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ON THE NATURE OF THE TWO TYPES OF RESPONSE IN THE NEUROMUSCULAR SYSTEM OF THE CRUSTACEAN CLAW.

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THE claw muscles of the crustaceans respond to stimulation with two distinct types of contraction. The response may be slow, of moderate intensity, and maintained for a long period, or it may be quick and very strong. The first type bears a certain resemblance to the contraction of smooth muscle, while the second type has more the characteristics of a single twitch in vertebrate striated muscle. The important evidence in this connection dates from the publication of the work of Lucas [1917], who made a careful analysis of the nature of the stimulus, bringing forth each of the two types of contraction in the crayfish (*Astăcus*). He showed that in order to elicit quick contraction, through a stimulus applied to the nerve, a relatively strong stimulus is required, and that the excitation time is shorter. The change from the slow to the quick type was shown to be a discontinuous one, each being of uniform magnitude through a considerable range of stimulation intensity or duration. Previous to this Lucas [1907] had reported the results of a study of the relationship between the duration and intensity of the exciting stimulus, applied to the nerve, on the response of the claw muscle of the lobster (*Homarus*). A break in the continuity of the curves occurred at the same point as the change in the nature of the mechanical response. These findings on *Astacus* and *Homarus* led Lucas to postulate the existence of two separate conducting mechanisms in the nerve trunk. A second factor which was not studied by Lucas, but which we have found to have an important bearing on the nature of the response, is the frequency of stimulation. The slow contraction alone is brought forth by

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stimulation of low frequency provided the intensity is not too great, while a sudden change to the quick type occurs as the result of a simple increase in frequency. The observations described herein were made in the course of a study designed to throw further light on the conditions determining the two types of response in crustacean nerve-muscle preparations.

Various crustaceans were used, including the lobster (*Homarus*), the edible crab (*Cancer*), and a form of spider crab (*Maia*), all of which gave essentially similar results. One of the large claw limbs was removed from the animal and fixed in a clamp, while the movable segment (dactylopodite) was connected with an isometric lever by means of strong silk cord or wire. The attachment of the abductor muscle was severed, so that the lever recorded the tension of the adductor muscle alone. Silver electrodes were applied directly to the nerve which was exposed in one or the other of the two limb segments most distant from the muscle. As a source of stimulation two Harvard induction coils were employed with motor driven contact breakers (Palmer) in the primary circuit, by means of which stimuli at the desired frequency were obtained. Break shocks only were used, the make shocks being short-circuited. In order to change as quickly as possible from one stimulating frequency to another, two contact breakers were used, either of which could be brought into the stimulating circuit with a double throw key. In most of the experiments the secondary coil was placed in the zero position, the intensity of the stimuli being controlled by a potential divider in the primary circuit. In certain experiments we employed the Neon tube stimulator made on the principle described by Briscoe and Leyshon [1929], which provided a simple and effective means of obtaining stimuli through a wide range of frequencies.

The influence of the frequency of stimulation on the response is typically as follows: If a nerve be stimulated with low intensities a contraction occurs which reaches its maximum very slowly and in a perfectly smooth curve. The tension developed depends upon the frequency of stimulation, *i.e.* the faster the rhythm of stimulation the higher the level of contraction reached. Within a certain range no change occurs with increasing intensity, but with relatively high stimulating frequencies, at a constant intensity, the type of contraction is abruptly altered: the muscle now contracts quicker and reaches a much higher tension. The curve is no longer smooth, but the individual contractions are clearly seen, resembling that of an incomplete tetanus in vertebrate striated muscle. The second type of contraction is usually obtained only above a certain frequency which is related to the intensity of the stimulus, and varies in different preparations. It is our impression that the better the preparation the lower is the frequency at which it is possible to observe the quick type of contraction, and rarely single stimuli of sufficient intensity gave twitches.

As a preliminary to further experimentation a strength and a frequency of stimulation were selected which gave a moderate sustained response (slow contraction) to the adductor muscle of the claw when applied to the

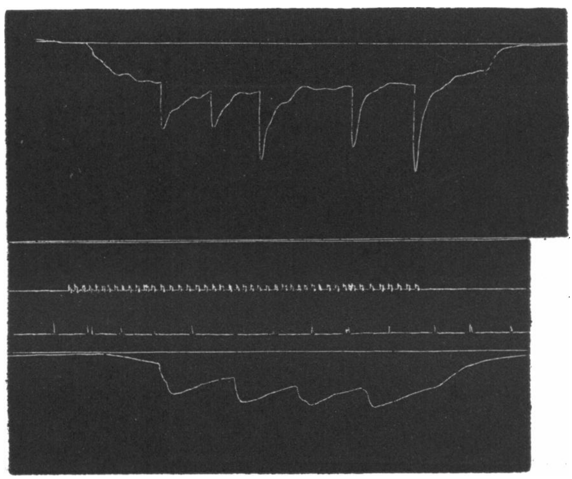

Fig. 1. Tracings from two experiments on *Cancer*, showing twitch-like response to extra stimuli applied during sustained activity. Lower record made with kymograph turning at a higher speed. In this and subsequent records where more than one tracing is shown, the upper line shows the individual stimuli, the middle line the time in seconds, and the lower line the record of the tension changes in the muscle.

nerve[1]. If, while the low-frequency stimulation is effective in maintaining the sustained contraction, a single stimulus is applied to the same nerve, the muscle will now respond with a single twitch, *i.e.* a contraction of the quick type, although this stimulus alone is quite without effect. Records showing this result are reproduced as Fig. 1. In this connection it should be pointed out that usually a single stimulus applied to the nerve results in no response of the muscle, although twitches can readily be obtained upon direct stimulation. Certain preparations do respond to single stimuli, the conditions for which will be discussed later, but the

[1] The exact relationship between these variables necessary to give the desired result has not been determined. It varies greatly in different species and from preparation to preparation, and is also subject to change during the course of an experiment.

present observations have been made in preparations in which the single stimulus alone has been shown by control observations to be absolutely without effect.

In an endeavour to explain the mechanism a number of variants on the experiment described above have been carried out. In many instances one pair of electrodes has been inserted directly into the adductor muscle while the second pair was applied to the nerve. If, while a series of twitches is being recorded in response to induced shocks applied to the muscle, the nerve is stimulated by a frequency giving alone a barely perceptible response of the slow type, there results a sudden increase in the

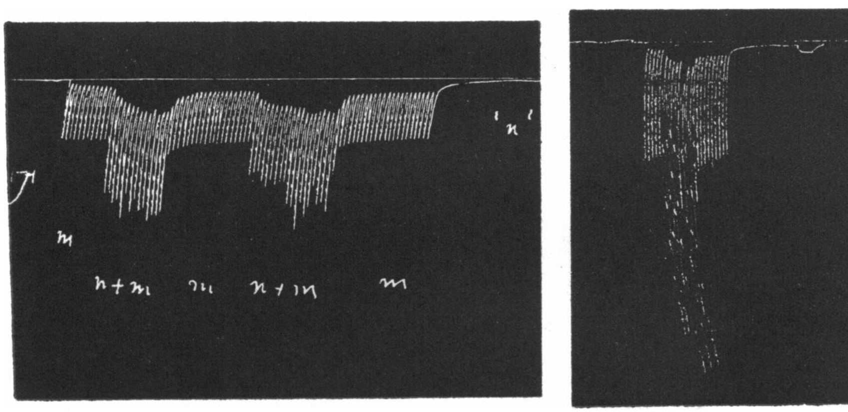

Fig. 2. The effect of low-frequency stimulation applied to the nerve (in *Maia*) on the response of the muscle to direct stimulation. The portions of the tracing giving the increased tension correspond to the time during which the nerve was stimulated. The given stimulus applied to the nerve alone in the absence of direct stimulation was without visible effect in the first tracing, and produced but a slight effect in the second.

tension developed during each twitch. Examples of this effect are given in Fig. 2. The magnitude of the increased tension is irregular in successive twitches, but usually quite large, amounting to a value several times that recorded previous to the application of the stimulus to the nerve. This effect suggests some influence of the nerve on the muscle fibres such that they respond to a direct stimulus with a stronger contraction. It must be remembered, however, that in applying a stimulus directly to the muscle we cannot exclude the stimulation of the nerve fibres in the vicinity. This being the case the conditions are as described in the first experiment where both electrodes were applied to the nerve. The stimuli sent directly to the muscle give a response which, because of the slow rate, is quite independent of any influence on the contained nerve endings. When a stimulus, alone giving but a barely perceptible slow response, is applied to the nerve it becomes irritable to single stimuli as described above, and the muscle responds with a greatly augmented twitch which therefore presumably has its origin in the nerve. Thus the effect of applying to the nerve a stimulus giving a slow response is to bring about the effective excitation, through the electrodes in the muscle, of the contained nerve elements, and thus to excite the quick contraction. This should result in a better distribution of the stimulus to the muscle fibres and would thus account for the augmentation of the twitches.

The production of a twitch by an extra stimulus applied to the nerve while it is being rhythmically stimulated as described above, is not dependent upon some local change, for the extra stimulus may be applied at any point on the nerve. In some experiments, for example, each pair of electrodes was placed on the nerve in different segments of the limb (carpopodite and meropodite). Nor is the effect dependent upon any peculiarity of the extra stimulus, as was shown by applying it through the same coil and electrodes which were giving the rhythmic stimulation for the slow tonic contraction. Under these conditions the character of the extra stimulus must have been indistinguishable from those giving the tonic contraction, nevertheless it caused the muscle to give a twitch of the type illustrated in Figs. 1 and 4. Evidently the effect bears some relation to the spacing of stimuli sent into the nerve. A single extra stimulus is sufficient to change the slow contraction, produced characteristically at low rates of stimulation, to the quick type, obtained at higher frequencies, or possibly to superimpose the second type upon the first.

It has been demonstrated experimentally by Hoffmann [1914] in the crayfish and by Knowlton and Campbell [1929] in the lobster (*Homarus*) that the inhibitory fibres to the adductor muscle are found in a bundle of fibres (excitatory to the abductor) which can readily be separated from the main nerve. The effect of the extra stimulus does not depend upon the presence of the inhibitory fibres, for in five experiments carried out on the claw of the lobster, complete removal of the nerve elements containing these fibres did not result in the least influence on the response. A tracing illustrating this is reproduced in Fig. 3.

Another feature of the response to the extra stimulus, well shown in the records reproduced, is the irregularity in the tensions developed, and the occasional failure to obtain any response whatever. This accords with expectation on the grounds that the effect depends upon the grouping of the extra stimulus with those of the rhythmically repeated series, for a stimulus falling within the refractory period would, of course, be without effect, and the influence of those falling later might well depend upon their relation to the other stimuli of the series.

The extra stimulus is, as a rule, without effect when the rhythmic series is reduced in intensity or frequency to a point giving no tonic contraction in the muscle. One or two exceptions have been noted, but these are presumably to be explained by a failure of the recording apparatus to respond to slight changes of tension in the muscle, for when a response is obtained from a single stimulus under these conditions, the rate of stimulation is very close to the threshold giving a mechanical record. This fact and the other experimental findings related above by themselves favour the hypothesis that both types of contraction are mediated

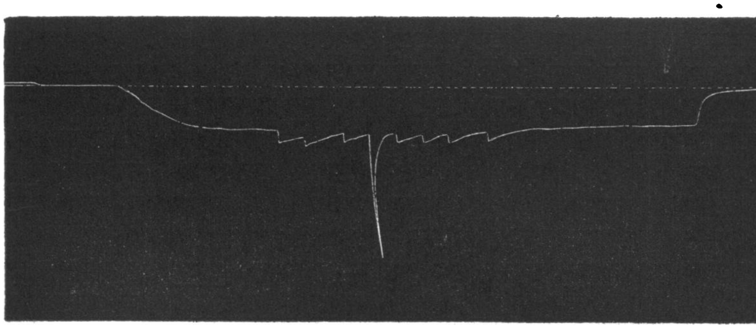

Fig. 3. The effect of a series of "extra" stimuli applied to the nerve of *Homarus* in which the "thin system" inhibitory to the adductor had been excised.

through the same morphological structures. They are not, however, inconsistent with the well-supported conception of Lucas [1917] that two separate excitable substances are present in the nerve trunk, each of which brings forth its specific kind of muscular contraction. A study of the action potentials in the nerve under these conditions should throw important light on the problem.

Our records show that the effects of excitation may persist for a considerable period. This phenomenon was discovered and carefully studied in the crayfish by Richet [1879] over fifty years ago. He pointed out that the effects of repeated stimulation are exactly the opposite of those occurring with fatigue, *i.e.* excitability and response are augmented, a phenomenon which Richet called addition latente. A demonstration of this is given in the record shown in Figs. 4 and 5. It will be seen that there is no response to the stimuli until a certain number have been applied, but finally, with the low-frequency stimulation employed, the

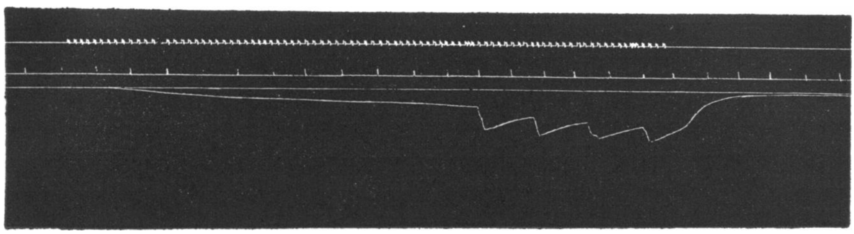

Fig. 4. Tracing obtained from *Cancer*, illustrating the gradual development of tension in response to low-frequency stimulation, and the effect of superimposed extra stimuli upon it.

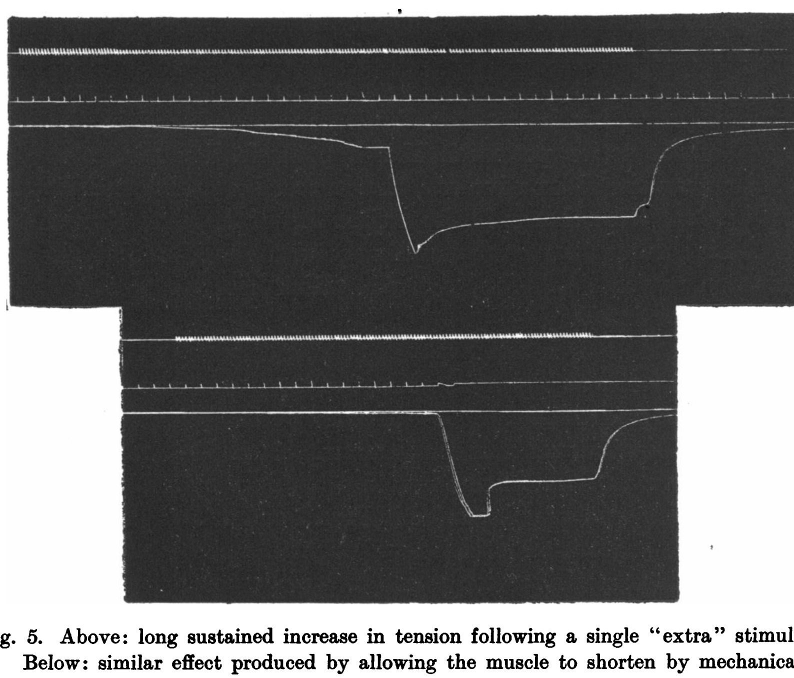

Fig. 5. Above: long sustained increase in tension following a single "extra" stimulus. Below: similar effect produced by allowing the muscle to shorten by mechanically closing the claw against the tension of the lever.

slow type of contraction gradually develops, the tension continuing to increase for a considerable period of time. This type of response is of interest in connection with Lucas's study of the summation of two stimuli in *Astacus* [1917]. It was shown, even when no response was elicited from the first of two stimuli, that nevertheless a propagated disturbance was set up in the nerve, and that the passage of this impulse produced a change such that the following stimulus became effective in causing a contraction. Since the optimum interval corresponded with the time at which the nerve was most excitable following previous activity, it was concluded that two properly spaced stimuli produce an effect because the second stimulus is aided in its passage to the muscle fibre by travelling in the supernormal phase of recovery of the first impulse. Lucas did not study the effects of more than two stimuli, and our experiments with a series of stimuli put the matter in a different light. The greater effectiveness of a series of stimuli, extending over a period of several seconds, as compared to two, clearly cannot be explained entirely on the basis of the relationship of the individual disturbances to each other, *i.e.* to their position in the recovery cycle. If the phenomenon is due to an influence on the conductivity of the nerve fibre, there must be a persistent change in the fibre resulting from its activity which is not wiped out by the response. That a change actually may occur in the crustacean nerve has been shown by Furusawa [1929] in the persistence of the action potential. This hypothesis, however, seems less probable than the alternative one, that successive propagated disturbances produce some change in the excitability of the muscle which persists and is accumulative, such that if a sufficient number of impulses are received a response will finally result, which becomes greater as the stimuli are continued.

Following the twitch-like response, produced either by a single extra stimulus or a series at higher frequency, any pre-existing tension of the slow type is augmented, *i.e.* the low-frequency stimulation maintains the tension at a higher level than that existing before the quick response, although previously the tension caused a barely perceptible movement of the lever. The effect is illustrated in Figs. 4 and 6. It is of course possible that the augmented tension level might ultimately be reached by the gradual increase in tension which commonly occurs during the application of the low-frequency stimulation, although in our experience the effect of the quick contraction has always been greater. The elevation in tension following the quick contraction is not the result of a specific influence brought about by activity, because an exactly similar result is secured when the muscle is assisted by mechanically closing the claw—compare the second tracing of Fig. 5. Fulton [1926, p. 188] has made a similar observation in frog's muscle, *i.e.* assuming that the lever employed did not distort the picture, his records show that a muscle can maintain a greater tension than it can set up.

A single stimulus applied to the nerve usually does not result in a response of the muscle. With an excessive shock a response is frequently obtained, especially if the preparation be fresh. Two considerations have a bearing on this behaviour. Firstly, when an excessively strong shock is given it is quite probable that a succession of nerve impulses results from a single stimulus, much as Forbes and Gregg [1916] found to be the case in mammalian nerves. Weaker stimuli (which give rise to propagated disturbances in the nerve, as can be proved by repetition when well-marked responses occur) are without effect on the muscle, indicating some peculiar action of the strong stimulus such as that just suggested. Secondly, the observation of Barnes [1930] that the crustacean nerve continues to propagate a series of impulses for a considerable period after cutting it from the rest of the animal, may have a bearing on the question. Frequently good responses to single stimuli were obtained early in the experiment, but after a short period excessively strong stimuli were required, although a good response to repeated stimuli might be obtained for hours. The same thing was observed by Richet [1879] and by Lucas [1917]. This result suggests that at the beginning of an experiment the single stimulus may have been applied to an already somewhat active nerve, and hence that the conditions were comparable to those experiments in which the extra stimulus was applied to a nerve responding to a rapid series of stimuli. In a few experiments in which a stimulus was applied to the nerve in an intact animal a response was usually obtained, and here there can be no question but that a certain background of activity was already present. In our experience a single stimulus is more likely to be effective in producing a response in the edible crab (*Cancer*) than in *Maia*.

The foregoing observations indicate an interesting mechanism controlling the claw muscles of the crustaceans tending to economize the expenditure of energy. It has been shown by Furusawa [1929] that complete "depolarization" of the crustacean nerve may be brought about with great rapidity by a tetanizing stimulus, and Hill [1929] has found a rapid decline in the heat production of the nerve during the application of such a stimulus. This is of interest in connection with the well-known fact that the claw muscles of the crab are frequently called upon to exert great tensions which are well sustained for considerable periods of time; facts which led Hill to suggest the probability of some mechanism enabling the contraction to be sustained without great demands on nervous conduction. In the experiments just cited the frequency of stimulation was in the neighbourhood of 50 make and 50 break shocks per second, produced by the vibrating contact breaker on the Harvard coil. The present experiments show that a stimulation frequency of from 2 to 10 per second is sufficient to bring forth a tonic contraction, and in this condition the muscle is ready to respond to a single extra stimulus with a quick contraction. Following the extra stimulus the given slow rhythm of stimulation may hold the tension at an increased level, and a series of extra stimuli spaced at intervals of several seconds would be all that would be required to build up a strong sustained contraction such as that illustrated in Fig. 6. On this conception one need only postulate the existence of a tonic activity of the muscle, such as is known to exist in the

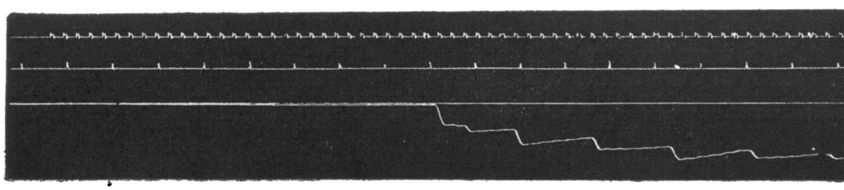

Fig. 6. Tracing from *Cancer* with a very light lever, showing failure of the adductor muscle to develop an appreciable tension at the given stimulation frequency and the gradual building up of tension with repeated extra single stimuli.

vertebrates; the rest of the mechanism has been demonstrated to occur by the present experiments. On such a basis far less demands would be made on the mechanism than was the case in the experiments of Furusawa and of Hill, in which activity of the nerve was maintained by constant high-frequency stimulation with resulting rapid fatigue.

In reporting the experimental results of the present investigation an attempt has been made from time to time to relate them to the work of others. We feel, however, that a great deal more information must be acquired before a comprehensive theory can be safely advanced for the mechanism involved in the control of the crustacean claw. In the present state of our knowledge it is dangerous to describe the results in terms of the classical physiology of vertebrate tissues. For this reason we regard this as a preliminary study and have been content to record the observed facts, omitting a theoretical discussion.

SUMMARY.

By a simple increase in the frequency of stimulation applied to the nerve the response of the adductor muscle of the claw in various crustaceans may be changed from a sustained contraction of moderate intensity to a quick twitch-like effect. This transition may be brought about by the addition of a single extra stimulus to those of the frequency series, *i.e.* an extra stimulus, differing in no way from those producing the sustained response and which alone is without effect, now brings forth a twitch-like contraction. Following such a quick contraction the tension is maintained at a higher level than that existing before the extra stimulus was applied. There thus exists a mechanism through which the organism can maintain a continuous strong tension and which makes comparatively small demands upon the nervous system.

The experiments described in this paper were carried out during the summers of 1929 and 1930 at the Marine Biological Laboratory, Plymouth, under the kind guidance of Prof. A. V. Hill, to whom we are also indebted for the apparatus employed. Our thanks are due to the Director, Dr E. J. Allen, for placing at our disposal the facilities of the laboratory.

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