UNIQUE ARRANGEMENT OF GLIAL MEMBRANES BETWEEN ADJACENT NEUROMUSCULAR SYNAPSES IN A SPIDER MUSCLE

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INTRODUCTION

During the course of comparative studies of the physiology and fine structure of neuromuscular systems in chelicerate arthropods, certain ultrastructural details of the synaptic regions in a spider leg muscle were noted that have not been reported previously for any muscle. The motor innervation of this muscle was found to be typical for arthropods in general, but in addition there were multiple layers of glial cell processes situated between adjacent neuromuscular synapses.

This report describes first some of the details of the innervation of the spider muscle under study and then the unique arrangement of glial membranes between the multiple neuromuscular synapses.

MATERIALS AND METHODS

Adult specimens of the tarantula spider *Eurypelma marxi* Simon were purchased from Southern Biological Supply Co., McKenzie, Tenn. Methods for maintaining the animals in the laboratory, a description of the claw elevator muscle, and the procedures employed in preparing the muscle for electron microscopy are given in detail elsewhere (Sherman and Luff, 1971). Muscle fibers were fixed in cacodylate-buffered 4% glutaraldehyde and 1% osmium tetroxide, stained with ethanolic uranyl acetate and lead citrate, and embedded in Spurr's embedding medium.

The number of motoneurons innervating the claw levator muscle was determined by recording with a Grass FT.03 transducer (Grass Instrument Co., Quincy, Mass.), the increments in whole muscle tension elicited upon external electrical stimulation of the peripheral leg nerves. The innervation of individual fibers was investigated with conventional microelectrode recording techniques, using methods similar to those described by Rathmayer (1965).

RESULTS

Muscle fibers to be examined for details of synaptic ultrastructure were analyzed first for the number of nerve inputs. Fibers studied were located at the surface regions of the muscle. Of a total of 30 fibers from four different preparations, all appeared to be innervated solely by a slow motoneuron, even though whole muscle tension recordings indicated that both a fast and a slow motor axon supply the muscle (Fig. 1). Only a single kind of excitatory postsynaptic potential (EPSP) in regard to amplitude and duration could be recorded even when the stimulus strength exceeded the thresholds for both motor axons (Fig. 2). This EPSP was evoked simultaneously with the slow, graded contractions, and several EPSP's in rapid succession were required to initiate a contraction, indicating that the EPSP's arose from slow motor axon activity (Fig. 3). No decrements in whole muscle tension and no inhibitory postsynaptic potentials were seen at stimulus strengths up to 100 V and 0.1 ms duration and stimulation frequencies up to 50 Hz. Consequently, no evidence for the presence of inhibitory innervation was obtained in these experiments.

The electron micrograph in Fig. 4 shows one of the main branches of the slow motor axon as it extends across two different muscle fibers. A small branch arising from the main branch also can be seen between the two fibers. The axon forms synaptic connections at numerous discrete sites along the surface of the muscle fibers. This multiterminal pattern of synapse formation is characteristic of arthropods (Atwood, Smyth, and Johnston, 1969; Edwards, 1959; Sherman and Atwood, 1972; Sherman and Fourtner, 1972). At each synapse, the plasma membranes of the axon and muscle fiber are separated by a cleft of about 300 Å. The synapses occur at points where the sarcoplasm projects outward from the contractile regions of the fiber. The sarcoplasm at these sites has a granular appearance and is devoid of myofilaments. The most prominent synaptic vesicles are of the spherical, agranular type with a diameter of about 400 Å. They tend to be clustered near the presynaptic membrane, although this feature is not particularly evident in the electron micrographs included in this report. Larger (ca. 1,000 Å) dense-cored vesicles are also present in the presynaptic element.

The most notable feature of the ultrastructure of the claw levator neuromuscular junctions is the occurrence of multiple glial processes interposed

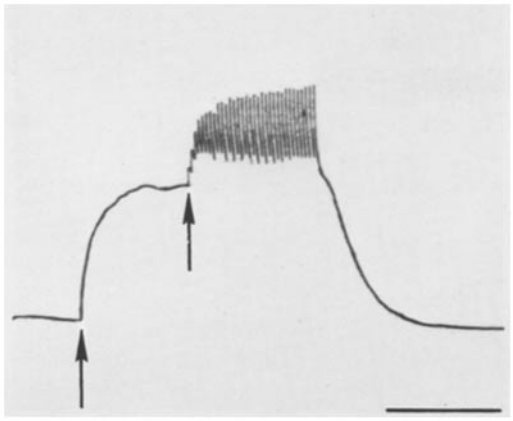


FIGURE 1 Whole muscle tension recording made while gradually increasing the strength of stimulation of the motor nerves. Two distinct tension increments were seen (arrows). A slow, graded contraction always occurred at the lowest effective stimulus strength. Upon increasing the stimulus strength by a factor of 10, fast or twitch contractions were elicited (second arrow). Stimulation frequency: 3 Hz. Scale: 10 s.

FIGURE 2 Typical excitatory postsynaptic potential (EPSP) recorded from the levator muscle fibers sampled upon stimulation of the motor nerves at the lowest effective stimulus strength. Vertical scale: 5 mV. Horizontal scale: 10 ms.

between the adjacent synapses (Figs. 4 and 5). These structures are identified as glial cell extensions primarily because they are: (*a*) distinct from the plasma membranes of the axon and muscle fiber, (*b*) situated externally to both the axon and muscle fiber, and (*c*) absent from the synaptic clefts.

The organization of the glial processes imparts a ladder-like appearance to the nonsynaptic regions beneath the axon. The plasma membrane of the muscle fiber invaginates along the margins of the "ladders" to accommodate the glial processes (Fig. 5). These invaginations often continue inward between the myofibrils to form part of the transverse tubular system. At a number of discrete locations, the glial processes form gap junctions with the muscle fiber (Figs. 4 and 5). Here, the distance between the plasma membranes of the glial and muscle cells narrows to about 150 Å. The gap between the membranes is filled with an electron-dense material (Fig. 5).

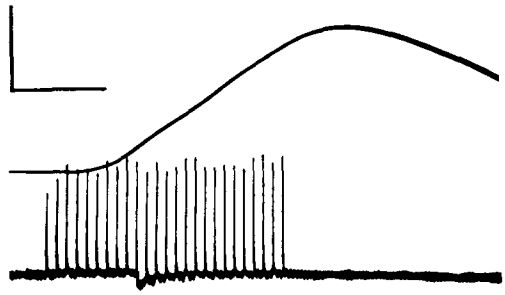


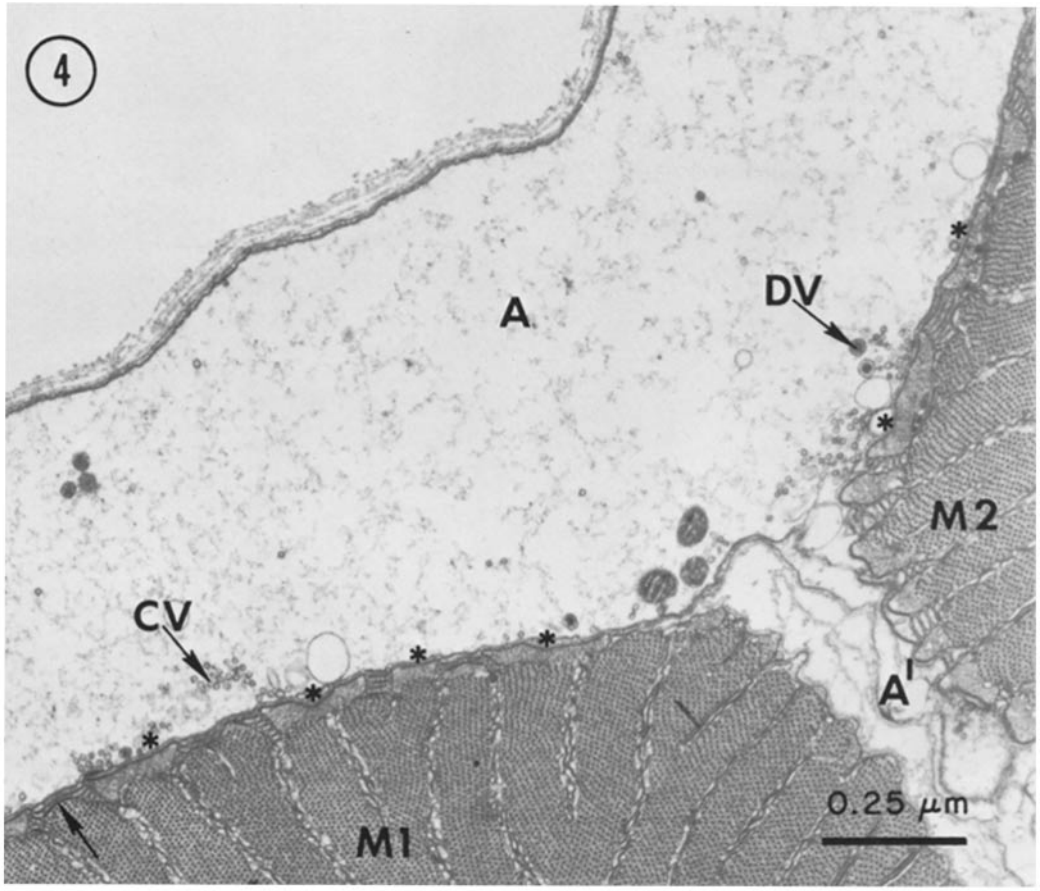
FIGURE 3 Simultaneous recording of whole muscle tension (upper trace) and excitatory postsynaptic potentials (EPSP's) of a single muscle fiber (lower trace) at the lowest effective stimulus strength. A slow, graded contraction follows several successive EPSP's. Stimulation frequency: 10 Hz. Vertical scale (lower trace): 5 mV. Horizontal scale (both traces): 1 s.

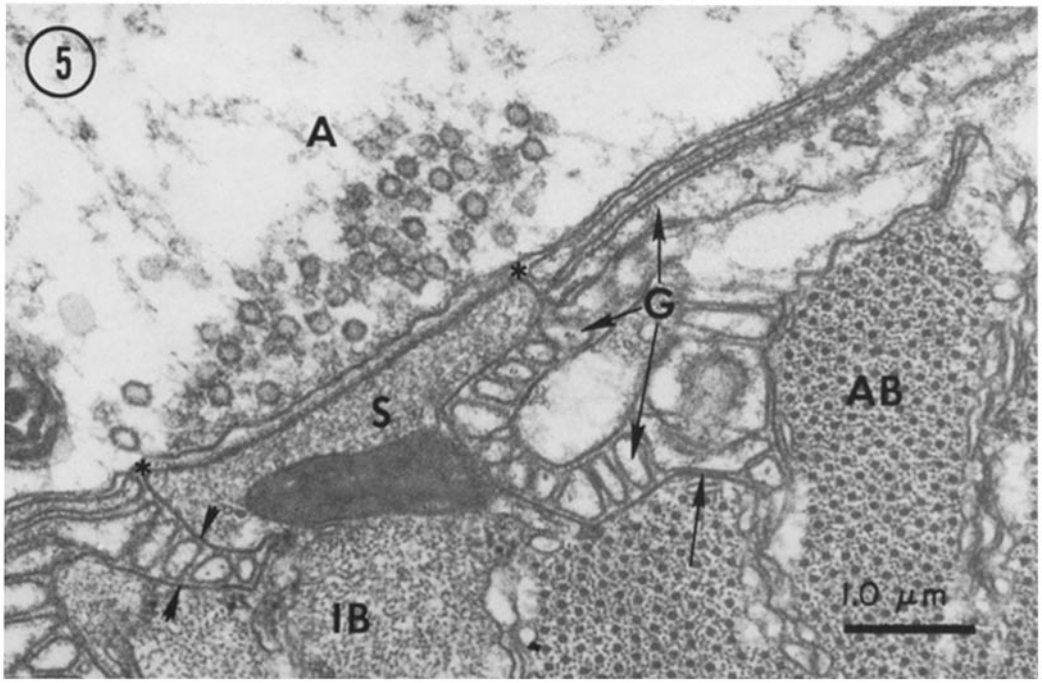
DISCUSSION

Differentiation of the motor innervation of the tarantula claw levator muscle into fast and slow neurons is comparable to the situation present in many insect and crustacean muscles (Atwood, 1967; Usherwood, 1967). In these animals, the slow neurons are tonically active and are thought to be responsible for controlling the postural activities of the muscles. The fast neurons are only phasically active and are involved in initiating quick movements of the appendages, such as those in escape or attack reactions.

Though the claw levator muscle and certain other arachnid muscles appear to lack inhibitory innervation (Gilai and Parnas, 1970; Rathmayer, 1965), peripheral inhibition is commonplace in insect and crustacean muscles (Atwood, 1968; Pearson, 1973). Recently, the first evidence of inhibitory innervation of an arachnid muscle was reported by Brenner (1972). Whether or not peripheral inhibition in arachnids is the exception or the rule remains to be assessed.

The fine structural features of neuromuscular junctions in the claw levator muscle are very similar to those of junctions described in insect and scorpion skeletal muscles (Atwood et al., 1969; Edwards, 1959; Faeder and Salpeter, 1970 *a*; Rees and Usherwood, 1972; Smith, 1971). However, the striking occurrence of the extensive ladder-like arrangement of glial processes interposed between adjacent synapses appears at present to be unique to the claw levator muscle. This feature was not reported in studies of other spider muscles (Kawaguti and Kamishima, 1969; Melamed and Tru-





jillo-Cenoz, 1971; Zebe and Rathmayer, 1968) or of muscles in general. The functional significance of the ladders of ensheathing elements around each synapse remains to be determined. Perhaps they serve to insulate the synaptic sites by obliterating the extracellular spaces nearby. Another possibility is that they may aid in anchoring the axon branches to the muscle fibers. Finally, the glial ladders may be intimately involved in the metabolism of the neurotransmitter chemical (cf. Faeder and Salpeter, 1970 b).

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FIGURE 4 Electron micrograph of neuromuscular junctions formed by a motor axon branch and two muscle fibers in the claw levator muscle. The main axon branch (*A*) gives off a smaller branch (A^1) between two muscle fibers (*M1, M2*). Numerous discrete neuromuscular synapses (asterisks) are formed along the surface of the muscle fibers. Glial cell extensions form a ladder-like complex between adjacent synapses. Both dense- and clear-cored vesicles (*DV* and *CV*, respectively) occur in the presynaptic element. A glial-muscle fiber gap junction, consisting of a close apposition of glial and muscle fiber plasma membranes separated by electron-dense material, is designated by the unlabeled arrow. This feature is shown at higher magnification in Fig. 5. Scale: 1 μm $\times$ 19,000.

FIGURE 5 Enlarged view of neuromuscular junctional area in the claw levator muscle. The axon (*A*) is surrounded by glial elements (*G*) except at the synapse (asterisks). The sarcoplasm (*S*) beneath the synapse has a granular appearance and lacks myofilaments. The plasma membrane of the muscle fiber (arrow heads) invaginates to accommodate the glial ladders. A gap junction formed by glial and muscle fiber membranes is designated by the unlabeled arrow. I band and A band contractile regions of the muscle fiber are denoted *IB* and *AB*, respectively. Scale: 0.25 μm $\times$ 68,000.

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