J. Exp. Biol. (1966), **45**, 475–488
With 11 *text-figures*
Printed in Great Britain

THE REPETITIVE RESPONSES OF ISOLATED AXONS FROM THE CRAB, *CARCINUS MAENAS*

BY R. A. CHAPMAN*

The Gatty Marine Laboratory, and the Department of Natural History, The University of St Andrews, Scotland

(*Received* 29 *June* 1966)

INTRODUCTION

Hodgkin (1948) demonstrated that the duration of the relative refractory period following the action potential in isolated axons of the crab *Carcinus* was too short to determine the duration of the spike intervals of a repetitive response to a maintained depolarizing current. He found that the interval between the onset of the stimulating current and the first spike was more closely related to the intervals between the later successive action potentials. He therefore suggested that the processes leading to excitation were similar throughout the response and that the influence of the relative refractory period was only significant at high repetition frequencies.

With the formulation of the Hodgkin–Huxley equations the factors involved in the genesis of the repetitive response have been more closely defined and the temporal pattern of the discharge which would be expected at different strengths of applied current have been computed (Fitzhugh, 1961; Agin, 1964). The responses obtained experimentally from the eccentric cells of *Limulus* eye, the squid axon, cat and rat spinal motoneurones, and the crab axon are not consistent with the predictions of the equations (Fuortes & Mantegazzini, 1962; Hagiwara & Oomura, 1958; Granit, Kernell & Shortness, 1963; Hodgkin, 1964). Fuortes & Mantegazzini have suggested that this divergence can be related to accommodation and to the cumulative after-effects of the action potentials themselves, which they in turn related to accumulating refractoriness.

The present investigation is concerned with the factors which determine the form of the repetitive response in isolated crab axons, although such influences as those caused by the accumulation of extracellular potassium ions (Frankenhæuser & Hodgkin, 1956; Chapman, 1963*a*) will not be dealt with in detail.

METHODS

Motor axons, of between 15 and 40 μ, were isolated from the leg nerve of the crab *Carcinus maenas*, obtained locally. Two to four cm. of axon was prepared and cleaned while still in continuity with its muscle. The axons were tested to see if they responded well and were at the same time identified according to the muscle they innervated (Wiersma & Ripley, 1952). The axon was then separated from the leg and each end of the axon was gripped in separate heavily insulated forceps.

The experimental system used enables an isolated segment of an axon to be bathed

* Present address, Department of Biophysics, University College, London.

by physiological solution (sea water) while electrical measurements are made upon it. The technique itself is similar to those that have been employed on other axons, (Stampfli, 1954; Hodgkin & Keynes, 1955; Tomita, Saimi & Toida, 1961; Julian, Moore & Goldman, 1962*a*). The axon after isolation is washed in isotonic sucrose solution (0·724 M) by repeated change of the bathing medium. This procedure completely abolishes excitability from the axon. The axon is then threaded with the aid of a length of fine silver wire through two holes (50–100 μ) which lie opposite each other in a piece of polythene tubing (inside diameter 1·0 mm.). The two ends of the axon are then returned to the insulated forceps. A length of chloride-coated silver wire is

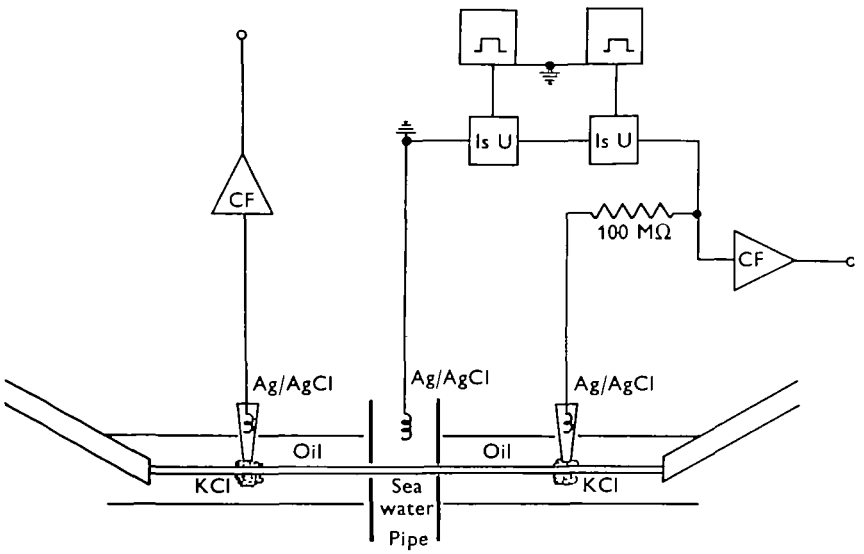

Fig. 1. Schematic diagram of the stimulating and recording system employed. Abbreviations: Ag/AgCl, silver–silver chloride; CF, unity gain cathode follower; Is U, stimulus isolation unit; KCl, isotonic potassium chloride Agar wick electrodes.

passed down one limb of the polythene tube until it comes close to the axon. This limb of the tubing dips into a reservoir of physiological solution, the other is attached to a water vacuum pump via a trap bottle. The slight negative pressure applied by the pump draws the solution from the reservoir along the tubing and past the axon. The negative pressure also draws some of the sucrose solution into the tube through the holes to provide an isolated area of the axon bathed by physiological solution to which full excitability is rapidly restored. At this point paraffin oil is substituted for the sucrose solution in the experimental bath and two KCl wick electrodes are brought into contact with the axon on either side of the tube and at about 5 mm. from it. Currents are applied to the artificial node via one of these KCl wicks and the central earthed pipe electrode, while the potential changes of the node are recorded between the other KCl wick and the central pipe (Fig. 1). This technique may produce preparations that respond as if hyperpolarized and in these cases the demarcation potential is indeed increased. Similar hyperpolarization have been reported by Julian, Moore & Goldman (1962*a*). Stampfli (1963) has suggested that hyperpolarizations occurring with sucrose-gap techniques applied to unmyelinated axons may be due to a liquid-

junction potential at the artificial node caused by the differential loss of sodium and chloride ions from the surrounding internodal regions resulting from the markedly different ionic mobilities of the two ions. Spyropolous (personal communication) has pointed out that in the region of the axon exposed to the sucrose solution the membrane potential will be much changed, and the resulting current flow between it and the artificial node is likely to influence the resting potential of the latter.

The preamplifier used throughout these experiments was of a type similar to that described by Bak (1958), with an input resistance of 10^{10} Ω and a grid current of less than 10^{-12} amps. Constant current stimulation was achieved by applying the current to the axon through a 100 MΩ resistance. Changes in membrane resistance were determined by measuring the potential displacement of the membrane caused by a short current pulse (0·01–1·0 msec.) of known strength.

RESULTS

Crab axons can yield types of repetitive behaviour that do not conform to the three original classes described by Hodgkin (1948). These wayward responses, however, occur under rather special experimental conditions, as in hyperpolarized axons (Chapman, 1963*b*), or in axons previously subjected to prolonged anodal current (Chapman, 1963*a*). Therefore, the classification adopted by Hodgkin will be used throughout the present paper. The somewhat improved technique has enabled certain other features typical of each axon class to be ennumerated, and for brevity these can be included in a set of modified definitions, as follows:

Class I axons. Axons in which the recovery cycle shows no significant supernormality (Fig. 3*a*), and which are capable of often very prolonged repetitive activity over a wide range of frequencies (less than 1/sec. to 150/sec.). The frequency of the response increases with increasing strength of applied current, while the critical level of depolarization for the spike and the amplitude of the action potential remain constant over a wide range of stimulus currents. The spike intervals during the repetitive response increase smoothly and progressively until the spikes cease. The demarcation potentials are high, 50–70 mV.; the critical level of depolarization for the spike is low, 10–15 mV.; the action potential is large, 80–90 mV., and the preparation resistance per unit area of node is high, 8500–7900 Ω/cm.^{2}.

Class II axons. Axons in which the recovery cycle shows an early marked supernormality that develops immediately after the action potential (Fig. 3*b*). When stimulated by maintained depolarizing current these axons yield a train of relatively high-frequency action potentials (50–200/sec.), which end abruptly without showing a stable low-frequency discharge. The spike amplitude is reduced only during the passage of high-intensity currents, but the critical level of depolarization for the spike rises progressively throughout the repetitive response with all suprathreshold currents (Fig. 2). The demarcation potentials, the critical level of depolarization, the preparation resistance and the spike amplitudes are not significantly different from those shown by Class I axons.

Class III axons. Axons in which the recovery cycle shows a prolonged subnormality (Fig. 3) and which yield only a short repetitive response to sustained depolarizing current. The action potential amplitude falls progressively during this response and

the critical level of depolarization for the spike shows a progressive rise. These axons are capable of only short initial latencies (less than 20 msec.) as compared to axons of the other classes, This type of response is typical of axons in poor condition, since the demarcation potential is low, 35–55 mV.; the action potential amplitude is reduced, 40–60 mV.; the critical level of depolarization for the spike is high, 20–30 mV.; and the preparation resistance per unit area is low, 6800–3500 Ω/cm.^{2}.

Hodgkin (1948) noted that any division of this type was essentially arbitrary for intermediate responses are not uncommon; this is further emphasized by the fact that different regions of the same axon may yield different classes of repetitive behaviour. We are, therefore, dealing with different types of responsiveness and not with different types of axons. As a further precaution each axon was identified according to the muscle it innervated, and no physiological differences were observed between them.

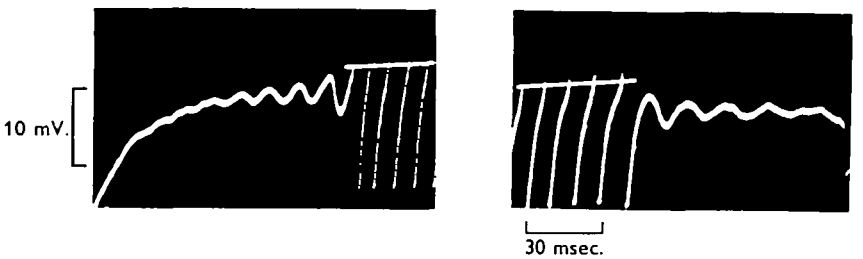

Fig. 2. The progressive rise of the critical level of depolarization for the spike during a repetitive response of a class II axon. The oblique white line is drawn through the threshold potential for each successive spike. Note also the subthreshold potential oscillations that precede and follow the train of action potentials. 20° C.

The response to maintained depolarization

In axons yielding responses typical of classes I and III, when the potential displacement of the membrane caused by a weak maintained depolarizing current just failed to exceed the critical level of depolarization for spike generation, the potential eventually falls back after a plateau even if the stimulating current is continued, due to the development of rectification. A similar process occurs at the end of a repetitive response. The situation observed in type II responses is complicated by the marked oscillatory nature of the local potential (Fig. 2).

Trains of spikes several seconds long are consistently evoked by maintained depolarizing currents just above threshold in axons of classes I and II. Occasionally the smooth increase in the spike intervals is interrupted when the local potential falls back without evoking an action potential and after a time rises again. Sometimes this process occurs several times before resulting in a further action potential. Following a break of this sort, the intervals between the next group of action potentials are shorter than those that preceded the break. Such behaviour with near-threshold currents is, however, uncommon in the best preparations. In axons of classes II and III there is a progressive rise in the threshold potential for the generation of each successive spike during a repetitive response (Fig. 2).

A method found convenient for comparing these present observations with those made upon sensory cells (Fuortes & Mantegazzini, 1962) and with computed theoretical relationships (Fitzhugh, 1961; Agin, 1964), is to plot the instantaneous frequency of

the spike intervals against the strength of the applied current. Fig. 3 shows the relationships obtained from an axon typical of each class of repetitive behaviour for the reciprocal of: (*a*) the latency from the onset of the stimulating current until the foot of the first action potential; (*b*) the mean interval between the subsequent spikes; and (*c*) the last spike interval appearing during the current pulses of 1 sec. duration (Fig. 3 *a* only), over a range of stimulus intensities. The reciprocal latency is linearly related to the current intensity in all the axons studied, varying only near to threshold. The reciprocal mean interval (mean frequency) shows a similar but not identical relationship in classes I and II responses, but unlike the reciprocal latency there is an upper

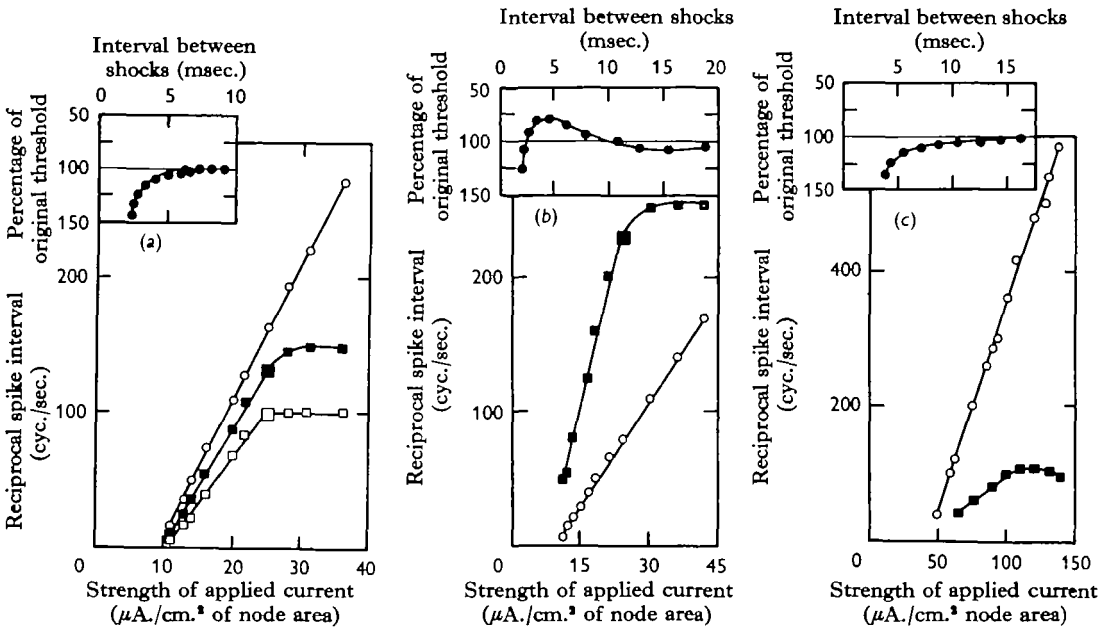

Fig. 3. Comparison of the reciprocal latency (○—○) with the reciprocal mean spike interval (■—■) and the reciprocal last interval (□—□), in response to a maintained depolarizing current of 1 sec. duration and of increasing strength, for: (*a*), a class I axon; (*b*), a class II axon; (*c*), a class III axon. The inset graphs show the form of the recovery cycle of each axon as determined by a two-pulse technique.

frequency limit, so that beyond a certain frequency (enlarged symbol in Fig. 3) increasing the current intensity is not accompanied by a rise in the mean response frequency.

The effects of depolarization beyond 5 times rheobase are not shown in Fig. 3. The general nature of the changes caused by strong cathodal current is similar in each type of response, but is most marked in weakly repetitive axons. If the current intensity is increased beyond the point where the mean spike frequency no longer increases, the threshold potential for spike generation increases more rapidly so that the repetitive response terminates early (Fig. 4), while during the response there is a progressive decline in the amplitude of the successive spikes. Still further increases in the strength of the applied current enhances the effects described above until only a single action potential develops following the make of the stimulating current. Over

the whole range of depolarizing currents the initial spike is of unchanged amplitude, its potential threshold remains the same, and the reciprocal latency is always proportional to the strength of the applied current. After the last action potential in a repetitive response curtailed by the passage of a strong cathodal current the potential displacement of the membrane exceeds the original critical level for spike generation (that for the first spike) without further spikes developing. The size of this potential displacement, however, is not proportional to the applied current strength but varies more slowly, indicating the development of rectification (see levels in Fig. 4).

Fig. 4. The progressive effects of strong sustained depolarizing current on a class I axon. This behaviour is typical of all of the axons discussed in this paper. 19° C.

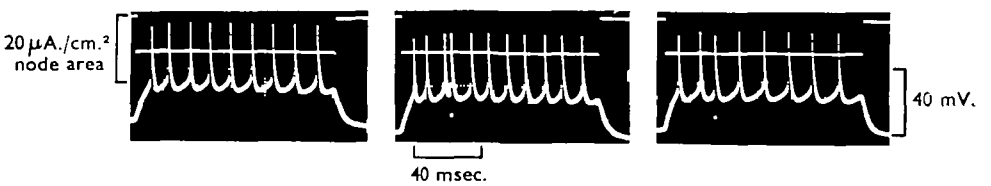

Fig. 5. The introduction of an extra action potential into a repetitive response of a class I axon causes a lengthening of the next spike interval. This effect is greatest when the additional impulse is applied during the relative refractory period of an action potential in the repetitive response. 19° C.

Stimulation by trains of short current pulses

When stimulated by sustained depolarizing current, the highest maintained response frequencies are: 150/sec. in class I responses, 250/sec. in class II responses, and 50/sec. in class III responses; whereas stimulation by discrete trains of short-duration current pulses can elicit maintained response frequencies as high as 550/sec. This difference in behaviour has been investigated by applying trains of short stimulating pulses of current over a wide range of frequencies and current intensities. A strength/frequency curve is obtained by determining the current strength required to elicit complete responses over a wide range of stimulus frequencies. This strength/frequency relationship is much more sensitive to changes in the stimulating current intensity than the strength/mean response frequency relationship for the direct current mode of stimulation (Fig. 6). As a result the curves for the two relationships cross, so that beyond a certain frequency the strength of the sustained current must exceed that of the individual pulses that compose a stimulating train at the same response frequency. No real significance can be placed on the actual frequency at which the strength/frequency relationships cross in any particular axon because it depends on the actual duration of the short current pulses that form the train. An explanation cannot be derived by comparing the responses in terms of their respective rheobases, as done by Fuortes &

Mantegazzini (1962), because of the influence of the transient current pulse duration, i.e. if the duration of the individual transient stimuli applied as a regular train of pulses is increased so that the current also flows during repolarization of the action potentials, an originally complete train of responses will now show interruptions.

'Extra' action potentials

In class I axons, when an extra action potential is evoked during a repetitive response by an additional strong transient current, the interval to the next spike is always longer than the interval normally expected at that point in the repetitive response (Fig. 5). The extent to which the subsequent spike interval is lengthened depends on how soon after a normally evoked action potential the extra impulse is

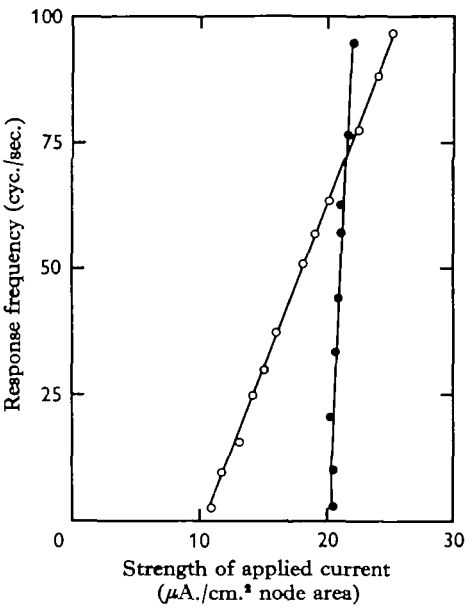

Fig. 6. Contrast of the response frequency/strength relationships of a typical class I axon for direct current stimulation (○—○), and for trains of short current pulses applied over a range of frequencies (●—●). 19° C.

elicited, being greatest when the extra spike falls within the relative refractory period of the preceding repetitive spike. The recovery cycle that follows after a repetitive action potential can be crudely determined using extra impulses and is of the same general form as that occurring after the single action potential. However, interpolated action potentials of reduced amplitude are evoked after a spike in a repetitive response, for a period longer than the relative refractory period that comes after the single spike. In the particular case of the axon shown in Fig. 5, the recovery of spike amplitude following the single spike was complete in 6·4 msec., but during a repetitive response extra impulses of reduced amplitude were evoked as late as 15 msec. after a repetitive spike (third record in Fig. 5). The time required for recovery of the action potential amplitude after repetitive spikes progressively increases as the response continues.

An extra action potential introduced during a class II repetitive response resets the

response, while in class III axons further repetitive action potentials seldom develop after the introduction of an extra action potential.

Transient currents applied during repolarization of the action potential

A very marked increase of the following spike interval occurs (up to 50% longer than the expected interval) if an additional cathodal pulse is applied during the repolarization phase of the preceding action potential. This effect varies with the strength duration and timing of this pulse (Fig. 7). To investigate this phenomenon further,

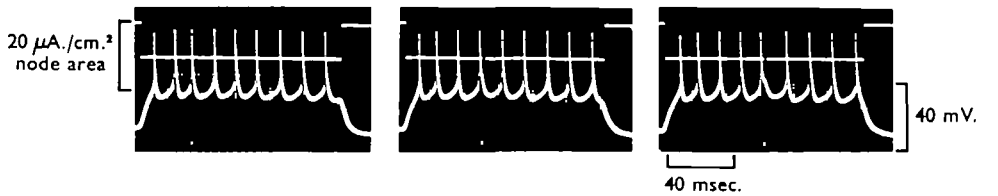

Fig. 7. The effect upon the following spike interval of introducing an additional short cathodal current pulse at various times during the repolarization phase of an action potential. Class I axon. 19° C.

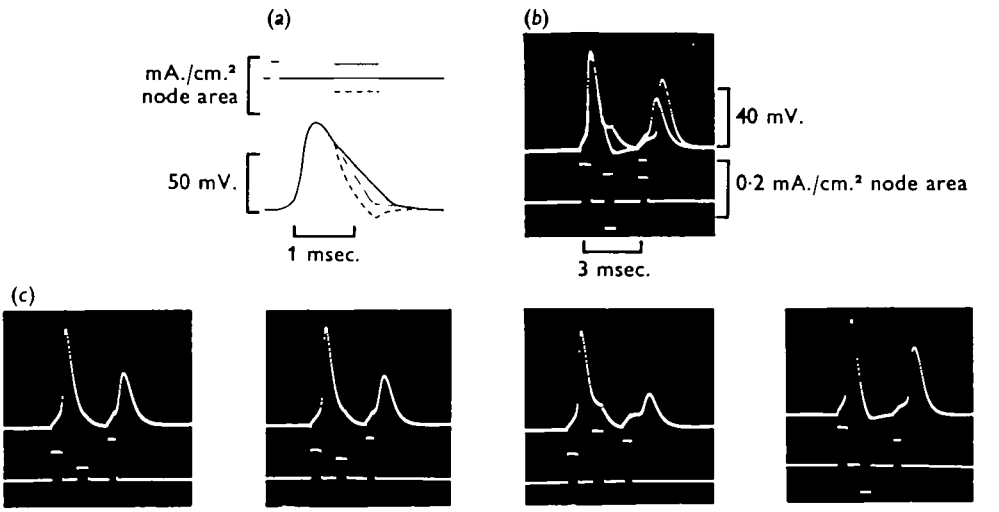

Fig. 8. (*a*). Tracings from original records of the hastening of repolarization phase of an action potential by anodal current (dashed line), and the slowing of repolarization caused by cathodal current (thick solid line). The normal course of repolarization is shown by the thin solid line. 19° C. (*b*). Two superimposed records showing the changes in recovery resulting from cathodal and anodal currents applied during the repolarization of an action potential. Recovery is more rapid after anodal current passage, as indicated by the lower threshold current, the greater spike amplitude and the lower threshold potential for the second spike. 19° C. (*c*) The slowing of recovery of the axons after an action potential shows some proportionality to the strength of the cathodal current applied during repolarization of that action potential, so that the amplitude of a second spike elicited during the relative refractory period falls as the strength of this earlier pulse is increased. For comparison a similar record of the hastening of recovery after the application of anodal current is shown. 18° C.

transient current pulses of either polarity were applied during the repolarization of an action potential evoked by a short shock, while the recovery cycle was determined by a third cathodal test-pulse technique. Transient currents of less than 50 μsec. duration

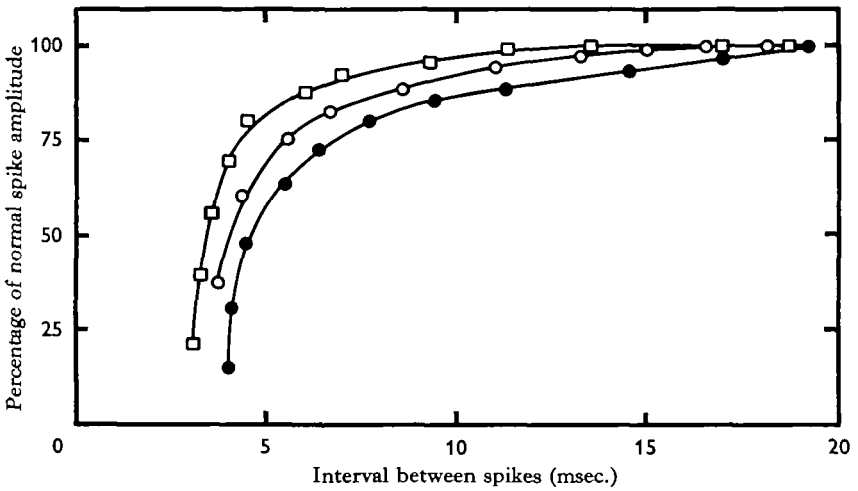

Fig. 9. The duration of the relative refractory period (○—○) is reduced following anodal current pulses (□—□) and is prolonged following cathodal current pulses (●—●) when these pulses are applied during the repolarization phase of the action potential. Pulse duration 250 μsec.; strength of pulse 100 μA./cm.^{2} of node area.

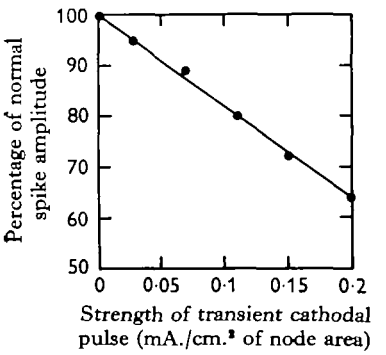

Fig. 10

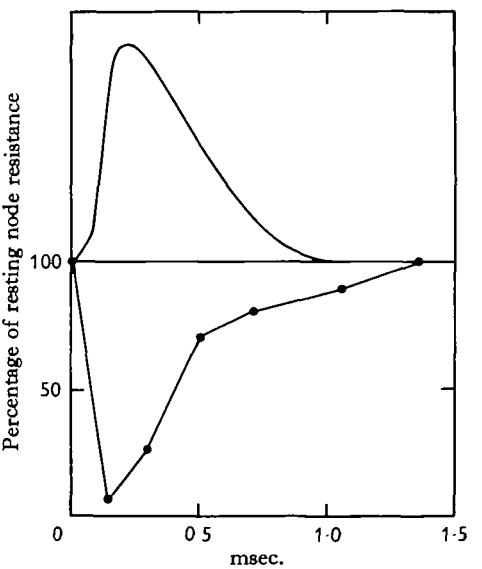

Fig. 11

Fig. 10. The reduction of the amplitude of an action potential evoked 3·5 msec after an earlier action potential shows proportionality to the strength of a cathodal current pulse (300 μsec. duration) applied 400 μsec. after the onset of the first spike. 20° C.

Fig. 11. The approximate change in the membrane resistance during an action potential as determined by applying short-duration (150 μsec.) square constant current pulses at various times during the action potential. For comparison a tracing of the action potential is also shown. 21° C.

need to be quite excessive (over 1 mA./cm.^{2} of node area) to produce any significant change in repolarization owing to the presence of the membrane capacity. Pulses longer than 100 μsec. are more effective, and pulses of either polarity displace the potential in a simple ohmic manner, with cathodal currents slowing repolarization and anodal currents hastening it (Fig. 8*a*). The later it occurs during repolarization of the action potential the greater is the potential displacement produced by a current of given intensity. The duration of the relative refractory period is shortened by anodal current and prolonged by cathodal current applied during the repolarization phase of the action potential (Figs. 8, 9). These changes in the recovery time are also proportional to the strength of the imposed currents and to their duration (Fig. 10), while the effect upon the subsequent recovery is more marked the later these currents are applied during repolarization.

The technique of applying short additional current pulses during an action potential enables the resistance change that accompanies the action potential to be mapped out. However, as relatively long pulses are required to reduce the contribution of the membrane capacity, the accuracy of this method is somewhat limited at room temperature. For an action potential of 1 msec. duration the membrane resistance falls to a very low value, reaching 5–10 % of the resting value at the peak of the potential change, after which the resistance rapidly increases achieving 70–80 % of the resting value after 500 μsec. The resistance of the 'node' then recovers quite slowly finally reaching the resting value some 0·5–1·0 msec. after the end of the action potential (Fig. 11). The resistance change therefore closely resembles that reported by Julian *et al.* (1962*a*, *b*) for the lobster giant axon.

DISCUSSION

Hodgkin (1948) established that, at least in class I repetitive responses, the rise of the local generative process is decisive in setting the repetition frequency. Although the situation is complicated in classes II and III responses by the fact that repetitive action potentials always develop before the relative refractoriness has completely subsided, he was able to show that the response time was still of major importance in determining the repetition rate. However, it is still necessary to propose additional processes to account for the finite length of the repetitive response, and for the progressive lengthening of the spike intervals throughout the response. The presence of these additional processes is clearly demonstrated in Fig. 3, where although the reciprocal latency and the mean response frequency are both linearly related to the strength of the depolarizing current, they do not show identical relationships.

The course of the events predicted by the Hodgkin–Huxley equations have been recently computed for the application of maintained currents (Fitzhugh, 1961; Agin, 1964). The equations predict that that infinite trains of action potentials should result from constant current steps above a certain intensity, with the interval from the current onset to the first spike (the latency) of the shortest duration and with the later spike intervals of equal duration. The frequency of the later impulses is proportional to the logarithm of the intensity of the applied current. The figures calculated for the strength/duration relationship yield a linear relationship to the current intensity (data from Fitzhugh & Antosiewicz, 1959). The reciprocal latency/strength relationships of the types of repetitive responses shown in Fig. 3 agree well with this latter prediction,

the only variations occurring close to threshold where small fluctuations in the stimulus current or in the membrane properties would be most effective. However, the predictions regarding the logarithmic relationship to the later impulses to the current strength, and the appearance of infinite trains of impulses, are not borne out. However, two important considerations must be taken into account when applying the Hodgkin–Huxley equations, namely; (i) that the equations when first formulated were not intended to account for prolonged activity; and (ii) that the equations may require some modification when applied to other excitable tissues. Important in this context are some unpublished observations I made on the squid giant axon. Under ideal conditions with a good fresh axon and a good current clamp, the predictions of the equations for maintained depolarization are obeyed. Very long (20–40 sec.) trains of action potentials are obtained with sustained depolarization, with the reciprocal latency proportional to the current strength, but with the mean frequency of the later impulses proportional to the logarithm of the current strength. Therefore, in the squid axon, it is not the response time that is the major factor determining the repetition frequency as it is in other tissues (crab axon and motoneurone).

The accumulation of the potassium ions released during the action potential, in the near vicinity of the axon membrane, has been proposed as a factor acting to influence the form of the repetitive response (Fuortes & Mantegazzini, 1962; Chapman, 1963*a*). Most probably this accumulation of potassium ions and its influence on the membrane resistance (Hodgkin & Huxley, 1947) is a major determinant of the slope of the strength/frequency relationship for the application of trains of pulses (Fig. 6). Comparison of the response frequencies evoked by sustained depolarization and by trains of stimuli show that a further factor is operating to affect the form of the repetitive response, because maintained current stimulation requires a larger increase in the current intensity to evoke higher response frequencies than trains of current pulses require when applied at various stimulus frequencies (Fig. 6). As the number of action potentials evoked by each mode of stimulation is the same, the different sensitivities to increases of the stimulus strength cannot be attributed to a differential accumulation of potassium ions unless the additional depolarization per unit time due to direct current stimulation causes a greater accumulation of potassium ions. The situation where trains of stimulating pulses are applied to a nerve cell or axon is not as simple as suggested by Fuortes & Mantegazzini (1962), because of the influence of the duration of the individual pulses that compose the train. This observation leads to the suggestion that it is the interaction of the sustained current and the action potentials that underlies the depression of excitability described by Fuortes & Mantegazzini (1962), and found in the present study. This suggestion is supported by the following facts:

(1) The reciprocal latency shows no signs of depression, being linearly related to the strength of the applied current over a wide range, while the subsequent spike intervals are longer in types I and III responses and the later spikes can be totally suppressed by strong cathodal currents.

(2) The shortening of the spike intervals after a break has occurred in the spike train of a repetitive response.

(3) Additional transient cathodal currents applied during the repolarization phase of an action potential delay the development of the next spike in the repetitive response.

The delay of membrane restoration (repolarization and recovery) by cathodal currents applied during repolarization of the action potential (Figs. 8, 9), clearly demonstrates this interaction, and the failure of the repolarization to achieve the resting potential during a repetitive response shows that similar processes are occurring during this response (Fig. 5). While a basis for the different types of repetitive responses described for crab axons may well lie in the fact that the sensitivity of the processes of membrane restoration to imposed currents is lowest in the highly repetitive axons and highest in the non-repetitive ones. It can be seen from the 'extra' action potential experiments that the duration of the relative refractory period increases progressively throughout the repetitive response, and a small prolongation of the relative refractory period would significantly influence the spike intervals at all repetition frequencies because the local potential develops more slowly during the relative refractory period (Hodgkin, 1938). It appears, therefore, that the passage of sustained cathodal current, apart from its depolarizing action, acts to progressively delay membrane restoration after each action potential in a repetitive response and that this leads to a slowing of the development of the local potential thereby slowing the response frequency until the final slowest subthreshold potential is reduced by the development of delayed rectification, and the response terminates.

The change in the membrane resistance that accompanies the action potential in crab axons (Fig. 11) closely resembles that already described for the lobster giant axon (Julian *et al.* 1962*a*), with the membrane resistance returning close to its resting value during the action potential and not remaining at a much reduced level as it does in the squid giant axon (Cole & Curtis, 1939). This difference between the crab and squid axons may well go towards accounting for their dissimilar behaviour when subjected to sustained depolarization.

The rapid recovery of the membrane resistance during the action potential of the crab axon suggests that during repolarization the sodium conductance increase is shut off early, and that the increase in the potassium conductance is small (as in the lobster giant axon, Julian *et al.* 1962*b*). This being the case, a mechanism similar to that suggested by Huxley (1959) for the abolition of the action potential at the amphibian node of Ranvier seems the most likely. This would mean that the changes in repolarization of the action potential by the application of current are due to changes in the sodium conductance. For hastening of repolarization can only be achieved by increasing the potassium conductance or by decreasing the sodium conductance (vice versa for delayed repolarization). The direction of the current flow that produces the changes in the rate of repolarization makes a change in the sodium conductance more acceptable (Huxley, 1959). The changes in the recovery of excitability following an action potential which has had its repolarization changed by the application of current would require a secondary change in the sodium inactivation and/or the potassium conductance if it were to be consistent with the basic Hodgkin–Huxley analysis.

SUMMARY

1. A method is described that enables the electrical responses of motor axons isolated from the leg nerve of the crab *Carcinus* to be studied close to or at the site of imposed electrical currents, while this area is continuously bathed by physiological solution.

2. The three classes of repetitive responses originally described by Hodgkin (1948) have occurred during the present work and additional features of these responses have been described.

3. The results support Hodgkin's original thesis that the development of the spike generating mechanisms determine the response frequency during a repetitive response, but a progressive lengthening of the relative refractory period occurs during this response and is considered to be the agency that causes the gradual slowing down of the response frequency, i.e. the adaption.

4. The processes of membrane restoration (repolarization and recovery) have been shown to be sensitive to applied currents; anodal current hastening and cathodal current slowing it. These phenomena provide a basis for interpreting the change in the duration of the relative refractory period observed during the repetitive response.

5. The differences between the form of the repetitive response in the crab axon and the predictions of the Hodgkin–Huxley equations is discussed and it seems likely that the rapid recovery of the membrane resistance during the repolarization phase of the crab axon action potential underlies this difference.

REFERENCES

AGIN, D. (1964). Hodgkin–Huxley equations: Logarithmic relation between membrane current and the frequency of repetitive activity. *Nature, Lond.* **201**, 625–36.

BAK, A. F. (1958). A unity gain cathode-follower. *E.E.G. Clin. Neurophysiol.* **10**, 472–85.

CHAPMAN, R. A. (1963*a*). 'Pre-pulse' and 'extra impulse' experiments on a type of repetitive crab axon. *J. Physiol., Lond.* **168**, 17–18*P*.

CHAPMAN, R. A. (1963*b*). Crab axons showing a prolonged repolarisation phase. *Nature, Lond.* **199**, 701–2.

COLE, K. S. & CURTIS, H. J. (1939). Electric impedance of the squid axon during activity. *J. Gen. Physiol.* **22**, 649–70.

FITZHUGH, R. (1961). Impulses and physiological states in theoretical models of nerve membrane. *Biophys. J.* **1**, 445–66.

FITZHUGH, R. & ANTOSIEWICZ, A. H. (1959). Automatic computations of nerve excitation—detailed corrections and additions. J. *Soc. Indust. Appl. Math.* **7**, 447–58.

FRANKENHAEUSER, B. & HODGKIN, A. L. (1956). The after-effects of impulses in the giant nerve fibre of *Loligo*. *J. Physiol., Lond.* **131**, 341–76.

FUORTES, M. G. F. & MANTEGAZZINI, F. (1962). Interpretation of the repetitive firing of nerve cells. *J. Gen. Physiol.* **45**, 1163–79.

GRANIT, R., KERNELL, D. & SHORTNESS, G. K. (1963). Quantitative aspects of repetitive firing of mammalian motoneurones by injected currents. *J. Physiol. Lond.* **168**, 911–31.

HAGIWARA, S. & OOMURA, Y. (1958). The critical depolarisation for the spike in the squid giant axon. *Jap. J. Physiol.* **8**, 234–45.

HODGKIN, A. L. (1938). The subthreshold potentials in a crustacean nerve fibre. *Proc. Roy. Soc.* B **126**, 247–85.

HODGKIN, A. L. (1948). The local electric changes associated with repetitive action in a non-medullated axon. *J. Physiol., Lond.* **107**, 165–81.

HODGKIN, A. L. (1964). *The Conduction of the Nervous Impulse*, p. 67. Liverpool University Press.

HODGKIN, A. L. & HUXLEY, A. F. (1947). Potassium leakage from an active nerve fibre. *J. Physiol., Lond.* **106**, 341–66.

HODGKIN, A. L. & KEYNES, R. D. (1955). Active transport of cations in giant axons from *Sepia* and *Loligo*. *J. Physiol., Lond.* **128**, 28–60.

HUXLEY, A. F. (1959). Ion movements during nerve activity. *Ann. N.Y. Acad. Sci.* **81**, 221–46.
JULIAN, F. J., MOORE, J. W. & GOLDMAN, D. E. (1962*a*). Membrane potentials of the lobster giant axon obtained by use of the sucrose gap technique. *J. Gen. Physiol.* **45**, 1195–216.
JULIAN, F. J., MOORE, J. W. & GOLDMAN, D. E. (1962*b*). Current-voltage relations in the lobster giant axon under voltage clamp conditions. *J. Gen. Physiol.* **45**, 1217–38.
STAMPFLI, R. (1954). A new method for measuring membrane potentials with external electrodes. *Experientia* **10**, 508–9.
STAMPFLI, R. (1963). Conduction and transmission in the nervous system. *Ann. Rev. Physiol.* **25**, 493–521.
TOMITA, T., SAIMI, T. & TOIDA, N. (1961). Repetitive hyperpolarising responses of the nerve fibre of the crayfish. *Nature, Lond.* **190**, 271–2.
WIERSMA, C. A. G. & RIPLEY, S. H. (1952). Innervation patterns of crustacean limbs. *Physiol. Comp. Oecol.* **11**, 391–405.