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CHELICERATE NEUROMUSCULAR SYSTEMS: THE DISTAL FLEXOR OF THE MERO-CARPOPODITE OF *LIMULUS POLYPHEMUS* (L.)

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Abstract—1. Each of the three groups of the distal part of the mero-carpopodite flexor in *Limulus* is innervated by at least eleven excitatory axons.

2. A single muscle fiber may be innervated by as many as six excitatory axons.

3. No direct inhibitory innervation to these muscle groups was observed.

4. The muscle fibers appear to be quite similar in their physiological response to indirect stimulation.

5. It is suggested that the neuromuscular system in *Limulus* is rather a non-specialized system when compared to its counterpart in other arthropods.

INTRODUCTION

AMONG the arthropods the neuromuscular junction has been extensively studied in the appendages of only a few mandibulates, more specifically the decapod crustaceans (Atwood, 1967) and the orthopteran insects (Hoyle, 1965; Usherwood, 1969). Thorough studies on the chelicerates are almost totally lacking.

In the mandibulates at least three types of axons—fast, slow and inhibitory—innervate the appendage musculature. The fast axon produces rapid contractions and there is little or no facilitation of either tension development or of junction potentials; the slow axon produces a slow contraction and there is a large facilitation in both the tension developed and the junction potentials. Stimulation of the inhibitory axon alone produces no visible change in tension but does produce inhibitory postsynaptic potentials (Wiersma, 1961). In addition to the different types of motor axons innervating the muscles, the muscles fall into several categories on the basis of their facilitatory ability and their structure (Atwood, 1963; Dorai Raj, 1964). Thus in the mandibulates, though the total number of axons innervating a particular muscle is limited, the complexity in nerve fiber types as well as muscle fiber types allows extremely fine and well co-ordinated movements.

Whether this complexity in nerve fiber types and muscle fiber types is also present in the chelicerates is at present unclear. Fast and slow axons (but no

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inhibitory axons) innervate the appendage musculature of the tarantula, *Dugesiella hentzi* (Rathmayer, 1965, 1967) but all the muscle fibers appear uniform (Zebe & Rathmayer, 1968). In the scorpion, *Leiurus quinquestriatus*, there are also fast and slow axons but no inhibitory axons; the muscle fibers also appear to be of only one type (Gilai & Parnas, 1970). Hoyle (1958) reported that the flexor muscle of the dactylopodite of *Limulus polyphemus*, the horseshoe crab, is innervated by a single fast axon and a single slow axon but no inhibitory axon. Parnas *et al.* (1968), however, reported finding that this muscle is innervated by at least one inhibitory axon and by at least six excitatory axons, which cannot be categorized as fast or slow.

Because of the few studies made on chelicerate muscles, in general, and the conflicting results reported on the innervation pattern in the horseshoe crab, we have undertaken this study of the distal flexor of the mero-carpopodite of *L. polyphemus*.

Flexion of the mero-carpopodite is accomplished by a pair of associated muscles; the proximal muscle originates in the basipodite and the distal muscle originates in the ischiopodite. Both of these muscles insert on a single apodeme. The distal muscle is composed of three distinct groups (1, 2, 3) of muscle fibers, which can be studied independently of one another (Fourtner, 1971). In addition, Vachon (1945) and Ward (1969) have demonstrated that there is no extensor of the mero-carpopodite. This allows one to test the Hoyle–Smythe hypothesis (1963), that one would not expect to find inhibitory input to a muscle lacking an antagonist.

A preliminary report of this study has appeared elsewhere (Fourtner, 1970).

MATERIALS AND METHODS

Horseshoe crabs, *Limulus polyphemus*, (carapace width 18–23 cm) were obtained from the Gulf Specimen Co., Panacea, Florida. The animals were stored in an artificial seawater aquarium (Dayno Co., Model 703) and were maintained at a temperature of 13–17°C.

The third and fourth pairs of walking legs were used in these experiments. A leg was removed from the body by severing the leg just distal to the coxopodite. A plug was then inserted in the coxopodite to stop excessive bleeding.

The ventral articular membrane between the mero-carpopodite and the ischiopodite was carefully severed exposing both the internal pedal nerve (IPN) and the external pedal nerve (EPN). Each nerve was ligated with a thread and cut distal to the ligature. Two parts of the flexor of the propodite originate on the dorsal exoskeleton of the ischiopodite (Group 3 of propodite flexor). These muscles were carefully cut as near their origin as possible. The propodite and the mero-carpopodite were removed. The preparation now consisted of the basipodite and the ischiopodite.

Two cuts were made along the ventral lateral margins of the basipodite and the ventral exoskeleton was lifted and removed thus exposing the IPN and EPN. The nerves were ligated near their proximal ends and then were carefully lifted and dissected free from the surrounding tissue. The basipodite was then removed by cutting along the articular membrane joining the basipodite to the ischiopodite.

The exoskeleton of the ischiopodite was cut along its entire dorsal midline. The preparation was then placed in a paraffin tray and covered with saline. The exoskeleton was very carefully pinned back exposing the distal muscle of the mero-carpopodite flexor. The remnants of the propodite flexors were very carefully dissected away from the Group 1

and Group 2 muscles. Also the muscles (flexors and extensors of the ischiopodite) in the proximal part of the ischiopodite were carefully removed. The preparation now consisted of the exoskeleton of the ischiopodite, the three muscle groups of the distal flexor of the mero-carpopodite, the IPN and EPN and the apodeme.

The particular muscle group to be used was isolated and the IPN and EPN were carefully dissected free from the two muscle groups to be discarded. These muscles were then cut at their insertions along the apodeme, and removed from the preparation. The distal part of the apodeme was dissected free from the articular membrane, thereby leaving the apodeme attached to only the muscle group to be studied. A small hole was then drilled through the apodeme. The preparation now consisted of the muscle group to be investigated, the apodeme, the IPN and EPN and a small part of the exoskeleton of the ischiopodite from which the muscle group originated.

The IPN and EPN are surrounded by the leg blood vessel (Dumont *et al.*, 1965). In most cases the blood vessel was left intact; this provides good protection to keep the nerve from drying when stimulated. In a few cases, however, the blood vessel was removed from around the nerve bundles.

Mechanical records were obtained by means of a mechano-transducer attached to the muscle via the hole bored in the apodeme. Intracellular muscle activity was recorded via 3 M KCl glass microelectrodes (resistances of 3–20 MΩ) and amplified by a W.P. Instruments M4A electrometer.

Stimulation of the nerves was accomplished by lifting them onto a pair of platinum hook electrodes. Unless otherwise noted, all stimuli were single with at least 15 sec allowed between each stimulation. Durations used were between 0·05 and 0·10 msec, strengths between 0·5 and 15 V.

The physiological saline used was a 10 per cent *Limulus* blood solution; nine parts Millecchia (Millecchia & Mauro, 1969) saline (424 mM NaCl, 9 mM KCl, 20 mM $CaCl_2$, 20 mM $MgCl_2$ and 25 mM $MgSO_4$) to one part *Limulus* blood. The blood was collected, allowed to clot, filtered and stored at 0°C. Before use the saline was refiltered and the filtrate was added to the saline. The pH of this final solution was always between 7·0 and 7·2.

Using the 10 per cent blood solution a mechanical response to a single stimulus could be recorded for at least 5 hr. Whereas, when using either artificial sea water or Millecchia saline, the response would deteriorate and become very weak after 3 hr of experimentation.

RESULTS

Mechanical recordings

Single stimulus response. Single stimuli applied to the internal pedal nerve (IPN) produce a twitch in each of the three muscle groups. The amplitude of the twitch is dependent on the stimulus strength (Fig. 1). As the stimulus strength was gradually raised above just threshold value, a series of discrete increases in twitch amplitude could be seen. These discrete increases are presumed to indicate recruitment of additional axons. On this basis it appears that there may be as many as eleven axons innervating each of the three muscle groups (Table 1).

This single stimulus response is not easily fatigued. Even following repetitive stimulation (50 Hz) for 1 min the single stimulus response was not notably altered.

At no time in any of these muscle preparations was there recorded a decrease in the tension developed as the stimulus strength was increased.

Single stimuli applied to the external pedal nerve (EPN) never evoked a recordable response in any of the three muscle groups, nor did stimulation of the EPN

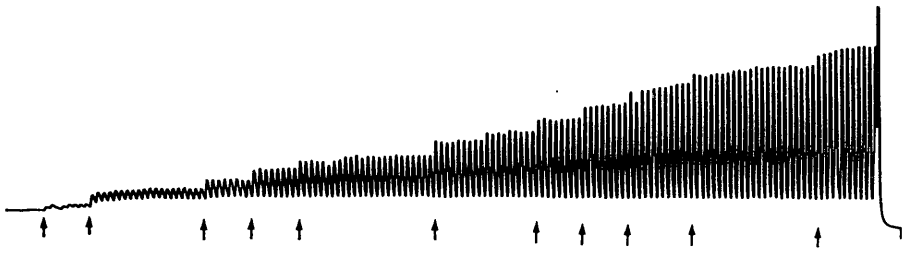

FIG. 1. Recording of mechanical activity showing threshold dependent increases in tension. The arrows indicate the threshold dependent increases in tension observed as the stimulus strength was gradually increased. The frequency of stimulation is 0·3 Hz.

TABLE 1—MECHANICAL RESPONSES OF MUSCLE GROUPS OF MERO-CARPOPODITE FLEXOR

	Group 1	Group 2	Group 3
No. of thresholds	9	7	8
(range)	(7–11)	(3–11)	(3–11)
Twitch tension (g)	4·7 ± 2·3 S.D.	1·9 ± 1·4 S.D.	3·0 ± 2·6 S.D.
(range)	(1·4–8·0)	(0·3–4·7)	(0·5–6·5)
Tetanic tension (g)	315 ± 88 S.D.	205 ± 79 S.D.	191 ± 87 S.D.
(range)	(108–440)	(87–339)	(89–351)
Twitch response			
67% of rise (sec)	0·24 ± 0·01 S.E.	0·29 ± 0·04 S.E.	0·23 ± 0·02 S.E.
67% of decay (sec)	1·11 ± 0·08 S.E.	2·28 ± 0·50 S.E.	2·18 ± 0·25 S.E.
Duration			
(to 67% of decay) (sec)	1·66 ± 0·09 S.E.	3·08 ± 0·60 S.E.	2·79 ± 0·29 S.E.

simultaneously with stimulation of the IPN produce any change in the response of the muscle groups to IPN stimulation. Total tension, rise time, decay time and duration of responses to single supramaximal stimuli are given in Table 1.

Facilitation of the twitch response. By using the 9 : 1 saline to *Limulus* blood solution the twitch response in *Limulus*, in contrast to most mandibulates muscle, does not fatigue or deteriorate but can be recorded for at least 5 hr after removal of the leg from the animal. Because of this it is possible in *Limulus* to determine to what extent the twitch response can be facilitated. This was done by comparing the magnitude of the mechanical response to pairs of closely spaced pulses to the magnitude of the single stimulus response. Paired pulses were applied using various intervals. All stimuli were supramaximal and a 30-sec interval was allowed between each stimulation.

Figure 2 gives the ratio of mechanical response to closely spaced pulses to that obtained by a single pulse. In this figure ratios of 2 : 1 or less indicate a simple summation; ratios of greater than 2 : 1 indicate varying degrees of facilitation. On

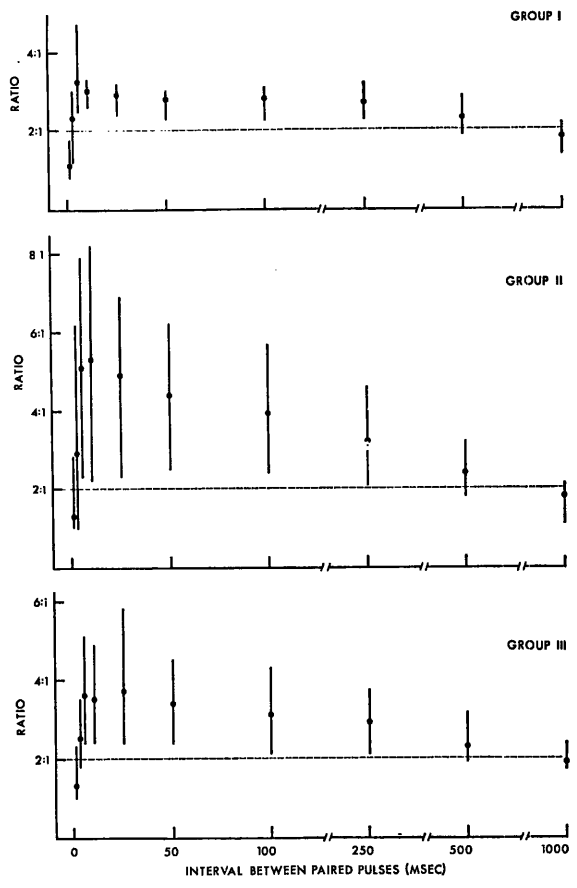

FIG. 2. The ratio between the tension developed by paired pulses and that developed by a single pulse as the interval between the pulses of the pair are changed. Each point represents the mean calculated from at least seven different preparations. The solid lines represent the ranges at each interval. The dashed line at the 2 : 1 ratio represents the amount of tension expected if the mechanical responses merely summated.

this basis it appears that facilitation is greatest between 5 and 50msec following a stimulus, the facilitated response sometimes being eight or more times greater than the non-facilitated response.

Intracellular recordings

EPSPs. The mechanical recordings described above indicate that each of the muscle groups may be innervated by up to eleven excitatory axons. In order to determine the least number of axons innervating a single muscle fiber, the intracellular activity evoked by single stimuli was also investigated. A total of 141

TABLE 2—CHARACTERISTICS OF THE EXCITATORY POSTSYNAPTIC POTENTIAL

	Group 1	Group 2	Group 3
Resting potentials			
(mV ± S.E.) (*N*)	50·4 ± 0·9 (15)	50·5 ± 0·6 (10)	50·9 ± 0·9 (10)
(range)	(34–73)	(33–70)	(34–70)
EPSP			
Amplitude			
(mV ± S.E.) (*N*)	9·9 ± 0·4 (31)	9·9 ± 0·6 (29)	9·5 ± 0·5 (27)
67% rise time			
(msec ± S.E.) (*N*)	8·4 ± 0·3 (31)	9·0 ± 0·3 (27)	8·9 ± 0·3 (27)
67% decay time			
(msec ± S.E.) (*N*)	138·0 ± 13·6 (28)	98·9 ± 10·6 (19)	107 ± 9·0 (24)
Rate of rise			
(V/sec ± S.E.) (*N*)	0·51 ± 0·02 (31)	0·52 ± 0·04 (27)	0·47 ± 0·03 (27)
(range)	(0·27–0·77)	(0·26–0·92)	(0·24–0·70)

muscle fibers from all three muscle groups was investigated. In response to single stimuli one records typical excitatory postsynaptic potentials (EPSPs). The results of these experiments are summarized in Table 2. As the strength of the stimulus is increased one records a variable number of discrete increases in the sizes of the EPSPs. If these discrete increases in the sizes of the EPSPs give an indication of the number of axons innervating a particular muscle fiber, then in our experiments 4 per cent of the fibers are innervated by at least two axons; 18 per cent, by at least three axons; 44 per cent, by at least four axons; 27 per cent, by at least five axons; and 7 per cent, by at least six axons.

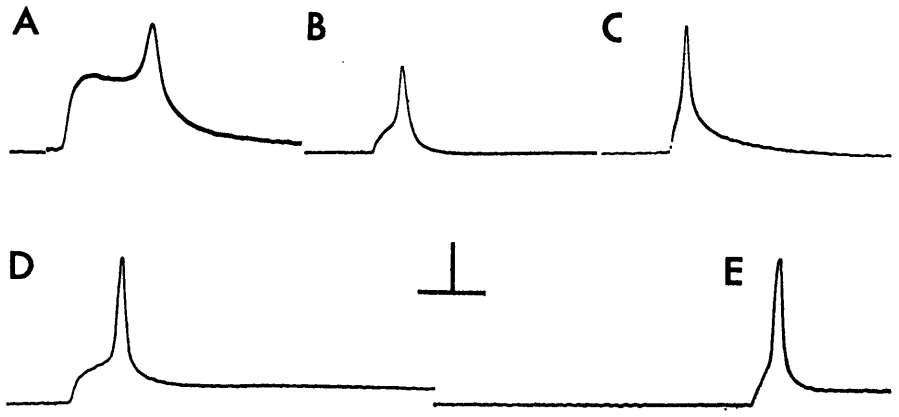

FIG. 3. Examples of graded-spikes. A, B and C are from different muscle fibers. D and E are consecutive recordings from the same muscle fiber. Vertical calibration: A, 5·0 mV; B, 25 mV; C, D and E, 12·5 mV. Horizontal calibration: 100 msec.

Graded-spikes. In nearly one-half (166 of 381) of the muscle fibers investigated single stimuli above the minimal level produced a spike-like potential. This potential always arises from an EPSP and can occur on either the rising or falling phase of the EPSP. However, it is not an "all-or-none" response since, in any particular muscle fiber this potential may have an amplitude of only a few mV, or, as is generally the case, it may be large enough to approach zero potential (Fig. 3).

The wave form of the graded-spike was analyzed to determine the total amplitude of the response (i.e. the EPSP and the spike), the 67 per cent decay time of the total response and the rate of rise of the spike. At least three responses, and in most fibers five responses were recorded and the means for the above characteristics were determined. Graded-spikes with amplitudes of greater than 10 mV were used in this analysis since there is some difficulty in measuring graded-spikes of only a few mV. These data are summarized in Table 3.

TABLE 3—CHARACTERISTICS OF THE GRADED-SPIKE

	Group 1	Group 2	Group 3
Amplitude of total response (mV ± S.E.) (*N*)	34·8 ± 2·0 (17)	34·9 ± 1·9 (19)	29·2 ± 1·5 (7)
(range)	(22·0–50·8)	(22·8–46·5)	(24·5–34·1)
67% decay of total response (msec ± S.E.) (*N*)	24·6 ± 3·5 (15)	16·6 ± 1·5 (18)	23·5 ± 7·9 (6)
Rate of rise of the "graded-spike" (V/sec ± S.E.) (*N*)	1·83 ± 0·18 (17)	1·95 ± 0·15 (19)	1·70 ± 0·22 (7)
(range)	(1·03–4·22)	(0·93–2·99)	(1·11–2·72)

Paired pulse stimulation. The effect of paired pulse stimulation on the intracellular activity was also investigated. In every muscle fiber it was possible with proper selection of intervals between pulses to evoke a graded-spike (Fig. 4). Intervals between 10 and 50 msec are usually most effective. The specific pulse intervals necessary to evoke a spike response differed in individual fibers. Thirty-six fibers of Group 3 muscles were investigated to determine which pulse pair intervals would evoke a spike to the second stimulus. All thirty-six fibers produced a graded-spike when a pulse pair with a 10-msec interval was applied; thirty-one fibers, at 25 msec; nineteen fibers, at 50 msec; seven fibers, at 100 msec; and one fiber produced a graded-spike at 250 msec. In one Group 1 muscle fiber a graded-spike was evoked by the second pulse when a pulse interval of 500 msec was used. When pulse intervals greater than those required to produce a graded-spike (but less than 500 msec) were used, one always obtains a second EPSP which was facilitated to varying degrees. This tends to confirm the facilitation seen in the mechanical responses to paired stimulation.

In those fibers displaying a graded-spike to a single stimulus, the response to the second stimulus of a pulse pair is variable. In approximately 50 per cent (twelve of twenty-seven) of the spiking fibers investigated, the response to the

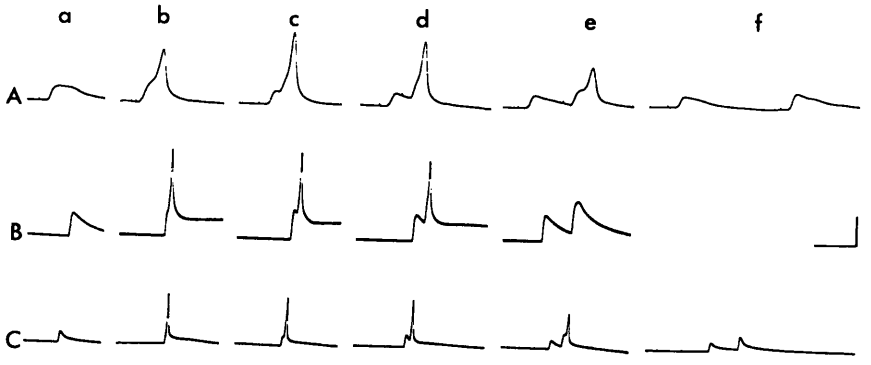

FIG. 4. Intracellular recordings of electrical activity evoked by paired pulses. Results from three different fibers are shown (A, B and C). Pulse pair intervals are as follows: a, single stimulus; b, 10 msec; c, 25 msec; d, 50 msec; e, 100 msec; f, 250 msec. Vertical calibration: A and B, 15 mV; C, 30 mV. Horizontal calibration: A, 100 msec; B, 200 msec; C, 400 msec.

second pulse at intervals of 25 msec was also a graded-spike. At intervals of 10 msec no change in potential was observed unless the graded-spike to a single stimulus did not approach zero potential. In these fibers there was an increase in the spike amplitude when a 10-msec pulse pair was delivered.

In the remaining fibers (fifteen of twenty-seven) the response to the second stimulus was a smaller graded-spike or at times there was only an EPSP recorded. This decrease in or "instability" of the spiking activity has been reported previously by Hoyle (1958) in the *Limulus* dactylopodite flexor.

Ostensibly one could classify *Limulus* skeletal muscle fibers into two groups, those producing only an EPSP (slow) to a single stimulus and those producing a graded-spike (fast) to a single stimulus. However, since the fibers producing EPSPs can produce graded-spikes to paired pulse stimulation, it would appear that all fibers are similar and differ only in the amount of stimulation (be it due to transmitter release or membrane depolarization) necessary to produce graded-spike activity.

DISCUSSION

Innervation patterns

In the crustaceans and the insects, the skeletal muscle is innervated by only a few excitatory axons, less than five, and one to three inhibitory axons (Wiersma, 1961; Hoyle, 1965; Pearson & Iles, 1971). The excitatory axons can be classified as either fast or slow depending on their conduction velocity and the response they evoke in muscle (Wiersma, 1961). The muscle fibers in a particular muscle are not uniform, but fall into several physiological and morphological populations (Atwood, 1967).

From our studies of the distal flexor of the mero-carpopodite and from previous studies on the closer of the dactylopodite, there appear to be differences between

the innervation patterns typically seen in the crustaceans and insects and that seen in *Limulus*. Up to six axons may innervate a single muscle fiber and as many as eleven axons may innervate a particular muscle group. Therefore, in *Limulus* the neuromuscular system differs from other arthropods studied in that the muscle are innervated by a larger number of axons. However, these axons all appear to be similar in function (Parnas *et al.*, 1968) and cannot be classified as being fast and slow.

Parnas *et al.* (1968) demonstrated inhibitory innervation to the flexor of the dactylopodite and Hoyle (1969) also implies that he found an inhibitory input to this muscle. I found no inhibitory axons innervating the distal flexors of the merocarpopodite. Since this muscle has no antagonist (Ward, 1969), this study supports the Hoyle–Smyth hypothesis (1963) that one would expect to find inhibitory axons innervating only those muscles which have an antagonist.

Physiological responses

A mechanical response to a single stimulus could always be recorded in these muscles even at the lowest threshold; this twitch response could be recorded throughout the course of an experiment. Parnas *et al.* (1968) found that each of the six excitatory axons innervating the flexor of the dactylopodite of *Limulus* produces a measurable amount of tension following a single stimulus. This differs markedly from crustacean skeletal muscle systems in that the twitch following stimulation of the fast axon is either easily fatigued (Lucas, 1917) or usually lost after an hour of experimentation (Atwood *et al.*, 1965).

The twitch response in both crustaceans (Wiersma, 1961) and insects (Usherwood, 1962) is much more rapid (rise times less than 15 msec) and of shorter duration (half-decay time less than 20 msec in insects and 50 msec in crustaceans) than that reported here for *Limulus*. It should also be noted that a single stimulus applied to the slow axons innervating crustacean and insect muscle produce virtually no measurable tension.

In *Limulus* the intracellular response of a single muscle fiber to a single supramaximal stimulus evokes either an EPSP alone (56 per cent of the fibers) or a graded-spike (44 per cent of the fibers). The EPSPs have a slower rise time, rate of rise and decay time than EPSPs evoked by the fast axons innervating crustacean muscle (Hoyle & Wiersma, 1958), and the EPSP evoked by both the fast and slow axons innervating insect muscle (Hoyle, 1955; Pearson & Iles, 1971).

The graded-spike response to a single stimulus is found much more commonly in *Limulus* skeletal muscle fibers than in crustacean fibers since stimulation of the fast axon in crustacean system usually evokes large EPSPs instead of a graded-spike (Wiersma, 1961). The spike seen in insect muscle has been shown to be "all-or-none" (del Castillo *et al.*, 1953; Wilson, 1954). The rate of rise of the graded-spike in *Limulus* muscle is much slower than that found in either the fast system of crustaceans (20 V/sec) (Fatt & Katz, 1953).

Thus from the above discussion, *Limulus* muscle appears to be intermediate between the fast and slow systems in other arthropods. Furthermore, since there

is such a similarity among the *Limulus* muscle fibers as to their intracellular activity, it would suggest that these fibers may be of a single type as opposed to individual muscle fibers in crustaceans in which there is a great diversity in the types of physiological responses to indirect stimulation (Atwood *et al.*, 1965).

As shown above *Limulus* skeletal muscle is innervated by a larger number of axons than has yet been reported for other arthropods. It also appears that these axons are of one type and cannot be designated as either fast or slow (Parnas *et al.*, 1968). It has also recently been observed that the muscle fibers which compose the skeletal muscle of the walking legs of *Limulus* are similar in their sarcomere lengths and ultrastructure (Fourtner, 1971; Fourtner & Sherman, in preparation).

Therefore, this information suggests that the neuromuscular system in the walking legs of *Limulus* is a rather non-specialized system when compared to its counterpart in other arthropods.

REFERENCES

ATWOOD H. L. (1963) Differences in muscle fiber properties as a factor in "fast" and "slow" contractions in *Carcinus*. *Comp. Biochem. Physiol.* **10**, 17–32.

ATWOOD H. L. (1967) Crustacean neuromuscular mechanisms. *Am. Zool.* **7**, 527–551.

ATWOOD H. L., HOYLE G. & SMYTH T. (1965) Mechanical and electrical responses of single innervated crab muscle fibers. *J. Physiol., Lond.* **180**, 449–482.

DEL CASTILLO J., HOYLE G. & MACHNE X. (1953) Neuromuscular transmission in a locust. *J. Physiol., Lond.* **121**, 539–547.

DORAI RAJ B. S. (1964) Diversity of crab muscle fibers innervated by a single motor axon. *J. cell. comp. Physiol.* **64**, 41–54.

DUMONT J. N., ANDERSON E. & CHOMYN E. (1965) The anatomy of the peripheral nerve and its ensheathing artery in the horseshoe crab *Xiphosura* (*Limulus*) *polyphemus*. *J. Ultrastruct. Res.* **13**, 38–64.

FATT P. & KATZ B. (1953) The electrical properties of crustacean muscle fibers. *J. Physiol., Lond.* **120**, 171–204.

FOURTNER C. R. (1970) Some aspects of neuromuscular transmission in *Limulus polyphemus*. *Am. Zool.* **10**, 304.

FOURTNER C. R. (1971) Morphological and physiological studies of the walking leg muscles of the horseshoe crab, *Limulus polyphemus* (Linnaeus). Ph.D. dissertation, Michigan State University.

GILAI A. & PARNAS I. (1970) Neuromuscular physiology of the closer muscle in the pedipalp of the scorpion, *Leiurus quinquestriatus*. *J. exp. Biol.* **52**, 325–344.

HOYLE G. (1955) Neuromuscular mechanisms of a locust skeletal muscle. *Proc. R. Soc.* B. **143**, 343–367.

HOYLE G. (1958) Studies on neuromuscular transmission in *Limulus*. *Biol. Bull.* **115**, 209–218.

HOYLE G. (1965) Neural control of skeletal muscle. In *Physiology of Insecta* (Edited by ROCKSTEIN M.), Vol. 2, pp. 408–449. Academic Press, New York.

HOYLE G. (1969) Comparative aspects of muscle. *A. Rev. Physiol.* **31**, 43–84.

HOYLE G. & SMYTH T. (1963) Neuromuscular physiology of giant muscle fibers of a barnacle, *Balanus nubilus* Darwin. *Comp. Biochem. Physiol.* **10**, 291–314.

HOYLE G. & WIERSMA C. A. G. (1958) Excitation at neuromuscular junctions in crustacea. *J. Physiol., Lond.* **143**, 403–425.

LUCAS K. (1917) On summation of propagated disturbances in the claw of *Astacus*, and on the double neuromuscular system of the adductor. *J. Physiol., Lond.* **51**, 1–35.

MILLECCHIA R. & MAURO A. (1969) The ventral photoreceptor cells of *Limulus*—II. The basic photoresponse. *J. gen. Physiol.* **54**, 310–330.

PARNAS I., ABBOTT B. C., SHAPIRO B. & LANG F. (1968) Neuromuscular system of *Limulus* leg closer muscle. *Comp. Biochem. Physiol.* **26**, 467–478.

PEARSON K. G. & ILES J. F. (1971) Innervation of the coxal depressor muscles in the cockroach, *Periplaneta americana*. *J. exp. Biol.* **54**, 215–232.

RATHMAYER W. (1965) Neuromuscular transmission in a spider and the effect of calcium. *Comp. Biochem. Physiol.* **14**, 673–687.

RATHMAYER W. (1967) Elektrophysiologische Untersuchung der Membraneigenschaften eines Spinnenmuskels. Verh. Dtsch. Zool. Ges. Heidelberg. *Zool. Anz. Supplement*, **31**, 616–622.

USHERWOOD P. N. R. (1962) The nature of "slow" and "fast" contractions in the coxal muscles of the cockroach. *J. Insect Physiol.* **8**, 31–52.

USHERWOOD P. N. R. (1969) Electrochemistry of insect muscle. *Adv. Insect Physiol.* **6**, 205–278.

VACHON M. (1945) Remarques sur les appendices du prosoma des *Limulus* et leur arthrogenèse. *Archs Zool. exp. gén.* **84**, 271–300.

WARD D. V. (1969) Leg extension in *Limulus*. *Biol. Bull.* **136**, 288–300.

WIERSMA C. A. G. (1961) The neuromuscular system. In *The Physiology of Crustacea* (Edited by WATERMAN T. H.), Vol. 2, pp. 191–240. Academic Press, New York.

WILSON V. J. (1954) Slow and fast responses in cockroach leg muscle. *J. exp. Biol.* **31**, 280–290.

ZEBE E. & RATHMAYER W. (1968) Elektronenmikroskopische Untersuchungen an Spinnenmuskeln. *Z. Zellforsch. mikrosk. Anat.* **92**, 377–387.

Key Word Index—Limulus polyphemus; neuromuscular system; chelicerate; innervation patterns; EPSP; graded-spike; facilitation.