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FURTHER STUDIES ON SYNAPTIC TRANSMISSION IN INSECTS

II. RELATIONS BETWEEN SENSORY INFORMATION AND ITS SYNAPTIC INTEGRATION AT THE LEVEL OF A SINGLE GIANT AXON IN THE COCKROACH

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INTRODUCTION

One of the major problems in modern neurophysiology concerns the mechanism by which information transmitted from receptors is transformed in the central nervous system into a more or less complex coded information, and in addition, how it is conducted to other parts of the nervous system (interneurones) or to muscles (motoneurones). The analysis of these integration processes has been carried out most often in relatively simple preparations such as ganglia in the nervous system of invertebrates or sympathetic nervous systems of vertebrates, for reviews, see (Tauc, 1967; Kandel & Kupfermann, 1970). Only relatively little information is available concerning integration of 'unit' presynaptic impulses (Maynard, 1966; Kandel & Wachtel, 1968; Kennedy, 1968; Gorman & Mirolli, 1969). The main difficulty most frequently encountered in this kind of study is the lack of a sufficient control of the sensory afferences, especially when the effect of single presynaptic impulses on the postsynaptic activity is studied.

Another inconvenience of many synaptic preparations is that their integrative properties are limited. A few presynaptic spikes are often sufficient to trigger a postsynaptic action potential.

The last abdominal ganglion of the cockroach provides a particularly suitable preparation for integration analysis. In the neuropile of this ganglion a large number of sensory fibres conducting information from very well-defined cercal mechanoreceptors are synaptically connected with a small number of giant axons which play some part in the escape reflex of this insect (Roeder, 1948; Dagan & Parnas, 1970).

A puff of air of sufficient strength applied to the cerci leads to a reflex escape of the animal. According to Pumphrey & Rawdon-Smith (1937) and Roeder (1948) this reflex involves one monosynaptic relay in the sixth abdominal ganglion between the cercal sensory fibres and the giant interneurones. This first abdominal relay has been used for the study of synaptic transmission in insects (see review by Boistel, 1969). It was not until 1966, however, that, using very fine tipped capillary microelectrodes (0·1–0·2 μm), we have been able to study some of the electrical characteristics of this transmission, when the cercal nerves are stimulated electrically (Callec & Boistel,

1966*a*, *b*). Recent experiments involving the use of an oil-gap technique to record postsynaptic potentials in a single postsynaptic giant axon (Pichon & Callec, 1970) has allowed for considerable progress to be made in this field. We will describe in this paper the characteristic features of the input–output relations across this synaptic relay. They deal with the postsynaptic events associated with: (1) a single presynaptic impulse; (2) a volley of impulses corresponding to the stimulation of a single cercal mechanoreceptor; (3) the asynchronous (mechanical stimulation) or synchronous (electrical stimulation) activity of a variable number of presynaptic fibres.

These results will be analysed and discussed in terms of integrative properties of the synaptic junctions.

A preliminary account of this work has already appeared (Callec, *et al.* 1969).

MATERIALS AND METHODS

(1) *Anatomy*

The sensory input to the 6th abdominal ganglion consists in volleys of nerve impulses generated essentially at the level of the cercal mechanoreceptors and conducted by the cercal nerves X and XI.

Although a complete mapping of all the homolateral or contralateral receptors which are connected to a given giant axon has not yet been made, it has been demonstrated that the number of these receptors is rather high (we have for example shown a relation between a dozen receptors and the median ventral giant axon).

These receptors are long (up to 700 μm) and thin (6 μm) sensilla with (most often) one single nerve cell. The axonal processes of these cells constitute the sensory cercal nerves. The firing pattern of these receptors can be classified in two classes corresponding to morphologically different receptors.

(*a*) *Phasic-tonic patterns associated with long and flexible hairs*

The longer ones (700 μm) give phasic-tonic patterns which are predominantly tonic; they will be termed 'tonic patterns' in this paper. This first category is extremely sensitive to air movements (the response rises 40% of maximum when the hair describes only a two degree arc along the preferential plane, Nicklauss, 1965. The others (less than 350 μm) adapt more or less rapidly to the stimulation and give responses which will be termed 'phasic-tonic patterns'.

(*b*) *Phasic patterns associated with the shorter hairs*

These responses are characterized by a short burst of a few spikes to each stimulation. The maximum frequency which can be reached during the phasic component of these different responses is of about 360 spikes/s.

The output from the 6th abdominal ganglion is carried by different kinds of interneurones. Some of them, the giant axons, are relatively large in diameter. These giant internuncials are distributed within each connective in two groups: a ventral consisting of three larger giant axons (up to 40–45 μm in diameter), and a dorsal group consisting of five to seven smaller axons (20–25 μm in diameter) (Roeder, 1948).

It appears from electromicroscopical studies (Farley & Milburn, 1969) that in the 6th ganglion each giant fibre bears numerous ramifications analogous to a dendritic

ee on which most of the afferent cercal fibres seem to make synaptic contacts. These features are similar to those found in other invertebrates as in the crayfish (Kennedy, Selverston & Remler, 1969), in the crab (Sandeman, 1969) and in the leech (Stuart, 1970; Nicholls & Purves, 1970).

The cell bodies of these giant axons are located at the periphery of the 6th ganglion. According to Farley & Milburn (1969) one cluster of lateroventral somata are related to each fibre. Preliminary intracellular microelectrode recordings have shown that cell bodies in this region cannot give rise to full-sized action potentials, and it therefore seems that they cannot directly affect the electrical activity of the giant axons.

(2) *Stimulation*

The stimulation of the cercal mechanoreceptors can be achieved in different ways. Most often some of them seem to be 'spontaneously' active, thus giving a ground activity which can be recorded on the cercal nerve. This activity results in fact from the stimulation of some of the receptors which are bent by Ringer or dust; it can be at least provisionally suppressed by a careful drying and cleaning of the cerci (Fig. 2).

Each receptor can be stimulated separately using a small glass rod held by manipulators. Several receptors can be activated more or less simultaneously by puffs of air of various strengths and durations, and the synchronous activity of the cercal fibres can be achieved by a direct electrical stimulation of the cercal nerves.

(3) *Recording*

The arrangement diagrammatically shown in Fig. 1 allows simultaneous recordings of the presynaptic and of the postsynaptic activity. We can thus follow with a good accuracy and at the unitary level the different patterns of information arising from a well-defined receptor until its integration at the first synaptic relay.

Presynaptic activity conducted in the cercal nerves is recorded between two baths of saline separated by a vaseline seal, care being taken to make this seal as light as possible to avoid any conduction block in the superficial fibres due to the compression. Unit spikes are of small amplitude (0·2–1 mV) but can usually be distinguished from the 'noise'. It is possible to get greater potentials by using a microelectrode placed at the base of a given receptor, but this technique does not generally permit a long-duration recording and damages the receptor. Thus, the former method has been preferred except for the records of Fig. 6.

Postsynaptic events have been recorded using the previously described 'oil-gap' technique (Pichon & Callec, 1970). This technique provides some advantages: a low impedance preparation (1–5 MΩ) and thus a low noise level, a very good stability whenever the preparation is stimulated mechanically, and the unequivocal identification of the postsynaptic element at the level of which the recordings are made. Most of the experiments described here have been made on the median ventral giant axon, few of them on the lateral ventral giants. The ganglion is continuously irrigated with an oxygenated physiological saline (composition: Na^+ 214 mM, K^+ 3·1 mM, Ca^{2+} 1·8 mM, $H_2PO_4^-$ 0·2 mM, $H\text{-}PO_4^{2-}$ 4·8 mM, in which the synaptic transmission could be maintained unimpaired during several hours. In the last experiments the Ca^{2+} concentration has been raised to 5·4 mM to provide a better stabilization of the fibre membrane and to enhance slightly the synaptic potentials.

(4) *Experimental procedure*

The preparation consists of the cerci, the cercal nerves and the abdominal ganglion. The single giant axon is dissected over 1–2 mm between the 5th and the 6th ganglia as close as possible from the 6th ganglion using the previously described technique (Pichon & Boistel, 1966; Pichon & Callec, 1970). It is then transferred to the recording chamber shown in Fig. 1. The cerci are then carefully cleaned and dried until all 'spontaneous' activity in the cercal nerves has completely disappeared. The level of

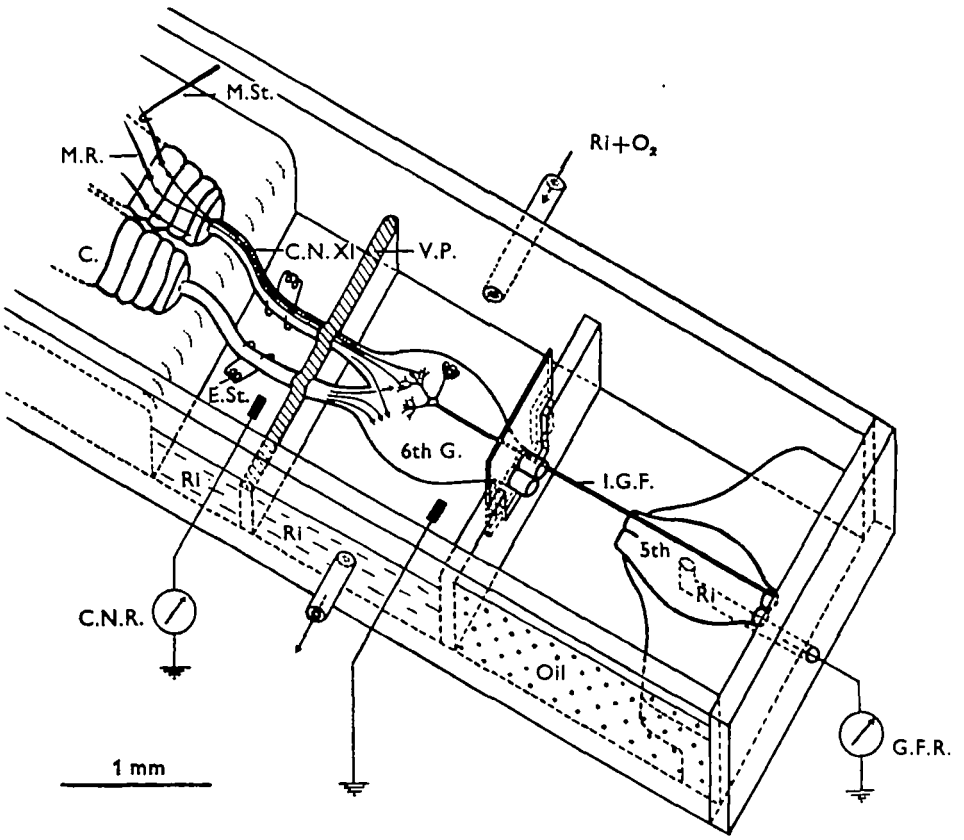

Fig. 1. Semischematic representation of the experimental arrangement. M.R., mechanoreceptor; C., cercus; C.N. XI, cercal nerve XI; 6th G., sixth abdominal ganglion; I.G.F., isolated giant fibre; 5th, fifth abdominal ganglion; $Ri+O_2$, oxygenated Ringer; M.St., mechanical stimulation; E.St., electrical stimulation; C.N.R., cercal nerve recording; G.F.R., giant fibre recording. Scale is only given as an indication for the region of the 6th abdominal ganglion. The cercal nerve X is not shown. Dashed area = Ringer; dotted area = mineral oil.

polarization of the postsynaptic fibre membrane is continuously monitored by a pen recorder. The mechanical stimulation and the lack of oxygenation during the dissection lead to a more or less important depolarization of the postsynaptic membrane. After the irrigation is started the preparation is left unstimulated during a variable period of time to allow the normal polarization to be reached again. The duration of the repolarization is variable (5–15 min.) depending essentially upon the time between

e beginning of the dissection and the beginning of the irrigation. It is only after the postsynaptic membrane has reached a stable level that one begins to look for receptors related to the dissected axon, this being done using a fine glass rod mounted on a manipulator.

RESULTS

(1) *'Spontaneous' postsynaptic activity: EPSPs and IPSPs*

When the preparation is placed in the recording chamber, one can usually record an important electrical activity as shown in Fig. 2A. This activity which consists essentially in so-called 'spontaneous' excitatory postsynaptic potentials (EPSPs) can be, as already mentioned, related to the mechanical stimulation of the cercal receptors. Usually it is suppressed by a careful drying of the cerci. In these conditions one can also record, in most cases, small hyperpolarizing waves which will be referred to as inhibitory postsynaptic potentials (IPSPs).

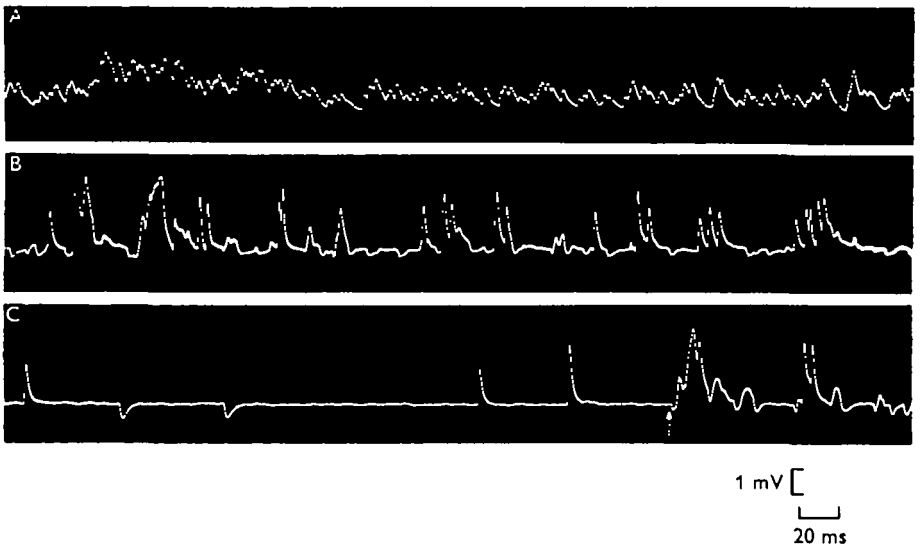

Fig. 2. 'Spontaneous' postsynaptic potentials in a giant axon. (Unitary EPSPs and IPSPs.) A careful drying of the cerci (from A–C) induces a clear decrease in the 'postsynaptic activity'. $[Ca^{2+}]$ = 5·4 mM. A, large activity at the beginning of the experiment. It corresponds to the firing of numerous mechanoreceptors bent by a thin layer of physiological saline. B, lower activity following a moderate drying of the cerci. Some receptors have ceased to fire. IPSPs become more clear. C, after a careful drying only a few receptors (2 or 3) are still in activity and we can see clearly isolated EPSPs together with IPSPs. The arrow indicates a small activity induced by a slight puff of air.

(*a*) *EPSPs.* The mean amplitude of these spontaneous EPSPs varies between 0·15 and 0·9 mV with a mean value of 0·5 mV (in Ringer 1·8 mM-Ca^{2+}). A histogram of the amplitudes of these potentials does not show any characteristic peak as shown on Fig. 3A. The time to peak can vary between 1·7 and 3·4 ms, but presents a sharp peak for 2·0 ms (Fig. 3B). The time necessary for these EPSPs to decrease by $\frac{2}{3}$ of their peak value ranges between 3·9 to 5·3 ms.

(*b*) *IPSPs.* The IPSPs (Fig. 4), can be observed only in special conditions, when

the excitatory activity is reduced or suppressed (Fig. 2) and when the polarization of the postsynaptic element remains below a certain level. Experiments have shown, therefore, that the polarization of the postsynaptic element can slightly increase after 2 or 3 h of experimentation. As the reversal of these IPSPs can be obtained for hyperpolarizations of about +5 mV from the 'normal' resting level, one can understand why these potentials have not been observed in our previous experiments, in which the time for dissection and mounting was longer than an hour and a half.

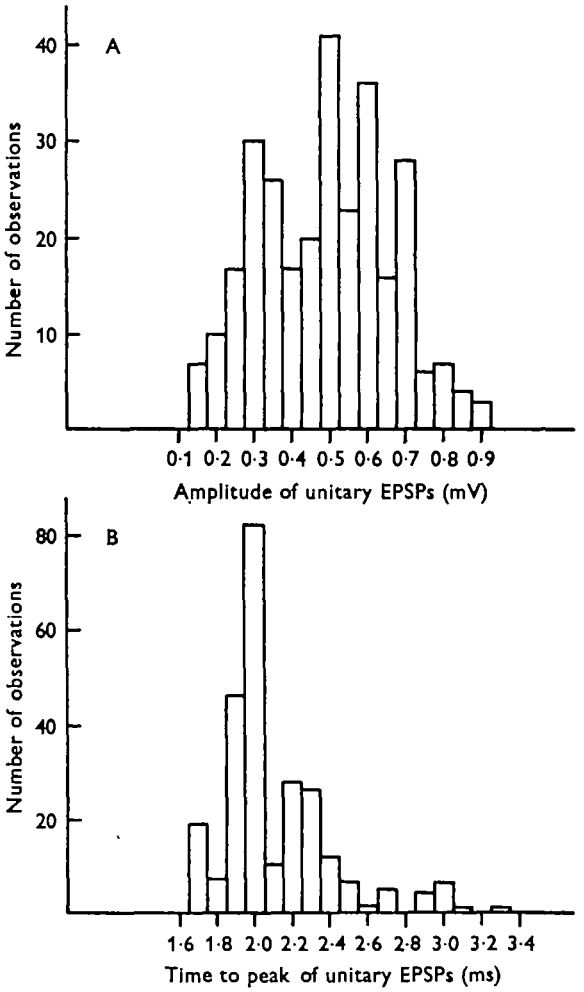

Fig. 3. Histogram of the amplitude (A) and the time to peak (B) of mixed unitary EPSPs (medial ventral giant axon). $[Ca^{2+}] = 1{\cdot}8$ mM.

The mean amplitude of these IPSPs varies generally between 40 and 320 μV (in Ringer 1·8 mM Ca^{2+}). The histogram of the amplitudes of these IPSPs shows two peaks in Fig. 5. Their shape is essentially similar to that of the EPSPs, but they are generally slower (time to peak ranging from 3·3 to 5·0 ms; total duration ranging from 10 to 15 ms). Their frequency remains low (4–20 s). These potentials still occur in completely de-afferented preparations showing that they must be related to an in-

aganglionic presynaptic activity. One does not know whether this activity originates in axonal regions or in cell bodies. We have reported earlier (Callec & Boistel, 1966*a*) that some cell bodies located in the mediodorsal part of the ganglion may fire spontaneously, and it does not seem impossible that this spike activity might be the origin of at least some of the 'spontaneous' IPSPs. These intraganglionary IPSPs cannot be considered as sensory information despite their evident role in modulating the excitatory postsynaptic responses. They will be studied in more detail in another paper.

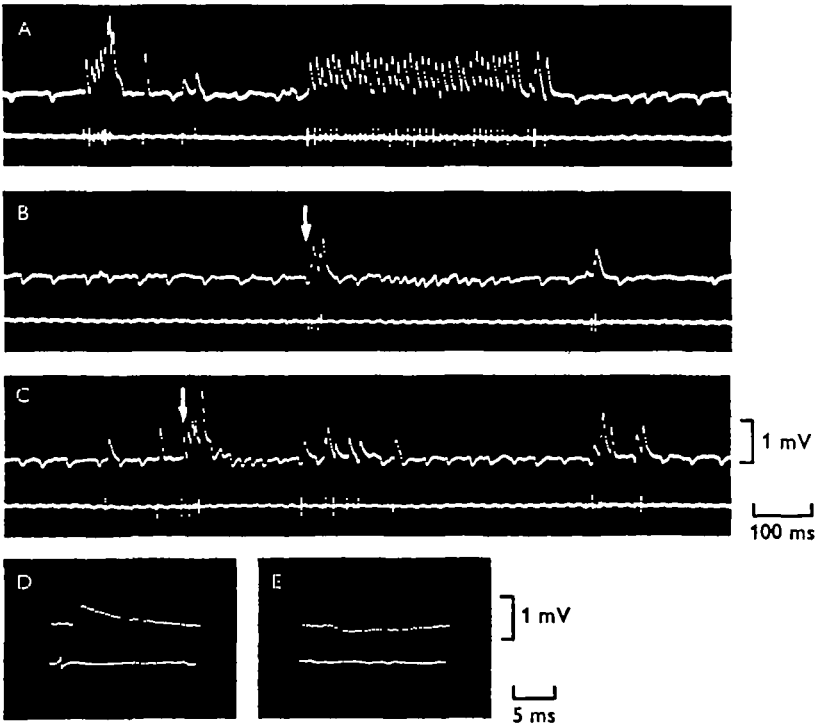

Fig. 4. A–C, postsynaptic activity in a medial ventral giant axon when the cerci have been carefully dried. Bursts of EPSPs are generated by slight puffs of air applied to the cerci. In A the long sequence of EPSPs is likely to belong to the same mechanoreceptor. Numerous 'spontaneous' IPSPs sometimes occurring in bursts do not correspond to spikes on the afferent nerve. However, bursts of IPSPs sometimes follow bursts of EPSPs (arrows). D and E. Higher sweep-speed recording of a unitary EPSP (D) and a unitary IPSP (E). Note that in D the EPSP is clearly related to a presynaptic spike. Upper trace, postsynaptic potentials; lower trace, external presynaptic activity recorded from the cercal nerve.

(2) *Relations between a single presynaptic spike and one EPSP*

Simultaneous recording of presynaptic and postsynaptic activity have shown that, in most cases, each spike at the receptor triggers an EPSP (Fig. 6).

The amplitude of the EPSPs corresponding to various receptors might be different (Fig. 7). Furthermore, the amplitude of the EPSPs corresponding to a single receptor might vary by a factor of three, whereas the time to peak remains relatively constant (about 2 ms) (Fig. 8).

The delay between the presynaptic spike recorded where it enters the ganglion and

the rising phase of the corresponding EPSP is of about 0·85 ms (mean for five preparations). This would correspond after correction for the conduction time in the presynaptic element (about 350 μm at 2 ms, that is 0·17 m/s) to a mean synaptic delay of 0·68 ms (Fig. 7).

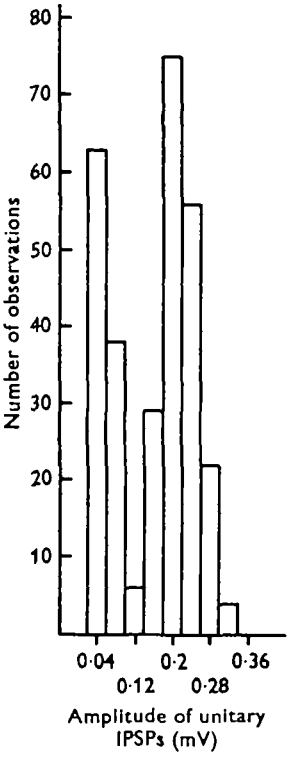

Fig. 5

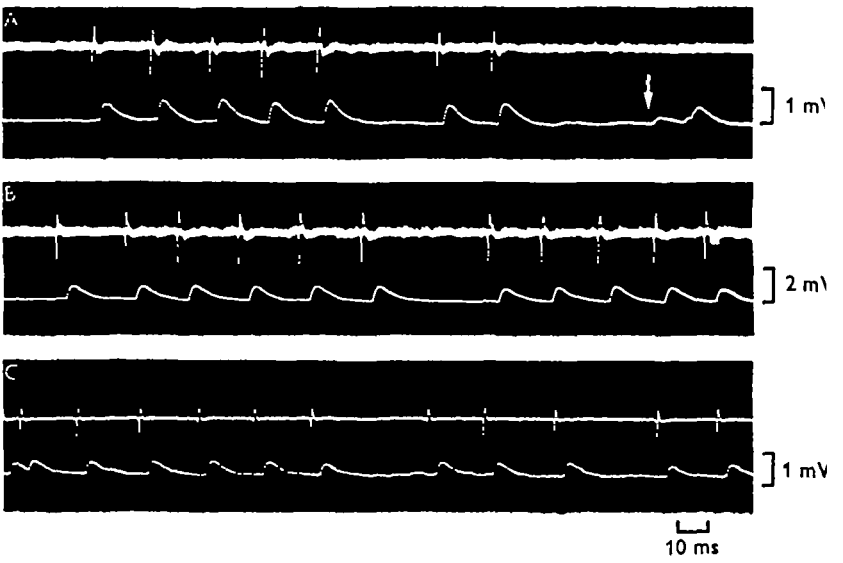

Fig. 6

Fig. 5. Histogram of amplitudes of unitary IPSPs in the medial ventral giant axon of the cockroach. $[Ca^{2+}] = 1{\cdot}8$ mM.

Fig. 6. Simultaneous recording of unitary afferent spikes (upper trace) and the corresponding EPSPs. These records are taken from three different preparations but they concern the same giant fibre; one afferent spike triggers one EPSP. Note that in each of these three sequences, the frequency being relatively low, the amplitude of EPSPs remains nearly constant (recordings have been taken during the steady state, see Fig. 20). The arrow in A shows a complex EPSP corresponding to another afference. In this figure the large presynaptic spikes have been recorded at the receptor level by a microelectrode.

(3) *Postsynaptic events associated with the mechanical stimulation of a single cercal receptor*

The preceding recordings have usually been obtained with receptors firing at a rather low frequency so that each spike could be considered as isolated. This is far from being general, and one knows that the frequency of the spikes initiated in one cercal mechanoreceptor can reach 360/s. It was thus important in a second step to analyse the evolution of the postsynaptic events when the unitary presynaptic input consisted in bursts of spikes.

These postsynaptic responses, associated with the different patterns of presynaptic activity, differ somewhat according to the simulated receptor.

(*a*) *Response following a phasic-tonic pattern* (*Fig.* 9)

The time course of the postsynaptic response approximately follows the changes in the frequency of the afferent volley. The level of the induced postsynaptic depolarization increases with increasing frequency. Nevertheless, the amplitude of the unitary EPSPs decreases with increasing depolarization; this is a limiting factor so that the maximum depolarization which could be obtained following natural stimulation of

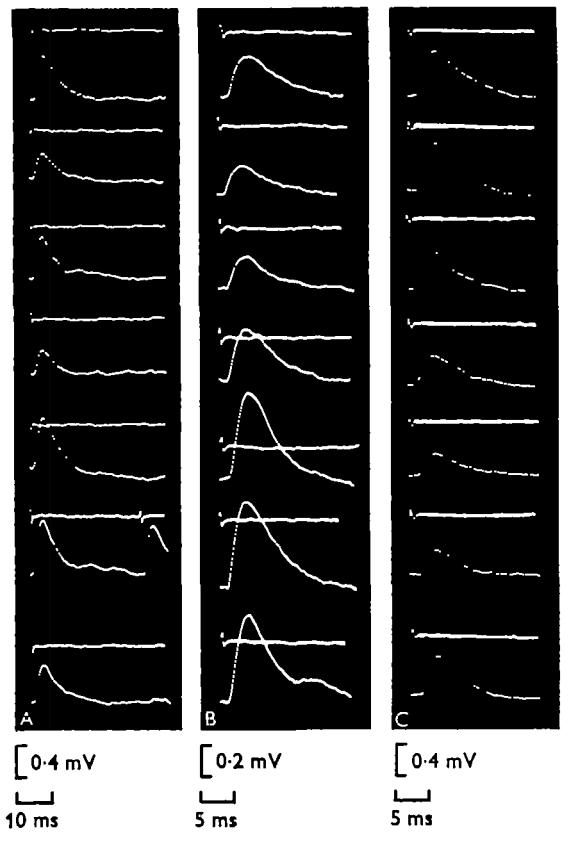

Fig. 7

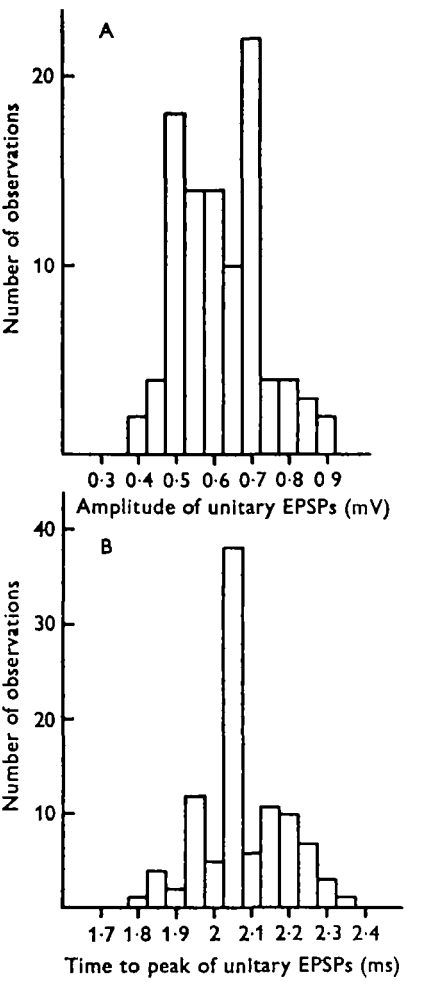

Fig. 8

Fig. 7. Variations in amplitude of a given unitary EPSP. Three examples (A, B, C) are taken from three different preparations. The mechanoreceptors are stimulated at a low frequency to avoid any fatigue. The presynaptic spike (upper trace) is used to trigger the sweep. The vertical scale refers to the postsynaptic potentials.

Fig. 8. Histogram of the amplitude (A) and the time to peak (B) of unitary EPSPs corresponding to the activity of a single receptor. (Medial ventral giant axon.) $[Ca^{2+}] = 1{\cdot}8$ mM.

these receptors (corresponding to a presynaptic frequency of 300/s in this example) is not sufficient to trigger a postsynaptic spike. Parameters, such as potentiation or depression, have little or no effect; for a given level of polarization the amplitude of unitary EPSPs remains nearly constant. When the frequency undergoes a rapid and brief variation, we observe a subsequent increase in the depolarization.

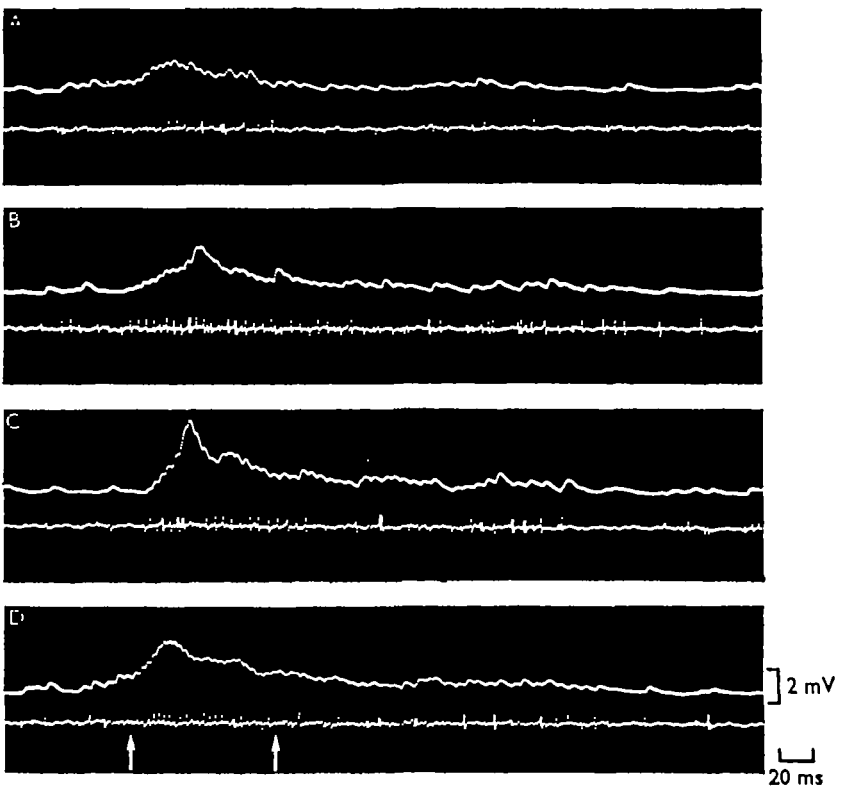

Fig. 9. Postsynaptic activity (upper trace) following the stimulation of a phasic-tonic receptor. The time course of the depolarization brought about by the summation of successive EPSPs apparently follows variations in frequency of firing of the receptors. This is particularly striking in examples labelled by a star; small rapid bursts give rise to obvious increases in the depolarization. In D a nearly constant frequency occurring at the beginning of the sequence (between arrows) clearly shows a summation phenomenon. The resulting depolarization, however, is limited by a decrease in the amplitude of unitary EPSPs.

(*b*) *Response following a 'tonic' pattern* (*Fig.* 10)

As far as we know, purely tonic receptors (that is, receptors whose frequency of firing remains constant throughout the stimulation) are only sparsely distributed. Almost all the receptors we have used for this study were in fact phasic-tonic but showed a very slow adaptation to the stimulus, so that for short times the frequency of their firing could be considered as constant during the tonic component.

The time course of the postsynaptic response following this kind of afferent volley begins by a rapid depolarization followed by a progressive repolarization whenever the frequency remains constant (this is particularly obvious in Fig. 10B, where the sequence has no initial phasic component).

The amplitude of the unitary EPSPs decreases during the first phase and then remains smaller and nearly constant, during the whole of the tonic phase.

If the stimulation is temporarily stopped at the end of the depolarizing phase (Fig. 10D), we can observe a slight and slow hyperpolarization. It is also present in the other records (Fig. 10A–C), but in part masked by the background activity. These events can be related to some kind of fatigue or depression. This phenomenon is

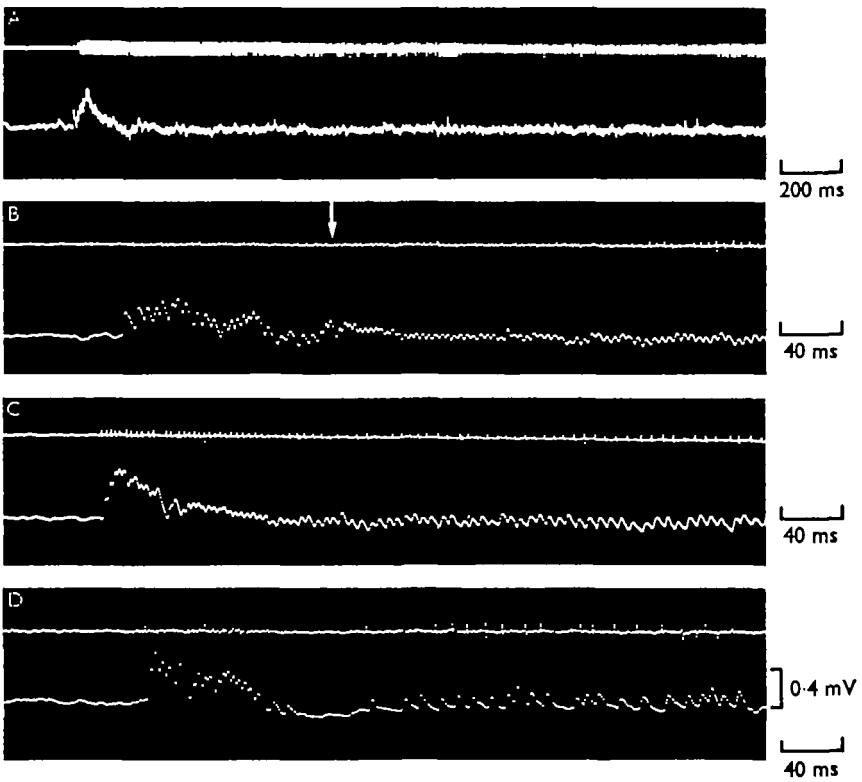

Fig. 10. Postsynaptic responses to the stimulation of a so-called tonic receptor. The same receptor has been stimulated in the four sequences (A–D). One can see a summation phenomenon in the early phase followed by a fairly rapid repolarization. This effect is particularly striking in sequence B where an increase in the frequency fails to enhance significantly the depolarization (arrow). Note: in D the occurrence of a clear hyperpolarizating phase following the first burst of presynaptic spikes. This hyperpolarization is less obvious in the other records (A–C) where the stimulation is maintained. Upper trace, presynaptic spikes; lower trace, postsynaptic activity.

particularly striking at the beginning of each sequence and also during the tonic component of Fig. 10B, where an increase in the frequency is no longer followed by a larger depolarization.

(*c*) *Response following a phasic pattern* (*Fig.* 11)

The mechanical stimulation of the receptors of this category gives rise to short volleys of one to a dozen of spikes. The frequency within one volley is relatively high (between 180 and 300/s). As for the other receptors, this frequency is high enough to

induce summation phenomena as can be seen in Fig. 11. This summation is often accompanied by some potentiation or depression, depending upon the interval between the presynaptic spikes (Fig. 12). When this interval ranges between 3 and 8 ms, one can observe an increase of the second EPSP which could be over 20%; for intervals reaching 10–12 ms the phenomenon is inverted and the second EPSP is smaller than the first (10% decrease after about 32 ms). This depression lasts up to 50 ms.

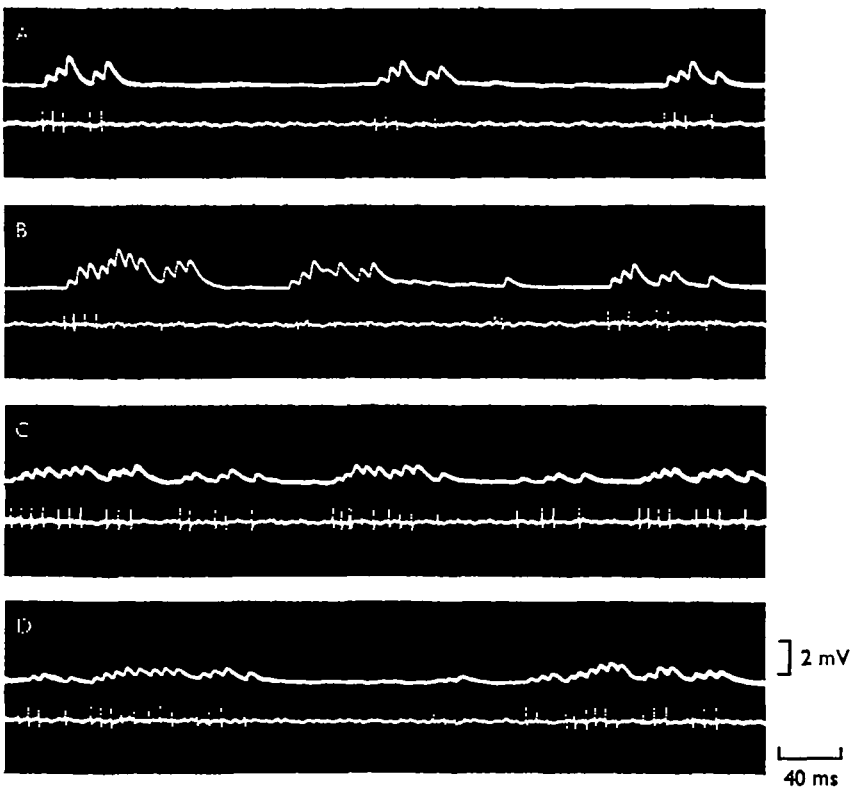

Fig. 11. Postsynaptic responses following the stimulation of the same phasic receptor by brief repeated movements. A–D, are selected in a total sequence of 6 s (see Fig. 13). Note that potentiation is visible in each burst whenever fatigue, which appears progressively, affects the amplitude of each EPSP. Upper recordings, postsynaptic events; lower recordings, presynaptic activity.

Repeated stimulation of this same receptor results in a reduction of the size of the unitary EPSPs as illustrated in Fig. 13. This can safely be referred to EPSP-habituation at the cellular level as defined by Bruner & Tauc (1966).

The restoration of the full amplitude is complete after a rest of 1–2 min.

(4) *Effect on postsynaptic activity of the stimulation of an increased number of presynaptic fibres*

We have seen in the preceding sections how a single postsynaptic giant axon is affected by afferent activity coming from different mechanoreceptors stimulated isolately and how complex the response can be. In this section we shall study the

fect of asynchronous (mechanical) or synchronous (electrical) stimulation of several presynaptic fibres (which correspond to the integrative properties of the giant axons).

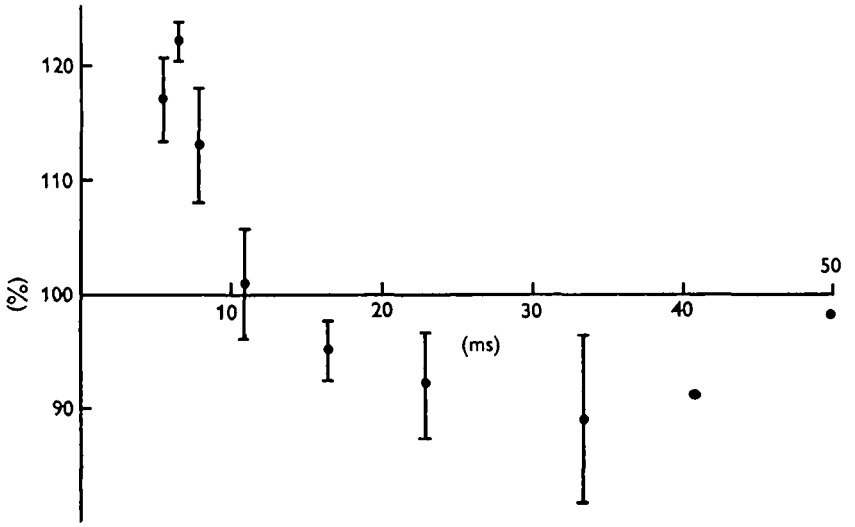

Fig. 12. Potentiation and depression following a phasic pattern of presynaptic activity (partly illustrated in Fig. 11). The size of the second unitary EPSP relative to that of the first of each sequence (taken as 100%) is plotted against the interval between the two. All points except the last two are the means ± S.E. for a least 30 measurements.

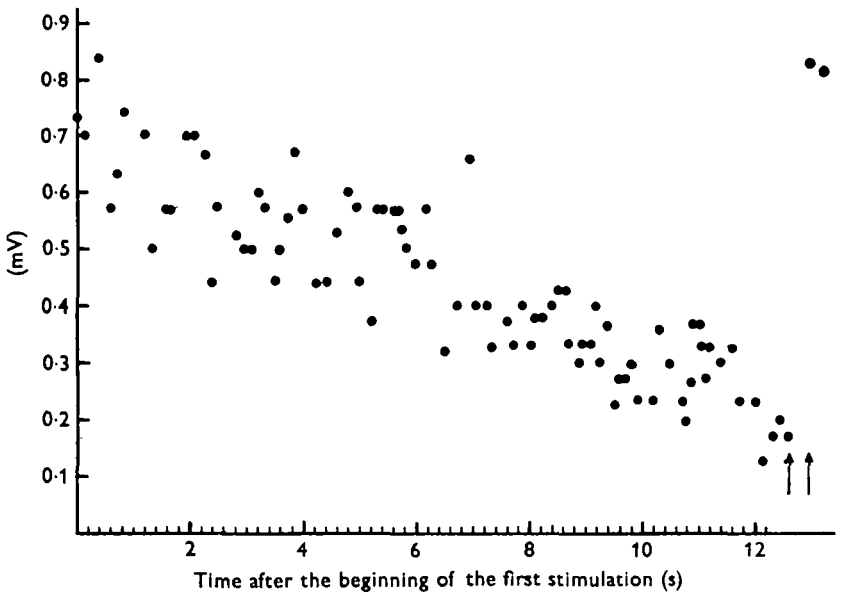

Fig. 13. Fatigue phenomenon following repetitive mechanical stimulation of a phasic mechanoreceptor (partly illustrated in Fig. 11). The variations in the size of the first unitary EPSP in each burst are plotted against time. The two arrows indicate an interval of 3 min which makes possible a complete restoration.

(a) Effects of mechanical stimulation of the cerci

The effect of stimulation of the cerci by puffs of air is illustrated in Fig. 14. For a slight stimulation (A) there is only a small infraliminal depolarization corresponding to the summation of EPSPs related to a very small number of receptors. In B a rather strong puff of air gives rise to a complex response; there is an early rapid summation which nearly reaches the threshold followed by a second step which is sufficient to trigger a propagated action potential. With a longer and stronger puff the depolarization is strong enough to induce several action potentials (6 in C).

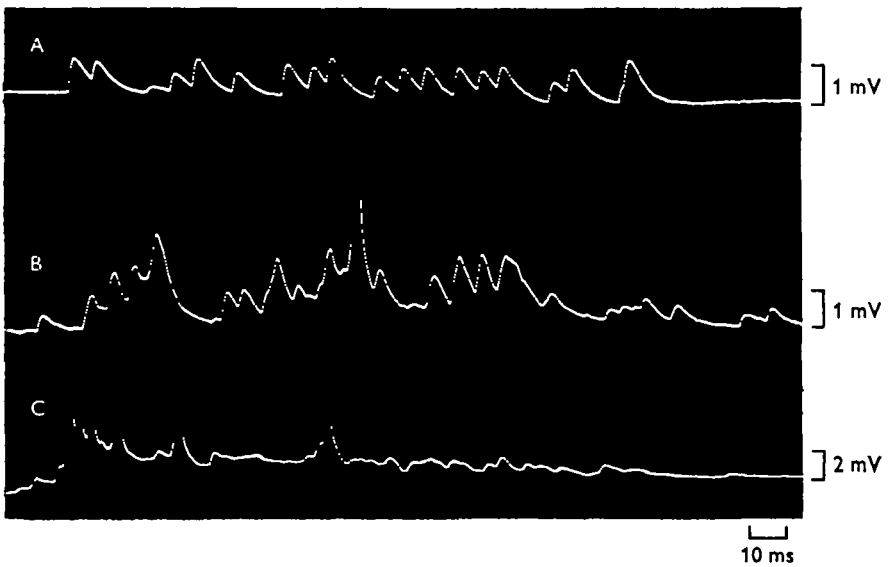

Fig. 14. A–C. Postsynaptic responses induced by increasing mechanical stimulation (puffs of air) on the cerci. In A a very small number of receptors are stimulated. In B a larger number of receptors are active and the threshold is reached, giving rise to a full-sized postsynaptic spike. In C a long and strong puff induces several postsynaptic action potentials. The spikes are truncated.

When these puffs of relatively constant intensity are repeated at regular intervals (2/s) one can see (Fig. 15A) a progressive but rapid decrease in the size of the postsynaptic response. This reduction can be related to the already mentioned habituation phenomenon to natural stimulation. The response always returns to its full amplitude after a rest of only a few minutes (Fig. 15B). It has never been possible to alter this phenomenon, for instance by the interpolation of an extra stimulus applied to the homolateral or contralateral cercal nerve (Pumphrey & Rawdon Smith, 1937).

Sometimes, as for the stimulation of a single tonic receptor (cf. Fig. 10), the response which consists essentially in a depolarization is followed by a long-duration hyperpolarization (Fig. 16).

(b) Effects of electrical stimulation of the cercal nerves

The postsynaptic response to an electrical stimulation of the cercal nerves is complex, and different arguments which will be discussed later have led us to divide our

scription into monosynaptic and polysynaptic phenomena. Furthermore, the frequency of stimulation has been revealed to be critical and we will describe the effects of this parameter in a separate paragraph.

Monosynaptic phenomena. When the electrical stimulation of the homolateral cercal nerve is increased, one can see a very smooth increase in the amplitude of the EPSP

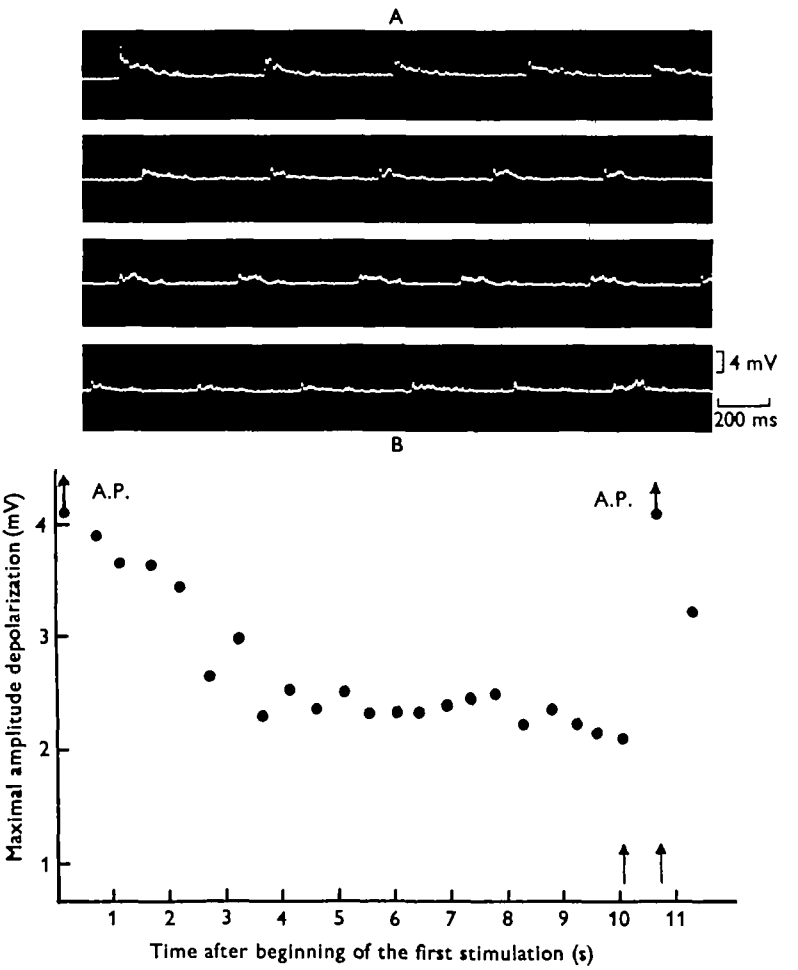

Fig. 15. Habituation at the cellular level following strong repetitive stimulation of the homolateral cercus. A, successive postsynaptic responses following nearly constant stimulation by puffs of air. Note that only the first volley gives a propagated spike. B, curve obtained from the upper records. The two arrows correspond to an interval of 5 min during which the preparation was left unstimulated and which makes possible full restoration. A.P., action potential.

(Fig. 17). When the postsynaptic depolarization is larger than 3–10 mV (the threshold is different in different preparations) it gives rise to a full-size (100–120 mV) action potential.

The EPSPs are often preceded by a small potential which increases with the intensity of the stimulation. It is likely to correspond to an electrotonic recording of the cercal

(presynaptic) potential. This seems to be the only electrotonic coupling between th afferent cercal nerves and the giant interneurone.

The synaptic delay can be measured with relatively good accuracy between this electrotonic presynaptic potential and the EPSP. In this example it ranges between

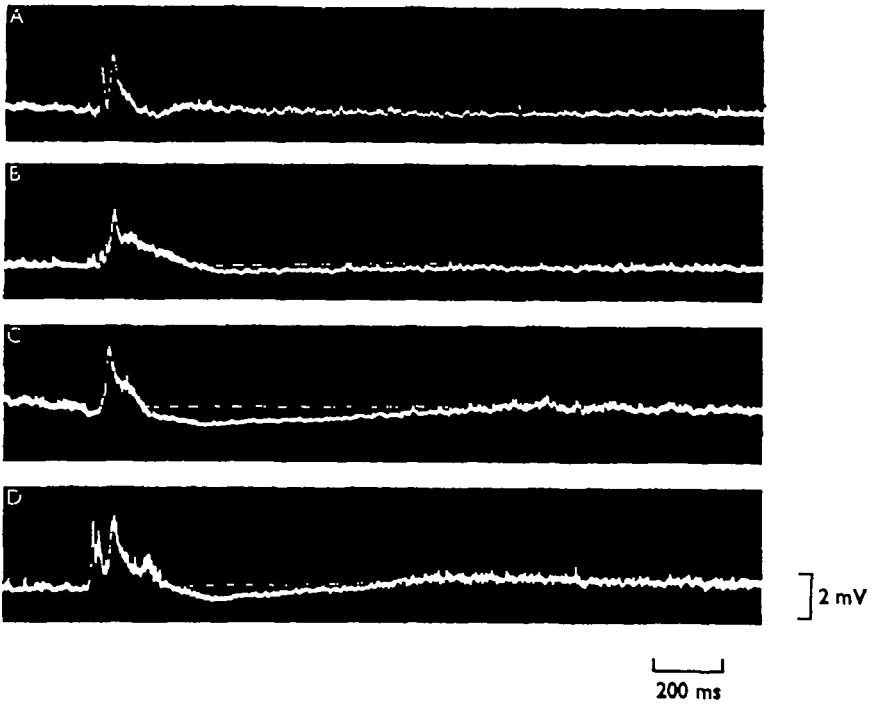

Fig. 16. A–D, long-duration postsynaptic after-hyperpolarizations (which sometimes follows the depolarizing wave) induced by a mechanical stimulation (puff of air) of the cerci. The four recordings have been made from the same preparation. The dashed line shows the resting level.

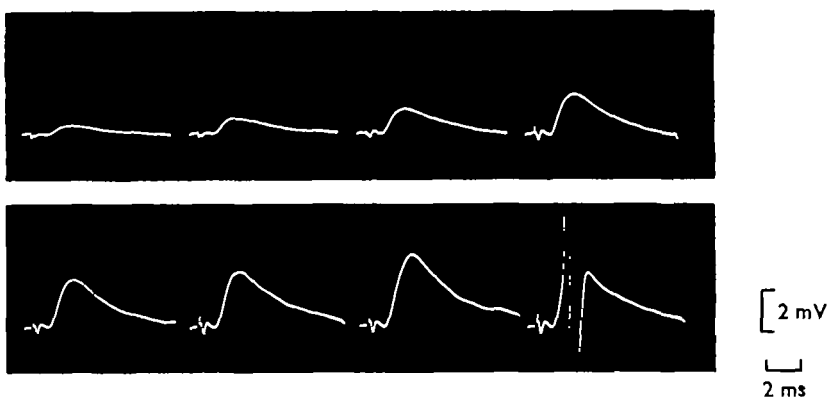

Fig. 17. Evolution in the postsynaptic response following increasing electrical stimulation of the homolateral cercal nerve XI. The EPSP increases smoothly with the intensity of the stimulation until it reaches the threshold and gives rise to a spike. It is preceded by a small diphasic potential which also increases with the intensity of stimulation and is likely to be a presynaptic potential recorded electrotonically.

83 and 0·91 ms. Whenever in a few other experiments it has been possible to note greater delays (up to 1·73 ms) and some variations, we think that the delay is short enough and nearly constant to argue for a monosynaptic relay.

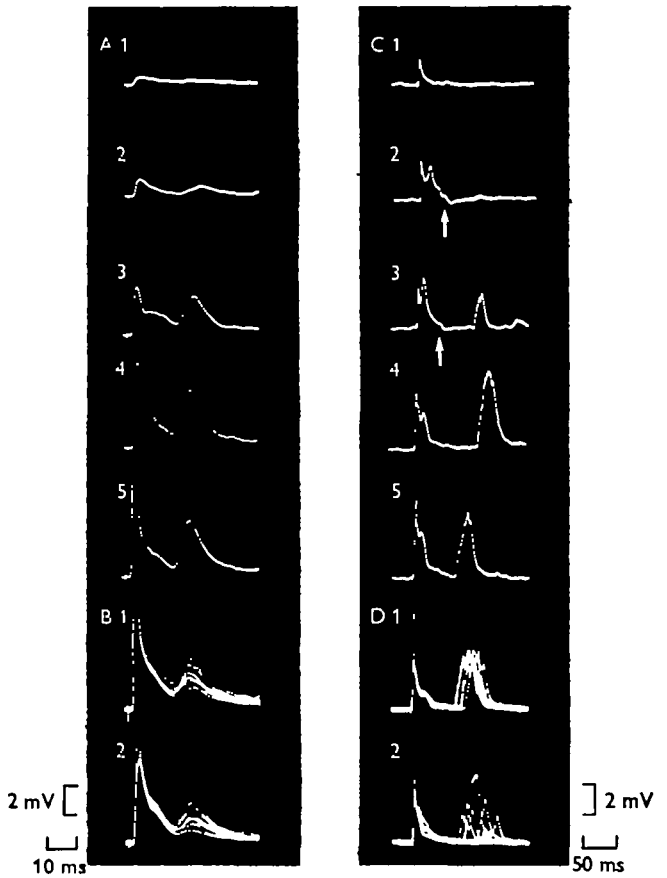

Fig. 18. Postsynaptic events induced by the electrical stimulation of the cercal nerve XI. A, progressive increase of stimulation. Note gradually increasing EPSPs. The first is monosynaptic and gives rise (in *A*5) to a conducted spike; the second, which appears after a delay of about 20 ms, is likely to be polysynaptic. On some occasions it can also induce a spike (see Fig. 19 C 2). B, effects of repetitive stimulation on this complex EPSP, at a frequency of 5/s in B 1. The first EPSP and spike remain relatively constant, while the second phase is more labile. For greater frequency (10/s in B 2) the first EPSP also shows some fatigue. C, recordings at a lower sweep speed show the occurrence of a third delayed (polysynaptic) wave (delay between 60 and 100 ms). Its amplitude is related to the frequency rather than to the intensity of stimulation. D, stimulation at a very low frequency (1/10 s) modifies only the delay (D 1). Higher frequency (5/s in D 2) quickly reduces the size and then abolishes completely this phase. Note also (arrows) a small IPSP which seems to be related to the stimulation.

Stimulation of the contralateral cercal nerve also gives rise to an EPSP, which means that the cercal fibres are also connected contralaterally; but it is not possible to trigger a postsynaptic action potential. However, the increase of the EPSP with the progressive increase in presynaptic stimulation was smooth enough to show that the number of synaptic contacts with the contralateral giant fibre is rather important.

Polysynaptic phenomena (*Figs.* 18, 19). In good conditions (for instance after a long period of rest), the electrical stimulation of the homolateral cercal nerve gives rise to a complex response which in Fig. 18C consists essentially in three depolarizing waves or EPSPs.

The first is the already described EPSP which is thought to be monosynaptic. The second, whose delay is about 20 ms (Fig. 18A), increases with increasing stimulation and can give rise (but very rarely) to a conducted postsynaptic spike (Fig. 19C2). The third EPSP, well separated from the others (60–100 ms, Fig. 18C), has a slightly higher

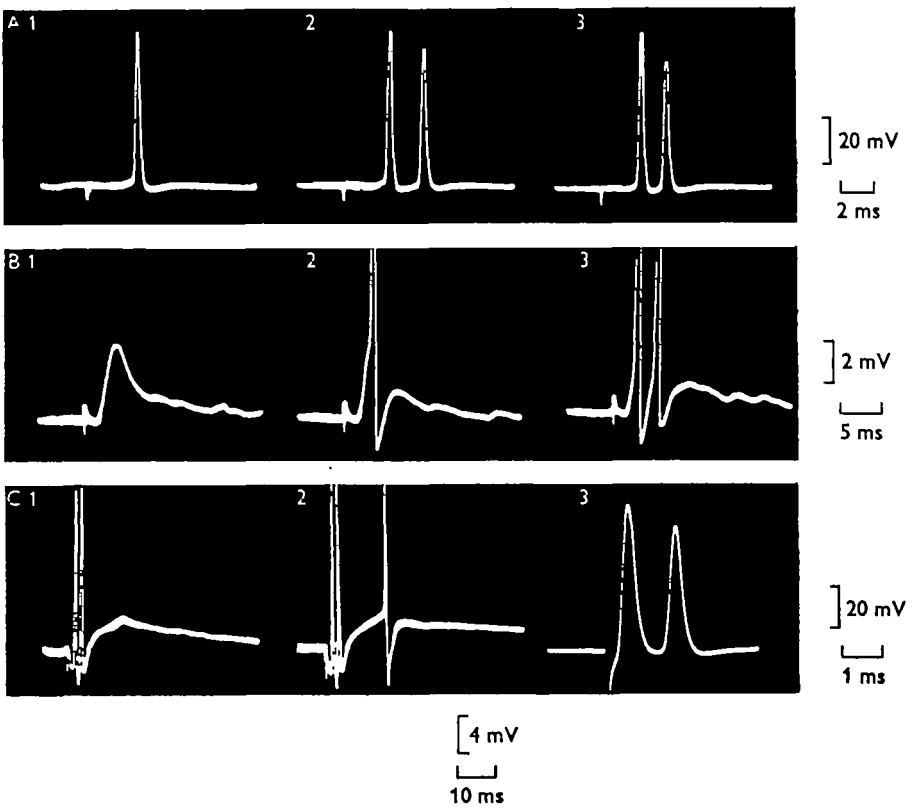

Fig. 19. Externally recorded postsynaptic potentials following electrical stimulation of the homolateral nerve XI. A, a suprathreshold stimulation gives rise to one or two action potentials, the second being smaller. The delay after the stimulation artifact decreases with an increased stimulation. B, postsynaptic potentials showing at a higher amplification the EPSP alone (B1) and spikes superimposed on it (B2–3). The second spike which corresponds to an increased stimulation seems to be generated by the same EPSP as the first one. C, the second EPSP which may occur (Fig. 18) gives rise to a third action potential (C2). In some experiments (C3) a stronger stimulation is able to give rise to an undelayed action potential due to direct stimulation of the postsynaptic element.

threshold. Its amplitude is related rather to the frequency than to the intensity of stimulation. The study of these two last EPSPs shows that their delay, amplitude and time course are essentially variable. This variability, together with the high value of delay, argue for their multineuronic origin.

The delay of the second EPSP could be explained by slower conduction speed along

me cercal fibres. It seems, however, that this alternative explanation is unlikely because of the relative homogeneity of the diameters of these fibres. In addition to these three kinds of EPSPs other intermediate or later depolarizations sometimes occur, but with much less regularity.

Effects of repetitive stimulation. To study the effects of repetitive stimulation with volleys of given frequencies three main precautions are taken: the stimulation is sub-threshold to avoid spikes, the ganglion is isolated from 'spontaneous' sensory inputs by

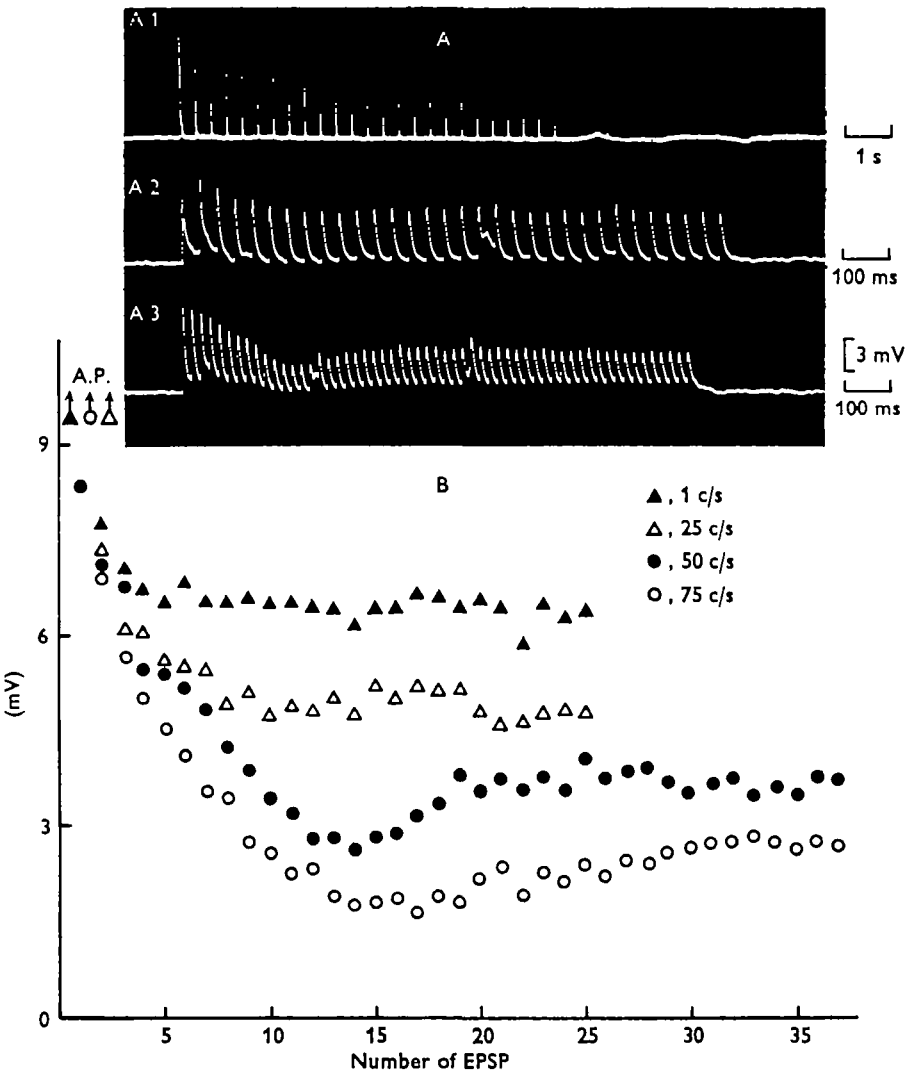

Fig. 20. Action of repetitive electrical stimulation at different frequencies on the monosynaptic EPSP. The first response is liminal or just sub-liminal. A, Typical recordings obtained for stimulation at a rate of 3/s (A 1) 25/s (A 2) and 50/s (A 3). The first EPSP in A 2 gives rise to a spike, the rapid phase of which is not shown. B, plot of the size of each individual EPSP against its position in the burst. Four different frequencies have been tested. The size of individual EPSPs decreases with time, even for low frequency (2/s) and then increases ('rebound' see text) after 10–20 stimulations if the frequency is higher than 25/s. Note that an action potential (A.P.) is then superimposed on the first EPSP of three series.

cutting the cerci and the rest interval between two volleys is maintained for 3–5 min suppress as far as possible interference with depression or potentiation.

We have already mentioned that repetitive simulation results in modification of the postsynaptic events. In Fig. 20 one can see the evolution of the first (monosynaptic) EPSP to repetitive stimulation at different frequencies. For frequencies less than about 25/s there is first a more or less fast decline of the size of the EPSP followed by a plateau. The higher the frequency, the faster is this decline and the lower is the plateau. This steady state is reached after about ten stimulations. For higher frequencies (more than 25/s) the amplitude of the first EPSPs falls rapidly, then subsequently increases before falling again very slowly to the steady state. This 'rebound' phenomenon will be discussed later.

The second and the third (polysynaptic) EPSPs are far more labile than the first. The second disappears after about 3 s at a rate of stimulation of 5/s whereas the first (monosynaptic) EPSP remains nearly unimpaired (Fig. 18B). In other experiments one can observe some variations in its delay during the repetitive stimulation. The third is still more labile and completely disappears after about five stimulations at a frequency of 5/s (Fig. 18D2). It remains nearly constant at a lower frequency (1/10 s in Fig. 18D1), whereas it shows great variations in its delay. If the preparation is unstimulated for a period of about 3–5 min, these polysynaptic EPSPs are completely restored.

Other observations may be mentioned here (Fig. 19). If one increases further the stimulation of the afferent cercal nerve, the slope of the monosynaptic EPSP becomes steeper. Thus the threshold is more rapidly reached and the delay of the postsynaptic spike decreases. Sometimes we observe a second action potential of smaller amplitude following the first one after about 1·7 ms (about the minimum refractory period at room temperature). Strong electrical stimulation can induce an action potential appearing with no delay, probably corresponding to an electrotonic stimulation of the postsynaptic element and called direct action potential (Fig. 19C3). The second spike on the monosynaptic EPSP and this kind of 'direct' action potential have already been described by Pichon & Boistel (1965) using capillary microelectrodes introduced in the connective between the 3rd and 4th ganglia.

DISCUSSION

Our recordings from a single giant axon of the postsynaptic phenomena associated with the activity of the cercal fibres in different experimental conditions give a particularly good insight in the integrative properties of the synaptic junctions between cercal fibres and giant axon. The possible connexions between a given postsynaptic giant axon and the main different inputs which seem to be involved can be synthesized as in Fig. 21. This diagram has been elaborated using our electrophysiological findings and the electron microscopical data of Farley & Milburn (1969). It will be used throughout the discussion in which we shall successively analyse the unitary postsynaptic activity and the mode of triggering one postsynaptic action potential, then the factors influencing synaptic transmission and finally the behavioural significance of the synaptic integration in this preparation.

(a) *Unitary postsynaptic activity: EPSPs and IPSPs*

'Spontaneous' EPSPs are in fact related to receptor activity. The careful analysis of the recordings has shown that each EPSP is preceded by a presynaptic spike. These unitary potentials are analogous to those described in the motoneurone of the cat (Kuno, 1964*a*; Burke, 1967), in the abdominal ganglion of *Aplysia* (Tauc, 1965), in the 6th ganglion of the crayfish (D. Kennedy, personal communication) and in insect neuropile (Callec & Boistel, 1966*a*), but one special feature of this preparation is that the afferences are controlled at the receptor level thus giving valuable indications of the precise relationship between a presynaptic spike and one EPSP.

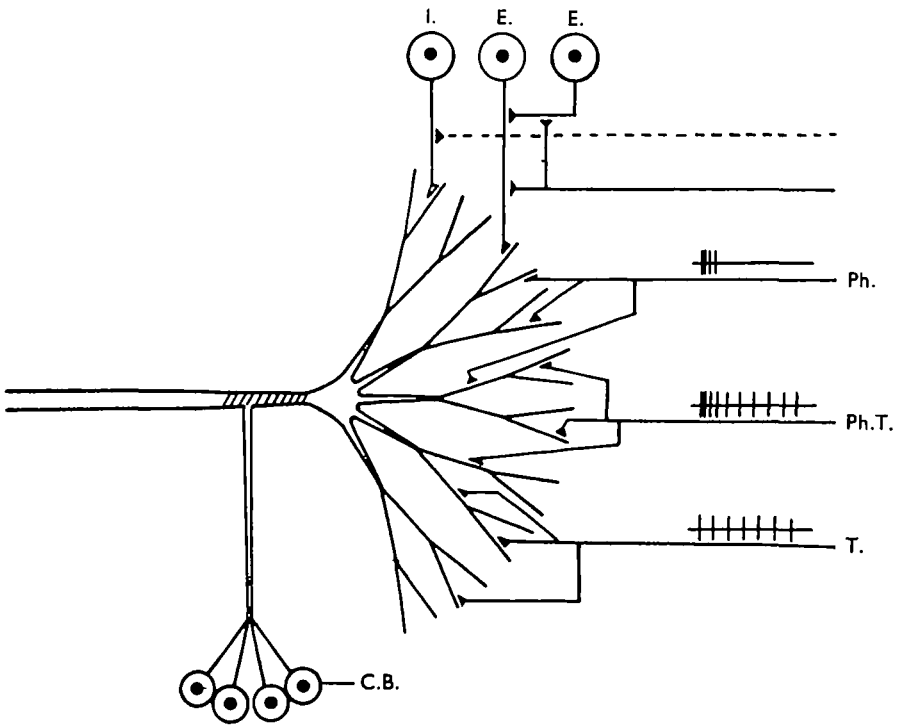

Fig. 21. Schematic representation of the synaptic portion of a giant axon with its ramifying axonal processes (dendritic tree) and its main inputs. All afferences are supposed to have their synaptic contacts only on the thinnest branches while the site of initiation (dashed area) of the propagated spike may be near the convergence region (the largest dendritic ramifications would be able to generate non-propagated spikes). E, cell bodies of two excitatory interneurones; I, cell body of an inhibitory interneurone; Ph., Ph.T., T., inputs consisting in burst of action potentials resulting from the stimulation of phasic, phasic-tonic or tonic receptors. C.B., cluster of cell bodies belonging to the same giant axon. Dotted line, possible afferent cercal pathway triggering the inhibitory interneurone activity (see Fig. 18).

An EPSP in relation with a given receptor varies in amplitude whereas its time to peak remains practically constant. This can be explained by the fact that a single presynaptic fibre might make several synaptic contacts with the dendritic tree of a given postsynaptic axon, some of them being sometimes ineffective. An alternative explanation might involve synaptic contacts more or less remote from the recording

site, but this would involve a variation in the time to peak which seems in contradiction with experimental evidence. A statistical analysis on a larger number of recordings has to be carried out for a further understanding of this point.

We have never observed so-called miniature potentials. It is possible that they are too small to be separated from the noise. In some occasions a few EPSPs (0·5–0·9 mV) remain after the ganglion has been completely isolated from all sensory input. It is, however, more likely that they are given by intraganglion interneurones for they also disappear when tetrodotoxin is applied to the whole ganglion (J. J. Callec, unpublished observations).

Unitary IPSPs subsist sometimes after the afferences have been cut. It is thus likely that they have their origin in spontaneous firing of intraganglion interneurones. They have already been recorded in *Aplysia* (Tauc & Gerschenfeld, 1962) and in motoneurones of the frog (Katz & Miledi, 1963).* They have been previously observed in some occasions using intraganglion microelectrodes (J. J. Callec, unpublished observations). In fact we have only few data on inhibitory potentials in insect ganglia; they have been recorded in the neuropile (Callec & Boistel, 1966*b*; Rowe, 1969) or in undetermined cell bodies (J. J. Callec, unpublished observations; Kerkut, Pitman & Walker, 1969). The size and number of the unitary IPSPs in the 'isolated fibre' preparation depend upon the quality and the rapidity of the dissection. They are sensitive to oxygen concentration. Furthermore, they can be inverted by a small hyperpolarization which takes place spontaneously after about 2 h.

(*b*) *Triggering of a postsynaptic spike*

It seems in our experiments that the depolarization induced by the maximal activity of one receptor stimulated mechanically is not able to reach the threshold. Thus, to trigger an impulse it is necessary to stimulate several receptors simultaneously and strongly.

The electrical stimulation of the cercal nerves shows that there is a great number of homolateral and contralateral sensory fibres connected with a given giant axon, for the EPSP grows progressively with an increase of the stimulation intensity. But in these conditions we can obtain only one action potential, rarely two. The stimulation by a puff of air applied to the cerci gives the longest depolarization which results from the summation of more or less synchronized unitary EPSPs. Nevertheless, it also gives rise to a small number of spikes, generally one or two (maximum six in our experiments). Thus for this kind of integrative synapse it is necessary to summate the action of many sensory impulses to trigger a propagating action potential; it remains possible, however, that the resting level *in vitro* is not exactly the same as *in vivo* and that, for instance, the already-mentioned slight hyperpolarization which occurs *in vitro* increases the threshold.

As in the crayfish (see Preston & Kennedy, 1960), when the action potential appears the subjacent EPSP does not disappear. This shows that the site of initiation of the EPSP and of the spike are likely to be differently located (see Fig. 21). All the excitatory and inhibitory afferences (Fig. 21 E, I) are supposed to have their connexions

* Recent experiments have shown that some unitary IPSPs can be elicited by presynaptic stimulation of cercal nerve XI (see Fig. 4, 18). Furthermore, stimulation of the cercal nerve X provides a strong inhibition in the giant axon (J. J. Callec, in preparation).

the dendritic tree while the site of initiation of the propagated action potential is thought to be at the beginning of the giant axon near the convergence site of the main dendritic arborizations. This is related to a higher threshold* or even a complete inexcitability of the thinner ramifications. This geometric arrangement is very well adapted to summate over a wide range a large number of presynaptic influences without giving a spike. These spikes would be blocked at the dilated point of convergence which, in that way, would act like the soma in a vertebrate motoneurone. Similar potentials have also been found in the crayfish (Takeda & Kennedy, 1965), where the anatomical features of the dendritic tree are comparable to those in the cockroach.

(*c*) *Factors influencing synaptic transmission.*

In our experiments we have seen that the transmission can be modulated by two principal phenomena: facilitation and depression. These phenomena which have a great importance in the integrative properties of this synapse have been studied at the unitary level by a stimulation of the different kinds of mechanoreceptors. This stimulation was carried out in such a way that it simulated as close as possible the natural patterns of firing.

Facilitation (or potentiation) has been found in many preparations: at the neuromuscular junction (Hubbard, 1963; Braun, Schmidt & Zimmerman, 1966; Mallart & Martin, 1967; Katz & Miledi, 1968; Rahamimoff, 1968; Maeno & Edwards, 1969) or in the central nervous system (Curtis & Eccles, 1960; Kuno, 1964*b*; Epstein & Tauc, 1970). In the cockroach potentiation seems to be only correlated with phasic receptors, so that their firing, whenever it is brief, is very effective and the threshold of the giant axon is easily reached (see later).

On the other hand the fatigue (or depression) decreases the potentialities of transmission of a coded message or a series of messages. This phenomenon is very frequent. It is often called depression when it follows a previous facilitation (Curtis & Eccles, 1960; Kuno, 1964*b*) or habituation when one can see a progressive but rapid decrease in the size of the behavioural response. This habituation has been studied by two principal methods; electrophysiological methods, often at the cellular level such as in *Aplysia* (Bruner & Tauc, 1966; Pinsker *et al.* 1970), in the crayfish (Krasne, 1969), in the squid (Horn & Wright, 1970), or behavioural methods on the whole animal, as in the cockroach (Zilber-Gachelin, 1966) and in an acridid (Fraser-Rowell & McKay, 1969). Our experiments show that, in the cockroach, this fatigue phenomenon occurs very frequently. It appears during repetitive afferent volleys induced by the mechanical stimulation of receptors. It can be seen as well after the stimulation of a single phasic receptor (Fig. 13) as after the stimulation of a great number of them by puffs of air (Fig. 15). In this last case it has been called EPSP habituation in the text. Repetitive electrical stimulation of afferent fibres (Fig. 20) shows also the same phenomenon complicated by a 'rebound' for high frequencies. The analysis of the fatigue phenomenon in these three kinds of experiments has given nearly exponential curves of somewhat comparable size. It can be advanced that in the last two cases we observe

* This hypothesis is strengthened by the fact that in some occasions, we can record spikes of a smaller amplitude (20–25 mV) coming from the main dendritic arborizations.

summation of the fatigue of individual fibres and that they are all supported by t[illegible] same fundamental mechanism. It is also visible after a tonic pattern (Fig. 10) where the postsynaptic depolarization decreases in amplitude even if the frequency remains nearly constant. After a phasic pattern the potentiation wave has been shown to be followed by a depression wave. The curve of Fig. 12 is comparable to the one obtained in the motoneurone of the cat (Curtis & Eccles, 1960). The fatigue is still more striking in polysynaptic pathways (Fig. 18B–D) where EPSPs are very labile and disappear rapidly even at low frequencies.

We think that the fatigue may be due essentially to a depletion of the available transmitter as in the cat motoneurone, but some other factors may also be present, namely, desensitivation of the postsynaptic membrane, modification 'at some stage of the secretory process', relative refractory period in the presynaptic terminals (Bennett, 1968). The last factor has some importance when a receptor gives high-frequency volleys (up to 360 c/s). We therefore know that during refractory period the size of the presynaptic spikes decreases, thus decreasing the quantity of transmitter release for each presynaptic spike (Takeuchi & Takeuchi, 1962). A quantitative study of these phenomena is now being carried out using an artificial (electrical) and well-controlled stimulation of a receptor for a wider range of frequencies. The first hypothesis is reinforced by the observed 'rebound' which has already been described by Elmqvist & Quastel (1965) on the human intercostal muscle. These authors have explained this fact by the onset of a transmitter mobilization following the more or less rapid 'depletion of a store of transmitter immediately available for release'. This hypothesis has been also mentioned in a recent review (Hubbard, 1970).

Two other limiting factors of less importance have been described in this paper. The first is related to the hyperpolarization component following a burst of great activity coming from a tonic receptor (Fig. 10) or following the mechanical stimulation of the whole cercus (Fig. 16). The second is in correlation with the summation of unitary EPSPs which, during high frequency, drives the resting potential towards the equilibrium potential, thus decreasing their amplitude.

(*d*) *Behavioural signification of these integratory phenomena*

We have seen in this paper that a giant axon in the sixth abdominal ganglion of the cockroach receives many inputs, thus giving rise to a very intense activity consisting in small unitary EPSPs or IPSPs, most of them related to presynaptic spikes in correlation with cercal stimulation. Nevertheless only few postsynaptic spikes are generated. The question then arises: what is the meaning of all this sensory information for a living animal?

The tonic receptors are continuously stimulated by dust, light puffs of air, slight contact with support or other animals, so they provide information about the environment. Their activity is certainly very important and would give a great synaptic depolarization of the giant axon if this was not, in fact, minimized by fatigue or habituation phenomena. It seems that these receptors give essentially a basic tonus and are not able to trigger a propagated action potential.

The phasic receptors, on the other hand, respond to a sudden strong puff of air or greater contacts and induce postsynaptic potentials which may be reinforced by poten-

tion. In these conditions a spike may be generated on the great depolarization which corresponds to the summation of the unitary activities including also the initial component of the tonic response. Then, all the limiting factors (fatigue, habituation, hyperpolarization, . . .) intervene to shorten the burst of postsynaptic propagated action potentials. The synapses have to be at rest for some time before giving rise again to other spikes. These spikes are rapidly conducted to higher centres (thoracic or cerebral ganglia) where they seem to develop and control the escape response.

SUMMARY

1. Some integrative properties of the chemical synapses between the sensory cercal afferent fibres and a giant axon are studied at the unitary level by external recording.

2. Unitary monosynaptic EPSPs are related to cercal receptor activity, one presynaptic spike inducing one EPSP. Unitary IPSPs are not directly related to cercal activity. They seem to originate within a ganglion.

3. Many receptors make synaptic contacts with a given giant axon. When a single receptor is strongly stimulated, the summation of elementary induced EPSPs gives a postsynaptic depolarization which is not able to reach the threshold. The simultaneous stimulation of several receptors provides a considerable amount of postsynaptic depolarization, thus triggering one or two spikes, rarely more.

4. The postsynaptic responses appear to be more or less different according to the afferent firing pattern: phasic, phasic-tonic and tonic. Some factors modulate these schemes: (*a*) Potentiation, which occurs only with phasic patterns, thus increasing their postsynaptic effect, is followed by a longer component of depression. (*b*) Limiting factors, such as fatigue phenomenon, are thought to be essentially related to depletion of an available transmitter.

5. Synchronous electrical stimulation induces a monosynaptic response, later followed by a complex polysynaptic phenomenon. These responses decrease with repetitive stimulation, but the former is far less labile than the latter.

6. These studies seem to indicate that a giant interneurone in the cockroach fires only in special conditions, namely when a strong mechanical stimulation occurs after a noticeable period of rest. This spike is then rapidly conducted to higher centres without relay.

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