SLOW AND FAST RESPONSES IN COCKROACH LEG MUSCLE

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INTRODUCTION

The multiple innervation of arthropod muscle has led to the suggestion that much of its electrical and mechanical activity is local in nature. It has also been shown by stimulation of isolated motor axons that several neuro-muscular systems may exist in the same crustacean muscle. Rapidly contracting fast systems, slow facilitating systems, and inhibitory mechanisms have been described (see Wiersma & Ripley, 1952). Wiersma & Wright (1947) maintain that all activity is non-propagated, but Katz & Kuffler (1946) have indicated that both local and propagated responses may be present. Katz (1949) considers that rapid twitches are brought about by stimulation of a fast, non-facilitating system, while graded activity depends on facilitating end-plate-like potentials, occurring in the vicinity of numerous nerve endings and associated with graded contractions. That propagation can take place within single fibres has been shown by Fatt & Katz (1951), who measured condition velocities in directly stimulated single muscle fibres with microelectrodes. While it is possible that different types of activity take place in the same muscle fibres (Wiersma & van Harreveld, 1938), the possibility remains that different muscle fibres are involved in the different types of contraction.

Neuro-muscular transmission in insects has been likened to the system described for Crustacea. Pringle (1939) described a non-facilitating fast system and a facilitating slow system in the extensor tibiae of Periplaneta, and believed that the difference between the responses was due to the number of muscle fibres involved in the contraction. Roeder & Weiant (1950), using external recording, have shown only an all-or-none fast system in the tergal muscle of the trochantin of Periplaneta, and believe that all electrical activity is local, with excitation being distributed over the muscle fibres by numerous nerve endings. Their reasoning is based on the positive sign of the externally recorded potentials (see also Wiersma & Wright, 1947), the presence of a multiple innervation (Roeder 1953), and the inexcitability of denervated muscle. However, the occurrence of propagation is suggested by the characteristics of the potentials recorded intracellularly from the flight muscles of flies by Boettiger & McCann (1953).

Since the muscles of insects as well as of Crustacea are innervated by a small number of motor neurones, the grading of contraction cannot depend on the involvement of large numbers of motor units. The different sizes of potentials

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recorded from the flexor tibiae of the roach by Hughes (1952) suggest that a small number of units are present in this muscle. Since the grading of activity may therefore depend largely on the interplay of slow and fast responses, it is of interest to study further the different responses first described by Pringle (1939). This is especially true in the light of recent work which has shown that slow and fast responses are present in other forms such as *Phascolosoma* (Prosser, Curtis & Travis, 1951), the frog (Kuffler & Williams, 1953), and the cat (Hunt & Kuffler, 1951). In the frog it has been shown with intracellular recording that the slow and fast responses take place in different muscle fibres (Kuffler & Williams, 1953). It seemed possible, therefore, that the responses of insect muscle could fit into a general scheme of motor activity, and the experiments to be described are an attempt to shed further light on neuro-muscular phenomena in *Periplaneta* by the use of intracellular recording techniques.

**PROCEDURE**

The flexor tibiae of *P. americana* was used in all experiments. The muscle was stimulated indirectly through the crural nerve in the coxa and the responses were recorded from exposed areas of the muscle from which the cuticle had been removed. The saline used throughout was the same as that used by Roeder (1948).

Both external and internal recording electrodes were used. In the former case recording was by means of fine platinum electrodes. In most instances recording was monophasic, the inert lead consisting of a platinum loop in contact with the saline in which the animal was bathed. The signal was amplified by a Grass P-4 condenser-coupled pre-amplifier and observed on a Dumont 208 oscilloscope. For internal recording the electrodes and circuits used were similar to those described by Nastuk & Hodgkin (1951). Fine electrodes with tips smaller than 1 μ were drawn from soft glass tubing and filled with 3M-KCl. The signal was passed through a cathode follower stage and DC pre-amplifier, and photographed from the screen of a two-beam oscilloscope, consisting of two Dumont 304H oscilloscopes driving a common tube. In some instances microelectrodes too large for penetrations were used for recording of external activity.

**RESULTS**

(a) **External recording**

When the crural nerve was stimulated with single shocks of gradually increasing intensity the electrical response of the muscle was seen to grow in stepwise fashion. Three, and sometimes four, heights of potential were seen. In this type of response, shown in Fig. 1A, it is clear that the increases in potential size are stepwise and not graded.

In some experiments the intensity of stimulation was kept constant while the frequency was increased. As a rule, little facilitation was seen in the frequency range used (up to eighty per second).

The externally recorded potentials are positive in many cases, but at other times negative potentials have been recorded (Fig. 1B). The positive sign of the potentials
was used by Roeder & Weiant (1950) as evidence for the absence of propagation. However, since both positive and negative potentials can be recorded, and since the shape of the potentials as well as their polarity varies with the placement of recording electrodes, it seems dangerous to attach too much significance to potential polarity.

Fig. 1. External recording with large tip capillary inadequate for penetrations. A, gradual increase in intensity of stimulation of crural nerve. Four different steps in the height of the response can be seen. B, negative potentials obtained at low intensities of stimulation. Upward deflexion positive, time marker 10 msec.

(b) Internal recording

(1) Criteria for penetration

Three main criteria were employed in judging the validity of the experiments involving penetration of single fibres: (i) The first criterion was a sharp drop in the potential which was observed while the muscle mass was repeatedly stabbed with the electrode, and which was taken to indicate penetration of a fibre. (ii) The penetration was not counted as valid unless the potential, once recorded, remained steady while under observation. Although some drifting of the beam occasionally took place, on withdrawal of the electrode from the fibre the beam always returned to a point quite close to the zero level. (iii) Another criterion which was helpful in determining whether the electrode had indeed penetrated a fibre was the difference in the action potentials recorded externally and internally. The former were invariably small and complex, while the latter were large and simple, as will be described below.

(2) The resting potential

Records from a number of fibres showed that the resting potential varied from approximately 30 to 70 mV., with a mean of 45 ± 9 mV. (see Fig. 2A). It seems likely that this spread is due to some extent to the disparity between the ionic concentrations (especially potassium) of the saline used and the haemolymph. This will be discussed further, p. 288. It is interesting to point out that the saline used is a widely accepted one for the roach, although there is a tenfold difference between the saline and haemolymph potassium concentrations.
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Fig. 2. Potentials obtained in microelectrode experiments. A, distribution of resting potentials. B, distribution of action potentials, fast-type potentials without overshoot omitted.

(3) The action potential

When the crural nerve was stimulated, different types of action potential were recorded in a series of penetrations. In forty-six cases large potentials were observed, varying in size from 40 to 85 mV. with 60% of the potentials between 55 and 70 mV., and showing an overshoot over the resting potential. These type one potentials, shown in Fig. 3A, had a duration of 3–4 msec., were invariably all-or-none, and showed no change when the frequency or intensity of the stimulus was changed within the same ranges which brought about changes in the externally recorded potentials.

Another group of action potentials was observed in seventeen cases, or approximately 18% of the penetrations. These type two potentials (Fig. 3B) were much smaller than the ones described above, being spread between 8 and 20 mV. Their time course varied, but was generally in the order of 6–8 msec., or twice the duration.
of the responses previously described. As will be shown later, these potentials are graded rather than all-or-none.

In addition to these two fairly well-defined groups of responses, twenty-nine potentials, varying in amplitude from 22 to 62 mV., were also observed. These potentials had an appearance similar to that of the potentials of larger size, except for the fact that the depolarization was not complete, and no overshoot was noted.

The time relations of these all-or-none potentials were the same as those of the type one potentials. The most reasonable explanation of these potentials of intermediate size is that they are actually the same response as the large potentials with overshoot, recorded under different conditions. It is likely that with some electrodes, although the fibre is penetrated, damage to the membrane occurs in the process of penetration, the potentials recorded therefore being smaller as a result of injury. Such potentials without overshoot have been observed in frog sartorius fast muscle fibres (Kuffler, 1953), and it has been previously pointed out that in nervous tissue the overshoot is a very labile phenomenon, disappearing rapidly on damage or penetration with a large electrode (Brooks & Fuortes, 1952). Another important similarity between these potentials and the type one response will be brought out in the experiments below.

The distribution of the type one and type two potentials is shown in Fig. 2 B.

(c) Experiments with paired shocks

In an effort to clear up the relationships of the responses discussed above, a group of experiments were run in which paired shocks were applied to the nerve at different intervals.
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(1) Responses to paired shocks, recorded externally

Fig. 4 shows the two potentials which are recorded from the flexor tibiae when the two stimuli are both just at threshold. As the two shocks are moved closer together, the second potential is seen to become reduced in size, disappearing when the two stimuli are about 2 msec. apart. This response is the same as that described in another muscle by Roeder & Weiant (1950).

Fig. 4. Refractoriness following potential, recorded externally. In this experiment both stimuli were just of threshold intensity. The stimulus artifact preceding the first response is just visible at the beginning of the sweep, the second artifact can be seen super-imposed on the base-line and then on the first potential as the stimuli are brought closer together. The second potential disappears when the interval between the stimuli is 2 msec. Upward deflexion positive, time marker, 10 msec.

(2) Responses to paired shocks, recorded internally

In view of the complexity of the responses recorded externally from the muscle as a whole, it is much more profitable to study the behaviour of single fibres as they respond to two stimuli. Such experiments show that the type one potentials are definitely followed by an absolute and a relative refractory period. As the two stimuli are brought temporally closer and closer together the second response gradually decreases in size, no longer overshoots the resting potential, and disappears completely when the interval is decreased to about 2 msec. (Fig. 5). It is interesting to observe that the group of potentials which resemble the type one potentials in all respects except for the absence of an overshoot show exactly the same characteristics with regard to refractoriness. This is another of the reasons for the assumption that these two groups of potentials actually belong to the same type of response.

A sharp difference, however, is seen in the response of the type two potentials to the same kind of stimulation. While the type one responses are followed by a refractory period, and the second potential is therefore extinguished as it is brought closer to the first one, the type two potentials behave in a different manner (Fig. 6).
Here we see that as the two potentials move closer summation takes place. It is clear, therefore, that these potentials are not followed by a refractory period, but rather that facilitation takes place when stimuli are separated by very short intervals.

Fig. 5. Internal recording of double pulse experiment, showing refractory period of type one potential. First stimulus simultaneous with beginning of sweep. Note decrease in size of the second potential as the stimuli are moved closer together. Upward deflexion positive, time marker, 10 msec.

Fig. 6. Internally recorded double pulse experiment showing type two potentials. First stimulus artifact seen at the beginning of the sweep. Note lack of refractoriness as the potentials are moved progressively closer together. Upward deflexion positive, time marker, 10 msec.

DISCUSSION

Two divergent opinions exist concerning the responses of arthropod muscle. According to Wiersma & Wright (1947) and Roeder & Weiant (1950) all responses in crustacean and insect muscle are local in nature. Other authors contend that, while much of the activity is local, propagated electrical and mechanical events also occur (Katz & Kuffler, 1946; Fatt & Katz, 1951). The question of the locus of the diversified responses observed in crustacean muscle (Wiersma & Ripley, 1952) and to a lesser extent in insect muscle (Pringle, 1939) has not been conclusively settled. While there exists evidence that the difference between the slow and fast responses of crustacean and insect muscle is purely quantitative and
 involves the amount of contractile substance affected at one time within the same group of muscle fibres (van Harreveld, 1939; Pringle, 1939), the possibility remains that in insects as in the frog (Kuffler & Williams, 1953) the different responses are obtained by the excitation of specialized groups of muscle fibres.

There seems little doubt that the type one potentials described above are propagated. Such an assumption is based not on direct evidence, but on the all-or-none appearance of the potential recorded intracellularly, the presence of an overshoot over the resting potential, the short duration of the potential, and the presence of a refractory period following the response. The presence of an overshoot has invariably been associated with propagation in other tissues (Hodgkin, 1951). Furthermore, there has never been described a local response followed by a refractory period, or a propagated one without subsequent refractoriness.

Different lines of evidence have been used in an attempt to show that the potentials recorded from roach muscle are entirely local. First, Roeder & Weiant (1950) suggested that the positive polarity of the potentials recorded externally was indicative of local activity (see also Wiersma & Wright, 1947). As shown above, positive and negative potentials can be recorded from the flexor tibiae. In view of this fact, and of the fairly complex geometry of the system under study, it may be dangerous to attach decisive significance to potential polarity. Secondly, it has been shown that when the motor nerve is allowed to degenerate, insect muscle becomes unresponsive to stimulation, although its outward appearance remains unchanged (Roeder & Weiant, 1950). While this may indicate that excitation can be spread to all parts of each muscle fibre only by fine nerve branches, few conclusions can be drawn from this experiment because denervation is known to cause drastic changes in the excitability of many tissues (Cannon & Rosenblueth, 1949). Finally, just as has been done for Crustacea, it has been argued that the presence of a multiple innervation of muscle fibres in the muscles studied (Roeder & Weiant, 1950) makes propagation within single fibres unlikely, since excitation can easily be spread through the numerous fine branches of the motor nerve. According to Weiant (unpublished, see Roeder 1953) the nerve endings are separated by approximately 40 μ. Should a similar innervation exist here, and should all activity consist of local responses around these endings similar to the end-plate potentials of vertebrate muscle, it might be expected that the size of the recorded potentials would vary with the locus of insertion of an electrode less than 1 μ in diameter at the tip. An overshoot (representing the greatest possible response) would then be seen only on insertion right at a region of nerve ending, although it is hard to see why an overshoot, always associated with propagation, would be seen even then. The high frequency of occurrence of large potentials with an overshoot on random penetrations makes it even more unlikely that the potentials recorded are local.

On the whole it seems reasonable to assume that the type one potentials recorded in these experiments represent propagated activity in single fibres of the flexor tibiae of the roach, and that the same holds true for the similar potentials recorded from the flight muscle of flies by Boettiger & McCann (1953).

An entirely different interpretation presents itself in the case of the type two
potentials previously described. Not only their small amplitude and somewhat longer duration, but also the fact that no refractoriness but rather some facilitation is seen when paired shocks are applied at close intervals, suggest that these potentials are local phenomena representing the activity of a slow system in the muscle studied. While it could be argued that these potentials are either due to damage of the fibre or abolition of the fast response through fatigue with only a local end-plate potential remaining, both of these possibilities are unlikely. The former is unlikely because it seems clear that on damage of the fast fibre with a large electrode the potential subsequently recorded is a fast-type potential without overshoot but followed by a refractory period similar to that of the undamaged fast response, and does not resemble the type two potential. The second possibility also seems improbable because the two types of potentials have been recorded from the same muscles while the preparation was in good condition and no evidence of fatigue had been seen. Finally, the difference between the two responses is emphasized by the fact that they have not been recorded in the same fibre, or at least with the same penetration, and that at no time has there been a shift from one type of response to the other. In general, it is likely not only that the type one and type two responses actually represent different phenomena taking place in the muscle, but also that these phenomena occur in different muscle fibres, some of which normally conduct while others do not.

Such a neuro-muscular system as described above is quite similar to the one described for the frog by Kuffler & Williams (1953). However, while those authors demonstrated that in the frog sartorius the fast, propagating fibres have a higher resting potential than the slow fibres giving only local responses, no such difference has been observed in the roach flexor tibiae.

An attempt can now be made to arrive at a picture of the grading of activity in the muscle studied. External recording has shown that three to four different heights of potential can be obtained from the flexor tibiae (see above, and Hughes, 1952). The changes from one height to another occur suddenly rather than gradually, and clearly represent the involvement of distinct motor units. The small number of motor units makes fine grading of activity according to the vertebrate plan impossible. Thus the fast system of this muscle is essentially divided into a small number of units which individually respond in an all-or-none manner, and are brought in as the intensity of stimulation of the nerve trunk is increased. The lack of facilitation in the fast system was pointed out by Pringle (1939), and is again seen here with both external and internal recording. Grading of activity in the fast system seems to depend entirely on the separate or joint activation of a small number of motor units, and on mechanical summation at raised frequencies of stimulation.

Finer grading of movement is evidently made possible by the presence of a facilitating slow system which, as in the frog, may be mainly involved in 'tonic' movements.

That the resting potentials show such a wide range of distribution is probably due largely to the disparity between the potassium concentrations of the haemolymph and of the saline used in these experiments (27 mM./l. as against 2.7 mM/l.).
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This would lead to uneven mixing between these two media, with the result that some muscle fibres would be exposed to higher external potassium concentration than others. It has been shown recently that in *Locusta* muscle resting potentials depend to some extent on the external potassium concentration (Hoyle, 1953), and the values obtained in the experiments described above fall within the range expected from the graph presented by Hoyle.

On the whole, therefore, it seems that the neuro-muscular system of the roach is similar to that described in other forms. The slow and fast systems in *Periplaneta* seem to be restricted to specialized muscle fibres, and the same is now found to be true in the frog (Kuffler & Williams, 1953), although it had been previously thought that in both these animals the same fibres were involved in the two responses (Pringle, 1939; Kuffler & Gerard, 1947). This suggests that a re-examination of crustacean neuro-muscular preparations with intracellular electrodes might be of great value, especially in the claw closer preparation described in the crayfish by van Harreveld & Wiersma (1936), where the easy separation of the slow and fast motor axons makes conditions ideal for a study of the two neuro-muscular systems. Evidence from comparative studies on several forms, as well as the work of Fatt & Katz (1951) and Katz & Kuffler (1946), suggests that the responses of Crustacea may be quite similar to those observed in insects and Amphibia. The main difference noted until now between the fast system of insects and that of Crustacea is that while the potentials in the former are followed by a refractory period (above, and Roeder & Weiant, 1950) those in Crustacea are not (van Harreveld & Wiersma, 1936). This may indicate an important difference between neuro-muscular phenomena in these two classes, and in any case shows that generalizations from higher Crustacea to insects are, on present evidence, quite unjustified.

**SUMMARY**

1. Intracellular recording from individual muscle fibres of the flexor tibiae of the cockroach shows two types of action potentials. One of these represents fast fibre activity, while the other represents slow fibre activity in this muscle.

2. The fast response consists of an all-or-none potential varying from 40 to 85 mV., with 60% of the responses between 55 and 70 mV. This potential has an overshoot over the resting potential, and is followed by an absolute and a relative refractory period. For these reasons it is considered likely that the potential is a propagated one. The slow response is a small, facilitating potential, varying from 8 to 20 mV., and is quite likely a local phenomenon.

3. All the evidence indicates that the two types of activity do not take place in the same muscle fibres, but rather represent the responses of two different types of fibres, as is the case in some amphibian muscles.

4. Gradation of activity in the fast system seems to depend solely on the activation of a very small number of motor units and on mechanical summation. The response of this system shows little, if any, facilitation as the frequency of stimulation is increased. The facilitating slow system may be concerned with slower, more finely graded movements.
5. The resting potential in roach muscle has an amplitude of 45 ± 9 mV., which falls within the range expected on the basis of the intracellular and extracellular potassium concentrations.

6. Slow and fast fibre activity in insects and Crustacea is discussed, and a revision of the earlier work on Crustacea with the use of intracellular recording is suggested.

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