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MECHANISM OF HETEROSYNAPTIC FACILITATION IN THE GIANT CELL OF THE ABDOMINAL GANGLION OF *APLYSIA DEPILANS*

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In certain cells in the right upper quadrant of the abdominal ganglion of *Aplysia depilans*, a small excitatory post-synaptic potential (EPSP), produced by a weak stimulus to one pathway (test stimulus), underwent a prolonged facilitation when it was paired several times with a very strong stimulus to a second pathway (priming stimulus). In some cells this heterosynaptic facilitation was dependent upon the concomitant pairing of the two stimuli; in the right upper-quadrant giant cell the facilitation was independent of pairing (Kandel & Tauc, 1965). The stimulus parameters for optimal heterosynaptic facilitation in the giant cell were analysed in the preceding paper (Kandel & Tauc, 1965). The analysis of the mechanism underlying this facilitation is presented in this paper. This analysis was facilitated by the reliable identification of the right upper-quadrant giant cell, as well as its large size (300–800 μ). A preliminary note on this work has already been published (Kandel & Tauc, 1964).

METHODS

The techniques for dissecting the connective tissue overlying the giant cell and for obtaining intracellular recordings were described in the preceding paper (Kandel & Tauc, 1965). In some experiments, a second KCl micropipette was inserted into the giant cell under direct vision, for purposes of stimulation. This electrode was initially also connected to a cathode follower and the resting and action potentials of the giant cell recorded by the electrode were monitored on the oscilloscope to insure a satisfactory impalement. Stimulating pipettes were selected for their low resistance (less than 10 MΩ). The current, injected through the electrode, was measured as a voltage drop across a current-limiting resistance (usually 200 MΩ but in some cases 500 MΩ) and was displayed on the second beam of the oscilloscope. When curare was used, a solution of 10^{-4} g/ml. was introduced into the chamber through a simple perfusion system which permitted a rapid change of solutions without interfering with stable intracellular recording.

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The stimulus sequence utilized in the earlier experiments (Kandel & Tauc, 1965) was retained because it was optimal for producing facilitation in the giant cell. In this cell, as in the unidentified cells, several intermittently repeated presentations of the priming stimulus produced greater and more prolonged facilitation than did a single presentation. Similarly, brief trains of 1 sec duration given intermittently (1/10 sec) were more effective as priming stimuli than a single long train. The presentation of the test stimulus before the priming stimulus permitted the measurement of changes in test EPSP amplitude during the presentations of the priming stimulus.

RESULTS

As a first step in this analysis, we examined the contribution of the post-synaptic unit to the development of heterosynaptic facilitation. This part of the analysis focused on two different processes: (1) conductance changes in the non-junctional membrane, and (2) spike activity in the giant cell. A detailed analysis of the non-junctional membrane was made particularly necessary by the recent demonstration of anomalous rectification in the metacerebral cells of the snail (Tauc & Kandel, 1964). In the snail metacerebral neurones, changes in membrane potential produced changes in membrane conductance which, in turn, produced changes in the amplitude of the synaptic potential. Membrane hyperpolarization caused a decrease in the amplitude of the synaptic potential while membrane depolarization caused an increase in the EPSP.

Changes in the post-synaptic unit

The passive electrical properties of the non-junctional membrane. In the previous paper (Kandel & Tauc, 1965), the depolarization that frequently accompanied the facilitation was described. This depolarization had a shorter time course than the facilitation and therefore cannot be primarily responsible for it. But the depolarization could contribute to the peak amplitude of the facilitation by producing an increase in the resistance of the non-junctional membrane. The experiment illustrated in Fig. 1 was designed to test this possibility. Figure 1*A* shows the changes in membrane potential produced by a series of current steps of constant magnitude. The giant-cell membrane did show rectification which is in someways similar to that described for the snail metacerebral cells. However, this rectification was not within the physiological range. This is illustrated in Fig. 1*B* which shows in greater detail the change in membrane potential on either side of the resting level. Within this physiological range, the potential changes were fairly constant. The graph of Fig. 1*C* illustrates the complete current–voltage curve. The curve is linear through zero; the rectifying conductance does not turn on until the membrane is hyperpolarized by 10 mV.

Figure 1*D* illustrates the results of another experiment designed to

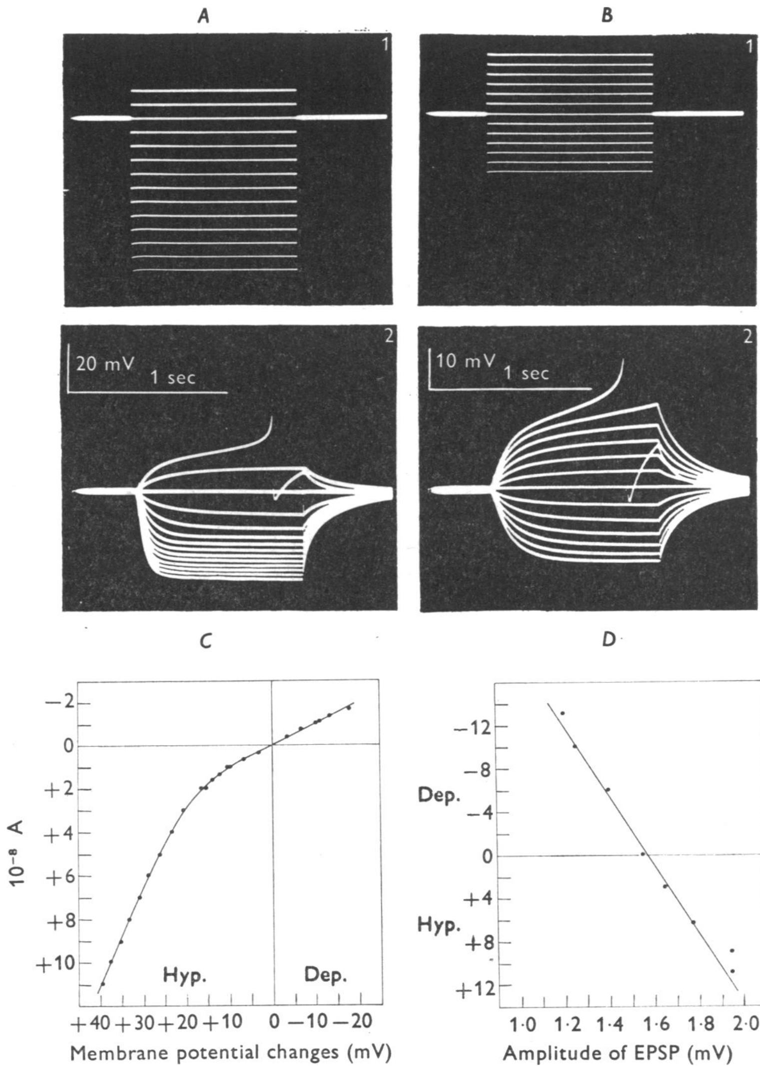

Fig. 1. Current–voltage relations in the giant cell. *A* and *B* are records from the same cell obtained at different gain settings to show the modifications in trans-membrane potential (Part 2) produced by constant changes in polarizing current (Part 1). Each current step is 1×10^{-8} A in *A* and $3{\cdot}3 \times 10^{-9}$ A in *B*. *C* illustrates the whole current–voltage curve based upon the data in *A* and *B*. Note that the current–voltage relation is linear in the subthreshold physiological range but that in the 10 to 20 mV hyperpolarizing range the curve is non-linear indicating the onset of anomalous rectification. *D* illustrates the changes in EPSP amplitude with changes in membrane potential. Within the physiological range used the relation is linear.

investigate this point. It is a graph of the amplitude of a small test EPSP as a function of membrane potential. If, throughout the physiological range, the membrane conductance of the non-junctional membrane is constant and the equilibrium level of the EPSP is closer to zero than is the resting potential, then the EPSP should decrease in amplitude with depolarization of the membrane and increase with hyperpolarization (Fatt & Katz, 1951; Eccles, 1964). The graph of Fig. 1*D* shows that this relation holds true for the giant cell. The EPSP behaved according to expectation within this range of potential changes.

A third type of experiment is illustrated in Fig. 2. The slope conductance of the membrane was measured with a square hyperpolarizing pulse before (*A* 1) and after stimulus pairing (*A* 2). There was no change in the electrotonic potential during peak facilitation of the test EPSP.

The depolarization accompanying the facilitation appeared to be due, in part, to the repetitive discharge of interneurones activated by the priming pathway. When a very powerful priming stimulus was used, this interneuronal activity caused an increase in membrane conductance which lasted 10–20 sec. Figure 2*B*, taken on moving film, illustrates a transient decrease of about 20 % in the electrotonic potential. Despite this increase in conductance, facilitation is evident. This finding is also important because it shows that an intrasomatic electrode is capable of measuring conductance changes in the synaptic region in the axon, some distance from the cell body. This is consistent with previous data on the giant cell (Tauc, 1962) indicating a low transverse resistance between the soma and the synaptic region in the axon.

These experiments indicate that the facilitation is not due to a change in the passive electrical properties of the giant cell membrane. The two changes which did occur, the depolarization and the occasional brief conductance increase, work against the facilitation of the PSP by masking the peak augmentation of the synaptic current.

Spike generating activity in the giant cell. The absence of appropriate changes in the passive properties of the membrane does not, however, completely exclude a contribution of the giant cell to the facilitation. It is possible that the facilitation is due to the generation of spikes by the giant cell and its axons. For example, there could be a facilitatory interaction of the giant-cell spike and a presynaptic terminal of the test pathway, or recurrent collateral facilitation converging on an interneurone in the test pathway.

This type of interaction seemed unlikely from work in other preparations; it was excluded for the giant cell in the series of experiments illustrated in Fig. 3. Rows *A*, *B* and *C* represent three different experimental conditions; columns 1, 2 and 3 represent identical time periods sampled before pairing, at the 5th pairing trial, and 2 min after pairing, respectively. Row *A* illustrates a conventional pairing situation. The priming stimulus was a train to the siphon nerve which produced repetitive discharge of the giant

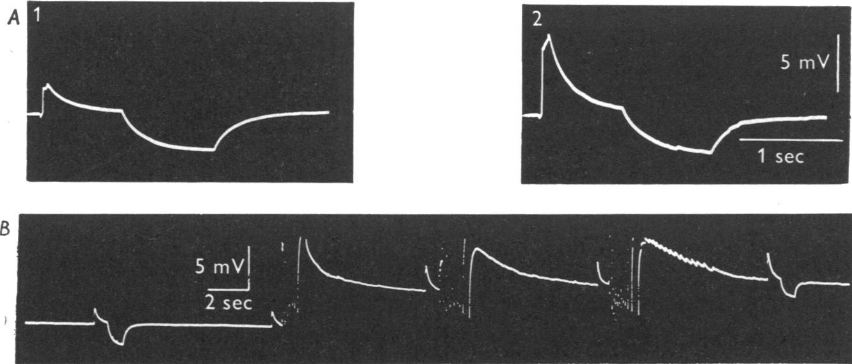

Fig. 2. Conductance measurements during facilitation. *A* 1 before pairing and A 2 during peak facilitation after pairing. Note increase in the PSP and absence of change in the electrotonic potential. *B* (same cell as in *A* but another run) illustrates a slight (20 %) decrease in electrotonic potential during the first post-pairing presentation. Oscillations in transmembrane potential after pairing are due to repetitive bombardment by interneurones.

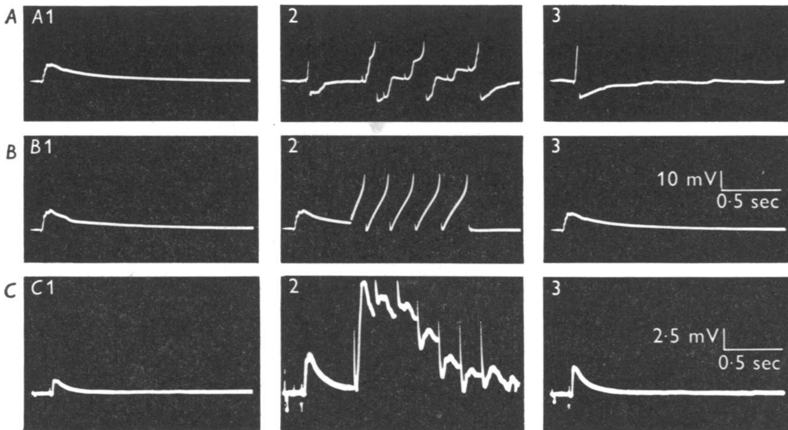

Fig. 3. Role of the action potential in facilitation. *A* and *B* are from two consecutive experiments in the same preparation using the same test PSP. In each case the genital nerve served as a test input. *C* is from another experiment in which the left connective was the test input. The columns represent control (1), fourth pairing trial (2), and 1 min after pairing (3), respectively. The priming stimulus, indicated in column 2, was varied in each of the three experiments. In A 2 it was a 5/sec train of 1 sec duration to the siphon nerve. In B 2 it was a directly initiated train of 1 sec duration producing five spikes; and in C 2 it was a 7/sec train of 1 sec duration to the siphon nerve which failed to trigger a spike because the membrane was hyperpolarized. The experiments indicate that the action potential of the giant cell is not essential for facilitation.

cell and an increase of the test EPSP. Row *B* is from a different experiment in the same cell as in *A* and with the same test EPSP; instead of using a stimulus to another nerve as a priming stimulus, a directly initiated train of spikes, produced by a second intracellular microelectrode, was substituted for the effective priming stimulus. As shown in Fig. 3*B*2 and 3, the direct train produces no change in the test EPSP indicating that spike generation on the part of the post-synaptic cell was not critical to the facilitation. This point was also illustrated in another experiment (Fig. 3 *C*1–3). The siphon nerve was stimulated to produce a suboptimal priming stimulus and the giant-cell membrane was hyperpolarized in order to prevent spike generation. Despite the absence of spike production in the giant cell, facilitation could be demonstrated.

These experiments exclude the contribution of the post-junctional unit and suggest that the facilitation is due to a change in the synaptic impingement upon the giant cell resulting either from an increase in the synaptic excitatory drive or a decrease in synaptic inhibitory drive (disinhibition).

Changes in the synaptic drive of the test input

Change in the inhibitory synaptic drive. The pharmacological properties of the synaptic organization of this ganglion permitted a ready examination of the role of disinhibition. A previous investigation had shown that almost all the post-synaptic inhibition in the ganglion but only some of the synaptic excitation is cholinergic (Tauc & Gerschenfeld, 1962). In particular, all the synaptic excitation on the giant cell is non-cholinergic. It was, therefore, possible to eliminate a major portion of the post-synaptic inhibition in the ganglion with the use of curare. The data for Fig. 4 were obtained after the ganglion had soaked for 20 min in 10^{-4} w/v of D-tubocurarine. As the graph shows, facilitation was still present. These findings suggested that the facilitation was due to a change in the excitatory input to the cell.

Changes in the excitatory synaptic drive. A change in the synaptic excitatory drive could occur either by recruitment of new elements into the test pathway, or by increase in efficacy of elements initially constituting the test response. To distinguish between these two possibilities, the configuration of different test responses were compared before and after pairing (Fig. 5). To a fair degree, the EPSP of a given test stimulus kept its general configuration during peak facilitation. In multicomponent EPSPs, each component was facilitated (Fig. 5; also Fig. 2*A*).

The finding of a symmetrical increase in the test PSP suggested that the facilitation occurred as a result of an increase in synaptic efficacy of the elements initially constituting the test response. Such an increase in

synaptic efficacy could occur by one of two mechanisms; (1) presynaptic facilitation, or (2) post-tetanic facilitation. The first of these mechanisms would depend upon a presynaptic interaction of the axon terminals of the priming and test pathways so that repetitive activity in the priming pathway could produce a prolonged increase in the transmitter release of the test pathway. The second mechanism would depend upon the priming stimulus setting into repetitive activity a set of interneurones that it shared with the test pathway.

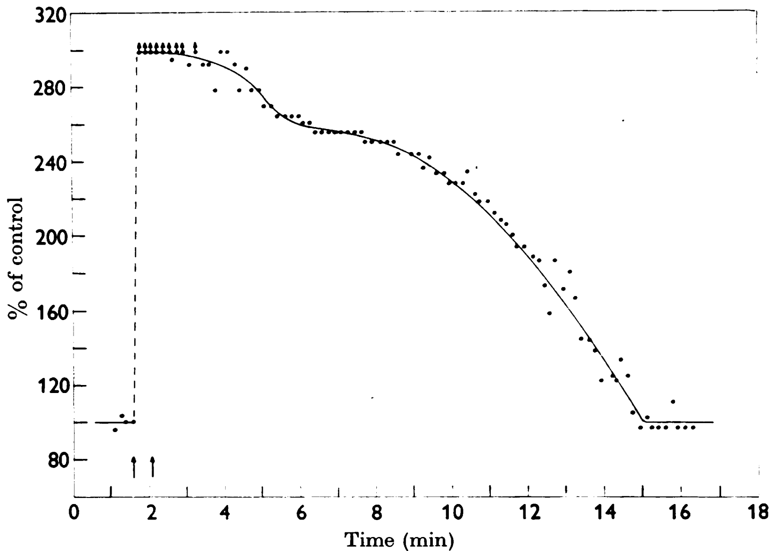

Fig. 4. Role of disinhibition in facilitation. The data for this graph were obtained after the abdominal ganglion had been exposed for 20 min in a 5×10^{-4} solution of D-tubocurarine in order to block cholinergic inhibitory synaptic transmission. The genital nerve was the test input and the siphon nerve the priming stimulus. The priming stimulus was a 6 sec train of 1 sec duration comparable to that used in other experiments. In this and all subsequent figures the percentage change in the test EPSP is plotted as a function of time and of pairing. The period of pairing is indicated by two arrows on the abscissa. Note that facilitation occurred despite post-synaptic inhibitory blockade.

The possibility of presynaptic facilitation was tested by using essentially the same criteria first used by Frank and Fuortes in their study of presynaptic inhibition of motoneurones: the demonstration of a change in a monosynaptic EPSP and the absence of a conductance change in the post-synaptic membrane (Frank & Fuortes, 1957). The relevant conductance data have already been discussed (Fig. 3). Since there are no adequate histological data on the synaptic organization of *Aplysia*, criteria for monosynaptic connexions were developed which were of necessity completely electrophysiological.

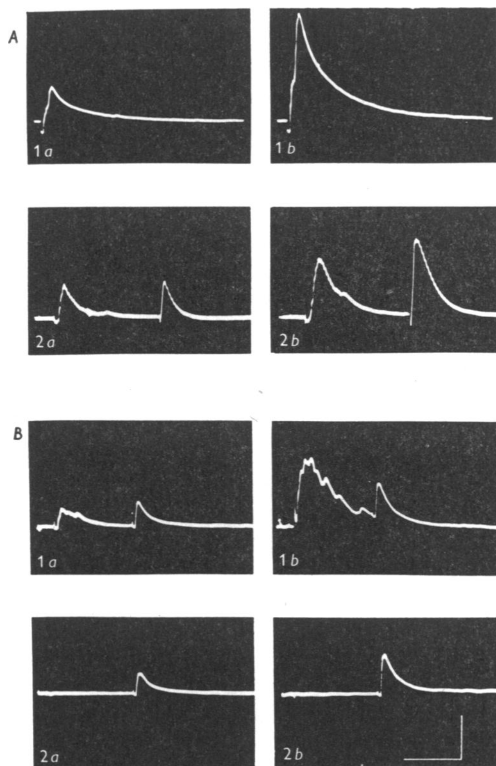

Fig. 5. Test EPSP configuration before and after pairing. In both parts *A* and *B* the records 1*a* and 2*a* are before pairing and 1*b* and 2*b* during maximum post-pairing facilitation. In A 1 the left connective was the test and the siphon nerve the priming input. In A 2 the genital nerve and left connective were the two test inputs and the siphon nerve the priming input. In B 1 the pericardial branch of the genital nerve and the left connective were the test inputs, and the branchial nerve was the priming stimulus. In B 2 the left connective was the test and the branchial nerve the priming stimulus. The calibration for all records is 5 mV and 0·5 sec.

The criteria are illustrated in Fig. 6. They included:

(i) *Short and constant latency.* The latency of the EPSP was constant and shorter than the antidromic conduction time of the giant-cell axon which is the second most rapidly conducting axon in the connective (Fig. 6, parts 1 to 4). The conduction velocity of the giant-cell axon in these experiments was approximately 0·8 m/sec, while that of the fibre mediating the PSP was about 1·2 m/sec. Tauc (1957), Goldman (quoted in Bullock, 1961), and Hughes & Tauc (1963) describe a small spike in the left and right connectives produced by axons that conduct more rapidly than the giant axon. The conduction velocity of these 'fast' axons is closely comparable to that mediating the presumably monosynaptic PSP.

(ii) *Ability to follow frequencies of 8–10/sec without change of latency.* (Fig. 6, 6*a–c*). These frequencies are close to the upper limit that axons in the connective can follow (Tauc, 1957 and unpublished observations). Moreover, the frequencies which could be observed may not be a true index of the maximum following frequencies of the EPSP. This limit was determined by technical reasons. Higher frequencies encroached upon the relative refractory period of the test axon and required stronger stimuli; these in turn tended to bring into activity additional synaptic elements which confounded the analysis.

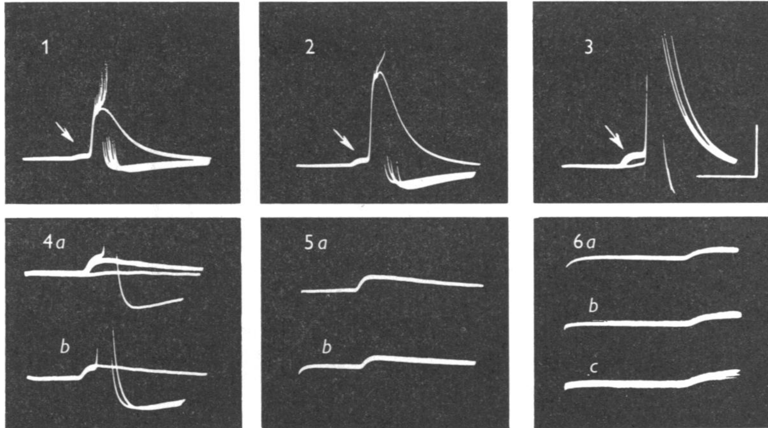

Fig. 6. Criteria for unitary, presumably monosynaptic, EPSP. 1–4. Comparison of latency of EPSP and antidromic spike (right connective). Superimposed sweeps to show antidromic A and AS spike. At the foot of the A spike is a small EPSP (arrow) whose all-or-none properties are illustrated in 3 and whose time course is evident in 4. Note progressive increase in amplification in 1–3. 5. Comparison of latency of unitary EPSP from right (5*a*) and from left (5*b*) connective. 6. *a–c* EPSP following 6/sec, 7/sec, and 8/sec stimulation without change in latency (left connective). All data are from the same cell. The voltage calibration is 50 mV in 1; 25 mV in 2; and 10 mV in 3–6. The time calibration is 50 msec in 1–5 and 25 msec in 6.

(iii) *All-or-none responsiveness.* This property, which derives from the unitary nature of elementary PSPs in *Aplysia* (Tauc, 1958, 1959), permitted study, in relative isolation, of an individual synaptic potential produced by the terminations of a single axon (Fig. 6, records 3 and 4).

These elementary PSPs are similar to the monosynaptic PSPs observed in *Aplysia* in the relatively rare instances when pace-maker follower couples have been successfully impaled (Tauc, 1959). They are also similar to the PSPs that have been observed to occur spontaneously and in response to cord stimulation in the crayfish (Furshpan & Potter, 1959).

The alternative technique of developing a monosynaptic input by impaling two directly connected cells is much the more difficult (Tauc, 1959). In six experiments in this series, in

which a systematic attempt was made to impale a second cell that made a direct connexion with the giant cell, about 20 such elements (mostly axons) were transiently impaled but only one survived more than a few seconds. This unit was severely damaged and lasted only 10 min but it also showed heterosynaptic facilitation.

These elementary, presumably monosynaptic, PSPs are also in several ways similar to those seen in vertebrate preparations such as cat motoneurones and rabbit sympathetic ganglion cells. The experiment shown in Fig. 7 was designed to compare the frequency sensitivity of the unitary EPSPs in *Aplysia* with comparable published data on motoneurones and on sympathetic ganglion cells (Figs. 30*C* and 31*B* in Eccles, 1964). At any given frequency, the unitary PSP reached a steady-state amplitude which was constant for many impulses (see inset in Fig. 7*A*). The steady-state amplitude was smaller at higher than at lower frequencies. Although the range of frequencies differ, the curve of steady-state amplitude as a function of frequency (Fig. 7*A*) is qualitatively similar to that for motoneurones. Eccles has suggested that the amplitude of the PSP may be taken as an index of the amount of transmitter released per impulse. Assuming that no modifications have occurred in either the chemosensitivity of the post-junctional membrane or in the passive properties of the extra-junctional membrane an approximate index of transmitter release per unit time may thus be obtained by multiplying the PSP amplitude by its frequency. A graph of this sort is illustrated in Fig. 7*B* and there is again a quantitative similarity between the data from *Aplysia* and those from motoneurones and from the sympathetic ganglion cells. The frequencies used in *Aplysia* differ from those employed in studies of motoneurone and sympathetic ganglion by a factor of 50 and 10 respectively, but all the sets of data show a threefold increase in transmitter release along the steepest linear portion of the curve.

One distinctive unitary EPSP fulfilling these criteria could usually be obtained from either connective by appropriate adjustment of the stimulus parameters (Fig. 6, part 5). The latencies of these two PSPs were practically identical. Since the connectives were of equal length, the fibres initiating the PSPs had the same conduction velocity. As was indicated above, these fibres fall into the group of most rapidly conducting fibres in the connectives.

Figure 8, parts A1–A3 and A4–A6 are from two different experiments and show the facilitation of a unitary, presumably monosynaptic, EPSP before and after four pairing trials with a priming stimulus to the siphon nerve. Throughout the duration of the facilitation the all-or-none elementary nature of the PSP could be demonstrated (Fig. 8 A3). The time course of the facilitation is illustrated in the graph of Fig. 8*B*. The facilitation had a maximum amplitude of 100 % above control, and a maximum

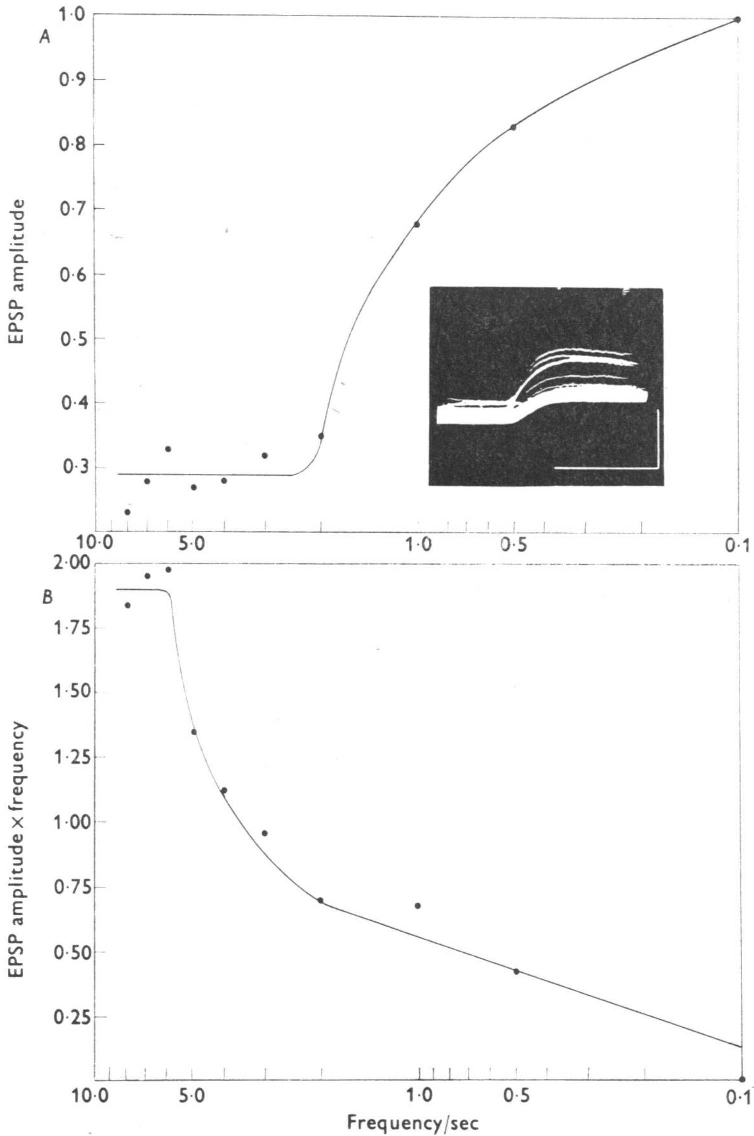

Fig. 7. *A* illustrates the changes in steady-state of the unitary PSP amplitude as a function of frequency. The inset is a superimposed sweep record at 8/sec to show the initial changes and the steady-state amplitude of the PSP. Note that the latency of the PSP remained constant. Calibration is 3 mV and 25 msec. In *B* the steady-state amplitude of the EPSP has been multiplied by its frequency. This index of transmitter release was plotted as a function of frequency of stimulation (see text for details).

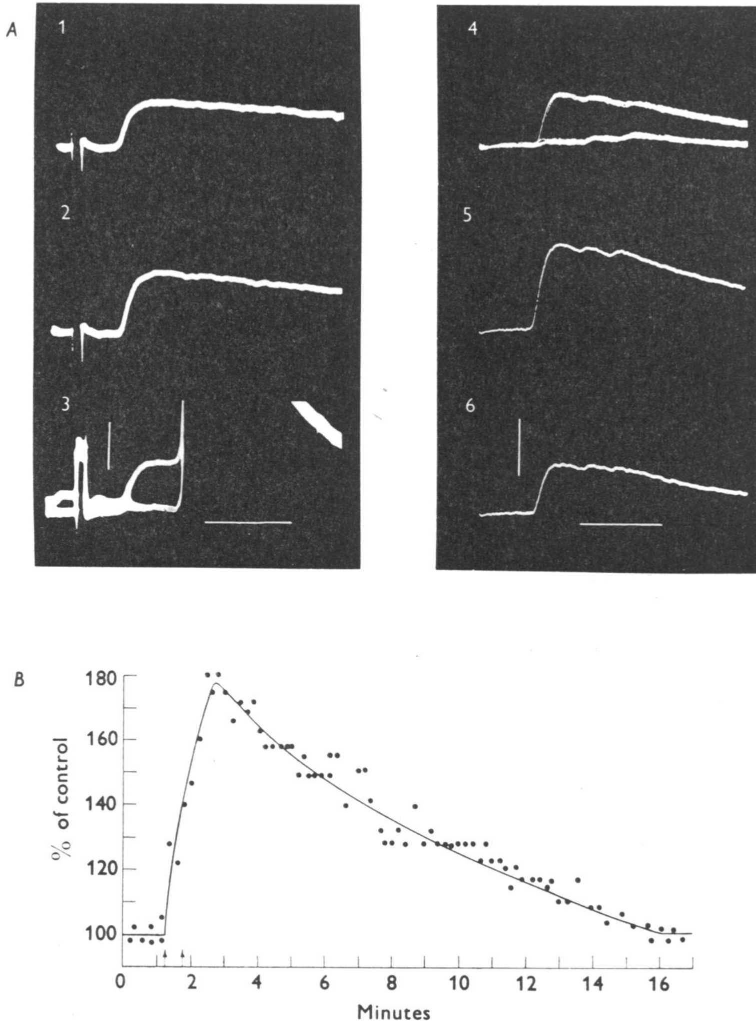

Fig. 8. *A*. Heterosynaptic facilitation of unitary, presumably monosynaptic EPSP. Parts 1–3 are from one experiment and 4–6 from another. Both experiments utilized an EPSP from the right connective; repetition rate in each is 1/12 sec. 1, Control; 2, 2 min after pairing; 3, 8 min after pairing, stimulus polarity was reversed and two sweeps superimposed to reveal latency relation of EPSP to antidromic A spike and all-or-none properties of PSP during facilitation; 4, control, superimposed sweeps illustrating all-or-none property of PSP and the presence of two late synaptic components; 5, peak facilitation after pairing; the late components have also increased; 6, return to control 14 min after pairing. Voltage calibration is 2·5 mV. Time calibration 2·5 msec. *B*. Percentage increase in a unitary, presumably monosynaptic, test EPSP (right connective) during and after four pairing trials with a suboptimal priming stimulus to the siphon nerve (arrows). Same unit as in *A* 1–3 but an earlier run.

duration of 15 min post-pairing. This compares reasonably well with the average values obtained with complex EPSPs. The maximum values of the complex response were not reached by the unitary PSPs. This may, in part, have been due to the necessity of using priming stimuli with unitary EPSPs. Optimal priming stimuli tended to initiate prolonged repetitive discharge of interneurones whose activity interfered with the accurate measure of the amplitude of the unitary test PSP during the early phase of facilitation.

In several experiments, it was also possible to measure the changes in amplitude of late unitary synaptic components in addition to the short latency, presumably monosynaptic, PSP. In some cases these late components were also facilitated, but in others they were not. The two synaptic components in Fig. 8 *A* 4 had a fixed and constant latency and did show an increase with facilitation. The late synaptic component in Fig. 9 had a variable latency and this PSP did not increase during facilitation.

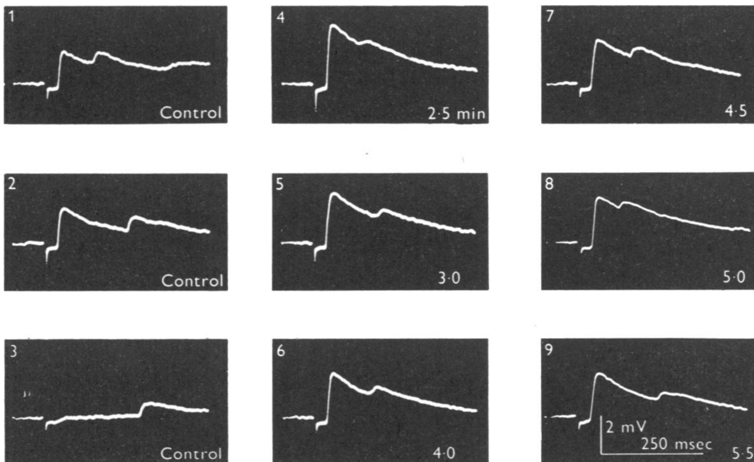

Fig. 9. Effects of heterosynaptic pairing on unitary PSP and on late synaptic potential. 1–3, control before pairing. Note all-or-none appearance of early PSP (left connective) and variable latency of late PSP. 4–9, facilitation of early unitary PSP 2·5 to 5·5 min after pairing.

The post-tetanic facilitation hypothesis was also examined with both complex and unitary test PSPs. Figure 10 illustrates the results of some of these experiments. Figure 10*A* shows the increase and decline in the same complex test PSP after five pairing trials with a priming stimulus to the siphon nerve (*A* 1) and after homosynaptic tetanization for 10 sec at 20/sec. Figure 10*B* illustrates a similar comparison with a unitary test PSP. *B* 1 shows the facilitation and decline of the unitary PSP after four heterosynaptic pairing trials and *B* 2 shows the results on the same

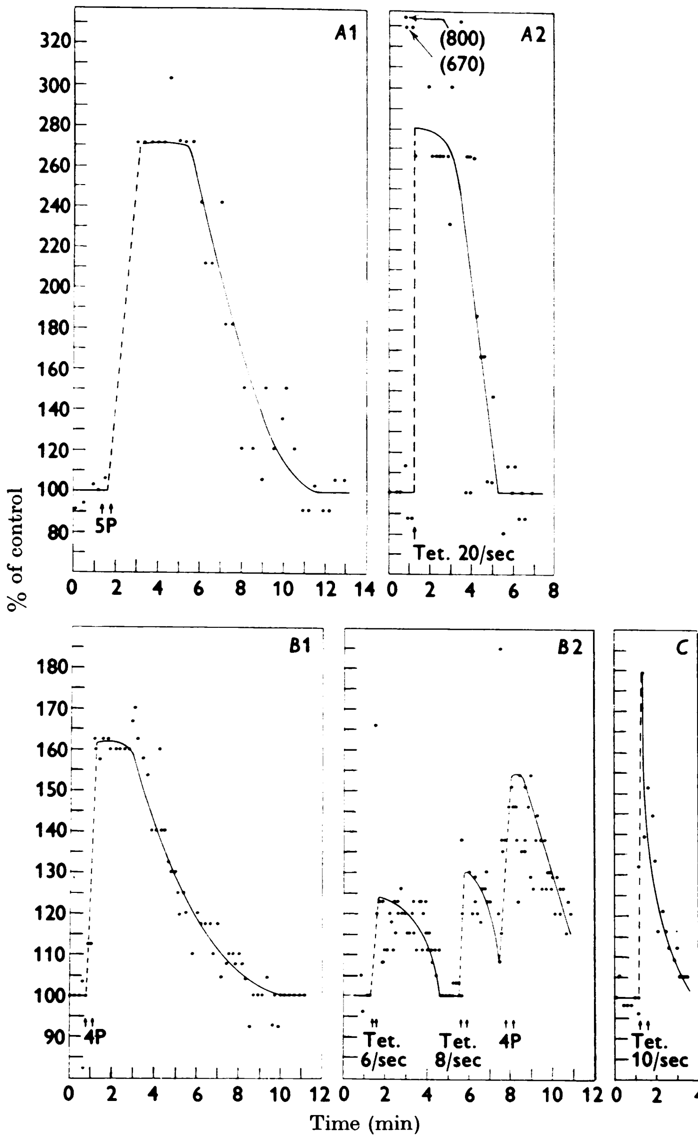

Fig. 10. Heterosynaptic and post-tetanic facilitation. A 1 and A 2 are from the same experiment with the same test PSP. A 1 shows the changes in a global test PSP following five heterosynaptic pairing trials with a priming stimulus (1) to the siphon nerve (6/sec for 1 sec). A 2 shows the changes in the test PSP following a 10 sec homosynaptic train of volleys at 20/sec to the test nerve. B 1 shows the changes in a unitary PSP following four heterosynaptic pairing trials. B 2 shows the change in the same elementary PSP following a homosynaptic train of 6/sec (for 5 sec) followed by a homosynaptic train of 8/sec (for 10 sec) followed by four hetero-synaptic pairing trials. *C* shows the changes in another experiment in a unitary PSP following a homosynaptic train of 10/sec for 5 sec.

elementary PSP of two consecutive periods of homosynaptic tetanization at 6/sec for 5 sec and 8/sec for 10 sec followed by four heterosynaptic pairing trials. With both complex and elementary PSPs, the increase in PSP amplitude after tetanization could be large and was comparable to the average values with heterosynaptic facilitation. But the time course of decay was consistently shorter. While other patterns of homosynaptic stimulation might have given a time course more comparable to that of heterosynaptic facilitation, brief intermittent homosynaptic trains at low frequency (similar to that used with the priming stimulus) do not cause significant facilitation (Segundo, Moore, Stensaas & Bullock, 1963 and personal communication).

DISCUSSION

The analysis that has been presented above and the discussion that follows are based upon the explicit assumption that, in the absence of alterations in the passive membrane properties of the giant cell, changes in EPSP amplitude reflect changes in release of the excitatory transmitter substance, and not changes in the chemosensitivity of the synaptic membrane. While this assumption has been repeatedly employed in studies of other systems (see Eccles, 1964 for review) its correctness has not been tested in most instances. This assumption could also not be tested in the current experiments since the excitatory transmitter for the giant cell has not been identified. However, in experiments with acetylcholine, which is the inhibitory transmitter for the giant cell and for other H cells of this ganglion, as well as the excitatory transmitter for D cells (Tauc & Gerschenfeld, 1962), response facilitation to iontophoretic injection of ACh was never encountered although response decrements (desensitization) were readily demonstrable (Tauc & Bruner, 1963).

Evidence has been presented to indicate that heterosynaptic facilitation is due to a change in the excitatory drive of the test input. Neither changes in the passive properties nor in the generation of spikes by the giant cell contribute to the facilitation which therefore cannot be due to changes in the post-synaptic unit. The possibility of disinhibition was made unlikely by experiments in which facilitation was demonstrated despite a post-synaptic inhibitory blockade by curare. Since test PSPs maintained their pre-pairing configuration during facilitation and the facilitation could also be demonstrated with a unitary test PSP, the possible mechanisms are restricted to one of two: (1) post-tetanic or (2) presynaptic facilitation. Either mechanism could explain the finding that the PSP of an elementary test unit is facilitated. In this sense, both mechanisms are 'presynaptic', since there is good reason to believe that the amplitude of the PSP is a measure of the amount of transmitter

released and that the control of transmitter release resides in the axon terminals (see Eccles, 1964). With post-tetanic facilitation, the increase in transmitter release would be due to repetitive activity initiated in the test unit by the priming stimulus. With presynaptic facilitation the firing frequency of the test unit would be unaltered but the priming stimulus would produce a change in the presynaptic terminals of the test pathway causing it to release more transmitter substance per test impulse than before pairing.

The available data show that post-tetanic facilitation tends to have a different time course from heterosynaptic facilitation. However, the data on the unitary PSP are limited since it was not possible to use frequencies higher than 10/sec. Also, the *pattern* of stimulation was not varied.

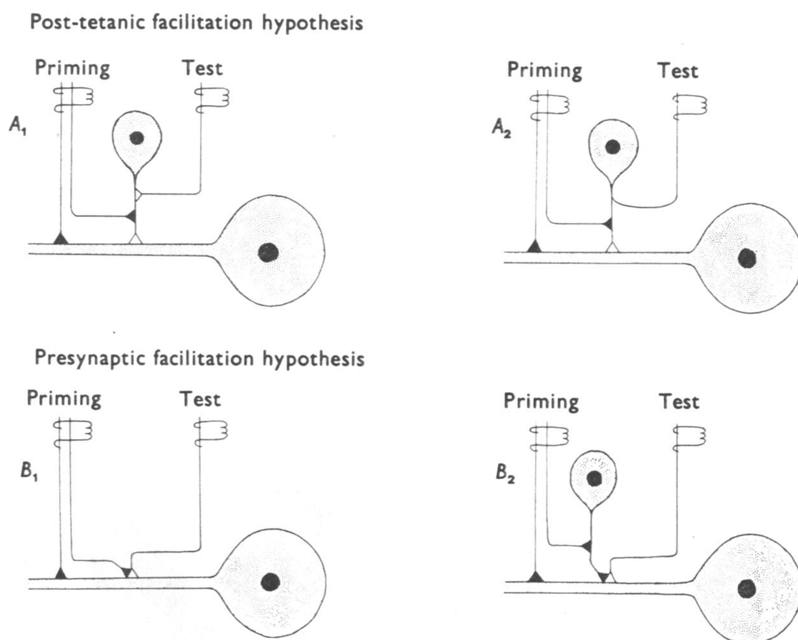

Fig. 11. Schematic diagrams to indicate alternate mechanisms for facilitation of the unitary PSP following heterosynaptic pairing (see text for details).

Therefore, the possibility of post-tetanic facilitation cannot be excluded on the basis of the available parametric evidence alone. There are, however, several additional reasons for considering post-tetanic facilitation as the less likely of the two possible mechanisms. The diagram of Fig. 11 illustrates two possible variants of the post-tetanic facilitation hypothesis as well as a schematic representation of the presynaptic facilitation hypothesis. The results will now be discussed in relation to these diagrams.

Post-tetanic facilitation by convergence on a common interneurone is illustrated in Fig. 11 *A* 1. This possibility can probably be excluded on the basis of the properties of the elementary presumably monosynaptic test PSP. The latency of the unitary test EPSP was consistent with it being mediated by the most rapidly conducting fibres in the connectives; this argues against an extrasynaptic delay. The ability to follow high frequencies without a shift in latency demands that an interneurone follow one for one without 'stutter'. In our experience with this ganglion, we have *never* encountered a neurone with this property. Moreover, the *constellation* of these criteria is strong evidence that these elementary PSPs is monosynaptic thereby excluding the possibility of an interneurone.

The evidence for monosynaptic transmission of the test response, however, does not conclusively demonstrate an interaction between nerve terminals unless one can be certain that the test axon is afferent to the ganglion. The possibility of monosynaptic transmission through an efferent axon is illustrated in Fig. 11 *A* 2. If the test axon were efferent to the ganglion, stimulation of the connective could produce an action potential in the test axon which would propagate antidromically toward the ganglion and might then invade a branch making synaptic contact with the giant cell. In this case the test EPSP would be monosynaptic but since the synaptic region of the test neurone is located *within* the abdominal ganglion it would be accessible to the priming stimulus which might initiate repetitive activity in the test unit and thereby create a condition of post-tetanic facilitation.

While this possibility has not been excluded, previous experiments with this ganglion have shown that antidromic volleys in one axon of a ganglion cell usually fail to invade the other branch, owing to blocking at the branch point, at repetition rates well below 10/sec (Hughes & Tauc, 1963; Tauc, 1957 and unpublished observation). On the basis of these results, it would be most unusual for one branch to follow faithfully frequencies of 10/sec applied to another branch without evidence of block.

The critical point of the post-tetanic facilitation hypothesis is that the test unit is set into repetitive activity by the priming stimulus. We have made a few attempts to investigate this point with relatively weak priming stimuli and elementary PSPs and in these experiments the test unit could be seen to respond *only* to the test volley.

In summary, the experiments are consistent with the hypothesis that the facilitation occurs as a result of a presynaptic interaction between the test and the priming pathway (Fig. 11 *B* 1 and *B* 2). However, the data are incomplete and the alternate possibility illustrated in Fig. 11 *A* 2, although less likely, has not been completely excluded.

It should be emphasized that independently of the mechanism involved

the available results do establish that in the giant cell a prolonged increase in the synaptic efficacy of an elementary test unit occurs after heterosynaptic pairing and that the locus for this type of facilitation is either in the presynaptic terminals of the test pathway (Fig. 11 *A* 2 and *B* 1) or in a neural circuit in which these terminals are involved (Fig. 11 *B* 2). The possibility of a 'circuit' mediating presynaptic facilitation is suggested by analogy to that proposed by Eccles for presynaptic inhibition of motoneurones (Eccles, 1964). This possibility is illustrated in Fig. 11 *B* 2 in which an interneurone has been interposed between the priming and the test pathway. With this circuit the duration of the facilitation may in part be determined by sustained firing of the interneurone.

Any detailed consideration of specific presynaptic changes associated with the postulated presynaptic mechanisms of facilitation is clearly premature, but it is of interest to evaluate briefly some additional anatomical and physiological data. Since heterosynaptic facilitation involves all inputs to the giant cell one would predict that presynaptic interdigitation should be quite common in the synaptic field of the giant axon. The corroborative electromicroscopic evidence for presynaptic interaction would be the demonstration of contacts between synaptic endings in apposition to each other as well as to the initial portion of the giant axon.

Presynaptic inhibition has recently been demonstrated in several synaptic systems (Frank & Fuortes, 1957; Dudel & Kuffler, 1962; Eccles, 1964). A form of heterosynaptic depression has also been shown to occur in certain cells of *Aplysia* (Fessard & Tauc, 1958) and results have now been obtained which indicate that this is due to presynaptic inhibition (Tauc, 1965). In the motoneurone, presynaptic inhibition occurs as a result of a depolarization of the presynaptic terminals. In suggesting a second and opposite presynaptic influence on synaptic efficacy, it is pertinent to recall that an increase in the release of transmitter substance may be caused by hyperpolarization of the presynaptic terminals (Hubbard & Willis, 1962) or by processes that increase sodium influx into the terminals (Birks, 1963). The recent demonstration of a hyperpolarization in the terminals of A fibres of the cat spinal cord (Mendell & Wall, 1964) by volleys in afferent C fibres is therefore particularly relevant since it suggests that presynaptic facilitation may also occur in the vertebrate central nervous system.

It should be emphasized that the mechanism of heterosynaptic facilitation which is specific to pairing and to the paired input is still undetermined (Kandel & Tauc, 1965). A non-specific presynaptic process such as that postulated here could, however, exhibit specificity if there were axon terminals which could undergo presynaptic facilitation only when they themselves had been invaded by an action potential some several hundred

milliseconds before the impingement of the priming stimulus. Specificity could also be achieved by means of appropriate convergence and divergence in a complex neural circuitry.

SUMMARY

1. An analysis of the cellular mechanisms of heterosynaptic facilitation in the right upper quadrant giant cell of the abdominal ganglion of *Aplysia depilans* was undertaken.

2. Possible contributions by either the passive properties or the spike-generating activity of the post-junctional cell were excluded by the demonstration that (*a*) the priming stimulus did not produce a significant conductance change in the giant cell, and (*b*) a train of directly initiated spikes could not serve as a priming stimulus.

3. Facilitation could be demonstrated despite a blockade, by curare, of post-synaptic inhibition, thereby eliminating the possibility of disinhibition.

4. The response configurations to different test stimuli were compared before and during facilitation. In most cases, the test PSPs retained their configuration during peak facilitation indicating an increase in the efficacy of the units initially making up the test pathway.

5. A presynaptic facilitation hypothesis was tested by use of an elementary, presumably monosynaptic, PSP. Such an elementary PSP was facilitated by 100% and for periods up to 15 min after pairing with a priming stimulus. The data are therefore consistent with this hypothesis.

6. The presynaptic facilitation hypothesis is, however, based on the additional assumption that (*a*) the test input is monosynaptic and (*b*) the test axon is afferent to the ganglion. Failure to meet either requirement would permit the priming stimulus to initiate repetitive activity in the test unit and thereby create a condition for post-tetanic facilitation. The criteria used and our experimental observations make it unlikely that repetitive firing of the test unit occurred, but this possibility cannot be fully excluded.

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