

## MOTOR NERVE ENDINGS OF TWITCH MUSCLE FIBERS IN *HIPPOCAMPUS HUDSONIUS*

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### INTRODUCTION

The structure of the motor end plate of striated skeletal muscle has been studied in considerable detail by light and electron microscopy (1-3). However, it has been only recently that electron microscope studies have shown that the end plates on twitch and slow tonic striated skeletal fibers have a distinctive structural organization which may serve to distinguish the two types of muscle fibers. In brief, Hess (4-6) and Hess and Pilar (7) have shown that twitch muscle fibers possess one or more rather circumscribed nerve endings which can be readily recognized by their highly folded postsynaptic membrane (sarcolemma). This is the "en plaque" type of motor nerve ending. On the other hand, Kuffler and Vaughan-Williams (8, 9), Hess (4-6), Hess and Pilar (7), Pilar and Hess (10), Page (11), and others have shown that slow tonic muscle fibers have multiple motor nerve endings which are strung out along the length of the muscle fiber. The postsynaptic membrane or sarcolemma does not exhibit the extensive folding found in the twitch fiber. This type of nerve ending has been designated as the "en grappe" type.

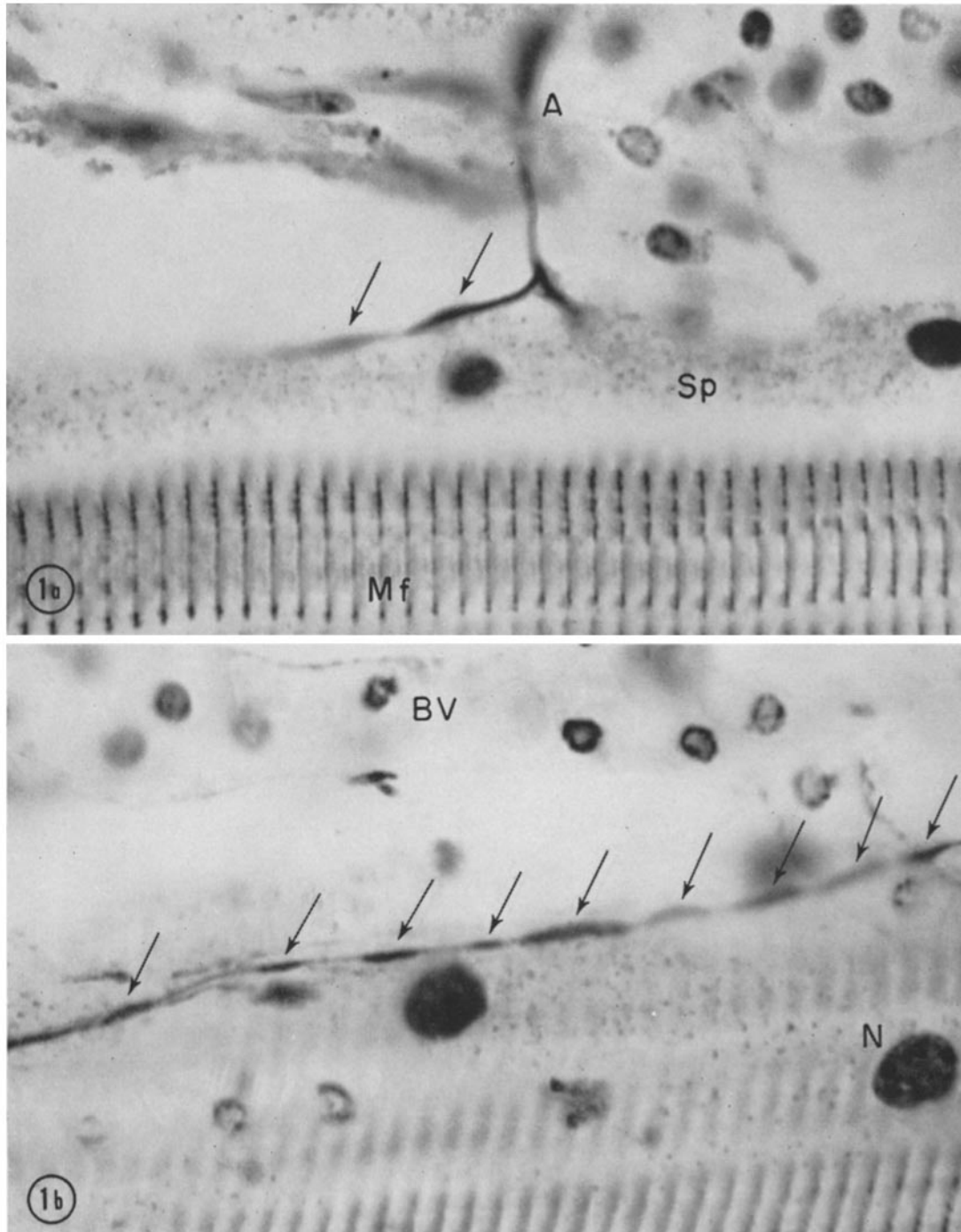
Although the work cited above illustrates the neuromuscular pattern found in many species, several variations have been reported. For examples, Gray (12) has demonstrated that the motor end plates of twitch fibers may have a more extensive distribution than the multiple endings associated with the slow tonic fibers in the same muscle. Nakajima (13) has shown in a study of snake fish red and white muscles that the motor nerve endings are similar to the endings typically found on slow tonic muscle fibers. Similar findings

were reported by Nishihara (14) for carp red and white muscles. In addition, Reger (15) has found motor nerve endings of the same type in the extra-ocular muscle fibers of the killifish, *Fundulus*. In an abstract, Hess (16) has also reported that the striated muscle of the avian iris has an internal structure similar to that of twitch muscle fibers, but the motor nerve endings are similar to those found on slow tonic muscle fibers (the *en grappe* type). As yet, no functional studies have been reported to determine the physiological properties of either the muscle fibers or the motor nerve endings.

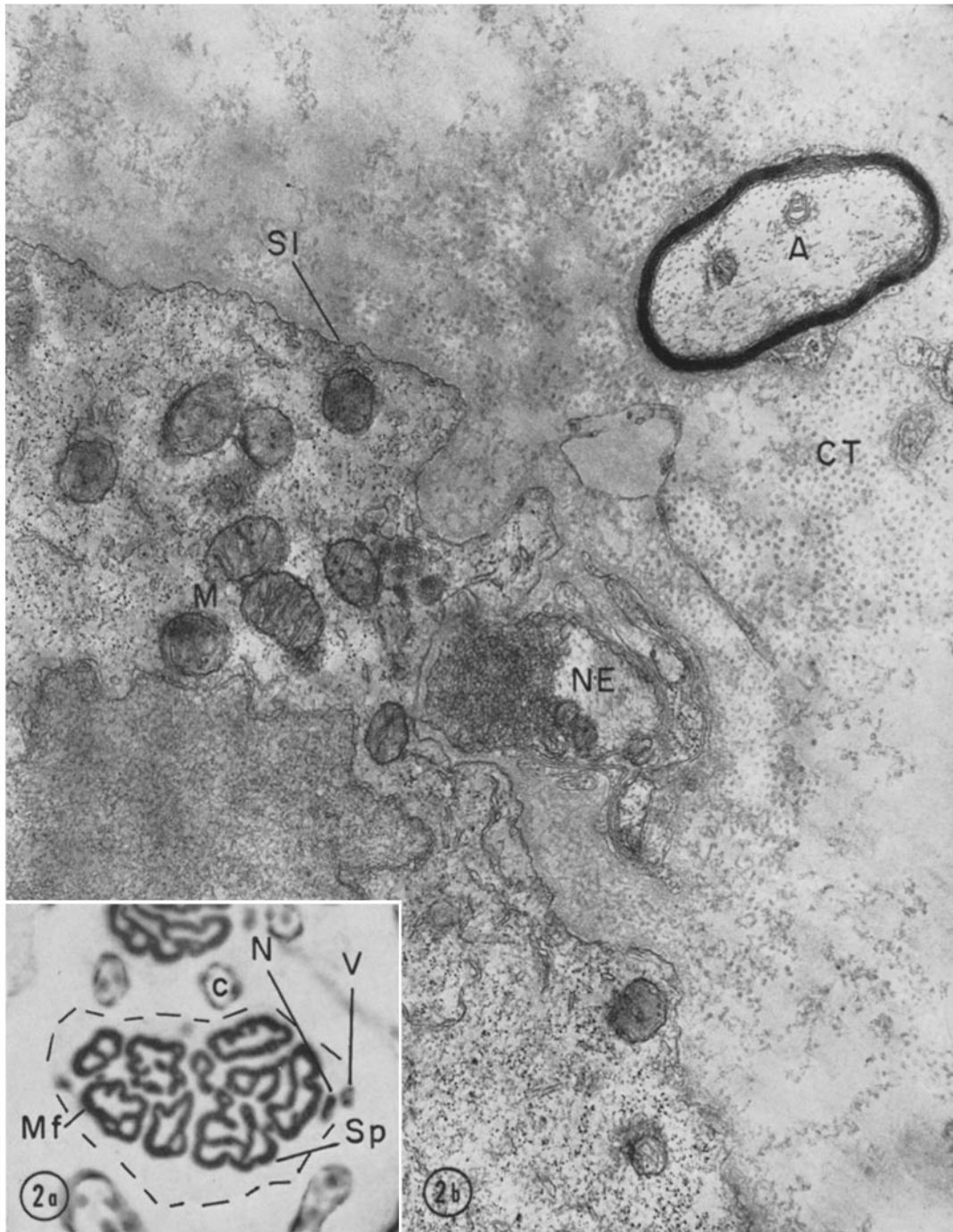
Recent investigations of the structure of the twitch muscle fibers which drive the dorsal fin of *Hippocampus* also suggested that the motor nerve endings are of the *en grappe* type and are strung out along the muscle fiber and lack any infoldings of the postsynaptic muscle membrane characteristic of other twitch muscles (17-19). That these muscle fibers are twitch fibers has been verified by their mechanical properties and fine structure (19, 20). In addition, the muscle fibers of which the dorsal fin musculature is composed appear to be structurally homogeneous and vary primarily in fiber diameter.

### MATERIALS AND METHODS

Five sea horses (*Hippocampus hudsonius*) were anesthetized with 0.1% tricaine methanesulfonate (MS222, Sandoz), were dissected, and the dorsal fin muscle fibers were fixed in aceto-formalin (1-10%) in artificial sea water for light microscopy and in 1% osmium tetroxide in artificial sea water for electron microscopy. The tissues were dehydrated in graded alcohols and embedded in paraffin and Epon 812 for light microscopy and in Epon 812 for electron micros-



FIGURES 1 *a* and *b* Light micrographs of a longitudinal section of a muscle fiber demonstrating a branched axon (Fig. 1 *a*) and the distribution of multiple nerve endings (arrows) along the fiber surface (Fig. 1 *b*). The size of the endings corresponds to those found by electron microscopy as illustrated in Figs. 2 *b*, 3, and 4. *A*, axon; *BV*, blood vessel; *MF*, muscle fiber; *N*, nucleus; *Sp*, sarcoplasm. Bodian's protargol, 10  $\mu$  section.  $\times 2667$ .



FIGURES 2 *a* and *b* The insert (2 *a*) is a light micrograph of a typical muscle fiber of the dorsal fin musculature of *Hippocampus hudsonius*. The fiber is circumscribed by a broken line. The dark myofibrils (*Mf*) are irregularly patterned in a voluminous sarcoplasm (*Sp*). The nucleus (*N*) and motor nerve endings (*NE*) are found in close apposition; this same relationship can also be seen in the larger electron micrograph (Fig. 2 *b*). The electron micrograph illustrates details of the nerve ending which resides in a shallow depression of the muscle fiber. The ending is rich in synaptic vesicles (*V*) and occasional mitochondria (*M*). The postsynaptic membrane is without the deep infoldings characteristic of other twitch muscle fibers. *A*, axon; *c*, capillary; *CT*, connective tissue; *Sl*, sarcolemma. Fig. 2 *a*,  $\times 950$ ; Fig. 2 *b*,  $\times 20,000$ .

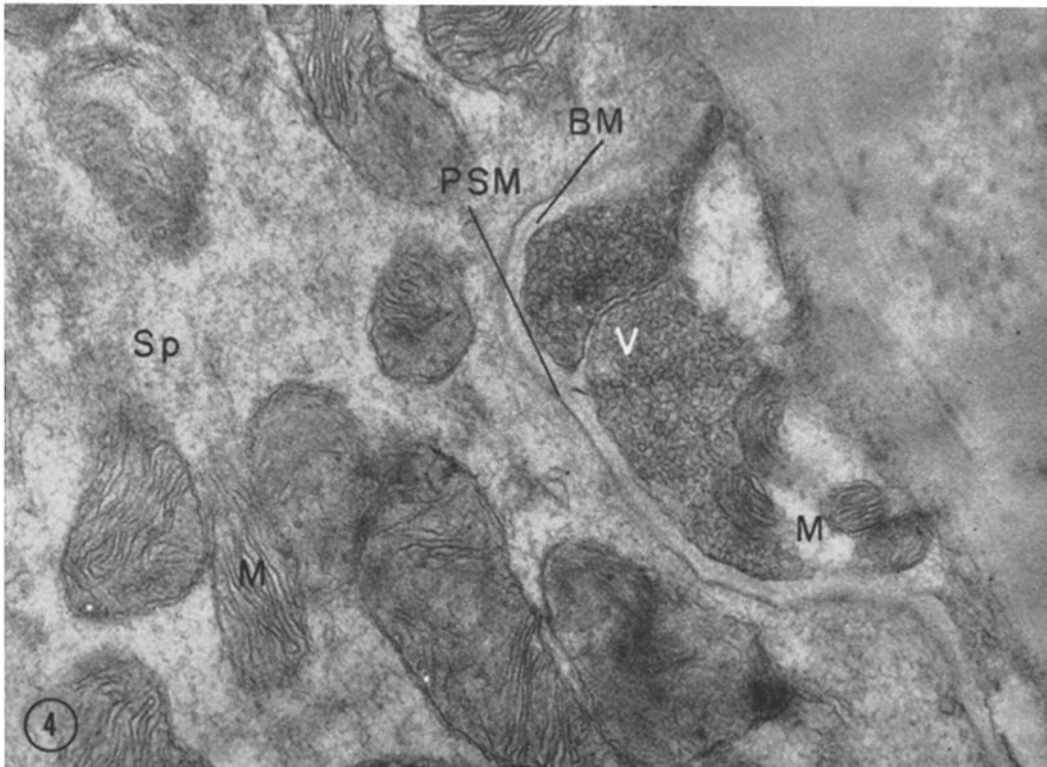
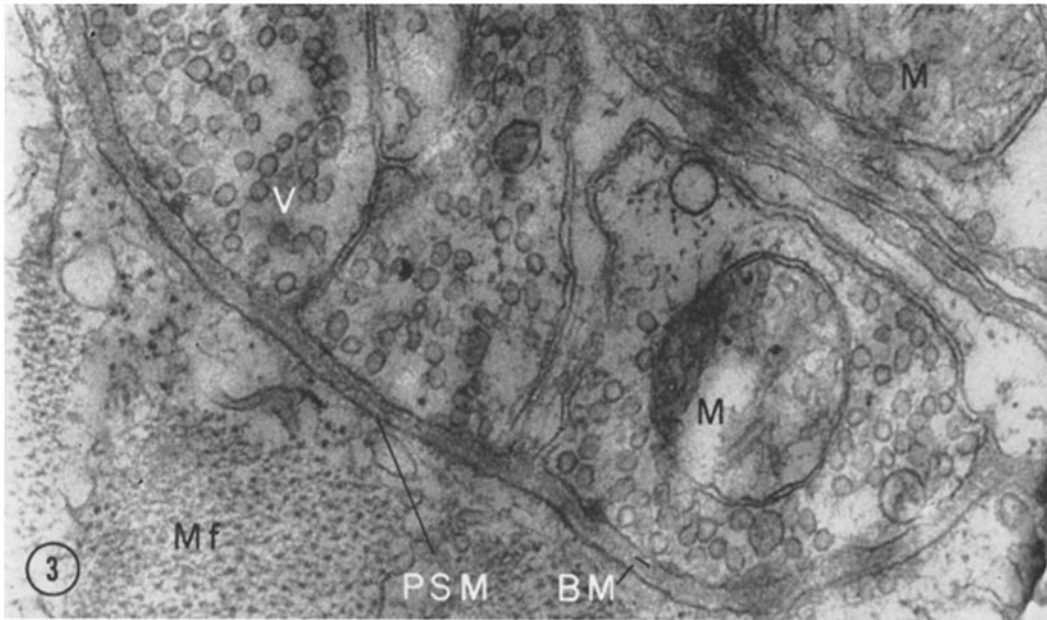


FIGURE 3 Similar to Fig. 2 *b*, but illustrating in addition the rare juxtaposition of a nerve-muscle junction to the myofibrils shown in cross-section. The nerve ending is seen as three separate elements of a branching nerve fiber. Unlike those in the nerve endings in Figs. 2 *b* and 4, the synaptic vesicles are not tightly packed. *M*, mitochondria; *Mf*, myofibril; *V*, synaptic vesicles; smooth postsynaptic membrane (*PSM*) and basement membrane (*BM*) are evident.  $\times 70,000$ .

FIGURE 4 The basement membrane (*BM*) is clearly seen in the 750 Å space separating the pre- and postsynaptic membranes. *M*, mitochondria; *PSM*, postsynaptic membrane; *Sp*, sarcoplasm; *V*, synaptic vesicles.  $\times 21,000$ .

copy. For light microscopy, sections both 2 and 10  $\mu$  thick were stained with Ehrlich's hematoxylin and eosin and with Bodian's protargol. In addition, 1  $\mu$  plastic sections were stained with toluidine blue. For electron microscopy, thin sections were obtained (LKB ultratome), mounted on copper screens, and stained with lead for study (RCA EMU 3 G).

#### OBSERVATIONS

The muscle fibers which drive the dorsal fin are, for the most part, 50–90  $\mu$  in diameter and 4.5 to 6 mm in length. These fibers which are "red" in the unstained condition (as compared with the bulk of the muscle in other parts of the animal) have an extremely high sarcoplasm-to-myofibril ratio. In cross-section, the rectangular myofibrils are irregularly patterned so that they appear as ribbon-like coils in a voluminous sarcoplasmic mass. By light microscopy and employing Bodian's protargol, it was determined that motor nerve fibers branch near the midpoint of the muscle fibers. From these branches multiple motor nerve endings are found. Although the exact number of endings per muscle fiber has not been ascertained, nine endings have been found in a "string" over a distance of 60  $\mu$ , whereupon the nerve left the plane of the 10  $\mu$  section. Almost without exception, in each muscle fiber cross-section prepared for light and electron microscope study the presence of a motor nerve ending can be demonstrated. By electron microscopy several hundred sections were examined from 20 blocks and five animals. The nerve endings are located in a shallow depression in the long axis of the muscle fiber and are approximately 0.75–1.5  $\mu$  in diameter. The postsynaptic membrane is smooth and without infolding or other specializations. The nerve terminal is filled with synaptic vesicles and occasional mitochondria. The pre- and postsynaptic membranes are separated by a basement membrane and a 750 Å space.

The motor endings are most frequently found juxtaposed to muscle fiber nuclei.

The results are illustrated in Figs. 1–4.

#### DISCUSSION

In contrast to the observations of Couteaux (18) on other species of *Hippocampus*, the motor nerve endings of the dorsal fin musculature of *Hippocampus hudsonius* may be found in close association with muscle fiber nuclei. Couteaux has reported that, in fish, the existence of muscle nuclei directly associated with the neuromuscular junction seems

doubtful or even improbable. Thus, the apposition of muscle nuclei to the motor end plates found in the present study may represent a variation of the usual neuromuscular pattern in fish.

In addition, evidence is presented that twitch muscle fibers may possess nerve endings which are structurally similar to the type of endings which heretofore were found on slow tonic muscle fibers in amphibians, reptiles, and mammals. The observations of Hess (16) would also suggest that these observations may also be extended to birds. Hence no rigid rule may apply and both structural and functional criteria must be employed to determine the nature of a specific neuromuscular system. Further, in fish, on the basis of the few existing studies cited in the Introduction, it would appear that both twitch and slow tonic muscle fibers receive motor nerve endings structurally similar to the endings on slow tonic muscle fibers in other species cited above. Both Nakajima (13) and Nishihara (14) reported that in the specific fish muscles studied by them the red muscle fibers were slow tonic muscle fibers from which a conducted action potential could not be elicited, whereas the white fibers were of the twitch variety from which muscle action potentials were demonstrable. In all instances, the motor nerve endings appeared structurally similar and of the type reported in this and other papers. Nishihara also noted that, based on the physiological evidence, the multiple motor nerve endings located on the white (twitch) muscle fibers were monofocused or more circumscribed than those on the red (slow) muscle fibers. In the present study, the red muscle fibers of the dorsal fin musculature are twitch fibers and widely distributed (18–20). Although direct physiological data are not available for the dorsal fin musculature of *Hippocampus*, it is useful to consider the possible role of multiple motor nerve endings and their extensive distribution on this twitch muscle system. Kuffler and Vaughan-Williams (9) noted that "in a twitch fibre a propagated wave of activation spreads rapidly in both directions from the neuromuscular junction so that the whole fibre contracts within a few milliseconds. . . . In slow fibres propagation is absent, yet the whole fibre contracts almost simultaneously because its different parts are individually stimulated by the numerous neuromuscular junctions distributed over the whole fibre length." Katz and Kuffler (21) have reported that the conduction of a muscle

action potential to fully activate a whole muscle fiber may take as much as 5 msec at 25°C in the frog sartorius muscle. It is possible, therefore, that, in fish, and more specifically the dorsal fin musculature of *Hippocampus*, the presence of multiple nerve endings on twitch muscle fibers may serve to reduce the activation time and increase the rate at which tension is exerted by the whole muscle fiber (21). This would be analogous to the use of massive electrodes to activate simultaneously a whole muscle fiber as commonly employed in the in vitro study of the mechanical properties of twitch muscle fibers.

The dorsal fin is the primary means of propulsion for this unusual teleost which swims in a vertical position. The fin is driven in an undulatory manner as each group of muscle fibers is sequentially activated in a twitch response. Because of the large size and mass of the animal compared to its means of propulsion, the animal is capable of only slow movements. Behavioral observations suggest that regulation of these movements is determined by the duration of the burst of muscular activity of the whole organ.

The neuromuscular arrangement described for the sea horse dorsal fin is perhaps the most efficient mechanism for this animal. The type of motor nerve ending for any particular muscle fiber or animal may be adapted to the specific needs of the organism and thus may be quite variable both structurally and functionally.

Although the discussion in this report has been focused on the neuromuscular relationship found in striated skeletal muscle, it is of interest to note that the motor nerve endings found in cardiac and smooth muscle are similar in several respects to those reported here (22-25).

#### SUMMARY

The motor nerve endings of the twitch muscle fibers of *Hippocampus hudsonius* have been studied by light and electron microscopy. The postsynaptic membrane is without the folds or invaginations which are characteristic of the sole plate of other twitch muscle fibers. The postsynaptic membrane is, however, similar to that found heretofore on many slow tonic muscle fibers in other species. The nerve endings are found in a shallow depression in the long axis of the muscle fiber and are approximately 0.75-1.5  $\mu$  in diameter. The pre- and postsynaptic membranes are separated by a 750 Å space within which a basement

membrane is found. The physiological significance of the extensive distribution of *en grappe* type motor nerve endings found in the *Hippocampus* dorsal fin musculature is discussed. In this situation, the presence of multiple nerve endings appears to be the morphological basis for the observed mechanical activity of the dorsal fin musculature which is the sole means of propulsion of this unusual teleost which swims slowly in a vertical position.

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