

A BIFUNCTIONAL SINGLE MOTOR AXON SYSTEM OF A CRUSTACEAN MUSCLE

By C. A. G. WIERSMA

*From the Kerckhoff Laboratories of Biology, California Institute of Technology
and the Marine Station of the Nederl. Dierk. Ver., Pasadena and Den Helder*

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(With Four Text-figures)

INTRODUCTION

The muscles of decapod crustaceans have been shown to possess a number of special features. Among these is the remarkable property which enables many of them to contract in two distinct ways, by fast and by slow contractions. Several explanations have been offered to account for this property (Lucas, 1917; Blaschko, Cattell & Kahn, 1931; Wiersma, 1933; Pantin, 1936*a, b*; Katz, 1936; Wiersma & Van Harreveld, 1938*b*; Katz & Kuffler, 1946).

It was shown that in all muscles which have both a slow and fast contraction, and which have been investigated by the method of isolating and stimulating single axons, it is possible to obtain one or other contraction type by the stimulation of one or other of the two motor fibres which form the sole motor innervation for most of the leg muscles. In the case of the main flexor muscle of the carpopodite which receives, instead of two, four motor fibres, each of these four was found to elicit a contraction with specific properties.

Hence it became likely that each contraction type of a given muscle was caused by a special motor fibre.

Openers (abductor of the dactylopodite) and stretchers (extensor of the propodite) were found to receive a single motor fibre in every case in which single-fibre analysis was made. This would then indicate that these muscles possessed only a single type of contraction. Most of the results obtained supported this view, as there was in most species no noticeable difference between the opener contractions and the slow contractions of the doubly motor-innervated muscles of the same species. However, in older experiments with the hermit crab (Wiersma & van Harreveld, 1934*a, b*, 1935), it was shown that two types of contractions were performed by its opener. Since this species had never been analysed for motor fibres, it was possible that its opener did in fact receive two motor fibres.

This assumption remained unquestioned until recent evidence (Wiersma & Adams, 1950) indicated that the general rule need not apply to openers. The present investigation was undertaken to determine the number of motor fibres innervating the opener of *Eupagurus bernhardus* L., and to obtain further information concerning the two contraction types shown by this muscle.

METHODS

Single motor fibres of the leg muscles of *E. bernhardus* L. were prepared according to the usual technique (Wiersma, 1941), and stimulated through micromanipulated platinum electrodes. In these experiments both claws and the first two walking legs have been used. For mechanograms of the opener the large claw was most satisfactory, since it showed the desired phenomena more clearly than the other legs. The differences were of a quantitative nature only.

Low-frequency stimulation was obtained with a variable interrupter in the primary circuit of an induction coil. Extra shocks were intercalated by a second coil, the secondary of which was connected to the same stimulating electrodes through which the low-frequency-background-stimulation (3–20 per sec.) was given. A modified Lucas contact breaker with four contacts was used in a number of experiments, but in many of these only one or two of its contacts were connected. No exact time calibration was available for this instrument.

Contractions were registered isotonically, the lever being fastened to the dactylopodite. Closer muscles have been used as a control in some cases. All claws were obtained from freshly caught animals in excellent condition during the summer of 1949 at the Marine Station in Den Helder.

RESULTS

A. *Staining with methylene blue*

Opener muscles (as well as other muscles) of the leg were stained with methylene blue in order to determine the number of nerve fibres innervating them. Staining was often satisfactory, though never so good as in the coconut crab, *Birgus latro* L. (Wiersma, 1949).

Three fibres were regularly observed in the closer, the bender and the extensor, as found in *Birgus*. Both in the opener and in the stretcher only two nerve fibres stained with certainty. Many preparations of the opener have been made and stained for different lengths of time with different concentrations of dye. In examining them, high-power magnification was sometimes used in order to try to find a third fibre as is present in *Birgus*. In *Birgus* this third fibre is small and acts as an inhibitor; it could be prepared functionally in the ischiopodite, but not in the meropodite because it branches there. The conclusion that in *Eupagurus* the opener does not receive a branch from this fibre, though supported both by anatomical and physiological evidence (see below), nevertheless cannot be regarded as certain. Thin fibres are sometimes very difficult to stain, and the course of a fibre may preclude its functional isolation in the meropodite. Yet it is not unlikely that such differences exist in innervation schemes among Anomura (Wiersma, 1949).

B. *Determination of the number of fibres innervating the opener and other muscles*

It was of primary importance for this investigation to decide whether or not one motor fibre formed the sole innervation of the opener muscle. In Anomura as in

Astacura, there occur in the meropodite two clearly separated bundles of nerve fibres, one thick and the other thin. Stimulation of the latter causes opening. In previous investigations (Wiersma & Van Harreveld, 1934*a*) on *Eupagurus*, the thick bundle had been cut. The opener contractions obtained were thus due to the stimulation of the whole thin bundle. The results might have been influenced either by the presence of two (or more) motor fibres for the opener in this bundle, or possibly by the presence of an inhibitor for the opener. Axon isolation in this bundle was therefore carried out with special care. The results were absolutely uniform; only one fibre could be found in this bundle which had any effect on the opener muscle. This fibre, which has been isolated many times, always caused a simultaneous contraction of opener and stretcher. The only other efferent fibre found in this bundle is the stretcher inhibitor, which runs side by side with the opener-stretcher motor axon. The stimulation of this fibre has no influence at all on the opener. It must therefore be concluded that stimulation of the thin bundle as a whole will have exactly the same effect on the opener as stimulation of the isolated motor axon (see also below).

A search has also been made for the efferent axons in the thick bundle. As expected, two motor axons for the closer and two for the bender were found. Stimulation showed that each of these muscles is innervated by one fast and one slow motor fibre. In addition, a fibre was prepared whose sole function was inhibition of the opener muscle, and a further fibre which, on simultaneous stimulation of the appropriate motor axons, could be shown to inhibit both closer and bender contractions. But stimulation of the latter fibre did not influence either opener or stretcher contractions. It was also found that stimulation of the isolated stretcher inhibitor from the thin bundle was without result on either closer or bender contractions.

C. Stimulation of the isolated opener-stretcher motor axon with long-lasting direct current

Since only one motor axon is present in the thin bundle, stimulation of the isolated single fibre should give the same effect as similar stimulation of the whole bundle. As found in previous experiments (Wiersma & Van Harreveld, 1934*a*), stimulation of the whole bundle did not give the smooth tetanus that is usually obtained when only one motor fibre is stimulated with a long-lasting direct current. Rather, it gave a veratrine-like contraction, consisting of a fast rise and a slight fall, followed by a slow rise before relaxing. Such contractions occur in doubly motor-innervated muscles only when both motor fibres are stimulated together. Then the first quick rise is due to the fast fibre, which usually fires for a very short time, so that relaxation occurs before the shortening caused by the continued firing of the slow fibre.

But long-lasting direct-current stimulation of the totally isolated motor axon of the opener of *Eupagurus* did in fact give rise to a veratrine-like contraction (Fig. 1). The large claw showed this phenomenon more clearly than did the other legs, because the relaxation between the fast and the slow phases of the contraction was more pronounced. On repetition of stimulation the relaxation became less (Fig. 1),

and eventually the slow part of the contraction followed the fast without any intermediate relaxation.

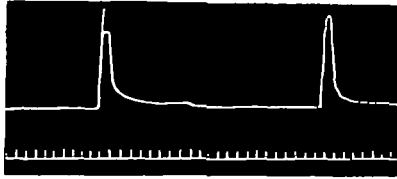


Fig. 1. Opener, prepared axon. Stimulation with constant current of 1 sec. duration, near threshold of twitch contraction. Veratrine-like shape, isotonic. On repetition the slow part becomes relatively more pronounced. Time in seconds.

D. *The effect of intercalated shocks*

Since stimulation of the whole thin nerve bundle gives contractions which are identical with those obtained after the isolation of the motor fibre, some of the experiments under D and E were carried out by stimulating the undivided thin bundle. This was done in order to prevent any fatigue of the opener muscle. Frequent checks with isolated fibre preparations showed that for this type of experiment also the results were the same in the two cases.

In a previous paper (Wiersma & Adams, 1950) it was shown that when a slow fibre is stimulated at a constant frequency and a single extra shock is given, there is never any marked effect of such intercalation and often no sign of it can be detected. When this same type of experiment is performed with a fast fibre, however, the intercalated shock often causes a very noticeable twitch. These extra twitches are, in some cases, immediately followed by a relaxation to the tetanus level; or, in others, followed by a complete or partial maintenance of the newly reached level of shortening. In openers of different species the results were different: in the crab, *Pachygrapsus*, intercalated shocks had practically no effect on the mechanogram, whereas in the crayfish, *Cambarus*, there was a distinct effect in many cases.

If in *Eupagurus* the first phase of the veratrine-like contraction is comparable to a fast contraction, intercalated shocks should have a noticeable effect. This proved, indeed, to be the case (Fig. 2). The amount of shortening achieved by a given intercalated shock will, of course, depend on its time relations with the shocks preceding or following it, and this factor is not controlled. Thus some of such shocks will have no effect, because they fall within the refractory period of a previous one, or blot out a following shock by their own refractory period. Also, when a nerve impulse does result, there will be an optimum interval, at which the largest 'trigger' effect occurs. It was found that a noticeable relaxation after the twitch-like contraction was obtained only when the muscle shortened considerably in response to the intercalated shock; smaller contractions were sustained (Fig. 3). In no case was a twitch followed by a relaxation to the 'base' line, as was the case in several fast systems.

Different frequencies have been used for the background stimulation, and trigger effects have been found with every frequency that gave a visible contraction. It is

important to note that very small, but complete or almost complete, tetanus was obtained with a frequency as low as 2 per sec. (Fig. 2), which is a feature of slow systems.

Some experiments have been performed with the isolated slow and fast closer axons. As expected, intercalated shocks were without effect in slow contractions and in the fast contractions gave noticeable trigger responses, of a magnitude comparable with those in the opener.

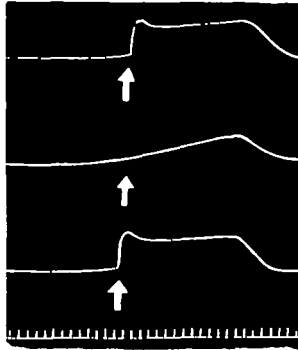


Fig. 2. Opener, isolated axon. Stimulation frequency 3 per sec. for 20 sec. At the arrows, 10 sec. after start of faradic stimulation, a single opening induction shock is given, which results, in first and third trials, in a veratrine-like shaped increase of contraction, but is without effect in the second trial.

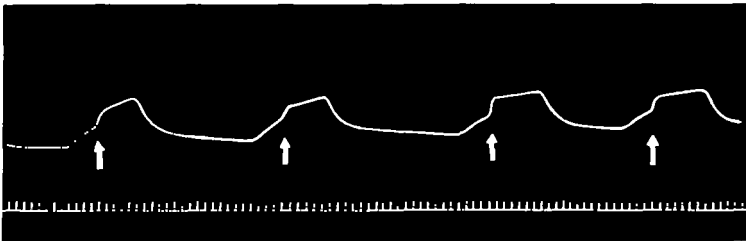


Fig. 3. Opener. Stimulation of thin bundle, with a frequency of about 10 per sec. At the arrows a single intercalated opening shock is given. Notice differences in effect in the different contractions, the third trial giving a slightly veratrine-like shape, whereas the others are followed by a sustained contraction of different magnitudes.

E. Effect of spacing of stimuli

In the paper quoted (Wiersma & Adams, 1950) it was shown that in cases where intercalated shocks had a large effect, the spacing of stimuli in time is of very great importance. This means that if, for instance, 20 stimuli are given per second the result will be different according to whether the intervals are uniformly $\frac{1}{20}$ sec. or whether the intervals are, say, alternately, $\frac{1}{80}$ and $\frac{7}{80}$ sec. The contraction with the latter distribution will be considerably larger than that with the former. In slow systems, on the other hand, such changes in spacing do not give rise to any significant differences in the contraction.

In the present investigation the total number of spaced stimuli had to be restricted to four, which proved only just enough. Each contact of the contact breaker was made to break the primary of an induction coil. The four secondaries of these coils were connected to one pair of stimulating electrodes. The first and last contacts were given fixed positions; the interval may have been of the order of some 20 msec. The other two contacts were either spaced so that all intervals were equal, or so that the second stimulus came immediately after the first and the third immediately before the fourth. It was found that under the conditions of these experiments all four contacts were necessary to obtain a contraction; with three no visible response was obtained (Fig. 4; 4 and 5). The contractions with equal spacing were always smaller

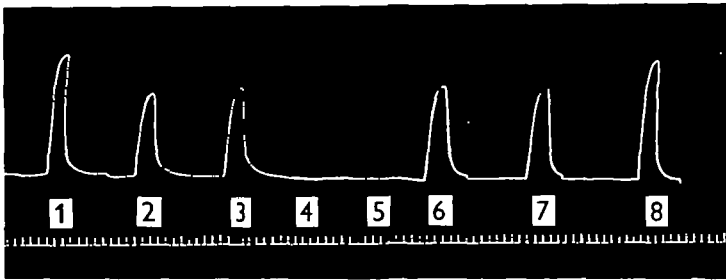


Fig. 4. Opener, single axon. Effect of spacing of shocks. 1, result of four shocks, spaced about as follows: interval 1st-2nd and 3rd-4th 2 msec., interval 2nd and 3rd 18 msec. 2, 3, 6 and 7, result of even spacing of four shocks, intervals 5 msec. 8, same spacing as in 1. Notice increased size of 1 and 8 as compared to other contractions. In 4, the first, in 5 the last of the shocks (equal spacing) were left out. No visible contraction on three impulses. Time $\frac{1}{2}$ sec.

(Fig. 4; 2, 3, 6, 7), often about half the size of those obtained by crowding the second near the first and the third near the fourth (Fig. 4; 1 and 8). This fact shows that spacing has in this muscle a very pronounced effect, as would be expected if a fast system were present.

DISCUSSION

From the foregoing results, it follows that the opener receives only one motor fibre and that this one fibre can elicit, depending on the way in which it is stimulated, two types of contraction, which resemble the fast and slow contractions of other muscles. This finding leads to a reconsideration of the problem of fast and slow contractions and necessitates a more extensive discussion.

The most obvious difference between fast and slow contractions is found in the closer muscle of the crayfish, where it was first described. Here, a single impulse in the fast fibre will cause a twitch contraction, whereas several impulses are needed before stimulation of the slow fibre will cause a visible smooth tetanus. However, this is a specialized case. In most doubly motor-innervated muscles, a number of impulses are needed in the fast fibre before a twitch is produced. In several cases this number is only two, but since in certain crabs (*Cancer*) a good twitch is obtained only with a larger number, the two systems are no longer easily distinguishable. At

low frequencies, the fast may even produce smaller contractions than the slow in these cases (Wiersma & van Harreveld, 1938*b*).

Another difference, which has been found so far to be reliable, is that intercalated shocks will cause a pronounced 'trigger response' in fast systems, but not in slow. Related to this is the fact that the spacing of impulses has a great influence in fast systems but not in slow; those fast systems which respond on a single impulse are, however, exceptions.

The muscle action potentials are also indicators of the type of contraction, for in slow systems they are smaller than in fast. But in both systems they show electrical summation in the way that contractions can show mechanical summation. This summation is considered to be related to the fact that these action potentials are local phenomena.

If we now consider, in the light of the above differences, the results obtained on the opener system of *Eupagurus* and described in this and previous papers, the conclusion seems inevitable that the contractions of this muscle are of a type which includes properties of both fast and slow neuro-muscular systems. It shares with slow systems the property that on low-frequency stimulation a gradual shortening develops. This shortening is perhaps somewhat more in the nature of an incomplete tetanus than in other slow systems, but it lacks the sudden increases and decreases in strength which are characteristic of fast systems when stimulated near the lowest effective frequency. However, as has been shown, it shares with fast systems the sudden increases in contraction produced by intercalated stimuli and the great sensitivity to pattern.

Again, in the muscle action potentials, it was previously found that at a relatively low frequency of stimulation there is a gradual growth from zero, as in slow systems, and not a growth from a considerable initial potential as in fast systems. In the case of the opener muscle, however, this growth may be rather more pronounced than in slow systems. Then again, a sudden burst of impulses will result in a big action potential 'spike', which looks exactly like that of such fast systems as are found in *Randallia*, and not like any known 'slow' potential.

The ability of a single motor axon to cause contractions with both slow and fast properties may be explained in several ways. According to Katz & Kuffler (1946), fast contractions are at least sometimes accompanied by conducted action potentials. If one accepted that the fast phase of the contraction was due solely to such conducted potentials in this muscle, the double contraction type would be readily explained. To the present author, there are great difficulties in the way of this hypothesis. Conducted action potentials would of necessity give rise to diphasic action potentials under most leading off conditions. From the previous results (Wiersma & Van Harreveld, 1935, fig. 3), it is obvious that the first part of the action potential configuration which accompanies a veratrine-like contraction, is just as monophasic as the later part. Monophasic action potentials have been found in other muscles as well as in the opener of *Eupagurus*, and the present author agrees with Wright (1949), that conducted action potentials have not been proved to exist in any crustacean system. Roeder & Weiant (1950) have also come to the conclusion that the action

potential accompanying fast contractions in insect muscles are not conducted, though the time relations of these contractions are certainly shorter than those of even the fastest twitches in crustacean muscles.

A second possibility would be that two types of contraction are due to two kinds of muscle fibres, each with its own kind of facilitation. This possibility is not excluded by direct evidence. However, it seems unlikely, because of the small number of muscle fibres in the opener and the fact that in doubly motor-innervated muscles the same muscle fibres are responsible for both contractions (Van Harreveld, 1939). The mechanisms by which the two fibre types are brought into contraction would still have to be the same as in the hypothesis which will be favoured here.

The third possibility is that there are two types of nerve endings on the same muscle fibre. The first type becomes activated when impulses at a low frequency reach it, since these endings have small but long-lasting facilitation. The second type becomes activated only when a burst of impulses at high frequency reaches it, because it has strong but short-lasting facilitation. In other words, the slow and fast endings as present in doubly motor-innervated muscles would here have a common axon instead of a fibre for each type. The postulation of two types of ending, instead of one which would combine the properties of a fast and slow ending, is based upon the veratrine-like contractions and the effects of intercalated shocks. If the same endings which are responsible for the slow phase of the contraction were also responsible for the twitch phase, the facilitation of the former by a twitch would be so large that no relaxation could be expected to precede the subsequent increase in tension. Also, the action potentials accompanying a veratrine-like contraction are clearly divided into two parts. After the first summated action potential, there is a sudden drop followed by a renewed action potential facilitation (see Wiersma & Van Harreveld, 1935, fig. 3). If only one process were present, one would expect a gradual diminution of the action potential, as the original high amount of facilitation disappears.

The two types of endings will, of course, be excited at the same time, but as stated, with low frequencies, contractions will take place only at the 'slow endings'. With higher frequencies, the 'fast endings' will also contribute to the contraction. On longer lasting stimulation it becomes impossible to decide what part each type of ending plays; but when only a few impulses follow each other quickly, the contribution of the slow endings will be negligible, and thus twitches will be the product of the fast endings almost exclusively.

It might seem that such a system of fast and slow, depending upon a single nerve fibre, would have advantages over one in which two nerve fibres are necessary. However, since systems of this type can only function when a relatively large number of impulses is given, sufficient to trigger the fast contraction, the delay in the onset of contraction is necessarily increased. Further, separation of the two systems may result in a much greater overall economy, especially of contractions due to an intermediate frequency. This may explain why the 'bifunctional single axon system' has developed only in muscles of relatively small size, such as the openers, which have a rather insignificant metabolism. These muscles are used

mostly in preparatory movements. Their antagonists, such as the closers, in which considerable power is developed, are doubly innervated.

SUMMARY

It is shown that the opener muscle of the hermit crab, *Eupagurus bernhardus* L., receives a single motor axon. Stimulation of this axon results, when appropriate stimuli are used, in two types of contractions comparable with the fast and slow contractions of doubly motor-innervated crustacean muscles. The theoretical implications of this finding are discussed and a hypothesis offered to explain the mechanism which makes the two contraction types possible. The physiological and anatomical features of the innervation of the four most distal muscles in the legs of *Eupagurus* are described.

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