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COMPARISON OF SLOW LARVAL AND FAST ADULT MUSCLE INNERVATED BY THE SAME MOTOR NEURONE

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SUMMARY

1. Muscles innervated by an identified set of motor neurones were compared between larval and adult stages.

2. The structure of the larval muscle is typically tonic: long sarcomeres, irregular Z-bands, and 10–12 thin filaments around each thick filament. The structure of the adult muscle is phasic: 3–4 μm sarcomeres, regular Z-bands, 6–8 thin filaments around each thick filament, and large mitochondrial volume.

3. The tensions produced by these muscles were correspondingly different. The larval twitch was about 7 times slower and the tetanus/twitch ratio 10 times greater than those of the adult.

4. No structural or physiological differences were observed in the neuromuscular junctions of the two stages.

5. The relatively unchanging functional relationship of a single motor neurone with two different muscle fibre types during two developmental stages is compared with the converse situation in which it has been reported that implantation of a different type of motor nerve into a muscle modifies contractile properties.

INTRODUCTION

Appropriate interaction between developing muscle and its innervating motor nerve is essential for the normal development of skeletal muscle in a variety of vertebrates (Close, 1972; Gutmann, 1977; Rubinstein & Kelly, 1978) and insects (Nüesch, 1968; Finlayson, 1975). Nerve-muscle interactions also contribute to the maintenance of adult muscle (Harris, 1974; Lewis, Kean & McGarrick, 1974; Gutmann, 1976, 1977; Lømo, 1976; Cangiano, Lutzemberger & Nicotra, 1977; Lømo & Slater, 1978). Changing the innervation (e.g. by denervating the muscle and reinnervating it with a foreign nerve) can alter muscle properties such as rate of contraction (Buller, Eccles

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& Eccles, 1960; Luff, 1975; Buller & Pope, 1977), acetylcholine sensitivity (Miledi, Stefani & Zelená, 1968), myosin ATP-ase activity (Bárány & Close, 1971), cholinesterase production (Grinnell & Rheuben, 1979), and membrane specializations (Ellisman, Brooke, Kaiser & Rash, 1978).

In the light of this evidence for specified neural influences on muscle properties, one would expect that a given motor neurone would be associated throughout its life with one type of muscle fibre. This expectation can be evaluated by studying different ages of an animal that possesses identifiable motor neurones. The neuromuscular system of moths provides particularly favourable material, because conversion of a caterpillar into a moth results in striking changes in body musculature and mode of locomotion, with many motor neurones persisting throughout the metamorphosis (Taylor & Truman, 1974; Truman & Reiss, 1976; Heinertz, 1976; Casaday & Camhi, 1976).

In the present paper we compare the ultrastructure and physiology of the larval and adult dorsal longitudinal muscle (dl_1) of *Manduca sexta* (L.). In the adult moth, this flight muscle is composed of five distinct bundles of muscle fibres (Nüesch, 1953; Eaton, 1971), and it is innervated by five motor neurones (Casaday & Camhi, 1976; Heinertz, 1976). These large motor neurones are also present in the same regions of the ganglia in larvae and pupae (Casaday, 1975; this paper). In caterpillars these motor neurones innervate dorsal muscles that are used in crawling and in other movements (Kammer & Rheuben, 1976*a*). During metamorphosis, some of the larval dorsal muscle fibres form the anlage of the adult dl_1 (Heinertz, 1976), and the five motor neurones that innervate them persist (morphologically) throughout metamorphosis and innervate the adult dl_1 (Casaday & Camhi, 1976).

The results described below show that the dl_1 motor neurones innervate two distinctly different types of muscle in the two developmental stages, while themselves retaining basically the same characteristics. Abbreviated accounts have been published (Rheuben, 1975; Kammer & Rheuben, 1976*a*).

MATERIALS AND METHODS

Larvae of *Manduca sexta* were reared with an artificial diet and kept at 26 °C on a regime of 16 h light and 8 h dark (after the methods of Yamamoto, 1969). The animals were maintained under the same temperature and light conditions throughout metamorphosis.

To examine the physiological properties of the adult dorsal longitudinal muscle (dl_1), we exposed either the lateral or the medial surface of the muscle and its motor nerve by dissecting away other muscles. (For anatomical details, see Nüesch, 1953 and Eaton, 1971.) The preparation was kept moist by a continuous flow of saline, which contained 25 mM-NaCl, 25 mM potassium methanesulphonate, 4 mM-$CaCl_2$, 33 mM-$MgCl_2$, and 150 mM Tris methanesulphonate, at pH 7. Oxygen was supplied to the tracheal system by sealing the external, lateral half of the thorax onto a hollow, truncated cone of wax. Oxygen was piped under low pressure into the cone and thereby forced into the tracheae via the spiracles. An adequate supply of oxygen is essential, since the membrane potential of the flight muscle fibres is dependent on metabolic activity (Rheuben, 1972). The motor nerve was stimulated with a fine suction elec-

trode. Intracellular recordings from muscle fibres were made with glass micropipettes, filled with 3 M KCl and of 10–20 MΩ resistance.

Tension in both larval and adult muscles was recorded with a strain-gauge transducer (Bionix) or an RCA 5734 tube in a bridge circuit (Lang, 1972). The time course of tension development was described in two ways. The rise time was defined as the time from the onset of tension to peak tension. Twitch duration was defined as the time from the onset of tension to 50% relaxation.

As a control for the effect of the experimental manipulations on the health of the dorsal longitudinal muscle, some tension measurements were made on moths in which dissection was minimal. A moth was immobilized with masking tape placed over the body and wings. The transducer was attached to the prescutum, and stimulating electrodes consisting of two insulated copper wires, 30 μm in diameter, were inserted into motor unit *b* of the dl_1 through two small holes in the cuticle. As another control, tension measurements were made on another fast flight muscle, the subalar (pv_4 and pv_5 of Nüesch, 1953), also in intact animals.

For studies of larval muscle, fourth and fifth instar caterpillars were used. A larva was dissected by making a posterior and then a longitudinal incision; the muscles on the right side of the mesothorax were then exposed by pinning the body wall to a wax block. The entire gut was then removed, taking care not to spill its contents.

The experimental techniques employed with larvae were as described above for adults, except for the following changes. The saline was modified to take into account the lower osmolarity of caterpillar haemolymph (Weevers, 1966; Jungreis, Jatlow & Wyatt, 1973); it contained 50 mM Tris methanesulphonate instead of 150 mM. The larval muscles are not extensively tracheated and oxygen supplied to the spiracles appeared to have little beneficial effect. Instead, care was taken to keep the tracheae fluid-free and a rapid flow of saline was used to keep the preparation healthy.

For electron microscopy the animals were perfused with an aldehyde fixative (4% paraformaldehyde, 1% glutaraldehyde in 0·1 M phosphate buffer and 0·1 mM-$CaCl_2$), and then small pieces of muscle were dissected out and carried through further aldehyde fixation (2–8 h) and post-fixed in 1% osmium for 2 h. Routine methods of block staining with uranyl acetate, dehydration, and embedding in Spurrs' medium or English Araldite were used. Sections were cut with glass knives on an LKB-III microtome and stained with uranyl acetate and lead citrate. The sections were examined at 60 kV on a Philips 300 or AEI 6B electron microscope.

RESULTS

Innervation of the adult and larval muscles

The adult dl_1 is innervated by five motor neurones (Fig. 1). The presence and positions of their somata in the thoracic ganglia of both larvae and adults, previously described by Casaday & Camhi (1976), were verified by backfilling with $CoCl_2$, and extended to include the contralateral mesothoracic soma seen in the adult but not the larvae by the above authors.

In the adult, the dl_1 is divided into five separate bundles of muscle fibres, with distinct origins and insertions. The contralateral mesothoracic motor neurone

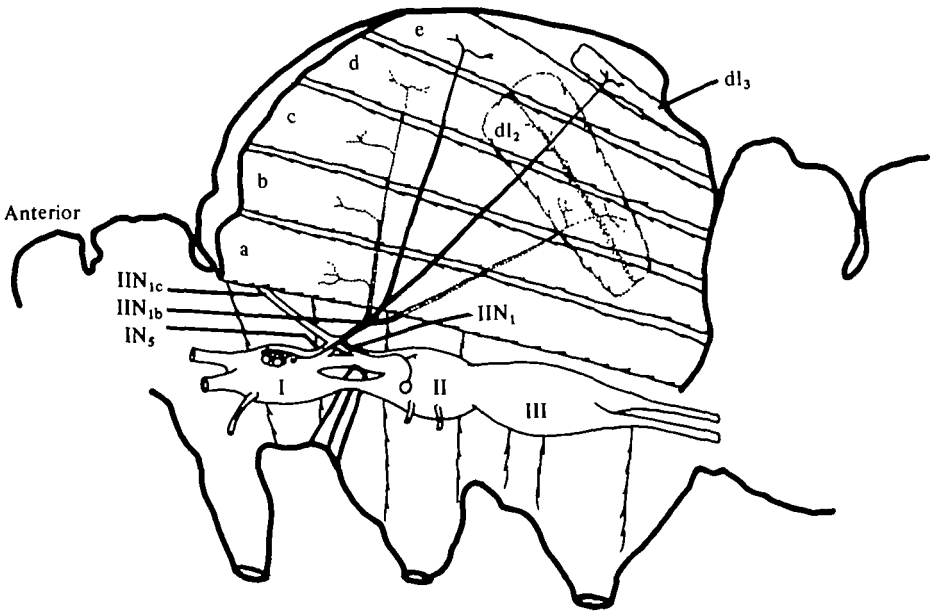

Fig. 1. Innervation pattern of the adult mesothoracic dorsal longitudinal muscle. The five bundles of this muscle are identified dl_{1a} to dl_{1e} going from ventral to most dorsal. Muscles dl_2 and dl_3 are also shown. The nerves are labelled: IN_5, the recurrent nerve; IIN_1, the main trunk of the nerve as it emerges from the mesothoracic ganglion; IIN_{1a}, large sensory branch to the wing; IIN_{1b}, carries motor axons to dl_1, dl_2, and dl_3. Four large neurones in the prothoracic ganglion send axons via the recurrent nerve to IIN_{1b}. Each innervates one of the bundles *a*, *b*, *c*, or *d*. Two smaller neurones innervate dl_2 and a third innervates dl_3 via the same route. Bundle *e* is innervated by a motor neurone whose cell body lies on the contralateral side of the mesothoracic ganglion. An unpaired medial cell, with branches in both left and right IIN_1, lies in the mesothoracic ganglion; its ultimate destination is unknown. I, II, III indicate the pro-, meso-, and metathoracic ganglia.

supplies the most dorsal muscle band (dl_{1e}), and the lower four muscle bands are innervated by neurones in the prothoracic ganglion. This conclusion is based on experiments in which the interganglionic connectives plus either the root from the prothoracic ganglion (IN_5, the recurrent nerve), or IIN_1 from the mesothoracic ganglion, was severed to eliminate input from one ganglion. The connectives distal to the other ganglion (anterior to the prothoracic or posterior to the pterothoracic) were then stimulated (Fig. 1). Intracellular recordings were made in each muscle bundle to search for innervation; in cases where there was response (excitatory junction potential, active membrane response, twitch) the nerve IIN_{1b} was cut or crushed to ensure that the response was not due to current spread across the ganglion to the nerve. Fibres in bundles *a* through *d* could be excited only via the prothoracic ganglion and the recurrent nerve, whereas unit *e* responded only when the mesothoracic pathway was stimulated. There was no indication of innervation of bundles *b*, *c*, and *d* by both ganglia as has been suggested by Nüesch (1968), nor was there any indication of double innervation of single muscle fibres. All fibres gave single, fast excitatory junction potentials to nerve stimulation.

In the larva (Fig. 2) the five motor neurones, as well as several others, supply muscle bands in the dorsal half of the mesothorax. Methylene blue staining revealed a

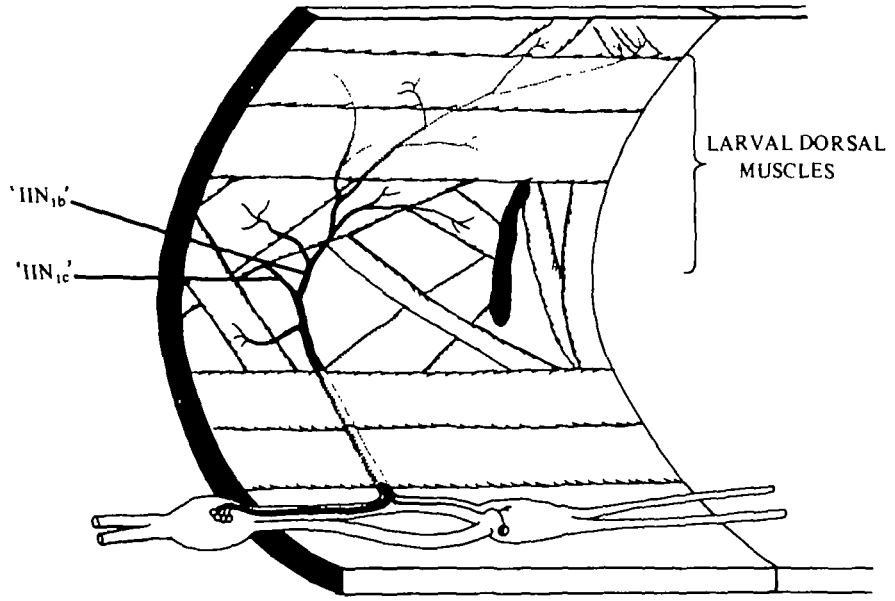

Fig. 2. Internal view of the surface layer of muscles and nerves of the larval mesothoracic segment, shown in a way comparable to Fig. 1. At the point indicated, just above the branch point of the major sensory trunk to the skin (presumably comparable to IIN_{1c}), there are at least six axons in addition to those belonging to the five large motor neurones. Subsequent branches contain the motor innervation of a stretch receptor muscle fibre and the innervation of a pair of muscle bands that may be related to dl_1. The exact fate and innervation of the individual larval muscle bands has not yet been determined.

major sensory branch of IIN_1, presumably corresponding to the wing nerve IIN_{1c} in the adult. The branch distal to this sensory trunk, 'IIN_{1b}', contained axons of the five large motor neurones as well as several others (determined by back filling with cobalt at this point). They innervate muscles that would be designated A, B, C, D, E, F, G, H, I, K, Q, R and α according to the description of the larval musculature of a similar species (Lyonet, 1762). The exact distribution of the innervation that would be comparable to that of the adult dl_1, dl_2, and dl_3 has not yet been determined for the larva. All of the above muscle bands had fast e.j.p.'s; no double innervation was seen.

Fine structure of the adult dorsal longitudinal muscle

The ultrastructure of the adult dl_1 has been described previously for another moth, *Antheraea pernyi* (Bienz-Isler, 1968), so we shall discuss primarily those features to be compared with larval muscle structure. The muscle fibres are 40–80 μm in diameter and have short sarcomeres (3–4 μm) (Fig. 3*a*). The Z-bands of adjacent myofibrils are aligned and each of a pair of T-tubules lies between the middle of the sarcomere and the Z-bands at either end. Mitochondria encircle the fibrils and extend longitudinally in columns, making up a large percentage of the muscle mass (Fig. 3*a*; Fig. 4*a*). Six to eight thin filaments surround each thick filament, forming an array in which the thin filaments lie on lines connecting adjacent thick filaments (Fig. 4*c*). This arrangement, which differs from that in vertebrate skeletal muscle, occurs in a variety of insects

(Elder, 1975) and was described by Bienz-Isler (1968) for the dl_1 of *A. polyphemus*. The T-tubules form either dyads or tryads with the sarcoplasmic reticulum, which runs longitudinally between the myofibrills and the columns of mitochondria (Fig. 3*c*). Tracheoles penetrate the muscle fibre within the lumina of the T-tubules (Fig. 4*a*). The basal lamina is thin, typically 0·004 μm.

Fine structure of the larval dorsal muscles

The larval muscle fibres that are innervated by the dl_1 motor neurones and that developmentally precede the adult dorsal longitudinal muscle fibres have the fine structure characteristic of insect 'tonic' or slow-contracting muscle. The sarcomeres of larval dorsal muscles are much longer than those of adult dl_1 fibres. The Z-bands are poorly defined and irregularly arranged (Fig. 3*b*). Ten to twelve thin filaments surround each thick filament (Fig. 4*d*). The size of larval muscle fibres is very variable and increases with age, reaching several hundred microns in the later instars. The plasma membrane of these large fibres is often deeply infolded (Fig. 4*b*). Tracheoles are seen in the clefts of the muscle fibre, but, unlike those of the adult, they rarely penetrate the T-tubules. The T-tubules are less commonly seen than in adult muscle, and they may be observed to run longitudinally as well as traversely. The T-tubules form dyadic junctions with the sarcoplasmic reticulum; tryads were uncommon (Fig. 3*d*). The sarcoplasmic reticulum surrounds the irregularly shaped myofibrils (Fig. 3*c*). The mitochondria are smaller and occupy a much smaller cross-sectional area than they do in the adult. The basal lamina is much thicker than in the adult muscle fibres, about 0·06 μm. A thinner layer is found in the clefts (Fig. 4*b*).

Neuromuscular junctions

In both adult moths and caterpillars, a motor neurone makes multiple synaptic contacts with each muscle fibre that it innervates. The fine structure of the adult junction has been described previously (Rheuben & Reese, 1978). In the present study, thin sections of larval and adult junctions were examined for differences in the following structures of physiological interest: the active zone in the nerve terminal, the postsynaptic membrane specialization, and the 'rete synapticum', a proliferation of glial cell and muscle cell processes that cover the nerve terminal. No clear qualitative differences were found between the larval and adult neuromuscular junctions. Both have a presynaptic specialization consisting of an electron-dense bar, about 0·2 μm long (as determined from longitudinal sections), around which clear vesicles are clustered. Both have a subsynaptic membrane specialization in which the inner leaflet of the muscle membrane is more densely stained than adjacent areas, and the outer leaflet appears to have regularly spaced fine protrusions. These postsynaptic membrane specializations are found on foot-like processes of the muscle fibre in the larval junction as well as in the adult. Likewise the 'rete synapticum' is equally elaborate in both stages, the glia intertwining with muscle processes in great profusion (Fig. 5).

Electrical responses of the muscle fibres

The resting membrane potential of muscle fibres of the moth *Antheraea polyphemus* has been found to be about -60 mV (Rheuben, 1972). In the present study, similar

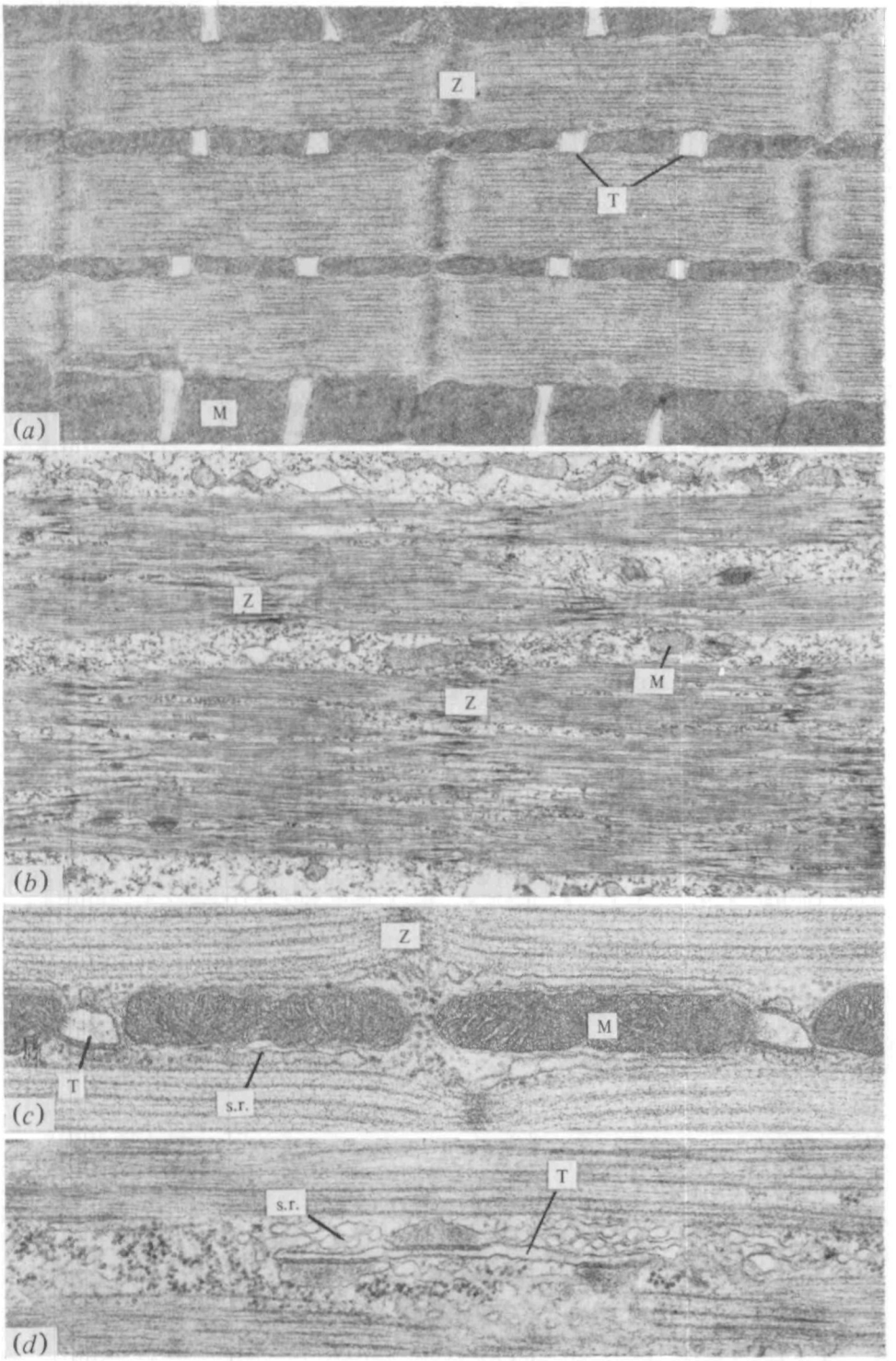

Fig. 3. Longitudinal sections of adult and larval muscle. (*a*) Adult muscle, showing two sarcomeres. × 17000. (*b*) Larval muscle, also showing roughly two sarcomeres. Note the irregularly aligned Z-bands and paucity of mitochondria. × 8500. (*c*) Junctions between the T-tubules and the sarcoplasmic reticulum, adult muscle. × 40000. (*d*) Junctions between the T-tubule and sarcoplasmic reticulum, larval muscle. × 37000. M, mitochondria; Z, Z-band; T, T-tubule; s.r., sarcoplasmic reticulum.

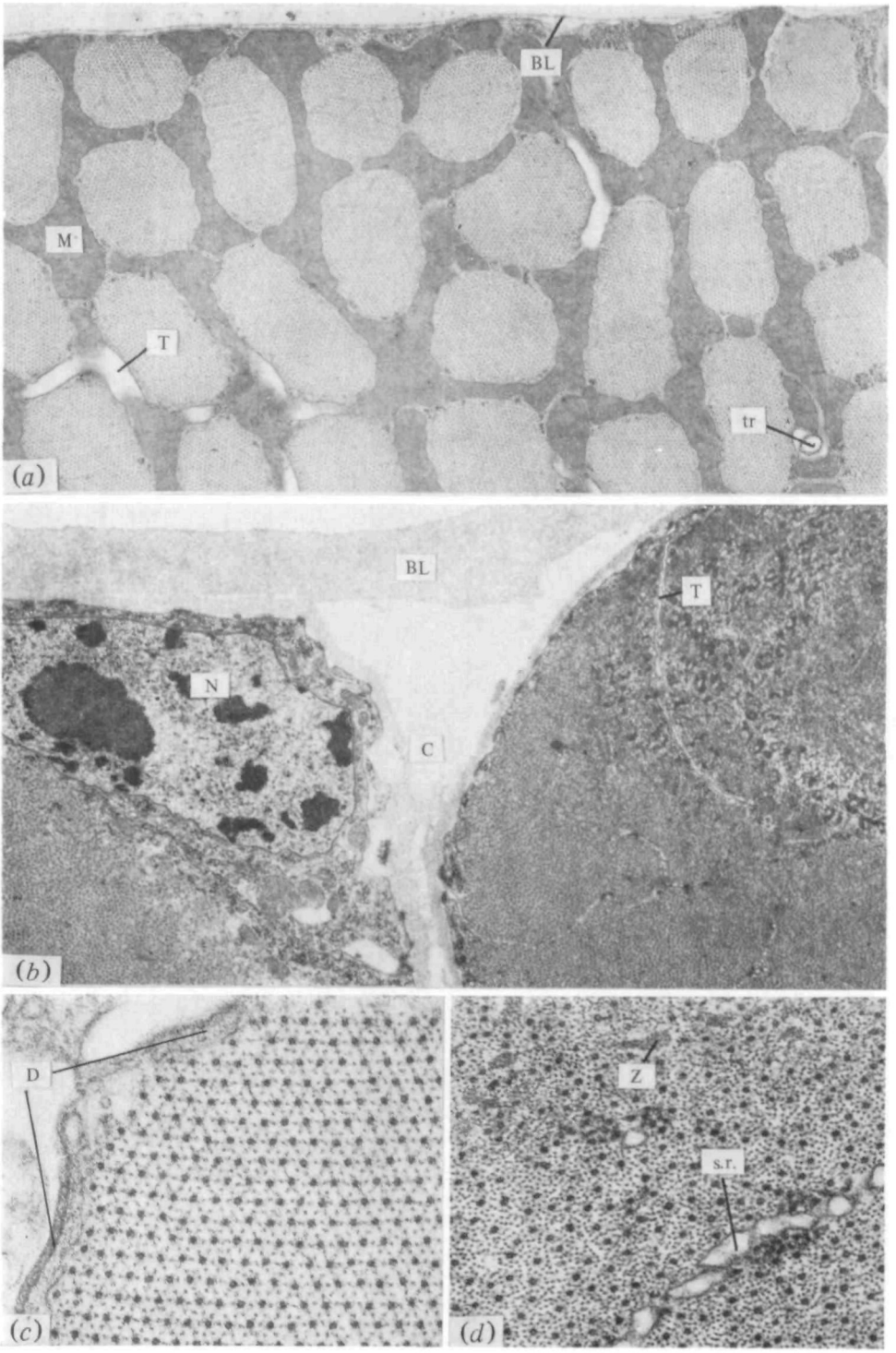

Fig. 4. Cross-sections of adult and larval muscle. (*a*) Adult muscle, showing myofibrils well separated by mitochondria and thin basal lamina. ×17000. (*b*) Larval muscle, showing clefts and thick basal lamina. Few mitochondria are seen in this section. ×13500. (*c*) Adult muscle showing the array of thick to thin filaments and two dyads. ×60000. (*d*) Larval muscle, with a higher ratio of thin filaments to each thick filament. There are areas where the section has passed through Z-disc. ×58000. BL, basal lamina; M, mitochondria; T, T-tubule; tr, tracheole; D, dyad; s.r., sarcoplasmic reticulum; Z, Z-band; N, nucleus; C, cleft.

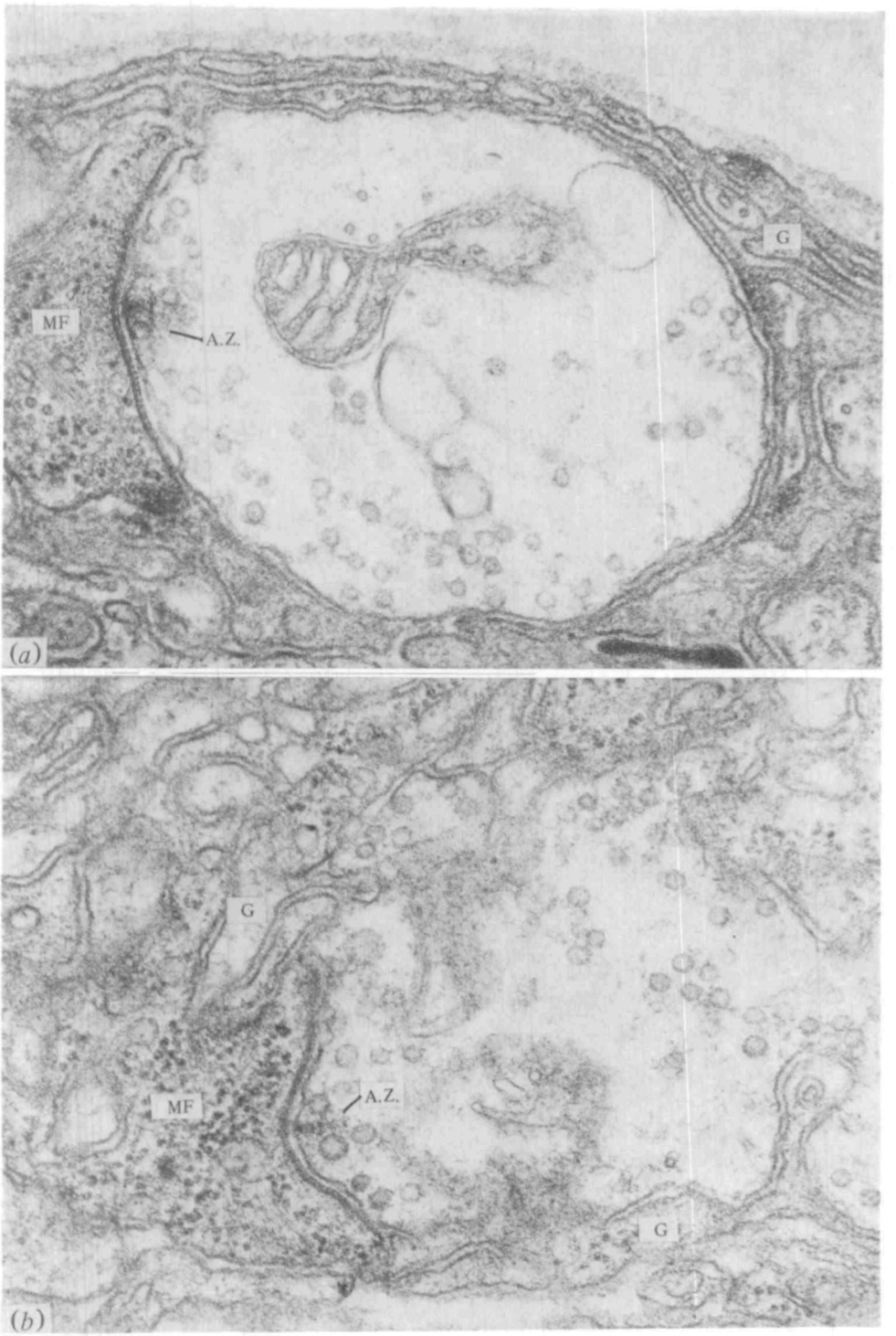

Fig. 5. Neuromuscular junctions. (*a*) Adult junction, section passing through pre- and post-synaptic specializations. (*b*) larval junction, similar planes of section. G, glial cell processes; A.Z., active zone, indicated by electron dense fuzz in nerve terminal cytoplasm; MF, process of the muscle fibre.

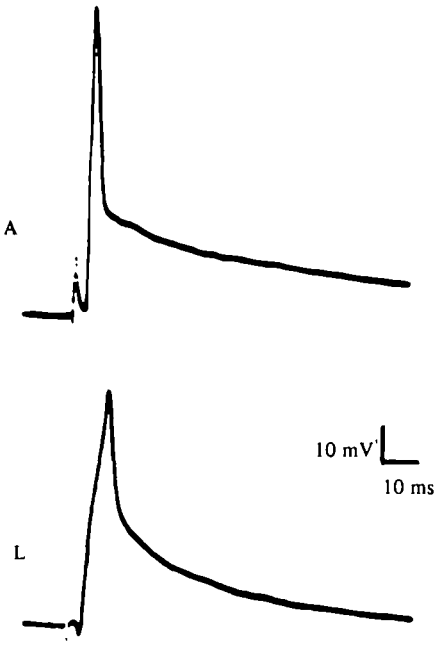

Fig. 6. Intracellular recording from adult (A) dl_1 and larval (L) dorsal muscle fibres, showing similar electrical responses to stimulation of their respective motor nerves. Both include the active membrane response.

values were found for *Manduca sexta* adult dl_1 muscle fibres; typical values were 55–65 mV. (In one preparation, the mean was 59·7 mV (S.D. 7·3; $n = 36$ fibres.) Resting potentials of larval fibres were similar to those of adult fibres, typically 50–60 mV.

In both caterpillars and moths the postsynaptic potential was large, 20–30 mV in amplitude, and it gave rise to an active membrane response (Fig. 6) that in healthy preparations exceeded 0 by a few millivolts. The apparent duration of the postsynaptic potential was prolonged by a long falling phase in both larva and adult. The duration of the active membrane response was slightly longer in the larva than in the adult. Such large-amplitude, short-duration (30–40 ms) postsynaptic potentials are typical of crustacean and insect fast neuromuscular junctions [also called phasic by Atwood (1976)]. There was an absence of facilitation to paired stimuli (Fig. 7), which is also characteristic of fast junctions. Smaller and longer postsynaptic potentials were not observed in the adult dl_1 nor in the larval dorsal muscles innervated by IIN_{1b}, although they could be recorded from other adult and larval muscles. These slow postsynaptic potentials had a duration of about 80 ms; their amplitude was usually smaller and below threshold for an active membrane response; and they sometimes facilitated with repeated stimulation.

The lack of any change in the electrical properties of the neuromuscular junction coupled with the lack of marked change in morphological features of the junction suggests that the motor neurone forms essentially the same kind of synapse on the two different forms of muscle at the two developmental stages.

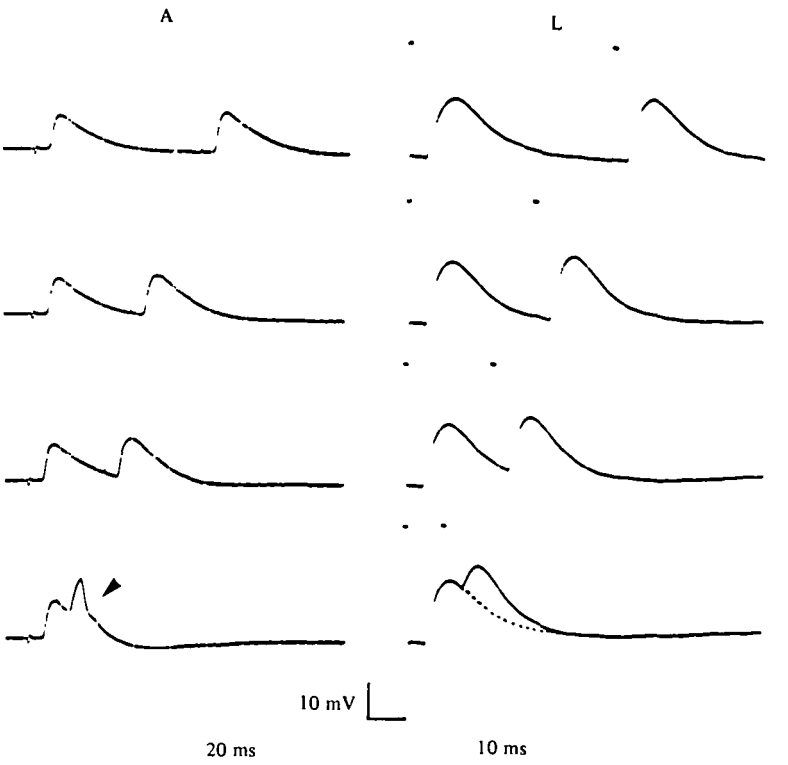

Fig. 7. Subthreshold postsynaptic potentials from adult (A) and larval (L) muscle fibres stimulated with pairs of shocks at decreasing intervals. The responses do not facilitate, but at the shortest interval the adult muscle membrane produced a small active membrane response (arrow).

Mechanical responses

The adult dl_1 responded to a single nerve stimulus with a rapid, vigorous twitch (Fig. 8). In intact preparations at room temperature the rise time was 14 ms (range 12–17 ms; S.D. 1·7 ms; $n = 48$ values from three preparations). Stimulation of the subalar muscle in intact moths gave similar values (mean 13 ms, S.D. 1·1 ms; three preparations). The mechanical response of the dl_1 in hemisected preparations was slightly slower and more variable (mean rise time 19 ms, S.D. 4·8 ms, 9 values from three preparations). These flight muscles also relaxed rapidly. The twitch duration was 26 ms (S.D. 2·7; $n = 49$) in the intact dl_1 preparation and 28 ms (S.D. 2·4; $n = 9$) in the subalar muscle.

The tension parameters varied with temperature (Fig. 9). At the flight temperature of 35 °C, the muscle reached peak tension twice as fast as at 20 °C. At these higher temperatures (32–37 °C), the twitch duration is compatible with the wingbeat cycle-time of 40 ms produced during flight at a thoracic temperature of 35–40 °C (Heinrich, 1971).

Comparisons between larval and adult muscle were based on measurements made at room temperature. The twitch of the larval muscle to nerve stimulation was markedly

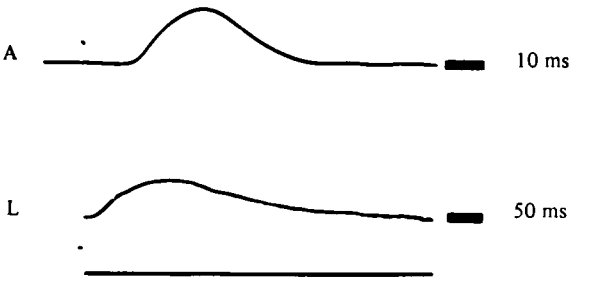

Fig. 8. Tension produced to a single stimulus in an adult (A) or a caterpillar (L) motor unit. The twitch of the larval muscle is about 7 times slower than that of the adult. The maximum tension produced during an adult twitch was at least 25 times greater than that of the larval twitch when comparing single motor units. (Values are not presented for tensions in this and subsequent figures because the *in vivo* resting lengths could not be determined exactly and the angle of force varied from preparation to preparation, especially among the larvae.)

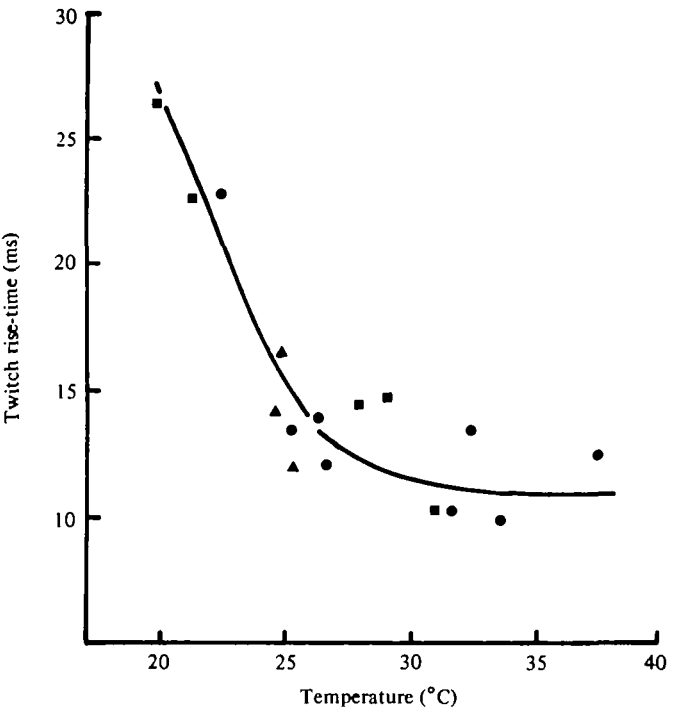

Fig. 9. Effect of temperature on twitch rise-time of adult flight muscle. (Ordinate: time from onset of tension to peak tension; ●, subalar muscle, intact animal; ▲, dorsal longitudinal muscle, intact animal; ■, dorsal longitudinal muscle, hemisected preparation.)

slower than that of the adult flight muscle (Fig. 8). In larval muscle, the mean tension rise time was 88 ms (S.D. 25·9 ms; in five preparations 59–117 ms), about 5 times longer than that of the adult. The larval twitch duration was 203 ms (S.D. 60·8 ms; range 171–312 ms), approximately 7 times longer than in the adult.

Adult and larval muscles also differed in their responses to repeated stimulation. At room temperature adult muscles, both the dl_1 and the subalar, produced only partially fused twitches when stimulated at 20/s; a tetanic contraction required more than 40 stimuli per second (Fig. 10). The ratio of tetanus tension to twitch tension was

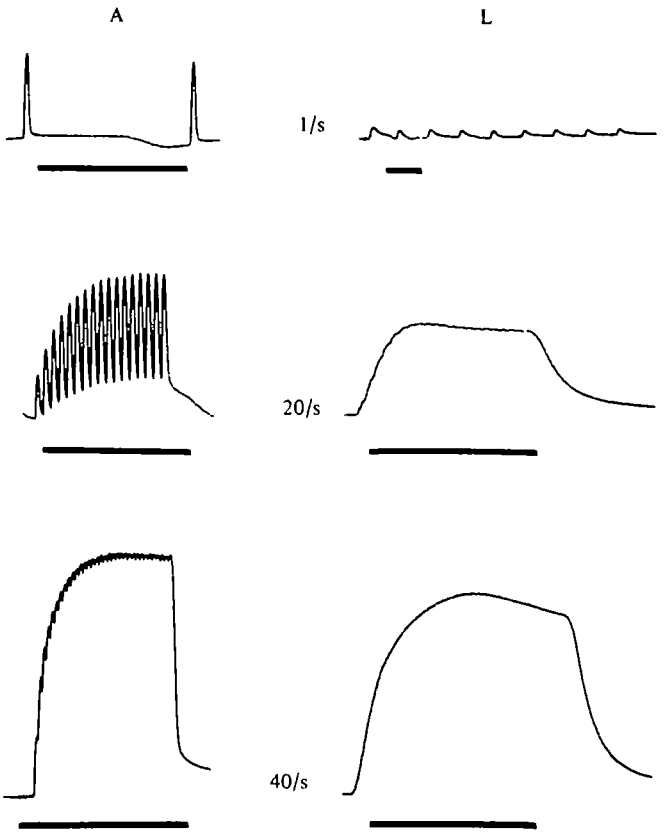

Fig. 10. Tension produced by adult (A, dl_1 muscle *b*) and larval (L, muscle band *a*) at the frequencies of stimulation indicated. Time mark for all records, 1 s. Larval and adult tensions not to the same scale. The larval muscle produced still more tension at stimulus frequencies greater than 40/s.

small, 2–6 in different preparations (7 subalar, 6 intact dl_1, 4 hemisected dl_1). Larval muscle was tetanized at a lower frequency, 20/s and the tension continued to increase with increasing frequency of stimulation up to 80/s (Fig. 11). The tetanus/twitch ratio (at a stimulus frequency of 40 or 50 per second) was about 10 times greater than that of an adult muscle under comparable conditions. The tetanus/twitch ratio in larvae ranged from 13 to 62 in five preparations; much of the variation was due to the difficulty of measuring accurately the small amount of tension produced by a single twitch of the larval muscle.

DISCUSSION

The results of the present study indicate that the adult and larval dorsal longitudinal muscles differ greatly from each other in structure and in physiological function. The properties of each muscle are well adapted to the different modes of locomotion employed by moths and caterpillars. In free flight *Manduca* beats its wings 25 times per second (Heinrich, 1971). The dorsal longitudinal muscle, which provides most of the power for the downstroke, must contract and relax rapidly. The larval muscles,

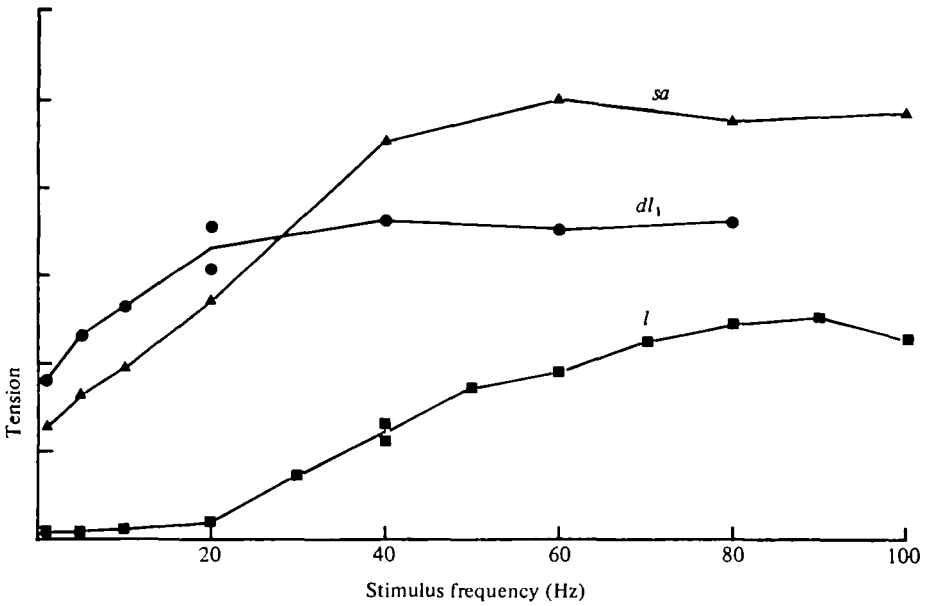

Fig. 11. Tension as a function of stimulation frequency in adult and larval muscles (Muscles: *sa*, subalar; *dl*, dorsal longitudinal; *l*, larval dorsal muscle). Ordinate: tension in arbitrary units. Scale for larval muscle is 10 times greater than for adult; no correction was made for cross-sectional area. Although larval muscle was tetanized at 20/s, increasing the stimulus frequency increased the tension produced.

on the other hand, contract slowly, and the tetanus tension is 13–62 times greater than the twitch tension. Strong, slow contractions, produced by multiple synaptic potentials, manipulate the body wall and sustain the hydrostatic skeleton that the caterpillar maintains for locomotion. The motor patterns generated by the central nervous system and transmitted via the five motor neurones during the two developmental stages are appropriately different: there are long bursts of excitatory junction potentials delivered to the caterpillar dorsal muscles and a single e.j.p. or a pair per wingbeat cycle in the adult (Kammer & Rheuben, 1976*a*).

Comparison with other arthropod and vertebrate muscles

The rapid, vigorous contraction of adult moth muscle, occurring in response to a single synaptic potential, is a property typical of 'phasic' arthropod muscles in general (Usherwood, 1962; Atwood, 1965, 1973, 1976; Parnas & Atwood, 1966; Cochrane, Elder & Usherwood, 1972) and is similar in function and contraction time to that of mammalian fast-twitch muscles (Close, 1972). The twitch duration of 24–31 ms and the tetanus/twitch ratio of 2–6 in *Manduca* at 25 °C are comparable to those of the locust dorsal longitudinal muscle (50 ms at 25 °C and tetanus/twitch ratio of 1·3–2·0 (Buchthal, Weis-Fogh & Rosenfalck, 1957; Neville & Weis-Fogh, 1963) but slower than the twitch duration of the homologous muscle used for singing in katydids (14·3 ms; Stokes, Josephson & Price, 1975). These phasic muscles in insects and crustaceans all have relatively short sarcomeres, 6–8 thin filaments surrounding each thick filament, a well-developed sarcoplasmic reticulum and transverse tubular

system, and abundant large mitochondria. Furthermore, quantitative differences in these structures can be correlated with differences in performance (insects: Elder, 1975; Josephson, 1975; crustaceans: Jahromi & Atwood, 1971).

The contractile properties and structure of the tonic muscle of the caterpillar described here are likewise similar to those of the tonic muscles of crustaeans and other insects (Jahromi & Atwood, 1971; Parnas & Atwood, 1966; Atwood & Dorai Raj, 1964; Cochrane, Elder & Usherwood, 1972; Hoyle, 1955). All these tonic muscles have long sarcomeres, unevenly aligned Z-bands, 10–12 thin filaments around each thick filament, and less sarcoplasmic reticulum. They also have similar types of contraction, giving either no response to a single stimulus, or a small prolonged contraction (insect, up to 1 s, Hoyle, 1978; crustacean, Atwood & Dorai Raj, 1964). They give extremely strong contractions to tetanic stimulation (tetanus/twitch ratio 100:1 in the locust; Hoyle, 1978).

Tonic and phasic muscles can differ both in the type of innervation they receive and in the properties of the muscle fibre membranes; these differences modify muscle performance. For example, some crustacean tonic muscle fibres are innervated by a slow axon that produces only a small junction potential to a single stimulus and there is no spike or active membrane response to large depolarizations. Spikes are usually produced, however, in the sarcolemmae of phasic muscle fibres and they are most often innervated by fast axons with large suprathreshold e.j.p.'s (Atwood & Dorai Raj, 1964; Govind & Lang, 1974). In vertebrates, non-spiking membranes are also found in certain slow muscles: chicken anterior latissimus dorsi, slow frog muscle, etc. (see Prosser, 1973), whereas the fast-contracting muscles normally produce spikes. The larval muscle described here is unusual in being innervated by a fast axon that elicits large junction potentials and an active membrane response. This difference in innervation explains the observation that the crustacean tonic muscles require a pair of stimuli to twitch, whereas the moth tonic larval muscle can produce a small twitch to a single nerve impulse.

The fast-contracting arthropod and vertebrate muscles and the slow contracting arthropod and vertebrate muscles are analogous in many respects, but they also differ in important features. To avoid the implication of identity when it does not exist, we are using the terms fast, slow-twitch, and slow to refer to the three major vertebrate muscle types, and the terms tonic and phasic for slowly and rapidly contracting arthropod muscle. In both groups of animals 'slow' and 'fast' have also been used to designate motor axons according to their conduction velocity and release properties, although this terminology also obscures the known diversity.

Properties of the motor neurones and muscle potentials

In spite of the fact that in larval and adult moths a single set of fast motor neurones impinge sequentially on tonic and phasic muscle fibres, there is little evidence for a change in their release properties. Even though small postsynaptic potentials that facilitate in response to repeated stimulation are features of the slow motor neurones that more commonly innervate tonic muscle fibres (Parnas & Atwood, 1966), in neither the larval (tonic) nor the adult (phasic) muscles did the p.s.p.'s show any marked facilitation. The postsynaptic potentials were large and relatively brief in both stages.

The fast postsynaptic potential and active membrane response that we recorded in the larval muscles of *Manduca* differ slightly in amplitude and time course from those described for other lepidopteran larvae. The time to peak (from onset of e.j.p. to peak of active membrane response) is about 5 ms for *Manduca*, *ca.* 10 ms for *Antheraea* (Weevers, 1966), *ca.* 8 ms for *Galleria* (Belton, 1969) and 2 ms for *Ephestia* (Deitmer, 1977). However, the experiments of Weevers (1966), Deitmer & Rathmayer (1976), and Patlak (1976) indicate that the amplitude and time course of the postsynaptic potential and the active membrane response are highly sensitive to small changes in extracellular Ca^{2+} and Na^{+} concentrations. The range of rise times from 2 to 10 ms could, therefore, be accounted for by the differences in the salines used or in membrane capacitance. These differences are slight relative to the characteristic differences in junction potentials of slow and fast nerve terminals.

Nerve-muscle interactions

There is considerable evidence (references in Introduction) that nerve-muscle interactions can have an effect on muscle growth and on development of certain muscle fibre properties, including contractile speed. In studies on insects, similar to those performed on vertebrates, denervation of immature muscles results in development of fibres that are smaller than normal (Nüesch & Bienz-Isler, 1972; Thommen, 1974). Similarly, denervation of mature muscle is followed by an increase in extrajunctional sensitivity to glutamate, the presumed excitatory transmitter (Usherwood, 1969; Cull-Candy, 1975). The mechanisms underlying these influences are still incompletely understood. However, the sequential innervation by a particular set of fast motor neurones of the radically different tonic and then phasic muscle fibres in *Manduca* suggests that the nerve-muscle associations in insects, though basically similar to those of other animals, can show greater diversity than previously described.

The denervation experiments of Nüesch & Bienz-Isler (1972) on a developing moth imply that the phasic morphology of the adult muscle can develop without the nerve. However, since distal nerve processes are embedded prior to denervation within the muscle anlagen (Stocker, 1974; Stocker & Nüesch, 1975; Heinertz, 1976; Rheuben, 1977), it can be argued that the nerve was in contact with the muscle during an early and critical stage of its development and thus may have 'specified' the adult muscle structure before denervation. This hypothesis requires that the nerve change its specification message to obtain the observed differences between the larval and adult stages.

Developmental plasticity

Structural changes such as shortening of sarcomere length between larval and adult muscle fibres may represent an extreme example of developmental plasticity described in other animals. Lang, Govind & Costello (1978) and Govind & Lang (1978) have shown that in the lobster there is a change in the composition (relative numbers of long and short sarcomere fibres) of closer muscle with age and with the development of the claw into either a cutter or a crusher. There is no evidence for degeneration of individual fibres (F. Lang, personal communication); hence, it is possible that in this preparation there is direct transformation of fibres from one type to the other.

The sequential changes seen during development in arthropods can be compared to those observed in mammals. There is an increase in contraction velocity during the first few weeks after birth (extensor digitorum longus; Buller, Eccles & Eccles, 1960) that is accompanied by a change in the amount of fast-twitch myosin that is synthesized (Gauthier, Lowey & Hobbs, 1978), for example. However, in vertebrates no morpholical changes have been observed to accompany the change in contraction speed.

There is some evidence that the activity produced in a muscle by differing amounts of neuronal excitation is important in modulating contractile properties. This conclusion is derived from muscle changes after crossing fast and slow nerves (Buller, Eccles & Eccles, 1960) and stimulating the nerve trunk to the reinnervated muscle (Salmons & Sréter, 1976). Since the motor pattern to the larval tonic muscles is very different from the flight motor pattern to the adult muscles, and since during metamorphosis there is both a developmental change and much electrical activity in the behaviourally quiescent pupa (Kammer & Rheuben, 1976*b*), one should consider the possibility that activity plays an important role in the development of the adult structure. However, since the adult thick to thin filament ratios and sarcomere lengths do develop in denervated moth muscles, patterned activity is likely only to affect muscle structures quantitatively or to influence characteristics not considered here.

In summary, these motor neurones are associated with different types of muscle in larva and adult. Their release properties and neuromuscular junctions are not markedly changed, although the motor patterns they transmit are different. The available evidence would indicate that motor activity *per se* is not responsible for the change in muscle structure. Other kinds of trophic specification and influences cannot be ruled out.

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