

## FURTHER STUDIES ON SYNAPTIC TRANSMISSION IN INSECTS

### I. EXTERNAL RECORDING OF SYNAPTIC POTENTIALS IN A SINGLE GIANT AXON OF THE COCKROACH, *PERIPLANETA AMERICANA* L.

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#### INTRODUCTION

During recent years the use of the micro-electrode technique has resulted in considerable progress in the study of synaptic transmission at the unitary level. This technique has, for example, been intensively used for giant somata such as the motoneurons of the anterior horn of the spinal cord of the cat (Coombs, Eccles & Fatt, 1955 *a, c* and others), for giant ganglion cells of *Aplysia* (Tauc, 1955; Tauc & Gerschenfeld, 1961, 1962), for giant axons in the stellate ganglion of the squid (Bullock & Hagiwara, 1955; Hagiwara & Tasaki, 1958) and in the ventral nerve cord of the crayfish (Furshpan & Potter, 1959; Watanabe & Grundfest, 1961; Preston & Kennedy, 1960). This technique has also allowed some progress to be made in the knowledge of the mechanisms of synaptic transmission in insects (Callec & Boistel, 1966 *a, b*, 1967).

In most cases, however, the small dimensions of the synaptic structures render intracellular recording very tedious and difficult and long-term experiments nearly impossible. Furthermore, the fact that, for those experiments carried out in small synaptic structures, one must use very fine-tipped micro-electrodes (which are necessarily of very high resistance) in order to avoid too great an injury of the nerve cell membrane results in a limitation of the high-frequency response of the recording apparatus, and a decrease of the signal-to-noise ratio (this effect is particularly important when 'spontaneous' or 'miniature' synaptic potentials are to be recorded). Moreover, since relative movements of the micropipette and of the preparation must be avoided, mechanical stimulation of some part of the preparation is virtually impossible and many difficulties are encountered in maintaining a continuous perfusion of the synaptic regions, as is often required in pharmacological studies.

Many of these difficulties may be overcome by the use of external electrodes. Yamasaki & Narahashi (1960) used such a technique to analyse the electrical phenomena associated with synaptic transmission in the last abdominal ganglion of the cockroach; the global post-synaptic responses were in this case recorded between the origin of the connective from this last abdominal ganglion and an indifferent electrode in contact with the third thoracic ganglion previously crushed with forceps to make the action potential monophasic. A refined version of this technique including the use of a 'sucrose-gap' to reduce the shunt between the electrodes has been recently applied to the study of the superior cervical ganglion of the rabbit (Kosterlitz & Wallis, 1966; Kosterlitz, Lees & Wallis, 1968). However, despite their interest the

results thus obtained remain far less precise and far less complete than those obtained with intracellular micro-electrodes.

It is obviously desirable to be able to record, with external electrodes, the synaptic potentials corresponding to a single post-synaptic element, for this technique would allow the same results to be obtained as with intracellular micro-electrodes without many of the above-mentioned inconveniences. We have recently developed such a technique for the study of synaptic transmission in the last abdominal ganglion of the cockroach, *Periplaneta americana* L. The purpose of this paper is to describe this technique and to show, from some typical results, some of its possibilities. The results of our study on synaptic transmission will be reported and discussed in a subsequent paper (Callec, Pichon, Guillet and Boistel, in preparation).

## METHODS

### 1. Principle

The principle of the recording technique is diagrammatically shown on Fig. 1. Post-synaptic electrical events, whether or not associated with pre-synaptic activity (Pre), are recorded as the potential difference between the active post-synaptic end (Post) and the other inactive end of an isolated axon. The external resistance between the electrodes was increased with the use of an 'oil-gap', according to our previously described technique (Pichon & Boistel, 1966, 1967).\*

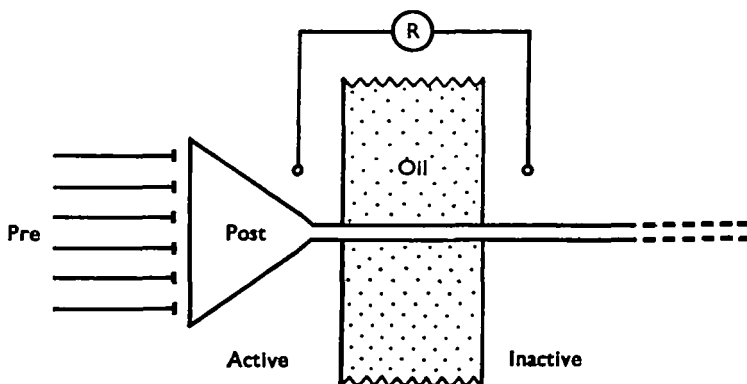


Fig. 1. Schematic diagram illustrating the principle of the recording technique: post-synaptic events are recorded across an 'oil-gap' as the potential difference between the active post-synaptic end (Post) and the other inactive end of a single giant axon.

### 2. Preparation

A portion of the ventral nerve cord, including the three last abdominal ganglia, the cercal nerves and the cerci, was dissected from adult male cockroaches reared at a constant temperature of 25° C. It was then placed on a glass slide in a drop of physiological saline. A single giant axon (generally the median ventral giant) was isolated over 1.5–2 mm. between the 5th and the 6th (last) ganglia as close as possible to the 6th ganglion. It was then transferred to the recording cell.

\* This technique is in some way similar to that used for the recording of generator potentials of muscle spindles (Katz, 1950) or the Pacinian corpuscle (Gray & Sato, 1953).

### 3. Recording cell

The cell consisted of two chambers separated by a thin Perspex partition (Fig. 2). The first chamber (ganglionic chamber) contained the cerci, the cercal nerves and the 6th ganglion. The cerci were laid on a small platform contiguous to a groove where the cercal nerves and the ganglion were continuously irrigated with the physiological saline flowing at a regulated rate. A three-way tap, placed upstream, allowed rapid changes to modified solutions or solutions containing drugs. This groove was in contact with the first electrode A. The second chamber was filled with mineral oil and contained the

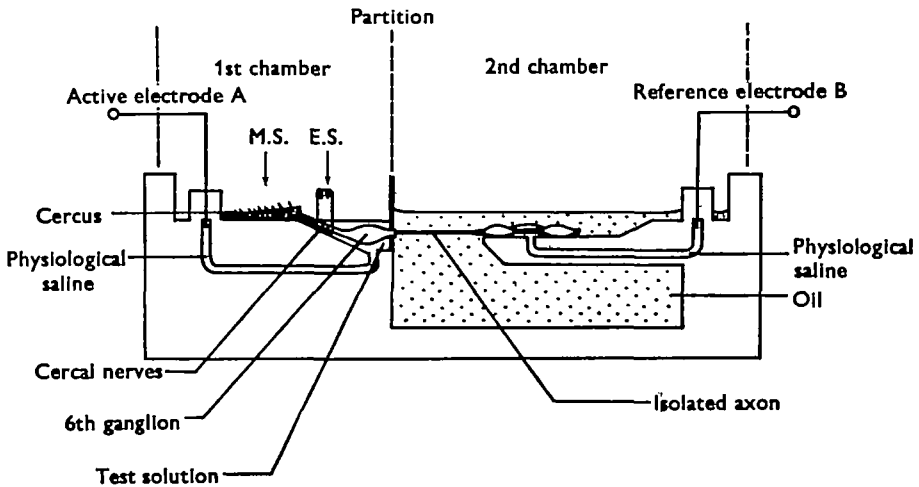


Fig. 2. Diagrammatic representation of the nerve chamber used for recording synaptic potentials with external electrodes. For explanation see text.

remainder of the preparation. The beginning of the connectives from the 6th ganglion passed through a small hole in the partition and served as a plug to avoid passage of oil into the first pool. The cut end of the nerve cord including the 4th and the 5th ganglia was supported on a second platform where it was in contact with the second electrode B. The isolated portion of the giant axon floated in oil, hanging between the hole in the partition and this platform.

### 4. Stimulation and recording

The preparation was stimulated pre-synaptically either by mechanical stimulation of the cercal sensilla (M.S.) or by short-duration (0.01 msec.) rectangular current pulses applied to the cercal nerves via a stimulus-isolation unit (E.S.). It was also possible to pass current through the isolated axon by means of a Wheatstone bridge connected between the two recording electrodes A and B.

The two Ag-AgCl electrodes A and B were connected to an oscilloscope and also to a chart pen-recorder via a high-impedance negative-capacitance amplifier.

### 5. Solutions

The physiological saline which has been used for those experiments was the 'modified Ringer's solution' of Yamasaki & Narahashi (1959*a*). It contained 210.2 mM-NaCl, 3.1 mM-KCl, 1.8 mM-CaCl<sub>2</sub>, 0.2 mM-NaH<sub>2</sub>PO<sub>4</sub> and 1.8 mM-Na<sub>2</sub>HPO<sub>3</sub> (pH 7.2). Experiments were carried out at room temperature (20–25° C.).

## RESULTS

We will limit ourselves to few characteristic examples showing some of the possibilities of this single-fibre preparation and allowing a comparison to be made between extracellular and intracellular recordings.

### 1. Without stimulation

In the (apparent) absence of stimulation an arrhythmical ground activity was recorded in most cases. It consisted of small depolarizing waves, with a mean rising phase of 2 msec., which decayed in an approximately exponential manner with a time

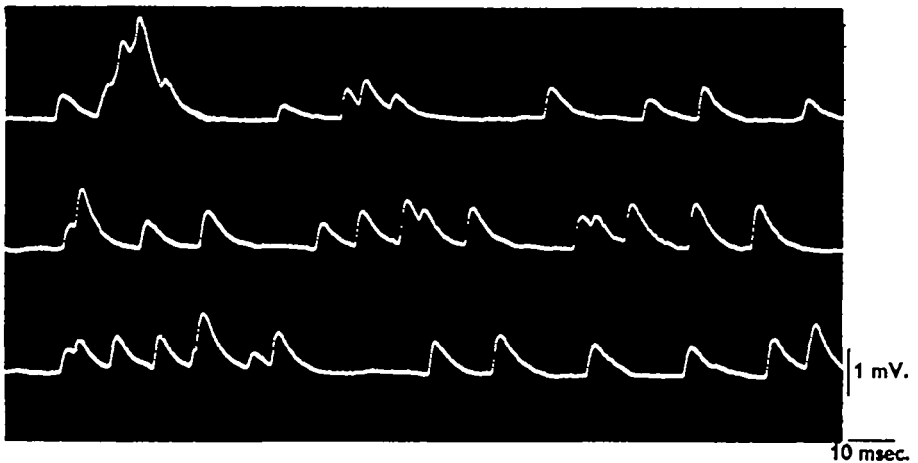


Fig. 3. Examples of externally recorded 'spontaneous' excitatory post-synaptic potentials (SEPSPs) in a giant axon. Notice different amplitudes and summation phenomena.

constant of 4–6 msec. (Fig. 3). The amplitude of these potentials never exceeded 2 mV. and was most frequently of 0.2 to 0.9 mV. There is some evidence that these are so-called excitatory post-synaptic potentials (EPSP) and they will be referred to, in this paper, as 'spontaneous' EPSPs or SEPSPs.

This pattern of activity has been recorded on some occasions by means of intracellular micro-electrodes (Callec & Boistel, 1966*a, b*). It seemed to depend upon a satisfactory physiological state of the synapses and it disappeared within a few minutes when the irrigation of the ganglion was stopped. It was also related to the activity of pre-synaptic cercal nerve fibres, for it ceased almost instantaneously when the cercal nerves were cut. Moreover, careful drying of the cerci considerably reduced the number of these SEPSPs (see (Fig. 5).

## 2. Following mechanical stimulation of the cercal mechanoreceptors\*

Mechanical stimulation of the cercal receptors by a puff of air resulted in a transitory increase in the number of post-synaptic potentials (Fig. 4A). In some cases full-sized action potentials originated from this stimulation when the depolarization brought about by the summation of these EPSP's reached or exceeded the threshold (Fig. 4B).

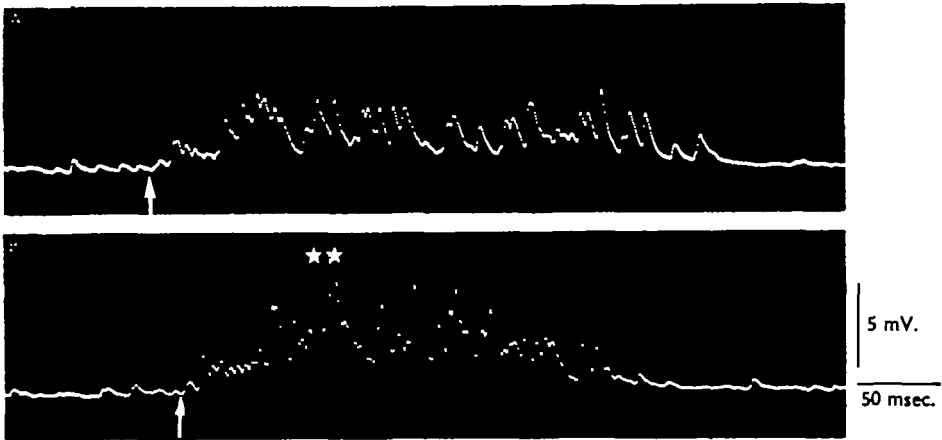


Fig. 4. Effects of mechanical stimulation of the cerci by a puff of air, beginning at the arrow, upon post-synaptic activity. Two post-synaptic action potentials (stars) were produced by this stimulation in B. These action potentials are truncated.

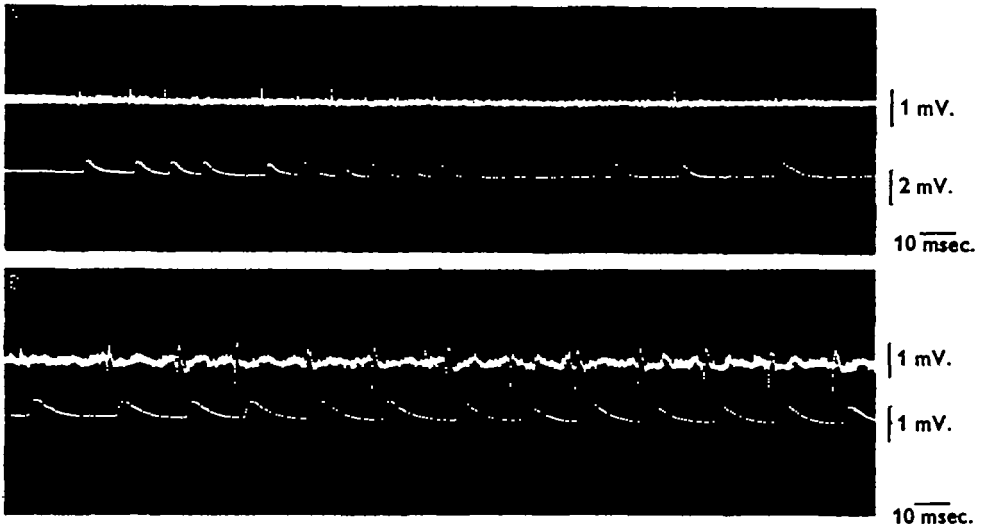


Fig. 5. Correspondence between electrical activity of a cercal mechanoreceptor (upper tracings) and EPSPs (lower tracings) in two different preparations (A and B). 'Spontaneous' post-synaptic activity has been nearly suppressed by a careful drying of the cerci.

\* During the dissection of the single axon, the cerci were generally dipped into the physiological saline, a thin layer of which clung to the cerci when the preparation was placed in the recording chamber. This thin layer was very effective in bending, and thus stimulating, the cercal mechanoreceptors.

Simultaneous recording of the electrical activity of a single cercal mechanoreceptor and of post-synaptic potentials showed for some receptors a fairly good correspondence between the two phenomena, each spike at the receptor being followed by one EPSP\* (Fig. 5).

### 3. Following electrical stimulation of the cercal nerves

Slow depolarizing waves roughly analogous in shape and in duration to the previously described SEPSPs could be obtained by electrical stimulation of the cercal nerves (Fig. 6). These 'electrically induced' EPSPs increased smoothly with increasing stimulation and gave rise above threshold to one or sometimes two spikes (Fig. 6 A-C).

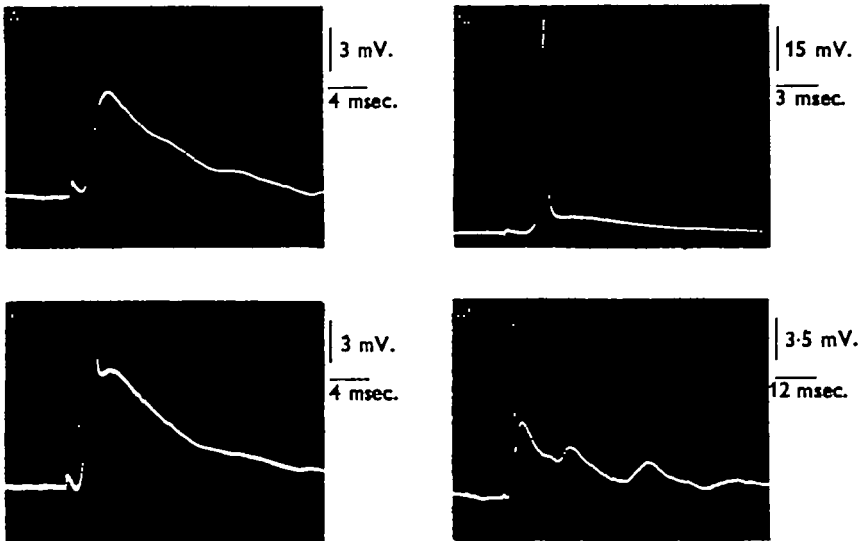


Fig. 6. Externally recorded post-synaptic potentials following electrical stimulation of the homolateral cercal nerve XI. A. Subthreshold EPSP. B. Slightly above threshold: a post-synaptic action potential is generated from this 'EPSP'. This action potential is truncated. C. The same post-synaptic action potential at a lower gain and a higher sweep speed. Note the negative after-potential. D. A complex EPSP analysed with a lower sweep speed.

The critical firing level (i.e. the level of depolarization at the threshold) varied from one preparation to another between 3 and 8 mV. On some occasions the shape of the 'electrically induced' EPSP was more complex and its duration longer (Fig. 6D), suggesting the existence of some polysynaptic connections between cercal fibres and the single fibre.

The amplitude of the spikes might exceed 110 mV. but was generally lower (70–100 mV.). Such 'electrically induced' EPSPs and post-synaptic spikes had also been recorded with intracellular micro-electrodes (Callec & Boistel, 1966b).

### DISCUSSION

The extracellular method, described here, of recording post-synaptic potentials in a single giant axon gives interesting results. Some of these results are new, such as those

\* We have shown that this correspondence may be altered in some cases (Callec, Guillet, Pichon & Boistel, 1969).

following mechanical stimulation of the cercal receptors. Others have already been obtained by means of intracellular micro-electrodes (Callec & Boistel, 1966*a, b*); they concern 'spontaneous' EPSPs, 'electrically induced' EPSPs and post-synaptic action potentials. If one compares the two sets of results (Table 1) one can see significant differences between the two techniques: (1) extracellularly recorded EPSP's are nearly three times smaller than, and twice as long as, intracellularly recorded ones; (2) extracellularly recorded post-synaptic spikes may be larger than intracellularly recorded ones.

Table 1. *Intracellularly and extracellularly recorded post-synaptic potentials*

	Intracellular	Extracellular
'Spontaneous' EPSPs		
Amplitude	0.7-2.3 mV.*	0.2-0.9 mV.
Time to peak	0.9-1.3 msec.*	2.0-3.4 msec.
Duration	5-10 msec.*	More than 10 msec.
'Electrically induced' EPSPs		
Threshold amplitude	15-20 mV†	3-8 mV.
Post-synaptic action potentials		
Amplitude	Up to 75 mV.†	Up to 115 mV.

\* Callec (unpublished results).

† Callec & Boistel, 1966*b*.

If one assumes that extracellular and intracellular recordings were made in the same or in similar post-synaptic fibres, the decrease in amplitude of externally recorded EPSPs could be related to an electrotonic decay of the (local) post-synaptic potentials in a cable-like structure (the giant axon) between the point of insertion of the micro-electrode ( $RP_1$ , most often in the middle of the ganglion) and the point where the isolated axon leaves the ganglion ( $RP_2$ ). If  $\Delta Em_1$  and  $\Delta Em_2$  are the amplitudes of the EPSPs recorded respectively in  $RP_1$  and  $RP_2$ , the relation between these two values would be given by

$$\Delta Em_2 = \Delta Em_1 \exp(x/\lambda), \quad (1)$$

where  $x$  is the distance between the two recording points ( $RP_1$  and  $RP_2$ ) and  $\lambda$  the space constant of the giant axon between those two points. One can calculate  $\lambda$  from equation (1):

$$\lambda = \frac{-x}{\Delta Em_2/\Delta Em_1},$$

taking the values of the largest spontaneous EPSP's as a reference (i.e.  $\Delta Em_1 = 2.3$  mV and  $\Delta Em_2 = 0.9$  mV) and  $x = 750 \mu$  (mean value in our experiments),  $\lambda = 796 \mu$ . This value is slightly lower than the space constant of the giant axons in the connectives ( $860 \mu$  from Yamasaki & Narahashi, 1959*b*;  $1300 \mu$  from Boistel, 1959). This was to be expected from the shrinkage of the giant axon in the ganglion, the space constant being proportional to the square root of the fibre diameter if the electrical properties of the plasma and axon membrane are held constant. It is thus logical to conclude that the lower amplitude of externally recorded EPSPs may be interpreted in terms of cable properties of post-synaptic giant axons inside the ganglion. The slowing of the

rate of change of membrane potential resulting in an increased duration of EPSPs may be related in the same way to an increased distance from the post-synaptic membrane.

Unlike EPSPs, post-synaptic action potentials are propagated, thus keeping their full amplitude from their site of initiation to  $RP_2$ .

Although it involves the reduction of the amplitude of the EPSPs, external recording of synaptic potentials has the following advantages:

(1) A tenfold reduction of the 'noise' level ( $30 \mu V$ . instead of  $300 \mu V$ .), corresponding for EPSPs to an increase of more than threefold of the signal-noise ratio.

(2) Preservation of the synaptic preparation for hours without significant changes in its characteristics.

(3) Continuous irrigation of the ganglion with a saline which may be changed within seconds for modified solutions, thus allowing a pharmacological study of the synaptic zones to be carried out in well oxygenated conditions.

(4) Stability of the recording system, permitting a physiological stimulation of the cercal mechanoreceptors to be made.

(5) Precise identification of the dissected post-synaptic giant axon, enabling a systematic study of the input to this particular axon to be made.

This single-fibre preparation is being used for the study of the input-output relations across the 6th abdominal ganglion (Callec *et al.* 1969). It will be utilized for a study of the influence on synaptic transmission of the electrical activity of nerve cell bodies (located at the periphery of the ganglion) which have been shown to fire spontaneously in many cases (Callec & Boistel, 1966*a*). It seems also possible at the present time to study with this technique the effects on post-synaptic activity of electrophoretic injections of pharmacological substances in the vicinity of the synaptic areas.

A similar technique may be applied to other post-synaptic structures either in insects (such as large motor axons in the thoracic ganglia of some insect species) or in other animals.

#### SUMMARY

1. The dissection of a small length of a giant axon between the 5th and the 6th ganglia of the abdominal nerve cord of the cockroach *Periplaneta americana* allows extracellular recordings of excitatory post-synaptic potentials (EPSPs) to be made in particularly good conditions.

2. 'Spontaneous' or 'induced' EPSPs are smaller and slower than those recorded with conventional micro-electrodes.

3. Post-synaptic action potentials are very similar to, or even larger than, those recorded by means of intracellular electrodes.

4. The low impedance of the recording system, together with its stability, allows numerous experiments to be made which were nearly impossible with the micro-electrode technique.

5. It is suggested that this technique might be used for other post-synaptic structures.

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