

HETEROSYNAPTIC FACILITATION IN NEURONES OF THE ABDOMINAL GANGLION OF *APLYSIA DEPILANS*

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The cellular mechanisms whereby a neural network undergoes a long-lasting change in its response to a given stimulus, when the latter is repeated or combined with other stimuli, are still mainly unexplored. An important beginning has been the investigation of the effects of physiological use and disuse and of tetanic nerve stimulation on the mono-synaptic spinal reflex (Eccles, 1958, 1964). This work has shown that limited potentialities for synaptic plasticity can be demonstrated, even in this simple system, with repeated homosynaptic stimulation. The plastic changes so produced manifest themselves as an alteration in the amplitude of the excitatory post-synaptic potential.

The neurophysiological application of complex intermittent and hetero-synaptic stimulus patterns, modelled on those used in psychological learning experiments, have usually been limited to experiments in which behavioural conditioning was established in the whole animal. While some important electrical correlates and neural determinants of the conditioning process have been described in these studies, it seems unlikely that the electro-encephalographic methods employed will yield much information about the mechanisms underlying the observed electrical changes (see reviews by Morrell, 1961; Doty & Guirgea, 1961). A more detailed analysis of the long-term neural consequences of these complex stimulus sequences would seem to require the use of analytic techniques capable of distinguishing between different cellular mechanisms as well as the selection of simpler preparations than those currently used in conditioning the whole animal (Eccles, 1958; Spencer, Thompson & Neilson, 1964).

This paper and the one that follows (Kandel & Tauc, 1965) describe an attempt to apply a heterosynaptic stimulus sequence, analogous to that used in psychological learning experiments, to a numerically simple and

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isolated preparation, the abdominal ganglion of the sea slug *Aplysia depilans*. The advantages of this preparation for the study of cellular neurophysiology are well known (Arvanitaki & Chalazonitis, 1958; Tauc, 1958). The ganglion contains only several hundred cells most of which are large, clearly visible and accessible to micro-electrode investigation. A few of these cells, such as the right upper-quadrant giant cell, are readily identifiable in each preparation (Arvanitaki & Cardot, 1941; Arvanitaki & Tehou-Si-Ho, 1942). The biophysical properties of some of these cells, their pharmacology and their ability to participate in simple forms of plastic changes have been established (Tauc, 1958; Fessard & Tauc, 1958; Tauc & Gerschenfeld, 1961; Frank & Tauc, 1963). Also, it has been shown that certain molluscs can be conditioned (Thorpe, 1956).

The stimulation procedure employed in these experiments represents an attempt to simulate the procedure used to produce Type I (classical) behavioural conditioning. Classical conditioning results from the temporal association of two stimuli. An initially ineffective stimulus becomes effective after a period during which it was applied together with an effective stimulus. In the present experimental situation two different afferent pathways to a quiescent ganglion cell were selected. The parameters of the stimulus applied to one of these pathways were adjusted so that the stimulus produced only a small excitatory post-synaptic potential (test stimulus). The other pathway was stimulated strongly enough to produce a train of action potentials in the cell (priming stimulus). When the priming stimulus was presented, it followed closely upon the test stimulus ('pairing' of stimuli) and the effects of several repetitions of this procedure on the response to subsequent test stimuli were examined.

The present paper describes two types of heterosynaptic facilitatory effects which were produced when this stimulus-pairing sequence was applied to different unidentified cells and to a constantly identifiable giant cell in the right upper quadrant of the abdominal ganglion. The subsequent paper will examine the mechanism of the one type of heterosynaptic facilitation that occurred in the giant cell. Preliminary reports of these investigations have already been published (Kandel & Tauc, 1963, 1964).

METHODS

The abdominal ganglion of *Aplysia depilans* was dissected from the animal along with the three main peripheral nerves which emerge from the lower portion of the ganglion. These connectives and nerves are constant in position and easy to identify (see Figs. 2 and 9). The left and right pleuroabdominal connectives are mixed association tracts containing both afferent and efferent fibres. The three peripheral nerves are mixed nerves, the siphon nerve (called n. abdominales by Eales (1921) and the anal nerve by Arvanitaki & Chalazonitis (1958) and by Segundo, Moore, Stensaas & Bullock (1963)) supplies the anal region, the genital duct, the accessory reproductive glands and the spout or siphon. The branchial nerve

supplies the purple gland, the osphradium and the body wall, and the genital nerve supplies the genital apparatus, the pericardium, heart, kidney, midgut and some body wall.

The ganglion was pinned through the edges of the connective-tissue capsule to the paraffin base of a chamber and was covered with sea water. In early experiments, the chamber contained only two pairs of Ag-AgCl electrodes but in later experiments a chamber containing five pairs of Ag-AgCl stimulating electrodes was used.

The connective tissue overlying the ganglion was carefully dissected under a microscope with a microscalpel. For work on the giant cell the microscalpel was positioned by a DeFonbrune micromanipulator. This method permitted a small opening to be made in the connective tissue overlying the giant cell while leaving the rest of the ganglion essentially undisturbed. Micro-electrodes containing 3 M-KCl or 0.6 M- K_2SO_4 were led through a unity gain negative capacitance preamplifier to the differential amplifiers of a double-beam oscilloscope. The amplifiers were either d.c. or a.c. coupled; in the latter mode of operation the input time constant was 0.5 sec. A simple Wheatstone bridge (Kandel, Spencer & Brinley, 1961) was used for passing current through the recording electrodes.

Only cells which showed no 'spontaneous' spike activity and in which a stable test input could be obtained were used in these experiments. The giant cell never showed spontaneous spiking in the isolated ganglion unless damaged. Unidentified cells were often spontaneously active and were hyperpolarized when necessary to prevent spontaneous firing. Only test or priming inputs containing purely excitatory components, as manifested by the PSPs in the impaled cell, were utilized. The stability of the input was judged by its ability to maintain a fairly constant amplitude excitatory post-synaptic potential (EPSP) for long periods of time. In some cells, the EPSP amplitude was a function of the repetition rate. The PSP was larger at slower frequencies and reached its maximum height at a repetition rate of about 1/2 min. In these cells, the PSP amplitude was two to three times greater with slow repetition rates (1/2 min) than with the repetition rates generally used (1/10 sec). Since repetition rate was held constant throughout most of the experiments, this factor did not generally enter into the analysis. However, in some instances the test input was purposely turned off for a period of time. In these cases the resultant change in the repetition rate had to be taken into consideration. This was done by determining the response decrement curve for the input, i.e. by measuring changes in test EPSP from its maximum to its new steady-state value as a result of a determined control period of 'rest'. Figure 1 shows the response decrement following two consecutive periods during which the test stimulus was not presented for 2 min. Note that the PSP returned to the same steady-state level. The response decrement had a fairly typical time course which was generally much faster than that following optimal heterosynaptic facilitation. In a considerable number of cells the test input was not sensitive to changes in the repetition rate and showed little or no amplitude change following a period of rest (see Figs. 7 and 8).

In many of the later experiments on the giant cell, the polarity of the stimulating current to the test nerve was reversed after each shock. This was done to improve the constancy over time of the test response by minimizing local tissue damage and by preventing electrode polarization. The reversal of polarity produced alternating test responses of different size. In the analysis of these experiments, the EPSPs were treated as two separate populations of test responses and this permitted certain additional comparisons to be made (Fig. 15).

Once stability of the test input was assured, it was paired for 1-5 min, but occasionally for shorter or longer periods, with the priming stimulus. The test input preceded the priming stimulus by about 300-500 msec. This stimulus interval was selected from behavioural data (Hilgard & Marquis, 1961). The repetition of the test stimulus was usually 1/10 sec but shorter (1/5 sec) and longer (1/20 sec) intervals were also used in some experiments.

In these experiments, no attempt was made to determine to what extent, if any, the PSPs produced by the test volleys were monosynaptic.

Experiments on the whole animal were carried out according to the technique of Hughes & Tauc (1962). The animal was fixed in a fully extended position on a large dissecting dish by pinning the margins of the head, parapodia and the foot. The haemocoelomic cavity was

opened by a ventral incision which ran from the mantle cavity to the head. The dissection was washed and then kept submerged in sea water. The left connective was cut and the end in contact with the abdominal ganglion was placed on stimulating electrodes. The activity of the right upper quadrant giant cell axon was recorded in the connective with bipolar silver chloride electrodes. A submerged coil of silver wire served as an earth (Fig. 11).

In this paper and the one that follows, we use 'right upper-quadrant giant cell' and 'giant cell' interchangeably. The former is, however, a preferable terminology since the ganglion has other 'giant' cells.

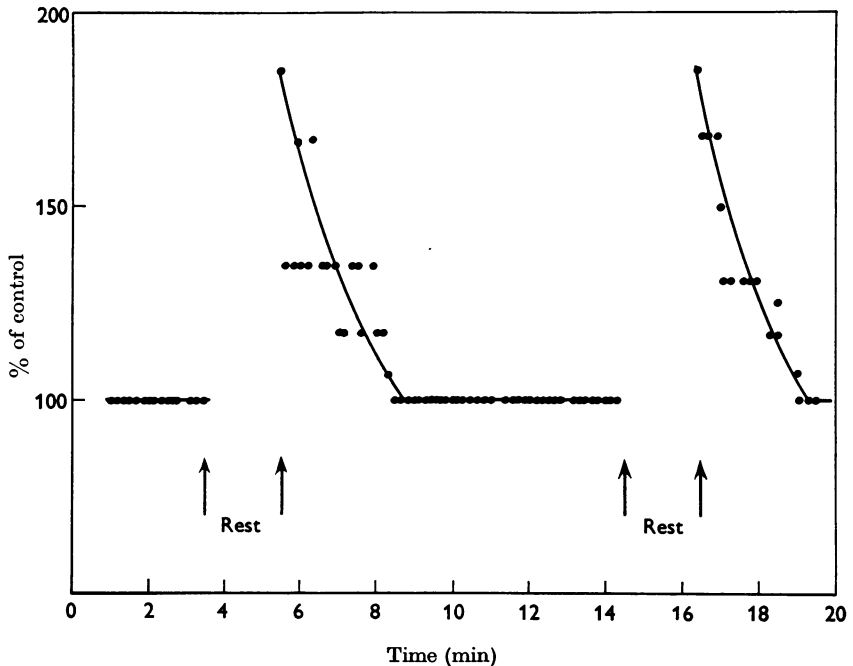


Fig. 1. Response decrement in a test EPSP. Effect of interruption of stimulation (rest) on amplitude of the test EPSP. Left connective stimulated at 6/min except for 2 min periods of rest which are followed by a transient increase in the EPSP.

RESULTS

Experiments were carried out on the dorsal surface of approximately 140 abdominal ganglia. In half of these only the right upper-quadrant giant cell was studied; in the remaining ganglia a total of about ninety different, essentially unidentified cells, were examined. These unidentified cells almost certainly represent a biased sample of the total population of the neurones in the ganglion since no attempt was made to survey systematically all regions of the ganglion.

In most cells examined, heterosynaptic pairing of stimuli produced no facilitation of the test response. However, in the right upper-quadrant giant cell and in fifteen of ninety unidentified cells, most of which were

also located in the right upper quadrant, the test EPSP was significantly augmented during pairing and declined only slowly after pairing. The facilitation in the giant cell differed quantitatively and in several instances qualitatively from that observed in the unidentified cells. In addition, the right upper-quadrant giant cell was readily identifiable in each preparation and gave fairly consistent results. It was therefore possible to study the facilitation which occurred in the giant cell in much greater detail than was possible for the unidentified cells. The studies on the giant cell will be described in the second part of this paper.

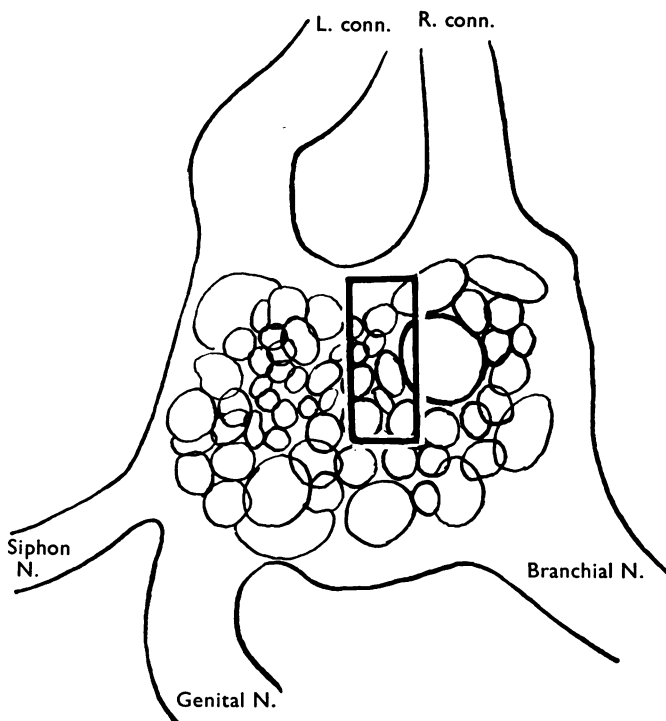


Fig. 2. Schematic view of the dorsal surface of the abdominal ganglion of *Aplysia depilans*. The area containing cells showing heterosynaptic facilitation is outlined. The connectives emerge from the upper corners of the ganglion, the nerves from the lower margin. Magnification approximately $30\times$.

PART I

Unidentified cells

Unidentified cells showing heterosynaptic facilitation tended to be found between the medial border of the giant cell and the mid line. Figure 2 is a drawing of the ganglion in which the area containing most of

these cells has been marked out. The maximum facilitation of the test PSP in these cells was usually about 100% and the facilitation declined over an average period of 9 min and a maximum period of 20 min after pairing (Table 1).

Figures 3 and 4 show the result of an early experiment in this series in which an unusually long period of pairing was used. A single strong shock served as a priming stimulus. Parts 1 and 2 of Fig. 3 show the response to the test and to the priming stimulus, respectively, before pairing. Parts 3, 4, and 5 show the increment in the test response following 5.5, 9.5 and 10 min of pairing. Pairing was carried out for a total of 20 min; and after

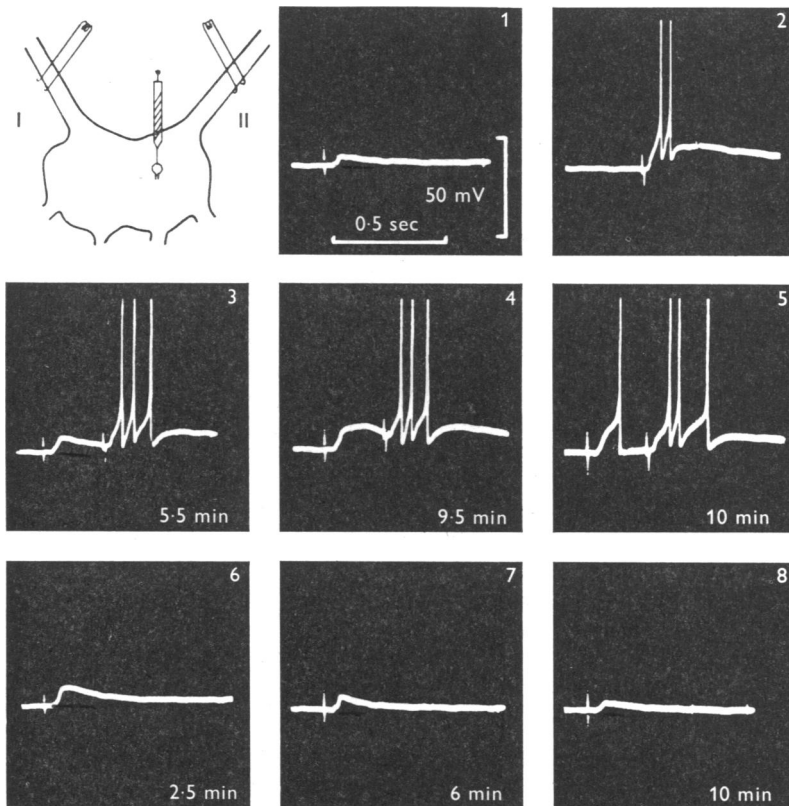


Fig. 3. Heterosynaptic facilitation. The experimental arrangement is indicated in the inset. The test stimulus was a single shock to the left connective, the priming stimulus a single shock of stronger intensity to the right connective. Parts 1 and 2 illustrate the responses to the test and priming stimulus respectively before pairing. Parts 3 to 5 show the changes in the test PSP following 5.5, 9.5 and 10 min of pairing. Note that the augmentation in the test PSP has also produced a slight facilitation in the priming stimulus. Parts 6 to 8 illustrate the decline in the test PSP following pairing. The action potentials have been retouched.

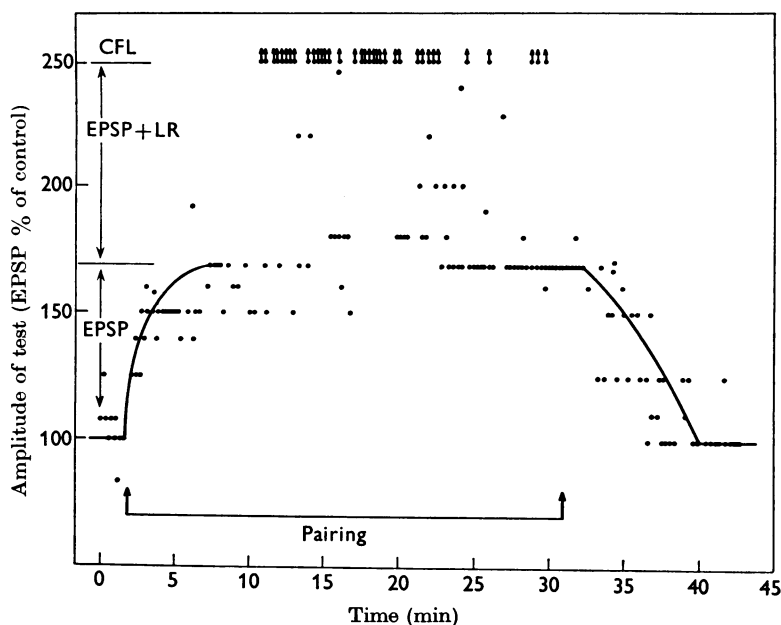


Fig. 4. Time course of heterosynaptic facilitation. Same experiment as illustrated in Fig. 3. In this and subsequent graphs, the percentage change in the test PSP is plotted as a function of time and of pairing. The period of pairing is indicated by the two arrows on the abscissa. The points with arrows indicate the generation of an action potential. LR indicates local response and CFL critical firing level. Note discontinuities in the curve due to the local response and to the generation of spikes.

TABLE 1

Cell	Duration of pairing (min and sec)	Peak facilitation (%)	Duration of post-pairing facilitation (min and sec)
1	26.00	150	8.50
2	12.30	460	7.30
3	5.30	100	19.30
4	3.00	100	7.30
5	3.30	80	5.00
6	2.30	40	4.20
7*	1.50	50	6.30
8*	1.50	80	4.20
9*	2.20	60	18.40
10†	1.50	110	4.00
11†	1.40	120	6.40
12	1.50	140	8.60
13	1.50	44	5.00
14	1.10	110	11.20
15	3.10	170	17.00
Average		122	9.00

* Cells in which specificity to pairing was controlled.

† Cells in which specificity to paired input was controlled.

All cells located in the right upper quadrant, except 2-6 which were either located elsewhere or note was not taken of their position.

the first 6 min of pairing, about 50 % of test stimuli began to produce spikes (Fig. 4). Parts 6, 7, and 8 of Fig. 3 show the decline of the facilitated test PSP during 10 min following the end of pairing. The time course of this facilitation is illustrated in Fig. 4. This graph also emphasizes the discontinuities in the data produced by the local response and the spike (see figure legend for further details).

In this experiment the EPSP in response to the less effective test input increased in amplitude as a result of pairing and reached the critical level for spike generation. To avoid the generation of a spike, which interfered

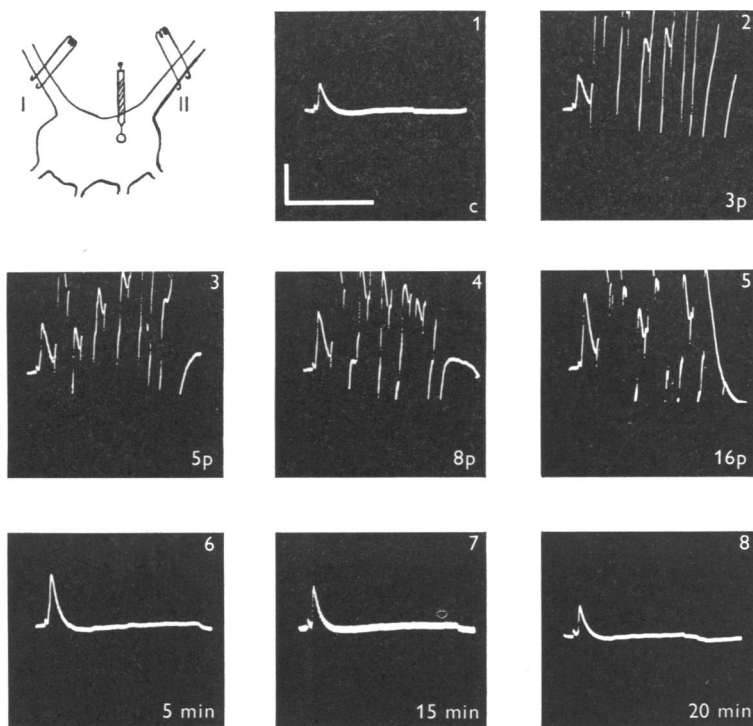


Fig. 5. Heterosynaptic facilitation with a train as a priming stimulus. Inset indicates experimental arrangement. Part 1 illustrates test PSP before pairing. Parts 2 to 5 show the progressive increase in the test PSP following the 3rd, 5th, 8th and 15th of thirty-five pairing trials with a priming stimulus (a 1 sec train at 8/sec). Parts 6 to 8 show decline of facilitated PSP during 20 min following pairing. a.c. recording. Input time constant 0.5 sec.

with the quantitative measurement of changes in the test EPSP, two experimental precautions were usually employed: (1) weak test volleys were used which produced small EPSPs, and/or (2) the post-synaptic membrane was hyperpolarized. Also, it soon became clear that the potentiation, when it occurred, was more pronounced when the priming

stimulus was highly effective. Consequently, in most experiments, a 1 sec train of shocks (usually 6–8/sec) was used as a priming stimulus and shorter periods of pairing (2–5 min) were employed.

Figure 5 illustrates the results from an experiment of this sort. The test response is indicated in Part 1. The gradual increase in the test EPSP after 3, 5, 8 and 15 of 35 pairing trials is indicated in Parts 2 to 5. Parts 6 to 8 indicate the gradual return of the test EPSP to control level during a period of 20 min following the end of pairing.

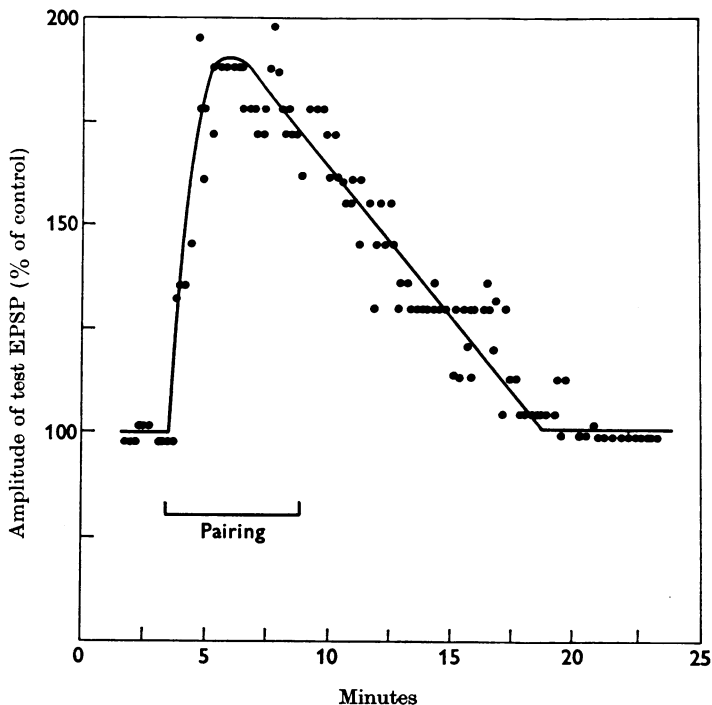


Fig. 6. Time course of facilitation. Same cell as in Fig. 4 but an earlier run. The priming stimulus was a 1 sec train of 8/sec. Pairing lasted 5 min.

Under these experimental circumstances, the changes in the test EPSP are continuous and can readily be plotted as a function of time and of pairing. A graph of this sort is illustrated in Fig. 6 which is based upon results from another run in the same cell illustrated in Fig. 5. Note that the increase of the test EPSP during pairing is fairly rapid and that the decline is much slower.

The facilitation seemed to be related to two independent factors: (1) pairing of the test and priming stimulus and (2) the efficacy of the priming stimulus. Figure 7 is a graph from a cell in which four different experiments were performed to illustrate these points. In the first two experiments a weak

priming stimulus was used and in the second two experiments a strong priming stimulus was used. The parameters to the test stimulus were held constant. During the second and during the fourth experiment, the test stimulus was shut off during the period in which the priming stimulus was presented (unpaired trials). During the first and third experiment the test

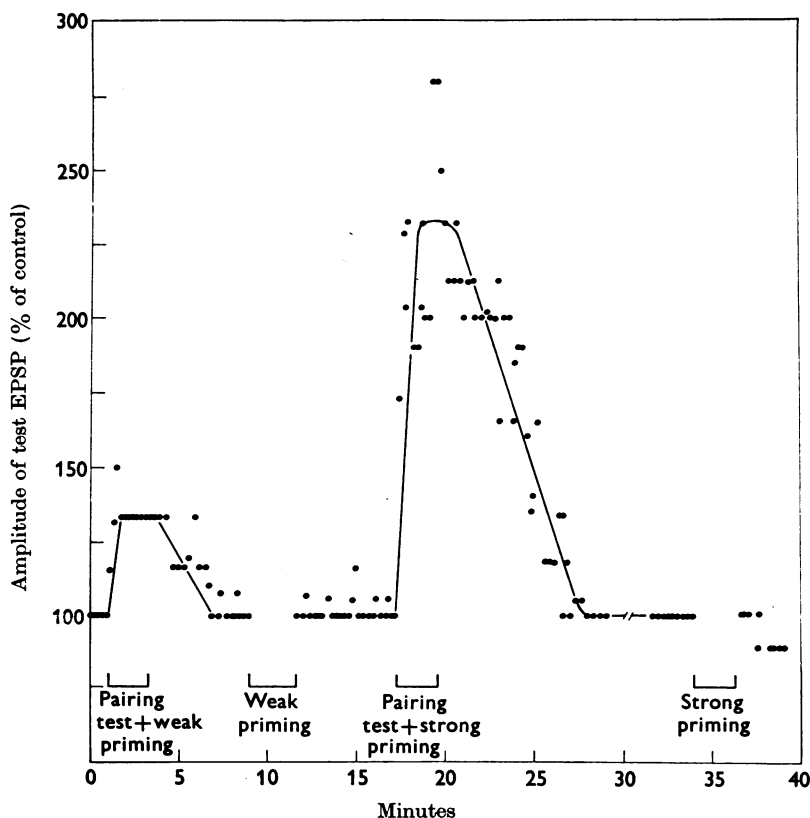


Fig. 7. Effects of paired and unpaired priming stimuli of differing efficacy. Graph illustrates four consecutive runs with the same test input. The first two runs were with a weak priming stimulus (a single shock producing a single spike). This priming stimulus was first paired with the test EPSP and subsequently presented alone. Note that the facilitation only occurred when the test input was paired with the priming stimulus. The next two runs were with a more effective priming stimulus (a train of 6/sec for 1 sec); the facilitation was greater than that with the weak priming stimulus and again the facilitation only occurred with pairing.

and priming stimuli were presented together as usual (paired trials). As Fig. 7 illustrates, in the first experiment, the test EPSP was paired 9 times with a weak priming stimulus (single shock to the left connective). Note a small facilitation which lasted only 3-4 min. When the test response had returned to control level, it was discontinued and the same priming stimulus was

now presented 9 times by itself (unpaired trial). After the presentation of the unpaired priming stimulus, the test EPSP was again introduced, but it showed no facilitation. In the third experiment the test EPSP was

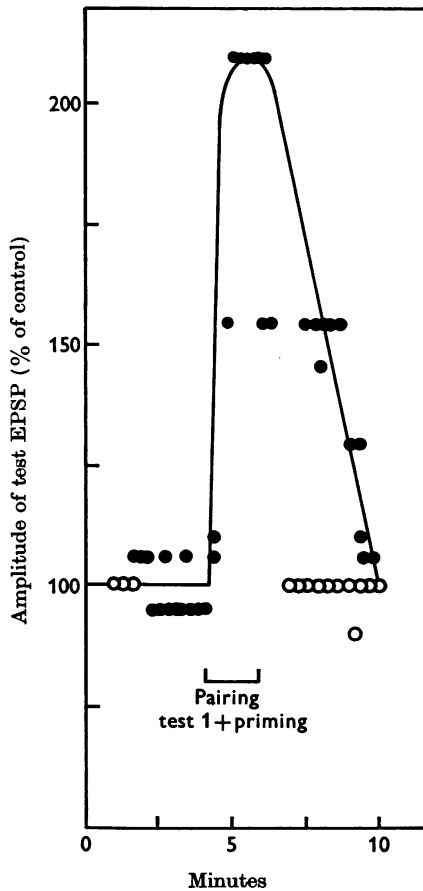


Fig. 8. Two different test nerves were used and the parameters of stimulation applied to each were adjusted to give comparable sized EPSPs. One EPSP was presented throughout the experiment (●) while the stimulus producing the other EPSP (○) was turned off during the period in which the priming stimulus was presented (period of pairing). Consequently, one EPSP (●), test 1, was paired with the priming stimulus, the other EPSP (○), test 2, was not. Facilitation increase occurred only in the EPSP which was paired (●).

again paired for nine trials but now with a more effective priming stimulus (6 shocks/sec for 1 sec). The potentiation is larger than that of the first paired experiment and the decline is slower. In the fourth experiment, the test stimulus was again omitted during the presentation of the strong priming stimulus (unpaired trial) and again the test EPSP was

subsequently not facilitated. The next paired run with the same priming stimulus (not indicated on the graph) again produced facilitation.

In view of the tendency for the priming stimulus to decrease in efficacy with time, only those experiments in which the efficacy of the priming stimulus remained constant in consecutive paired and unpaired runs could be used for comparison. The examination of comparable paired and unpaired runs was performed on only three cells, but in each case the test EPSP was facilitated only when paired with the priming stimulus. This experiment served as an electrophysiological parallel to the control for sensitization (or quasi conditioning) used in behavioural experiments. It indicates that in some cells the facilitation is due to the pairing process *per se*. The question of pairing sequence was not, however, investigated.

This problem was also approached in another way in two other cells. Two test EPSPs of comparable size were used (each produced by a stimulus to a different nerve). One test EPSP was paired with the priming stimulus and the other was not. As Fig. 8 indicates, only the paired EPSP was potentiated.

The findings from these two types of control experiments suggest that the facilitation does not reside at the level of the post-synaptic cell but rather in the pathways shared by two inputs.

PART II

Right upper-quadrant giant cell

To overcome some of the technical limitations encountered in work on the unidentified cells, a more detailed series of experiments was performed on one of the fixed cells of this ganglion, the right upper-quadrant giant cell.

In addition to its position, which was usually but not invariably constant, the right upper quadrant giant cell could also be identified by its great size (300–800 μ), its distinctive dark-orange pigmentation and electrophysiologically by antidromic activation following stimulation of the right connective. In small animals, the right upper-quadrant giant cell was clearly the largest cell in the ganglion; in larger animals there were occasionally one or more other cells of almost comparable size. However, there was rarely any difficulty in the clear identification of the cell.

The course and branchings of the giant axon were known from earlier work (Hughes & Tauc, 1963); we have therefore focused here only on specifying the synaptic contributions of the five major nerves. These are summarized in Fig. 9 in which the usual position of the giant cell is also indicated.

Organization of the synaptic input to the giant cell. The left and right pleuro-abdominal connectives are mixed association tracts containing both afferent and efferent fibres. The right connective carries the giant-cell axon; this fact could readily be demonstrated by the appearance of a typical antidromic action potential upon stimulation of this connective (Fig. 9 superimposed sweeps). The right connective also carries fibres that

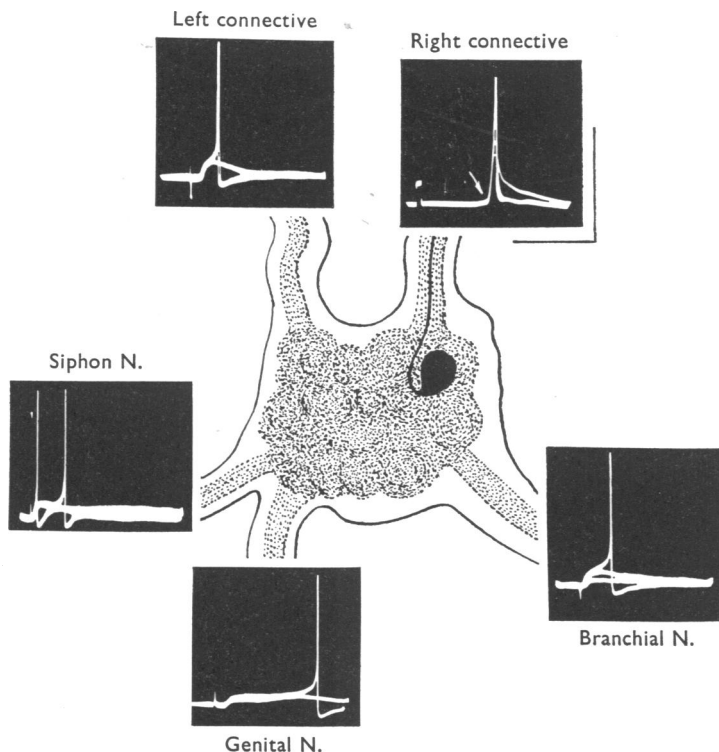


Fig. 9. Input organization of the right upper-quadrant giant cell. The drawing illustrates the typical position of the giant cell. The course of the giant cell axon is only indicated diagrammatically; actually, it runs more centrally into the synaptic neuropile before emerging into the right connective (Tauc, 1962). The insert above the right connective shows the antidromic action potential recorded intracellularly following stimulation of the giant cell axon in the connective. The arrow at the foot of the action potential indicates a small EPSP mediated by fibres whose conduction velocity is faster than the giant axon (see Kandel & Tauc, 1965). The remaining figures indicate the configuration of the PSPs at the critical firing level for each of the afferent inputs. In each case, two sweeps have been superimposed. Note the relatively greater effectiveness of the input from the siphon nerve; this frequently produced repetitive firing to single shock stimulation. (In all records several sweeps have been superimposed.) The voltage calibration is 100 mV for all records. The time calibration is 100 msec for the record of the antidromic action potential (right connective) and 500 msec for the remaining records. The action potentials in all but the antidromic record have been retouched.

produced orthodromic excitation on the giant cells. A particularly interesting afferent contribution, whose latency was even shorter than that of the antidromic spike, is indicated by the arrow. Its properties will be described in the next paper.

The left connective and the three peripheral nerves also produced large EPSPs in the giant cell (Fig. 9). The siphon nerve was the most effective of the several inputs (Fig. 9). Even single electrical stimuli or, in the whole animal, stroking the skin over the siphon frequently set up a repetitive train of spikes in the giant cell. It was for this reason that this nerve was almost invariably used as a priming stimulus in these experiments.

The remaining peripheral nerves, the branchial and the genital nerves, were primarily used as test nerves together with the left connectives. However, each of the nerves could also serve as priming stimuli.

The giant cell is an H cell according to the criteria developed by Tauc and Gerschenfeld (1961). It receives a cholinergic inhibitory input from several interneurons and its excitatory inputs are non-cholinergic (L. Tauc and H. M. Gerschenfeld, unpublished observation).

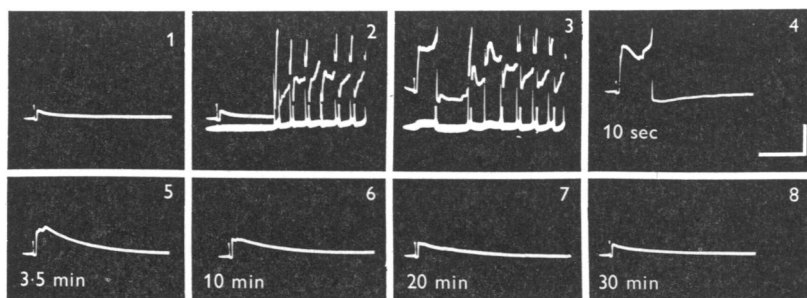


Fig. 10. Heterosynaptic facilitation. 1. Test EPSP produced by stimulation of genital nerve before pairing. 2. First of nine pairing trials of test EPSP and response to priming stimulus (6/sec train of 1 sec duration to the siphon nerve). 3. Seventh pairing trial. Note accompanying depolarization and increase in test PSP. 4-8. Test PSP 10 sec, 3.5, 10, 20, and 30 min after pairing. The lower trace in 2 and 3 is a lower gain record. The voltage calibration is 10 mV for the upper and 100 mV for the lower trace. The time calibration is 500 msec.

Heterosynaptic facilitation. Figure 10 illustrates the effect of heterosynaptic stimulus presentation in the giant cell. Part 1 shows the synaptic potential produced by a single test shock to the genital nerve. Part 2 illustrates the first of nine pairing trials of the test EPSP with a highly effective priming stimulus, a train to the siphon nerve (6/sec for 1 sec). The bottom trace is the simultaneously recorded low-gain record. Part 3 shows the increase in the test EPSP in the fifth pairing trial; the EPSP had increased sufficiently to generate a spike. Parts 4 to 8 illustrate the slow decline in test EPSP in the 30 min after pairing.

The time course of the facilitation and the accompanying decrease of the membrane potential are illustrated in the graph of Fig. 11. By comparison with the 'unidentified' cells, the facilitation in the giant cell rose more rapidly, was usually greater and lasted longer after pairing. In the giant cell, the test EPSP increased by 100–700 % during pairing and decayed over 10–40 min after pairing. Clear effects could usually be seen after one or two trials but three to nine pairing trials were necessary for optimal facilitation.

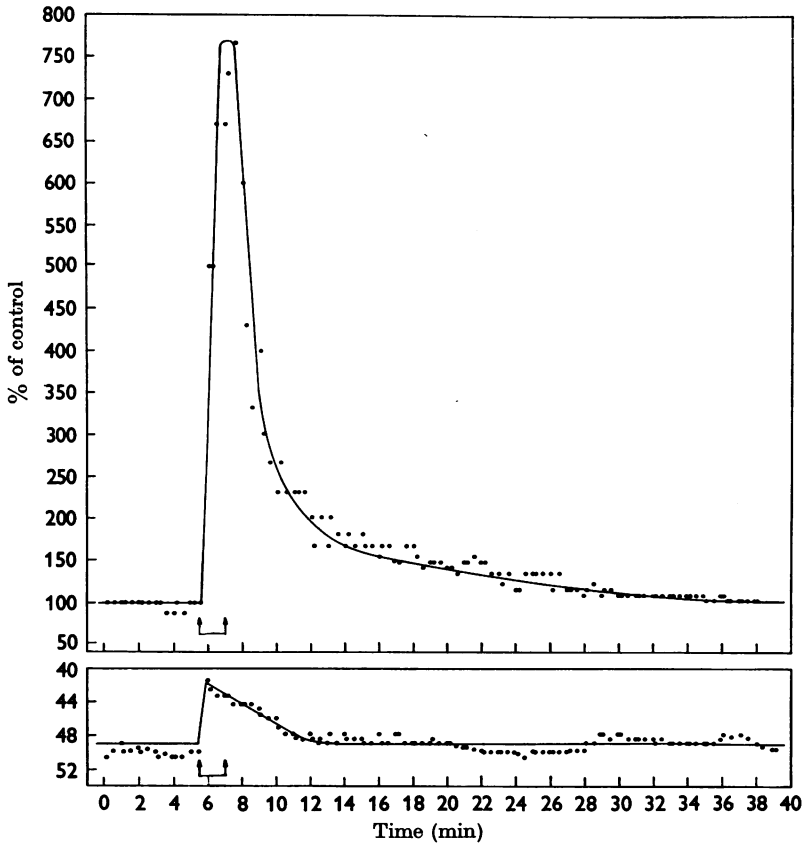


Fig. 11. Time course of heterosynaptic facilitation. Same experiment as in Fig. 10 but a different run. In this and subsequent graphs, the amplitude of the test EPSP is plotted as a function of time and of pairing; the period of pairing is indicated by arrows. The graph at the bottom indicates the average level of membrane potential. Note the time course of the depolarization accompanying the facilitation.

Properties of the priming stimulus. For a constant test PSP, facilitation was a function of the effectiveness of the priming stimulus (Figs. 12, 13 and 14). The 'efficacy' of the priming stimulus could be altered in two ways, (1) by changes in stimulus intensity (Fig. 12) and (2) by changes in stimulus

frequency (Figs. 13 and 14). Figure 12*A* illustrates that a weak priming stimulus, in which the response in the giant cell was only slightly greater than the test response, produced no effect. As the intensity to the priming stimulus was progressively increased (Fig. 12*B* and *C*) the test response showed progressively more facilitation.

Comparable effects could be demonstrated when intensity was held constant and the frequency of the priming stimulus was increased

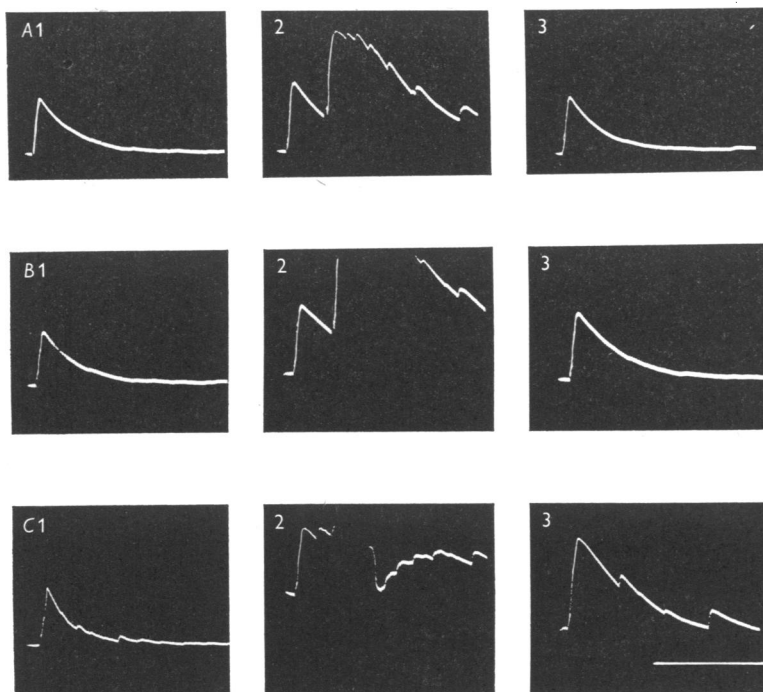


Fig. 12. Amplitude of facilitation as a function of strength of the priming stimulus. All records are from the same experiment. The three columns indicate comparable time periods and the three rows different runs using progressively stronger priming stimuli. 1, Control records; 2, 3rd of seven pairing trials; 3, 30 sec after pairing. In all instances, the test input was a single shock to the left connective, the priming stimulus a single shock to the siphon nerve. In *A* and *B* the priming stimulus produced only a PSP (much larger in *B* than in *A*), in *C* it triggered a spike. At weak stimulus strengths the priming stimulus produced no facilitation. Facilitation only occurred when the priming stimulus was considerably more effective than the test input. There is slight facilitation in *B* and greater facilitation in *C*.

(Figs. 13*A* and *B*). As is illustrated in Fig. 13 and in the graphs of Fig. 14, any increase in the efficacy of the priming stimulus affected the duration of the facilitation as well as its amplitude.

As with unidentified cells, the facilitation could not be repeated very often in the same preparation. While it was frequently possible to produce

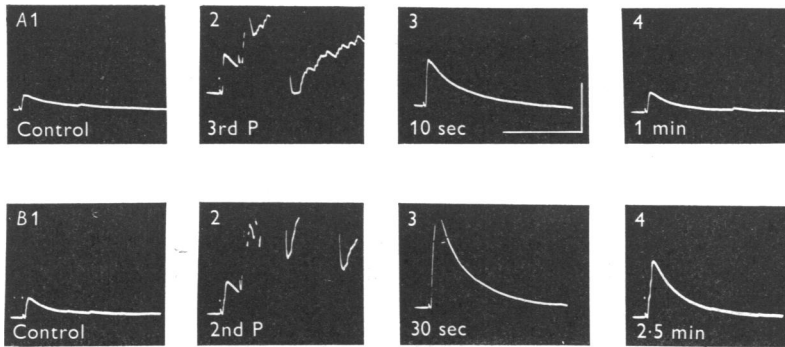


Fig. 13. Amplitude of facilitation as a function of frequency of the priming stimulus. Parts *A* and *B* are from the same series of experiments. The test input was the left connective and the priming input was the siphon nerve. A1 and B1 are control records. A2 to A4 shows the facilitation during and after four pairing trials with a single shock to the siphon. B2 to B4 shows the facilitation during and after four pairing trials with a double shock. The stimulus strength to the priming nerve was held constant in *A* and *B*. Voltage calibration is 10 mV; time calibration is 1 sec.

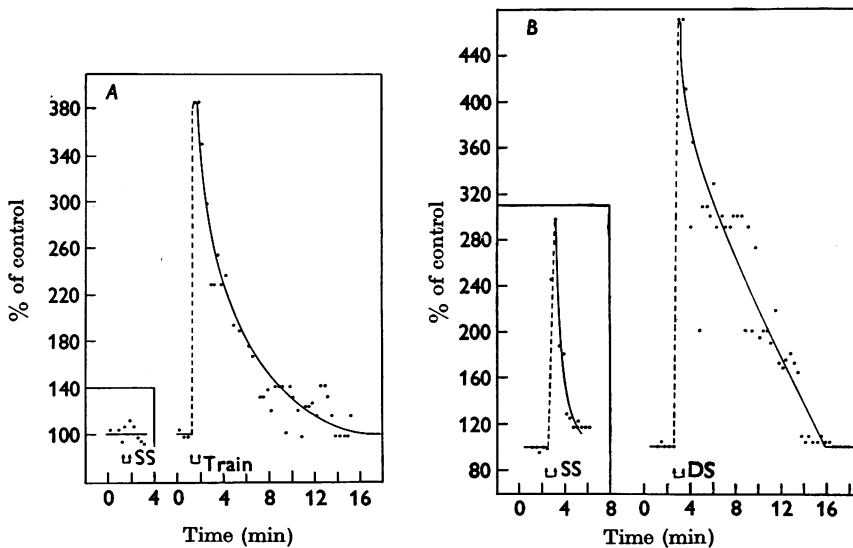


Fig. 14. Time course of facilitation as a function of frequency of the priming stimulus. The experiments shown in *A* and *B* are from different preparations. In each experiment the stimulus strength to the priming stimulus was held constant and the frequency of the priming stimulus was varied. In *A*, the test input was the genital nerve and the priming stimulus the siphon nerve. In the inset graph four pairing trials were given with a single weak shock (SS) as a priming stimulus and no facilitation occurred. When the same priming stimulus was presented as a 6/sec train (for 1 sec), four pairing trials produced significant facilitation. In *B* the left connective serves as a test input and the siphon nerve as a priming stimulus. The two graphs illustrate the facilitation following four pairing trials with a single strong shock (SS) as a priming stimulus and following brief trains of two such stimuli (DS) at 6/sec.

facilitation five or six times in the same experiment, the facilitation tended to become less effective with time and irreversibly so. This seemed, at least in part, to be related to some apparently irreversible or at least very long-lasting (more than 2 hr) change in the effectiveness of the priming stimulus. The cause of this prolonged decrease in the response to the priming stimulus is obscure. On the basis of our experiments, it was difficult to know whether the decline represented the deterioration of the preparation or a physiological and potentially reversible response decrement of unusually long duration (see experiments on whole-animal preparation).

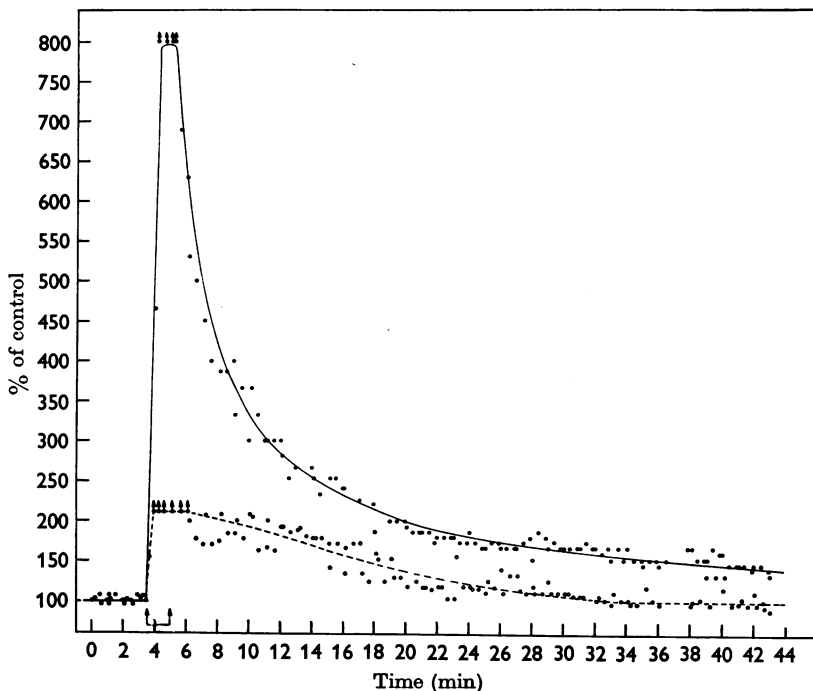


Fig. 15. Facilitation as a function of test response. The data for the two graphs were obtained concurrently. The stimulus polarity to the test input was reversed every ten sec. One polarity produced a small PSP (2.8 mV; filled circles) and the other polarity a large PSP (12.5 mV; open circles). Both PSPs were paired for the same number of times (5) with the same priming stimulus. The absolute value reached by both PSPs during peak facilitation was comparable and in each case triggered action potentials (arrows). But the percentage increase was almost 4 times greater for the small PSP. The time course of the facilitation was, however, similar. Test input: genital nerve; priming stimulus; siphon nerve.

Properties of the test stimulus. In contrast to the priming stimulus, the amplitude of the test stimulus was not critical for the occurrence of facilitation. Both large and small EPSPs were facilitated by being paired

with an appropriate priming stimulus. However, the *relative* increase was several-fold larger for small test PSPs than for large ones. This was investigated by comparing the effects of the same priming stimulus on test responses of different amplitude produced by reversing the stimulus polarity each 10 sec (see Methods). As the graph given in Fig. 15 shows, the peak facilitation was 4 times greater for the smaller EPSP. The duration of the facilitation was comparable.

This examination of small and large test PSPs does not involve an exactly comparable situation. The large PSP is closer to the equilibrium level for excitation than is the small one and therefore less likely to show an increase in potential for a constant increase in synaptic current. Therefore,

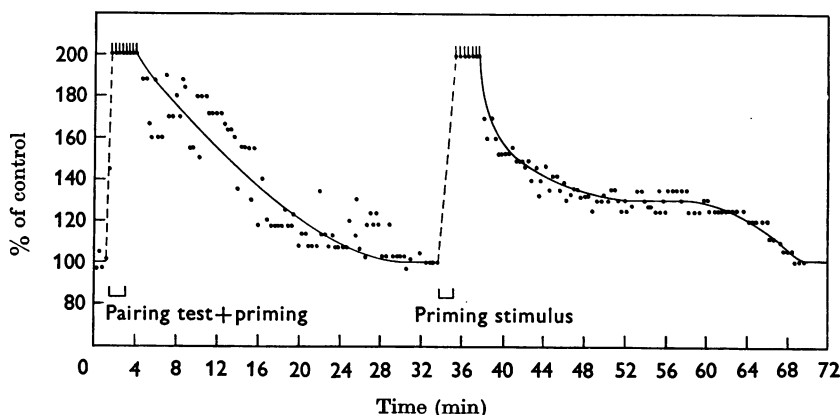


Fig. 16. Percentage increase of test EPSP as a function of time with and without pairing of the test with the priming stimulus. The arrows at the bottom of the graph indicate the period during which the priming stimulus was presented. Each arrow at the top represents an action potential triggered by the test EPSP. The two graphs are from two consecutive runs in the same cell. Note that the graphs, although not identical, are quite similar.

while the increase in synaptic *potential* is relatively greater for smaller than for larger test PSPs, the increase in synaptic *current* may actually be comparable; thus the possibility remains that the amplitude of the initial test PSP may not be a significant determinant of the magnitude of the facilitation process.

Specificity to pairing. Unlike the facilitation in some of the unidentified cells, the facilitation in the giant cell was not dependent upon pairing. Control experiments similar to those illustrated in Figs. 7 and 8 were done on the giant cell. These experiments showed that the time course of the potentiation of the test EPSP was similar whether or not the priming stimulus was presented alone or paired with the test. This point is illustrated in the graph of Fig. 16 which is based upon two consecutive runs

in the same cell. In the first run the test EPSP was paired with the priming stimulus and in the second it was not. Although the two curves are not superimposable, their peak and general time course is similar.

Non-specificity could also be demonstrated with different test inputs (Fig. 17). When two test inputs were used, one of which was paired with the priming stimulus and the other of which was not, both test EPSPs were potentiated (see figure legend for details).

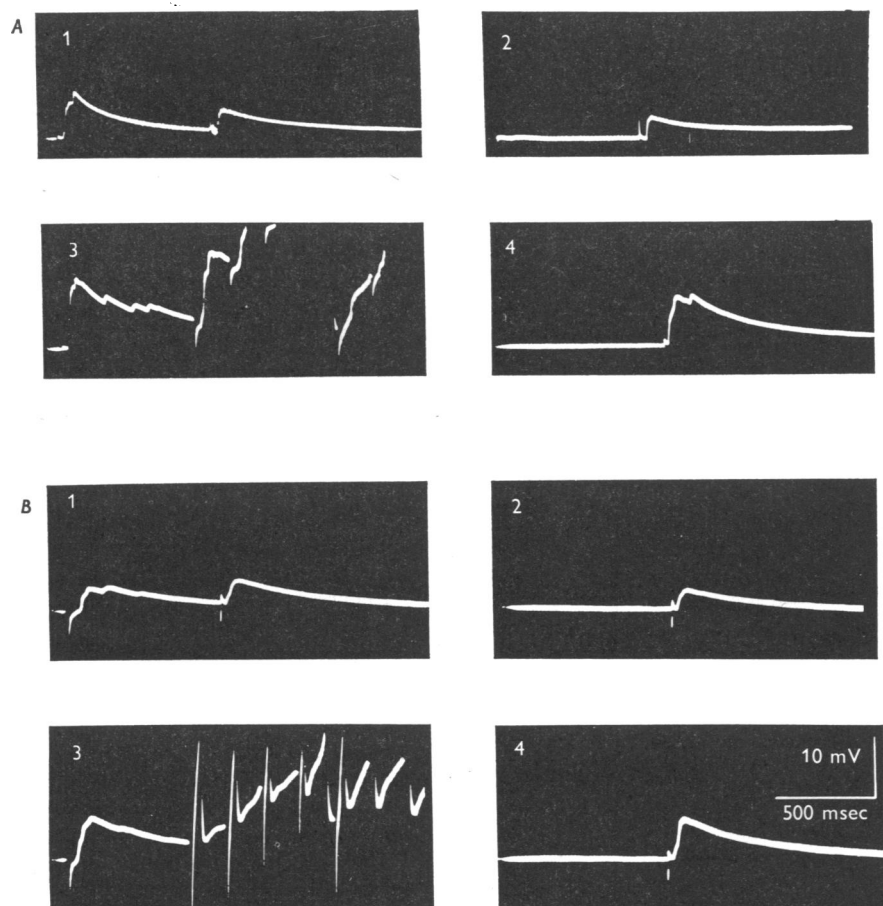


Fig. 17. Facilitation of the non-paired input. *A* and *B* are from two different experiments. In *A* the first (paired) test input was the genital nerve and the second (unpaired) test input was the left connective. The priming input was the siphon nerve. In *B*, the first (paired) test input was the siphon nerve and the second (unpaired) test input was the left connective. The priming input was the genital nerve. Records 1 and 2 in each part illustrate both inputs (1) and the unpaired input alone (2) before pairing. Records 3 and 4 illustrate the facilitation of the paired input during pairing (3) and of the non-paired input after pairing (4).

Heterosynaptic facilitation in the whole animal. An additional advantage offered by the right upper-quadrant giant cells was the opportunity of studying heterosynaptic facilitation in the whole animal with the use of a physiological stimulus to the siphon as a priming stimulus. For this purpose, intracellular recordings were abandoned and the activity of the giant cell axon was recorded in the right connective. This axon produces an action potential which is several times larger than any other in the connective and is therefore readily recognized (Hughes & Tauc, 1962).

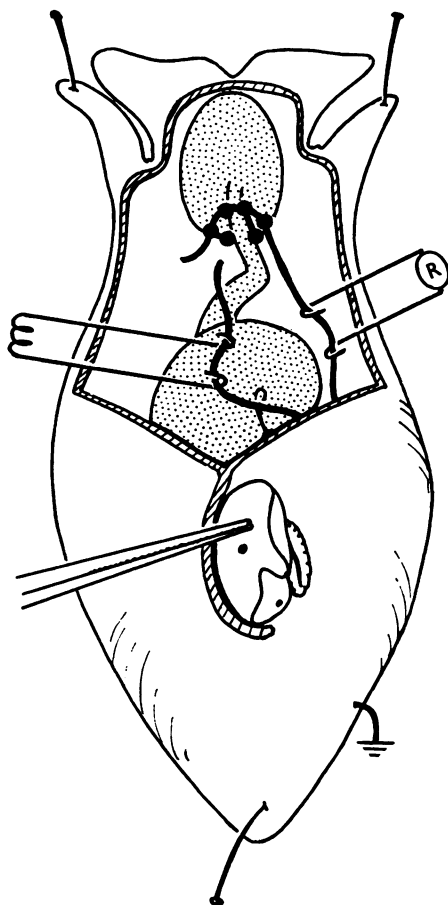


Fig. 18. Diagram of the experimental arrangement in the whole-animal preparation.

Experiments were conducted with the whole animal when only the left connective was cut and its central end stimulated as a test input. The area of the spout, innervated by the siphon nerve, was stroked manually (4 times per pairing trial) with a pair of blunt forceps. These experiments were set up as indicated in Fig. 18 and the results of an experiment are

shown in Fig. 19. Parts 1 and 2 of Fig. 19 illustrate latency shifts in the giant axon spike with suprathreshold (1) and barely-threshold stimuli (2). The stimulus strength was then reduced to a control level which was safely below threshold to ensure that the stimulus did not trigger the giant axon spike (3 to 5 are sample records from this control period). Parts 6, 7 and 8 represent the response to the first, third and fourth of four pairing trials with the priming stimulus. The priming stimulus was not

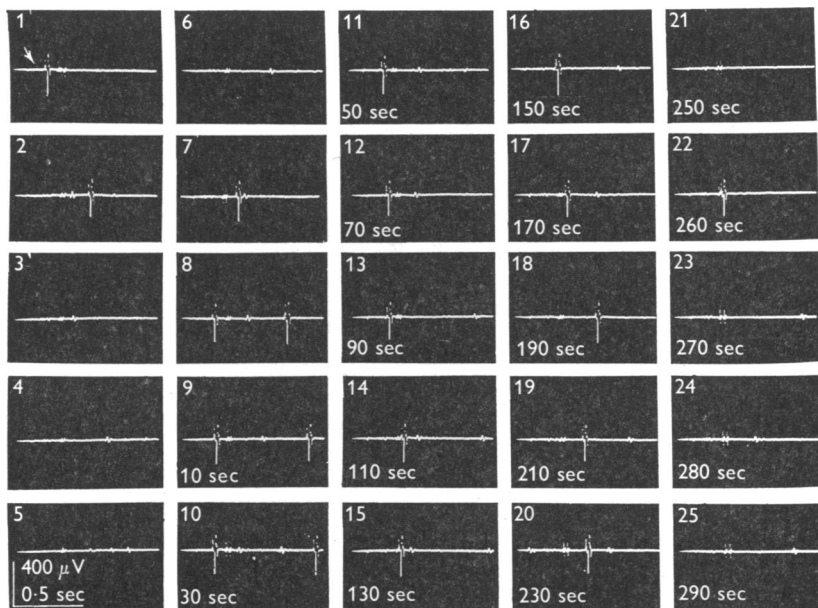


Fig. 19. Heterosynaptic facilitation in the whole-animal preparation. The left connective served as a test input and a natural stimulus to the siphon (stroking) as a priming stimulus. The action potentials have been touched-up. See text for details.

synchronized with the sweep and the repetitive firing initiated by it was not evident at this sweep speed. The priming stimulus facilitated the response to the test stimulus which produced spikes for about $4\frac{1}{2}$ min following pairing (9–25 min). Note the increase in spike latency as the facilitation declined.

The facilitation in the whole-animal experiment only measured the peak of the facilitation curve; it indicated the period during which the test EPSP was sufficiently facilitated to trigger a spike. In the absence of intracellular recordings, it is difficult to compare the time course of this phenomenon with that seen in the isolated preparation. However, these experiments do suffice to indicate that heterosynaptic facilitation can occur with a physiological priming stimulus.

Several additional findings relevant to the properties of the priming stimulus were also replicated in the whole animal. The duration of the facilitation was here also a property of the effectiveness of the priming stimulus and, as in the isolated ganglion, the physiological priming stimulus became less effective with time and with repeated stimulations. As the priming response became less effective the facilitation became less marked and finally failed to occur. These results form one argument for thinking that decline in the response to the priming stimulus may be in part an adaptation or habituation to the stimulus. A similar type of response decrement in the whole animal has previously been noted to other natural stimuli (Hughes & Tauc, 1963).

As in the isolated ganglion, heterosynaptic facilitation of the giant cell in the whole animal was not specific to pairing.

DISCUSSION

An input-pairing sequence derived from Type I behavioural conditioning has been applied to different cells of the abdominal ganglion of *Aplysia depilans*. In most cells examined the response produced by one pathway was not significantly facilitated by activity in another pathway. However, in some cells, which tended to be located in a restricted portion of the ganglion, a significant and fairly prolonged facilitation occurred. This facilitation was sufficient to make an ineffective EPSP transiently effective in producing a spike.

When heterosynaptic facilitation was further examined two types could be distinguished: one type occurred in some of the unidentified cells, the other occurred in the giant cell. In the unidentified cells, more pairing trials were required for maximum facilitation and the facilitation was generally smaller and less prolonged than in the giant cell.

The distinction between the two types of facilitation was further supported by the findings that in some of the 'unidentified' cells the facilitation was dependent upon pairing of the test and the priming responses and was specific to the particular test-response which was paired. This specificity to pairing and to the paired input is in some ways analogous to classical behavioural conditioning. By contrast, facilitation in the giant cell was independent of pairing; this facilitation is in some ways analogous to behavioural quasi-conditioning (Hilgard & Marquis, 1961).

While the two types of heterosynaptic facilitation observed bear certain analogies to behavioural conditioning and quasi-conditioning, it is preferable to look at these experiments primarily from a neurophysiological point of view. Here they represent an attempt to produce a long-lasting change in the neuronal behaviour of single cells in an isolated ganglion

by repeated and concomitant pairing of two inputs of differing efficacy. The results show that relatively few intermittent presentations of a highly effective priming stimulus to one pathway are capable of producing a significant and prolonged alteration in the amplitude of the synaptic potential produced by another pathway. This finding demonstrates the possibility of an efficient 'transfer' of facilitation from one pathway to another. The facilitatory 'transfer' so produced appears to be able to generate more prolonged increments in synaptic efficacy than do repeated homosynaptic volleys presented over a comparable time period. Moreover, fewer stimuli and lower stimulus frequencies seem to be required for hetero- than for homosynaptic facilitation (Eccles, 1964; Martin & Pilar, 1964; Kandel & Tauc, 1965) suggesting that temporal factors other than synaptic use, or highly regular repetition, may be important for establishing prolonged alterations in synaptic efficacy.

The fact that EPSPs can be facilitated for over half-an-hour, with an input patterning schema designed to simulate a behavioural conditioning paradigm, also suggests that the concomitant changes in the efficacy of synaptic transmission may underly certain simple forms of information storage in the intact animal.

Further results obtained from the unidentified cells and from the giant cell will now be considered.

Unidentified cells

In the molluscan nervous system only a very few cells are sufficiently distinctive in their appearance and invariant in their location to be readily recognizable in each preparation (Bullock & Horridge, 1965). The data in the first part of the Results section probably come from different cells within the ganglion. Since the facilitation could generally not be well demonstrated more than two or three times in any given cell it was difficult to explore, in detail, the phenomenon encountered. Consequently, the results from the unidentified cells are fragmentary. Little more can be said except that heterosynaptic facilitation occurs in some cells and that in a few of these it has been demonstrated to be dependent upon pairing and specific to the paired input.

Despite the fact that specificity was found among unidentified cells in all instances examined, the number of cells examined was small and the possibility that some of the unidentified cells show non-specific facilitation or that transitional forms exist has not been excluded.

It seems likely that the facilitation in the unidentified cells is not due to a change in the properties of the post-synaptic neurone otherwise even an unpaired test input would have been facilitated (Fig. 8). There also appears to be some relation between the magnitude of the facilitation and the

strength of the priming stimulus (Fig. 7). The factors contributing to the decline of the facilitation with subsequent pairing trials could not be clearly determined. There did seem to be a tendency, which was better documented in experiments on the giant cell, for the priming stimulus to decrease in efficacy with time.

Cells which showed this type of heterosynaptic facilitation tended to be located in a restricted region of the ganglion near the medial border of the giant cell. The functional significance of this finding is obscure but it accords with other observations which indicate that distinctive physiological 'cell types' (e.g. cells showing spontaneous elementary IPSPs, inhibition of long duration, different spontaneous firing rates) tend to occur in specific locations in the ganglion (Arvanitaki & Chalazonitis, 1958; Tauc, 1958, 1960; G. P. Moore and J. P. Segundo, personal communication).

Giant cell

In this cell it proved possible to examine in some detail the parametric properties of the non-specific form of facilitation. These results show that the magnitude and duration of the facilitation were functions of the effectiveness of the priming stimulus. More effective priming stimuli produced greater and more prolonged facilitation. The amplitude of the test stimulus was not critical for the occurrence of facilitation. However, the relative increase was greater for small PSPs than for large ones.

The generation of a large action potential by the giant cell axon, which can readily be recorded in the left connective, permitted experiments to be done in the whole animal. In these experiments, it was possible to demonstrate that a physiological stimulus to the area of the siphon could serve as a satisfactory priming stimulus. These results strengthen the validity of the present experimental approach in the isolated ganglion by demonstrating that this type of heterosynaptic facilitation can occur under more natural circumstances. The giant cell, therefore, offers the additional advantage of permitting an experimental comparison between the somewhat more simple analogue in the isolated ganglion and the whole-animal preparation.

SUMMARY

1. A stimulus-pairing sequence based on classical (Type I) behavioural conditioning was applied to the isolated abdominal ganglion of *Aplysia depilans*. Intracellular recordings from single cells were obtained, and the stimulus parameters to two different afferent nerves were controlled so that one produced a relatively small excitatory post-synaptic potential (the test stimulus) and the other, usually a brief train, produced a burst of spikes (the priming stimulus). The two stimuli were paired (once every 10 sec) for several minutes with the test preceding the priming by about 300 msec.

2. In most of the cells examined, input pairing produced no facilitation of the test EPSP. However, in the right upper-quadrant giant cell of each ganglion and in fifteen out of ninety unidentified cells located near the medial borders of the giant cells, the test EPSP was augmented during pairing. The facilitation declined only slowly following the pairing procedure.

3. In the unidentified cells, the test PSP was augmented by about 100 % during fifteen to thirty pairing trials and the facilitation declined within an average of 9 and a maximum of 20 min after the pairing procedures.

4. In three unidentified cells, it was possible to demonstrate that the facilitation was specific to the pairing process since the test EPSP was not augmented after repeated but unpaired presentations of the priming stimulus. In two additional unidentified cells, it was possible to show that the facilitation was specific to the paired input.

5. In the right upper-quadrant giant cell the PSP was facilitated 100-700 % following three to nine pairing trials and the facilitation declined over periods lasting up to 40 min after pairing.

6. In contrast to some of the unidentified cells which showed a heterosynaptic facilitation specific to pairing, the type of facilitation which occurred in the giant cell was not specific to pairing and was not limited to the paired input.

7. In the whole-animal preparation, a physiological stimulus to the area of the siphon could serve as a satisfactory priming stimulus for the giant cell indicating that this non-specific form of heterosynaptic facilitation can also occur under more physiological conditions than those afforded in the isolated ganglion.

8. These results demonstrate that in certain cells the amplitude of the post-synaptic potential produced by a weak stimulus to one pathway is capable of being facilitated for a prolonged period of time as a result of the repeated and concomitant pairing with a more effective stimulus to another pathway.

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