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# A SIMPLE TECHNIQUE FOR MONITORING THE SYNAPTIC ACTIONS OF PHARMACOLOGICAL AGENTS

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## INTRODUCTION

Many pharmacological and insecticidal agents are known to act at the level of the synapse in the central nervous system. The actions of such compounds on synaptic transmission in insect ganglia have been reviewed by several authors (cf. Colhoun, 1963; Boistel, 1968; Pitman, 1971; Callec, 1972; Narahashi, 1971; Gerschenfeld, 1973; Pichon, 1973). A variety of electrophysiological techniques have been employed in these studies and their relative merits and drawbacks have been discussed in an earlier paper (Pichon & Callec, 1970). A method for recording synaptic events which is simple to perform and also provides detailed information concerning the site of action of drugs applied to the ganglion has not yet emerged. In this paper a simplification of the 'oil-gap' system introduced by Pichon & Callec (1970) is described in an attempt to fulfil such requirements.

The 'oil-gap' method of recording from a single postsynaptic fibre offers several improvements over the conventional microelectrode technique. For example, extracellular recording of the spontaneous and evoked excitatory postsynaptic potentials (EPSPs) and the inhibitory postsynaptic potentials (IPSPs) is possible (Callec, 1972). Although these potentials are somewhat smaller and slower than those recorded using microelectrodes located within the ganglion, they can be maintained for extended periods enabling long-term experiments to be performed. Irrigation of the ganglion is possible, allowing the application of drugs under controlled conditions. This method also enables the recording of both postsynaptic action potentials comparable in magnitude to those obtained with microelectrodes, and changes in the resistance of the postsynaptic membrane. The low-impedance of the recording system and its stability allow experiments to be performed which would be impossible with microelectrode techniques.

The 'oil-gap' method, as originally devised, involves the isolation by microdissection of a single giant axon from the connective linking the fifth and sixth abdominal ganglia of the cockroach *Periplaneta americana* (L.) (Pichon & Callec, 1970). Such a preparation is not readily achieved without considerable experience. The simple recording technique described here is a modification of the 'oil-gap'

system and does not involve a complex dissection. It nevertheless incorporates many of the advantages of the original method. This report describes some typical results obtained using a whole-ganglion preparation in conjunction with a 'mannitol-gap' recording technique (cf. Pichon & Treherne, 1970). In order to illustrate the potential usefulness of this system, the action of acetylcholine on synaptic transmission in the terminal abdominal ganglion of *Periplaneta* has been studied.

The elucidation of the chemical nature of transmitter substances in the insect ganglion relies on biochemical, ultrastructural and electrophysiological evidence. All the criteria for the identification of transmitter substances (cf. Curtis, 1961, 1970; Werman, 1966; Gerschenfeld, 1966; Hebb, 1970) have not been satisfied in this preparation, largely due to the difficulties of working with a complex tissue. The available evidence, nevertheless, points clearly towards a cholinergic mechanism for excitatory synaptic transmission (cf. Colhoun, 1963; Smith & Treherne, 1963; Kerkut, Pitman & Walker, 1969*a*, *b*; Pitman & Kerkut, 1970; Shankland, Rose & Donniger, 1971; Callec & Boistel, 1971*a*; Callec, 1972). In spite of this, the techniques employed to date have not described the relationship between the concentration of applied acetylcholine and its effect on synaptic transmission. The application of our technique to the pharmacological investigation of the terminal abdominal ganglion of the cockroach *Periplaneta americana* enables the characterization of this and other drug responses by means of such a dose-response curve.

## METHODS

### (1) *Fundamental features of the anatomy and physiology of the sixth abdominal ganglion of* Periplaneta americana

Certain features of the anatomy and physiology of the terminal abdominal ganglion of *Periplaneta americana*, which have been reviewed in detail elsewhere (Callec, 1972, 1974), are relevant to these experiments. Micro-anatomical investigations of the cockroach giant axons by means of fluorescent-dye injection have been performed by Milburn & Bentley (1971) and by Harris & Smyth (1971). These studies reveal that the dendritic tree of a giant fibre extends throughout a considerable proportion of the sixth abdominal ganglion. Such findings have been confirmed recently using the cobaltous ion technique (Pitman, Tweedle & Cohen, 1973).

The main input of sensory information to this ganglion is derived from the cercal mechanoreceptors and conducted via nerves X and XI (cf. enumeration of Roeder, Tozian & Weiant, 1960). Nerve X contains excitatory and inhibitory fibres, the latter providing important inhibitory effects at the level of the sixth ganglion. The IPSPs are apparently bisynaptic and the first synaptic contact appears to be located in the sixth ganglion (Callec & Boistel, 1971*b*; Callec, 1972).

The fibres of nerve XI are predominantly excitatory in function, though inhibitory phenomena can sometimes be recorded (cf. Callec, 1972). Electrophysiological studies, using the 'oil-gap' method on giant axons, have provided clear evidence of a component of the complex EPSP which is composed of unitary monosynaptic EPSPs each of which is related to cercal receptor activity (Callec *et al.* 1971). Further evidence for the existence of such monosynaptic connexions is provided by ultrastructural studies on degenerating cercal nerves carried out by Farley & Milburn (1969). These

authors have shown that the cercal nerve endings occur in rows along the branches of what appear to be giant fibres.

### (2) *Preparation and recording chamber*

A preparation comprising the abdominal nerve cord, the cercal nerves (X, XI) and the cerci was dissected from the adult male cockroach (*Periplaneta americana*) and placed in a drop of physiological saline on a glass slide. Extraneous tracheae and remnants of abdominal cuticle were then removed. This procedure is the same as for the first part of the dissection described by Pichon & Callec (1970) prior to the isolation by micro-dissection of a single axon. The preparation was finally transferred to a Perspex recording chamber containing 5 compartments isolated by petroleum jelly seals (cf. Fig. 1A, B). The subdivisions of the chamber are designated *a–e* and contain: *a*, the cerci, mounted in air on a raised platform; *b*, the cercal nerves, under Ringer, surrounded by very fine platinum stimulating electrodes; *c*, the sixth abdominal ganglion, under perfusion by either Ringer or test solution; *d*, a portion of the connective linking the fifth and sixth abdominal ganglia, under perfusion by a mannitol solution; *e*, the remainder of the ventral nerve cord, under Ringer. In a limited number of experiments the chamber of Callec (1972) was employed using the preparation described above.

For the demonstration of excitatory phenomena, the X nerves were cut and stimulation was via the XI nerves alone. Inhibitory phenomena were demonstrated following the severance of the XI nerves and stimulation via the X nerves (cf. Methods, section 1). For pharmacological experiments, the sixth abdominal ganglion was 'de-sheathed' (cf. Boistel & Coraboeuf, 1954).

### (3) *Stimulation and recording*

The disposition of the preparation within the chamber facilitated stimulation by two main methods. For example, by the application of a puff of air directed towards the carefully dried cerci in compartment *a*, mechanical stimulation of the cercal receptors was readily achieved. Electrical stimulation of the cercal nerves was achieved via the adjustable platinum hook-electrodes which were inserted into compartment *b*. Pulses of short duration, provided by either a Grass or Farnell pulse-generating system, were delivered to the preparation via a stimulus-isolation unit.

Electrical potential changes were recorded using a 'nonelectrolyte-gap' system (cf. Stämpfli, 1954). The region of high resistance was achieved in this case by perfusion, with a mannitol solution (cf. Pichon & Treherne, 1970), of the connectives adjacent to the sixth abdominal ganglion. In order to enhance the penetration of the extracellular spaces by mannitol, the connectives were sometimes 'de-sheathed' between the fifth and sixth abdominal ganglia. Excitatory (EPSP) and inhibitory (IPSP) synaptic potentials were recorded by means of Ag–AgCl electrodes linked to compartments *c* and *e* by flexible Ringer–agar bridges (Fig. 1). Electrical signals were led via an impedance converter of high input impedance to a Tektronix 502 or 549 (storage) oscilloscope and filmed using a Grass oscilloscope camera. Slow D.C. potential changes were monitored on a Smith Servoscribe potentiometric recorder. With good chloridation a potential of less than 1 mV was recorded between the two Ag–AgCl electrodes. Under these conditions changes in the polarization of the test

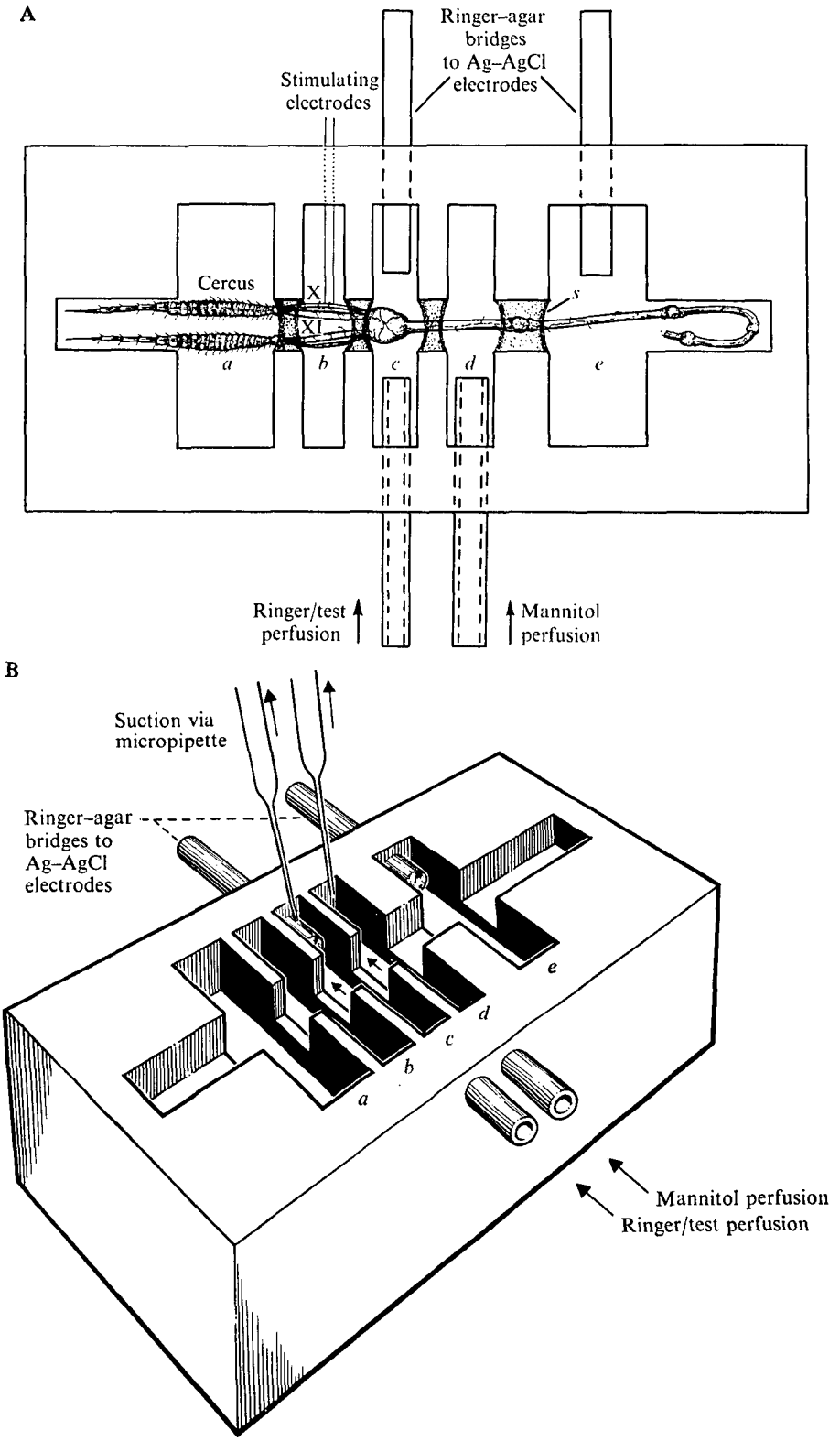


Fig. 1 (A) and (B). For legend see opposite.

compartment were measured (for example during drug action) on the assumption that the polarization of the other compartment remained unchanged.

By the 'oil-gap' method changes in the resistance of a single postsynaptic membrane can be measured (Callec, 1972). Preliminary experiments revealed that resistance changes could be observed, accompanying the action of applied drugs, with the 'mannitol-gap' recording technique in conjunction with a Wheatstone bridge. In a whole-ganglion preparation, however, these changes could be taking place in presynaptic, postsynaptic or axonal elements. They reveal little information concerning the site of action of the applied drug and have not, therefore, been pursued.

### (4) *Perfusion system and physiological saline*

Solutions were delivered by flexible polythene tubes from elevated reservoirs to the inlets of the recording chamber. Compartment *c* was connected via a multi-way non-return valve to eight elevated saline reservoirs (cf. Holder & Sattelle, 1972). The valve was closely applied to the recording chamber to minimize the dead space of the system, and the appropriate test solution was determined by a mechanical selector device. Compartment *d* was linked directly to a reservoir containing a solution of mannitol (87 g/l).

The physiological saline employed in these experiments contained 208·6 mM-NaCl; 3·1 mM-KCl; 5·4 mM-$CaCl_2$; 2·0 mM-$NaHCO_3$ (pH 7·0) and was able to maintain synaptic transmission for several hours. Solutions of acetylcholine chloride (Sigma and BDH) were prepared in this Ringer solution.

## RESULTS

### (A) *Synaptic potentials*

#### (1) *Without stimulation*

In the (apparent) absence of stimulation it was possible to record a dense spontaneous activity from preparations in which the cerci had not been dried (Fig. 2*A*). Careful drying of the cerci resulted in a decline of this background activity to the point where occasional spontaneous depolarizing waves were discernible. The amplitude of these potentials was typically 250–300 $\mu$V, but in a few preparations reached values of 0·5 mV. By analogy with recordings from a single postsynaptic element (Pichon & Callec, 1970), these potentials appear to represent spontaneous unitary EPSPs. Small hyperpolarizing waves of less than 200 $\mu$V were occasionally recorded corresponding to the spontaneous unitary IPSPs observed using the single-fibre technique. However, the alternative view, that these potentials represent spike activity at some distance from the recording electrode, cannot at this stage be discounted.

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Fig. 1. (A) A plan view of the recording chamber illustrating the position of the preparation. The compartments *a–e* (for description see text) are isolated by petroleum jelly seals (*s*). Arrows indicate the direction of perfusion. Cercal nerves (X, XI) are indicated. Normally, the electrode linked to compartment *c* is connected to the earth. (B) A three-dimensional representation of the recording chamber showing its 5 compartments, the end two of which contain a raised platform to support in *a* the cerci, and in *e* the bulk of the ventral nerve cord. The direction of perfusion is indicated by the arrows and suction via the micropipettes maintains constant fluid levels and speeds of flow in chambers *c* and *d*.

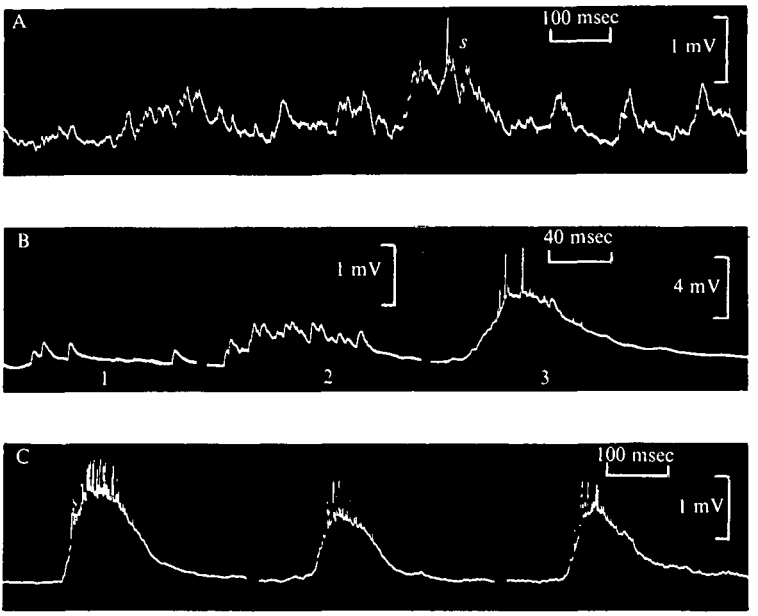



Fig. 2. Trace *A* is an example of spontaneous postsynaptic potentials recorded from the sixth ganglion. The depolarizing waves sometimes reach the threshold for spike initiation (*s*). This background activity can be suppressed by careful drying of the cerci. Trace *B* shows the effects of mechanical stimulation on such a silent preparation. The frequency of the unitary EPSPs shown in $B_1$ increases with the intensity of the mechanical stimulation (puff of air) applied to the cercal mechanoreceptors. The resulting summation of EPSPs ($B_2$) may give rise, at higher stimulation, to a depolarization which sometimes reaches the threshold for spike generation ($B_3$). Trace *C* illustrates the abundant spike activity that can follow very high stimulation.

(2) *Mechanical stimulation of cercal receptors*

Mechanical stimulation, by means of a light puff of air directed towards the cerci, resulted in a transitory increase in the number and frequency of EPSPs (Fig. $2B_1$) and summation of these potentials gave rise to a brief wave of depolarization (Fig. $2B_2$). Stronger stimulation resulted in a greater depolarization, sometimes reaching the threshold for spike generation (Fig. $2B_3$). Normally one or two spikes were initiated by a strong puff of air, but occasionally this resulted in a train of many spikes (Fig. $2C$).

The summation of unitary EPSPs and the subsequent initiation of spikes clearly rules out any possibility that these potentials could be interpreted as spikes recorded at a distance. Similar results to those reported have been obtained using the 'oil-gap' technique (Pichon & Callec, 1970; Callec, 1972).

(3) *Electrical stimulation of cercal nerve XI*

Electrical stimulation of the eleventh (XI) nerve (cf. Fig. 1A) was carried out following severance of the tenth (X) nerve. Remnants of the latter were reflected back into the ganglion compartment (*c*) to avoid stimulation by this pathway. A small presynaptic spike, which was related to cercal nerve activity, preceded slow depolarizing waves which increased in amplitude and duration with small increases in electrical

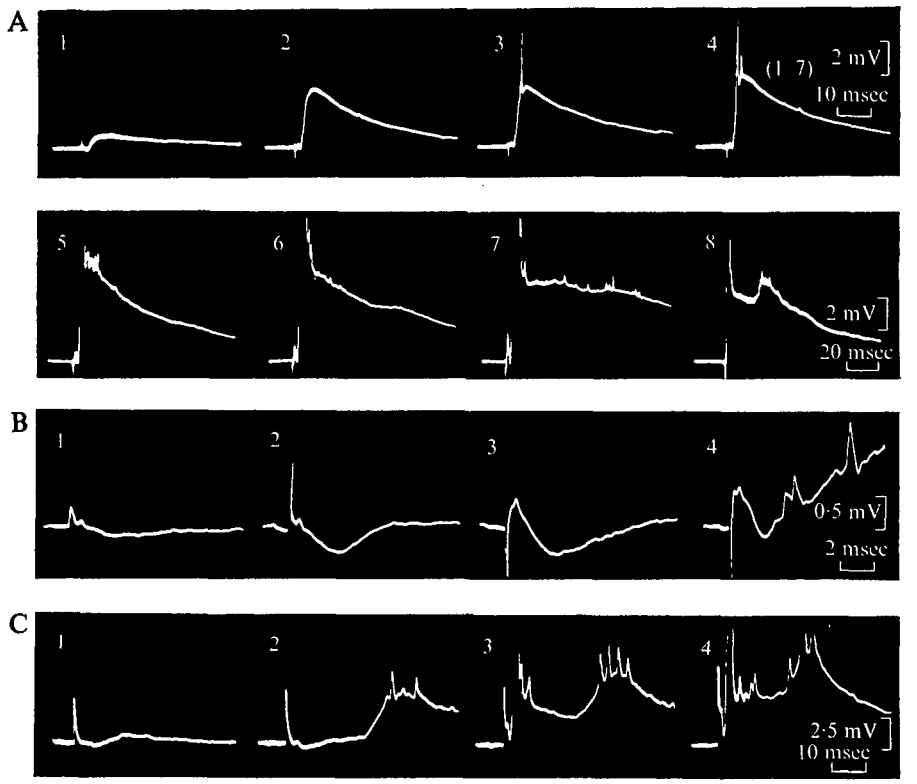
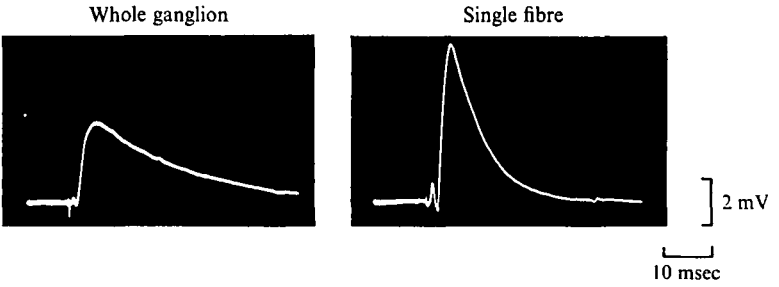



Fig. 3. Postsynaptic events following the electrical stimulation of cercal nerves XI, X. *A*, *B* were recorded using the 'mannitol-gap' chamber. *C* was recorded using the 'oil-gap' chamber (Callec, 1972). *A* illustrates the effects of progressively increasing the stimulation applied to nerve XI. $A_1$, $A_2$, monosynaptic EPSP increasing in amplitude with higher stimulation. $A_3$, monosynaptic EPSP at threshold of single giant axon spike. $A_4$–$A_8$ show evolution of polysynaptic response and compound spike. *B*, *C* show the effects of progressively increasing the stimulation applied to nerve X. $B_1$–$B_3$ show compound IPSP which increases in amplitude with higher stimulation. $B_4$ shows IPSP followed by polysynaptic component of EPSP. *C*, as *B* but at lower gain and sweep speed. $C_1$, $C_2$ show IPSP and development of polysynaptic component of the EPSP. This precedes the appearance of the monosynaptic component of the EPSP and the compound spike ($C_3$, $C_4$).

stimulation (Fig. $3A_{1,2}$). These corresponded in shape and duration to the spontaneous unitary EPSPs, and rapidly followed the presynaptic spike. Such depolarizations appeared, therefore, to represent a monosynaptic response. A small increase in stimulation resulted in the appearance of a compound spike which increased in amplitude to a maximum of between 30–35 mV as the applied current was increased (Fig. $3A_{3-8}$). At the same time a slower, polysynaptic EPSP (Fig. $3A_{5-8}$) and finally a very sensitive delayed response became discernible (Fig. $3A_8$). Small spikes were seen superimposed upon both polysynaptic and delayed components of the EPSP at high stimulation (Fig. $3A_{5-8}$).

(4) *Electrical stimulation of cercal nerve X*

Electrical stimulation of the tenth (X) nerve was carried out following the severance of the eleventh (XI) nerve, care being taken to avoid any stimulation via the latter

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|---|---|---|
| Amplitude of spontaneous unitary EPSP | 200–500 $\mu$V | 0·5–2 mV |
| Amplitude of electrically evoked EPSP at spike threshold | 2–3 mV | 3–8 mV |
| Amplitude of postsynaptic action potential | Up to 35 mV (compound spike)<br>1·5–3·0 mV (giant axon) | Up to 115 mV |

Fig. 4

route. Not infrequently a response similar to that described for nerve XI was obtained. It was, however, possible in other preparations to obtain responses such as those illustrated in Fig. 3*B*, *C*. These were characterized by the appearance at low stimulation of slow hyperpolarizing waves preceded by a delay of about 3 msec. Such waves increased in amplitude and duration with small increases in stimulation (Fig. $3B_{1-3}$). The identification of these potentials was possible when stimulation was further increased. Under these conditions the polysynaptic EPSP first appeared and at higher stimulation this gave rise to small spikes (Fig. $3B_4$, $C_{1,2}$). As the stimulating current was increased the hyperpolarizing wave was replaced by a monosynaptic EPSP which gave rise to a compound spike (Fig. $3C_{3,4}$). The observed polarity of the first recorded potentials and the increase in threshold of the giant-axon response confirms that these hyperpolarizing potentials are IPSPs. It is clear that inhibitory phenomena are not quite so readily demonstrated with the 'mannitol-gap' technique as are excitatory phenomena. However, the response, when it is recorded, is sufficiently well defined to merit investigation of the necessary conditions for obtaining inhibition.

(5) *Synaptic potentials recorded from whole-ganglion and single-fibre*

Fig. 4 summarizes data on the amplitude of postsynaptic potentials obtained both from the whole ganglion using the 'mannitol-gap' method, and from the isolated postsynaptic fibre by means of the 'oil-gap' technique. It is clear, from this and the results presented earlier, that studies on the whole-ganglion preparation provide results which, although attenuated in amplitude, reproduce all the elements of the response recorded from a single postsynaptic unit. An extension of the time-course of the electrically induced EPSP, recorded from the whole-ganglion preparation is noted, when the shape of this potential is compared to that of the single-fibre response.

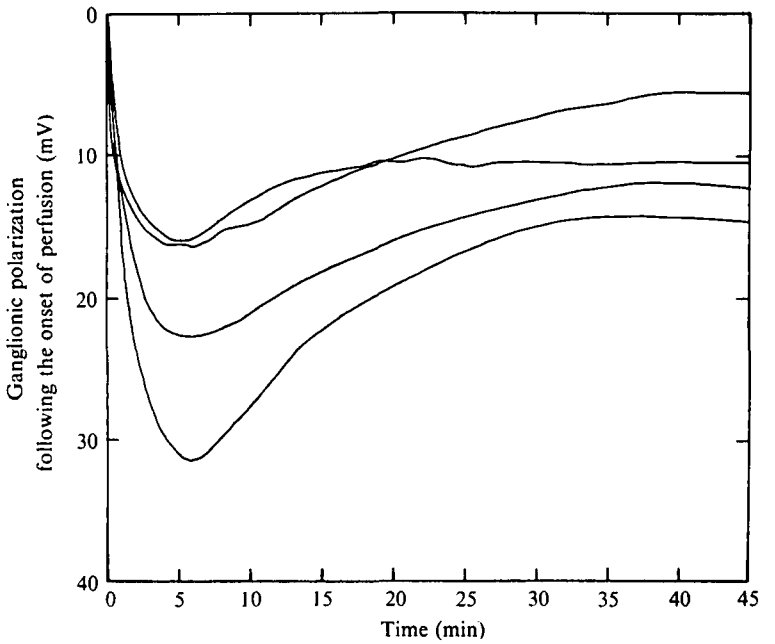


Fig. 5. Graphs of the changes with time of ganglionic polarization following the onset of perfusion by normal saline. The recovery curves of 4 preparations are illustrated. In each case a period of 40 min elapsed between the start of the dissection and the initiation of perfusion.

Values of 22 msec (whole ganglion) and 7·5 msec (single postsynaptic fibre) are obtained for the time-constants of the decay of the illustrated EPSPs (Fig. 4).

### (B) *Polarization*

Slow D.C. potential changes were recorded from the sixth ganglion by means of Ag–AgCl electrodes (cf. Methods, section 3). In addition to the quality of the chloridation of the electrodes, the stability of the recorded potential was also strongly dependent upon the quality of petroleum jelly seals between compartments and the correct positioning and dimensions of the suction micropipettes (Fig. 1B). When the preparation was newly transferred to the recording chamber, it was found to be somewhat depolarized and without synaptic activity. On the commencement of perfusion by physiological saline, a recovery process began typically resulting in a strong hyperpolarization. Under continuous perfusion the potential returned to a steady level (Fig. 5). Polarization was usually stable 30–40 min after the start of perfusion and remained so for many hours.

Similar results have been obtained with the 'oil-gap' technique (Callec, 1972). It has been suggested that deoxygenation of the preparation during dissection, presumably resulting in the cessation of sodium-pump activity, accounts for the observed depolarization. Similarly, the hyperpolarization during recovery may result from hyperactivity of the putative sodium-pump during deoxygenation (cf. Callec, 1972). It is of interest to note that the ability to monitor this recovery process is another feature common to the whole-ganglion and single-fibre preparations.

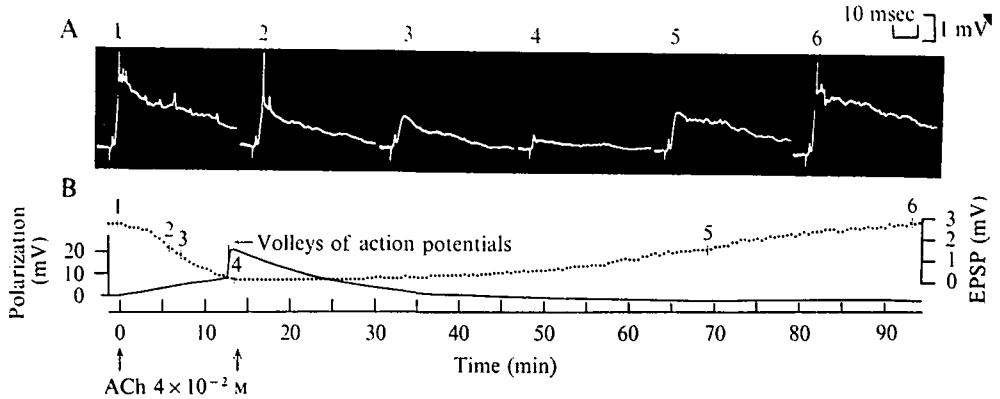


Fig. 6. Effects of the application of acetylcholine ($4 \times 10^{-2}$ M) to a 'de-sheathed' ganglion. *A* shows the changes in the electrically-evoked synaptic potentials. *B* illustrates the changes with time of both ganglionic polarization and the amplitude of the monosynaptic EPSP, during the action of acetylcholine and recovery in normal Ringer. The numbered vertical bars on the EPSP curve in *B* refer to the corresponding traces shown in *A*.

### (C) *The action of acetylcholine*

When acetylcholine in normal Ringer was applied to the 'de-sheathed' sixth abdominal ganglion at concentrations above $10^{-3}$ M, a depolarization of the ganglion ensued, accompanied by a reduction in the amplitude of the recorded EPSP. The detailed form of the response was found to vary slightly with the quality of the 'de-sheathing' and more strongly with the applied concentration. An experiment demonstrating the response of a preparation to $4 \times 10^{-2}$ M acetylcholine is illustrated (Fig. 6). The application of the drug resulted in the onset of an initial ganglionic depolarization which continued steadily for *ca.* 12 min, reaching a value of 7 mV. At this point a second, very rapid, phase of depolarization was observed, which as it approached its maximum value resulted in the initiation of volleys of spikes. The peak change in polarization (*ca.* 22 mV) was reached 13 min after the application of the test solution. The amplitude of the monosynaptic component of the EPSP declined slowly for a period of about 3·5 min following the application of acetylcholine, after which a more rapid decline was observed (Fig. 6). The EPSP was completely blocked 13 min after the application of the drug, co-incident with the point of maximum depolarization.

A change to normal Ringer in the bathing medium resulted in a repolarization which continued slowly until the initial level of polarization was restored. This return to normal saline did not produce any consistent recovery in the EPSP for a period of 20 min. Thereafter, recovery was slow and continued long after ganglionic repolarization was complete. Restoration of the original amplitude of the EPSP was achieved 81 min after re-bathing the preparation in normal Ringer.

A number of such experiments were performed in which varying doses of acetylcholine were applied to the sixth ganglion. By recording the depolarization in each case a dose-response curve was constructed. Fig. 7 summarizes the results obtained on 5 separate preparations. An S-shaped curve was obtained when the depolarization (in mV) was plotted against the logarithm of the applied concentration of acetylcholine.

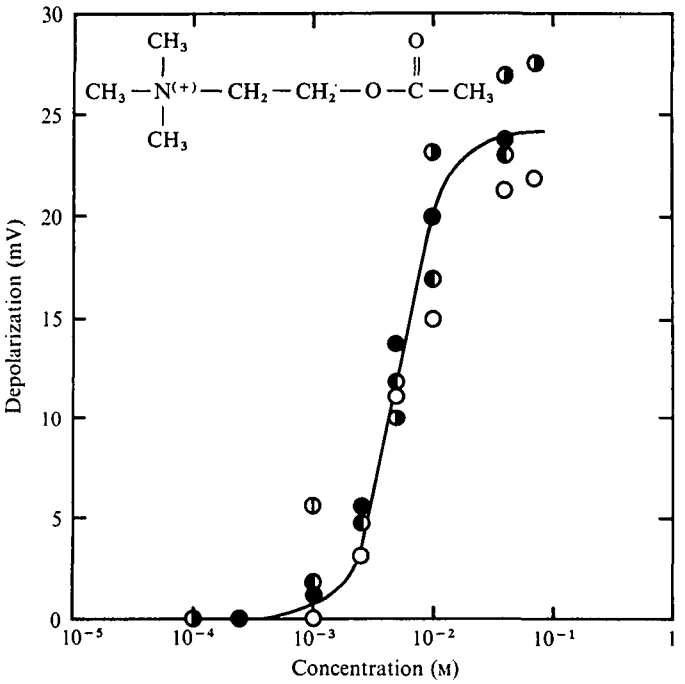


Fig. 7. The effects of various concentrations of acetylcholine on ganglionic polarization. Results of experiments on 5 preparations are illustrated.

## DISCUSSION

The technique described in this report enables the recording of synaptic events by means of extracellular electrodes. The use of external electrode recording to monitor accurately nervous activity was greatly facilitated by the introduction of the 'sucrose-gap' technique by Stämpfli (1954). By interposing a nonelectrolyte gap (oil, sucrose or mannitol) between the recording electrodes, the short-circuiting factor was reduced. This enabled studies to be made using external electrodes which had hitherto only been possible with intracellular microelectrodes. The 'nonelectrolyte-gap' technique has been applied extensively to peripheral nerve (cf. Pichon & Callec, 1970), but has not frequently been employed for the investigation of central nervous ganglia. The only detailed study available has been performed on the superior cervical ganglion of the rabbit (Kosterlitz & Wallis, 1966; Kosterlitz, Lees & Wallis, 1968, 1970). These authors have analysed in detail the complex polysynaptic potentials recorded by means of this technique.

The fact that so few attempts have been made to obtain such recordings reflects perhaps some of the problems that must first be overcome. A major difficulty is to record such local synaptic potentials by means of external electrodes placed at some distance from the site of electrogenesis. This can be overcome by the construction of a chamber with a narrow ganglionic compartment and a very thin barrier between this and the compartment containing the nonelectrolyte. The importance of these dimensions can be seen when the space-constant of a cockroach giant axon in the sixth ganglion is considered. A value of about 1 mm has been obtained using data

from synaptic potentials (Pichon & Callec, 1970), and the distance of the site of origin of such potentials from the anterior end of the ganglion can be taken as about 0·5 mm. With this in mind a chamber has been designed in which the separation of the synaptic site and the mannitol-containing compartment is as small as possible. The preparation described here (cf. Methods) can be dissected without difficulty and when transferred to the recording chamber it gives stable recordings of synaptic phenomena for long periods.

This combination of preparation and recording chamber enable certain features of synaptic activity to be recorded. We consider that, collectively, they may represent a useful tool in pharmacological research. The aspects of synaptic transmission which can be monitored with this technique and which are of most pharmacological interest are summarized below.

*Presynaptic action*

The clearly recorded presynaptic spike is a useful monitor of the presynaptic action of a drug. Also, when the action is postsynaptic and involves a change in threshold, adjustment to a constant presynaptic spike height enables a more precise analysis to be performed. Presynaptic activity can also be detected by observing the changes in frequency of the readily recorded spontaneous EPSPs (cf. also Callec, 1972).

*Postsynaptic action*

The amplitude of the spontaneous unitary EPSPs and IPSPs, recorded after careful drying of the cerci, may represent a sensitive monitor for drug action. Such spontaneous activity is probably more useful as an indicator of the state and quality of the preparation. Of greater pharmacological interest are the electrically evoked monosynaptic EPSP and the bisynaptic IPSP (cf. also Callec, 1972). These can be followed for long periods in conjunction with ganglionic polarization. The changes in polarization recorded from the whole ganglion during the action of acetylcholine (and other drugs, cf. Sattelle & Callec, 1974) closely correspond to those obtained under similar conditions from a single postsynaptic fibre (cf. Callec, 1972). Such changes in potential can, therefore, be considered as essentially postsynaptic in origin. Thus the dose-response curve, relating ganglionic polarization to the concentration of applied acetylcholine, primarily represents the postsynaptic action of this drug. The possible existence of a small presynaptic component of this response cannot, however, be completely ruled out.

The postsynaptic response to iontophoretically applied acetylcholine has been studied in the sixth abdominal ganglion of *Periplaneta* by Callec (1972). A plot of iontophoretic current against the postsynaptic depolarization has provided confirmatory evidence for a cholinergic mechanism for excitatory transmission. It does not, however, enable a ready determination of the postsynaptic response to a given concentration of acetylcholine. Furthermore, as the iontophoretic current is increased, the expelled acetylcholine will diffuse over greater distances, thus increasing the population of receptors under investigation. The technique described in this paper involves perfusion of the whole ganglion, which enables the production of a dose-response curve with reference to a fixed number of receptors. In addition, the amplitude of the response at a given concentration can be readily determined.

The ease with which this technique can be performed renders it suitable for teaching purposes and for investigations in which the synaptic action of a large number of drugs needs to be tested (cf. Sattelle & Callec, 1974). Apart from its usefulness for the detailed pharmacological investigation of synaptic transmission in insects, it also represents a potential research tool in the pharmaceutical and agrochemical industries.

SUMMARY

1. A simple electrophysiological method for the pharmacological investigation of synaptic transmission in insects is described.

2. By means of this technique synaptic potentials and ganglionic polarization can be recorded from the sixth abdominal ganglion of *Periplaneta americana* L. for long periods.

3. In the absence of stimulation, spontaneous excitatory (EPSP) and inhibitory (IPSP) postsynaptic potentials can be recorded. The mechanical stimulation of cercal receptors demonstrates the summation of EPSPs leading to spike generation.

4. Electrical stimulation of the cercal nerves (X, XI) enables the recording of a monosynaptic EPSP (XI) and a bisynaptic IPSP (X).

5. The action of acetylcholine on synaptic transmission, including a dose-response curve, is described.

6. The potential of this technique as a tool in pharmacological research is discussed.

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