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# HETEROSYNAPTIC FACILITATION AND POST-TETANIC POTENTIATION IN *APLYSIA* NERVOUS SYSTEM*

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SUMMARY

1. Heterosynaptic facilitation was defined as an increase of amplitude of a test excitatory post-synaptic potential (EPSP) after the activation of a pathway (heterosynaptic pathway) different from that which produced the test EPSP. This phenomenon has been studied in *Aplysia* central nervous system under conditions which excluded the participation of post-tetanic potentiation.

2. A unitary test was produced in the left and right giant cells, by indirect stimulation of an interneurone located in the peri-oesophageal ring.

3. During heterosynaptic stimulation, orthodromic and antidromic activation of the test interneurone was prevented by (1) isolating the synaptic afferent region of the test interneurone from the tested synapse on the right giant cell by a sucrose block applied on the left pleuro-visceral connective, and (2) using physiological stimulation of a piece of skin as a heterosynaptic stimulus.

Under these conditions which prevented any firing in the test interneurone, heterosynaptic facilitation is observed as a 200% increase of amplitude of the test EPSP in the right cell which lasted more than 15 min.

When instead of the physiological stimulus a supramaximal electrical stimulation of the nerves afferent to the abdominal ganglion was used, the increase of amplitude of the test EPSP could reach as much as 500% of its original amplitude. The effectiveness of such heterosynaptic stimulus was smaller when it was applied in the absence of a block of the left pleuro-visceral connective.

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4. It was possible to produce heterosynaptic facilitation when the preparation was cooled to 7–9° C or if $Li^+$ replaced $Na^+$ in the medium. Both of these changes suppressed post-tetanic potentiation.

5. It was concluded that heterosynaptic facilitation is a phenomenon different from post-tetanic potentiation. Heterosynaptic facilitation is similar to heterosynaptic inhibition seen in other cells in the same preparation, except for the polarity of action. Both phenomena seem to result from comparable mechanisms, probably acting on the quantity of transmitter released.

## INTRODUCTION

The generally known physiological mechanism by which synaptic transmission can be facilitated, in the sense of an increased transmitter output, depends on the repeated firing of the presynaptic terminal. Another mechanism was suggested by Kandel & Tauc (1965) and Tauc (1965) as a result of their observation of a phenomenon which was called heterosynaptic facilitation.

It was shown by these authors, in the abdominal ganglia of *Aplysia*, that the amplitude of unitary excitatory post-synaptic potential (EPSP)* which was produced in the right giant cell by the threshold stimulation of a given nerve could be increased by repetitive stimulation of several different nerves. This increase in amplitude outlasted the heterosynaptic stimulation by as much as 10–15 min and was not due to changes in the biophysical constants of the post-synaptic cell.

The parameters of this facilitation (duration, intensity) differed from the post-tetanic effect, which was obtained through repetitive stimulation of the same nerve which produced the test EPSP. It was therefore proposed that the heterosynaptic effect was due to a different mechanism, not related to the repetitive firing of the presynaptic terminal but probably acting on the release of the transmitter.

This hypothesis, however, could not be definitively tested by these authors, since the stimulation yielding the heterosynaptic effect gave rise to a complex synaptic, and occasionally antidromic, activation of the cell in which the EPSP was recorded. Against this background activity, it was impossible to assert the absence of the test EPSP, in order to demonstrate the independence between the heterosynaptic facilitation and the repetitive firing of the presynaptic terminal (Kandel & Tauc, 1965).

The results presented in this paper show that heterosynaptic facilitation

* Throughout this article the denomination EPSP will be used to designate unitary excitatory post-synaptic potentials as defined by Kandel & Tauc (1965), implying the potential due to the activation of only one presynaptic neurone making contact with the cell in which the record is obtained.

is still present when (1) the presynaptic terminal is functionally isolated in a way which should prevent the activation of the presynaptic test neurone through the heterosynaptic stimulation, and (2) after abolishing the post-tetanic potentiation. A preliminary report of this work has been published elsewhere (Tauc & Epstein, 1967).

## METHODS

The whole ganglionic central nervous system of the sea slug, *Aplysia*, was used (Fig. 1) without finding any evident functional differences between individuals of different origins or species (*Aplysia depilans*, *punctata* and *californica*). For practical reasons (length of the nerve), only medium to large animals were selected.

Several nerves were left attached to the ganglionic system, in particular the anterior tentacular and tegumentary nerves, some of the pedal nerves, and the main nerves to the abdominal ganglia (Fig. 1). In some of the experiments, a piece of skin approximately 2 $cm^2$ was dissected from the region innervated by the siphon nerve (cf. n. anal., Eales, 1921) together with the ganglionic system, taking care to maintain the innervation as complete as possible.

The preparation was pinned to the bottom of a lucite chamber which was covered with paraffin and in which several pairs of silver electrodes were embedded. Each nerve was stretched across a pair of these electrodes for stimulation.

The chamber was divided into several compartments by means of paraffin partitions with slots through which the nerves were placed. To seal these slots, 'Vaseline' was carefully applied after drying the corresponding segments of the nerves with cellulose tissue. Thus it was possible to expose given parts of the preparation to different solutions. The various compartments were designed for isolation of the peri-oesophageal ganglia, the abdominal ganglia, restricted portions of the pleuro–visceral connectives and the piece of skin. According to the needs of the experiment, inlet and outlet systems for the bathing solutions were provided for the different compartments.

The chamber was placed directly on the cold side of two Pelltier-type batteries which could be regulated to different temperature levels. A narrow slot between the mounted batteries permitted the transillumination of the ganglia. This was essential for a good electrode impalement since the connective tissue covering the ganglia was left intact. The experiments were usually performed at room temperature (about 22° C) except when stated otherwise.

The solutions utilized were: filtered natural sea water, artificial sea water (cf. the composition of the 'average' sea water: Prosser & Brown, 1961, chap. 3, Table 4, p. 60), artificial sea water with complete replacement of the NaCl with equimolar LiCl (Li-sea water), and a saccharose solution of the same osmolarity as the 'average' sea water (Prosser & Brown, 1961, chap. 3, Table 4, p. 60).

Capillar micro-electrodes were filled with 2·5 M-KCl or saturated $K_2SO_4$ solutions. The system for intracellular micro-electrode recording was standard and consisted of Bak-type amplifiers, oscilloscope and stimulators. To obtain a steady threshold stimulation of the nerves and minimize polarization effects, a stimulus consisting of two square pulses, of equal amplitude and duration but opposite polarity, was used. The amplitude and polarity of both pulses could be varied simultaneously, and the over-all duration kept at approximately 10 msec.

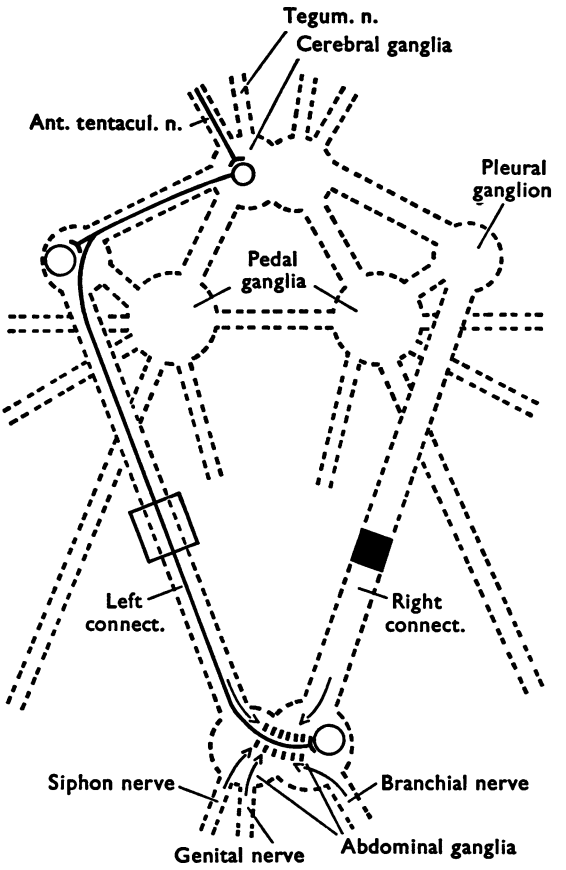


Fig. 1. Diagram of the ganglia composing the central nervous system of *Aplysia*: the peri-oesophageal ring of ganglia in the upper part is connected by two connectives to the abdominal ganglia. The two giant cells (left, LGC and right, RGC) are indicated and the common presynaptic neurone has been tentatively placed in the left cerebral ganglion. The afferents through which this neurone is synaptically activated are shown symbolically starting in the left anterior tentacular nerve. The two ganglionic groups were isolated from each other by cutting the right connective (filled rectangle) and reversibly blocking the left connective (open rectangle). The hypothetical presynaptic region at which the heterosynaptic stimulation could have a facilitating effect is indicated in the abdominal ganglion by small bars.

## RESULTS

### *Heterosynaptic facilitation of a synaptic ending functionally isolated from the cell body*

In the experimental situation used in the work by Kandel & Tauc (1965), the greatest difficulty was represented by the possibility that the pre-

synaptic neurone, which generated the test EPSP, and the post-synaptic cell in which this EPSP was recorded, were both in the same ganglion. In such an event, the heterosynaptic stimulation could induce repetitive firing of the presynaptic neurone, through a more or less direct pathway of synaptic activation, thus producing a post-tetanic effect.

The functional symmetry of the two 'giant' cells in the *Aplysia* ganglionic system (Hughes & Tauc, 1963), the right giant cell (RGC) in the abdominal ganglia, and the left giant cell (LGC) located in the left pleural ganglion (Fig. 1), prompted a search for a way to activate a neurone making synaptic contact on both the RGC and the LGC. Such an interneurone would need to be located either in a ganglion different from that containing the soma of one of the giant cells or in a ganglion containing neither of the giant cells.

*Localization of an interneurone presynaptic to both giant cells*

(*a*) *The common interneurone.* Using a preparation composed of the main part of the ganglionic system of the *Aplysia* (Fig. 1), it was found that the tentacular and tegumentary nerves frequently produced couples of unitary EPSPs in the RGC and LGC. In about 50–70 % of the preparations in which both giant cells were successfully penetrated with the microelectrodes, the left anterior tentacular nerve consistently produced a pair of post-synaptic potentials at a sharp threshold (left and right EPSP) of several millivolts of amplitude.

With the stimulus intensity used, the right EPSP was occasionally preceded by another small EPSP of less than 0·5 mV which did not interfere with the experimental requirements. In most cases the left EPSP was superimposed on a small wave of depolarization due to a complex post-synaptic potential. For this reason only preparations in which the left EPSP appeared alone or against a very stable background were considered. When the right or the left EPSP were difficult to recognize due to other post-synaptic activity the preparation was discarded.

The continuous stimulation of the left anterior tentacular nerve at a rate of 0·1/sec (the basic frequency of stimulation throughout the experiments) produces a simultaneous and strong habituation of both EPSPs (Bruner & Tauc, 1966). After 40–70 stimulations they attained a *quasi*-steady state at 50–30 % of their initial size. Discontinuing the stimulation permitted dishabituation (cf. Bruner & Tauc, 1966; Fig. 6*A*), but the period of rest required was much longer than the interruption of activation used in present experiments (see below).

The interval by which the right EPSP followed the left EPSP (Fig. 2) was always constant, and the two EPSPs always appeared together. This

strongly suggested that one presynaptic neurone gave rise to both potentials.

It has to be borne in mind that the pair of EPSPs has been maintained through experiments lasting several hours, submitting the preparation to various frequencies of stimulation and, in some experiments, to cooling and

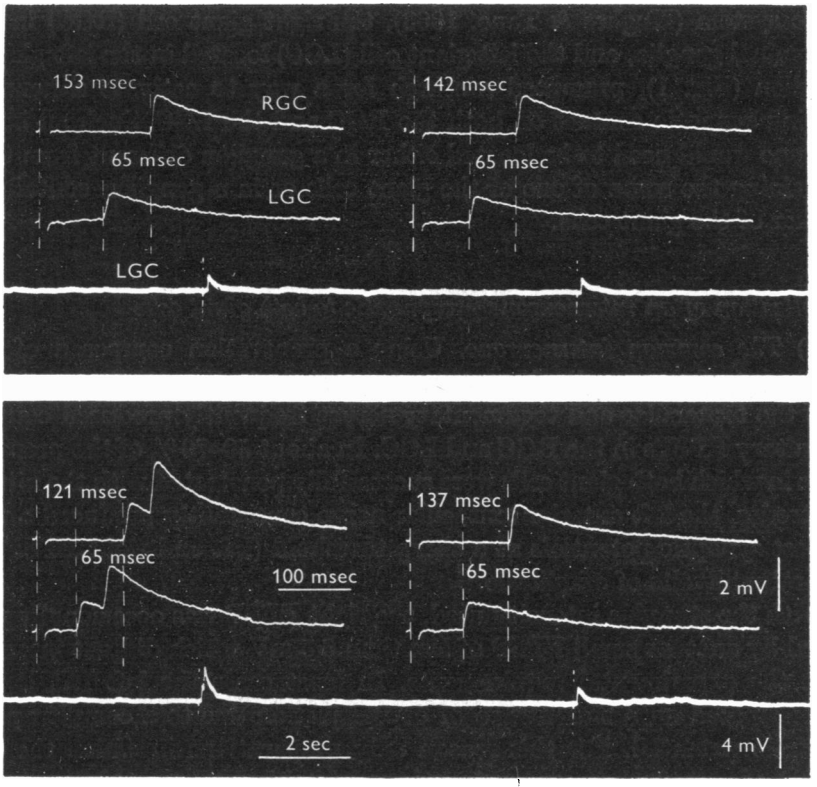


Fig. 2. Four consecutive records of the EPSP in the RGC (upper trace) and the EPSP in the LGC (central trace) following threshold stimulation of the left anterior tentacular nerve at 0·1/sec. The lower trace is a continuous record of the LGC activity. The latency of the EPSPs is highly variable with respect to the initiation of the stimulus. Upper values show latency of right EPSP with respect to stimulus. The interval between the two EPSPs remains constant, however (lower values).

to the change of bathing medium by Li-water. These last two procedures would clearly affect different neurones differently (cf. Murray, 1966; Carpenter, 1967) in a presynaptic chain and thus lead to a dissociation of the right and left EPSPs. The only variation seen was a progressive increase in interval between left and right EPSP with the 'aging' of the preparation and after repeated blocking of the left pleuro–visceral connective.

In contrast to the constant interval between the EPSPs, their latency with respect to the stimulus was quite irregular at the basic rate of stimulation, with changes of 15–20 % or more from one stimulation to the other (Fig. 2). This led to the conclusion that the activation of the common presynaptic neurone was achieved by an indirect pathway, comprising at least one synaptic step. Consistent with this viewpoint was the inability of the EPSPs to follow frequencies higher than 6–8/sec (Kandel & Tauc, 1965) and the transient diminution of the threshold of the left anterior tentacular nerve which appeared during the repeated stimulation of this nerve, referred to later as homosynaptic stimulation.

(*b*) *Localization of the soma of the common presynaptic neurone.* Though it was impossible to localize such a neurone with an intracellular microelectrode, the fact that we were stimulating the common presynaptic neurone through a synaptic step and not antidromically, made it quite easy to decide where its soma had to be placed. Since in our preparation only two nerves were connecting the peri-oesophageal ganglia with the abdominal ganglia, it was immediately established that the left pleuro–abdominal connective contained the axon conveying the impulse which yielded the right EPSP as previously mentioned. When this became clear, the right pleuro–abdominal connective was regularly sectioned at the beginning of each experiment.

When the left connective was blocked or cut, the left EPSP continued to appear unimpaired and unchanged, though the right EPSP was no longer seen (Fig. 4, trace 2). Thus, it was assumed that the soma of the common presynaptic neurone was located in one of the peri-oesophageal ganglia since the available evidence for the *Aplysia* neurones indicates that their spike-generating trigger zone or zones are located in the axonal regions near the soma (Tauc, 1962). This assumption also corresponded with the fact that the left EPSP had a shorter latency with respect to the stimulus than the right EPSP (Fig. 2). Cutting the peri-oesophageal ring medially showed that the common presynaptic neurone was probably located in the left half, but several trials with intracellular electrodes failed to identify the neurone.

*Comparison between heterosynaptic facilitation and post-tetanic potentiation*

In order to compare accurately the parameters of the post-tetanic potentiation and the heterosynaptic facilitation of the two EPSPs, the same stimulation pattern was used to produce both effects. This pattern usually consisted of two stimulus trains each of 1 sec duration and a frequency of 6–8/sec, separated by a 10 sec interval. The frequency used for a given preparation was the highest that the EPSPs would follow upon homosynaptic stimulation.

The intensity of the stimulus differed, however, for each kind of stimulation. Since the magnitude of the heterosynaptic effect depended, within limits, on that intensity, a level high enough to produce maximal effect was used. This level of stimulation always gave an important background activity in the giant cells, as in the experiments of Kandel & Tauc (1965). For the homosynaptic stimulation the stimulus intensity was kept as close to the threshold for the activation of the common presynaptic neurone as possible. This was difficult to achieve since, as previously stated, this threshold diminished transiently during and after repetitive stimulation. Two practical means were used to overcome this difficulty: either the stimulus amplitude was manually adjusted during the stimulation according to the variations of the threshold, or the trains were started at a barely subthreshold level which became effective after the first one or two stimuli. The point of restricting the homosynaptic facilitation in this way was twofold: to avoid the increase of the background synaptic noise through recruitment of other afferences to the giant cells which would have impaired the measurements, and to obtain a 'pure' post-tetanic effect. This type of control sometimes yielded incomplete trains of responses of the common neurone during the homosynaptic stimulation. These were disregarded for the quantitative analysis. The quantitative comparison was only performed on those preparations in which both EPSPs were easily measured due to a relatively or completely pure threshold response of the left anterior tentacular nerve.

The heterosynaptic facilitation due to the various nerves was different in intensity for each one of the EPSPs. The homosynaptic effect on the contrary produced a quite parallel augmentation of both potentials (Fig. 3, left and central part). The time course of the two effects was also quite distinct. The post-tetanic potentiation of the EPSPs was very rapid, reaching a maximum 20–40 sec after the start of the repetitive stimulation (Figs. 3 and 6). The heterosynaptic effect rose more slowly, frequently starting 1 min after initiating the stimulation, with a maximum in about 2–3 min (Figs. 3 and 4). The difference in duration of the two phenomena was quite marked: the post-tetanic effect lasted from 1 to 4 min, while the heterosynaptic stimulation yielded an effect which lasted 10–30 min (Fig. 3). This very important difference in duration had also been observed by Kandel & Tauc (1965), leading them to the assumption of two distinct mechanisms.

Another rather striking feature which differentiated the homosynaptic effect from the heterosynaptic one was that the former could be repeated as often as desired, while the latter one was in general difficult to reproduce at less than 10–15 min intervals, and usually a 25 min rest was necessary to get repeated maximal increases of the EPSP's amplitude. The

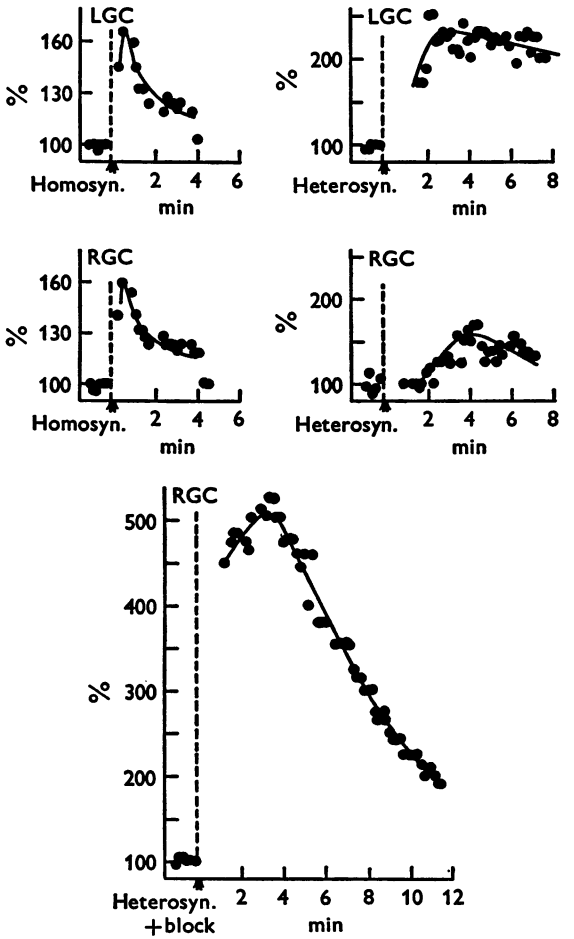


Fig. 3. Comparison of post-tetanic potentiation and heterosynaptic facilitation. The evolution of the amplitude of the left EPSP (LGC) and the right EPSP (RGC) as the result of two trains of stimuli applied to the left anterior tentacular nerve (homosynaptic stimulation) are shown in the two upper left diagrams. The two upper right plots show the changes in amplitude of the two EPSPs due to a similar stimulation of the siphon nerve (heterosynaptic stimulation). The bottom plot represents the variation in amplitude of the right EPSP after applying the same stimulation to the siphon nerve during a conduction block of the left pleuro–abdominal connective. No change of the left EPSP was observed in the latter case. Note the difference in the vertical axis scale between the homosynaptic and heterosynaptic stimulation. Due to the large amount of activity induced in both giant cells immediately following heterosynaptic stimulation, it was initially impossible to measure the EPSP amplitudes. When heterosynaptic stimulation was applied during conduction block of the connective, this block was maintained until all activity subsided in the RGC.

maximum amplitude change obtained homosynaptically varied from none to a 70–100 % increase. The heterosynaptic facilitation was also lacking in some preparations, but there was no correlation between the absence of the two effects. The resting level of both cells was between 55 and 60 mV and was constant during the entire experiment; this eliminated a possible effect of modification of membrane potential on the amplitude of the EPSPs.

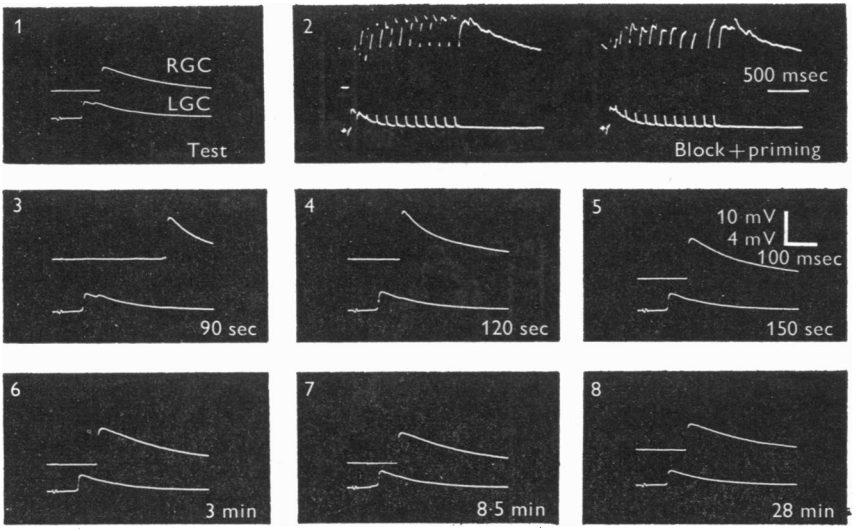


Fig. 4. Heterosynaptic facilitation of the isolated synapse on the RGC. Upper traces, recordings in the RGC; lower traces, recordings in the LGC. 1. Control amplitude of right and left test EPSPs. During the whole experiment, the test EPSPs were produced at a frequency 0·1/sec. 2. After blocking the left pleuro–abdominal connective, the EPSP in the RGC disappeared (not visible here, e.g. Fig. 5, trace 2) and two trains of heterosynaptic stimuli were applied to the siphon nerve. As a consequence EPSPs and spikes (only partially reproduced here) appeared in the RGC, but due to the block, only stimulation artifacts were seen in the LGC. 3. First right EPSP after interruption of the block (the figures in the lower left corner indicate time after the beginning of the heterosynaptic stimulation). Note the increase of the interval between EPSPs due to a residual conduction block. 4. Maximum amplitude increase of the right EPSP. Traces 5 to 8, progressive recovery of the initial amplitude levels of the right EPSP which was completed in 28 min (record 8).

*Heterosynaptic facilitation at an isolated presynaptic terminal*

Since it was established that the common presynaptic neurone had a branch extending caudally from the peri-oesophageal group of ganglia to the abdominal ganglia through the left pleuro-visceral connective, it was thus possible, by blocking the conduction in between caudal ganglia and

the abdominal ganglion, to produce a heterosynaptic facilitation restricted to the synaptic ending on the RGC in the abdominal ganglion. In such condition the stimulation used to obtain this effect could not reach synaptically the common interneurone. Moreover, it was also possible to control if the presynaptic neurone had been fired during the stimulation or afterwards by recording continuously in the LGC. In this way one of the major causes of uncertainty in the experiments reported by Kandel & Tauc (1965) could in principle be eliminated.

Throughout all the experiments reported in this section the common presynaptic neurone, defined above, was used. With the right connective cut at the start of the experiments, the left connective was the only remaining link between the peri-oesophageal and the abdominal ganglia.

When an isotonic sucrose solution was applied to a restricted segment of the left pleuro–abdominal connective (see Methods and Fig. 1), a conduction block occurred as shown by the disappearance of the right EPSP. Also no activity could be seen in the LGC when stimulating one of the nerves originating at the abdominal ganglia (Fig. 4), including that part of the left connective which was distal to the blocked region with respect to LGC. Thus the process of the common presynaptic neurone which terminated on the RGC was functionally isolated from the soma.

The routine procedure used to study the heterosynaptic effect was the following: after several minutes of control stimulation of the left anterior tentacular nerve, in order to establish a 'steady state' in the amplitude of the habituated EPSPs (Fig. 4, trace 1), the sea water in the compartment which isolated a segment of the left connective was replaced with isotonic sucrose solution. In about 1–2 min conduction in the process making contact with the RGC was abolished and the right EPSP disappeared (Fig. 5, trace 2). This was preceded by a short transitional period during which the conduction velocity diminished progressively, as shown by the lengthening of the interval between the EPSPs. The left EPSP continued to appear normally as the stimulation of the left anterior tentacular nerve was maintained (Fig. 4, trace 2).

Usually 1–2 min were allowed to secure a complete block of the whole connective and then the standard stimulation of two trains was applied to a given nerve of the abdominal ganglia. This stimulation produced a burst of synaptic potentials and spikes in the RGC (Fig. 4, trace 2). When this activity subsided (to avoid a background synaptic noise which could obscure the observations of the right EPSP), sea water was re-introduced in the compartment filled with sucrose solution. Conduction was re-established and the right EPSP reappeared in approximately 30–60 sec. A residual, partial block was observed during recovery which transiently increased the interval between the two EPSPs (Fig. 4, traces 3 to 5).

Under these circumstances an increase in the amplitude of the right EPSP was usually observed. This effect was, in general, composed of two elements: (1) a transient increase due to the dishabituation produced by the rest imposed through the block of the right presynaptic terminal, and (2) a long-lasting augmentation produced by the heterosynaptic stimulation. The increase in amplitude due to dishabituation was usually very

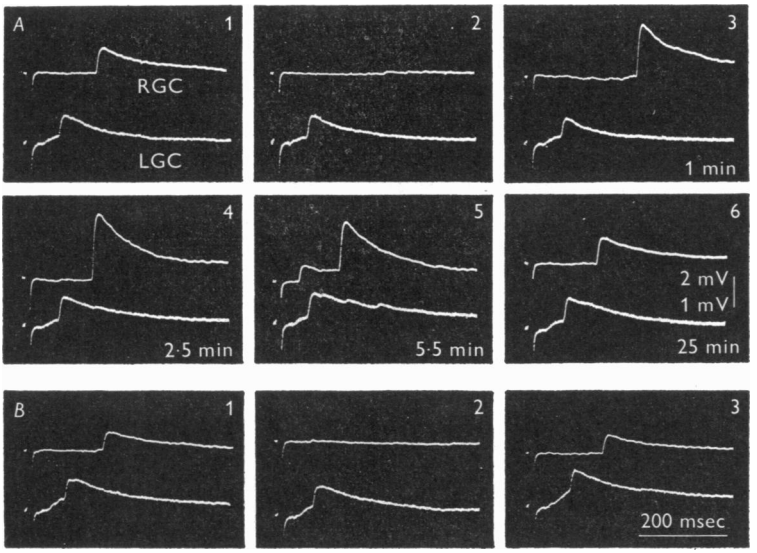


Fig. 5. Heterosynaptic facilitation due to mechanical stimulation of a piece of skin. Upper and lower records: RGC and LGC respectively. *A* 1: control. The left connective is already developing a conduction block. *A* 2: complete block of conduction. During this period mechanical stimulation of the skin innervated by the siphon nerve (see text) is applied to induce heterosynaptic facilitation. *A* 3: first EPSP reappearing in the RGC after partial removal of the conduction block of the left connective. *A* 4: maximum of heterosynaptic facilitation of the right EPSP. *A* 5 and *A* 6: gradual disappearance of the facilitatory effect. The time values indicated in the lower left corner of each frame are computed from the beginning of the heterosynaptic stimulation. *B*. Control of the dishabituation of the right EPSP due to the rest imposed on the synapse during the block. *B* 1: control before block. *B* 2: block of conduction of the same duration as in *A* 2 without mechanical stimulation of the skin. *B* 3: after recovery from block. Dishabituation was not apparent.

short (Figs. 6 and 8) and often there was no such effect at all (cf. Fig. 5). This especially was the case in the more prolonged experiments. In this case the habituation had accumulated to levels which were not counteracted by the short resting period.

In any case, it was easy to check the participation of the EPSP habitu-

ation by using the above described experimental procedure but omitting to apply heterosynaptic stimulation during the block (Fig. 5*A*, 6*Ab*).

The heterosynaptic facilitation of the right EPSP by stimulation of the abdominal nerves, during block of the left connective, had temporal parameters similar to those of the effect of the same stimulation without such a block, except for the intensity. Indeed the heterosynaptic facilitation applied during block of the left connective was very frequently much more important than the effect when the stimulation was applied without such block (Fig. 3). No specific explanation could be found for this fact but some of its implications are considered in the Discussion. No change was seen in the left EPSP for an effective heterosynaptic stimulation of the abdominal nerves when the left connective was blocked (Fig. 4).

These experiments still left open the possibility that the heterosynaptic stimulation was creating a post-tetanic effect through collaterals of the presynaptic process giving the right EPSP. Such branches could exist in the nerves used to produce the heterosynaptic effect and thus activate antidromically the terminal on the RGC. It was thought that if stimulation of peripheral mechanoreceptors was used such a possibility of antidromic stimulation would be avoided.

With this objective in mind a preparation of the whole ganglionic system was used with a piece of skin attached as described in Methods. The region of the skin dissected corresponded to the area innervated by the siphon nerve (n. anal., Eales, 1921). To avoid any direct activation of the neurones in the ganglia during the mechanical stimulation, the stimulated area was mounted in a separate compartment of the chamber.

The stimulation of the skin consisted of light scratching with a blunt glass rod or, in most cases, the projection of a jet of sea water. This stimulation was used in a sequence similar to the one described above for the electric stimulation of the nerve. Heterosynaptic facilitation was also obtained in these experiments under block of the left connective (Fig. 5), even if the maximum effect was weaker as compared with the effectiveness of the supramaximal electric heterosynaptic stimulation of the siphon nerve in the same experiment. The intensity of the facilitation obtained with the mechanical stimulation depended upon the region to which it was applied and its strength, i.e. the pressure exerted with the glass rod or the closeness of application of the water jet. Four to six stimulations, spaced over 10–15 sec, and repeated once or twice, were clearly more effective than a more frequent stimulation, and increased the amplitude of the right EPSP 50–100 %. More frequent stimulation reduced in effectiveness as did the repeatedly applied electric stimulation.

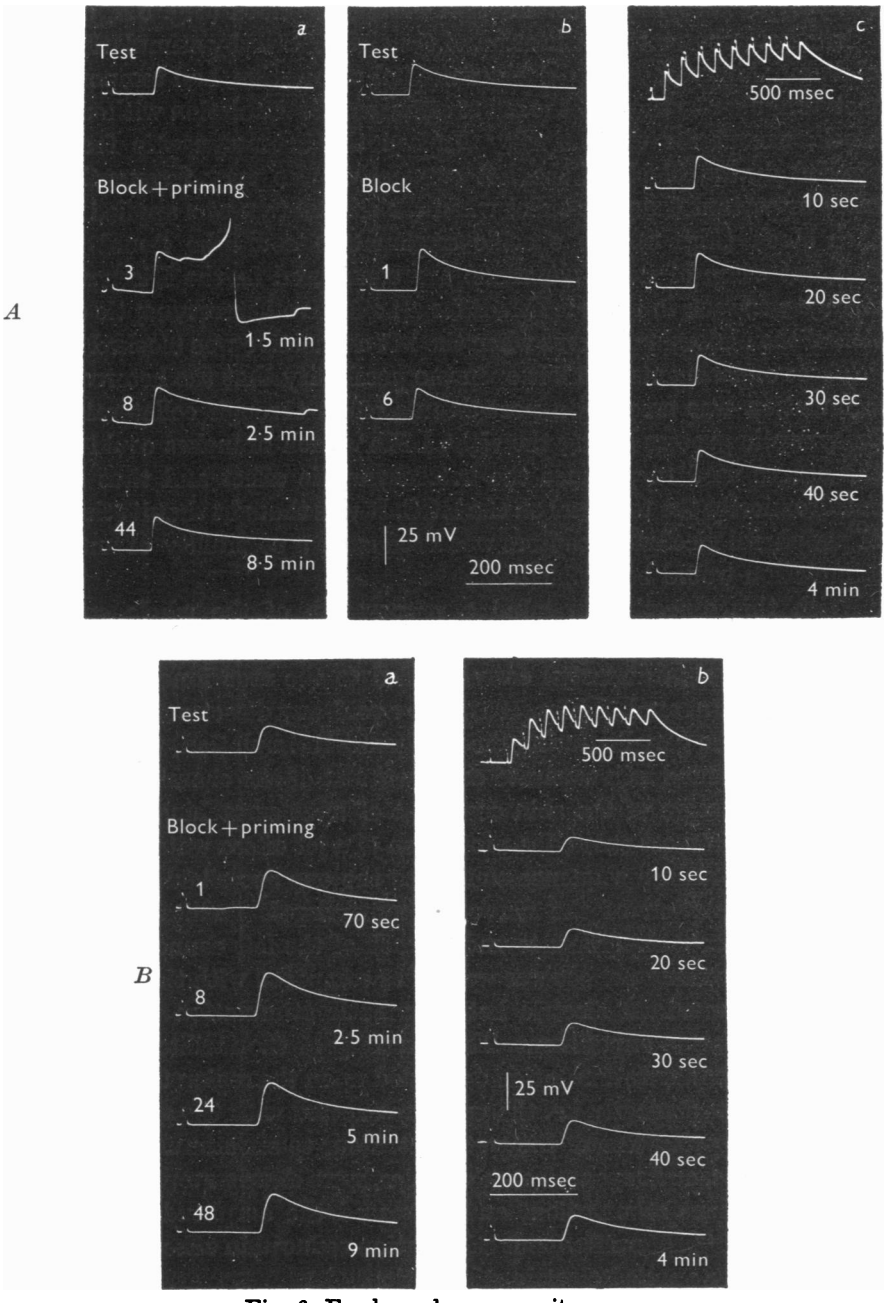


**Fig. 6. For legend see opposite page.**

### *Heterosynaptic facilitation in conditions of depressed post-tetanic potentiation*

Hubbard & Gage (1964) were able to depress post-tetanic potentiation of neuromuscular transmission in the rat by means of drugs known to affect the $Na^+$-pump mechanism. This suggested a quite different approach to demonstrate the difference between post-tetanic potentiation and heterosynaptic facilitation.

An attempt was made to abolish in our preparations the post-tetanic potentiation by exposing the preparation to a low temperature, and by replacing the $Na^+$ in the artificial sea water with an equimolar amount of $Li^+$. The observations were performed also on unitary excitatory inputs to the RGC activated by threshold stimulation of one of the nerves to the abdominal ganglia, usually the right or the left pleuro-visceral connectives (cf. Kandel & Tauc, 1965). This was possible since isolation of the presynaptic axon terminal was not necessary. Only those preparations showing a clear post-tetanic potentiation of the EPSPs at room temperature were used.

When the temperature of the bathing fluid was gradually diminished

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Legend to Fig. 6.

Fig. 6*A*. Heterosynaptic facilitation and post-tetanic potentiation at 22° C (room temperature). The EPSP in the RGC due to stimulation of the left anterior tentacular nerve. *a*: heterosynaptic facilitation produced by stimulation of the siphon nerve at 8/sec (two trains of 1 sec at a 10 sec interval). Maximum amplitude increase after starting heterosynaptic stimulation (150 % of control amplitude). After 44 stimulations and 8·5 min heterosynaptic facilitation is still present (120 %). *b*: dishabituation effect (135 %). After six stimulations (1 min) the EPSP is at its control level. *c*: post-tetanic potentiation after a tetanization of the left anterior tentacular nerve with the same stimulus parameters used for the heterosynaptic effect. Maximum effect at 20 sec after the tetanus (40 sec after the start of the stimulation) with 125 % of control amplitude (first EPSP of the train in the Figure). Recovery of the control amplitude at 40–50 sec without further amplitude variations.

*B*. Heterosynaptic facilitation and post-tetanic depression at 8° C. Same preparation as in 6*A*. *a*: heterosynaptic effect after stimulation of the siphon nerve. The maximum effect was seen at 2·5 min (160 % of control), at the 8th stimulation, and facilitation was still present after 9 min and 48 stimulations (140 % of control). No dishabituation due to the short block of conduction was seen at this stage of the experiment. *b*: repetitive stimulation of the left anterior tentacular nerve yielded a depression to 50 % of the control (first EPSP of the train) but with recovery to the original amplitude following a somewhat similar time course as the potentiating effect. Note the prolonged time course of the EPSPs at this temperature.

from room temperature (22–24° C), to about 18° C, a period of strong spontaneous activity appeared in the ganglion which frequently induced repetitive firing of the RGC. This contrasted greatly with the low level of synaptic noise at the higher temperatures. This high activity subsided when a temperature of about 10° C was reached. At temperatures between 7 and 9° C (i) the RGC was slightly depolarized (probably because of the partial inactivation of the electrogenic $Na^+$-pump; Carpenter, 1967), (ii) the conduction time, which had been increasing steadily, was approximately doubled, (iii) the threshold for activation of the test EPSPs was increased and (iv) the potentials, though unchanged in amplitude, had a considerably slower rising phase.

At the same time the post-tetanic potentiation of the test EPSPs disappeared (Fig. 6*Ac* and *Bb*). In several experiments, the post-tetanic effect was even reversed into a depression which lasted a short time (30–60 sec). This depression seemed to have the same duration as the potentiation obtained at room temperature (Fig. 6*Bb*). The heterosynaptic facilitation, on the contrary, was unaffected by the temperature reduction and maintained a relatively constant intensity and duration (Fig. 6*Aa* and 6*Ba*).

Similar results were obtained when the $Na^+$ in the bathing solution was replaced by $Li^+$, at room temperature. The post-tetanic potentiation was depressed or abolished, or changed to a post-tetanic depression, while the heterosynaptic facilitation remained unchanged.

The disadvantage of the latter method was that in general it took 90–150 min to abolish the post-tetanic potentiation, and frequently the test EPSP suddenly disappeared. This suggested a conduction block in the presynaptic fibre, and was rapidly reversed by reintroducing $Na^+$-artificial sea water. The amplitude of the EPSP was maintained practically unchanged or enhanced during the exposure to the $Li^+$-artificial sea water, while the duration of the rising phase was augmented.

In one experiment in $Li^+$-artificial sea water, a heterosynaptic facilitation could be produced with a stimulus intensity which evoked only a minimal activity in the RGC. In this case it was clearly seen that the test EPSP had not been fired by the heterosynaptic stimulus. Four trains of heterosynaptic stimuli induced a small complex wave of depolarization which reached the firing level of the RGC only once during the first train. The depolarization diminished progressively with each train (Fig. 7*A* 2). The facilitatory effect was quite large and lasted more than 9 min (Fig. 7*A* 3 to *A* 7). Homosynaptic trains of the same frequency as the heterosynaptic ones produced very little potentiation (Fig. 7*B* 1 to *B* 3). During these trains, the test EPSP could be identified very easily. Since the first EPSP of the first homosynaptic train (Fig. 7*B* 2) depolarized the RGC much more than the initial part of the wave produced by the hetero-

synaptic stimulation (Fig. 7*A* 2), it seems safe to assume that heterosynaptically induced depolarization did not contain the test EPSP. The depression of the excitability seen in experiments performed at low temperature or in $Li^+$-artificial sea water also gave the impression that no indirectly evoked post-tetanic effect was involved in the heterosynaptic facilitation.

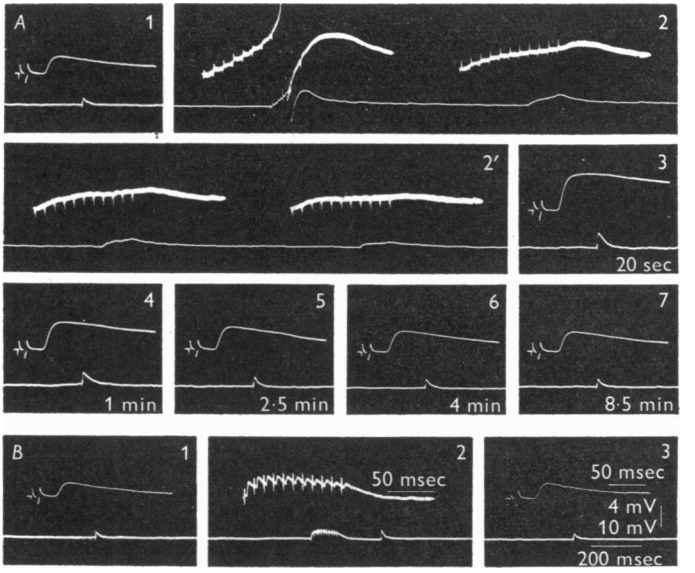


Fig. 7. Heterosynaptic facilitation in a $Li^+$-sea water-exposed abdominal ganglion. *A* 1: the EPSP was obtained by stimulation of the pleuro–abdominal connective. *A* 2: after 90 min of exposure to the $Li^+$-sea water, very little activity appeared in the RGC during heterosynaptic stimulation (branchial nerve) due to a general loss of excitability (see text). *A* 3: 20 sec after ending heterosynaptic stimulation, a maximum increase in EPSP amplitude (210% of control). *A* 4 to *A* 7: progressive return to the original amplitude. *B* 1: control. *B* 2: tetanization of the right pleuro-abdominal connective (stimulation with the same parameters as used on the branchial nerve). *B* 3: no post-tetanic effect was seen.

## DISCUSSION

A study of presynaptic mechanisms in the central nervous system of *Aplysia* suffers from practical limitations which require the use of rather indirect approaches, since the presynaptic terminal is practically inaccessible, and in many cases the presynaptic neuronal soma cannot be identified. These limitations obliged us to make some assumptions which require explicit analysis. We must first consider the conditions which led to the conclusion that we were dealing with an input that originated in a

neurone which had simultaneous synaptic contacts with both giant cells, the RGC and LGC.

Kandel & Tauc (1965) analysed the electrophysiological criteria for establishing that a unitary EPSP was monosynaptic. In the present case the criterion of a constant latency between the left and right EPSP was the main element in the assumption that one common presynaptic neurone produced an EPSP in each giant cell. If there were more than one synaptic step between (1) the last link that was common in the pathway producing the two EPSPs and (2) each of the neurones in which the EPSPs were recorded, then such an arrangement would give rise to loose temporal co-ordination of these synaptic potentials.

By the same reasoning, the variability of the latency with respect to stimulus led to the conclusion that the neurone generating the EPSPs in the giant cells was activated through a pathway containing at least one synapse. The large variability of latencies also eliminates the possibility that the common interneurone sends a process into the left tentacular nerve where it could be antidromically stimulated.

A further assumption was that the soma of the common presynaptic neurone is located in one of the ganglia of the peri-oesophageal ring. This was based upon (i) the temporal sequence of the two EPSPs and (ii) the maintenance of the left EPSP after separation of the abdominal ganglion. One can then say that any stimulation of the abdominal nerves producing heterosynaptic facilitation of the right EPSP could not induce a synaptically driven firing of the presynaptic terminal if the same was applied while these ganglia were functionally isolated from the peri-oesophageal ring. This was the core of the working hypothesis considered in the first group of experiments, which sought to induce heterosynaptic facilitation in a situation in which the facilitating stimulus had no possibility of synaptically inducing a repeated activation of the nerve ending giving rise to the test EPSP.

The possibility of an antidromic activation of the presynaptic ending via collaterals during heterosynaptic stimulation seemed unlikely for various reasons. First, a given pattern of repetitive stimulation was much more effective in inducing a heterosynaptic facilitation through various nerves than in the generation of a post-tetanic potentiation via the left anterior tentacular nerve. To explain this difference with a pure post-tetanic effect, it is necessary to claim that the hypothetical antidromic activation elicited a firing frequency higher than the stimulus frequency; however, we never saw such a burst of high frequency firing after the trains applied to the left anterior tentacular nerve. Furthermore, it has been shown (Tauc & Hughes, 1963) that antidromic conduction is in general easily blocked at branching points, and this would rather restrict the efficacy of antidromic stimula-

tion. Heterosynaptic facilitation due to indirectly evoked post-tetanic potentiation would also hardly explain the different degrees of facilitation attained with the same train of stimulus applied to various nerves and the differences in effectiveness for the stimulation of the 'isolated' or 'unisolated' abdominal ganglia (Fig. 3).

The effectiveness of mechanically stimulating the skin innervated by the genital nerve to induce heterosynaptic stimulation makes an antidromically induced post-tetanic potentiation still more unlikely. The lower excitability seen in the ganglia exposed to $Li^+$-artificial sea water or cooled to 7–9° C reduced and shortened the synaptic bombardment in RGC due to the heterosynaptic stimulation. With such a low level of synaptic noise it could be directly ascertained that there was no repetitive firing of the test EPSP to explain the amplitude increase through an indirectly evoked post-tetanic effect.

The most positive evidence of the independence between the post-tetanic potentiation and heterosynaptic facilitation was provided by the experiments under cooling or $Li^+$-artificial sea water, which selectively suppressed post-tetanic potentiation.

Though it was recently shown that the lack of correlation between the changes in the post-tetanic polarization of the terminals and the potentiation of the end-plate potentials contradicts a causal correlation between these two variables (Gage & Hubbard, 1966*b*), our results converge with some of the results of these authors to show that procedures which have been shown to depress the post-tetanic hyperpolarization due apparently to the activity of a $Na^+$-pump mechanism in various neural structures (Ritchie & Straub, 1957; Greengard & Straub, 1958, 1962; Connelly, 1959, 1962; Straub, 1961; Holmes, 1962; Nakajima & Takahashi, 1966; Rang & Ritchie, 1968; see also Gage & Hubbard, 1966*a*), also abolish the post-tetanic increase of post-synaptic potentials.

Two interesting points not directly related to the main subject of this paper can be discussed in relation to the action of $Li^+$-sea water on our preparation. These points concern the ionic mechanisms of both EPSP and the responsible presynaptic spike.

Both giant cells are of H-type, that is have inhibitory inputs conveyed by a cholinergic synaptic mechanism producing chloride permeability change (Tauc & Gerschenfeld, 1962). In such cells the EPSPs thus result most probably from a $Na^+$ permeability. As no immediate change of the aspect of the unitary EPSP has been observed when $Li^+$ sea water replaced normal sea water, it can be concluded that $Li^+$ forms in this process a perfect substitute for $Na^+$. On the other hand about 2 hr were necessary to $Li^+$-sea water to block the conduction in the presynaptic neurone, as can be concluded from the sudden disappearance of the EPSP at that time.

One has to admit either that $Li^+$ can be substituted for $Na^+$ in the spike-generating mechanisms or that $Na^+$ is not involved in the generation of spikes, which might be calcium-dependent as suggested for some other molluscan neurones (Meves, 1966; Gedulgig & Junge, 1968).

Little can be said with respect to the mechanism underlying the heterosynaptic facilitation. Kandel & Tauc (1965) already found that there were no changes in the electrical properties of the post-tetanic membrane to account for the increase of the EPSP amplitude. The other change of postsynaptic origin which could show up an enlarged EPSP is a sensitization of the subsynaptic receptors. The test of this possibility is ruled out as long as the transmitter involved remains unidentified, but such a phenomenon is not known to occur normally. Dudel (1965) has shown facilitating effects of 5-hydroxytryptamine on neuromuscular synapses of crustacea, and their mechanism could be relevant to our observations. Atwood (1967) has seen also a heterosynaptic facilitation in such type of synapses.

Mendell & Wall (1964) have postulated in the spinal cord the existence in one particular case of a depression of a tonic presynaptic inhibition which produced an enhanced synaptic potential. We never had any clear observation which could be interpreted as showing the existence of presynaptic inhibition. The RGC pertains to a group of cells clearly distinct from the one which receives synaptic activity regulated by such a presynaptic mechanism (Tauc, 1965).

Another way to rule out such a possibility arose from the observation that a given heterosynaptic stimulation was much more effective when applied to 'functionally isolated' abdominal ganglia. If a hypothesis such as the one suggested by Mendell & Wall could also apply to our case then different tonic levels of presynaptic inhibition in the 'isolated' and 'non-isolated' ganglia could explain variation in the effectiveness of heterosynaptic stimulation. The fact that the simple 'isolation' of the abdominal ganglia without heterosynaptic stimulation had either no effect on the right EPSP amplitude (Fig. 8) or only an effect which could be accounted for the concurring dishabituation (cf. page 19) due to the resting of the right terminal spoke against such a possibility.

The intervention of a tonic presynaptic inhibition is also not relevant to the lack of any important change in the efficiency of the heterosynaptic stimulation in the preparations at low temperature. The strong general effect of reduction on spontaneous and evoked activity in these cooled preparations would be expected to modify seriously the parameters of the heterosynaptic effect of a rather complex system involving a tonic activity. Moreover, the rather direct relationship between intensity of heterosynaptic stimulation and facilitation also refutes such an indirect mechanism. The limited curtailment of the conspicuous long duration of

the heterosynaptic facilitation in the preparations at low temperature suggests that this characteristic is probably unrelated to the long-lasting firing of certain neurones.

It seems likely that the heterosynaptic effect on the presynaptic terminals is mediated on more or less specific contacts ('episynapse'; Tauc, 1965, 1967) via specific transmitter substance. The duration could be explained by the prolonged action of the transmitter with peculiar cumulative effects, such as it has been observed in the same ganglion, in another

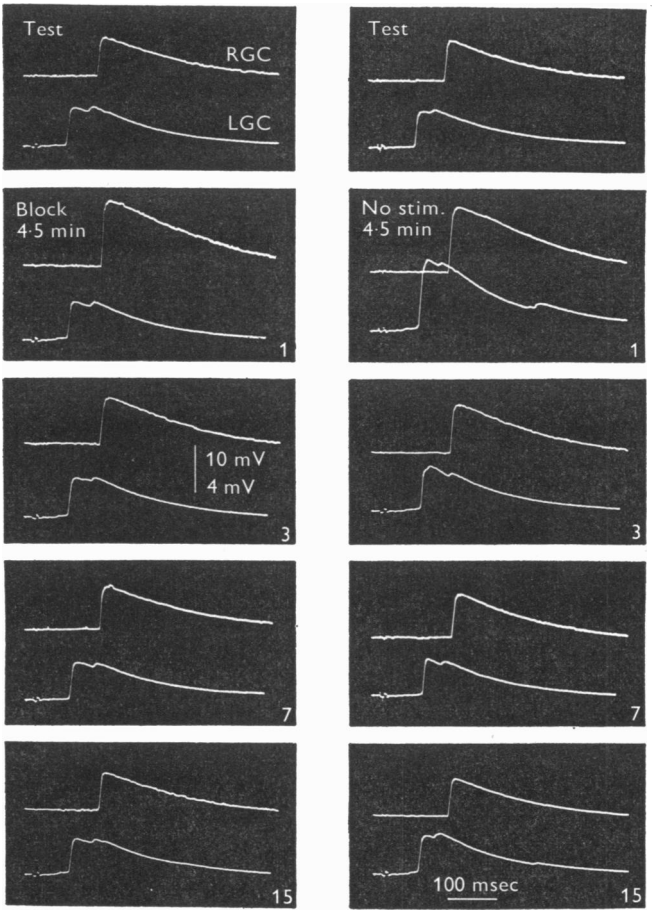


Fig. 8. Dishabituation of the EPSP in the RGC due to block of the left pleuro-visceral connective (left column) in comparison with the dishabituation due to interruption of the stimulation of the left anterior tentacular nerve (right column). A similar degree of dishabituation is seen for the right EPSP in both cases, while variations in amplitude of the left EPSP are seen only when the stimulation is discontinued. The isolation alone of the two ganglia during the block does not affect the left EPSP amplitude.

long-lasting transmission process, called inhibition of long duration (Tauc, 1968*a*, *b*). Except for the opposite polarity of effects, the heterosynaptic facilitation and heterosynaptic inhibition show comparable parameters, and it was suggested that their mechanisms are fundamentally identical (Tauc, 1965). Most probably they both act on the quantity of transmitter released and consequently can be called presynaptic. As during presynaptic inhibition, no obvious changes in the time-dependent parameters of the EPSPs during the facilitated state were noticed, suggesting that no changes in the elimination or the inactivation of the transmitter nor qualitative modifications in the mechanism of transmitter release should be expected.

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