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PRESYNAPTIC INHIBITION IN THE ABDOMINAL GANGLION OF *APLYSIA*

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The results of the experiments revealing the presence of heterosynaptic facilitation in cells of specific location in the abdominal ganglion of *Aplysia* (Kandel & Tauc, 1964, 1965*a*, *b*), have shed light, in an indirect way, on heterosynaptic inhibition. The latter was observed years ago in the same ganglion but only preliminary reports were published (Fessard & Tauc, 1958; Tauc, 1958*a*, 1960). It appeared as a decrease of amplitude of a test excitatory post-synaptic potential (EPSP) when preceded by stimulation of another afferent pathway different from that producing the test EPSP. It was thought that heterosynaptic facilitation and inhibition might be two phenomena resulting from a similar mechanism but opposite in effects, like excitatory and inhibitory synaptic action (Tauc & Gerschenfeld, 1961); the knowledge of one might possibly help the understanding of the other. To establish this comparison, it was, however, necessary to re-analyse the old results concerning heterosynaptic inhibition and develop them in new experiments, in order to find cells in which the time course and intensity of heterosynaptic inhibition would be comparable to that of heterosynaptic facilitation. Also, it seemed desirable to confirm in a more definite manner the presynaptic character of the heterosynaptic inhibition.

This paper describes and analyses heterosynaptic inhibition observed in a group of cells of definite location in the abdominal ganglion of *Aplysia* which have very similar properties and in which the phenomenon could be particularly strong evidence. The results have shown that heterosynaptic inhibition in *Aplysia* is a presynaptic phenomenon, essentially similar to presynaptic inhibition in the spinal cord of vertebrates (Frank & Fuortes, 1957; Eccles, 1964), but of much longer duration and presenting some special characteristics. It is in many respects a mirror image of heterosynaptic facilitation. A brief account of these findings has already been published (Tauc, 1964).

METHODS

The techniques were essentially similar to those utilized in the preceding papers (Fessard & Tauc, 1958; Kandel & Tauc, 1964, 1965 *a*, *b*). The cells in the isolated ganglia of *Aplysia depilans* and *Aplysia punctata* were penetrated with one or two intracellular micro-electrodes used for recording and pre-setting the membrane potential to any desired level. In some experiments two different cells were penetrated and their activities recorded simultaneously. In order to establish whether interneurones were firing spontaneously, a continuous record of the cellular activity was taken at slow speed. Faster phenomena were observed by triggering a faster beam. 140 cells in thirty-seven preparations were examined.

The nerves and connectives leading to the ganglia were stimulated by shocks of alternating polarity (see Kandel & Tauc, 1965*a*). In this way a very constant synaptic response could be maintained for very long periods of time. The two connectives as well as the branchial, genital and siphon nerves were used. The pairing method used in preceding papers (Kandel & Tauc, 1964, 1965*a*) was not employed since the experiments showed that a single short train of stimuli to a nerve or connective (called 'conditioning' or 'priming' stimuli) usually 6–10/sec (for 1 or 2 sec) is enough to produce an intense effect on a test EPSP from another nerve or connective which was presented every 10 sec or, exceptionally, every 5 sec.

RESULTS

Identification of cells

A preliminary investigation of the properties of neurones located in different parts of the abdominal ganglion has established that heterosynaptic inhibition occurs in a group of cells located in the lower central part of the abdominal ganglia. An especially intense effect was observed in a group of about a dozen cells of constant localization which we arbitrarily called P1 cells. Simultaneous recordings from the P1 cells have shown that they have a very similar synaptic input with a number of common interneurones (Fig. 1*A*), as shown by synchronously appearing unitary EPSPs and an identical response to stimulation of different nerves and connectives. Repetitive homosynaptic stimulation produced in some of these cells the long-lasting inhibition (Tauc, 1958*b*), which consists of a transient hyperpolarization of 10–30 sec or longer duration (Fig. 1*C*, upper trace; Fig. 2, 6). All these neurones belong pharmacologically to the CILDA type (Gerschenfeld & Tauc, 1964). They are characterized by the presence of a non-cholinergic excitatory input and by a hyperpolarizing response to catecholamines.

These cells send efferent axons to the branchial and siphon nerves and receive an intense afferent excitatory input from these nerves and from the genital nerve. The stimulation of the two connectives is much less effective. It is supposed that at least a part of the input coming from the stimulation of the three nerves is monosynaptic, as the composite post-synaptic potential has a short delay, smooth rise and decline of a duration which over a large range is not appreciably affected by the intensity of the

stimulation in spite of large differences in amplitude. Also repetitive stimulation of a nerve at short intervals increases the amplitude of the resulting composite EPSP. It thus shows homosynaptic facilitation by repetition (Fig. 11). A unitary monosynaptic excitatory input from the right connective was constantly observed in P1 cells. It is described on p. 291.

Because of the easy identification and powerful effect of the input, these

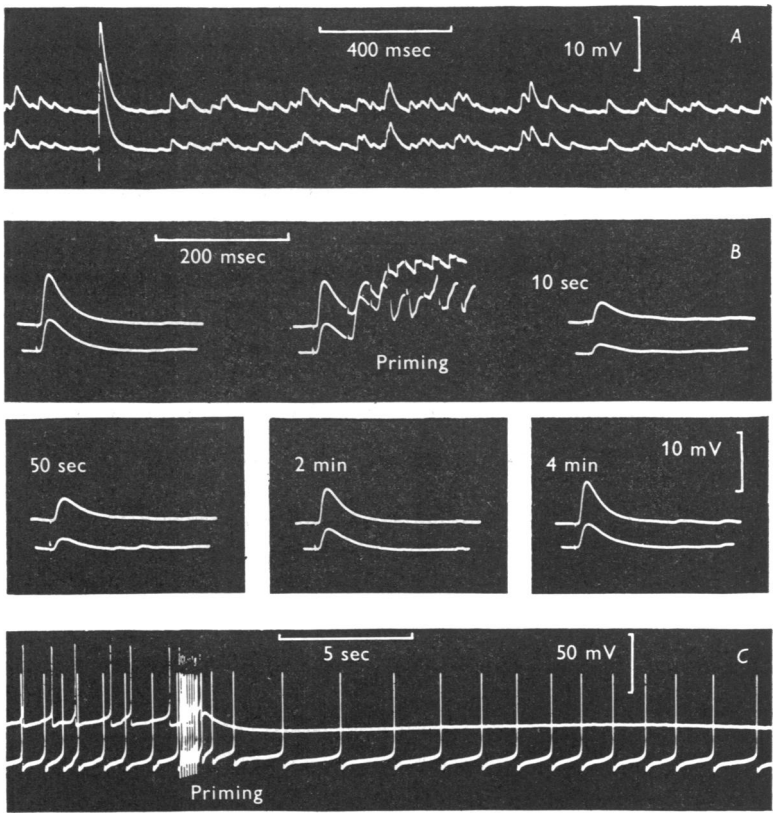

Fig. 1. Simultaneous intracellular recordings in two hyperpolarized P1 cells. *A*. Stimulation of the siphon nerve showing composite EPSP followed by unitary EPSPs from common interneurones. *B*. Composite test EPSPs from the branchial nerve are considerably depressed by a single short train of priming stimuli (frequency 10/sec) to the genital nerve. In this and following figures the test stimulus was presented every 10 sec: the recovery after priming stimulation is illustrated by records chosen at the times indicated. The cell in the lower record is less hyperpolarized than the other cell, which explains the difference in size of EPSPs. *C*. The same priming stimulation alone applied in absence of artificial polarization. Both cells show inhibition of long duration (ILD), much more intense in the upper cell, where it lasted 30 sec. But the total duration of ILD is considerably less than the time of recovery of EPSPs.

cells have been most often used in our experiments. Sometimes heterosynaptic inhibition was tried on cells in the vicinity of the P1 cells in order to obtain further information for comparison, but it was not possible to identify these from one preparation to another; in some of these, however, the effect was very strong (Fig. 2). Only cells in a limited region of the lower central part of the ganglia were affected by heterosynaptic inhibition.

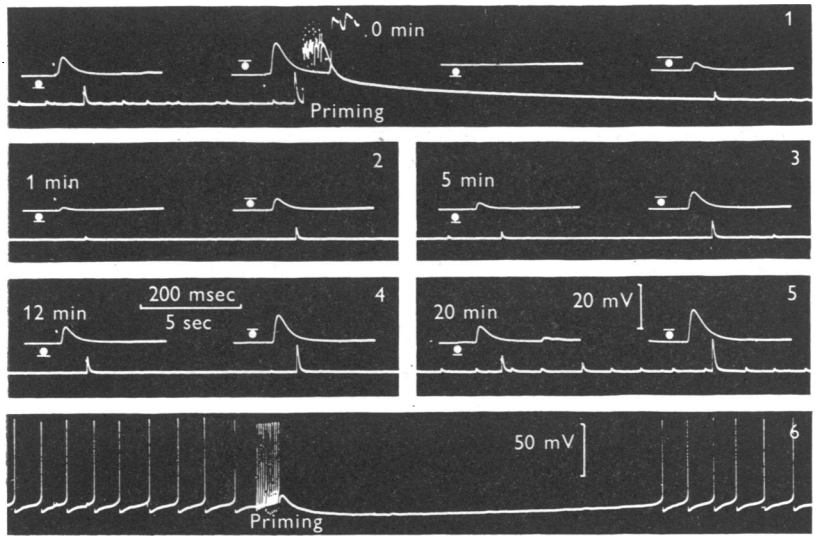

Fig. 2. Heterosynaptic inhibition shown in a cell in the vicinity of P1 cells. The lower trace represents a continuous recording. The upper trace shows records at higher speed during test stimulations. The polarity of stimulating electrodes is reversed after each stimulus as indicated by a dot above or below the base line. 1–5, At different times before and after priming stimulation (frequency 10/sec). Total recovery occurs in 20 min, cell hyperpolarized. The EPSP is presumably largely polysynaptic since its shape is modified during the heterosynaptic inhibition (test—branchial nerve; priming—siphon nerve). 6, Priming stimulus alone, in the same non-polarized cell showing spontaneous activity, produces ILD of 20 sec duration.

Heterosynaptic inhibition of a composite EPSP

When a short train (priming or conditioning stimulus) of stimuli is applied to a nerve or connective different from the one which is used for elaboration of the test EPSP in these cells, the amplitude of the EPSP after the priming stimulus is depressed.

Figures 1*B* and 2 illustrate such an effect. In Fig. 2 the test EPSP is clearly depressed for both polarities of stimulation and recovers its original amplitude only after 20 min. All P1 cells are affected in a similar manner by the priming stimulus, as shown in the simultaneous recordings from two

cells in Fig. 1*B*. The heterosynaptic depression can be quantitatively expressed as the percentage of amplitude of the test EPSP before (100 %) and after the priming stimulus. In Fig. 3 the test EPSP is depressed by a strong priming stimulus to 20 % of its initial value without full recovery after 30 min. A weak priming stimulus depressed the same test EPSP to only 60 % with a full recovery in 6 min.

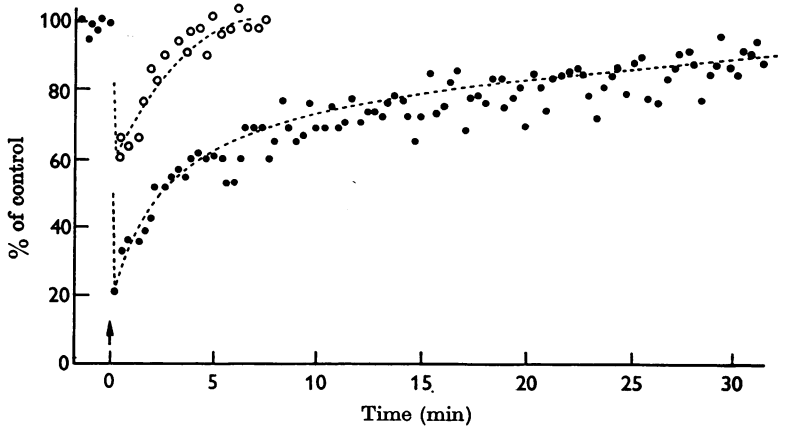

Fig. 3. Ordinate: amplitude of EPSP elicited by stimulation of branchial nerve expressed as percentage of amplitude before priming stimulating through the siphon nerve. Abscissa: time after onset of priming stimulus. ○, 2 priming stimuli at a frequency of 10/sec; ●, 16 priming stimuli at the same frequency. The recovery after strong priming was incomplete after 30 min.

The graph given in Fig. 4 shows typical curves of the heterosynaptic inhibition in one non-P1 cell produced by different intensities of the priming stimulus: 1, 2, 6 and 12 shocks, frequency 10/sec. Clearly both intensity of depression and the recovery time of the test EPSP depend upon the intensity of the priming stimulus. However, beyond a maximal priming stimulation the depression and recovery times do not increase.

Whereas the parameters of heterosynaptic depression are the same for all P1 cells in the same preparation (Fig. 1*B*), the parameters differed in cells from different preparations. The maximal recovery time in P1 cells was thus found to vary from a minimum of a few minutes to a maximum of 40 min. In two preparations P1 cells did not show any heterosynaptic inhibition. On the other hand, in some preparations, after a strong priming stimulus the recovery was not complete even after several hours. It is difficult to decide whether this is a prolongation of heterosynaptic depression or the consequence of an alteration in the condition of the preparation; it is, however, significant that these cells have retained an unaltered membrane excitability and the same height of spike. The

behaviour of non-P 1 cells was less uniform, big differences in the intensity of the depression being observable in different cells in the same ganglion.

The depression of test EPSPs does not immediately follow the priming stimulus, but occurs with a delay of several seconds. The delay of the depressive action is also indirectly dependent upon the intensity of the priming stimulus; with more intense priming stimulation the delay of the action decreases. The delay is generally longer in non-P 1 cells than in P 1 cells. But there is always a definite irreducible period before the effects of the depression are maximal. This is apparent from the graphs in Fig 4. The

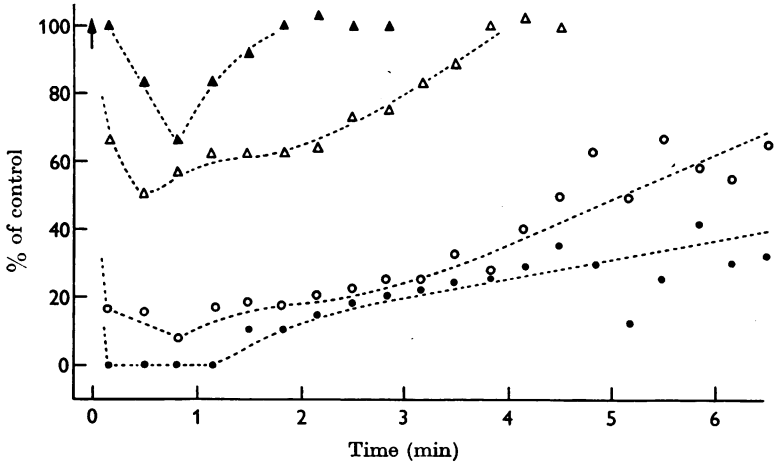

Fig. 4. Time course of heterosynaptic inhibition in a non-P 1 cell after different intensities of priming stimulation (1, ▲; 2, △; 6, ○; and 12, ●, stimuli, to siphon nerve (frequency 10/sec). The inhibition of test EPSP from branchial nerve occurs with a delay clearly visible after weak priming stimulations. The time required for full recovery increases with the intensity of priming and was 15 min after 6 stimuli and 28 min after 12 stimuli.

maximal depressive action in this non-P 1 cell is between 20 and 40 sec for the two weak priming stimuli. A clear demonstration is also given in the experiment illustrated in Fig. 5 which shows in a P 1 cell an EPSP at two different positions with respect to the priming stimulus. It is to be noted that the EPSP immediately following the priming stimulus is not diminished, but shows, rather, a small increase in amplitude. The depression occurs only later.

In all cells a clear diminution of the depressive heterosynaptic action and of the recovery time is observed when the heterosynaptic depression cycle is repeated several times. After several sequences on the same ganglion, the effectiveness of the priming stimulus in producing a depression of the EPSP is diminished after each cycle. Eventually, heterosynaptic depression may fail to occur. A partial restoration of the capacity

to produce heterosynaptic depression is observed after a long period of rest. It does not seem that this alteration of the capacity to produce the heterosynaptic depression by 'use' results from a deterioration of the preparation with time; a ganglion which has been pinned in the nerve chamber for the same period of time as a preparation that has lost the ability for heterosynaptic depression shows the depression with full intensity.

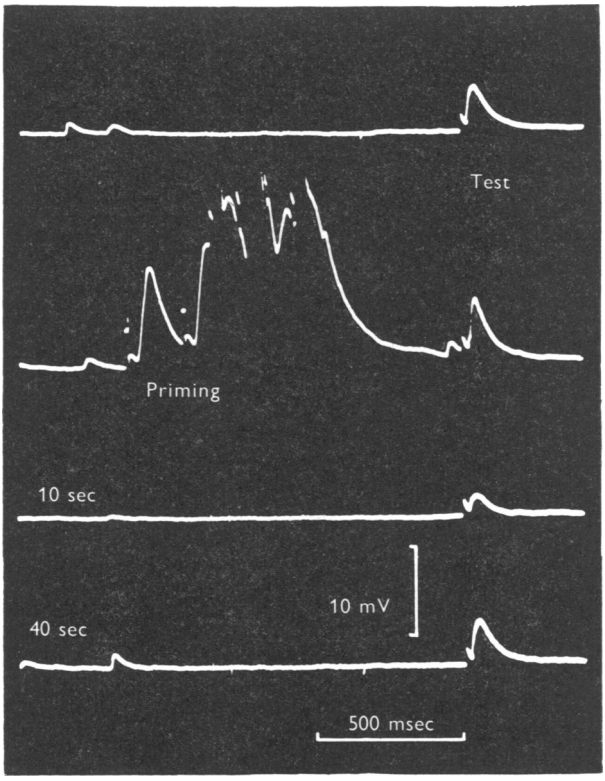

Fig. 5. Recordings from a hyperpolarized P 1 cell. The test stimulus (branchial nerve) presented immediately after weak priming stimulation (four shocks to siphon nerve) is not depressed. Maximal inhibition is seen after 10 sec; recovery occurs in 40 sec.

It has to be noted that in P 1 cells any nerves or connectives might be used as test pathway and as a priming stimulus. However, the most pronounced heterosynaptic effects are produced on test EPSPs from nerves and the nerves are also the most effective priming pathways. The EPSPs produced by the stimulation of the connective are relatively small and show a big polysynaptic component. Used as test EPSPs, they are less affected, and recover more rapidly, than the test EPSPs from nerves; used as priming pathways they produce little heterosynaptic depression.

Depression of test EPSP is sometimes observed when strong priming stimulation is applied to the nerve which receives the test stimulation. Owing to the heterogeneity of fibres in the nerves, it is supposed that, in these conditions, the priming stimulus activates more fibres than that of the test pathway and consequently acts as a heterosynaptic stimulus. The amplitude of the test EPSP would then result from the difference between the post-activation potentiation and heterosynaptic inhibition.

Site of the mechanism of heterosynaptic inhibition

The fall in the amplitude of the test EPSP must arise either from a change in the post-synaptic cell, from the diminution of recruitment of interneurones, or from the modification of efficacy of the presynaptic fibres of the test pathway.

Post-synaptic unit. In the search for a possible change in the post-synaptic unit, the passive membrane properties and excitability of ganglion cells were studied after the application of a priming stimulus. The conductance was measured directly with square hyperpolarizing pulses before and after applying the priming stimulus (cf. Kandel and Tauc, 1965*b*). In most of the cells conductance change was detectable; in some of the cells, especially in some of the P 1 cells, a small increase in conductance was observed. This conductance change is related to the long-lasting inhibition which was often observed in these cells. However, the duration of this conductance change was less than 30 sec and in a given cell it was much briefer than the recovery time of the heterosynaptic inhibition.

An easier way to detect a conductance change in the post-synaptic unit, requiring only a single intracellular electrode, is to use as indicator the amplitude of an antidromic A spike (Tauc, 1957, 1962). For this purpose the test EPSP was produced by stimulating a nerve which contained an axon of the examined cell; consequently the cell was stimulated both ortho- and antidromically. The somatic spike was eliminated and the size of the A spike depressed by hyperpolarizing the cell (a bridge was used for passing current through the recording electrode). If conductance changes were occurring under the influence of the priming stimulus, the amplitude of the A spike would be altered (see also Tauc & Gerschenfeld, 1962). Figure 6 shows such an experiment: whereas the occurrence of the priming stimulus does not alter the amplitude of the A spike, the subsequent EPSP is considerably depressed.

Many cells showed autoactivity and the rhythm then provided a more sensitive index of excitability than any direct measure. The effect of 'heterosynaptic' stimulation on spontaneous firing was therefore always tested. In all cases the modification of spike frequency was considerably shorter-lasting than the duration of heterosynaptic depression. Examples

of changes in spontaneous frequency in cells showing 'inhibition of long duration' (Tauc, 1958*b*) are shown in Figs. 1 and 2, 6.

It was always possible to induce heterosynaptic inhibition in cells without producing spikes by the priming stimulus in the post-synaptic unit. Also a directly evoked train of spikes in the cell never had any effect on the amplitude of the test EPSP.

It seems therefore clear that the post-synaptic unit is not involved in the mechanism of heterosynaptic inhibition.

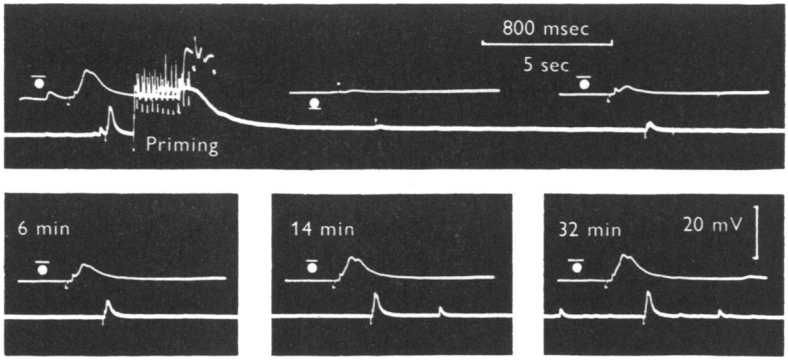

Fig. 6. Test for conductance changes during heterosynaptic inhibition in a P 1 cell by the method of antidromic A spike (see text). The test stimulus is applied to the branchial nerve, which contains both afferent fibres and the efferent axon of the cell; for one polarity of stimulation the test EPSPs are preceded by a small antidromic A spike, not invading the soma. The priming stimulus to the siphon nerve depressed the EPSPs, leaving the amplitude of the A spike unmodified.

Reseau. As in the experiments on presynaptic facilitation (Kandel & Tauc, 1965*a*) curare was utilized to eliminate the interference of synaptic inhibition. It was shown (Tauc & Gerschenfeld, 1961, 1962) that most of the synaptic inhibition (and part of the excitation) in the ganglia was cholinergic and consequently blocked by curare, whereas the excitation to the CILDA-type cells is not cholinergic (Gerschenfeld & Tauc, 1964). It is reasonable to suppose that, after soaking for half-an-hour in D-tubocurarine 5×10^{-4} g/ml., synaptic inhibition would be eliminated from the ganglia. This treatment affects neither the intensity nor the recovery time of heterosynaptic inhibition.

This experiment, however, does not exclude the explanation of heterosynaptic inhibition as a diminution in the recruitment of active interneurones by the priming stimulus. The synaptic inhibition is not the only factor diminishing the excitability of interneurones; indeed, the latter might be affected by the already mentioned inhibition of long duration, the effect of which is even more powerful than that of synaptic inhibition.

To exclude completely the role of interneurones in heterosynaptic inhibition it was necessary to choose for the test response a purely monosynaptic input.

Unitary monosynaptic input. It has been mentioned that the afferent fibres from branchial and siphon nerves to P1 cells are most likely monosynaptic in part. The amplitude of the test response from these nerves is very intensely affected by heterosynaptic inhibition, but the duration of the EPSP is not modified. In the absence of any anatomical evidence the criteria for the monosynaptic nature of test fibres to a P1 cell are, however, subject to criticism. To remove any doubt, the effect of heterosynaptic stimulation upon a clearly unitary and monosynaptic EPSP was tested. The criteria for the monosynaptic and unitary nature of the EPSP were essentially the same as those used in a previous paper (Kandel & Tauc, 1965*b*): constant latency, ability to follow high frequencies (10 or more/sec) without change in latency, all-or-none responsiveness.

It appeared that in P1 cells (and in some cells in the vicinity) such unitary EPSPs produced by terminations of a single axon might be easily evoked by liminal stimulation of the right connective. Repetitive stimulation produced EPSPs which appeared with a constant latency (Fig. 9, 6) in an all-or-none manner. However, in contrast to the unitary EPSPs in the giant cell, which show a decrease in amplitude during repetitive stimulation (see Fig. 7 in Kandel & Tauc, 1965*b*) the EPSPs in P1 and other CILDA cells are considerably potentiated (Fig. 7). Homosynaptic stimulation, applied at a frequency and duration reproducing that used for priming stimulation, was followed by an intense post-activation potentiation, lasting for several minutes. A post-tetanic depression was never observed, even with a stimulation of much higher frequency and duration than that used for priming stimulation.

Heterosynaptic stimulation of the branchial or siphon nerve was followed by a considerable depression of the unitary test EPSP amplitude without affecting its duration (Fig. 8). A strong priming stimulus was even able to suppress completely the test EPSP (Fig. 8*A*). The recovery time was several minutes; it was, however, shorter than the recovery time of test EPSPs produced by the branchial or siphon inputs. This is not surprising, as it is difficult to believe that all afferent fibres to the same cell would have the same properties or be affected in the same manner; the *Aplysia* brain is not a homogeneous structure. On the contrary, a heterogeneity of properties can be expected, especially for different inputs. This was already shown above (p. 287) with respect to the efficacy of different inputs. The same fibre, however, should have the same properties on whatever cell it synapses. This is indeed the case for the unitary EPSP resulting

from the liminal stimulation of the right connective activating a single fibre, which appeared to synapse with all P 1 cells. Simultaneous recordings from different cells in this group show that the unitary EPSP which appeared to be produced by the same afferent fibres is equally depressed in all cells and recovers in the same time (Fig. 9, 1–5), in spite of a different

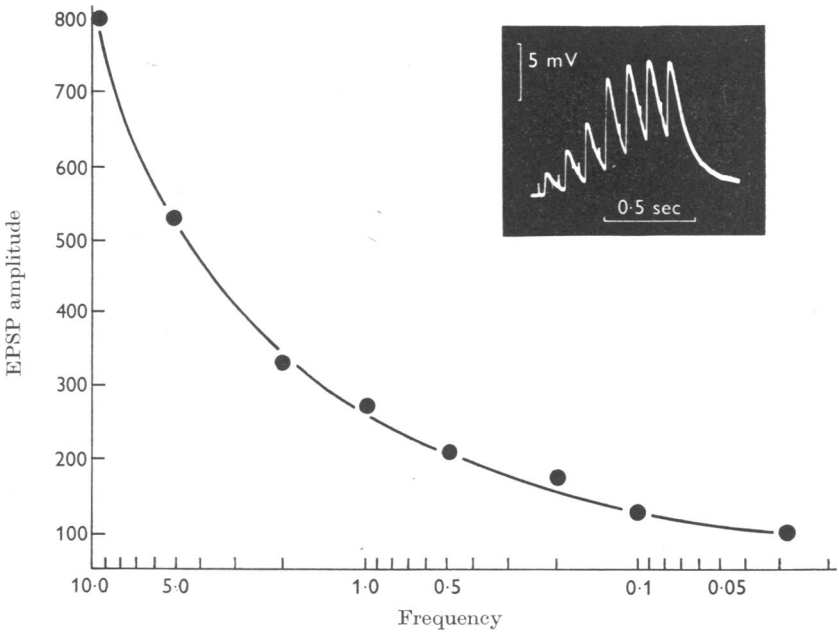

Fig. 7. The steady-state amplitude of the unitary and monosynaptic EPSP from the right connective in a P 1 cell as a function of frequency: semi-logarithmic scale. The insert shows the initial change of an EPSP stimulated after rest at 8/sec.

action of the priming stimulus on the two post-synaptic cells (Fig. 9, 7). Again, this figure shows that the priming stimulus produced a complete disappearance of this unitary test EPSP in both cells for about 20 sec.

It is to be noted that the amplitude of an elementary EPSP is not constant even if stimulated at slow frequencies. Variations occur continuously, owing to the events at the synapse (possibly changes in quantal release of the transmitter substance) and not in the presynaptic axon; simultaneous recording of unitary EPSPs in different cells but coming from the same presynaptic axon show EPSPs of unequally varying amplitudes (unpublished observations). Because of this constant variation around a mean value, it was often necessary, when the effect of a priming stimulus was studied, to consider not the momentary height of the EPSPs but the mean amplitude of a series (cf. Fig. 10).

The decrease in the capacity of the same priming stimulus to produce

heterosynaptic inhibition when the cycle priming stimulation/recovery is repeated has already been mentioned. Figure 10 illustrates such diminution in efficacy on a unitary and monosynaptic EPSP in a P 1 cell. After several cycles depression of the test EPSP is smaller and recovery time shorter. After fifteen trials the priming stimulus is practically ineffective. Yet the EPSP produced in the cell by the priming stimulation did not show any significant changes. It is therefore difficult to ascribe the diminution of

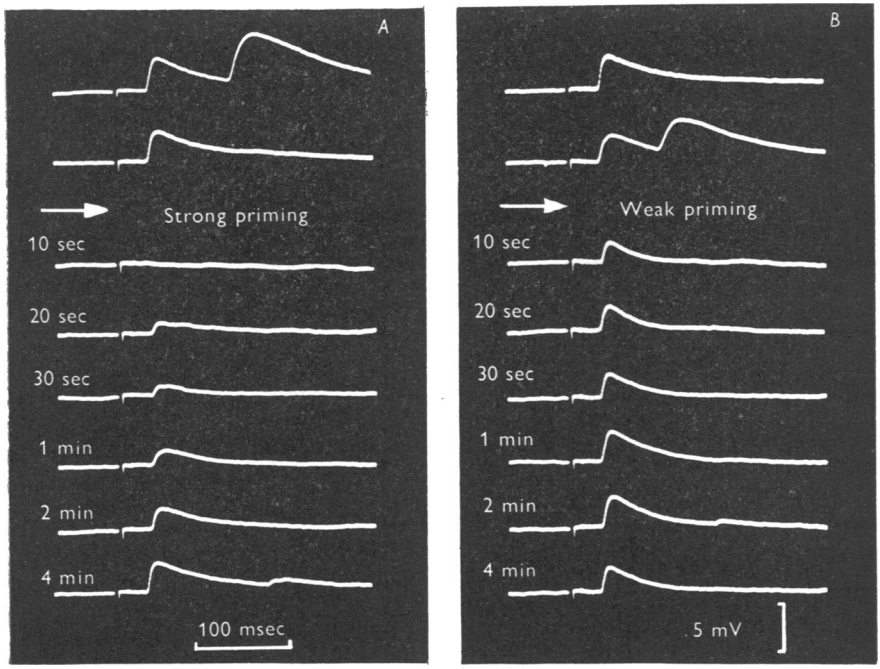

Fig. 8. Heterosynaptic inhibition on a unitary and monosynaptic EPSP from the right connective in a P 1 cell. Effects of strong (*A*) and weak (*B*) priming stimuli applied to the branchial nerve. Test stimulus every 10 sec.

capacity to produce heterosynaptic inhibition to a change in efficacy of the priming pathway, at least as far as its direct synaptic effects are concerned.

The differences in the recovery time of P 1 cells observed for the first maximal priming stimulations in different preparations might be due to the recent history of the animal, which had been previously affected by different quantities of natural 'priming' stimulation.

In some cells, not belonging to the P 1 cell type, unitary and monosynaptic EPSPs from fibres other than those shown in Figs. 8 and 9 were used as test EPSPs. In a few cases, for the same priming stimulus, the recovery time of these unitary EPSPs approach that of a global test EPSP

with a maximum of 8 min. It was, however, not possible to get unitary monosynaptic EPSPs from the branchial or siphon nerves.

The unitary monosynaptic test EPSPs from cells other than P 1 cells often showed the same delay in the maximal depression effects of the

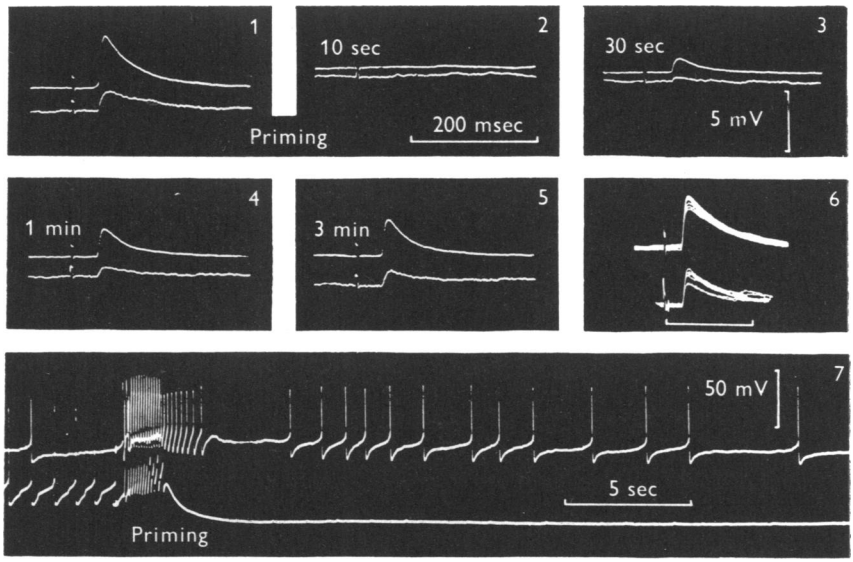

Fig. 9. Simultaneous recordings from two P 1 cells. 1–5, Unitary and monosynaptic EPSPs presumably produced by the same afferent fibre (right connective) in the two cells are both inhibited by the priming stimuli (10 stimuli to the branchial nerve) and recover in the same time. Test presentation, every 5 sec. 6, Superposed traces of these EPSPs show no change in delay. 7, Effects produced by the same priming stimulation alone on the two cells at normal polarization which permit the cell to fire spontaneously. The duration of ILD in the lower cell was 70 sec.

priming stimulus as that observed on global test EPSPs. The delay sometimes amounted to 40 sec (Fig. 11) with a mean value of 20 sec.

The P 1 cells and other cells in which heterosynaptic inhibition could be demonstrated often showed unitary EPSPs as a result of the activity of interneurones. This forms 'synaptic noise' in which, however, unitary EPSPs are identifiable (Tauc, 1958*a*). The priming stimulus usually depresses the synaptic noise in the cells for quite a long period. Such an effect was described by Fessard & Tauc (1958). However, the recovery time of these unitary EPSPs is usually shorter than the recovery time of complex test EPSPs from the branchial or genital nerve (Fig. 12). Some of the unitary EPSPs do not show depression but only a change in frequency. This again underlines the heterogeneity of properties of presynaptic fibres with respect to heterosynaptic inhibition. A similar specificity

of presynaptic fibres was noted with respect to heterosynaptic facilitation (Kandel & Tauc, 1965*b*).

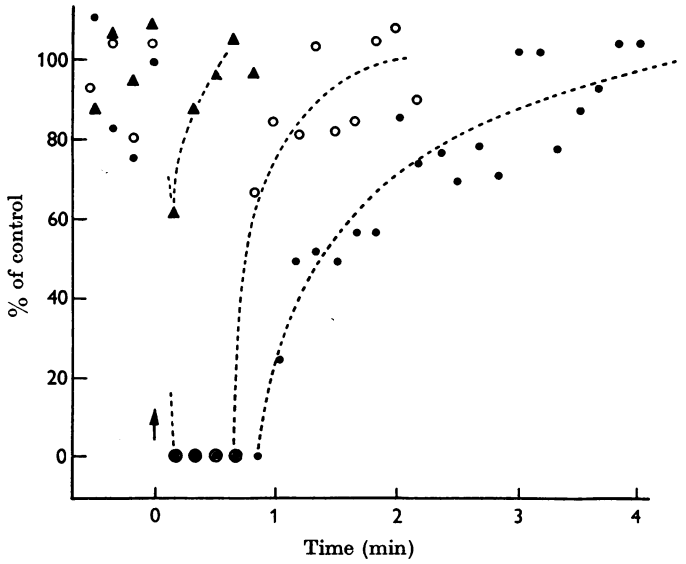

Fig. 10. Curves representing the diminution of heterosynaptic depression and recovery time of monosynaptic and unitary test EPSP (right connective) by repetition of the same priming stimulus (six shocks at a frequency 6/sec to the branchial nerve, applied at 4 min intervals). After the 15th trial the cell was given 1 hr rest. The priming stimulus applied after the rest period produced a heterosynaptic inhibition of intensity similar to that after the 8th trial. ●, 1st trial; ○, 8th trial; ▲, 15th trial.

Spontaneous heterosynaptic inhibition

The abdominal ganglion has a relatively complex nervous structure containing many interneurones. Some of these interneurones show spontaneous activity which may be tonic or phasic (Tauc, 1958*a*). It was assumed *a priori* that some interneurones would convey heterosynaptic inhibition and that one could expect the phenomenon of heterosynaptic inhibition to appear in the analyses of spontaneous activity. A method of discerning such a spontaneous action was to present a test EPSP over long periods of time and to analyse the record in the hope that some spontaneous phenomenon would have affected the size of the test EPSP. Surprisingly this happened very often.

Figure 13 illustrates the action of such a spontaneous interneurone on the test EPSP. The direct synaptic effect on the cell (see Spont. on the figure) is very small; yet the test EPSP, which results from the activity of many fibres, is clearly depressed for about 1 min. It is to be noted that

the post-synaptic membrane properties are not affected, as seen from the size and frequency of the spontaneous spikes after a strong priming stimulus (Fig. 13, 4). Another example is presented in Fig. 14. Although the cell in this figure is taken from a different preparation from that of Fig. 13, the short synaptic effects indicating the activity of interneurones

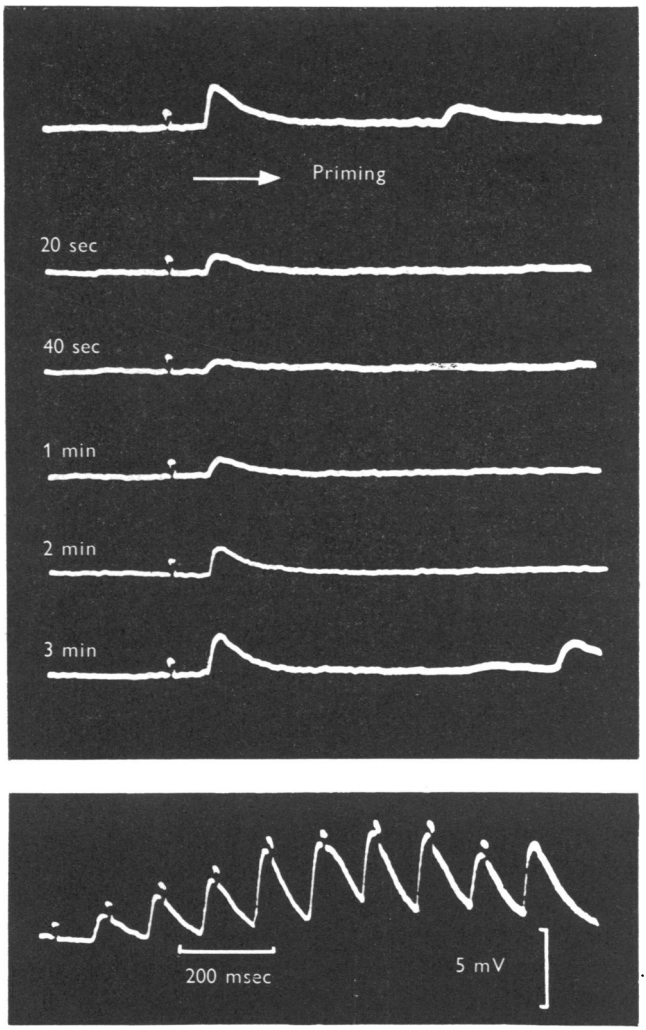

Fig. 11. Presynaptic inhibition on a unitary and monosynaptic test EPSP (right connective) occurring with a delay after the priming stimulation (six shocks at 6/sec to the siphon nerve). Maximal inhibition is observed 40 sec after the priming stimulus. A hyperpolarized non-P 1 cell. Lower record represents the primary potentiation of the same EPSP produced by a frequency of 10/sec. Note also the unmodified delay of each response.

responsible for the heterosynaptic inhibition are similar in both cells. The unchanged size of the A spike indicates that the depression of the test EPSP is not a post-synaptic phenomenon.

Heterosynaptic inhibitory action on unitary and monosynaptic test EPSPs observed in a cell not belonging to the P1 cell type is shown in Fig. 15. The small direct synaptic effect on the cell (Spont. in Fig. 15, 1),

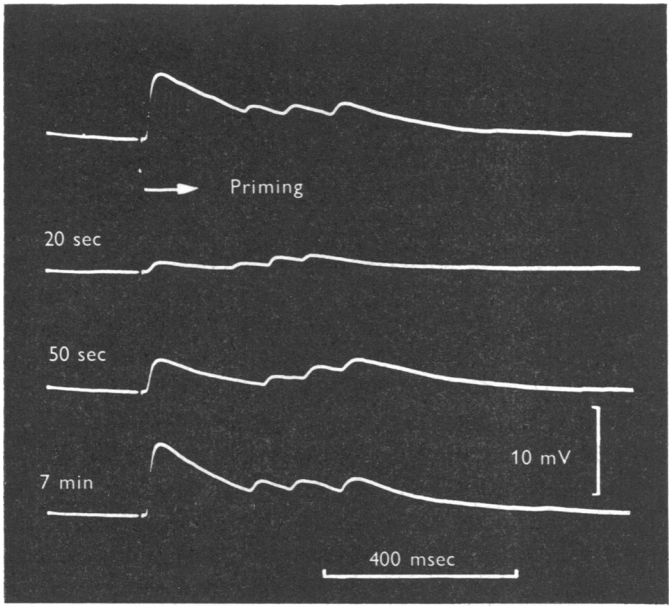

Fig. 12. Heterosynaptic depression of a composite EPSP and superposed unitary EPSPs from interneurones. The interneurone EPSPs are less depressed than the composite EPSP from the branchial nerve, and recover also more rapidly. Priming stimulation of the siphon nerve.

similar to that of Figs. 13 and 14, led to the complete disappearance of the test EPSP. This drastic depressive action occurred with a delay of several seconds (Fig. 15, 2). The first test stimulus applied after the 'spontaneous priming wave' produces an EPSP which is practically unmodified, but the two following stimuli give no potential change. The action is similar to that produced by the priming stimulation of a nerve (Fig. 15, 3). In spite of the fact that the total priming EPSP appearing in the cell is much bigger than the 'spontaneous priming wave', the total depression of the test EPSP is of about the same duration: the maximum depressive action appears in both cases with a delay which can be estimated at approximately 20 sec.

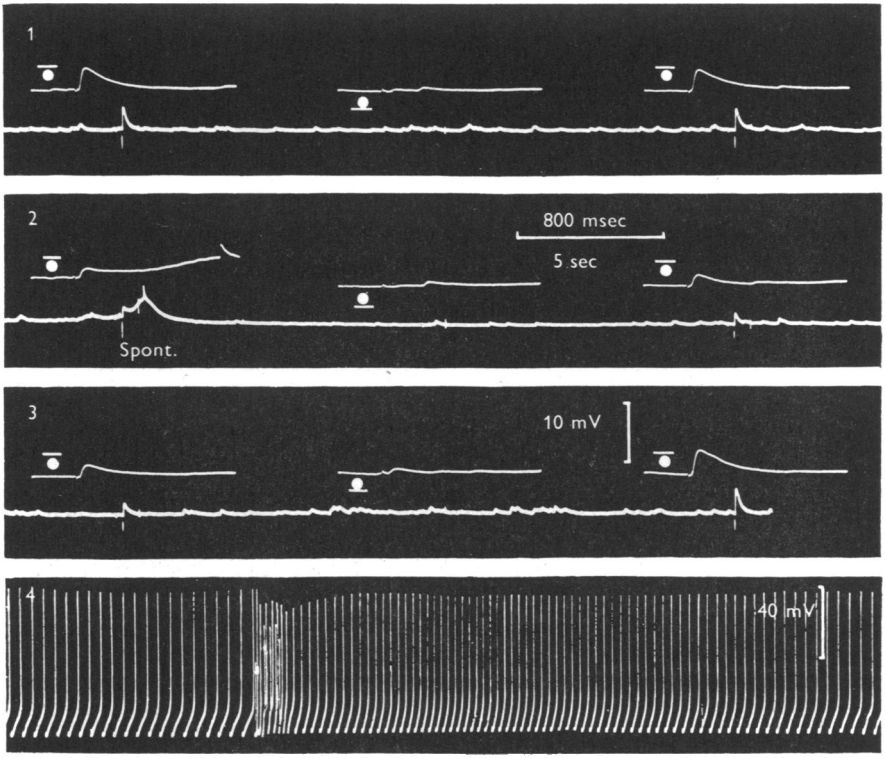

Fig. 13. 1–3, Spontaneous inhibition of a composite test EPSP from the branchial nerve preceded in the cell by a 'spontaneous priming wave' (Spont. in 2). Reversal technique was used for test stimulation. Continuous records, but 10 additional seconds are to be added between 1 and 2 and between 2 and 3. In 4 a strong priming stimulus (genital nerve) was applied to the same cell at normal polarization. The modification of frequency of spontaneous firing produced in this way did not last more than 10 sec, whereas the effect of 'spontaneous priming wave' on the test EPSP's lasted 60 sec.

DISCUSSION

Presynaptic nature of heterosynaptic inhibition

In many ways heterosynaptic inhibition resembles the inhibitory action occurring in the spinal cord due to the reduction in size of monosynaptic EPSPs of motoneurones by muscle afferent volleys (Frank & Fuortes, 1957; Frank, 1959). Frank and Fuortes first called this inhibition presynaptic inhibition and this term was accepted by the Canberra school, which has contributed considerably to the knowledge of this phenomenon (Eccles, 1964). Like the presynaptic inhibition in the spinal cord, the heterosynaptic inhibition in *Aplysia* does not result from any change in

the post-synaptic unit: there is no associated conductance change or excitability change in the post-synaptic membrane. The experiments with curare have shown that cholinergic post-synaptic inhibition is not involved in the process. The demonstration of heterosynaptic inhibition for monosynaptic and unitary EPSPs very clearly indicates that the reduction of the EPSP is due to the diminished efficacy of the presynaptic impulses in the test pathway. It seems therefore justified to consider the heterosynaptic inhibition in *Aplysia* as a presynaptic inhibition similar to that described for motoneurones in the spinal cord.

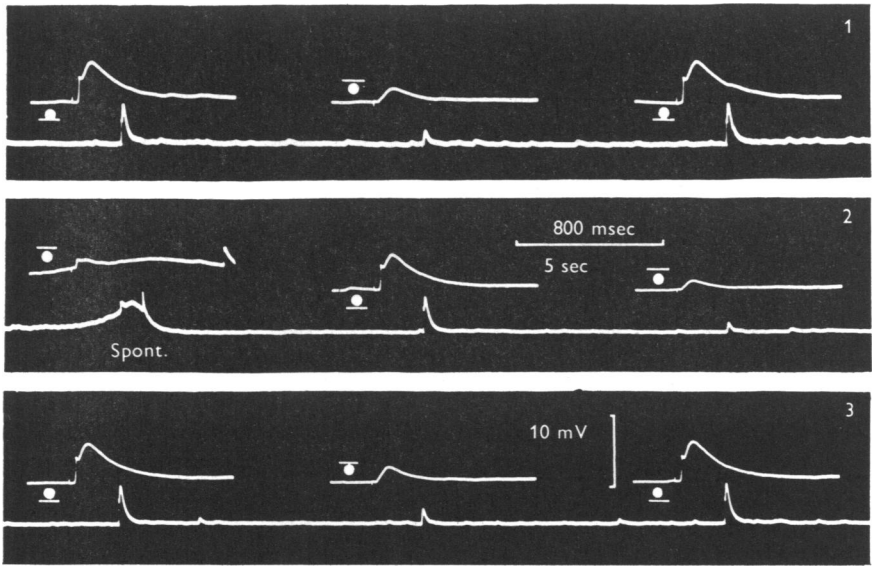

Fig. 14. Inhibitory effects on test EPSP (siphon nerve) following 'spontaneous priming wave' (Spont. in 2). Reversal technique for test EPSPs. The test response shows, at one polarity, antidromic A spikes the amplitude of which was not affected. 1–3 continuous recordings.

One of the interesting features of the presynaptic inhibition in *Aplysia* is its long duration, which in some cells and for strong priming stimuli can attain 30–40 min and perhaps more. In this and in the many other features already mentioned, it resembles the heterosynaptic facilitation observed in the same ganglia, the inhibitory character of the action excepted. A possible explanation of the heterosynaptic facilitation in *Aplysia* was discussed in the previous paper (Kandel & Tauc, 1965*a*). In the experimental work on heterosynaptic facilitation it was not possible to discard completely the possibility of access of the priming pathway to the test pathway. Such access would induce repetitive firing in the test pathway which would increase the amplitude of the test EPSP by a pro-

cess similar to post-tetanic potentiation. The interference of post-tetanic facilitation in heterosynaptic inhibition is *a fortiori* excluded. Indeed repetitive activity of a unitary test input was always followed by a post-tetanic facilitation; a post-tetanic depression was never observed. If the

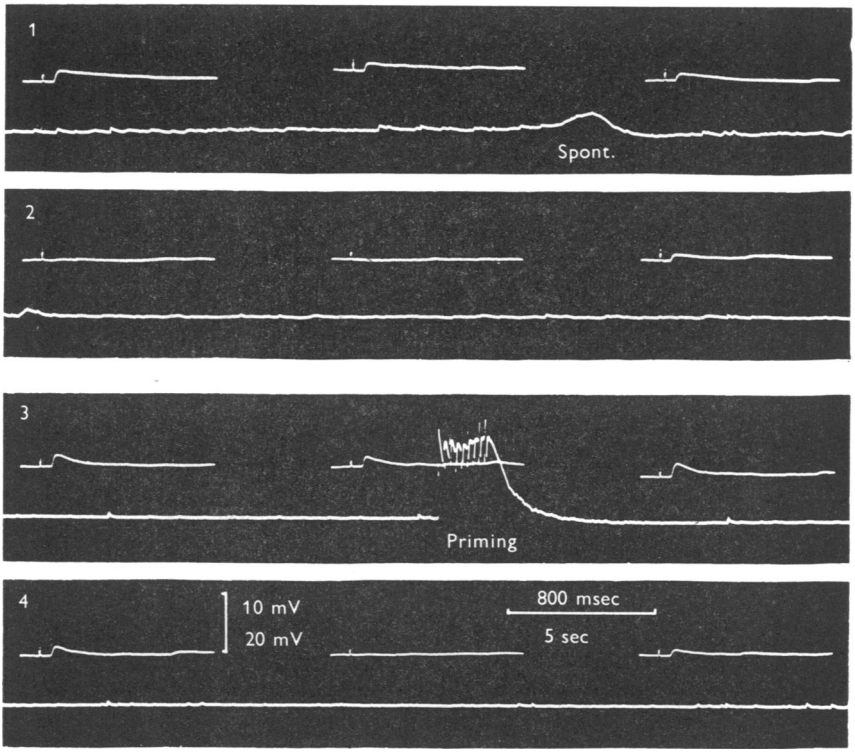

Fig. 15. Inhibitory effects on unitary and monosynaptic test EPSPs (right connective) following a 'spontaneous priming wave' (1–2) and a priming stimulation to branchial nerve (3–4) in the same non-P 1 cell. Both actions on the test EPSPs occur with a delay and lead to a complete abolishment of the monosynaptic EPSP. Continuous recordings between 1 and 2 and between 3 and 4.

similarity between heterosynaptic inhibition and facilitation is considered, the heterosynaptic facilitation could not result from post-tetanic potentiation. This argument by analogy which, by itself, would be of very little value adds to the series of others (Kandel & Tauc, 1965*b*) and strengthens, in a way, the concept of heterosynaptic facilitation as a presynaptic facilitation.

Structures and mechanisms acting on the presynaptic fibre

There is now quite good evidence that presynaptic inhibition in the spinal cord is due to the depolarization of the primary afferent presynaptic fibres. This is considered to be responsible for the depression of amplitude of the presynaptic spike, which would consequently liberate less transmitter at this terminal; hence the reduction of test EPSPs (Eccles, Eccles & Magni, 1961; Eccles, Magni & Willis, 1962; Eccles, Schmidt & Willis, 1962). It was postulated (Eccles, 1964) that the depolarization is produced by the release of a chemical transmitter by interneurones activated by the conditioning muscle afferent volley. In *Aplysia* the presence of interneurones which would change the efficacy of the test pathway was clearly demonstrated (p. 296). The presence of interneurones explains why all inputs might produce presynaptic inhibition or be affected by it. However, it was not demonstrated that all presynaptic inhibition is due to interneurones in the ganglia. It is not excluded that some fibres in the priming input might have the ability to act presynaptically on others. This possibility is most likely in the case of the fibres from the three nerves, the priming effects of which seem to be, within some limits, directly proportional to the number of stimuli. By contrast such a proportionality was not seen for the priming effects of the connectives, whose presynaptic action is more probably conveyed by interneurones.

There is a big disproportion in the duration of post-synaptic and presynaptic action. This big disproportion made very improbable the hypothesis that the long duration of the presynaptic inhibition is due to repetitive firing in the responsible interneurones. It seems rather that the long duration can be attributed to a prolonged action of a specific transmitter (cf. Eccles, 1964). In this respect the presynaptic inhibition in *Aplysia* fundamentally differs from the presynaptic inhibition in crustacean muscle fibre (Dudel & Kuffler, 1960, 1961) and that in the Mauthner cell (Furukawa, Fukami & Asada, 1963).

In other features also, like the decrease in efficacy after several trials (Fig. 10), the presynaptic inhibition (and possibly heterosynaptic facilitation) in *Aplysia* differs from direct synaptic action in such a manner that it does not seem possible to transpose the classical theory of synapses (Eccles, 1964) without notable modifications to explain events at presynaptic sites. In order to simplify the phraseology and to avoid any error of interpretation, we propose to designate the contact between the priming fibres and the afferent test fibres by the name of 'episynapse'. It is evident that as a result of priming stimulations of different intensities different quantities of episynaptic transmitter would accumulate on presynaptic test fibres either owing to repetitive firing (but for a short period

of time) of specific interneurones or directly liberated by afferent fibres of the priming pathway. But this transmitter does not seem to be destroyed in a fixed period of time. The differences in recovery time of the EPSP after priming stimulations of different intensities or after several cycles seem to indicate the absence of a transmitter-destroying enzyme such as we are used to consider in classical synapses. The duration of transmitter action on classical synapses is constant, independent of the quantity of transmitter liberated. This results from the dynamics of action of the enzymes present in excess. The transmitter action on the 'episynapse', however, seems either to occur in the presence of an insufficient quantity of destructive enzyme, or to be regulated by diffusion only. Also, the exhaustion of the specific transmitter reserves seems to be very fast as judged from the rapid decrease, in the priming pathway, of the capacity to produce presynaptic inhibition.

Different presynaptic fibres appeared to be more or less affected by the same priming stimulus (Fig. 12) and react with different delays (Figs. 8, 11). This might be explained by a specific sensitivity of each fibre to the episynaptic transmitter, by necessity of summation and by structural peculiarities of episynaptic contacts, interposing perhaps between the pre-episynaptic and post-episynaptic membranes a distance larger than that of the synaptic cleft.

It has to be noted that the activation of an interneurone exerting presynaptic inhibition (Figs. 13–15) is shown in the post-synaptic cell by the 'spontaneous priming wave' which seems to be a composite EPSP. If we admit that this post-synaptic potential is brought forth directly by the 'priming' interneurone, the same interneurone would be able to produce different variations on the post-synaptic cell and on the afferent fibre. However, the presence of an additional interneurone which would specifically carry 'episynaptic' effects cannot be discarded.

Modifications in the presynaptic fibre

The modifications induced by the priming stimulation on the test presynaptic fibre which would affect the amplitude of the spike and consequently the quantity of transmitter released might be of two kinds: variation of conductance by generating a flow of ionic current, and the resulting polarization change. Experiments on the giant synapse in the squid (Hagiwara & Tasaki, 1958; Takeuchi & Takeuchi, 1962), at the mammalian neuromuscular junction (Hubbard & Willis, 1962*a*, *b*, *c*), and at the motoneurone excitatory synapses in the spinal cord (Eccles, Kostyuk & Schmidt, 1962) have shown that hyperpolarization of the presynaptic fibres causes an increase in release of the transmitter and hence increases the measured post-synaptic potential, whereas depolari-

zation decreases the quantity of released transmitter and diminishes the size of the PSP. In the spinal cord, both depolarization and conductance change in the test pathway seem to be responsible for the presynaptic inhibition (Eccles, 1964).

In *Aplysia*, on the basis of present results, it is difficult to decide whether the reduction in post-synaptic EPSP amplitude is related to a depolarization or hyperpolarization of the presynaptic fibres. Indeed, the conductance changes in the presynaptic fibre seem to be very strong, as can be deduced from a complete disappearance of the unitary and monosynaptic EPSP, doubtless due to a block in conduction of the spike in the terminals. The block can be explained by a significant increase in the loading effect of the surrounding membrane on the spike generator. A lesser load would permit the spike to be conducted, but its amplitude would be smaller even in a hyperpolarized fibre and consequently less transmitter would be liberated at the terminal.

If the conductance change on episynapse were so important one would wonder how the hyperpolarization of the presynaptic fibres could explain heterosynaptic facilitation observed in other cells. A satisfactory explanation would be given if the site of hyperpolarizing 'episynapse' were located far enough from the terminal, so that the conductance change on the 'episynapse' would not be strong enough to exert an important change on the spike-generating mechanism at the terminal, but would affect its polarization. (A similar relation was observed in *Aplysia* neurones with inhibitory synapses located in an axonal region in the vicinity of the soma. The activation of these synapses produces clear hyperpolarization of the somatic membrane without affecting significantly its spike-generating mechanism, so that the relative size of the somatic spike increases.) The amplitude of the spike at the terminal would be increased and more transmitter liberated at the synaptic knob. Thus the position of the hyperpolarizing 'episynapse' would decide whether the presynaptic action would be inhibitory or excitatory.

Long-lasting changes in polarization were observed in some *Aplysia* cells. The so-called inhibition of long duration (ILD) was especially studied. It consists of a long-lasting hyperpolarizing wave produced by the stimulation of an excitatory input (Fig. 1*C*; see also Tauc, 1958*b*). It was shown that the ILD might follow a unitary EPSP (Tauc, 1957): previous publications have described the duration of ILD as being of 30–60 sec, but unpublished results have shown that in some cells this duration might reach 20 min or longer. Quite significant conductance changes were also taken as evidence in relation to ILD (unpublished results). On the other hand, long-lasting excitability changes probably related to long-lasting depolarizations have been shown in other cells (Fessard & Tauc,

1960; Tauc, 1960), and also in the giant cell of *Aplysia* (Kandel & Tauc, 1965*b*, see fig. 2; and Tauc, unpublished results). In spite of the fact that it is difficult to discard completely the possibility of increased excitatory input, the existence of a depolarizing phenomenon similar to ILD except in polarity is significant. It seems tempting to consider a correlation between these phenomena and the presynaptic facilitation and inhibition.

Specificity of fibres carrying heterosynaptic inhibition and facilitation

It is significant to note that cells showing heterosynaptic facilitation and presynaptic inhibition belong to two different pharmacological groups. It was shown that the upper quadrant giant cell, which shows heterosynaptic facilitation, belongs to the H group cells (Tauc & Gerchenfeld, 1962) which have cholinergic inhibitory input and are intensively depolarized by catecholamines (Gerschenfeld & Tauc, 1964), whereas the cells in which presynaptic inhibition was demonstrated in the present study (P1 and neighbouring cells) belong to the CILDA-type cells (Gerschenfeld & Tauc, 1964) which show ILD and a hyperpolarizing response to catecholamines. All the H or CILDA cells do not show presynaptic phenomena, but the pharmacological dissociation is very important since we now know (Gerschenfeld & Tauc, 1964) that the excitatory inputs to H and to CILDA cells are conveyed by pharmacologically different presynaptic fibres. The excitatory input to CILDA-type cells is affected by Bol 148 (bromolysergic acid), whereas this drug has no action on the excitatory input to H cells. We have also seen (in the results section) that excitatory inputs to these two kinds of cells show opposite reactions to the increase in frequency of stimulation: they decrease in amplitude in the giant cell, but are clearly potentiated in the P1 cells. Consequently heterosynaptic inhibition and facilitation are mediated by different fibres synapsing on different cells. This explains the co-existence of heterosynaptic inhibition and facilitation in the same ganglion as well as the fact that EPSPs in a cell show either heterosynaptic facilitation or inhibition, but never both.

It is remarkable that presynaptic inhibition is apparent in cells which show long-lasting inhibitory post-synaptic change (ILD), while long-lasting excitation is present in cells with long-lasting excitatory responses to the priming stimulus. Thus the presynaptic and post-synaptic phenomena in each case seem to reinforce one another's action.

Presynaptic inhibition in the central nervous system of *Aplysia* seems to be a property of a small group of cells. The functional significance of these cells and of the presynaptic inhibition is obscure. Eccles (1963) suggested that the presynaptic inhibition in the spinal cord provides the first stage in what he terms 'perceptual attention', which would suppress

any concurrent trivial and undesired synaptic activity for a limited length of time. The differences in recovery time, however, which are observed in preparations that have undergone the same experimental treatment, and the reduction in the intensity of presynaptic inhibition after repeated priming stimulation, indicate that the modifications of synaptic efficacy in the *Aplysia* brain are of much longer duration than simple presynaptic inhibitory action.

SUMMARY

1. Intracellular recordings in a group of cells located in the central dorsal part of the abdominal ganglion of *Aplysia* have revealed that the amplitude of a test excitatory post-synaptic potential (EPSP) produced by the stimulation of afferent fibres in a nerve or connective is depressed for many minutes if preceded by a short priming stimulation of another nerve or connective. The degree of depression of the test EPSP and the recovery time from this heterosynaptic inhibition depend upon the intensity of the priming stimulation.

2. During heterosynaptic inhibition the conductivity as well as the excitability of the membrane of the post-synaptic neurone are not modified. The intensity of heterosynaptic inhibition is not affected by a previous blocking of the inhibitory synapses in the ganglion by D-tubocurarine.

3. The amplitude of a unitary, monosynaptic test EPSP is equally depressed by the priming stimulation, but the duration of the EPSP not modified. A complete disappearance of the test unitary EPSP is observed for a limited time after strong priming stimulation.

4. The capacity of the priming stimulus for affecting heterosynaptically the test EPSP decreases when the cycle 'priming stimulation/recovery' is repeated, whereas the synaptic effects of the priming stimulus on the cell are not affected. This diminution of efficacy is reversible after rest.

5. Depression of the test EPSP was sometimes observed in the absence of external priming stimulation as a result of a spontaneous activity of interneurones in the ganglion.

6. The cells in which heterosynaptic inhibition was observed are of a different pharmacological type from those cells in the same ganglion in which heterosynaptic facilitation has been previously demonstrated. Moreover, their afferent fibres are of a different type.

7. Heterosynaptic inhibition in *Aplysia* is thought to be a presynaptic phenomenon similar to presynaptic inhibition observed in the vertebrate spinal cord. The mechanism of the process by which the priming stimulus affects the presynaptic fibres of the test pathway and decreases the efficacy of its synaptic action is discussed in the light of the present results.

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