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NEURAL ACTIVATION OF THE HEART OF THE LOBSTER *HOMARUS AMERICANUS*

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INTRODUCTION

The heart-ganglion preparation of the lobster is an independent nerve-effector system. It can be isolated from the rest of the animal and maintained for many hours, actively contracting, in a cooled saline bath. It is therefore an ideal system for studying the functional organization of a relatively simple organ – a pump – that is governed by a part of the nervous system composed of only a few units.

The single-chambered heart, made up of striated muscle fibres ranging from 11 to 39 μm in diameter, is enclosed within the pericardial cavity located just below the dorsal carapace of the animal (see Anderson & Smith, 1971, for details of fine structure). It is suspended from epimeral plates by elastic ligaments. Haemolymph enters the heart through two pairs of ostia and is driven out of the heart via anterior and posterior vessels.

The lobster heart is neurogenic (Alexandrowicz, 1932). It contracts only in response to activity produced by nerve cells in the Y-shaped cardiac ganglion that lies on its inner dorsal wall. The ganglion contains five large follower cells which provide efferent motor output to the muscle fibres and four small pacemaker cells which provide, via synaptic contacts, input to the large cells. The electrophysiology of the lobster cardiac ganglion has been studied in detail (for reviews of early work in both *Homarus* and *Panulirus* see Maynard, 1960; Hagiwara, 1961; and Bullock & Horridge, 1965; recent references: Cooke, 1966; Hartline, 1967; Connor, 1969; Hartline & Cooke, 1969; Mayeri, 1969; Rao *et al.* 1969; Livengood & Kusano, 1970). The small cells time the output of the events produced by the large cells (Hartline, 1967). The axons of the large cells branch profusely and innervate the muscle fibres of the heart (Alexandrowicz, 1932). They produce high-frequency bursts of impulses at regular intervals and each burst is associated with a contraction of the heart (Welsh & Maynard, 1951; Maynard, 1955).

A further step in understanding the organization of the heart-ganglion system is to find out how the information processed in the ganglion is transmitted to the muscle fibres of the heart. In this paper the nerve-muscle physiology of the heart of the lobster *Homarus americanus* is considered. Glass microelectrodes were used to record intracellular activity from single muscle fibres. Events were recorded when the heart-

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ganglion preparation was spontaneously active, when the heart was quiescent and when electrical stimuli were applied to the distal end of cut branches of the ganglion. Some of these observations have been published in brief notes (Anderson & Cooke, 1969, 1970).

MATERIALS AND METHODS

Animals

Lobsters weighing 1–1½ lb were obtained from a local fish market. They were kept in pans of aerated sea water at 8 °C for as long as a month.

Dissection

The claws were caused to autotomize; the ventral nerve cord was cut between the thorax and abdomen, and the abdomen and head region were then cut off. Two lateral cuts were made in the thoracic gill region to divide the thorax into ventral and dorsal halves. The ventral half was discarded; the gut, digestive gland and gonads were removed from the dorsal half to expose the heart within the pericardium. The heart attached to the dorsal carapace was pinned out, ventral side up, in a wax-bottomed dish filled with lobster perfusion solution. The ventral wall of the pericardium was cut away and a longitudinal slit was cut down the middle of the ventral heart wall. The flaps of the wall were pulled apart either with porcupine quills or with small stainless-steel hooks attached to elastic threads which were anchored by pins to the bottom of the dish. The preparation was bathed in a saline solution which was changed at a rate of approximately 15 ml/min. The temperature of the bath during an experiment was maintained between 9 and 12° C.

Perfusion solution

The perfusion solution had the following ionic composition, expressed in mM/l (Cole, 1941; Welsh & Smith 1960): Na^+, 479·6; K^+, 15·7; Ca^{2+}, 25·9; Mg^{2+}, 9·3; Cl^-, 548·9; SO_4^{2-}, 8·4. It was buffered with H_3BO_3 (8·8 mM/l); the pH was adjusted to 7·4–7·6 with NaOH (0·48 mM/l).

Electrodes and equipment

Suction electrodes were used to record from the proximal ends of cut branches of the ganglion and to apply stimuli to the distal ends. Glass tips with diameters of approximately 0·1 mm were connected to a syringe by polyethylene tubing. A silver wire was inserted through a small hole in the end of the polyethylene tubing and advanced into the glass tip. All junction points of the electrode components were sealed with nail polish. For recording, the wire within the electrode and the indifferent wire in the bath were connected through an a.c. pre-amplifier to a multiple-beam oscilloscope. For stimulating, the wires were connected to a pulse generator through an isolation unit.

Intracellular recordings were made from single muscle fibres using standard 3 M-KCl-filled glass (Pyrex) micropipettes of 10–15 MΩ resistance. The electrodes were

suspended from fine silver wires, in a floating arrangement, and connected through a neutralized capacity-input amplifier to the oscilloscope.

A passive RC differentiating circuit with a time constant of 1 ms was connected between the vertical output of the oscilloscope beam which monitored intracellular events and the input of a second beam. By using this circuit it was possible to distinguish inflexion points in the depolarizations that were not always obvious on the non-differentiated trace.

To record contractions of the whole heart, a thread was tied to the anterior vessels and attached to a transducer connected to the input of a polygraph. The contractions were also displayed at the oscilloscope.

Tetrodotoxin (TTX)

To record miniature junction potentials the perfusion was stopped and sufficient 10^{-6} M TTX solution (Sankyo Company Ltd, Tokyo) was added to the bath to abolish spike activity of the cells in the ganglion and, therefore, contractile activity of the myocardium. Miniature junction potentials were also recorded without TTX from the muscle fibres of hearts from which the ganglia had been removed.

RESULTS

Spontaneous activity

Fig. 1 illustrates events recorded from a spontaneously beating heart. In (*a*) the top trace is a mechanical record of six successive beats. The bottom trace is the intracellular record from a single muscle fibre. The electrode was removed from the cell to indicate the zero reference level. For a series of 148 measurements the mean value of the resting potentials recorded across the surface membrane of the muscle fibres was $51{\cdot}5 \pm 3{\cdot}2$ mV. The depolarizations were never seen to overshoot the zero reference level.

Preceding and accompanying the early phase of each heart contraction there is a depolarization of the muscle fibre. The depolarizations exhibit a fast rising phase which reaches a peak amplitude of 35–40 mV. A plateau of 20–25 mV follows the peak and then decays to the base-line. The duration of these potential changes is relatively constant in a given heart and varies from 400 to 700 ms in different preparations.

In (*b*) the top trace is the differentiation of the bottom (intracellular) trace. It is clear that, in this example, at least three components contribute to the fast rise of the depolarization.

In (*c*) the temporal relationship of extracellular, intracellular and mechanical events is shown. The extracellular record was taken from a cut proximal branch of the cardiac ganglion (the posterolateral nerve). The frequency of impulses is highest at the beginning of a burst. Shortly after the initiation of the burst the muscle fibre begins to depolarize. Contraction of the heart begins during the plateau. Depolarizations always preceded contractions by approximately 40 ms. Spontaneous bursts of impulses generated in the cardiac ganglion produced heart beats at a frequency of about 1/s.

Hartline (1967) showed that each of the two posterolateral nerves (PLNs) that leave the trunk of the ganglion contain three axon branches which arise from the three most anterior large cells in the ganglion. Each of these cells sends an axon in a caudal direction down the trunk of the ganglion. At the point where the PLNs join the trunk, each axon branches symmetrically and sends one process out the left PLN and another out the right PLN. One can therefore assume that, under normal circumstances, the outputs of both PLNs are identical.

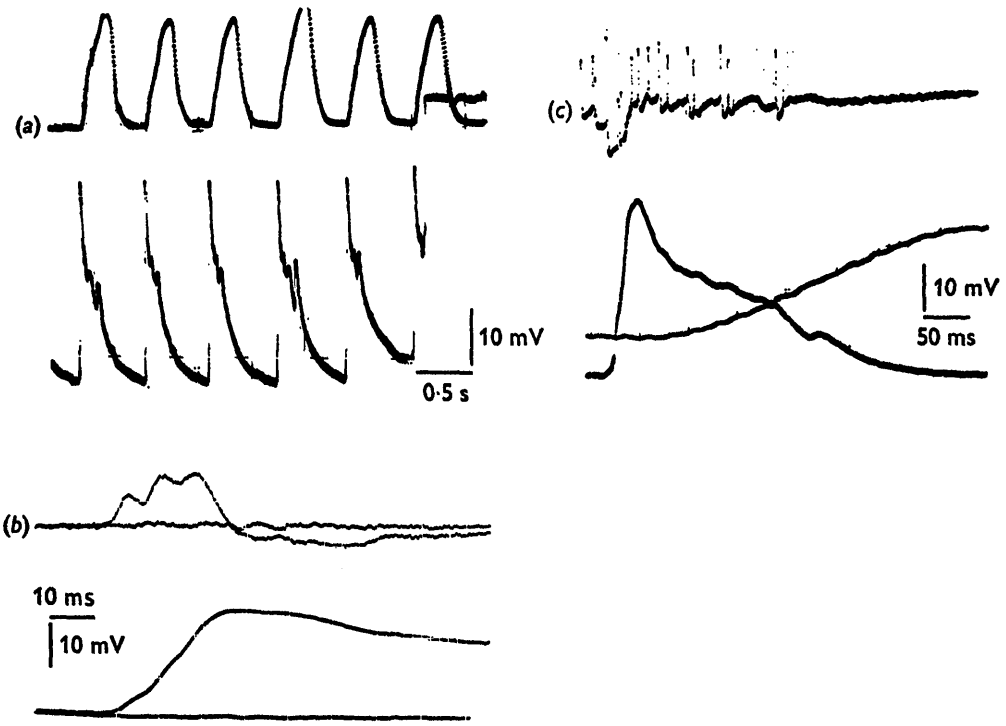

Fig. 1. Events recorded from a spontaneously active heart. See text for explanation. (*a*) Top trace, mechanical record of six heart beats; bottom trace, intracellular record from a single muscle fibre. The electrode was removed from the cell to demonstrate the zero reference level for the transmembrane potential. (*b*) Top trace, differential of the intracellular record; bottom trace, intracellular record from a single muscle fibre. (*c*) Top trace, extracellular record from a cut proximal branch of the cardiac ganglion; middle trace, mechanical record; bottom trace, intracellular record.

On the basis of this assumption the following experiment was performed. All of the branches of the ganglion except the PLNs were cut. Cutting these connexions, and thereby reducing the number of axons transmitting impulses to the heart muscle fibres, decreased the contractile activity of the heart. Instead of producing strong, maintained contractions, the heart pulsated weakly and failed to function as a pump. One of the PLNs was then cut, and spontaneous nerve impulses were recorded extracellularly from its proximal end (Fig. 2, top traces, (*a*) and (*b*)). Intracellular events (bottom traces, (*a*) and (*b*)) were recorded from a muscle fibre on the opposite side of the heart innervated by the intact PLN. In (*a*) the effects of cutting the branches of the ganglion anterior to the PLNs are shown. The duration of each burst is increased and the frequency of impulses within the burst is decreased. Except in the fast-rising portion it is possible to see that each depolarizing potential change is preceded by a nerve impulse.

In (*b*) the trunk of the ganglion was cut transversely half-way between the anterior bifurcation and the PLNs. Further weakening of the contractile activity resulted. In this intracellular record – taken from the same cell as (*a*) – there is a one-to-one correlation between each nerve impulse and each unit of depolarization.

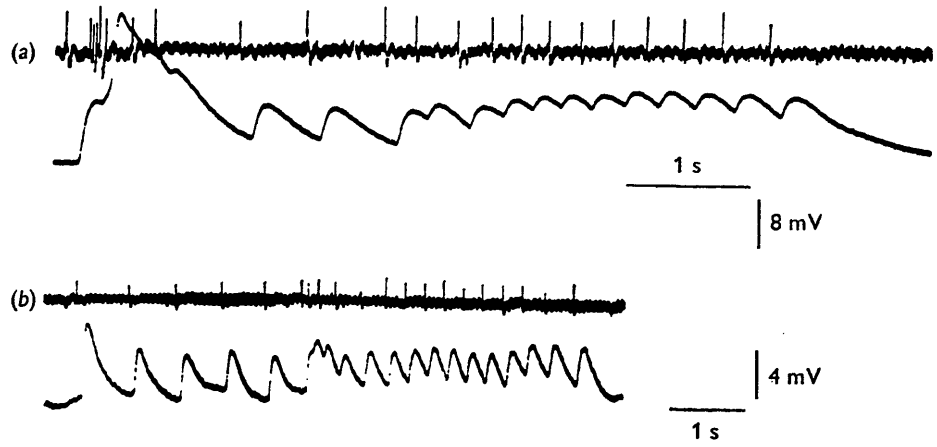

Fig. 2. One-to-one relationship between nerve impulses and muscle fibre depolarizations. See text for explanation. Top traces, (*a*) and (*b*), extracellular records taken from the proximal end of a cut posterolateral nerve (PLN). Bottom traces, (*a*) and (*b*), intracellular records taken from a muscle fibre located in a region innervated by the intact contralateral PLN. The spikes in the extracellular records were retouched.

On the basis of observations such as these we conclude that each unit of depolarization is produced by a single nerve impulse and can be interpreted as an excitatory junction potential (EJP). We further conclude that, in the intact, spontaneously active heart, each high-frequency burst of impulses generated in the ganglion produces many EJPs; these occur in rapid succession and produce a complex depolarization of the muscle fibre.

Current–voltage relationship

There is no evidence that the individual EJPs or the depolarizing complexes produced by summation of several EJPs give rise to regenerative membrane responses. The absence of regenerative responses was shown by current–voltage curves obtained from hearts made quiescent by removing the main trunk of the ganglion that contains the nerve cell bodies. Two microelectrodes were placed in the same muscle fibre. Current pulses of 100–200 ms duration and of either polarity were passed through one electrode while the resultant potential changes were recorded with the other. The amplitudes of the voltage changes (ordinate) were plotted against the intensities of the applied current pulses (abscissa). One such experiment is shown in Fig. 3.

The current–voltage relationship is linear, and the line passes through the origin of the co-ordinates. The maximum depolarization elicited in this experiment was about 34 mV, a value close to the peak amplitudes of the spontaneous depolarizations. Were these muscles fibres capable of producing regenerative responses, a departure from linearity should have been observed as an upward skew of the current–voltage curve in the depolarizing quadrant. This was never seen; instead, the current–voltage relationship is depressed by delayed rectification, as indicated in the figure.

Miniature potential changes

When the heart is made quiescent by adding TTX to the bath or by removing the trunk of the ganglion, a microelectrode placed in a single muscle fibre records small, spontaneously produced potentials. The inset of Fig. 4 is a sample record of these

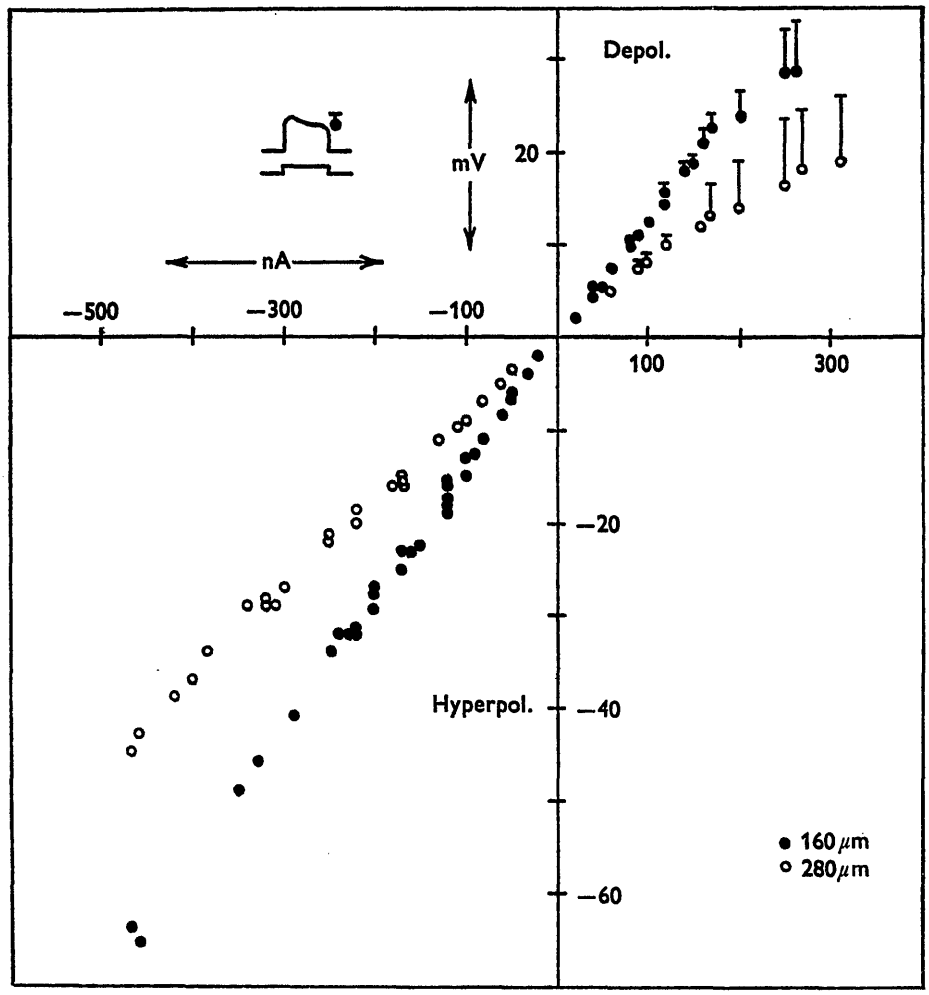

Fig. 3. Current–voltage relationship. See text for details. The amplitudes of the voltage changes in mV (ordinate) were plotted against the intensities of the current pulses in nA (abscissa). The current pulses were of 160 ms duration. While the current-passing electrode remained in the same position, the recording electrode monitored voltage changes at two different sites on the muscle fibre: 160 μm (closed circles) and 280 μm (open circles) away. The degree of delayed rectification (measured as indicated in the inset diagram) is indicated by the vertical lines above the points in the depolarizing quadrant.

small potentials. Their amplitudes range from about 50 μV (just detectable above the noise level) to about 0·5 mV. If it can be shown that these small potentials occur as independent events, it is likely, although not conclusive, that they are miniature junction potentials (MJPs) such as those described for some peripheral nerve–muscle junctions in crustaceans (crayfish: Dudel and Kuffler, 1961; Bittner, 1968; lobster: Atwood & Parnas, 1968).

The histogram in Fig. 4 shows the distribution of intervals between the potentials measured in one experiment. Each bar represents the number of intervals whose duration falls within a given 20 ms period. The smooth curve indicates the distribution expected for a sequence of independent events (Dudel & Kuffler, 1961). These observations suggest that each potential is an independent event; however, the fact that the

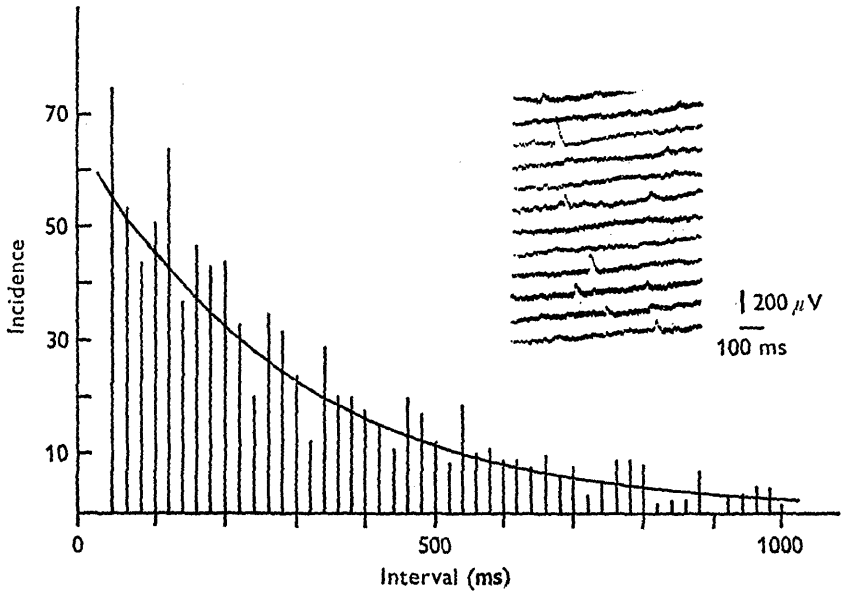

Fig. 4. Distribution of the intervals between small spontaneous potentials recorded from a single muscle fibre in the presence of TTX. Inset: a sample record of spontaneous potentials recorded in the presence of TTX. Histogram: the distribution of 932 intervals between potentials recorded in a single experiment. The record was of approximately 10 min duration. Each bar represents the number of intervals whose duration falls within a given 20 ms period. The smooth line indicates the expected distribution for a series of independent events. The fit of the curve to the data, on the basis of a χ^2 test, is significant at the 0·025 level.

muscle fibres are polyneuronally (see Fig. 5) and, possibly, multiterminally innervated complicates these results; an electrode inside a muscle fibre records events occurring at more than one junctional site (Dudel & Kuffler, 1961). Indeed, the miniature potentials recorded from muscle fibres of the lobster heart show different rates of rise. This suggests that there are nerve terminations on the muscle fibre at different distances from the recording microelectrode. Although the interval distribution shown in Fig. 4 suggests that the events are independent, some periodicity of potentials occurring at one or more sites may be masked.

To test this possibility, Mr Wayne Wiitanen* designed a computer program that used autocovariance tests (Cox & Lewis, 1966) to detect any hidden periodicities. The data of four separate experiments (372, 171, 694, 323 intervals) were analysed in this manner. He first demonstrated that each series of intervals was stationary; that is, any sample of data was representative of all the data obtained; the mean and variance were the same, with respect to the grand mean, regardless of what period in time the data sample was taken. The analyses failed to reveal any periodicities. This supports the suggestion that the potentials are indeed occurring independently.

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The spontaneous potentials recorded in the lobster heart therefore resemble MJPs in that they cannot be blocked by TTX and that they are independent. The data, however, are not sufficient to find out whether the recorded potentials represent single transmitter quanta.

Nerve stimulation

Experiments were performed using hearts without ganglia. Controlled electrical stimuli were applied with an extracellular suction electrode to the distal end of a cut PLN. Pulses of 0·5 ms duration were applied at frequencies of 0·5, 1·0, 2·0 and 5·0/sec. These pulses evoked activity from the axon branches in the PLN. The evoked activity produced EJPs in the muscle fibres innervated by the active axon branches. All tests were separated by a period of at least 2 min.

Fig. 5. Evidence for polyneuronal innervation. See text for explanation. Brief electrical stimuli were applied to the distal end of a cut PLN. The intensity of the stimuli was gradually increased. The responses (EJPs) of a muscle fibre to the evoked activity were monitored with a microelectrode.

Fig. 5 is a multiple-trace record showing polyneuronal innervation of a single muscle fibre. Stimuli were applied to the distal end of a cut PLN. As the stimulus intensity was gradually increased a distinct threshold was reached at which an EJP was recorded from the muscle fibre (lowest amplitude responses). The size of the response is constant until, upon a further increase in stimulus intensity, it changes in amplitude by a discrete increment (middle amplitude responses). Continued increase in stimulus intensity results in a third increase in the size of the response (largest amplitude responses). We interpret the responses of the muscle fibre to be the summation of EJPs which result from stimulating one, two and finally all three axons in the PLN. This experiment thus indicates that the muscle fibre was innervated by all three of the axons which leave the ganglion via the cut PLN. The fact that the rise times of the recorded potential changes are similar suggests that they terminate on the muscle fibre at approximately the same point. (A systematic study of the innervation pattern of the heart was not made; however, incidental observations suggest that each muscle fibre probably receives input from more than three axons.)

In the rest of the experiments described in this paper the intensity of the applied stimuli was adjusted to evoke activity from only one axon branch of the PLNs. There is no evidence to indicate that the lowest threshold unit corresponds to the equivalent axon branch in all preparations.

The record of Fig. 6 shows a series of responses of a muscle fibre to stimuli applied

to the distal end of a cut PLN. The successive responses in the train show a pronounced facilitation; they grow in amplitude up to a plateau level.

Facilitation can be defined as the fractional change in the amplitude of any response after the first response, with respect to the amplitude of the first response (Mallart &

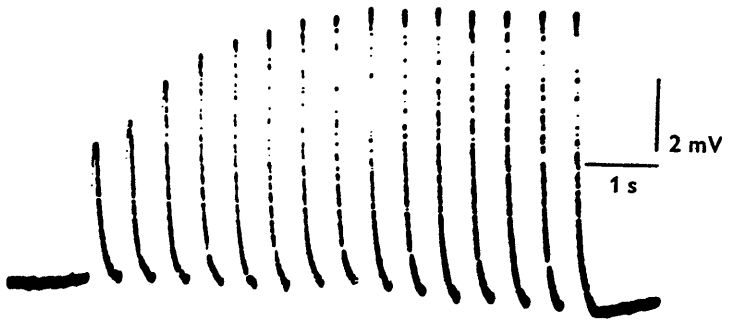

Fig. 6. Facilitation. A train of stimuli was applied to the distal end of a cut PLN. The intensity of stimulation was adjusted so that activity was evoked from only a single axon. The responses (EJPs) were recorded from a muscle fibre.

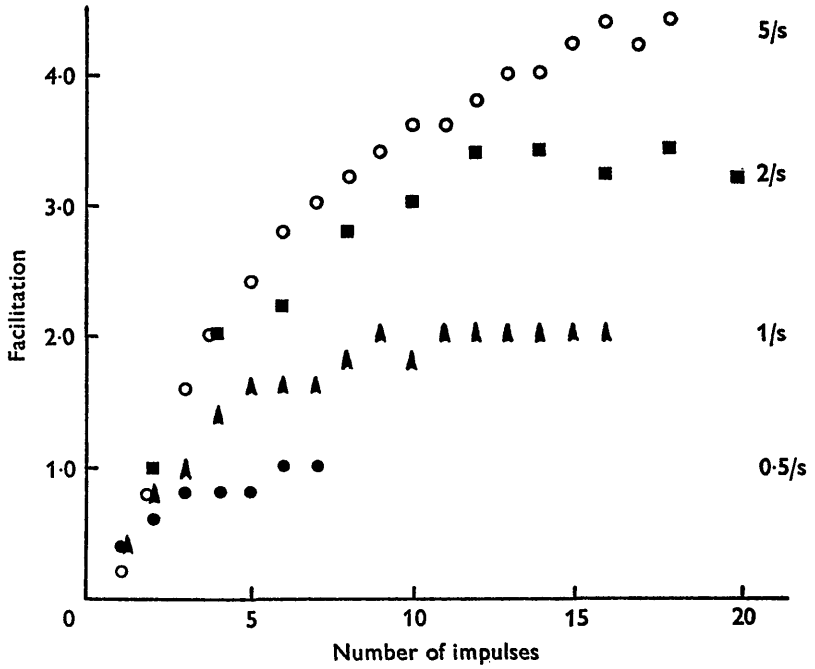

Fig. 7. Facilitation as a function of stimulus frequency. Trains of stimuli were applied to the distal end of a cut PLN at an intensity just sufficient to activate a single axon branch. The frequencies of the trains are indicated on the right of the figure. Facilitation was expressed as the fractional change in amplitude of any response after the first response, with respect to the amplitude of the first response. The facilitation values (ordinate) are plotted against the number of impulses in a train (abscissa).

Martin, 1967). In Fig. 7 the facilitation values calculated for each response in a train are plotted against the number of impulses in trains of stimuli applied at different frequencies. The facilitation for each train grows to a plateau. The level reached by the plateau depends on the frequency of stimulation.

Fig. 8 illustrates the type of experiment that was performed to demonstrate the decay of facilitation. Conditioning trains of stimuli applied at 5/sec were followed by test pulses at varying intervals after the conditioning trains. The amplitude of the test response decreases as the interval between the conditioning train and the test pulse increases. Fig. 9 shows the facilitation values of each test response plotted against

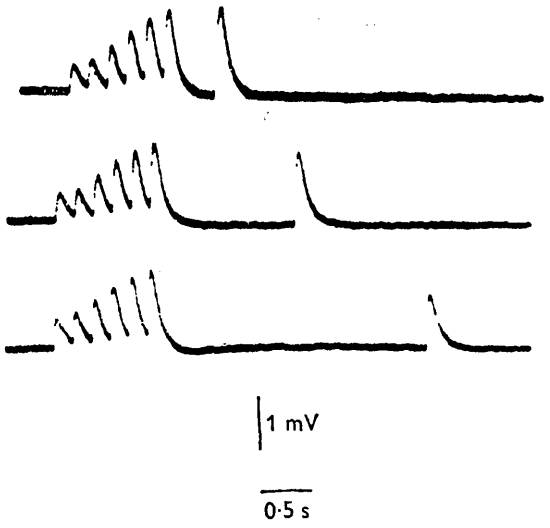

Fig. 8

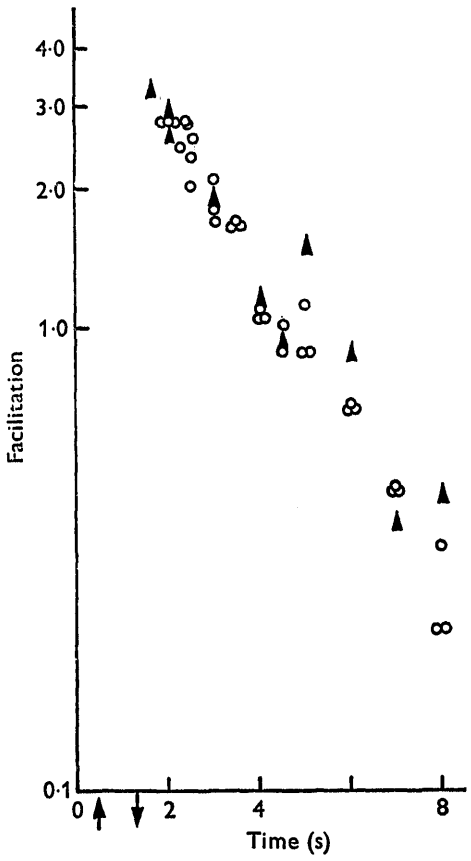

Fig. 9

Fig. 8. Decay of facilitation. Conditioning trains of pulses followed by test pulses at variable intervals were applied to the distal end of a cut PLN. The intensity of stimulation was adjusted so that activity was evoked from a single axon branch. The EJPs were recorded from a muscle fibre.

Fig. 9. Decay of facilitation plotted against time on semi-$\log_{10}$ paper. Conditioning trains of pulses followed by test pulses at variable intervals were applied to the distal end of a cut PLN at an intensity adjusted to evoke activity from a single axon branch (see Fig. 8). The facilitation values of the test responses were calculated with respect to the first responses of the conditioning trains. The period during which the conditioning train was applied is indicated by the arrows on the abscissa. The different symbols indicate two series of tests that were made at the same experimental site at different times (open circles, first series; closed triangles, second series). The plot results in an approximately straight line with a time constant of about 2·5 s.

time on semi-log paper. The results from two test series made at a single experimental site fall on an approximately straight line. The decay is an exponential process with a time constant of about 2·5 sec. The interpretation of this decay time is complicated by further evidence as described below.

Experiments were performed in which two pulses with varying inter-pulse intervals

were applied to a cut PLN. Examples of the EJP responses that were monitored in a muscle fibre during such an experiment are shown in Fig. 10. In Fig. 11 the facilitation values of the second pulses of a two-pulse experiment are plotted against the interpulse interval. The amplitude of the second response was depressed (facilitation expressed as negative values) when the interval was less than 0·5 s and slightly facilitated when it was greater than 0·5 s.

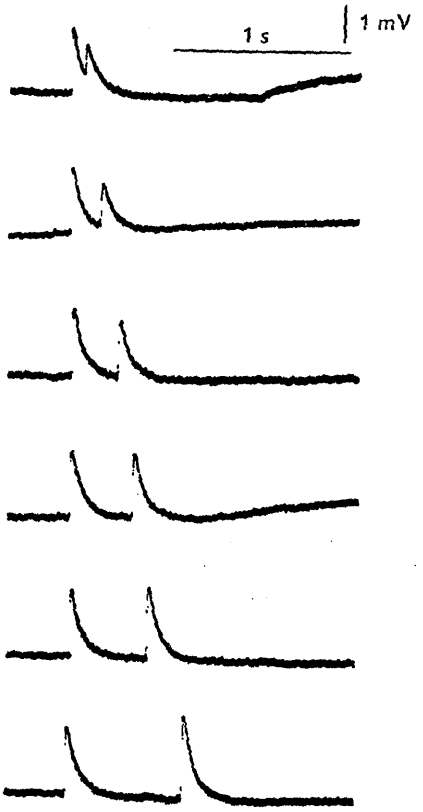

Fig. 10. Depression. Examples of the data plotted in Fig. 11. Conditioning pulses followed by test pulses at varying intervals were applied to the distal end of a cut PLN. The responses were recorded from a muscle fibre.

Usually, when trains of stimuli are applied to a cut PLN at a frequency $\geqslant$ 1 stimulus/s, the first recorded EJP is greater in amplitude than the second; this result is particularly clear when the stimuli are applied at a frequency of 5/s (Fig. 12). After the second response the facilitation process manifests itself. If the amplitude of the first response is not considered, the growth to the plateau amplitude is exponential. Even in Fig. 6 where the first response is smaller than the second, it is nevertheless larger than would be expected for an exponential rise.

Fig. 13 shows conditioning trains followed by test trains at varying intervals. In the top trace the inter-train interval is less than the inter-pulse interval. The first pulse of the test train is smaller in amplitude than the last pulse of the conditioning train (i.e. it is depressed). In the second trace the inter-train interval is nearly equal to the inter-pulse interval; consequently, the first pulse of the test train appears as if it were a continuation of the conditioning train. In the third, fourth and fifth traces the

inter-train intervals increase and the amplitudes of the first pulses of the test trains augment noticeably.

DISCUSSION

Rhythmic depolarizations have been intracellularly recorded from the muscle fibres of several spontaneously active neurogenic hearts (see Table 1). Many of these studies indicate that each depolarization is associated both with a burst of impulses from the cardiac ganglion and with a contraction of the heart. Most of the depolarizations

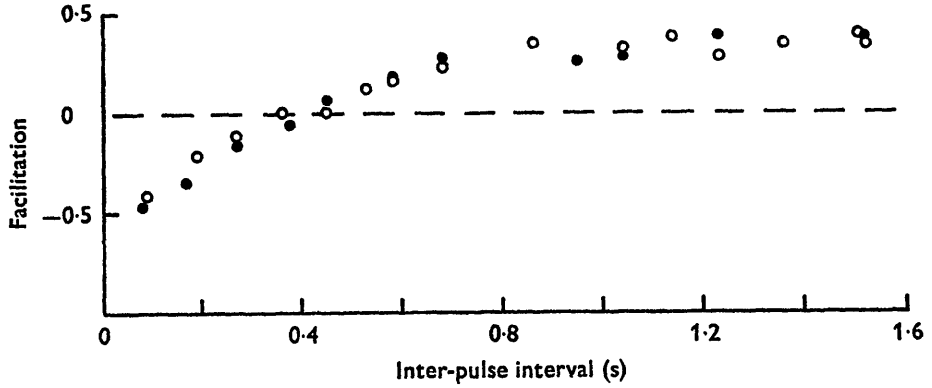

Fig. 11. Depression and facilitation as a function of inter-pulse interval. The values of the test pulses, calculated with respect to the amplitude of the conditioning pulses, were plotted against the inter-pulse interval. When the test pulse amplitude is depressed, the facilitation value is negative. The different symbols indicate series of tests that were made at the same site at different points in time (closed circles, first series; open circles, second series).

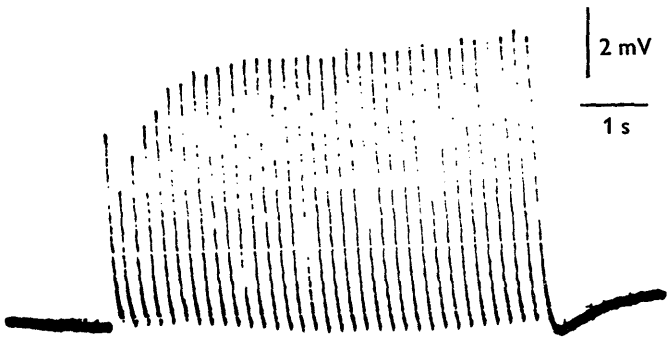

Fig. 12. Combination of facilitation and depression. A train of stimuli was applied to the distal end of a cut PLN at an intensity to evoke activity from a single axon branch. The responses were recorded from a muscle fibre.

show a fast rising phase, which often reaches a peak, followed by a plateau which may or may not exhibit accessory peaks. The depolarizations seldom overshoot the zero potential level. Since the level of the membrane potential determines tension development within muscle fibres (Orkand, 1962), one can assume that the fast rise of these depolarizatons insures a rapid and co-ordinated initiation of contraction while the plateau insures maintained shortening. Such a pattern of tension development appears to be required for an efficient pumping mechanism.

Orkand (1962) working with the crayfish contractor epimeralis muscle showed that

the fibres must be depolarized to a threshold value (to about −60 mV from a resting potential of about −80 mV) before contraction begins. We did not observe such a sharp threshold in the lobster heart. Muscle fibres developed tension with as little depolarizaton as 5 mV from the base-line. The contraction is graded and its strength depends on the degree of depolarizing displacement from the resting potential of the muscle fibres. The relationship of contractile process to membrane potential was not investigated in this study.

Table 1

Preparation	Reference	Resting potential (mV)	Duration	Configuration of depolarization	Over-shoot
Class Crustacea					
Order Isopoda					
Porcellio dilatatus	Holly & Salomon, 1965	−60 to −70*	~ 400 ms	Fast rise to a peak (40–50 mV) followed by a plateau	No
Order Stomatopoda					
Squilla oratoria	Irisawa *et al.* 1962	−42·8±2·5	~ 500 ms	Fast rise to 22·0± 2·0 mV followed by a plateau. Associated with nerve bursts and heart beat	No
S. mantis	Brown, 1964	−51·5 (av.)	500–600 ms*	Fast rise; several peaks on a sustained plateau of 27·6 mV (av.). Associated with burst of impulses from the ganglionic nerve trunk	No
Order Decapoda					
Palinurus vulgaris	Tricoche & Tricoche, 1966	Not given	150 ms	Fast rise to a peak (75 mV*) followed by a plateau. Potentials 'complicated by secondary potentials'	.
Homarus americanus	Anderson & Cooke, 1969	−50 to −60	400–700 ms	Fast rise to a peak (35–40 mV) followed by a plateau (20–30 mV). Associated with burst of impulses from the ganglion and contraction	No
	Van der Kloot, 1970	Not given	400 ms*	Initial spike of 30 mV* followed by long plateau. Associated with burst of impulses from the cardiac ganglion and contraction	.
Crabs (no genera given)	Kuriyama *et al.* 1960	(*a*) −53·0	180·2 ms	Peak amplitude 64·7 mV	Yes
		(*b*) −57·6	139·5 ms	Peak amplitude 49·3 mV	No

Table 1. (*cont*).

Preparation	Reference	Resting potential (mV)	Duration	Configuration of depolarization	Over-shoot
Carcinus moenas	Laplaud *et al.* 1961	Not given	100–300 ms	Peak amplitude about 100 mV; variations from very little to considerable plateau	
Common shore crab	Irisawa *et al.* 1962	Not given	Not given	Not described	Consistent over-shooting
Carcinus maenas	Brown, 1964	−62*	250–300 ms	Fast rise to a peak of about 50 mV* followed by a plateau of about 35 mV*	No
Maia squinado	Brown, 1964	−62*	~ 600 ms	Fast rise to a peak of about 56 mV* followed by a plateau of about 25 mV*	No
Palaemon serratus	Brown, 1964	−48*	~ 500 ms	Fast rise to a peak of about 46 mV* followed by a plateau with several smaller peaks	No
Cancer sp.	Anderson, unpubl.	−55 to −60	300–400 ms	Fast rise to a peak of 40–45 mV followed by a plateau ⩽ 10 mV that possesses one or two accessory peaks of up to 35 mV. Associated with each contraction	No
Procambarus clarkii	Van der Kloot, 1970	−55 to −70	800 ms*	Sharp spike of 40–55 mV followed by a plateau	Some-times
Pagurus argus	Van der Kloot, 1970	Not given	100 ms*	A large spike but no plateau	Almost always
Class Merostomata Order Xiphosura					
Limulus polyphemus	McCann, 1962	−45 mV (av.)	430 ms (av.)	A rapid upstroke and prolonged plateau. Associated with each contraction of the heart	'No significant overshoot'
	Robb & Rech, 1964	−40 to −50	~ 1·0 s	Fast rise to a peak of 35–40 mV followed by a plateau with transient oscillations. Associated with impulses from the dorsal median nerve and contractions	No

Table 1. (*cont.*)

Preparation	Reference	Resting potential (mV)	Duration	Configuration of depolarization	Overshoot
	Lang *et al.* 1967; Abbot *et al.* 1969*a*, *b*	−35 to −45	~ 1·5 s	Fast rise to a peak of about 40 mV followed by a plateau with oscillations. Associated with a burst of impulses from the ganglion and contractions	No
Tachypleus tridentatus	Tanaka *et al.* 1966	−35 to −45; −30 to −50 (two statements)	~ 3·0 s	Rapid rise to 25–35 mV followed by a plateau with oscillations. Associated with impulses from the ganglion	No
Class Arachnida Order Araneae *Geolycosa missouriensis*	Sherman & Pax, 1969	−45 (mean, S.E. 1·7)	~ 450 ms	Fast rise to peak of 22 mV (S.E. 3·8 mV) followed by a lesser oscillatory plateau. Associated with heart beat	No
Eurypelma marxi	Bursey & Sherman, 1970	Middle of heart: −65 (S.E. 3); ends of heart −48 (S.E. 3)	1·0 s*	Fast rise to prolonged plateau of 35 mV (S.E. 3 mV) in middle and 12 mV (S.E. 2 mV) at ends. Associated with burst of impulses in the cardiac ganglion and contraction	No
Order Scorpiones *Buthus occitalis* L.	Tricoche *et al.* 1960	Not given	~ 100 ms	Single peak to 100 mV*. Authors see inflections on rising phase	
Class Amphibia Order Salienta Toad	Kuriyama *et al.* 1960	28·6	239·6 ms	Peak amplitude 8·2 mV	No
Frog	Kuriyama *et al.* 1960	35·0	320·0 ms	Peak amplitude 26·5 mV	
Bufo marinus *Rana pipiens* *Eleutherodactylus portoricensis*	del Castillo & Sanchez, 1961	Up to −60	150–200 ms	Fast rise to a spiky wave of depolarization of up to 30 mV amplitude. Associated with burst of nerve discharges from XI spinal nerve and with contraction	No

* Estimated from the published figures.

The heart-muscle fibres of *Homarus* do not produce regenerative membrane responses (Fig. 3). The configuration of the spontaneous depolarizations recorded in the lobster heart can be accounted for by the combined summation and facilitation of the EJPs produced during a burst of impulses generated in the ganglion. The frequency of impulses at the beginning of the burst is greater than that at the end (Fig. 1*c*). Several impulses, presumably originating from several different axons, occur in rapid

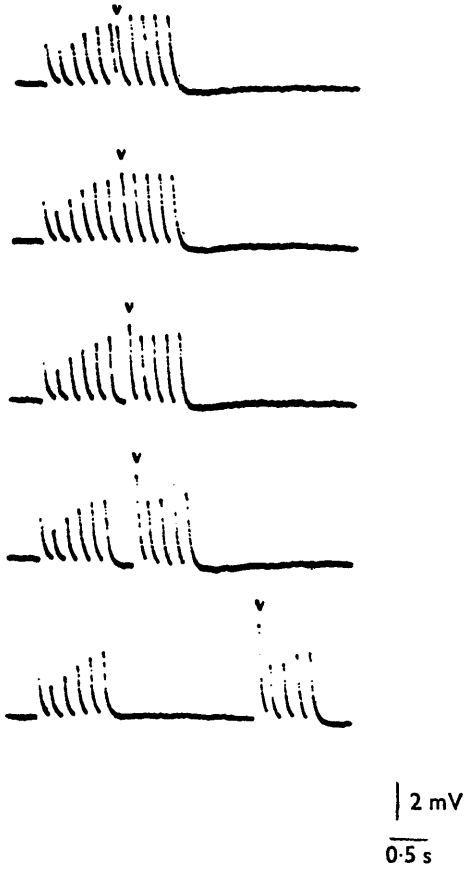

Fig. 13. Combination of facilitation and depression when conditioning trains are followed by test trains at varying intervals. The intensity of stimulation was adjusted to evoke activity from a single axon branch in the distal end of a cut PLN. Responses were monitored in a muscle fibre. Arrows indicate the first pulse of each test train.

succession and contribute to the fast rising phase of the depolarization (Fig. 1*b*). Mallart & Martin (1967) proposed for the frog nerve-muscle junction that a constant increment of facilitation accompanies each impulse. Each increment decays exponentially. During a series of impulses, facilitation grows exponentially; upon cessation of stimulation it decays exponentially. On a slower time scale, Mallart's and Martin's hypothesis can be applied to the lobster heart. Fig. 7 demonstrates that, in the lobster heart, facilitation increases with the frequency of stimulation. Fig. 9 shows that the decay of facilitation is probably exponential. Thus, during the early, high-frequency period of the burst there is a rapid depolarization; the increments of facilitation decay very little between impulses and therefore summate rapidly. As the frequency of impulses within the burst decreases, the amplitude of the depolarization decreases; each increment of facilitation decays before it is added to that facilitation already pre-

sent. Yet, due to the residual facilitation, a sustained plateau level is ensured. This facilitation may also minimize the effect of delayed rectification which results from depolarization (Fig. 3). When the burst stops, the depolarization, and the facilitation, decline to the resting level.

In addition to facilitation the fact that the second response of a train of impulses is smaller than the first (Fig. 10) must be taken into account. Fig. 1(*b*) illustrates that several EJPs make up the fast rising phase of the complex depolarization. Due to the high frequency of impulses in the burst, as compared to the time course of the individual EJPs, considerable summation of the responses takes place. Because of this rapid summation the depressed amplitude of the second response is not evident.

At this point in the study we can only describe how the presumed facilitation and depression processes (Figs. 10–13) interact. Several mechanisms, or combinations of mechanisms, may account for these results. In addition to changes in the probability of transmitter release (Mallart & Martin, 1967; Christensen & Martin, 1970), refractoriness of the fine terminal arborizations of the nerve, immediate availability of transmitter, or de-sensitization of the receptor site (Thesleff, 1955; Takeuchi & Takeuchi, 1964) may act to produce the observed combination of depression and facilitation. Bruner & Kennedy (1970) show some figures for a crayfish nerve-muscle junction in which trains of stimuli were applied at frequencies similar to those used for the lobster-heart experiments. In these instances the second response is smaller than the first, and facilitation occurs after the second response. In the crayfish the facilitated amplitude is not maintained, but instead declines during the train. No such decline occurs when trains of similar frequency and duration are applied to the lobster-heart nerve-muscle junction.

Both facilitation and depression are particularly pronounced in the lobster heart. Indeed, this may be an extremely favourable preparation to investigate the time courses and mechanisms underlying these processes. It is hoped that modelling experiments will indicate the most probable hypotheses to be tested using electrophysiological techniques. Experiments to determine the effects of magnesium and calcium on the depression and facilitation phenomena have been initiated.

It is very likely that the muscle fibres in the lobster heart are multiterminally innervated, as they are in crustacean peripheral muscle (Fatt & Katz, 1953); i.e., a single axon branch makes more than one contact on a given muscle fibre. However, this study has not demonstrated the presence of multiterminal innervation in the lobster myocardium. Considerable redundancy of information is provided by polyneuronal innervation. (During experiments in which stimuli were applied to the cut PLNs, which contain three axon branches, only occasionally was a muscle fibre found to be innervated by less than all three. On these occasions injury to the preparation, instead of paucity of innervation, was the more likely reason for the results.) Such redundancy ensures that the entire population of muscle fibres, directed by only five motoneurons, will contract in concert, efficiently squeeze the haemolymph into the vessels, and then relax synchronously so that the heart can fill again. Even greater redundancy would obtain were the muscle fibres multiterminally innervated.

The chemical nature of the nerve-muscle transmitter(s) is unknown. There is no evidence that different axons produce different effects at the nerve-muscle junctions; this similarity of synaptic effect suggests that there may be a single transmitter present.

Two pairs of excitatory fibres and one pair of inhibitory fibres from the central nervous system enter the cardiac ganglion and exert a regulatory action (see Wiersma & Novitsky, 1942, and Bullock & Horridge, 1965, for early references to regulatory nerves in crustaceans; Maynard, 1953*a*, *b*). The regulatory nerves have a marked effect on the activity of the ganglion cells; however, these nerves are not necessary for the normal, rhythmic activity produced in the ganglion (Welsh & Maynard, 1951; Cooke, 1966). There is no physiological evidence that the extrinsic fibres reach the myocardium directly (Mark Hallett, personal communication).

SUMMARY

1. Glass microelectrodes were used to record intracellular activity from the striated muscle fibres of the heart of the lobster *Homarus americanus*.

2. In spontaneously beating hearts bursts of impulses are generated at regular intervals by neurones in the cardiac ganglion. These bursts produce depolarizations of the muscle fibres. Each depolarization is associated with a contraction of the heart. The depolarizations consist of many depolarizing steps; each step is related in a one-to-one manner to a nerve impulse and is believed to be an excitatory junction potential (EJP). The depolarizations (400–700 ms duration in different preparations) exhibit a fast rise to a peak (35–40 mV) followed by a plateau (20–25 mV) which decays to the resting level ($51{\cdot}5 \pm 3{\cdot}2$ mV, $n = 148$).

3. Current-voltage curves indicate that the EJPs do not give rise to regenerative membrane responses.

4. Small, spontaneously produced potentials were recorded in the presence of TTX. Autocovariance tests show that the potentials occur independently and are likely to be miniature junction potentials.

5. Polyneuronal innervation of the muscle fibres was demonstrated by applying stimuli of gradually increasing intensity to the distal ends of cut nerves while recording the responses of a muscle fibre.

6. When a train of stimuli is applied at an intensity to evoke activity from a single axon, the first response of a muscle fibre is usually greater than the second; facilitation of the second and subsequent responses takes place. The degree of facilitation developed depends on the frequency of stimulation. Facilitation decays exponentially with a time constant of a few seconds.

7. In two-pulse experiments the second response was depressed when the interpulse intervals were $\leqslant 0{\cdot}5$ s.

8. Examples of combined facilitation and depression are presented.

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