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INNERVATION PATTERNS OF FAST AND SLOW MOTOR NEURONES DURING DEVELOPMENT OF A LOBSTER NEUROMUSCULAR SYSTEM

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SUMMARY

1. Relationships between motor innervation and muscle fibre type were examined in the closer muscle of the claws of the lobster *Homarus americanus*. The dimorphic claws of the adult–the cutter and crusher–were compared with the claws of the juvenile (stages 4–6) when differentiation takes place. The muscle is known to receive a fast and a slow motor axon. Previous findings have shown that the crusher contains mostly slow fibres; the cutter and juvenile claws have both fast and slow fibres.

2. In the adult, the majority of fibres in the crusher received fast and slow axons; in the cutter most fibres received the fast axon. Fast fibres in the cutter claw were innervated by the fast axon, alone or with the slow axon. Slow fibres in both claws could receive the slow axon only, or both axons. Some slow fibres in the crusher claw were innervated primarily or solely by the fast axon.

3. In the juvenile, most fibres in each claw were innervated by both axons. The juvenile synapses were immature; postsynaptic potentials fluctuated greatly with frequent failures. The homologous fast axons in these claws formed fatigue-resistant synapses.

4. In both adult and juvenile, regardless of fibre type, fast axon synapses had poor facilitation; slow axon synapses had moderate-to-high facilitation.

INTRODUCTION

The dynamic relationship between a neurone and its end organ has been investigated in mature, regenerating, and developing systems. Crustacean neuromuscular systems are useful in such studies because they are innervated by a small number of axons, each of which forms a heterogeneous population of synaptic endings on the muscle fibres (see Atwood, 1976). On any single fibre, there is a population of different types of synapse which is matched to the properties of that fibre. To explain this matching, two schools of thought have arisen (Atwood, 1976). The myogenic school proposes that the muscle is playing the active role in development (Frank, 1973), dictating the type of synapses which will form, e.g. high or low facilitation.

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† Dr Fred Lang died in an automobile accident while this paper was being written. His presence as teacher, colleague and friend is sorely missed.

The neurogenic school suggests that motor nerve synapses are formed as a result of some developmental sequence (Atwood, 1973). The synaptic properties would then play a decisive role in determining muscle fibre properties. Recent evidence shows that on a single fibre there can be a more heterogeneous population of synapses than was previously believed (Lang, Sutterlin & Prosser, 1970; Meiss & Govind, 1979).

We have studied the innervation of different fibre types in the closer neuromuscular system in the dimorphic claws of the lobster *Homarus americanus*–the crusher and the cutter. The nerve to each closer muscle contains two motor axons (a fast and a slow). It has been amply demonstrated that the closer muscle has different properties between the two claws. Wiersma (1955) found that the cutter claw would close completely if this nerve was stimulated with two closely spaced shocks. The closer muscle of the crusher claw required at least three such stimuli before any mechanical activity could be elicited. These differences in mechanical activity are matched with the fibre types found in the closer muscles. In the cutter, the closer muscle consists of short-sarcomere fast fibres and long-sarcomere slow fibres; in the crusher the muscle consists only of slow fibres (Jahromi & Atwood, 1971; Goudey & Lang, 1974; Lang, Costello & Govind, 1977).

In striking contrast, the claws of young lobsters are symmetrical. At stages 4 and 5 (first two post-larval stages), the claws are cutter-like in appearance (Herrick, 1896, 1911). As the lobster moults into successive stages, the external morphology of the claws diverges so that one resembles the crusher claw, the other the cutter claw. Similarly, the distribution of fibre types in the paired claws of young animals (stages 1–5) is the same (Govind & Lang, 1978). Each muscle contains short-sarcomere fibres (20–30%), long-sarcomere fibres (60–70%) and a few intermediate fibres. Commencing at stage 6 (or late stage 5), the closer muscle of the presumptive crusher claw progressively loses all short-sarcomere fibres while in the presumptive cutter claw there is an increase in the relative number of short-sarcomere fibres. While claw placement in the adult is essentially random, removal of a claw from an animal in stage 4 or early stage 5 (<2 days after moult) always results in the remaining claw becoming a crusher (Emmel, 1908; Lang, Govind & Costello, 1978). Claw removal prior to or after this time has no effect on subsequent claw replacement, the remaining claw becoming a cutter or a crusher with equal probability. Thus it is evident that the factors which influence claw placement and transformation of fibre types are active only in these early post-larval stages.

MATERIALS AND METHODS

Adult lobsters (*Homarus americanus*) were obtained localy from the waters around Woods Hole, Massachusetts. They were kept in tanks supplied with fresh sea water at ambient temperatures and were fed fresh squid or fish regularly. Juvenile lobsters employed in this study ranged from stage 4 to stage 6. These animals were kept in separate trays (Lang, 1975) to monitor their subsequent growth and were fed frozen brine shrimp every day.

The basic experimental procedure was the same for adult and juvenile animals. Only lobsters with normal claws were chosen for experiments. The lobster was induced to automize its claw. The exoskeleton covering the opener muscle was then

chipped away and the opener muscle was removed. In adults, the nerve bundle containing the fast and slow excitor axons and the inhibitor was exposed in the carpopodite (Govind & Lang, 1974).

Platinum wire hook electrodes were used for stimulation and a suction electrode for monitoring the axon spikes. In juvenile lobsters (stages 4–6) the stimulating electrode and the suction electrode could not both be placed in the carpopodite due to the small size of the claws; therefore, the nerve bundle was exposed in the ischiopodite for placement of the stimulating electrode. Each axon could be identified by certain characteristics. The fast axon has a larger diameter, hence a larger spike height, a faster conduction velocity (Govind & Lang, 1974) and a lower threshold than the slow axon when short duration (0·8 ms) stimuli were used (Wright & Colemen, 1954). However, by trial and error placement, the stimulating electrode could be positioned near the slow axon to make it fire at a lower stimulus voltage than the fast axon. Thus, it was possible to use two stimulating electrodes, one to stimulate only the fast axon, the other to stimulate only the slow axon.

The preparation was placed in a bath of physiological solution (Atwood & Dorai Raj, 1964), and kept at a temperature of 17–18 °C. Glass microelectrodes filled with 3 M–KCl (7–15 MΩ) were used for intracellular recording from the muscle fibres.

Innervation of a muscle fibre by an axon was established if an excitatory post-synaptic potential (e.p.s.p.) could be discerned when that axon was stimulated with paired stimuli (2–4 ms separation) at 10 Hz, which is the most effective stimulus (Govind & Lang, 1974). Generally, only innervation by the slow axon was weak enough to require this procedure. A signal averager was used to verify the presence or absence of potentials (16 or 32 sweeps). The degree of facilitation (Fe) of e.p.s.p.s. was measured as the ratio of the e.p.s.p. height at 10 Hz to that at 1 Hz (Atwood & Bittner, 1971).

Sarcomere length of muscle fibres was determined in some experiments in the manner of Costello & Lang (1979).

RESULTS

Innervation of adult dimorphic claws

The size of the closer muscle and the large number of fibres precluded identifying the innervation pattern for every muscle fibre of a claw. However, a good approximation of the distribution patterns of the axons was accomplished by studying a large number of claws and by identifying the innervation of morphologically distinct bundles–described and named by Costello & Lang (1979).

There was a large variation in the size of e.p.s.p.s. generated by either motor axon among closer muscle fibres of both claws. This diversity in size of e.p.s.p.s. appears to be a common phenomenon in crustacean muscles (Velez & Wyman, 1978; Mellon & Stephens, 1979). E.p.s.p.s. from fast axon firing were generally larger than those from slow axon firing. In a fibre innervated by the fast axon, the e.p.s.p. was usually present at 1 Hz stimulation (Fig. 1A). In contrast, in some muscle fibres innervated by the slow axon, e.p.s.p.s. could only be observed when the axon was stimulated with paired pulses at 10 Hz (Fig. 1B–C), and these e.p.s.p.s. were often small (<0·4 mV). The output from the synapses of either motor axon was stable in the adult closer muscles.

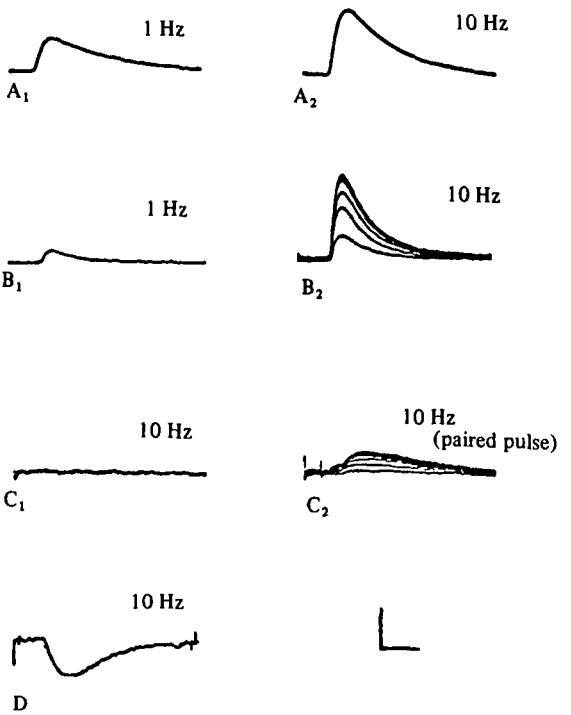

Fig. 1. Intracellular recordings from adult closer muscles. The amplitude at 10 Hz stabilized after reaching peak value. In A, the poor facilitation from fast axon excitation is seen (short-sarcomere fibre; in CuD). In B, the typical facilitatory response from high frequency stimulation of the slow axon is shown (long-sarcomere fibre; in CuD). In C, the effect of paired pulse stimulation (7 ms interval) is seen (short-sarcomere fibre; in CuD). In D, an i.p.s.p. is shown from a long-sarcomere fibre in CuD (average of 16 sweeps). Motor innervation was by slow axon only. These responses were rarely seen. Calibration: Vertical: A, 5 mV, B–C, 4 mV, D, 0·2 mV; Horizontal: A–C, 20 ms, D, 12·8 ms.

Few inhibitory postsynaptic potentials (i.p.s.p.s.) were observed in muscle fibres, although the closer muscle is innervated by an inhibitory axon (Wiersma, 1961). When present (Fig. 1 D), they were hyperpolarizing and small (<0·8 mV). Paired pulse stimulation enhanced the response. The fibres with postsynaptic inhibition were always innervated by the slow axon, either by itself or with the fast axon.

Cutter claw

The innervation of 552 muscle fibres was determined in 42 cutter claws. The majority (64 %) of closer muscle fibres was innervated solely by the fast axon (Fig. 2). Of the remaining fibres, 16 % were innervated only by the slow axon and 20 % by both axons.

Each muscle bundle had a characteristic innervation (Fig. 2). Fibres receiving only the fast axon were restricted to the largest bundle, CuD. All of CuPV and much of CuV were innervated solely by the slow axon. There was also a small region at the distal border of CuD-CuV receiving only this axon. Doubly innervated fibres were primarily found in CuV, its boundary with CuD, and in all of CuP.

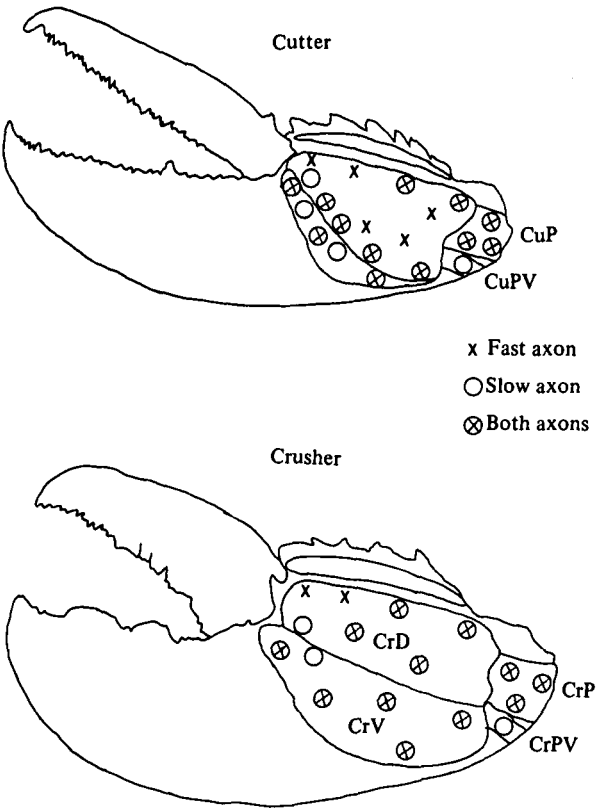

Fig. 2. Medial view of the cutter and crusher claws, showing excitatory innervation of the muscle bundles of the closer muscle. Each muscle has a large dorsal and ventral bundle (labelled CuD and CuV for the cutter and CrD and CrV for the crusher). Smaller proximal dorsal and ventral bundles are labelled, respectively, CuP and CuPV in the cutter and CrP and CrPV in the crusher. Fibre types: CuD and CuV are composed of fast muscle (sarcomere lengths < 4 μm); the other bundles in both claws consist mostly of slow muscle (sarcomere length > 6 μm) though a small number of fibres ($< 5\%$) have intermediate sarcomere lengths (4–6 μm).

Crusher claw

In 15 crusher claws, the innervation of 295 muscle fibres was determined. The regional distribution of the fast and slow motor axons was very different from that observed in the cutter claw (Fig. 2). Most fibres of the crusher closer muscle received both motor axons (67 %). Few fibres were innervated solely by the fast axon (15 %) or solely by the slow axon (18 %). The majority of the former type were in the dorsal distal portion of CrD; the remainder were found in the central region of CrD (too few to be shown in Fig. 2). Fibres innervated solely by the slow axon were located, as in the cutter, in CrPV and in a small area at the distal border of CrD and CrV.

Table 1. *Average amplitude of the e.p.s.p. at 1 Hz and the facilitation ratio (Fe) seen in fibres of the claw closer muscles in the adult, arranged by innervation of the motor axons (all bundles)*

Innervation in claw	Fast axon		Slow axon	
	E.p.s.p. (mV)*	Fe	E.p.s.p. (mV)*	Fe
Cutter:				
Fast only	3·1	2·0	—	—
Range	0·3–12·0	1·0–4·0		
N	83	83		
Slow only	—	—	1·9	6·1
Range			0·1–10·0	1·5–11·0
N			61	49
Both axons	1·7	1·9	0·3	6·4
Range	0·1–13·0	1·0–5·0	0·2–13·0	2·5–10·0
N	158	76	61	38
Crusher:				
Fast only	1·9	2·4	—	—
Range	0·4–6·0	1·2–4·2		
N	28	28		
Slow only	—	—	2·2	5·2
Range			0·1–7·5	1·5–13·0
N			56	37
Both axons	1·5	2·5	0·5	6·2
Range	0·3–7·5	1·0–6·0	0·1–4·0	2·5–10·0
N	134	132	35	33

* The size of e.p.s.p.s in a muscle fibre generated by the fast and slow axons varied with each other in a logarithmic manner:

Cutter – (e.p.s.p.:fast axon) = 1·17–1·41 $\log_{10}$ (e.p.s.p.:slow axon): $r = -0{\cdot}312$, $P < 0{\cdot}01$
Crusher – (e.p.s.p.:fast axon) = 2·57–0·94 $\log_{10}$ (e.p.s.p.:slow axon); $r = -0{\cdot}529$, $P < 0{\cdot}01$

Synaptic properties of the motor axons

The amplitudes of e.p.s.p.s. and facilitation values of the motor axons for the cutter and crusher claws are given in Table 1.

Fast axon. The e.p.s.p.s. in muscle fibres elicited by fast axon excitation usually were larger at low frequency stimulation than those elicited by the slow axon. Fibres innervated only by the fast axon had larger e.p.s.p.s. than those receiving both motor axons. In the cutter claw, e.p.s.p.s. from fast axon stimulation tended to be higher in amplitude than in the crusher claw. Facilitation in both claws was poor (average cutter Fe = 2·0; average crusher Fe = 2·5). Indeed, the Fe for fast axon synapses fell into a narrow range regardless of e.p.s.p. size (Figs. 3–4).

Slow axon. At 1 Hz stimulation the e.p.s.p. was larger in fibres receiving only the slow axon than in fibres also receiving the fast axon. Generally, e.p.s.p.s. from slow axon stimulation were greater in the crusher than in the cutter. A small region in both claws at the distal portion of the muscle (Fig. 2) contained long-sarcomere fibres innervated only by the slow axon. These fibres had the largest e.p.s.p.s. from slow axon stimulation in the entire muscle (average cutter = 3·7 mV; average crusher = 2·8 mV). The degree of facilitation by slow axon synapses in singly and doubly innervated fibres was usually large, but there was a widespread distribution; the modal Fe was the same (5·5) in both claws (Figs. 3–4).

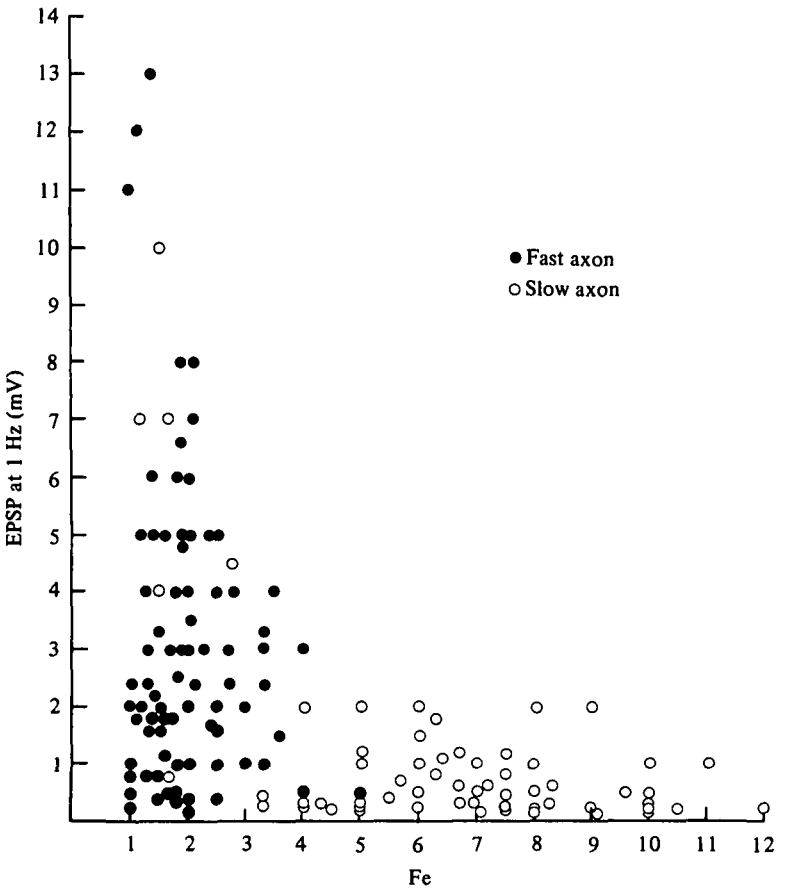

Fig. 3. Cutter claw closer muscle: plot of the e.p.s.p. elicited in muscle fibres by stimulating the motor axon at 1 Hz against the facilitation index (Fe). Note pre-eminence of fast axon values in the low Fe range and of slow axon values in the moderate-to-high range. Curve of best fit for the slow axon values was hyperbolic with equation $\gamma = 5{\cdot}37\chi^{-1{\cdot}43}$ ($r = -0{\cdot}573$, $P < 0{\cdot}01$). Note similarity of this equation to that for the slow axon in the crusher claw (Fig. 4). Curve of best fit for the fast axon was a power equation $\gamma = 1{\cdot}53\chi^{-1{\cdot}39}$ ($r = 0{\cdot}160$, $P < 0{\cdot}05$). The tendency of low amplitude e.p.s.p.s (< 1 mV) to show little or no facilitation greatly affected the calculations. When these values were removed (< 1 mV and < 2Fe) the curve became hyperbolic with equation $\gamma = 2{\cdot}90\chi^{-1{\cdot}34}$ ($r = -0{\cdot}151$, $P < 0{\cdot}10$). $N = 111$ fibres (slow axon); 157 fibres (fast axon).

Comparison between synaptic properties and fibre type in adult claws

Depending on which bundle was being examined, synaptic properties of short- and long sarcomere fibres could be compared (Costello & Lang, 1979). Table 2 shows the values for the e.p.s.p. and the Fe of the claw closer muscles arranged by fibre type.

Regardless of fibre type or whether the fibre was singly or doubly innervated, the degree of facilitation was generally small for fast axon synapses and moderate-to-high for slow axon synapses (Table 2; Figs. 3–4). The inverse relationship between e.p.s.p. and Fe typical of slow (tonic) systems (Atwood, 1976) exists here for the slow axon. For the slow axon, the relationship was strikingly similar between both types of claw (legend, Figs. 3–4).

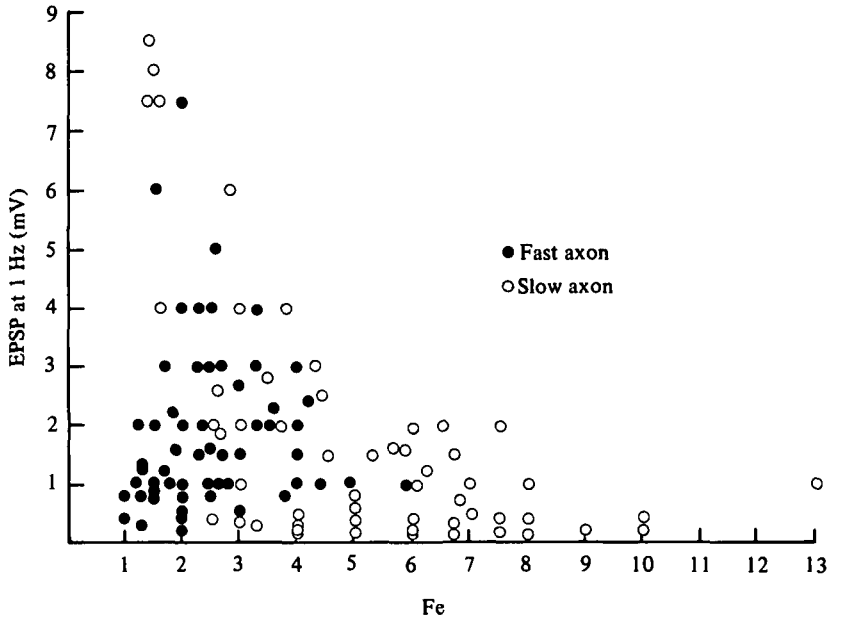

Fig. 4. Crusher claw closer muscle: plot of the e.p.s.p. elicited in muscle fibres by stimulation of the motor axons at 1 Hz against the facilitation index (Fe). Note that, as in Fig. 3, the fast axon Fe values are generally low while those of the slow axon are usually moderate-to-high. Curve of best fit for slow axon was hyperbolic with equation $\gamma = 5{\cdot}37\chi^{-1{\cdot}31}$ ($r = -0{\cdot}623$, $P < 0{\cdot}01$). This equation is similar to that in the cutter claw. Curve of best fit for the fast axon was a power equation $\gamma = -1{\cdot}04\chi^{1{\cdot}31}$ ($r = 0{\cdot}160$, $P < 0{\cdot}05$). As in the cutter claw, low amplitude e.p.s.p.s (< 1 mV) usually showed little facilitation, thus affecting the equation. However, this relationship did not change by removing these values as it did in the cutter claw ($\gamma = 1{\cdot}21\chi^{0{\cdot}08}$, $r = 0{\cdot}039$, no significant relationship). $N = 93$ fibres (slow axon); 168 fibres (fast axon).

Table 2. *Average values of e.p.s.p. at 1 Hz and the facilitation ratio (Fe) from closer muscle fibres, arranged by fibre type*

Fibre type	Fast axon		Slow axon	
	E.p.s.p. (mV)	Fe	E.p.s.p. (mV)	Fe
Fast (< 4 μm)	2·8	2·0*	0·3	6·3**
Range	0·3–13·0	1·0–5·0	0–0·8	3·3–10·0
N	143	139	25	24
Slow (> 6 μm)	1·4	2·4*	1·4	6·1**
Range	0·1–13·0	1·0–6·0	0–10·0	1·0–14·0
N	268	188	200	189

* No significant difference, 5 % level, between fast axon Fe's

** No significant difference, 5 % level, between slow axon Fe's (Kolmogorov-Smirnoff two sample test used)

E.p.s.p. sizes from fast axon stimulation tended to be larger in fast fibres than in slow fibres (Table 2). The reverse was true for the slow axon. Regardless of fibre type, an inverse, non-linear relationship existed between the relative sizes of e.p.s.p.s. produced by either motor axon (see Table 1).

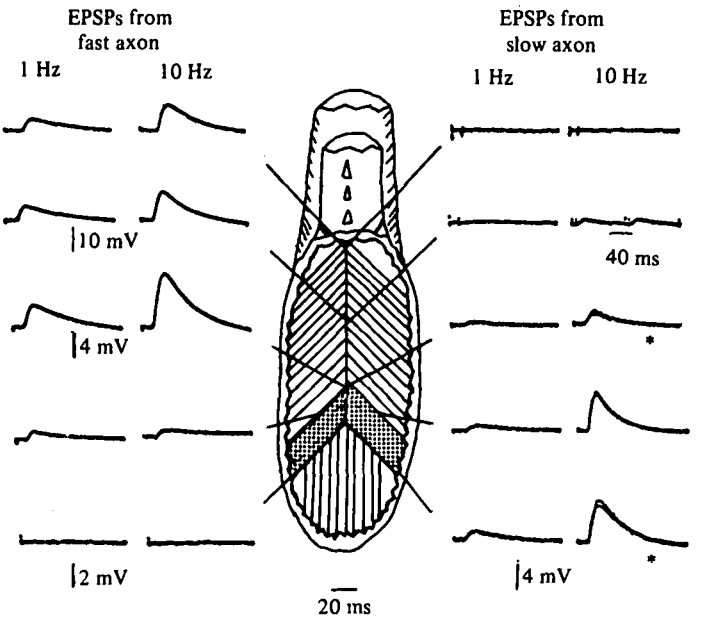

Fig. 5. Gradient of innervation by the motor axons in fibres on the dorsal surface of the cutter claw closer muscle. Intracellular recordings commence with the most proximal fibre of CuD (bottom traces) and progress to the most distal fibre (top traces). The pairs of e.p.s.p.s in the fast and slow axon columns at each level are from the same muscle fibre. Paired pulses were needed to elicit the e.p.s.p. in the slow axon column, second trace from top. Stippling denotes long sarcomere fibres (> 6 μm sarcomere length). The sarcomere length decreased from 10 μm for the most proximal fibre (*) to 6 μm for the most distal slow fibre (**).

In the dorsal region of CuD and CrD there appeared to be a proximal-distal gradient in fast and slow axon innervation. This is illustrated for CuD in Fig. 5. In the cutter, the proximal fibres had the most effective slow axon innervation (measured by size of e.p.s.p.) and contained long sarcomeres (6–10 μm). At the region where this amplitude had decreased (but not entirely disappeared) and e.p.s.p. amplitude from the fast axon had increased, there was a 'jump' to short sarcomere fibres (2–4 μm). No gradation of intermediate lengths (4–6 μm) was evident.

All possible innervation patterns were observed but one: fast fibres were never innervated solely by the slow axon.

Juvenile innervation

Most fibres in the juvenile (stages 4–6) closer muscles displayed fluctuating e.p.s.p. values when either motor axon was stimulated (Fig. 6A). Such fluctuation within a fibre is characteristic of newly formed synapses in vertebrate tissue culture (Robbins & Yonezawa, 1971) and in regenerating crab limb muscle (Govind, Atwood & Lang, 1973). An interesting feature was that the e.p.s.p.s. fluctuated at constant amplitudes (Fig. 6A). To achieve a degree of standardization for e.p.s.p. size, 10 pulses at 1 Hz were given to the axon, and the average e.p.s.p. amplitude was calculated.

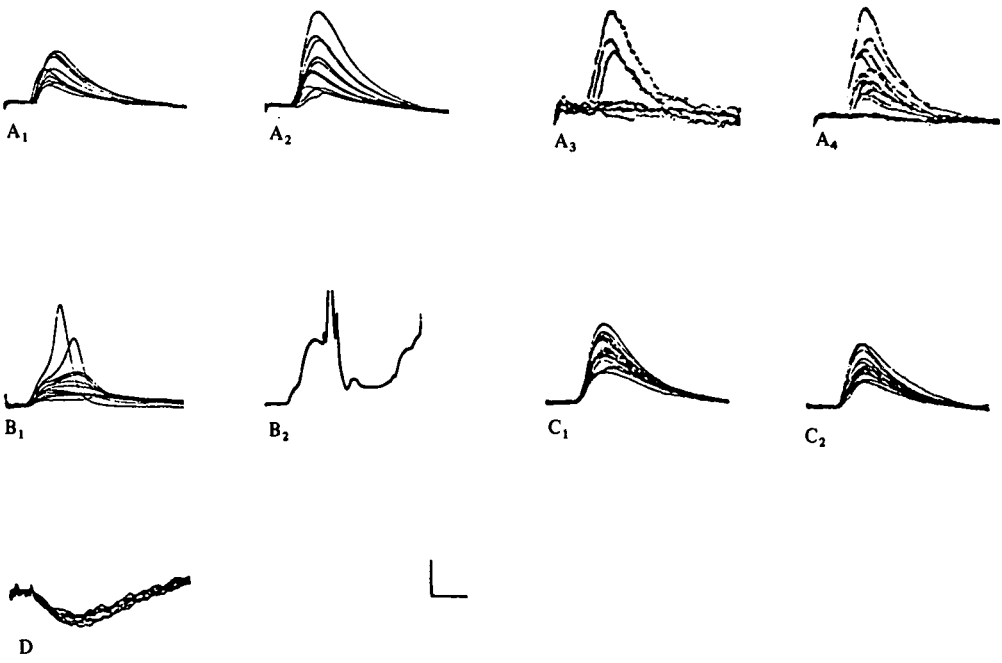

Fig. 6. Intracellular recordings from fibres in the closer muscle of juvenile animals. In A (from stage 5 claw), variability of output is shown by the different amplitudes of e.p.s.p. from excitation of fast axon (1–2) and slow axon (3–4). 1 and 3 are from 10 pulses to the axon at 1 Hz; 2 and 4 are from 10 Hz stimulation. E.p.s.p. values fluctuated randomly so that the last e.p.s.p.s in a train of stimulation were not necessarily the highest. In B_1, graded secondary responses from 10 Hz stimulation to fast axon in fibre from stage 5 closer muscle; in B_2, regenerative spike (90 mV) elicited by a single paired pulse stimulus to the fast axon in the adult cutter claw (fibre located in CuP). In C, recovery of fast axon synapses after being fatigued is shown for a stage 5 closer muscle. E.p.s.p.s elicited by fast axon stimulation at 10 Hz at start of impulse train (C_1). When no more e.p.s.p.s were elicited, the stimulation ceased. After a 3 min rest, the fast axon was again stimulated at 10 Hz with substantial recovery of e.p.s.p.s (C_2). In D, an i.p.s.p. obtained in stage 4 claw by stimulating the inhibitory axon with paired pulses at 10 Hz. As in adults, such post-synaptic inhibition was rarely seen, and only then in fibres receiving the slow axon as well. Calibration: A_{1-2}, 10 mV; A_3, 1 mV; A_4, 2 mV; B_{1-2}, 20 mV; C_{1-2} and D, 4 mV. Horizontal: A_{1-2}, B_1, and C_{1-2}, 4 ms; A_{3-4}, 8 ms; B_2 and D, 20 ms.

Fast axon stimulation elicited e.p.s.p.s. which were larger than those in adults though the average degree of facilitation was almost the same (Table 3). Usually no e.p.s.p. was seen in fibres upon low frequency stimulation of the slow axon. Even when present at low frequency there was a large number of failures (Fig. 6A). The Fe was smaller than in adults, but significantly larger than that of the fast axon. Paired pulse stimulation of the slow axon at 10 Hz was often necessary to elicit e.p.s.p.s. The maturation of the system in successive stages was seen by the decrease in number of slow axon innervated fibres requiring this patterned stimulation (from 24 % in stage 4 to 6 % in stage 6).

There was no difference in the pattern of innervation between paired claws in stages 4–6 (Fig. 7). The majority of fibres in each claw was innervated by both motor axons (stage 4, 86 %; stage 5, 77 %; stage 6, 82 %). The remaining fibres were singly innervated by either the fast axon (stage 4, 9 %; stage 5, 18 %; stage 6, 9 %) or the slow axon (stage 4, 5 %; stage 5, 5 %; stage 6, 9 %).

Table 3. *Average values of the e.p.s.p. at 1 Hz and the facilitation ratio (Fe) of fibres in the claw closer muscles of early postlarval animals*

Stage	Fast axon		Slow axon	
	E.p.s.p. (mV)	Fe*	E.p.s.p. (mV)	Fe*
Stage 4	6·8	1·8	1·8	3·5
Range	0–23·0	0·9–3·5	0–5·0	2·0–6·0
N	67	66	54	20
Stage 5	5·6	2·0	1·1	3·5
Range	0–24·0	0·9–5·0	0–3·0	1·0–9·2
N	113	108	75	17
Stage 6	5·9	1·8	1·4	3·4
Range	1·0–20·0	0·9–2·3	0–4·0	1·0–9·0
N	40	29	26	17

* Values significantly different (1 % level) between axons at each stage (Kolmogorov-Smirnoff test).

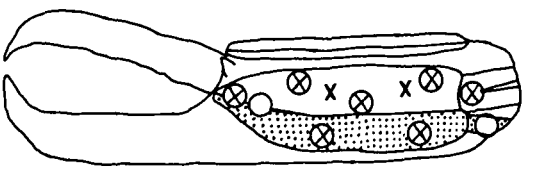

Fig. 7. Motor innervation of the closer muscle in a stage 4 lobster. Stippled areas denote the bundles (C-V and C-PV, precursors to CuV/CrV and CuPV/CrPV, respectively) containing no fast fibres. Clear areas (C-D and C-P, precursors to CuD/CrD and CuP/CrP, respectively) have mixed populations of fibre types (fast, intermediate, and slow) at this stage. During maturation all fast fibres and most intermediate fibres are lost in the presumptive crusher; in the presumptive cutter, primarily fast fibres remain in CuD/CuP. Legend: same as in Fig. 2.

Several characteristics of the neuromuscular system in juvenile claws were different from that in the adults. First, there was more spontaneous release ('mini's') which increased after a bout of stimulation. This might be due to higher input resistance since these fibres are smaller in diameter. Second, many muscle fibres produced secondary electrogenic responses when the fast axon was stimulated at 10 Hz (Fig. 6B). Fibres in the adult muscles can also generate these secondary responses, but generally only with specialized stimulation (paired pulses) (Fig. 6B). Third, the fast axon synapses in these stages were resistant to fatigue (Fig. 6C). This property was in great contrast to fast axon synapses in adult claws (Govind & Lang, 1974) where there was little or no recovery of e.p.s.p.s. after fatigue, especially in the cutter claw. Conversely, the synapses of the slow axon in these juvenile claws were more labile than those of the fast axon. After intense stimulation, though the fast axon synapses recovered, those of the slow axon often did not. I.p.s.p.s. were evident in many muscle fibres at these early stages (Fig. 6D). As in the adult, they were found only in fibres also innervated by the slow axon.

DISCUSSION

Sarcomere length and pattern of innervation

The results argue against the hypothesis that the motoneurone governs properties of the muscle fibre it innervates, at least for sarcomere length. At stage 4, two classes of fibres, short-sarcomere and long-sarcomere, are already established in the precursors of the distal bundles CuD/CrD and CuV/CrV (Costello & Lang, 1979; Fig. 7). But virtually all fibres in these precursor bundles at this stage receive both the fast and the slow axons in each claw. Even after claw differentiation (stage 6), no major difference in motor axon distribution is found, though profound changes in fibre type populations are underway (Govind & Lang, 1978).

Furthermore, in juvenile and adult claws both motor axons innervate all fibres of the proximal bundle CuP/CrP (Figs. 2, 7). Yet the fibre population in this bundle changes from mixed (short- and long-sarcomere) in the juvenile stages to all short-sarcomere in the cutter (CuP) or all long-sarcomere in the crusher (CrP) of the adult (Costello & Lang, 1979).

A small population of fibres in the adult crusher claw is always innervated solely or primarily by the fast axon, though all fibres in this claw have long sarcomeres. Conversely, in adult cutter claws the slow axon often innervates short-sarcomere fibres with the fast axon as well as long-sarcomere fibres (with or without the fast axon). This tends to discount the idea that the slow axon exerts an influence subliminally to form slow fibres (Atwood, 1976).

Sarcomere length and synaptic facilitation (Fe)

Our studies show that the degree of synaptic facilitation (Fe) is somewhat independent of fibre type: fast axon synapses usually have low values of Fe while slow axon synapses usually have moderate-to-high values whether they occur on short- or long-sarcomere fibres. It seems unlikely then that the muscle fibre regulates synaptic facilitation as has been previously suggested for a tonic mononeurone in the proximal accessory flexor muscle of the lobster (Frank, 1973).

Another interesting observation is the change in the facilitation properties of motor axons between juvenile and adult lobsters. The average value of Fe for slow axon synapses is lower in younger stages than in the adult, but significantly larger than the fast axon Fe. The average degree of facilitation of the fast axon synapses differs little in juvenile or adult. Apparently two significantly different populations of synapses are being formed by the fast and slow axons even at early contact with the fibre. In this case, timing of synaptic formation and subsequent maturation could be a major factor causing the wide range of facilitation values of the slow axon (Atwood, 1973). Such a process for fast axon synapses appears limited, there being little change in degree of facilitation of range from early stages to the adult.

Changes in homologous fast axons

In the juvenile the fast axon of both claws forms fatigue-resistant synapses. *In vivo* studies have also shown that the fast axon in both juvenile claws is very active, firing frequently in long bursts (unpublished observations). During maturation a disparity arises between the fast axons. The crusher fast axon synapses remain more resistant to

fatigue than those of the cutter (Govind & Lang, 1974). Also, the fast axon is more active in the closer muscle of the adult crusher, causing it to contract more often and for longer periods of time than in the cutter (Costello & Lang, 1977; Hill, Costello & Lang, 1977). Such activity might have an effect on muscle fibre properties, as demonstrated in vertebrate muscle (Drachman & Witzke, 1972). Differences in the characteristics of homologous motor axons have also been reported in the dimorphic claws of *Alpheus* (Mellon & Stephens, 1979). Apparently, the juvenile state is maintained by these axons in the small claw (pincer) which can change should the specialized snapper claw be removed. In the lobster this plasticity is lost after the early postlarval stages.

Functional aspects

The distribution of fibre types and patterns of innervation clearly differ in the closer muscles of adult cutter and crusher claws. Further, the muscle of each claw can be subdivided into bundles with distinct combinations of innervation, synaptic properties and fibre type. Thus, the large dorsal bundle in the cutter (CuD) has short-sarcomere fibres receiving only the fast axon which forms high output, poorly facilitating synapses. The dorsal bundle in the crusher (CrD) has long sarcomere fibres receiving both axons, the slow axon having low output synapses with high Fe values. As sarcomere length has been shown to be correlated to speed of contraction (Jahromi & Atwood, 1971) our findings established the physiological basis for the observation that the cutter claw is specialized for rapid closure with little resistance to fatigue while the crusher, closing more slowly, is fatigue-resistant (Wiersma, 1955; Govind & Lang, 1974). Thus CuD, considered a phasic unit, would represent the specialization of the cutter; the homologous CrD, as a tonic unit, would represent that of the crusher.

In contrast to the claws of the adults, those of the juvenile lobsters remain unspecialized. The claws of early postlarval lobsters are indistinguishable in morphology (Herrick, 1911; Costello & Lang, 1979), in fibre type and bundle shape (Lang, Govind & She, 1977; Govind & Lang, 1978; Costello & Lang, 1978), and as reported here, in innervation. The changes initiated at stage 4 (Emmel, 1908; Lang *et al.* 1978) produce the dramatic differentiation of the claws onto the highly specialized cutter and crusher. Thus, during maturation, CuD keeps its fast muscle fibres to become a phasic motor unit in the adult cutter claw. To become specialized as a tonic unit, CrD loses its fast muscle fibres while the remaining slow fibres maintain innervation by both axons.

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