MYONEURAL JUNCTIONS OF TWO ULTRASTRUCTURALLY DISTINCT TYPES IN EARTHWORM BODY WALL MUSCLE

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ABSTRACT

The longitudinal muscle of the earthworm body wall is innervated by nerve bundles containing axons of two types which form two corresponding types of myoneural junction with the muscle fibers Type I junctions resemble cholinergic neuromuscular junctions of vertebrate skeletal muscle and are characterized by three features: (*a*) The nerve terminals contain large numbers of spherical, clear, ~500 A vesicles plus a small number of larger dense-cored vesicles (*b*) The junctional gap is relatively wide (~900 A), and it contains a basement membrane-like material. (*c*) The postjunctional membrane, although not folded, displays prominent specializations on both its external and internal surfaces The cytoplasmic surface is covered by a dense matrix ~200 A thick which appears to be the site of insertion of fine obliquely oriented cytoplasmic filaments The external surface exhibits rows of projections ~200 A long whose bases consist of hexagonally arrayed granules seated in the outer dense layer of the plasma membrane The concentration of these hexagonally disposed elements corresponds to the estimated concentration of both receptor sites and acetylcholinesterase sites at cholinergic junctions elsewhere. Type II junctions resemble the adrenergic junctions in vertebrate smooth muscle and exhibit the following structural characteristics: (*a*) The nerve fibers contain predominantly dense-cored vesicles ~1000 A in diameter (*b*) The junctional gap is relatively narrow (~150 A) and contains no basement membrane-like material. (*c*) Postjunctional membrane specialization is minimal. It is proposed that the structural differences between the two types of myoneural junction reflect differences in the respective transmitters and corresponding differences in the mechanisms of transmitter action and/or inactivation.

INTRODUCTION

Previous studies of neuromuscular junctions (3, 6, 9, 13, 24, 25, 27, 28, 29, 30, 35) have demonstrated two morphological features which are common to junctions in a variety of muscle types and a variety of animals These constant components are: proximity of the nerve and muscle plasma membranes with no cellular processes intervening and the presence of large numbers of vesicles in the junctional region of the axon. Variable features include the exact dimensions of the gap, the presence of formed extracellular material within the gap, specializations of the junctional membranes, and variations in the morphology of the vesicles Attempts to correlate specific morphological patterns with specific transmitters or known physiological properties have been only partially successful, however, and in some instances have led to the conclusion that junctions known to have very different properties may be indistinguishable morphologically (e.g., reference 26)

The present report describes myoneural junc-

tions in earthworm longitudinal body wall muscle, whose cells are known to exhibit both hyperpolarizing and depolarizing end plate potentials (14) and are therefore assumed to be doubly innervated. A brief earlier paper (22) describes only one kind of myoneural junction in this muscle However, an extensive survey by electron microscopy demonstrates two very distinct kinds of myoneural junction formed in some cases on the same muscle process The two types can be identified on the basis of axonal vesicle morphology, gap width, and postjunctional membrane specialization, and are therefore readily distinguishable One type resembles the myoneural junctions of vertebrate skeletal muscle (6) but displays membrane specializations which are more prominent and in which regular mosaic patterns can be brought out by special staining methods The second type is similar to the adrenergic junctions of vertebrate smooth muscle (27) Since the two types of junction occur together in the same muscle, and indeed sometimes on the same cell, it is possible to exclude regional anatomical or species differences as causes of the obvious distinctions between them and to attempt to relate their structural differences instead to the physiological mechanisms that operate in the two cases

MATERIALS AND METHODS

Earthworms of the family Lumbricidae were collected locally in the New York City area. Some were anesthetized with 7 5% $MgCl_2$, slit open longitudinally behind the clitellum, eviscerated, pinned in maximal extension on a wax plate, and then flooded with fixative (2–3.5% glutaraldehyde in 0.1 M phosphate buffer, pH 7 5) Unanesthetized animals were fixed similarly at shorter lengths After 1–3 hr at room temperature, the tissues were rinsed in saline and postfixed in 1–2% OsO_4 in the same buffer. Care was taken to embed the tissues so that sections could be cut in known planes with respect to the axis of the animal 1 μ sections stained with toluidine blue were used for survey purposes and thin sections ranging from silver to purple in color were used for electron microscopy The thin sections were stained with uranyl acetate followed by lead hydroxide. In some instances pieces of earthworm body wall were soaked in 0 1% lead nitrate (9) in 0 65% NaCl for approximately 30 min before fixation in glutaraldehyde They were then dehydrated and embedded without postfixation. Sections of these specimens when examined unstained had extremely low contrast. Marked enhancement in contrast in these unosmicated specimens could, however, be accomplished by staining the thin sections either with 1% phosphotungstic acid (PTA) in 95% ethanol or, even more so, with 2% potassium permanganate ($KMnO_4$) followed by a 5% citric acid rinse and then 2% uranyl acetate in 75% ethanol for 15 min at 60°C (18). Electron micrographs were taken with a Philips EM 300 electron microscope operated at 60, 80, or 100 kv.

RESULTS

The earthworm body wall (Fig 1) is covered by a cuticle and epidermis externally (8) and contains outer circular and inner longitudinal muscle layers both of which are obliquely striated. The longitudinal layer, which is much the thicker of the two, is composed of flat pennate bundles within each of which the individual muscle cells are apposed to one another without intervening connective tissue and in some instances are interconnected by desmosomes Each such bundle is surrounded by a basement membrane and is separated from neighboring bundles of muscle fibers by connective tissue septa The circumferentially oriented segmental nerve rings are located at the interface between the circular and longitudinal muscle layers and give rise to peripheral branches which extend centripetally within the radially oriented connective tissue septa (Fig. 3)

A typical peripheral nerve branch (Fig 2) has axons of two types traveling together within it One type contains microtubules, flattened membranous cisternae, mitochondria, and neurofilaments The filaments differ from those of vertebrate nerve fibers, however, in that they are packed closely together (center-to-center distance ~100 A) rather than separated widely from one another The inner surface of the nerve fiber membrane is typically coated with a fine weblike material. The second type of axon has these same elements but in addition contains a population of dense-cored vesicles ~1000 A in diameter. The core itself is typically separated from the enveloping membrane by a light halo The concentration of dense-cored vesicles in the second type of fiber may be very high (Fig. 3) even in regions remote from myoneural junctions

The entire bundle of axons is completely or partially ensheathed by flattened supporting cells which exhibit prominent "gliosomes," and externally the nerve is separated from the surrounding connective tissue space by a distinct basement membrane Thus, in nonjunctional regions, earthworm peripheral nerve fibers are separated from neighboring muscle cells by the

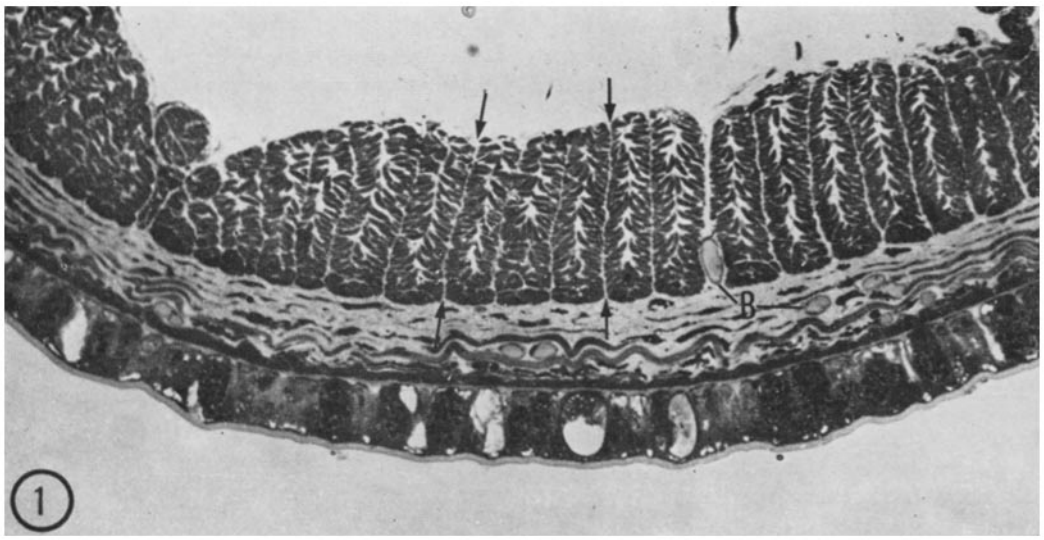


FIGURE 1 Photomicrograph of earthworm body wall (transverse section). The longitudinal muscle layer consists of pennate bundles separated from each other by radial connective tissue septa (arrows). Several sinuous fibers of the circular muscle are visible in the connective tissue beneath the scalloped base of the longitudinal muscle. *B*, blood vessels. × 430.

basement membranes of both the nerve and muscle bundles, by the intervening connective tissue, which contains both collagen fibrils and microfibrils, and in some cases by one or more supporting cell lamellae At intervals, however, all along the radial connective tissue septa, nerve fibers and muscle cells become closely approximated forming myoneural junctions of two distinct types corresponding to the two types of nerve fiber.

Type I Myoneural Junctions

Nerve fibers that are devoid of vesicles in nonjunctional regions can be traced to junctions formed either with muscle fibers themselves, i e directly over myofibrils, or with veil-like processes which may extend a considerable distance from the myofibrillar portion of the muscle cell. These processes tend to converge on nerve bundles in a manner reminiscent of the convergence of *Ascaris* muscle arms onto the nerve cord (30). In one location a single nerve bundle may form junctions with multiple muscle fibers and processes originating on both sides of a connective tissue septum (Fig. 4).

The most striking aspect of this type of junction is the appearance of hordes of clear vesicles ~500 A in diameter in the junctional region of the nerve fiber (Figs. 4–6) The vesicles cluster against the axon membrane and irregularities and concavities in the contour of that membrane are encountered frequently. Small densities associated with the prejunctional membrane occur occasionally. In addition to the clear vesicles, scattered dense-cored vesicles also appear in the junctional regions These are considerably larger than the clear vesicles and are of two types In one the dense core is significantly smaller than the vesicle itself and is surrounded by a halo, in the other the vesicle is filled uniformly with a fine punctate material of intermediate density. The ratio of dense-cored vesicles to clear vesicles in this type of ending is small and although it is usually the clear vesicles that cluster against the junctional membrane, dense-cored vesicles can also be found in this location (Fig 6) Microtubules and neurofilaments are absent from the junctional axoplasm

The second distinctive feature of this type of ending is the junctional gap. The pre- and postjunctional membranes parallel each other but are always separated by a large cleft of relatively constant width The gap is approximately 900 A wide and it contains a fine filamentous material of medium density which resembles basement membrane but is usually much thicker (Fig 5) Such junctions are seen occasionally deep within a

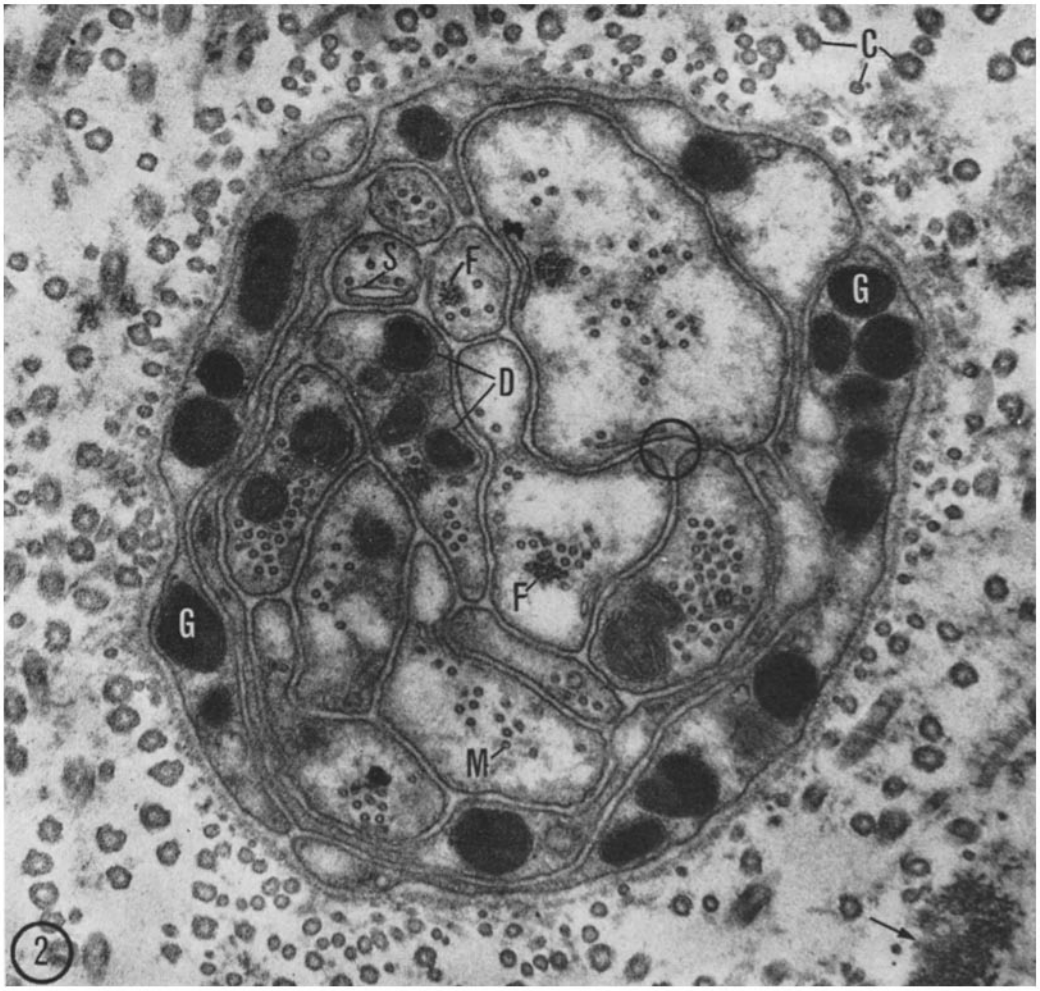


FIGURE 2 Peripheral nerve bundle. 12 nerve fibers are surrounded by supporting cell layers which contain prominent gliosomes (*G*). The nerve fibers contain microtubules (*M*), bundles of neurofilaments (*F*) and flattened membranous sacs (*S*). One of the nerve fibers contains in addition several dense-cored vesicles (*D*). The clefts between the nerve fibers are free of formed elements except occasionally at corners where some amorphous dense material can be found (circle). The entire bundle is enveloped by a basement membrane outside of which collagen fibrils (*C*) and bundles of microfibrils (arrow) are visible. × 59,000.

muscle bundle, at a distance from the connective tissue septa, and in these cases too the same junctional gap material is present At some junctions the gap material is distributed uniformly across the entire width of the cleft, in others, it can be resolved into laminae, which sometimes can be traced to the nerve and muscle basement membranes

The third and most distinctive component of this type of junction is the postjunctional membrane specialization. In specimens postfixed with osmic acid the postjunctional membrane, when cut transversely (Figs 7 and 8), exhibits a somewhat asymmetric trilaminar structure with an over-all width of ~70 A. The membrane is usually straight or concave facing outwards in contrast to the membrane at hemidesmosomes which is typically convex. At some of the junctions (Fig. 7) a prominent row of projections can be seen extending from the outer dense lamina of the postjunctional membrane into the junctional cleft These projections are approximately 200 A long and are

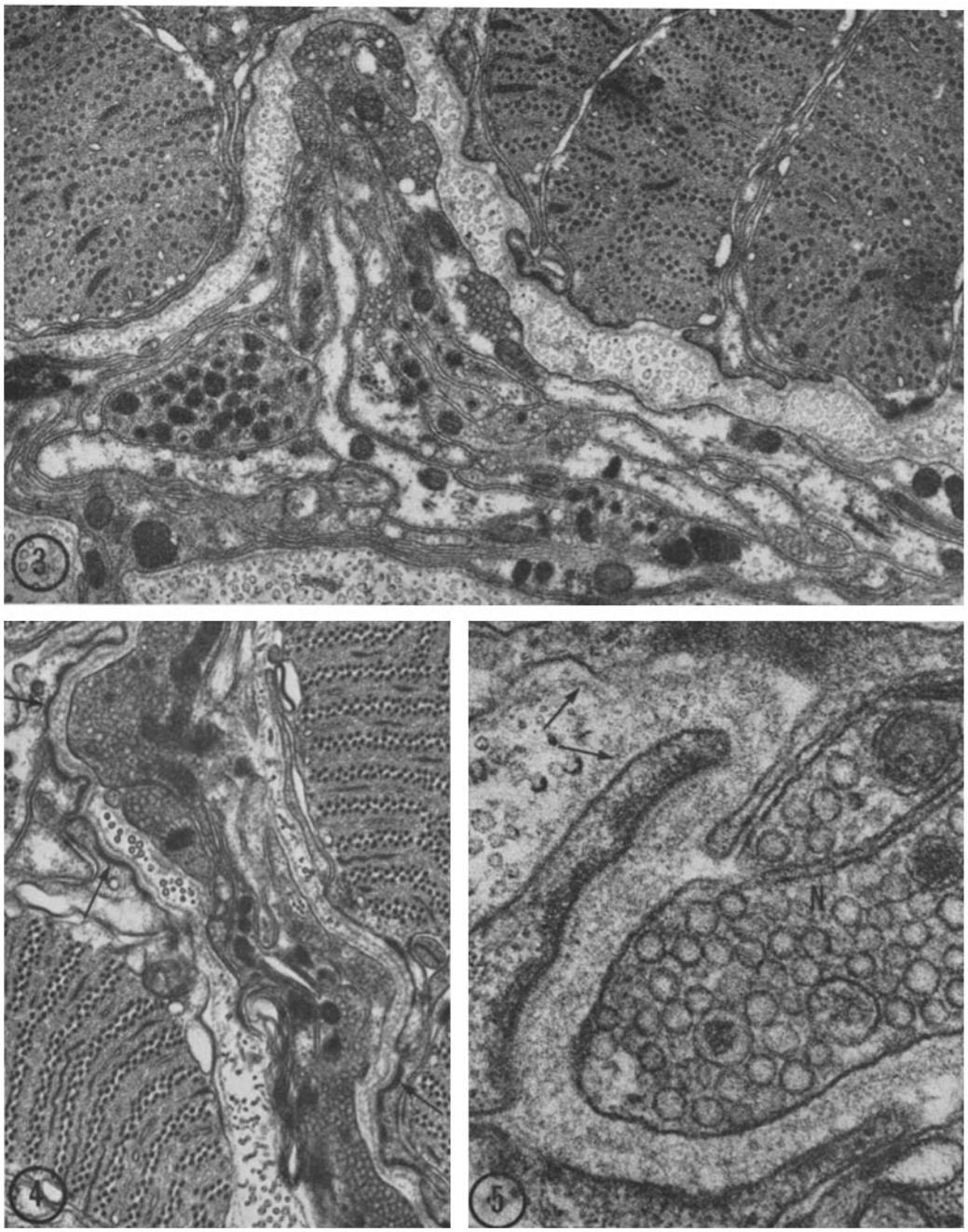


FIGURE 3 Segmental nerve bundle giving rise to a radial branch. Portions of two longitudinal muscle bundles are separated by a connective tissue septum which is penetrated by the radial nerve branch. Two kinds of nerve fibers are visible, one containing predominantly dense-cored vesicles ~ 1000 A in diameter and the second containing either no vesicles or clusters of predominately clear vesicles ~ 500 A in diameter. The thick myofilaments in the muscle cell at the extreme right are of larger caliber and are oval in cross-section as compared with those in the cell at the top center. × 24,000.

FIGURE 4 Relatively thick section showing radial nerve branch forming type I myoneural junctions with muscle processes on both sides of the connective tissue septum. Arrows indicate three instances in which a linear density is clearly seen in the junctional cleft parallel to the postjunctional membrane at a distance of ~200 A (cf. Figs. 8 and 11 *inset*). × 28,000.

FIGURE 5 Myoneural junction. A nerve fiber (*N*) is apposed to parts of three muscle fibers. The basement membranes of both the nerve and muscle bundles (arrows) appear to be reflected into the junctional gap. The junctional membrane of the muscle process at the left is scalloped and exhibits a cytoplasmic coating. The external coating is not clear, however. × 95,000.

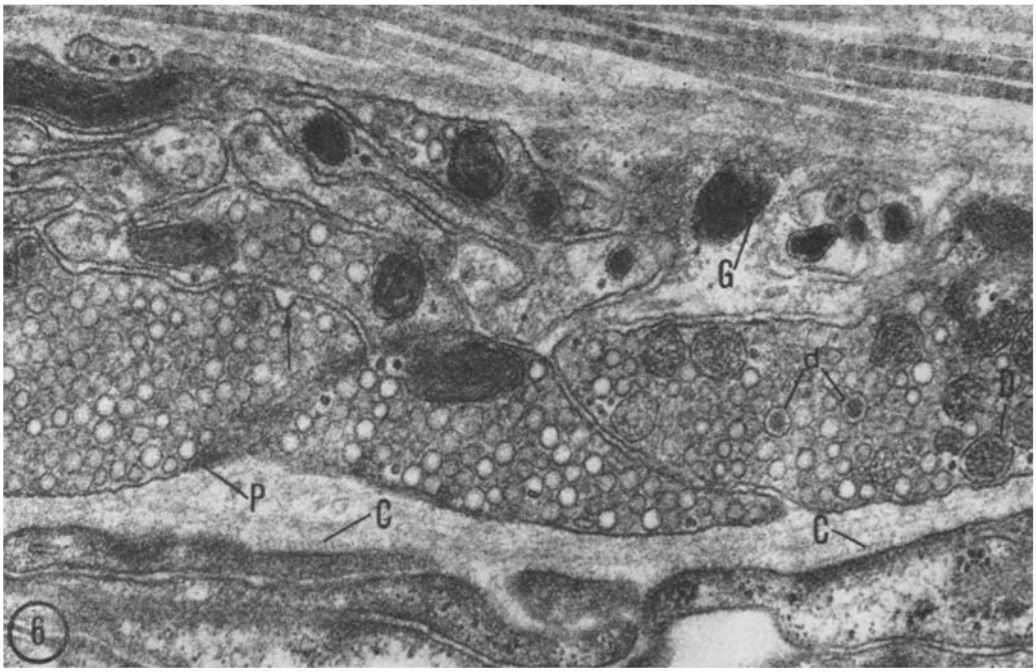


FIGURE 6 Type I myoneural junction. Collagen fibrils are visible within a radial connective tissue septum at the top. A bundle of nerve fibers lies between this connective tissue layer and muscle processes at the bottom. Most of these nerve fibers contain innumerable small clear vesicles together with a few small (*d*) and large (*D*) dense-cored vesicles. One of the latter is closely applied to the junctional membrane. A U-shaped profile is present at the interface between two nerve fibers (arrow). The junctional membrane of the nerve fibers is rippled and exhibits occasional dense patches (*P*). That of the muscle processes exhibits regularly spaced projections in two regions (*C*). *G*, gliosome. × 61,000

spaced at apparent intervals of ~140 A. Profiles of the individual projections are thickened at their distal ends, and near their basal ends, where they join the outer lamina of the plasma membrane, they flare somewhat giving them a dumbbell shape. In other instances (Fig. 8) the projections are seen faintly and are spaced at intervals of only ~80 A. Still other examples can be found in which the shafts of the projections cannot be distinguished at all (Fig. 5), their bulbous tips, however, may be visible, especially in relatively thick sections, apparently interconnected and forming a line parallel to the postjunctional membrane but seemingly separate from it (Figs. 4, 5).

When the plasma membrane is cut obliquely the projections tend to appear tilted and longer than in the normal views (Fig. 9) and in addition, they appear beaded. In tangential views of the membrane (Fig. 10), regularly disposed punctate densities appear, somewhat indistinctly, superimposed on the other dense components of the membrane. The linear patterns sometimes seen in such images form angles approximating 60° as would be expected from elements in hexagonal array.

In sections normal to the sarcolemma, the internal coating is usually seen as an amorphous layer ~200 A wide applied directly to the cytoplasmic surface of the postjunctional membrane. This coating appears to be the terminus for bundles of fine cytoplasmic filaments which approach the plasma membrane at an oblique angle (Figs. 7, 9).

Unosmicated specimens that have been stained, after sectioning, with either phosphotungstic acid or permanganate followed by uranyl acetate reveal additional details of these membrane specializations. The coating on the cytoplasmic surface of the postjunctional membrane is consistently dense (Fig. 11), resembling in this respect the densities associated with synaptic membranes in the central nervous system (1, 2) and the periodic densities associated with some muscle

dyads (31). Dense patches also appear occasionally on the presynaptic (axonal) membrane in such specimens The postjunctional plasma membrane appears markedly asymmetric The inner dense lamina is a continuous dense line, while the outer dense lamina is a faint line interrupted by a series of regularly spaced, very dense granules ~70 A wide. These granules, which appear to constitute an integral part of the outer dense lamina of the plasma membrane and indeed sometimes even extend part way across the middle lucent lamina of the membrane (Fig 11), represent the bases of the radial projections that arise from this membrane (Fig 11, inset). As in the case of some post-osmicated specimens (Fig 4), the distal ends of the projections often appear to be interconnected by a linear density which parallels the subjacent plasma membrane but which may appear detached from it since the shafts of the projections are frequently indistinct in preparations of this kind

In *en face* views (Fig 12) hexagonal arrays of densities ~70 A in diameter and spaced at intervals of ~160 A are sometimes very conspicuous and presumably correspond to the granules which are so prominent in normal views of the membrane. Thus the outer dense lamina of the postjunctional plasma membrane appears to be a mosaic, a significant component of which consists of regularly disposed granules which constitute the bases of the projections that extend into the junctional cleft

The assumption that these postjunctional elements are in hexagonal array is supported partly by direct observation of *en face* views and partly by measurement of the spacing of the projections as seen in side views, i.e , in sections perpendicular to the plasma membrane Thus, assuming that the projections are spaced at 160 A intervals and are in hexagonal array, at some orientations of the normally cut membrane superimposition of rows of nearest neighbors would produce an apparent period of 140 A in the section plane, and at certain other orientations, superimposition of next-to-nearest neighbors would produce an apparent period of 80 A. These calculated intervals correspond to the spacings actually observed Moreover, the relative faintness of the projections in the second case can be accounted for by the greater separation of the superimposed projections from one another within each row Only one or two could be accommodated within the thickness of a 500 A section, while in the first case three or four would fit At other orientations of the membrane no periodicity would be expected in side views either because the projections do not superimpose coherently or because the space between rows is too narrow to be resolved

The apparent elongation of the projections in oblique sections (Figs 9, 10) can be ascribed to an optical effect resulting from stagger in the overlap of the projections located at progressively deeper levels. The apparent tilting of the projections can be accounted for similarly. When the rows of partially superimposed projections in an oblique section are not quite perpendicular to the plane

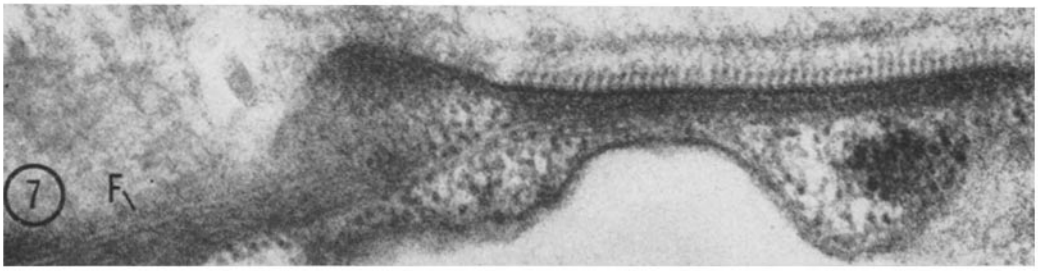
FIGURES 7–10 Details of type I postjunctional membrane specializations.

FIGURE 7 The plasma membrane is cut transversely and appears trilaminar. The projections extending from it are dumbbell shaped. On its cytoplasmic surface the membrane is coated by an amorphous layer ~ 200 A thick towards which a bundle of fine filaments (*F*) extends obliquely. × 111,000.

FIGURE 8 Concave unit membrane (left) showing continuous inner dense lamina and thinner outer dense lamina which is beaded (~80 A period) The shafts of the projections are indistinct in this region but their distal tips are visible as a line (*L*) parallel to the plasma membrane. Projections spaced at ~ 80 A intervals are faintly visible at the right (arrows). × 225,000.

FIGURE 9 Oblique section. The degree of obliquity increases from right to left and as it does so, the projections appear tilted and, at the extreme left, beaded. *F*, cytoplasmic filaments. × 99,000.

FIGURE 10 Multiple views of postjunctional membrane specialization. A bundle of nerve fibers (top center) is ringed by muscle processes which exhibit five regions of membrane specialization cut at various angles. In one case (upper right arrow) the projections appear to branch or interconnect; in another (lower right arrow) they appear linear and greatly elongated, in a third (left arrow) they appear as arrays of dots. *F*, cytoplasmic filament bundles. × 74,000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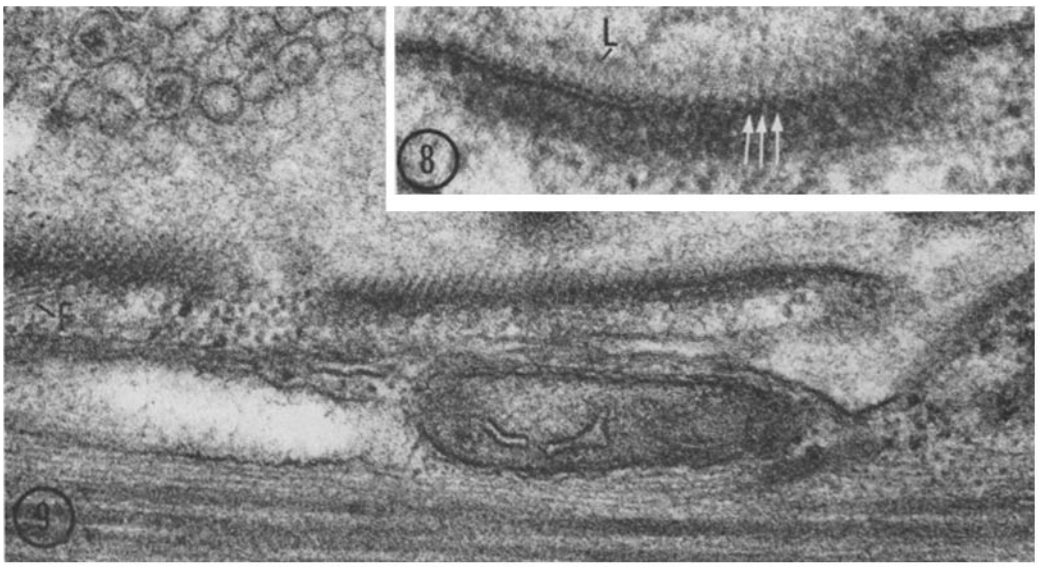
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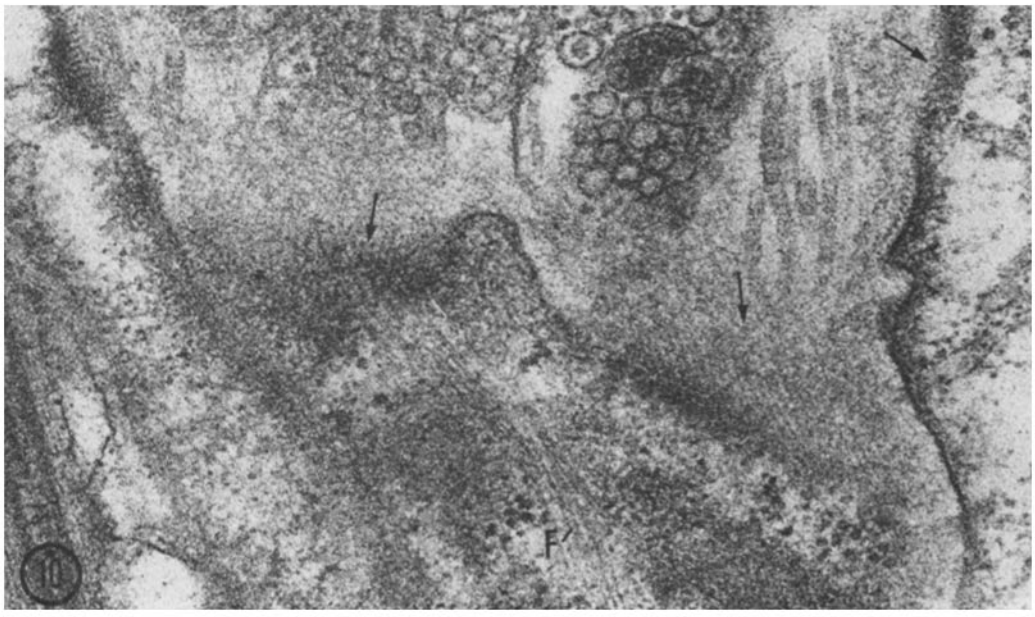
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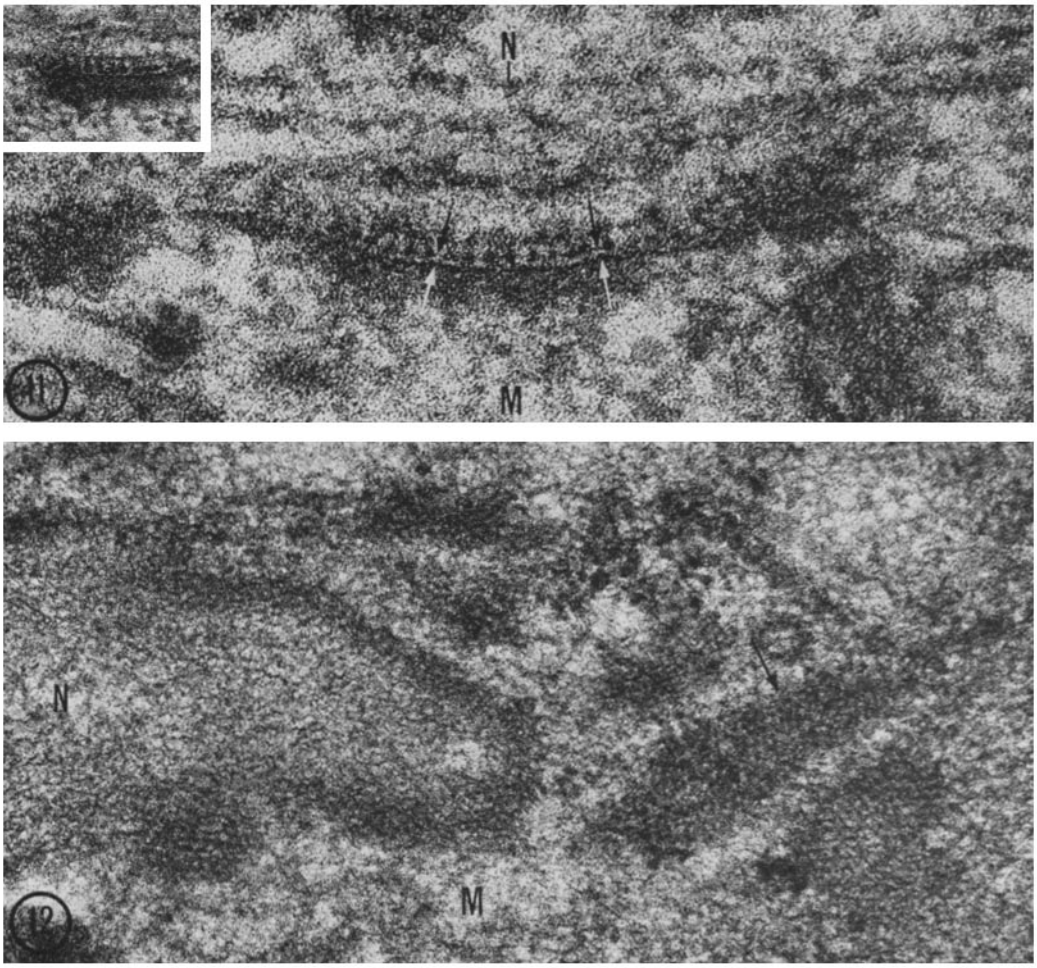

FIGURES 11 and 12 Details of type I postjunctional membrane specializations in unosmicated sections stained with potassium permanganate and uranyl acetate

FIGURE 11 White arrows indicate the continuous inner dense lamina of the postjunctional membrane Black arrows indicate the faint outer dense lamina which is interrupted by dense granules. *M*, muscle cell, *N*, prejunctional (axonal) plasma membrane. *Inset:* Postjunctional membrane showing continuity of projections with granules seated in the plasma membrane. The distal ends of the projections are interconnected by a thin linear density × 192,000; *inset*, × 107,000.

FIGURE 12 *En face* view of postjunctional membrane. In this preparation a regular lattice-like pattern is visible in several patches of postjunctional membrane. The linear patterns form angles of approximately 60° with each other. Arrow indicates cluster of granules in hexagonal array. *N*, nerve fiber; *M*, muscle cell × 86,000.

of section then the tips of the projections at progressively deeper levels are displaced laterally to an increasing degree, and the line connecting their staggered tips would be expected to appear tilted. These effects can be seen readily in a model consisting of hexagonally arrayed pegs in a board.

Type II Myoneural Junctions

Junctions of this type are encountered much less frequently than the type I junctions They resemble myoneural junctions of vertebrate smooth muscle (27, 28) and of many invertebrate

muscles (4, 26) in lacking pronounced junctional specializations even though the nerve and muscle plasma membranes are very closely apposed with neither connective tissue elements nor basement membrane material intervening (Fig 13) Junctions of this type differ from the type I junctions in three respects: the axonal vesicles are predominantly dense cored and larger and occur all along the length of the nerve fibers (Fig 2) and not just in the junctional regions, the junctional gap is narrow (~150 A); and the postjunctional membrane exhibits only minimal signs of specialization on either surface both in standard preparations (Fig 13) and in those stained with PTA or $KMnO_4$.

Along the contact region short peglike densities can sometimes be observed in the cleft between the respective plasma membranes (Fig 13, inset). These projections appear to arise from either of the two membranes and extend part way across the gap. On the cytoplasmic surface of the muscle fiber membrane a faint undercoating can sometimes be seen in the junctional region. The obliquely oriented sarcoplasmic filaments associated with the type I junctions have not been seen, however. On occasion the junctional nerve and muscle plasma membranes appear to approach each other very closely, perhaps reflecting the virtual absence of formed elements within the gap The respective membranes in such regions may be separated by as little as ~30 A

Junctions of this type also form either with muscle cell bodies or with veil-like muscle processes However, the latter have a smaller tendency to extend long distances towards the nerve bundles than do the muscle processes that converge on type I endings. Junctions are formed *en passant* (Fig. 13), the same nerve fiber contacting a series of muscle cells in its course along the radial connective tissue septum

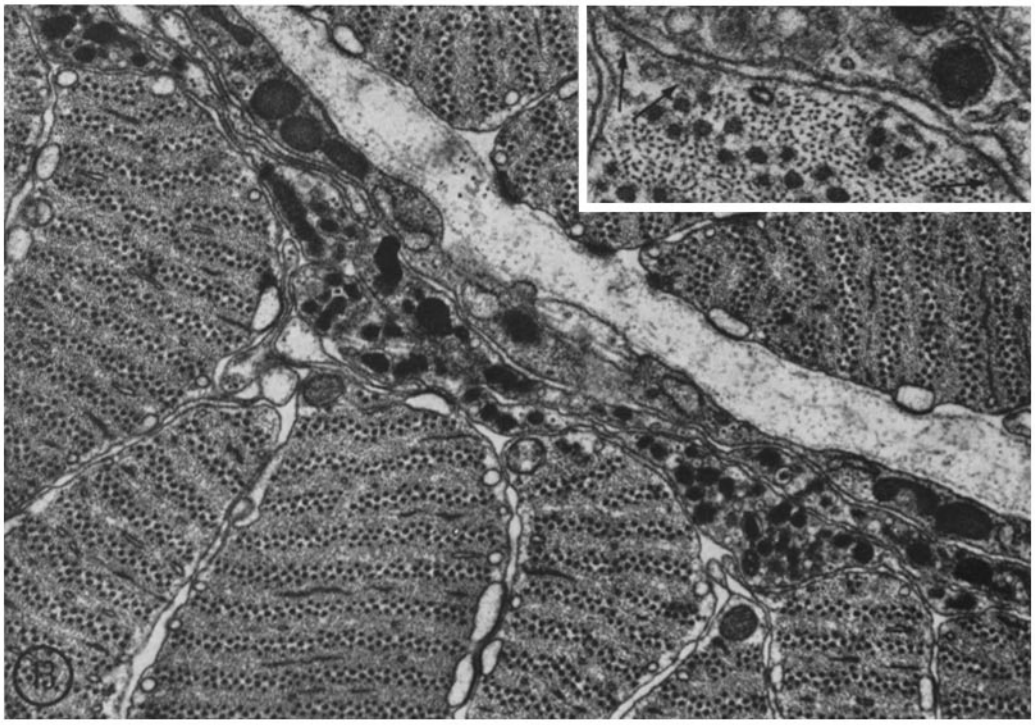

FIGURE 13 A bundle of type II nerve fibers is in intimate contact with a series of muscle cells. No connective tissue or supporting cells intervene between the nerve and muscle fibers in the junctional region. *Inset*· Detail of Fig 13 showing type II neuromuscular junction. The nerve and muscle cell membranes are separated by a narrow gap which contains no basement membrane. In three regions (arrows) there is a small amount of dense material applied to the cytoplasmic surface of the muscle cell membrane and within the junctional gap as well. × 27,000, *inset*, × 92,000.

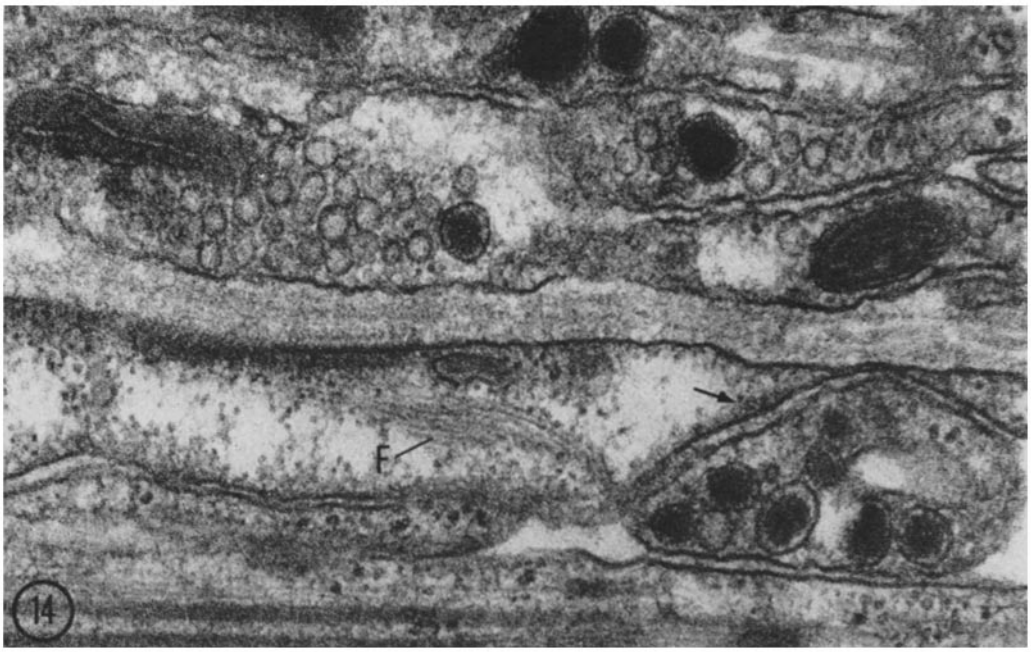

FIGURE 14 Type I and type II myoneural junction on the same muscle process. The nerve fiber at the top left contains small clear vesicles and is separated from the underlying muscle process by a wide gap which is filled with amorphous material. The muscle cell membrane has a dense coating on its cytoplasmic surface towards which a bundle of cytoplasmic filaments (*F*) extends An external coating is vaguely discernible also. The nerve fiber at the lower right contains predominantly dense-cored vesicles. It is separated from this same muscle process by a narrow gap A small amount of coating is visible on the cytoplasmic surface of the muscle membrane in one region of this junction (arrow). × 89,000.

The dense-cored vesicles in the nerve fibers that form type II junctions vary considerably in size, shape, and density, and different nerve fibers, even together in the same bundle, may appear to contain different classes of vesicles It need not be assumed, however, that these apparently different vesicle populations arise from correspondingly different cell types When nerve cell bodies from the nerve cord of the earthworm are examined, it is possible to find instances in which a single cell body contains a wide variety of dense-cored vesicles ranging from 600 to 1800 A in size and containing granules of different sizes, shapes, and densities.

The nerve fibers that give rise to type I and type II junctions travel together in the same bundles (Figs 2, 3), and in rare instances both types of junction can be found together on the same cell or process (Fig 14). Thus, the longitudinal muscle exhibits two morphologically distinct types of innervation and, at least in some cases, the same muscle cell receives both.

DISCUSSION

This study reports two clearly distinguishable types of myoneural junction which can be compared directly with one another in the same muscle.

Previous efforts to distinguish different types of myoneural junctions morphologically have been disappointing. Peterson and Pepe (26), for example, examined inhibitory nerve endings in crayfish and found them not to be significantly different in appearance from excitatory terminals Uchizono (35) and Atwood and Morin (4) reported quantitative differences in the size and shape of synaptic vesicles in inhibitory vs. excitatory crustacean nerve terminals but found no differences in the contents of the vesicles or in other components of the junctions Richardson (27, 28) showed that the smooth muscle of the rat vas deferens, which is known to receive an adrenergic nerve supply, exhibits neuromuscular junctions marked by large numbers of dense-cored

vesicles in the nerve endings, whereas in the iris, in contrast, which receives an almost purely cholinergic innervation, the nerve endings contain primarily clear vesicles. Other details of these junctions, i.e. gap width and postjunctional specialization, are, however, not noticeably different In *Mytilus* muscle (ABRM) two populations of nerve endings can be distinguished by their vesicle content but consistent differences in gap width and postjunctional membrane specialization are absent (20) In contrast to all of these, the earthworm junctions can be resolved into two types not only by differences in the size and content of the synaptic vesicles, but also by parallel differences in gap width and postjunctional specialization.

Type I Junctions

Except for the lack of junctional folds, junctions of this type in the earthworm are similar to those of vertebrate skeletal muscle Not only are the vesicles of approximately the same size and shape, but also the junctional gap is relatively wide (6, 24). Among invertebrates a comparable gap has been seen also at *Ascaris* neuromuscular junctions (30) Since the presently available evidence indicates that acetylcholine is the excitatory transmitter in the earthworm longitudinal muscle (14), and since the type I junctions resemble vertebrate skeletal myoneural junctions morphologically, it is most probable that type I junctions are sites of cholinergic transmission

The most intriguing component of these junctions is the sarcolemmal specialization consisting of regularly disposed ~70 A granules and associated projections The concentration of these granules in the membrane is approximately 5×10^3 per μ^2 based on the assumption that they are in hexagonal array and are spaced at intervals of ~160 A This figure is compatable with the estimates, which are in the range of 10^3–10^5, of the number of receptor sites per μ^2 in postjunctional membranes elsewhere which are known to be cholinergic (12, 17, 21) The fact that the granules reported here appear to constitute part of the outer dense lamina of the plasma membrane and may encroach on the middle lucent lamina as well is also consistent with the postulated location of receptor molecules (10, 11) The granules are also similar in size to the protomers of acetylcholinesterase (AChE) extracted from *Electrophorus* electric organs (23) and correspond in concentration to the estimate of 3.2×10^3 AChE sites per μ^2 at a vertebrate myoneural junction (34) Thus, the possibility that the hexagonally disposed postjunctional elements indicate the site of receptor protein, or acetylcholinesterase, or both together (cf 7) is consistent with previous data concerning the dimensions and concentration of these constituents in other postjunctional membranes Alternatively, the sarcolemmal specializations might represent a structural framework within which the functionally important molecules are situated

Type II Junctions

Junctions of this type resemble those seen in other invertebrate muscles, e.g. the ABRM (20), and also in the smooth muscles of vertebrates (27, 28). Since membrane specialization is minimal, the basis for the identification of the junctions as such is primarily the close proximity of the nerve and muscle processes in these areas in contrast to other areas where they are separated not only by wide spaces, but also by supporting cells, collagen fibrils, and basement membranes

If, as discussed above, the periodic postjunctional membrane specialization at type I junctions represents the location of receptor and/or acetylcholinesterase molecules, the virtual absence of such a specialization at the type II junctions may indicate that receptors and degradative enzymes are not heavily concentrated here The action of the transmitter therefore may not be localized to the junctional region, and elimination of the transmitter may depend more on reuptake than on degradation It is well established that rebinding is a significant mechanism for inactivation of catecholamine transmitters (5). Earthworm type II junctions are probably catecholaminergic in view of the abundance of dense-cored vesicles in the axon terminals together with fluorescence-histochemical data which have demonstrated the presence of catecholamine-containing nerve fibers traveling in the radial septa of the earthworm body wall (19).

The virtual absence of a postjunctional membrane specialization at the type II junctions raises a question about the need for visible membrane specialization in identifying synapses elsewhere. Such specialization may be inconspicuous in general at synapses which employ primarily a reuptake rather than a degradative mechanism for transmitter inactivation.

In the type II as in the type I nerve endings some of the apparent vesicle inhomogeneity may arise from the presence of secretory granules containing "trophic materials," or of organelles concerned with the metabolism or maintenance of the endings, or of vesicles containing deposits of substances taken up from the junctional cleft by pinocytosis, a process known to occur in both nerve cell bodies (33) and nerve endings (16). In addition, if a catecholamine is indeed present in these nerve endings, some of the vesicles may be concerned with its storage and others with its release

Physiological Implications

Previous physiological studies of earthworm muscle indicate not only that the same muscle cell may exhibit both excitatory and inhibitory junctional potentials (14), but also that something comparable to a "catch" state may be seen under some circumstances (15) Thus, different neural influences probably govern separate, nonparallel functions in the earthworm, which, like other invertebrates (36), probably relies on peripheral mechanisms in the control of its body muscle to a greater extent than the more highly cephalized vertebrates do in the control of their skeletal muscles.

Exactly how complex these peripheral control mechanisms are in the earthworm is not clear. The simplest assumption to make, based on the morphological findings, is that the type I and type II junctions represent excitatory cholinergic junctions and inhibitory catecholaminergic junctions, respectively. The possibility that the system is more intricate than this cannot be excluded, however. Each type of junction could include several subgroups that make use of different transmitters. In addition, the close apposition of the different kinds of nerve fibers in the prejunctional nerve bundles and the presence of occasional profiles compatable with exocytosis in these regions, open up the possibility that mechanisms equivalent to presynaptic inhibition or facilitation also operate in this system.

Conclusion

Nerve-muscle junctions have been used as simple models for understanding the vastly more complex synaptic interactions of the central nervous system. However, the very simplicity of vertebrate skeletal myoneural junctions limits the extent of their usefulness for that purpose Invertebrate neuromuscular transmission systems such as that of earthworm have enough additional diversity to illuminate somewhat more complex synaptic systems without being as unwieldy as the central nervous system itself. In addition some of the minute details of the earthworm junctional specializations may prove to be common to synaptic and myoneural junctions in a variety of animals

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