

THE MOTOR INNERVATION OF A TRIPLY INNERVATED CRUSTACEAN MUSCLE

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INTRODUCTION

PART of the muscles in the claw of *Cambarus clarkii* are innervated by two motor axons, the thicker one causing a "fast" contraction, the thinner one a "slow" contraction. Stimulation of a third fibre, thinner than the two motor ones, inhibits both kinds of contraction (van Harreveld & Wiersma, 1937). The question arises whether there are two motor units, with the two motor axons innervating separate parts of the muscle, or whether there is a double motor innervation of each muscle fibre, with each motor axon innervating each muscle fibre of a triply innervated muscle. Evidence has been brought forward that the latter is the case. It has been found that fibres of such a muscle receive branches of each of the three axons involved in its innervation, thus providing an anatomical basis for a double motor innervation of each muscle fibre (van Harreveld, 1939a). The observation has been made that a preceding fast contraction facilitates a following slow one (heterofacilitation); the reverse has also been demonstrated. This heterofacilitation can best be explained by assuming that both contractions are elicited in the same muscle fibre (Wiersma & van Harreveld, 1939). Furthermore, it has been found that the maximum force developed by the fast and slow contractions is about the same, which also supports the assumption that in both cases the same contractile substance functions (van Harreveld & Wiersma, 1936). Finally, Bergren & Wiersma (1938) found that the changes in phosphagen and lactic acid content in a muscle after slow and after fast contraction are comparable when the mechanical effects of the two contractions are similar. This observation excludes the possibility of a phasic and tonic substrate for the fast and the slow contraction either in the same or in different muscle fibres and is again consistent with the assumption that in both contractions the same substance functions.

The only observation which is not in agreement with this conception is the absence of any influence of exhaustion of one kind of contraction on the height of contraction of the other (Wiersma & van Harreveld, 1938). This has been explained satisfactorily, however, by the fact that during the stimulation which fatigues one kind of contraction the action currents are abolished, indicating that what is

exhausted is not the contractile substance, but the intermediary processes between the nerve impulse and the contractile substance. Since it was argued (Wiersma & van Harreveld, 1939) that these intermediary processes are different for the two kinds of contraction, there is no reason why the exhaustion of one contraction would influence the other.

Lately, quadruply motor innervated muscles have been described (van Harreveld, 1939*b*; van Harreveld & Wiersma, 1939) and arguments similar to the ones mentioned above for the triply innervated muscle have been presented to substantiate the opinion that in these muscles the individual muscle fibre is quadruply motor innervated.

Attempts to obtain direct evidence regarding the double motor innervation of the individual muscle fibres in a triply innervated muscle have met with unexpected difficulties, which could only be fully understood after study of the peculiar nerve supply of the crustacean muscle.

The nerve fibres in a crustacean muscle form a rather dense feltwork between the muscle fibres. From this feltwork arise the finer and finest branches which end on adjoining muscle fibres. Contrary to observations in vertebrates, the usually short fibres of crustacean muscles possess large numbers of nerve endings (multiple innervation). Physiological evidence (Wiersma & van Harreveld, 1938) and these anatomical findings (van Harreveld, 1939*a*) have led to the belief that in the crustacean muscle the impulse is not conducted along the muscle fibre by a special process of muscular conduction, but that the impulse is distributed by nervous conduction to the numerous nerve endings spread over the fibre surface. It is mechanically impossible to separate a part of a muscle fibre or even of a group of fibres in such a way that an individual fibre can be observed responding to stimulation of the nerve. This is due to the fact that each fibre receives several branches from the surrounding feltwork of fibres and the operation of isolating the muscle fibres inevitably cuts these. Unlike the vertebrate fibre, the muscle fibre of crustaceans when stimulated at one point does not set up a conducted muscular wave over the whole fibre, it only responds locally to excitation reaching it through each localized nerve ending. No contraction has ever been observed after indirect stimulation of a partially isolated crustacean muscle fibre, although the process of isolation is readily effected in view of the slight development of connective tissue within the muscle. In order to observe contractions of individual muscle fibres of crustaceans, the intact muscle must be used.

In each podomere of the claw two feathered muscles are present having their origins from the chitin. The muscle fibres insert on a central tendon. The only muscle surface that can be prepared without gross injury is the surface that faces the other muscle in the podomere. In various muscles (flexor of propodite, extensor of carpopodite) this surface was exposed as cautiously as possible, but unlike the deeper parts, the superficial fibres never contracted during indirect stimulation. It must be concluded that the slight displacement of the superficial layers of fibres during the preparation of this muscle surface suffices to damage their nerve supply.

The only place where it has been possible to obtain a superficial layer of muscle

fibres which contracted on indirect stimulation, has been the surface of the adductor muscle that faces the abductor. The chitin bounding the abductor was removed; the claw was opened as far as possible in order to stretch the adductor fibres and in this position the abductor muscle was slowly pulled out, being held by its tendon. Since the removal of the connective tissue and nerves between the ad- and abductor muscles always resulted in the abolition of the function of the superficial muscle fibres, these were left intact. The fibres were observed with light transmitted through the chitin and the adductor muscle. By focusing on the adductor fibres a satisfactory image can be obtained and the disturbance of the overlying tissue is slight.

The axons for the slow and fast contraction were prepared in the way described in an earlier paper (van Harreveld & Wiersma, 1936).

THE EFFECT OF INDIRECT STIMULATION

A few remarks must be made about the recognition of contraction in a muscle fibre. Usually the muscle fibres in the relaxed state are not straight, but more or less curved. Fibres that are strongly bent seem to be injured since they never contract on indirect (or direct) stimulation. When the muscle contracts, the tendon moves proximally during closing of the claw, thus increasing the angle included between the muscle fibres and the tendon. Since the origin and insertion of the fibres approach each other by this movement, the curvature of the non-contracting muscle fibres increases. If the muscle fibre contracts vigorously, however, it shortens and straightens out its curves. If a non-contracting muscle fibre is attached to a functional fibre it also becomes straighter, but in such a case a wrinkling of the edge of the fibre can usually be observed. Thus a muscle fibre was considered as contracting only if it straightened out its curves and at the same time showed smooth edges.

Many carefully made preparations did not show any sign of contraction in the superficial layer of muscle fibres, though the deep layers of the muscle functioned, since the claw closed during stimulation. In some preparations part of the fibres of the muscle surface contracted, and in a very few all of them did so.

Those preparations in which all the superficial muscle fibres contracted showed, in the relaxed state, an even layer of slightly curved superficial fibres. On stimulation of the nerve fibre for either the fast or the slow contraction this slight curvature was straightened out and the edges of the muscle fibres remained smooth. The differences between the two contractions were slight: the fast contraction was more vigorous and somewhat quicker.

Continued stimulation changed a preparation, in which the whole muscle surface contracted, into one in which only groups of fibres were still active. As mentioned above, most preparations were in this state at the beginning of the experiment. They showed an uneven muscle surface, with groups of fibres at different levels. Single functioning muscle fibres were never found situated between fibres that had stopped contracting, but always groups, sometimes con-

sisting of only a few fibres, could be seen between groups that remained relaxed. Often these groups of functioning fibres contract slower than the rest of the muscle. The deeper layers close the claw, moving the tendon proximally and increasing the curving of the still relaxed fibres of the superficial layer. An instant later, however, these fibres contract, and the curvature is completely straightened out. Thus in these preparations the curvature first increases and then disappears. This phenomenon must be considered as a sign of slight damage to the muscle fibre or its nerve supply.

The fibres of a functioning group contracted at the beginning of the experiment on stimulation of both motor axons. However, after some stimulations, faradization of the fibre for the fast contraction no longer caused any contraction in the superficial layers. This is in agreement with the previous observation, that the fast contraction is more easily exhausted than the slow one. No preparations have been found in which fibres which reacted on stimulation of the fibre for the fast contraction did not contract on stimulation of the axon for the slow contraction.

THE EFFECT OF DIRECT STIMULATION

The effect of direct stimulation of the muscle fibre depends on the amount of damage that has been done to them. If the muscle fibres that still contract on indirect stimulation are stimulated directly, the whole muscle contracts because apparently the nerve fibres have the lower threshold of stimulation. If the muscle fibres have stopped contracting on indirect stimulation, but are still in good conditions (only slightly curved), it is often possible to obtain contractions of the muscle fibres in the neighbourhood of the electrodes on direct stimulation. When, in such a preparation, a fibre is stimulated by microelectrodes which are close together and well isolated almost to their tips, making it possible to stimulate part (one-quarter to one-third of the total length) of the fibre, it was found that only the part of the fibre between the electrodes contracted. In more badly damaged groups of muscle fibres (strongly bent) and in all isolated single muscle fibres no contraction was observed with reasonable strength of the stimulus. Even in those fibres, however, a shortening may be seen on very strong faradization; this shortening is usually irreversible (contracture).

DISCUSSION

The observations described above are consistent with the conceptions of the crustacean nerve-muscle preparation communicated before (Wiersma & van Harreveld, 1938; van Harreveld, 1939*a*). The extreme vulnerability of the muscles to mechanical injury is well explained by the nerve supply by a feltwork of fine nerve fibres between the muscle fibres. It is clear that such a feltwork will be damaged very easily by any relative displacements of the muscle fibres. This is, at the same time, an argument for the lack of a muscular conduction process. It is hardly conceivable that a slight injury would damage all the nerve fibres surrounding a muscle fibre. One functional nerve ending would be enough to set up a contraction in the whole muscle

fibre if a muscular conduction process existed. If such a conduction process is lacking, however, the slight local contraction set up by a few functioning nerve endings will have such a slight effect on the whole muscle fibre that this will be hard to detect. The local contraction of a slightly damaged muscle fibre during direct stimulation may be considered also as an argument for the lack of a local conduction process. It is likely that the direct stimulation of such a preparation is really an indirect stimulation; though the feltwork between the muscle fibres is destroyed, as shown by the fact that the impulses do not spread over the whole muscle, it is probable that the finest branches, including perhaps the hypolemmal ones, are still intact and can be stimulated.

The phenomena observed in the same muscle fibre during the stimulation of the axon for the slow and for the fast contraction can be considered as strong evidence for a double motor innervation of the individual muscle fibres in a triply innervated muscle. This, together with the evidence mentioned in the introduction, makes the double motor innervation in these muscle fibres almost certain.

SUMMARY

The crustacean muscle is extremely sensitive to mechanical injury. This is due to the fact that the muscle fibres are innervated by a feltwork of nerve fibres which surrounds them. Apparently, there is a lack of a muscular conduction process in these muscles. Contractions have been observed in the same muscle fibres during stimulation of the axon for the fast contraction as well as during stimulation of the fibre for the slow contraction.

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