The Physiology of the

INSECT CENTRAL NERVOUS SYSTEM

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Papers from the 12th International Congress of Entomology held in London, 1964

Edited by

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Preface

The relative simplicity and anatomical disposition of the insect central nervous system makes it an ideal medium for the study of its physiology. This volume reports the first major Symposium to be attended by authorities in this field which was held at the 12th International Congress of Entomology in London in July 1964. The contributions are of two kinds: review articles, which are valuable to the general biologist as well as to the specialist, and papers dealing with the most recent discoveries in several aspects of the subject. These contributions represent a continuous spectrum, ranging from investigations on the physiology of giant axons, the chemistry of nervous tissues and the mechanism of synaptic transmission to such topics as the neuronal pathways in the central nervous system, the central control of physiological processes, and finally to the neurophysiological basis of learning and instinctive behaviour in insects.

As Professor Roeder emphasizes in his far-sighted epilogue, our progress in understanding the physiology of the central nervous system depends not only on advances in technique, but also upon our way of thinking analytically about it. It is hoped that the contributions to this volume will serve both as a useful summary of our present state of knowledge and also as a stimulus to further research and analysis in this subject.

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March, 1965 J. W. L. B.

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The Physiology of Insect Axons

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The neurophysiology of insects relies, in part at least, upon a knowledge of general neurophysiology, although there are certain fields in which work on insects has influenced the general interpretation of nervous functions. The physiology of insect nerve and muscle is important for two reasons: (1) because of the enormous variety of insects (in terms of morphology, physiology or behaviour) which provides us with excellent opportunity to study comparative neurophysiology, and (2) since many of the currently developed insecticides are neurotoxins, it is of great importance to know the basic aspects of insect nervous function in order to elucidate the mode of action of these insecticides.

The study of neurophysiology is based in many cases on the observations of potential changes occurring in nerve and muscle. The technique is of use for either of two purposes: (1) to learn at the cellular or even molecular level how nerve and muscle work, and (2) to learn how organ and animal are organized and work. For instance, the mechanism of action potential production belongs to the former category, while the behaviouristic responses induced by external stimulation belong to the latter. The cellular aspect provides the latter with the basic knowledge.

Neurophysiology has achieved remarkable progress in the recent two decades so that a variety of nervous functions can now be interpreted in physico-chemical terms. The first measurements of the action potentials of nerve and muscle were made using external electrodes, which only permitted observation of severely attenuated potential changes due to short circuit. Subsequently, however, Curtis and Cole (1940) and Hodgkin and Huxley (1939) were able to measure the absolute magnitudes of membrane resting and action potentials by introducing a longitudinal electrode into a squid giant axon. The action potentials recorded in these studies showed an overshoot beyond the zero potential level, a discovery which was not accounted for by the classical membrane theory of Bernstein (1912) which predicted a simple disappearance

of the membrane potential during activity. This discovery stimulated physiologists, especially those of the Cambridge group headed by Professor A. L. Hodgkin, to explore the mechanisms causing the overshoot of the action potential. Another technical achievement was the voltage-clamp method which was first developed by Marmont (1949) and Cole (1949) and then extensively used by Hodgkin's and other groups (e.g. Cole and Moore, 1960; Hagiwara and Saito, 1959a, b; Hodgkin, Huxley and Katz, 1949, 1952; Julian, Moore and Goldman, 1962b; Narahashi, Moore and Scott, 1964; Tasaki and Hagiwara, 1957). This technique permits us to analyse the sequence of events occurring

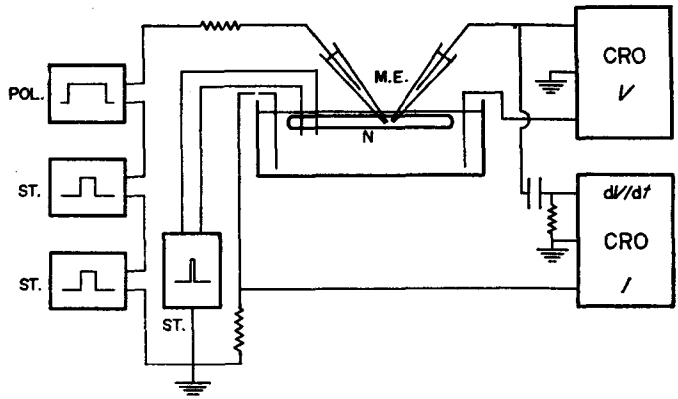

FIG. 1. Diagram of stimulation and recording by means of intracellular microelectrodes. CRO, cathode ray oscilloscope; dV/dt, recording of the rates of rise and fall of the action potential; I, current recording; M.E., microelectrode; N, nerve; POL., polarization; ST., stimulation; V, voltage recording.

at the nerve membrane in terms of membrane current and membrane conductance, which are otherwise too complicated to analyse. An additional tool, the radio-active isotope, has also been of value in the direct measurement of ionic fluxes across the nerve and muscle membrane at rest and during activity.

In the present article, the most fundamental aspects of insect cellular neurophysiology are described, and attempts are made to apply this knowledge to elucidating the mechanism of drug action on insect nerve, and to extend it to the molecular level of excitation mechanisms. Analyses have been made in cockroach giant axons by means of the intracellular microelectrode technique unless otherwise stated (Fig. 1).

I. Physiological Aspects of Nervous Functions

A. *Membrane Electrical Properties*

A nerve fibre is composed of an axoplasm surrounded by a very thin membrane, the "nerve membrane" or "excitable membrane". It is at this membrane that the nerve manifests various physiological functions such as impulse conduction. The nerve membrane, about 100 Å thick, is made of protein and phospholipid molecules. There is a potential difference of 50–100 mV across the nerve membrane, inside being negative with respect to outside; this is called the "resting potential". The nerve membrane shows a relatively high electrical resistance parallel with a capacity (Fig. 2). Since the resistances of the

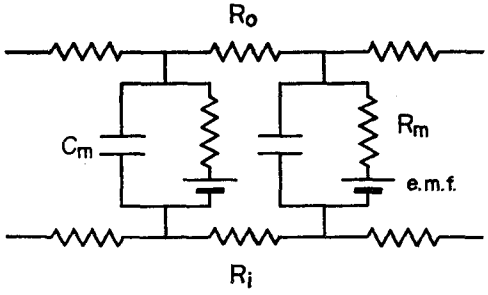

Fig. 2. Electrical equivalent circuit of the nerve membrane. C_m, membrane capacity; R_i, internal resistance; R_m, membrane resistance; R_o, external resistance.

axoplasm and of the external fluid are relatively low, a nerve fibre behaves electrically like a cable. Because of such a cable structure, a potential difference imposed at a point on the nerve fibre falls decrementally with distance along the fibre and also changes with time (Fig. 3). It is possible to estimate the values of the electrical constants

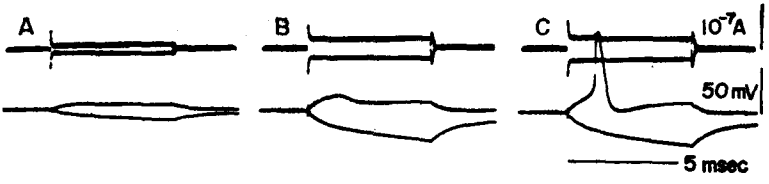

Fig. 3. Potential changes produced by passing square pulses of polarizing current through the membrane of a cockroach giant axon. Upper tracings represent current recording and the zero potential level, upward deflexion being outward current. Lower tracings represent potential recording, upward deflexion being depolarization. Responses to outward and inward currents of the same intensity are superimposed in each set of the records. Note the local response and delayed rectification in B and the action potential superimposed on a catelectrotonic potential in C. Preparation 61-219Ba. Temperature 20° C.

by applying square pulses of current to a nerve fibre and by recording the resultant potential changes. Table I gives results of such experiments, with some examples from other animals for comparison. It is apparent that the electrical properties of the cockroach giant axon in large measure resemble those of other nerves.

B. *Nature of Resting Potential*

The resting potential of the cockroach giant axons is of the same order of magnitude as that of other nerves (Table I). It is reduced by increasing the external concentration of potassium (Fig. 2 of Yamasaki and Narahashi, 1959a). As in other nerves and muscles, the potassium concentration is higher in the axoplasm than in the external fluid (Tobias, 1948; Treherne, 1961), so that the resting potential can be interpreted in terms of the potassium electrode property (e.g. Curtis and Cole, 1942; Huxley and Stämpfli, 1951; Ling and Gerard, 1950); that is to say, it is determined by the concentration gradient of potassium across the nerve membrane, and approaches the potassium equilibrium potential, E_K,

$$E_K = \frac{RT}{F} \ln \frac{[K]_o}{[K]_i}, \tag{1}$$

where R is the gas constant, T is the absolute temperature, F is the Faraday constant, and $[K]_o$ and $[K]_i$ are potassium concentrations outside and inside the nerve, respectively.

C. *Nature of Action Potential*

When a cockroach giant axon is subjected at one point to a weak pulse of current of either potentiality with respect to the membrane, a potential change develops whose time course is determined by the membrane time constant. Nothing happens when the polarizing current is increased in intensity in an inward (anodal) direction except for the proportionate increase in potential which is called the "anelectrotonic potential" (Fig. 3). On the contrary, when the outward (cathodal) current is increased in intensity, there is a critical point of depolarization beyond which the depolarization grows rapidly, crosses the zero potential level and then returns towards the resting potential level. These changes are known as the "action potential" (Figs. 3 and 6A). When the action potential occurs at one point of the axon the neighbouring region is subjected to outward membrane current, due to the potential difference established, so as to produce another action poten-

tial (Fig. 4). Because of the regenerative nature of the action potential production, the impulse is conducted along the axon without undergoing any decrement. When the properties of the axon are changed in such a way as to lower the safety factor of conduction, impulse conduction is blocked.

The action potential of the cockroach giant axon is of the same order of magnitude as that of other nerves (Table I). It is reduced by decreasing the external sodium concentration (Fig. 6 of Yamasaki and Narahashi, 1959a), the relation being again very similar to that found in other nerves and muscles (e.g. Hodgkin and Katz, 1949; Huxley and

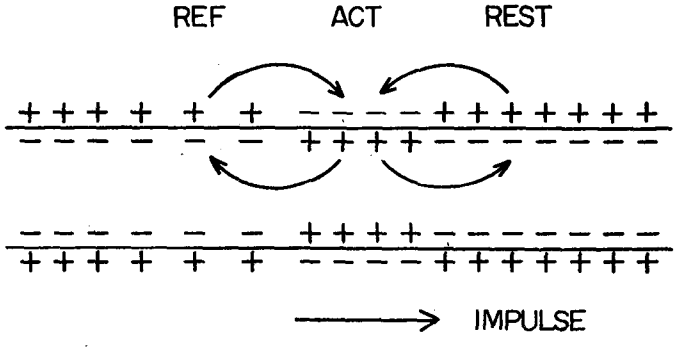

FIG. 4. Diagram of impulse conduction along a nerve fibre. ACT, active region; REF, refractory region; REST, resting region.

Stämpfli 1951; Nastuk and Hodgkin, 1950). This is to be expected when the action potential behaves as a sodium electrode, i.e.

$$E_{Na} = \frac{RT}{F} \ln \frac{[Na]_o}{[Na]_i}, \qquad (2)$$

where E_{Na} is the sodium equilibrium potential, and $[Na]_o$ and $[Na]_i$ are sodium concentrations outside and inside the nerve, respectively. Unlike potassium, the sodium concentration is higher in the external medium than in the axoplasm (Tobias, 1948; Treherne, 1961).

It is then reasonable to assume that the membrane resting and action potentials are explicable in terms of the ionic theory advanced by Hodgkin and his associates (Hodgkin, 1951, 1958). Figure 5 shows the schematic explanation of excitation. In the resting state, the membrane is permeable to potassium but only scarcely so to sodium, so that the membrane potential approaches the potassium equilibrium potential. When the nerve is stimulated, the sodium conductance rises quickly,

TABLE I

MEMBRANE POTENTIALS AND MEMBRANE ELECTRICAL CONSTANTS

Tissue	*RP* (mV)	*AP* (mV)	R_m (Ωcm^2)	R_i (Ωcm)	C_m ($\mu F/cm^2$)	τ_m (msec)	λ (mm)	Reference
Cockroach axon	77	99*	800		6·3	4·2	0·86	Narahashi and Yamasaki (1960a), Yamasaki and Narahashi (1959b)
	78	85†	610	46			1·3	Boistel and Coraboeuf (1954), Boistel (1959)
Squid axon	61	104	700	30	1·0	0·7	6·0	Cole and Hodgkin (1939), Curtis and Cole (1942), Hodgkin, Huxley and Katz (1949)
Crab axon	71–94	116–153	7700	90	1·1	6·8	2·0	Hodgkin (1947), Hodgkin and Huxley (1945)
Lobster axon	73	101	2300	60	1·3	2·3	1·6	Hodgkin and Rushton (1946), Tobias and Bryant (1955)

RP, resting potential; *AP*, action potential; R_m, specific membrane resistance; R_i, specific axoplasm resistance; C_m, specific membrane capacity; τ_m, membrane time constant; λ, membrane length constant.

* In 214 mM-Na Ringer. † In 154 mM-Na Ringer.

thereby causing the membrane potential to approach the sodium equilibrium potential; this is the rising phase of the action potential. At this time sodium enters the axon according to its concentration gradient. The sodium conductance then begins to decrease and the

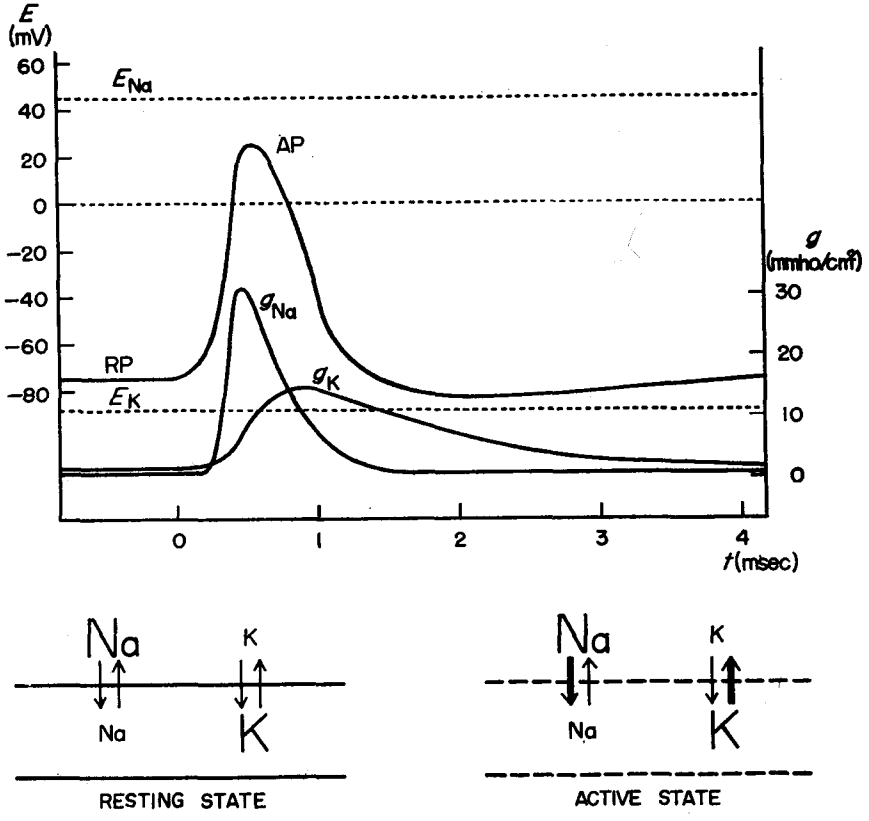

FIG. 5. Schematic representation of the excitation mechanism by the ionic theory. AP, action potential; E, membrane potential; E_K, potassium equilibrium potential; E_{Na}, sodium equilibrium potential; g, membrane conductance; g_K, membrane potassium conductance; g_{Na}, membrane sodium conductance; t, time. The upper scheme is adapted from Hodgkin (1958).

potassium conductance now begins to increase, both working to bring the membrane potential down to the resting level; this is the falling phase of the action potential. Potassium now tends to escape from the axon according to its concentration gradient. In both cockroach and squid giant axons, the falling phase may be followed by an undershoot,

which is called the "positive phase" (Fig. 6); this is not seen in lobster giant axons. The positive phase is attributable to the sustained increase in potassium conductance. Because the actual resting potential is slightly lower than the potassium equilibrium potential due to conductances to ions other than potassium, the membrane now becomes an ideal potassium electrode bringing its potential closer to the potassium equilibrium potential. The positive phase is very often followed by a small, sustained depolarization called the "negative after-potential" (Fig. 6). Analyses with cockroach giant axons have revealed that the negative after-potential is produced by an accumulation of

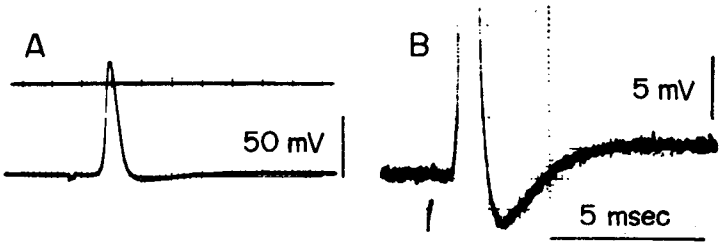

FIG. 6. Propagated action potentials produced by a brief pulse of current in a cockroach giant axon. The spike potential is followed by a positive phase which is terminated in a prolonged negative after-potential. The latter two phases are more clearly seen in B where the voltage amplification is 10 times greater. The upper tracing in A shows the zero potential level. Preparation 64–219Ba. Temperature 20° C.

potassium released during activity in the immediate vicinity of the nerve membrane (Narahashi and Yamasaki, 1960a). Electron microscope observations support this view (Smith and Treherne, 1963).

The negative after-potential is liable to be affected by various experimental conditions. It is augmented and prolonged by barium ions (Narahashi, 1961), DDT (Narahashi and Yamasaki, 1960b, c), allethrin (Narahashi, 1962a, b), high calcium (Narahashi and Yamasaki, 1960a), or high temperature (Boistel, 1960, 1962a, b; Narahashi, 1963). In such cases, large negative after-potentials are not necessarily attributable to the potassium accumulation. For example, the suppression of the potassium-activation mechanism is involved in the case of DDT (Narahashi and Yamasaki, 1960b, c), and the accumulation of an unknown depolarizing substance is suggested in the case of allethrin (Narahashi, 1962a, b).

II. Pharmacological Aspects of Nervous Functions

A. *Classification of the Conditions that Affect the Nervous Functions*

The properties of the nerve membrane can be altered by a variety of means. The following is a classification of the conditions based on the mechanisms whereby the membrane properties are changed:

(a) Those substances which change the resting and/or action potential by simply altering the e.m.f. of the membrane. High potassium and low sodium are typical examples.

(b) Those substances which combine with or are absorbed into the nerve membrane, thereby altering the membrane properties. This group may be further divided into two: (1) those which hinder membrane potential changes produced by various means and block conduction without affecting the resting potential, i.e. stabilizers (Shanes, 1958a, b) such as high calcium and cocaine; (2) those which enhance or accelerate membrane potential changes, and may cause repetitive activity, i.e. labilizers (Shanes, 1958a, b) such as DDT and veratrine.

(c) Those which block excitability through the inhibition of energy metabolism, e.g. rotenone (Fukami, Nakatsugawa and Narahashi, 1959).

The group (a) above has already been described. The group (b) is of special interest, because ions and drugs belonging to this group may be used as tools to explore the mechanisms of excitation. Here, some examples will be presented in which attempts have been made to account for the action of drugs in terms of membrane conductance changes.

B. *Possible Approaches*

It has become apparent that at least three factors govern excitability: (1) the ability of the membrane to undergo a sodium conductance increase upon depolarization, the "sodium-activation mechanism"; (2) the ability of the membrane to undergo a sodium conductance decrease during sustained depolarization, the "sodium-inactivation mechanism"; (3) the ability of the membrane to undergo a potassium conductance increase upon depolarization, the "potassium-activation mechanism". There is also evidence that, at least in certain nerves and muscles, the "potassium-inactivation mechanism" is operative (Frankenhaeuser and Waltman, 1959; Grundfest, 1960; Nakajima, Iwasaki and Obata, 1962).

In order to elucidate the mechanism of action of the agents which affect the membrane, it is necessary to interpret the action on the nerve membrane in terms of the above three, or possibly four, factors. The

ideal way of doing this would no doubt be to use the voltage-clamp technique, but so far no attempts have been made to apply this to insect nerves. This technique requires space clamping, which was formerly only possible by introducing longitudinal electrodes into an axon; and is very difficult, if not impossible, in the small insect axons. However, it has now become possible to establish a space-clamp condition by means of the "sucrose-gap" method without having any electrode inside the axon (Julian, Moore and Goldman, 1962a). This method was successfully used in voltage-clamp experiments with lobster giant axons which were only twice as large in diameter as cockroach giant axons (Fig. 7) (Julian *et al.*, 1926b; Narahashi *et al.*, 1964). It is hoped that this method will be applicable to insect giant axons in the near future.

FIG. 7. Voltage-clamp experiments in a lobster giant axon by means of the sucrose-gap method. A, voltage is not clamped. An action potential is produced by an outward current. The critical depolarization for firing is high because of hyperpolarization by sucrose. B, current recording when the membrane is depolarized by 90 mV from the holding membrane potential of −115 mV. Upward deflexion denotes outward current. Note the initial and transient inward current and the delayed and sustained outward current. C, current recording when the membrane is hyperpolarized by 40 mV. Note the simple inward current. Preparation 63–116Af. Temperature 10° C.

It is also possible, however, to analyse, by means of the conventional intracellular microelectrodes, changes in the above four factors by the action of membrane-attacking agents. Since the maximum rate of rise of the action potential is proportional to the inward sodium current at that moment in the propagating action potential (Narahashi, 1961), this parameter can be used as an indication of the sodium-activation mechanism. Delayed rectification revealed by cathodal current is known to be an expression of the sustained potassium conductance rise during depolarization (Hodgkin *et al.*, 1949; Yamasaki and Narahashi, 1959b), and can be used as a measure of the potassium-activation mechanism. The potassium-activation is also manifested as prolonged depolarizing responses in sodium-free potassium-rich media when the membrane potential is brought to appropriately high levels (Fig. 8) (Ooyama and Wright, 1962; Wright and Tomita, 1962). We have for the moment no direct way of estimating the sodium-inactivation mechanism, but the activity of the mechanism may be inferred to

some extent from observation of the maximum rate of fall of the action potential and of delayed rectification, because the maximum rate of fall is determined both by the sodium inactivation and by the potassium

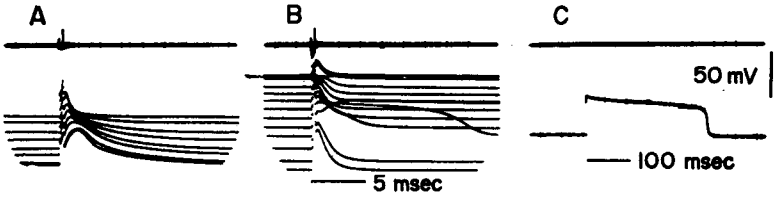

FIG. 8. Responses of a cockroach giant axon in Na-free 3·1 mM–K choline Ringer (A) and in Na-free 40 mM–K Ringer (B and C). A brief cathodal current is applied to the membrane which has been hyperpolarized to various levels by sustained anodal currents. Upper tracings show the zero potential level. Superimposed (A and B) and single (C) records. Note the appearance of prolonged depolarizing responses in high-K (B and C) and its absence in low-K (A). The responses are regarded as indicating the potassium-activation mechanism. Preparation 63–816Aa. Temperature 34° C.

activation. The potassium-inactivation mechanism, when present, may be estimated by observing the time course of change in delayed rectification or the current-voltage relations (Grundfest, 1960).

C. *Allethrin and Tetrodotoxin*

Allethrin, a derivative of pyrethrins, has at least three distinct effects on the cockroach giant axons: (1) increase in negative after-potential;

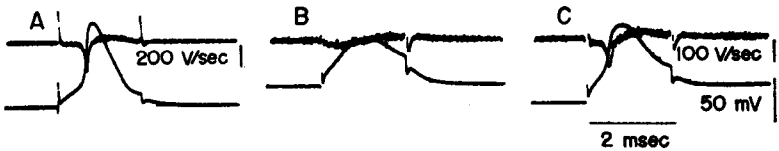

FIG. 9. The effect of allethrin 10^{-6} g/ml on the action potential of the cockroach giant axon. Upper tracings represent the rate of rise (downward deflexion) and the rate of fall (upward deflexion) of the action potential, and the zero potential level. Lower tracings represent the action potential produced by a pulse of cathodal current. A, before allethrin; B, 12 minutes after allethrin, note the slight depolarization and the disappearance of the action potential; C, as in B, but the action potential is restored by anodal polarization. Preparation 64–130Aa. Temperature 18° C.

(2) repetitive after-discharge; (3) conduction block. The first two effects have already been analysed and reported elsewhere (Narahashi, 1962a, b). Here the third effect will be described. Tetrodotoxin is an active principle of puffer poison, and in lobster axons and in frog muscle

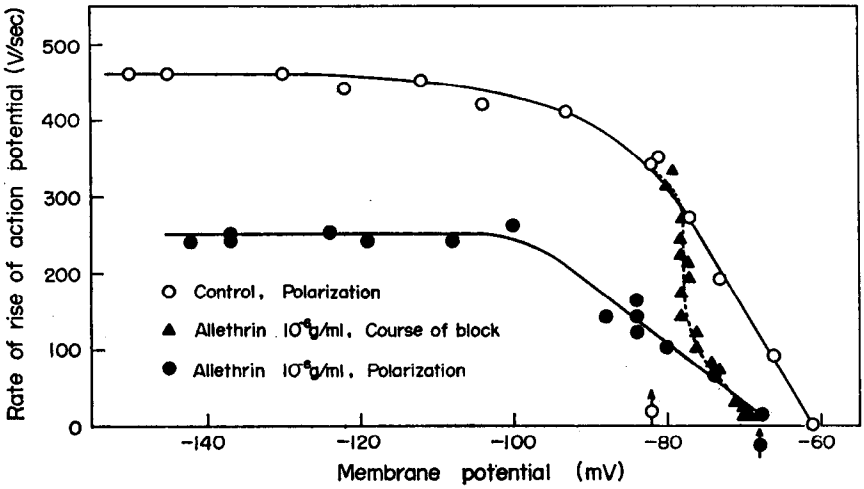

FIG. 10. The maximum rate of rise of the action potential of a cockroach giant axon plotted against the membrane potentials displaced by polarizing currents before (○) and 18 minutes after (●) allethrin 10^{-6} g/ml (sodium-inactivation curves). The arrows indicate the resting potentials. The values of the rate of rise during the course of blockade after allethrin are also plotted against the respective membrane potentials (▲). Preparation 64–128A. Temperature 23° C.

fibres has been known to block conduction through the selective inhibition of the sodium-activation mechanism (Narahashi, *et al.*, 1960, 1964). This is used here for comparison.

After exposure to allethrin at a concentration of 10^{-6} g/ml, the spike height declines and finally excitability is completely blocked (Fig. 9). The resting potential is also reduced, but this is not the sole cause of blockage. The decline of the spike height is greater than would

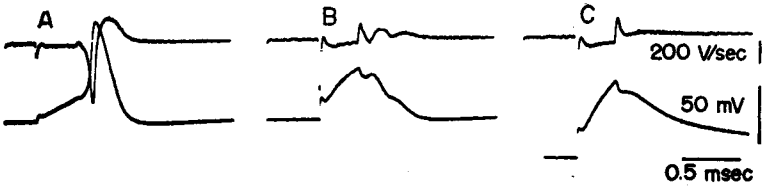

FIG. 11. The effect of tetrodotoxin 3×10^{-8} g/ml on the action potential of the cockroach giant axon. Upper tracings represent the rate of rise (downward deflexion) and the rate of fall (upward deflexion) of the action potential, and the zero potential level. Lower tracings represent the action potential produced by a pulse of cathodal current. A, before tetrodotoxin; B, 5 minutes after tetrodotoxin, note the drastic reduction of the action potential; C, as in B, showing the inability of anodal polarization to restore the action potential. Preparation 63–822Ba. Temperature 31·5° C.