

The Mechanism of Dual Responsiveness in Muscle Fibers of the Grasshopper *Romalea microptera*

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ABSTRACT Dually innervated *Romalea* muscle fibers which respond differently to stimulation of their fast and slow axons are excited by intracellularly applied depolarizing stimuli. The responses, though spike-like in appearance, are graded in amplitude depending upon the strength of the stimuli and do not exceed about 30 mv. in height. In other respects, however, these graded responses possess properties that are characteristic of electrically excitable activity: vanishingly brief latency; refractoriness; a post-spike undershoot. They are blocked by hyperpolarizing the fiber membrane; respond repetitively to prolonged depolarization, and are subject to depolarizing inactivation. As graded activity, these responses propagate decrementally.

The fast and slow axons of the dually responsive muscle fibers initiate respectively large and small postsynaptic potentials (p.s.p.'s) in the muscle fiber. These responses possess properties that characterize electrically inexcitable depolarizing activity. They are augmented by hyperpolarization and diminished by depolarization. Their latency is independent of the membrane potential. They have no refractory period, thus being capable of summation. The fast p.s.p. evokes a considerable or maximal electrically excitable response. The combination, which resembles a spike, leads to a twitch-like contraction of the muscle fiber. The individual slow p.s.p.'s elicit no or only little electrically excitable responses, and they evoke slower smaller contractile responses. The functional aspects of dual responsiveness and the several aspects of the theoretical importance of the gradedly responsive, electrically excitable component are discussed.

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This work was reported in preliminary abstracts (9, 10). It was carried out with partial support from the following: Muscular Dystrophy Associations of America, National Institutes of Health (B-389 C2), National Science Foundation (NSF G-5665), United Cerebral Palsy Research and Educational Foundation, and from the Marine Biological Laboratory under its ONR contract.

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Received for publication, April 24, 1959.

INTRODUCTION

Several grades of contractile activity, in many cases initiated by clearly different nerve supplies (polyneuronal innervation) are characteristic of arthropod muscles (*cf.* references 33, 34, 36, 44). In insect muscles a "fast" innervation typically evokes twitches, while a separate "slow" innervation induces smaller, summative contractions. The two varieties of mechanical responses are associated with different types of electrical activity, both of which may occur in the same muscle fiber (34, 44, 48). The fast nerve fiber elicits a large, relatively brief pulse which may have an overshoot and then resembles a spike. The slow nerve fiber evokes "junctional" potentials which, like the contractions, are small and graded. If the stimuli to the slow nerve are closely spaced they may produce "spikes" of various heights.

Beginning with the first studies with microelectrode recording in crustaceans (13, 14), it has been shown that various arthropod muscle fibers can be directly stimulated by electrical pulses (*cf.* references 11, 12, 15, 26, 27). It has also been noted that the evoked potential does not correspond to a spike (*cf.* references 12 and 13), in the classical sense; *i.e.*, as an all-or-none, decrementlessly propagated response. The electrically excited activity of the dually responsive muscle fibers appears, on the contrary, to propagate with decrement, as has been shown in the present work.

The theoretical problem involved was considered briefly in the initial formulation (1, 3, 16) of the distinction between electrically excitable, conductile activity and transmissional, electrically inexcitable postsynaptic potentials (p.s.p.'s). It was suggested that the dually responsive muscle fibers operate with a combination of two graded potentials, one a p.s.p., the other an electrically excitable response.

This paper reports experiments designed to explore the properties of the electrical activities of insect muscle fibers specifically for the nature of their dual responsiveness and with reference to the above suggestion. The problem gains general interest from the findings that eel electroplaques (1, 2), squid giant axons (25, 37, 38), and cardiac muscle (39) exhibit graded responsiveness under various experimental conditions. The theoretical aspects of graded responsiveness have been discussed elsewhere (17, 18, 21, 24).

Methods

Extensor muscles of meso- and metathoracic legs and also flexors of the metathoracic legs of the grasshopper *Romalea micropetera* were used in the present experiments. Many individual muscle fibers, usually on both sides of the 23 preparations, were probed with microelectrodes of the conventional variety (41, 43). The muscles were studied *in situ*, prepared according to methods previously described (31), with the

nerves containing the slow and fast axons exposed separately and placed upon individual pairs of stimulating electrodes. The trunks in which the slow and fast axons leave the ganglion are similar anatomically to, and appear to be homologous with those designated as 3b and 5 in locusts (32).

The stimuli delivered to the nerves and also directly to muscle fibers by an intracellular electrode were square pulses, controlled as to strength, duration, and frequency of repetition. They were applied through radio frequency isolation circuits. Two oscillographic traces were available for simultaneous intracellular recording. Coupling of the microelectrodes to the oscillograph was through transistorized negative capacity amplifiers with low input grid-current (4). The experiments were performed at room temperatures which ranged from 22 to 26°C. The saline solution used was similar to that employed by Hoyle (30) for locust preparations.

RESULTS

A. The Neurally Evoked Electrical Activity of Romalea Muscle Fibers

The membrane potential of the muscle fibers usually ranged between 50 and 70 mv., inside negative. However, smaller values, as low as 30 mv., were also encountered (Fig. 1 *g*) which may have been due to faulty penetrations or to damage resulting from muscle movements (Fig. 1 *i*).

Examples of the responses to stimulation of the slow and fast axons are shown in Fig. 1. Dually innervated muscle fibers (*g-i*) were encountered rarely in the main body of the extensor muscle of the metathoracic leg, but were common in the flexor muscle of this leg and in the extensors of the mesothoracic leg. Only the fibers attached to the proximal border of the femur in the metathoracic leg were commonly dually innervated. These were the fibers most frequently used in the present experiments.

Some of the proximal fibers responded only to the fast axon. A small number responded only to the slow nerve, these being the first fibers encountered in any insect not to have a fast innervation (33). They were distinguished anatomically by a very rich tracheal supply, and could not be excited to activity by intracellularly applied electrical stimuli. Some of these muscle fibers produced particularly large and long lasting potentials (Fig. 1 *a*). The slow potentials of the dually responsive muscle fibers were in general rather similar in form (Fig. 1 *a-c; g-i*). Sometimes the slow response of dually innervated fibers carried a small pulse-like component (Fig. 1 *c*).

The activity evoked by the fast axon always appeared spike-like (Fig. 1 *d-i*), but varied in amplitude. In some fibers the response developed an overshoot. This pulse-like response arose out of a longer lasting depolarization which varied considerably in magnitude and form, resulting in a greater

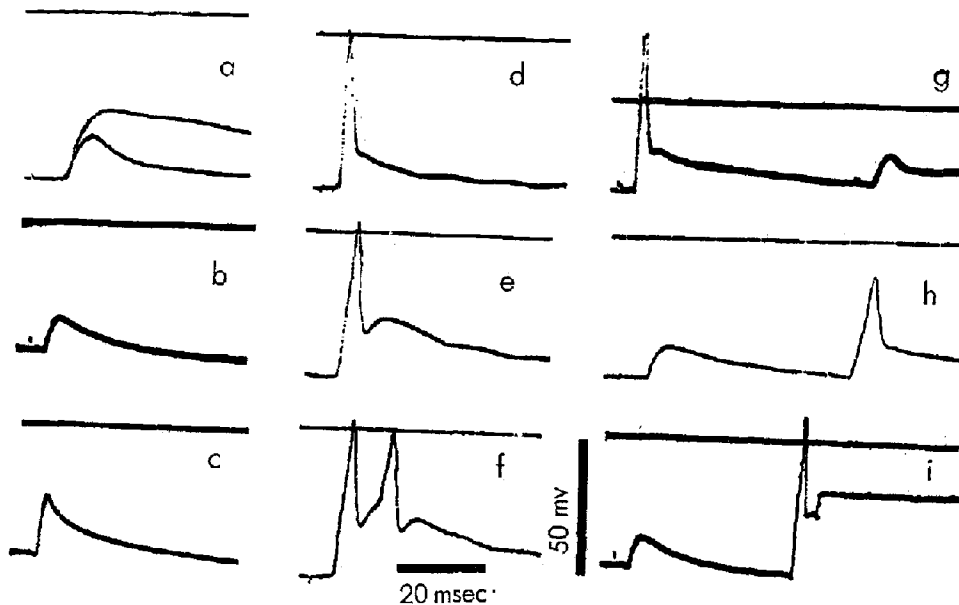


FIGURE 1. Varieties of "slow" and "fast" responses in different muscle fibers. *a*: Superimposed records of slow p.s.p.'s in two fibers of the same preparation. The larger response was from a muscle fiber that was electrically inexcitable and did not respond to stimulation of the fast axon. *b, c*: Another preparation, the response in *c* has a small pulse-like electrically excitable component. *d*: Fast response from a flexor muscle fiber. *e, f*: An extensor muscle preparation in which a large prolonged p.s.p. was seen, which on occasion evoked a second spike-like response. *g-i*: Examples of responses evoked by both innervations of the same muscle fiber; *g*, from a flexor muscle; *h*, a low amplitude pulsatile component; *i*, the muscle twitch evoked by the fast response reduced the resting potential. The zero reference is shown on the upper trace of each recording, except that the resting potential of the smaller p.s.p. in *a* was 40 mv.

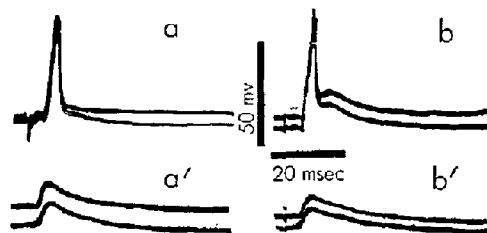


FIGURE 2. Similarity of neurally evoked responses at two recording loci in the same muscle fiber. Fast and slow responses *a, a'* are from different fibers of one preparation; *b* and *b'*, from another. Resting potentials: 40 mv. in *a*, 50 mv. in others. Recording electrodes 1.7 mm. apart in *b'*, about 1 mm. in others. Note identical onset of activity at both recording loci in all four responses.

variety of fast responses than was the case for the slow responses. The longer lasting component of the potential produced by the fast axon sometimes was so large as to elicit two "spikes" (Fig. 1 *e, f*). In other preparations (*cf.* Fig. 11) the long lasting response was small, or nearly absent.

When the neurally evoked responses were recorded simultaneously at two loci of the same muscle fiber they were found to be nearly identical (Fig. 2). Although the distances between the two recording electrodes in these experiments were comparable to the length constant of the muscle fibers,¹ the responses developed almost simultaneously at both loci. These results agree with the anatomical finding that the multiterminal innervation of insect muscle fibers by both fast and slow axons may be as closely spaced as 40 to 60 μ apart (33, 45, 48). The similarity of activities recorded at different sites suggests that the synaptic excitation of a given muscle fiber tends to be everywhere of about equal potency. Variations between the potentials recorded from different sites did occur, however, in the *Romalea* fibers as seen in record *a* of Fig. 2. This indicates that the synaptic activity evoked in different regions was not necessarily uniform.

B. Responses to Direct Stimulation

Brief intracellularly applied depolarizing stimuli, increasing in strength from subliminal to supramaximal, produced a series of graded responses (Fig. 3). The larger responses appeared to be diphasic because of an "undershoot" which developed with the depolarization of the membrane. The response reached a maximum value which was always appreciably less than the resting potential (*g, h*), usually about 30 mv. As the stimulus increased, the response was lifted on a raised baseline of applied depolarization, to give the false appearance of an overshoot. The response developed with vanishingly small latency when the stimuli were made very strong.

The pulse-like electrically excitable response was subject to refractoriness (Fig. 4). The absolutely refractory period lasted at least 7 msec. (*a, b*) and relative refractoriness some 25 msec. (*f, g*).

Stimulation of a fiber by longer depolarizing pulses (Fig. 5) evoked a train of responses. The frequency of these increased as the stimulus was increased. Initially, the responses were small and prolonged (*a*), having an appearance similar to that of local responses in cells which can also produce all-or-none spikes. The undershoot increased markedly and the pulse-like form of the first response of the train became clear (*b*), but the growth of the undershoot

¹ The electrical circuit properties of the muscle fibers were not studied systematically in the present work. The length constant in four experiments ranged between 1.5 and 2.0 mm. (average 1.7 mm.). The time constant was 5.6 to 8.3 msec. (average 6.6 msec.).

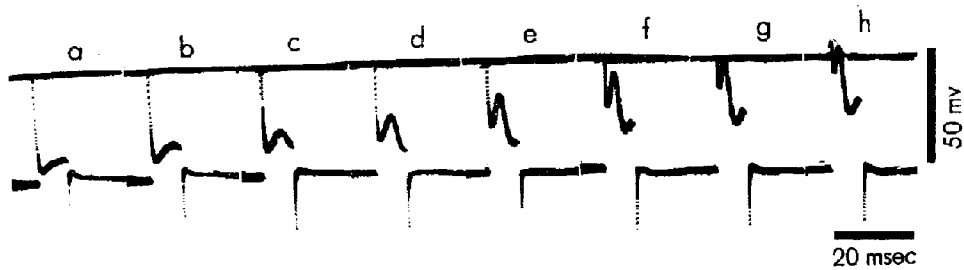


FIGURE 3. Graded responses produced in a muscle fiber by intracellularly applied depolarizing pulses. The magnitude of the depolarization was progressively increased in the series from *a* to *h*. The response in *h* was nearly of the same over-all amplitude (peak-to-peak) as that of *g*, but appeared to overshoot because it registered on a more elevated baseline produced by the stronger depolarizing stimulus. The latency of the response decreased with stronger stimulation and an "undershoot" gave the appearance of diphasicity.

and, indeed, of the amplitude of the repetitive responses was slower. The initial response reached a maximal value first, the repetitive activities continuing to grow.

The membrane depolarization produced by the intracellularly applied current was essentially "clamped" at a value between the critical firing level of the electrically excitable responses and the undershoots in which they terminated. However, the muscle fiber, during the course of stimulation, gradually lost this "clamping" capacity, behaving more and more like a generator of high internal impedance. With this loss there was also associated a diminution of repetitive responsiveness (*i-l*; *m-p*). The threshold

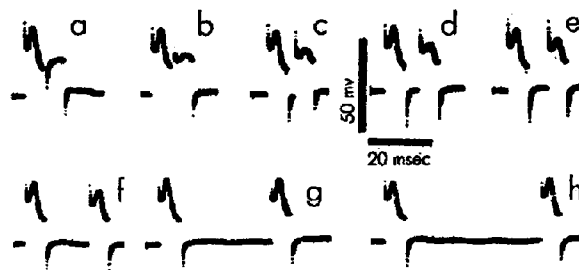


FIGURE 4. Refractoriness of directly evoked electrically excitable component of the muscle fiber response. Absolute refractoriness is seen in *a*. Relative refractoriness persisted for more than 25 msec. (*f*, *g*).

depolarization required to evoke an initial response of a given amplitude increased (*i-l*). The initial response retained its amplitude although the amplitude of the subsequent responses decreased. The train of activity therefore appeared as a damped oscillation on the baseline of the applied depolarization. With further experimental manipulation of the fiber these

changes progressed (*m-p*). The maximal initial response was still of approximately the same amplitude (*cf. k* and *p*, in which the depolarizing stimuli were nearly equal). Its undershoot, however, diminished and the pulse therefore appeared broadened. The repetitive oscillations became smaller (*n-o*) and tended to disappear with stronger depolarizing stimuli (*p*). Presumably these changes are linked with processes analogous to sodium inactivation in squid giant axons (28, 29).

Graded responses of electrically excitable membrane can, as a rule, propagate only decrementally, since the local circuit current of even maximal activity at one site is subject to attenuation in its electrotonic spread. The activity generated in the insect muscle fibers by an intracellularly applied stimulus was propagated in this way (Fig. 6). Depending upon the parameters of the stimulus and the state of the muscle fiber (as shown in Fig. 5) the response to a 20 msec. pulse, observed close to the site of stimulation, de-

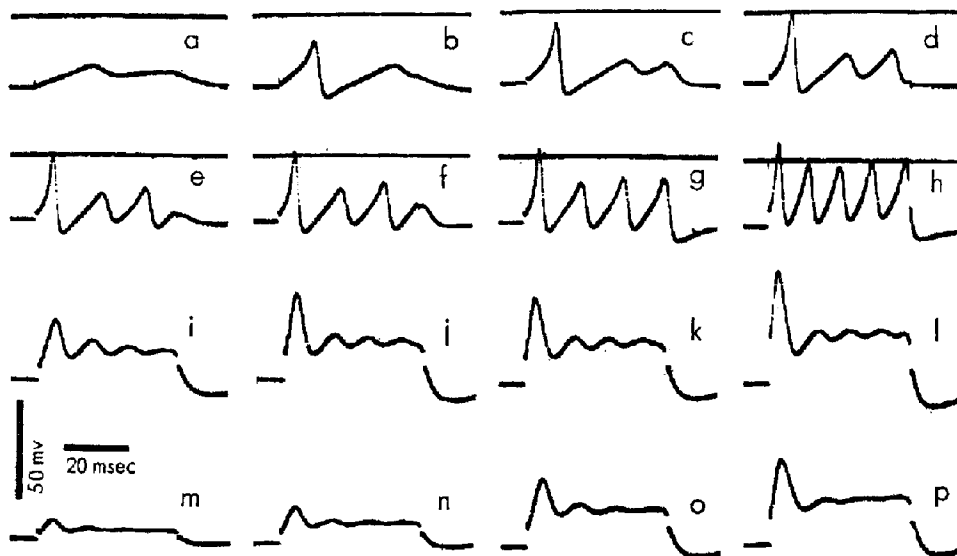


FIGURE 5. Direct excitation of muscle fiber by long depolarizing pulses applied through an intracellular electrode. Increasing intensities of stimulation (*a-i*). *a*: Oscillatory "local responses" became spike-like but graded activities with increasing stimulation. *b-h*: Frequency of pulses also increased. *i-l* and *m-p*: Two repetitions of the stimulations in same fiber led progressively to decrease in capacity for repetitive responses.

veloped with a pattern of various degrees of repetitive activity. The general pattern of these potentials was maintained at sites farther removed from the stimulus, but the amplitudes were markedly reduced, that of the first, pulse-like component being reduced the most. That the potentials were not merely spread passively by electrotonus is shown in the case of Fig. 6 C. The stimulus

was about twice as strong as for the experiments shown in *A* and *B*. The stimulus which evoked a maximal initial response at the stimulation site in *C* also evoked a maximal first response at a site 0.5 mm. away, where the local depolarizing stimulus was much smaller. Spread of excitation, not only from the stimulus itself, but also as an excitatory contribution by local circuit action from the graded response has also been found in eel electroplaques (1, 2) and may be observed in the local response of squid axons (*cf.* reference 23, Fig. 6).

The data presented above show that the directly evoked responses of *Romalea* muscle fibers, produced by intracellularly applied electrical stimuli, in a number of respects resemble spikes of axons or skeletal muscle fibers, tissues which also exhibit electrically excitable activity (20). They

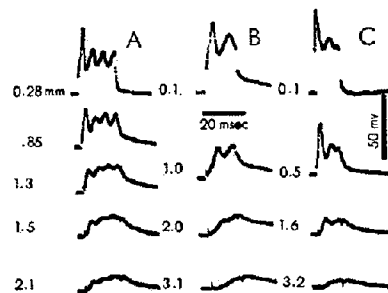


FIGURE 6. Decremental spread of electrically excitable activity. Three different experiments (*A*, *B*, *C*). An intracellular stimulating electrode delivered a constant depolarizing current, which was monitored on a second trace (records not shown). An intracellularly recording electrode was inserted in the muscle fibers at the distances from the stimulating site shown to the left of each column. Note that the general pattern of the response was maintained at the different distances, but the initial large pulse was attenuated more rapidly by electrotonic losses than were the subsequent components.

differ from the latter, however, in being graded rather than all-or-none, and therefore capable only of decremental propagation. It is also clear that the longer lasting depolarizing potentials evoked by neural stimuli are not produced by the electrical excitation. The next section presents data to show that the neurally evoked responses have properties which classify them as electrically inexcitable p.s.p.'s.

C. Differences Between Pulse-Like and Electrically Inexcitable Components of the Neurally Evoked Responses

These differences were demonstrated in several kinds of experiments. The slow response (Fig. 7 *a-d*) progressively augmented with increasing hyper-

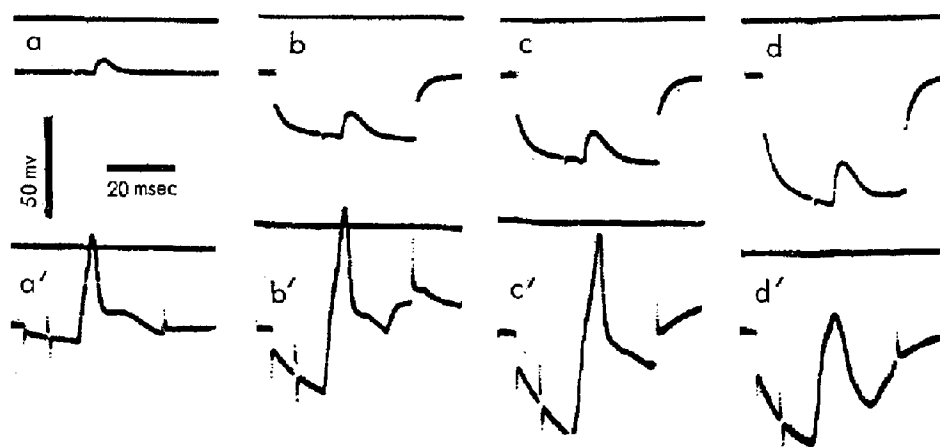


FIGURE 7. Different effects of hyperpolarization on p.s.p.'s and electrically excitable component of muscle fiber responses. *a-d*: The slow p.s.p. augmented progressively as the membrane potential was raised by current from an intracellular electrode. *a'-d'*: In another fiber, the fast response was fractionated by the same procedure. Hyperpolarization (*b'*, *c'*) at first augmented both the p.s.p. and the electrically excitable spike-like component. This response then developed later on the p.s.p., leading to a pronounced inflection in the rising phase. More intense hyperpolarization markedly diminished the pulse-like component, the p.s.p. remaining large.

polarization of the muscle fiber, but did not undergo any marked change in form or in latency. The fast response, however, under the same conditions clearly became separated into two components (*a'-d'*). One was progressively delayed with increasing hyperpolarization and then was nearly or entirely abolished. The remaining component increased in size when the membrane was hyperpolarized, but its latency was not affected. Thus, all, or nearly all, of the slow response represents a p.s.p. apparently too small to evoke the pulse-like electrically excitable activity. The latter is evoked, however, by the larger p.s.p. produced by the fast axon.

The growth of the fast p.s.p. with hyperpolarization appeared to be larger than the increase in the slow p.s.p. This, however, was probably due to the distributed nature of the innervation and the electrical characteristics of the system. The muscle fiber was most hyperpolarized in the immediate vicinity of the intracellular stimulating and recording electrodes which were close together. At some indeterminate distance, full spike-like responses must have developed. Their electrotonic spread into the hyperpolarized region was probably facilitated by the higher resistance of the hyperpolarized and inactive electrically excitable membrane.

Another of the properties characteristic of depolarizing p.s.p.'s is their

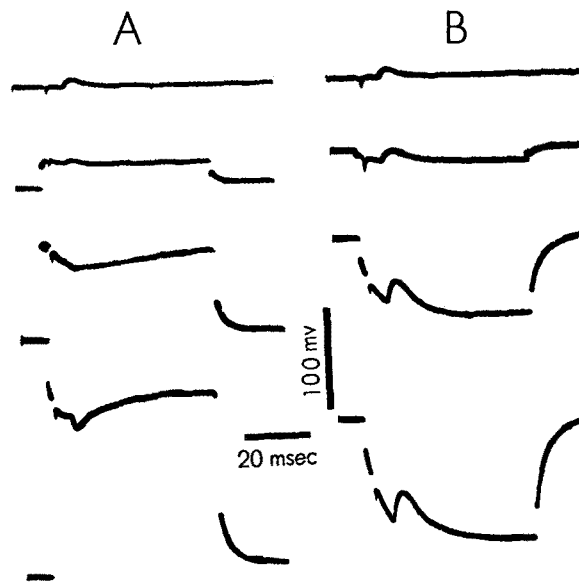


FIGURE 8. Modification of slow p.s.p. by changing membrane potential of the muscle fiber. *A*: Diminution and reversal of the p.s.p. by depolarizing the fiber through an intracellular stimulating electrode. *B*: Hyperpolarization of the same fiber augmented the p.s.p.

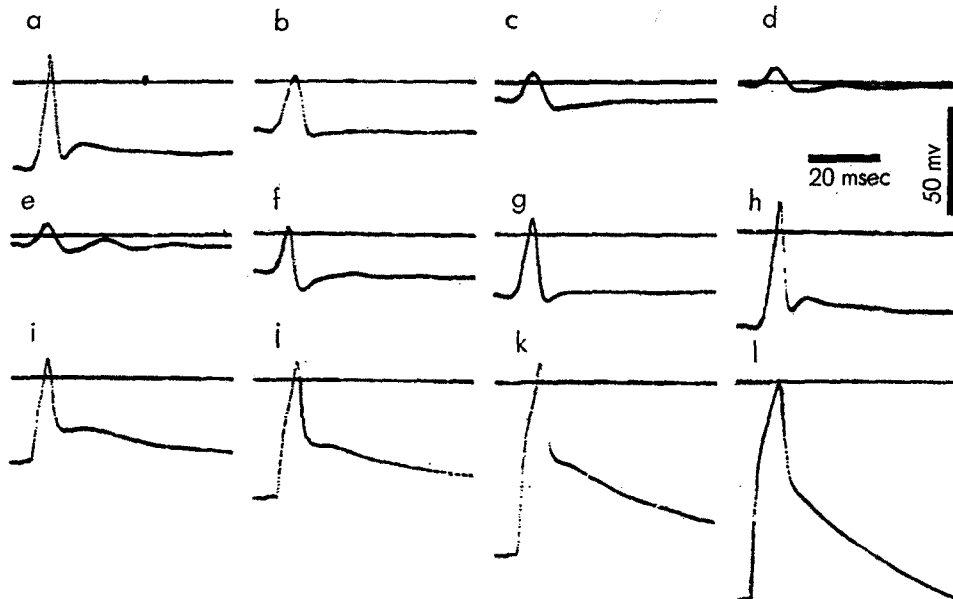


FIGURE 9. Effects of prolonged changes in membrane potential on the responses evoked by the fast axon. *a, h, i*: Responses at the resting membrane potential. *b-d*: Stepwise depolarization of the fiber with an intracellularly applied current. *e-g*: Stepwise return toward the resting potential: *j-l*: Progressively increasing hyperpolarization of the muscle fiber.

diminution and inversion when the membrane is depolarized (20). This effect was also observed in the case of the slow potential of *Romalea* muscle fibers (Fig. 8 A). The response was augmented by hyperpolarizing the same fiber (B). The latency of the slow p.s.p. was not altered despite the large changes of membrane potential in both directions. When the fast response was evoked during depolarization of the membrane (Fig. 9 a-h) two changes

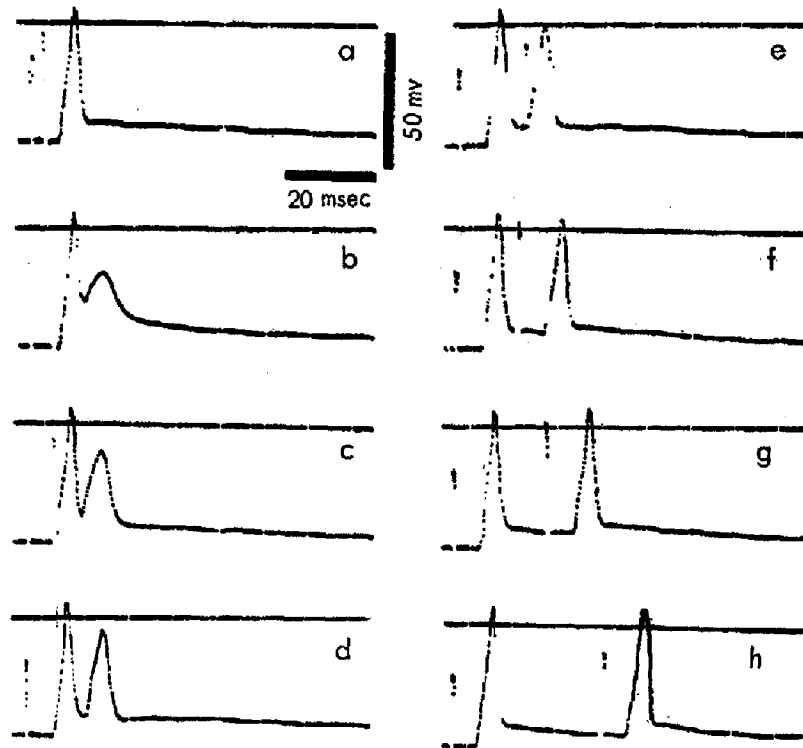


FIGURE 10. Refractoriness of the electrically excitable component evoked by stimulating the fast axon. *a*: Two stimuli were delivered within the refractory period of the nerve and only the fast response of the muscle occurred. *b*: The second stimulus evoked a p.s.p. *c*, *d*: The p.s.p. evoked a small, electrically excitable, pulse-like component during the relative refractory period. *e* to *h*: Progressive recovery of responsiveness of the electrically excitable component of the muscle fiber.

developed simultaneously. The spike-like electrically excitable component was inactivated (as in Fig. 5) at the same time that the underlying fast p.s.p. was diminished. The difference in the properties of the two components was therefore not well demonstrated by depolarization. That the fast p.s.p. was present was shown by hyperpolarizing the muscle fiber (*i-l*).

Absence of refractoriness is also a characteristic property of electrically inexcitable responses (20). The second of two stimuli to the fast nerve, de-

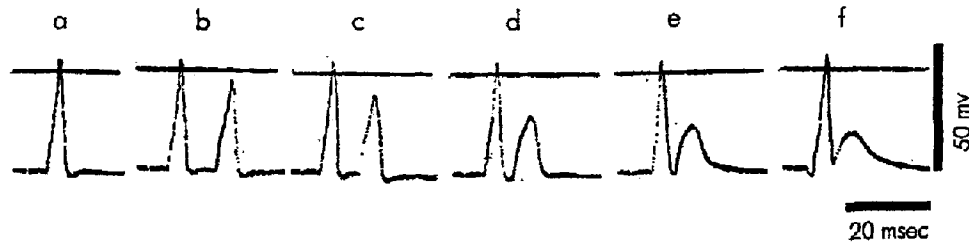


FIGURE 11. Disclosure of p.s.p. not seen in the individual fast response. As the response evoked by the second of two stimuli to the fast axon fell earlier in refractoriness after a conditioning response, the spike-like component was delayed (*c-e*) as well as diminished. A prolonged p.s.p. was then disclosed, and in *f* was probably present without an electrically excitable response component.

livered in the absolutely refractory period of the muscle fibers (*cf.* Fig. 4), evoked a second p.s.p. in the latter (Fig. 10 *a* and *b*). As the second stimulus fell later, the pulse-like response appeared and gradually augmented in amplitude (*c, d*). The individual responses shown in Fig. 10 carried a prolonged depolarization indicative of the p.s.p. out of, and on which, the spike-like component developed. The first p.s.p. was masked in the responses

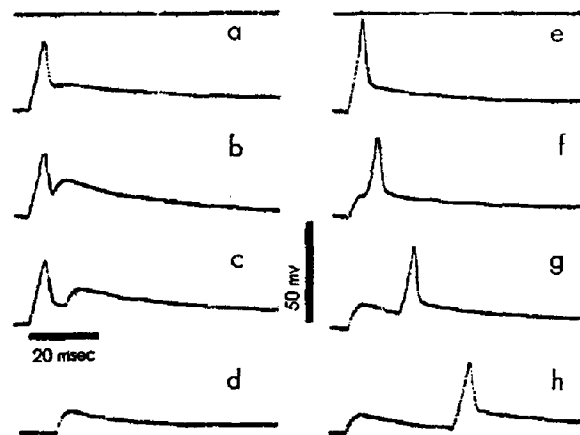


FIGURE 12. Summation of fast and slow responses. *a-d*: The fast response preceded the slow. *a*: Fast response in isolation; *d*: slow response. The two summed without refractoriness in *b* and *c*. *e-h*: The slow response preceded. *e*: When fast response occurred simultaneously with slow there was a slight increase of the spike-like component.

shown in Fig. 11 *a*, except for an inflection on the rising phase. The inflection became more marked when a second response was evoked during the relatively refractory period of the muscle fiber (*b, c*), and as the second electrically excitable response was diminished and abolished (*d-f*), the large p.s.p. was uncovered.

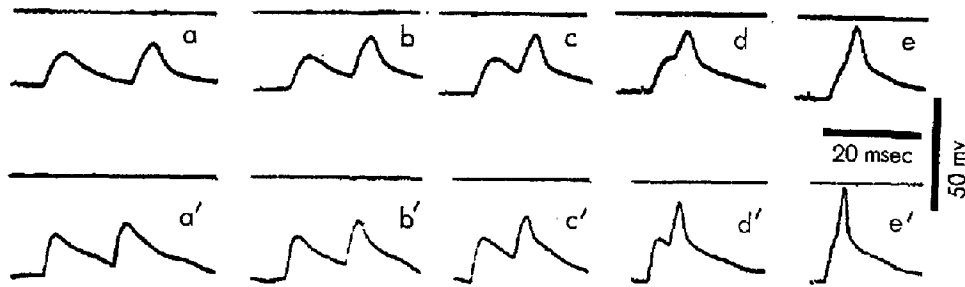


FIGURE 13. Summation of slow p.s.p.'s. *a-e*: Only a small pulse-like component was produced by the summated synaptic depolarization. *a'-e'*: Another fiber in which the spike-like electrically excitable response evoked by the summed p.s.p.'s was larger. This component became clearly noticeable in *e'* and the total response in *e'* resembled that evoked by the fast nerve fiber.

The slow p.s.p. also developed during the falling phase of the electrically excitable component of a conditioning fast response (Fig. 12 *b*). A slow p.s.p. preceding a fast response did not cause the electrically excitable membrane to become refractory. A pulse-like response evoked by the fast nerve therefore occurred when the two stimuli were simultaneous or at any interval apart. Indeed, the depolarization evoked by the fast and slow nerves summed as shown by the somewhat augmented pulse-like response in *e*. The interaction was surprisingly brief, however, while the summed excitatory effects of slow p.s.p.'s were more marked (Fig. 13).

DISCUSSION

Several kinds of evidence have demonstrated that in *Romalea* muscle fibers there exist the two fundamentally different kinds of electrical activity that are characteristically found (20) in cells which are excited by transmissional, synaptic processes, as well as by direct electrical stimulation.

Most of the muscle fibers studied reacted to intracellularly applied depolarizing stimuli (Figs. 3-5), with a response which in appearance was spike-like, but which differed from a spike in that it was a graded activity depending in magnitude upon the size of the stimulus (Fig. 3). As the stimulus was increased the latency of the response was diminished and became vanishingly brief. The response had a refractory period (Fig. 4); it was inactivated by a prolonged depolarization (Fig. 5) and blocked by hyperpolarization (Fig. 7). It propagated actively by local circuit excitation, but decrementally (Fig. 6). These properties all belong in the constellation that characterizes electrically excitable membrane (20). The chief difference between the properties of responses of *Romalea* muscle fibers and those of axons or verte-

brate skeletal muscle fibers is the graded responsiveness of the insect muscle fibers. However, there is ample evidence that cells which normally respond with all-or-none activity under various conditions may respond gradedly (17, 18, 24). In the original descriptions of graded responsiveness the generality of the phenomenon was indicated (3, 16) and it was suggested specifically that insect and crustacean muscle fibers might normally be gradedly responsive. It has been demonstrated recently also in heart muscle (39) and in supramedullary neurons of the puffer (5).

The second general class of electrogenic activity, that of p.s.p.'s, exists in *Romalea* muscle fibers in two grades, a small response produced by stimulation of the slow axon and a larger one evoked on stimulating the fast axon (Fig. 1). These p.s.p.'s were not evoked by direct stimulation of the muscle fibers, and in some fibers which did not respond to electrical stimuli the slow axon evoked a large, long lasting p.s.p. (Fig. 1 *a*). Other evidence also indicates that both types of p.s.p.'s of *Romalea* muscle fibers are electrically inexcitable. They were augmented by hyperpolarizing the fibers (Figs. 7-9) and diminished by depolarization (Figs. 8 and 9). The slow p.s.p. was inverted by polarizing the fiber membrane inside-positive (Fig. 8). The latency of both p.s.p.'s was independent of the membrane potential (Figs. 7 and 8). Both types of p.s.p.'s were free from refractoriness (Figs. 10-13). The slow and fast p.s.p.'s summed one with the other (Fig. 12) and slow p.s.p.'s added to produce the appearance of a fast p.s.p. out of which arose the electrically excitable component (Fig. 13). The various properties enumerated above belong together in the constellation associated with electrically inexcitability (20).

The combination of the two grades of p.s.p.'s evoked by different axons, and of graded electrically excitable responses allows the arthropod neuromuscular apparatus to operate over a wide range of performance characteristics despite the relatively few muscles, and a limited number of motor axons (33, 47). Probably, however, the range of performance is due not only to the large variety of electrogenic activity which is then made possible. It seems likely that the excitation-contraction coupling between electrogenic and contractile mechanisms also ought to be graded (1). Thus, the various grades of depolarization would permit graded mechanical responses. This appears to be also the case for the "slow" muscle fibers of frog (40) in which the electrical activity is chiefly or only of the electrically inexcitable variety (8). It is probably the situation also in muscles which show sustained contraction in response to depolarizing drugs (19), but not in muscles which relax during continued depolarization.

Indicated, but not studied in detail in the present work, are patterns of differences in the innervation of homologous muscles in different legs, of different muscles in the same leg, and even of various portions of one muscle.

Some muscle fibers, recognizable by their particularly rich tracheal supply, are innervated only by the slow axon and are electrically inexcitable. Thus, they resemble functionally the slow muscle fibers of frog (8, 40). These fibers and the large slow p.s.p.'s which some of them produce have not yet been studied adequately. The varieties of differently innervated and differently responding muscle fibers, and their presence in different proportions could play a role in the gradation of movements. In some crustaceans an even wider range of varieties is observed (15, 36). In the stick insect (*Carausius*) every fiber of the flexor muscles examined for this was found to be dually innervated (48).

Another adaptation is required to permit optimum operation of the gradedly responsive muscle fibers. After neural excitation has been initiated at one or several junctional sites of vertebrate twitch muscle fibers, the decrementless propagation of the all-or-none spike permits rapid involvement of the whole muscle fiber. In the gradedly responsive arthropod muscles, as well as in the slow muscle fibers of amphibia, involvement of the whole fiber by conduction is not possible. Since the latter fibers are electrically inexcitable, their electrical activity is non-propagating (20). This is also the case for the p.s.p.'s of the arthropod muscle fibers. The electrically excitable component, though it propagates actively, does so only decrementally. In both kinds of muscles the solution of this problem has been the same. Arthropod muscle fibers, like the amphibian slow fibers, are diffusely innervated (14, 33, 40, 45, 47, 48), and in insects the nerve terminals are applied as closely as 40 to 60 μ apart to the muscle fiber. As a result (Fig. 2), the electrical responses at different loci of the muscle fiber are essentially identical and arise nearly simultaneously. Presumably, parallel effects also occur in the local contractile systems.

Everywhere in the muscle fibers (Fig. 2), the slow axon produces a small p.s.p., while the fast axon causes a p.s.p. large enough to evoke the electrically excitable response. The reasons for the different effects of the two axons are not yet known. It seems unlikely that the two p.s.p.'s are generated by different kinds of membrane, since the two kinds of p.s.p.'s behave in a similar manner during membrane hyperpolarization. If they were different, they might be excited by different kinds of transmitter substances and ought to have different pharmacological properties. However, tests of this type (42) have not yet been successful since insect neuromuscular synapses are not affected by the commonly known synaptic agents (22, 42, 48). Another possibility is that the two innervations are structurally dissimilar. They could differ in density of presynaptic terminals, in the area of postsynaptic membrane which they cover, in the closeness of contact across the synaptic space, or in all of these. This question presumably could be decided by electron microscopy.

It has been suggested (18, 24) that graded responsiveness indicates an organization of the electrically excitable membrane into minute patches, each with a specific population of electrogenically active units. These populations are presumed to differ with respect to their excitatory thresholds, the latter being capable of modifications under various experimental conditions. Although its basis is now considered to be empirical (28), the ionic theory derived for the spike of the squid giant axon (29) can account qualitatively for graded responsiveness. In terms of this formulation, graded responsiveness could be produced if the potential-dependence of enhanced potassium conductance exhibited only a small time lag behind that of sodium conductance, and if when it was initiated, it quickly exceeded in magnitude the maximum sodium conductance (46). Consonant with this interpretation is the disproportionately large "post-spike undershoot" seen in the relatively small responses of *Romalea* muscle fibers (Figs. 3 and 5). This undershoot, indeed, sometimes appears able to obliterate the fast p.s.p. (Fig. 11). The occurrence of graded responsiveness as the normal electrogenesis of electrically excitable membrane and, indeed, as one which is incorporated into a physiological mechanism for grading muscular contraction, is of considerable importance to the development of a general theory of bioelectric activity (18, 24). This importance is enhanced further by the variations which can be produced in the responses of *Romalea* muscle fibers under different experimental conditions (42, 46).

The present data also bear on the evolutionary aspects of the functional properties of excitable cells (7, 18, 22, 23). The neuromuscular mechanisms of decapod crustacea and insects appear to have evolved differently with respect to cellular properties measured by two different functional scales, synaptic pharmacology and graded responsiveness. In their sensitivity to amino acid drugs and other agents, the neuromuscular synapses of lobster and probably of other decapods bear a curious doubly inverted relation to mammalian axodendritic synapses (22, 26). The neuromuscular synapses of *Romalea* (42) and of *Carausius* (48) are inert to the known synaptic drugs, in this respect resembling the squid giant axon synapse (6). With respect to graded responsiveness of their electrically excitable muscle fibers, however, decapod crustaceans and the insects appear to be closely related.

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