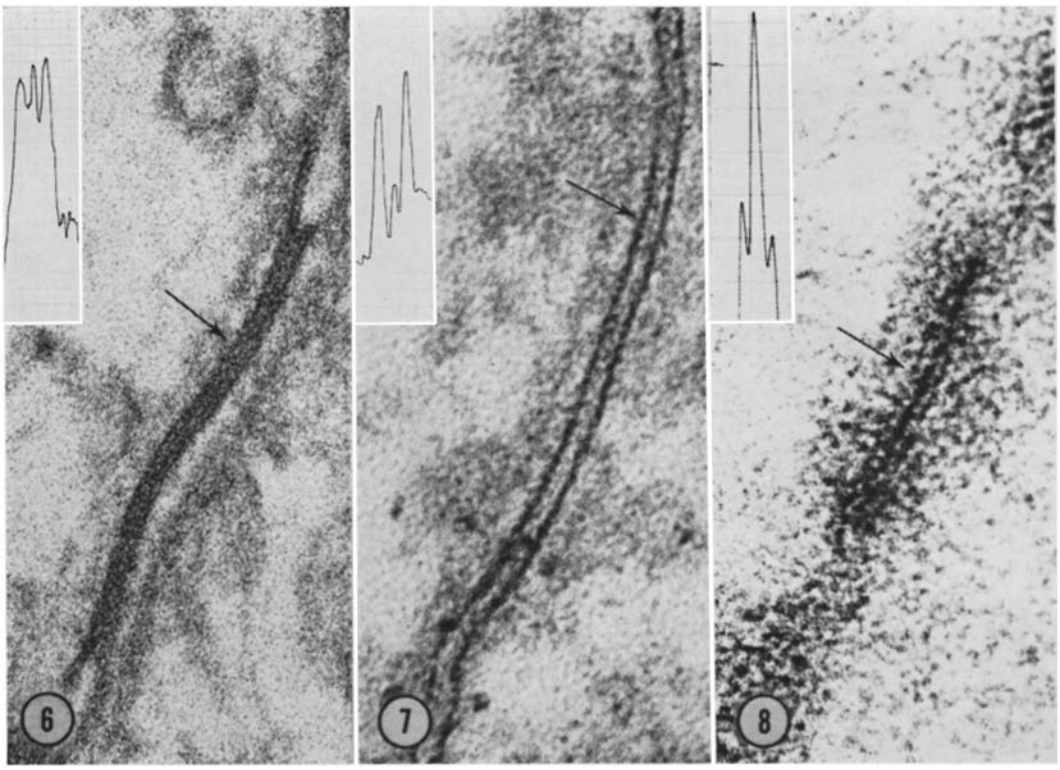

FIGURE 6 High-power electron micrograph of a synaptic disc of a specimen fixed with formalin–$0s0_4$. The external compound membrane measures by eye ~140 A in thickness. The inset shows the microdensitometer trace across the region at the arrow. The peak-to-peak measurement in this trace is ~100 A. × 240,000.

FIGURE 7 High magnification of a portion of a synaptic disc of a specimen fixed with glutaraldehyde–acrolein–OsO_4. Notice the middle line of the external compound membrane. The eye measurement of over-all thickness in this case is ~105 A. The inset shows the microdensitometer trace of the external compound membrane through the region at the arrow. The peak-to-peak measurement is ~150 A. × 240,000.

FIGURE 8 High magnification of a synaptic ECM of a specimen fixed with acrolein–$KMnO_4$. Notice that the middle line of the external compound membrane is clearly shown. The eye measurement in this case is ~165 A. The microdensitometer trace is characteristic of permanganate-fixed material. The peak-to-peak measurement in the direction of the arrow is ~140 A. × 240,000.

peak measurements in the microdensitometer traces in each case are, of course, lower than the over-all measurements by eye.

It is interesting to note that the over-all thickness measurements of the ECM after all fixatives, except in the case of our one fairly well fixed permanganate specimen, are between ~136 and ~160 A. The possible explanation for the eye measurements of ~165 A in the acrolein–permanganate specimen is poor fixation with some resultant artificial separation of the membranes. In this case, as mentioned before, the corresponding densitometer measurement is ~140 A.

The intervening SMC clefts between the ECM discs are widened and measure over-all between ~300 and ~340 A. There is a condensation of dense material in a zone extending about 400 A into pre- and postsynaptic cytoplasms all along the SMC (Figs. 3–5).

The SMC membranes are separated in some places by glial processes whose cytoplasm shows tubular organelle profiles in cross-section (Figs. 2 and 4). In such regions the condensations of dense material in pre- and postsynaptic cytoplasms is absent.

DISCUSSION

A considerable body of evidence is accumulating that suggests that closures of the synaptic cleft with the formation of external compound membranes, or tight junctions of some authors (5), such as we have observed in the spoon endings are characteristic of electrical transmission. The first inferences of this kind were made from correlations of physiological and morphological features of the crayfish median and lateral giant-to-motor synapses. Furshpan and Potter (12) showed that these synapses transmit by an electrical mechanism and that the synaptic membrane complex (SMC) has a rectifier property. At about the same time, Hama (16) demonstrated a narrowing of the synaptic cleft in these synapses, and Roberston (27) showed that they were characterized morphologically by complete closure of the synaptic cleft. Subsequent work (28) has verified these earlier studies, and it is now clear that the synaptic cleft in these crayfish giant fiber synapses is consistently closed. Similarly, it appears that the synaptic cleft is greatly narrowed or perhaps completely closed in the lateral giant septal synapses in the crayfish, as well as in the shrimp (28). Presumptive evidence is available for electrical transmission in the crayfish septal synapses, in that they show practically no synaptic delay (36). The septal synapses, however, transmit two ways, and so are physiologically different. The work on the Mauthner cell club endings by Robertson et al. (22) demonstrated that in those endings the SMC also is characterized by disclike regions of ECM formation. The ECM discs are quite localized in extent and alternate with regions in which the synaptic cleft is open. The arrangement resembles very much the one demonstrated in the spoon endings, but the ECM discs are more regular and uniform in size and their disc shape has been established. Working independently, Furshpan and Furukawa (11) have produced evidence of electrical transmission in the club endings.

A similar line of evidence that suggests a correlation between electrical transmission and ECM comes from the correlated physiological and morphological studies of Bennett et al. (2) on the synapses between pacemaker cells in certain fishes. These authors have put microelectrodes into adjacent cells and have demonstrated that electrical coupling exists between them. They showed in electron microscope studies that this phenomenon was associated with ECM discs between adjacent cells. Yet another instance of carefully correlated physiological and morphological studies is provided by the work of Barr et al. (1) on cardiac muscle. These authors showed that ECM in the intercalated discs are essential for electrical coupling between the cells. First, they demonstrated that ECM are present in the normal intercalated discs (7). This confirmed earlier work by Sjöstrand et al. (33). Having established the morphological features by electron microscopy, Barr et al. then showed physiologically that electrical coupling existed between adjacent cells and that this coupling could be broken by causing the cells to shrink in hypertonic media. The resulting failure of excitation to spread from one cell to another was shown to be morphologically accompanied by the opening of the ECM. By immersion of their preparation in hypotonic media, Barr et al. found that it was possible to reestablish the ECM and that simultaneously the electrical coupling and spread of excitation returned. Similar studies by Dewey and Barr (7, 8) on smooth muscle have permitted similar conclusions. The evidence thus seems overwhelming that some association exists between the ECM and electrical coupling between cells. Thus we propose that the morphological relationships

that we have described in the spoon type endings in the chick tangential nucleus are strongly suggestive of electrical transmission. It would be appropriate to test this hypothesis by physiological methods, which might provide another link in the chain of evidence suggesting that the ECM is associated with electrical synaptic transmission.

Other features of this synapse also deserve comment. First, the ECM observed in the spoon endings are not so regular as the disc shaped ones seen in the Mauthner cell club endings. In the present instance, the ECM are of irregular extent, varying widely from 0.04 to 0.47 μ. Second, the ECM are also often associated with accumulations of dense material on both the presynaptic and postsynaptic sides of the SMC, and such material is also seen in regions in which the synaptic cleft is open. In the Mauthner cell synapses, this feature is more notable in the regions of the SMC between discs. Another point of comparison concerns the distribution of neurofilaments and microtubules in the endings. Both the Mauthner cell club endings and the spoon endings are similar in showing relatively large numbers of these elements, compared to other kinds of endings, but the disposition of the elements in each is distinctly different. In the club endings the neurofilaments and microtubules terminate perpendicular to the SMC, whereas in the spoon endings they are mainly disposed parallel or at a small angle to the SMC.

It is interesting to note that certain features of synaptic ultrastructure associated with neurohumoral transmission are not found in the synapse described here. It is well established from the work of Katz and his associates (6, 10) that motor end plates transmit neurohumorally. Morphologically, motor endings are characterized by accumulations of large numbers of vesicles and numerous mitochondria, and by a paucity of neurofilaments and microtubules (3). In central nervous system synapses, as described by Palay (19, 20), Gray (13, 14), Gray and Guillery (15), and others, characteristically many axodendritic and axosomatic synapses have certain of these features, with large numbers of vesicles accumulated near the presynaptic membrane. Numerous mitochondria as well as neurofilaments and microtubules are sometimes seen, but not in immediate relationship to the SMC. Sometimes accumulations of dense material along the presynaptic and postsynaptic membranes occur with dense deposits of material between the synaptic membranes in the open synaptic clefts (13–15). In the spoon type endings very little dense material is accumulated in the open synaptic clefts, but dense material is accumulated next to the presynaptic and postsynaptic membranes. However, these accumulations are much more widespread and are not localized. These synapses are prominently characterized by a relative paucity of vesicles and mitochondria although some are present. The strikingly large numbers of neurofilaments and microtubules in immediate relationship to the SMC in the spoon endings are reminiscent of the situation in the Mauthner cell club endings (23). In the case of the electrically transmitting giant synapses in crayfish, again one sees a relative paucity of vesicles and the presence of neurofilaments in the synaptic region although in this case they are perhaps more pronounced on the postsynaptic side. Thus, apart from the closed synaptic cleft, there are other features of the spoon type synapse that are comparable to features found in other synapses that are known to transmit electrically. These are quite different from features commonly associated with synapses that are known to operate neurohumorally. This reinforces our belief that the spoon type endings described here transmit electrically.

Recently, Charlton and Gray (5) described ECM (tight junctions) in axosomatic and axodendritic synapses in the spinal cord of both fish and frog. These endings have some of the features of a neurohumoral synapse but in these cases are associated with ECM. They exhibit large numbers of synaptic vesicles, although usually accumulated out of the ECM region, numerous mitochondria, dense material accumulated in the area of the ECM, and neurofilaments with no relation to the ECM.

The implications of the correlation of the ECM with electrical transmission in a nerve ending deserve comment. This type of nerve ending was not recognized in early studies of synapses, possibly in part because of inadequate definition of unit membranes and failure to apply methods that would reveal external compound membranes. As techniques have improved, ECM are being recognized, in more and more instances, as important features of areas of cell contact in both neural and nonneural tissues (1, 2, 5, 7, 16, 22, 23). They may be much more widespread in brain tissue than heretofore recognized. This type of contact between neurons, if correctly identified as indicative of electrical transmission, offers certain unique

advantages such as rapidity and certainty of interaction, but perhaps the most important advantage is the opportunity, afforded uniquely by this type of synaptic contact, for chemical reactions to occur between apposed molecular constituents of adjacent membranes that could not occur if the membranes were separated by the usual 100–150 A gap. The existence of this type of synaptic contact may also be important because it suggests a way in which dynamic interactions may occur between neurons as a result of electrical activity (29, 30). It has been found that gaps of 100–150 A between apposed outside surfaces of cell membranes can be caused to close or open in response to very small changes in ionic milieu (28). The concentrations involved are small enough to allow one to consider that similar alterations could occur at the surfaces of neuronal membranes in correlation with electrical activity. If such changes occur, gap occlusions may result. Chemical interactions might follow that result in transitory or permanent local changes. Hence, the type of synapse described here may be of significance beyond the immediate implications regarding electrical transmission. While at present the evidence suggests that such synapses are primitive, the future may disclose that similar morphological operants are applied in higher neural function.

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BIBLIOGRAPHY

1. Barr, L., M. M. Dewey, and W. Berger. 1965. Propagation of action potentials and the structure of the nexus in cardiac muscle. *J. Gen. Physiol.* **48**:797–823.
2. Bennett, M. V. L., E. Aljure, Y. Nakajima, and G. D. Pappas. 1963. Electrotonic junctions between teleost spinal neurons: Electrophysiology and ultrastructure. *Science.* **141**:262–264.
3. Birks, R., H. E. Huxley, and B. Katz. 1960. The fine structure of the neuromuscular junction of the frog. *J. Physiol.* **150**:134–144.
4. Cajal, S. R. 1908. Sur un noyau spécial du nerf vestibulaire des poissons et des oiseaux. *Trav. Lab. Rech. Biol. Univ. Madrid.* **6**:1–20.
5. Charlton, B. T., and E. G. Gray. 1966. Comparative electron microscopy of synapses in the vertebrate spinal cord. *J. Cell Sci.* **1**:67–80.
6. Del Castillo, J., and B. Katz. 1956. Biophysical aspects of neuromuscular transmission. *Progr. Biophys. Biophys. Chem.* **6**:122–170.
7. Dewey, M. M., and L. Barr. 1964. A study of the structure and distribution of the nexus. *J. Cell Biol.* **23**:553–585.
8. Dewey, M. M., and L. Barr. 1967. Intercellular connection between smooth muscle cells: the nexus. *Science.* **137**:670–672.
9. Engström, H., and J. Wersäll. 1958. Myelin sheath structure in nerve fibre demyelinization and branching regions. *Exptl. Cell Res.* **14**: 414–425.
10. Fatt, P., and B. Katz. 1952. Spontaneous subthreshold activity at motor nerve endings. *J. Physiol.* **117**:109–128.
11. Furshpan, E. J., and T. Furukawa. 1967. Intracellular and extracellular responses of the several regions of the Mauthner cell of the goldfish. *J. Neurophysiol.* **25**:732–771.
12. Furshpan, E. J., and D. D. Potter, 1959. Transmission at the giant motor synapses of the crayfish. *J. Physiol.* **145**:289–325.
13. Gray, E. G. 1959. Axo-somatic and axo-dendritic synapses of the cerebral cortex: an electron microscope study. *J. Anat.* **93**:420–433.
14. Gray, E. G. 1961. Ultrastructure of synapses of the cerebral cortex and of certain specializations of neuroglial membranes. *In* Electron Microscopy in Anatomy. J. D. Boyd, F. R. Johnson, and J. D. Lever, editors. Edward Arnold (Publishers) Ltd., London. 54–73.
15. Gray, E. G., and R. W. Guillery. 1966. Synaptic morphology in the normal and degenerating nervous system. *Intern. Rev. Cytol.* **19**:111–182.
16. Hama, K. 1961. Some observations on the fine structure of the giant fibers of the crayfishes (*Cambarus virilis and Cambarus clarkii*) with special reference to the submicroscopic organization of the synapse. *Anat. Record* **141**: 275–293.
17. Kappers, C. U. A., G. C. Huber, and E. C. Crosby. 1960. The Comparative Anatomy of the Nervous System of Vertebrates, Including Man. Hafner Publishing Co., New York. 473–484.
18. Palay, S. L., S. M. McGee-Russell, S. Gordon, and M. A. Grillo. 1962. Fixation of neural tissues for electron microscopy by perfusion

with solutions of osmium tetroxide. *J. Cell Biol.* **12**:385–410.

19. PALAY, S. L. 1956. Synapses in the central nervous system. *J. Biophys. Biochem. Cytol.* **2**(Suppl.): 193–202.
20. PALAY, S. L. 1958. The morphology of synapses in the central nervous system. *Exptl. Cell Res.* **5**(Suppl.):275–293.
21. PALAY, S. L., and G. E. PALADE. 1955. The fine structure of neurons. *J. Biophys. Biochem. Cytol.* **1**:69–88.
22. ROBERTSON, J. D., T. S. BODENHEIMER, and D. E. STAGE. 1963. The ultrastructure of Mauthner cell synapses and nodes in goldfish brains. *J. Cell Biol.* **19**:159–199.
23. ROBERTSON, J. D. 1963. The occurrence of a subunit pattern in the unit membranes of club endings in Mauthner cell synapses in goldfish brains. *J. Cell Biol.* **19**:201–221.
24. ROBERTSON, J. D. 1956. The ultrastructure of the myelin sheath near nodes of Ranvier. *J. Physiol.* **135**:56–57.
25. ROBERTSON, J. D. 1959. Preliminary observations on the ultrastructure of nodes of Ranvier. *Z. Zellforsch. Mikroskop. Anat.* **50**:553–560.
26. ROBERTSON, J. D. 1958. Structural alterations in nerve fibers produced by hypotonic and hypertonic solutions. *J. Biophys. Biochem. Cytol.* **4**:349–364.
27. ROBERTSON, J. D. 1961. Ultrastructure of excitable membranes and the crayfish median-giant synapses. *Ann. N. Y. Acad. Sci.* **94**:339–389.
28. ROBERTSON, J. D. 1964. Unit membranes: A review with recent new studies of experimental alterations and a new subunit structure in synaptic membranes. Proceedings of the 22nd Symposium of the Society for the Study of Development and Growth. M. Locke, editor. Academic Press Inc., New York. 1–8.
29. ROBERTSON, J. D. 1965. Intracellular membranous structure. Proceedings of the First International Symposium for Cellular Chemistry, Ohtsu, Japan, 1963. S. Seno and E. V. Cowdry, editors Japan Society for Cell Biology, Okayma, Japan. 379–433.
30. ROBERTSON, J. D. 1965. The synapse, morphological and chemical correlates of function. *Neurosciences Res. Progr. Bull.* **3**:3–79.
31. SABATINI, D. D., K. BENSCH, and R. J. BARRNETT. Cytochemistry and electron microscopy. *J. Cell Biol.* **17**:19–58.
32. SANDBORN, E., P. F. KOEN, J. D. MCNABB, and G. MOORE. 1964. Cytoplasmic microtubules in mammalian cells. *J. Ultrastruct. Res.* **11**:123–138.
33. SJÖSTRAND, F. S., E. ANDERSSON-CEDERGREN, and M. M. DEWEY. 1958. The ultrastructure of the intercalated discs of frog, mouse and guinea-pig cardiac muscle. *J. Ultrastruct. Res.* **1**:271–287.
34. UZMAN, B. G., and G. NOGUEIRA-GRAF. 1957. Electron microscope studies of the formation of nodes of Ranvier in mouse sciatic nerves. *J. Biophys. Biochem. Cytol.* **3**:589–598.
35. VAN HARREVELD, A., J. CROWELL, and S. K. MALHOTRA. 1965. A study of extracellular space in central nervous tissue by freeze-substitution, *J. Cell Biol.* **25**:117–137.
36. WIERSMA, C. A. G. 1947. Giant nerve fiber system of the crayfish. A contribution to comparative physiology of synapse. *J. Neurophysiol.* **10**:23–28.