SYNAPTIC PLASTICITY AND THE MODULATION
OF THE Ca²⁺ CURRENT

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INTRODUCTION

'Every physiological teaching on the working of the brain based on localization, no matter how excellent, leaves us ignorant about the mechanism of mental activity. These actions are certainly accompanied by molecular modifications in nerve cells and preceded by complex changes in the relationship between neurones. To understand mental activity it is necessary to understand these molecular modifications and changes in neuronal relationships. One must know, of course, the complete and exact histology of cerebral centres and their tracts. But that is not enough; it will be necessary to know the energetic transformations of the nervous system which accompany perception and thought, conciousness and emotion.'

Ramon y Cajal (1911)

The study of the mechanisms of neuronal plasticity and the attempt to relate these mechanisms to actual instances of learning has accelerated in recent years as a result of the application of the techniques of biophysics and cell biology to central neurones and their interconnexions. Although support for it has been obtained only recently, the idea that learning might involve changes in the effectiveness of the connexions between neurones actually had its origins at the turn of the century. Following an earlier suggestion by E. Tanzi (1893), Ramon y Cajal postulated, in his Croonian Lecture to the Royal Society of London in 1894 (a lecture to which he was invited through the intervention of Charles Sherrington), that learning might involve changes in strength of connexions between neurones. An almost identical idea was put forth by Sigmund Freud also in 1894 in a fragmentary manuscript published only in 1950. A first requirement of this postulate is that some synapses have *plastic* properties, that they can change their efficacy following simple use or following more complex patterns of stimulation. This basic requirement has now been fully satisfied. A variety of experiments have shown that chemical synapses can undergo changes in effectiveness as a result of activity or inactivity in a given pathway (homosynaptic change) or as a result of activity in other pathways (heterosynaptic change). Some of the best evidence has come from studies of simple synaptic systems such as the synapses between vertebrate motor neurones and skeletal muscle (for reviews, see Katz, 1962; Eccles, 1964; Kandel & Spencer, 1968).

Soon after the initial discovery of the end-plate potential by Gopfert & Schaefer (1938), Schaefer, Scholmerich & Haass (1938) and subsequently Feng (1941) found that repeated (tetanic) stimulation increased the amplitude of the end-plate potential without producing any obvious changes in the action potential of the presynaptic axon. The increase in the end-plate potential was not restricted to the period of tetanic

stimulation but persisted for several minutes after the tetanus. Feng called the synaptic enhancement that persisted after the tetanus *post-tetanic potentiation*. He also found that longer tetani produced greater potentiation than did shorter ones. In 1947, Larrabee and Bronk found post-tetanic potentiation at a peripheral neurone-to-neurone synapse between preganglionic and postganglionic cells of the stellate ganglion. In 1949, Lloyd described similar potentiation in the monosynaptic reflex of the spinal cord, thereby showing that these plastic changes also occur in central neurones. Lloyd found (as had Larrabee and Bronk) that post-tetanic potentiation produced by stimulating one afferent pathway did not increase the response of the postsynaptic cell to synaptic activation via another, unstimulated, afferent pathway. These experiments indicated that post-tetanic potentiation is *homosynaptic*: it is restricted to the stimulated pathway and results from a change in the synapse itself. Lloyd (1949) also described a second type of plastic change when he found that low frequencies of stimulation produced a decrease in synaptic effectiveness. This form of plastic change he called *low-frequency* or *homosynaptic depresssion*.

Analysis remained at this stage for a number of years because it was difficult to determine whether these changes were due to a presynaptic mechanism (a change in transmitter release) or to a postsynaptic mechanism (a change in receptor sensitivity). The solution to this problem was facilitated in 1954 when del Castillo & Katz (1954a) demonstrated that release of acetylcholine at the nerve muscle synapse is not graded but quantized. An action potential releases about 200 multimolecular packets of transmitter – called quanta – and each quantum contains several thousand molecules of ACh. Quantal transmission was soon found to be the general mode of transmitter release at chemical synapses (see Dudel & Kuffler, 1961a; Eccles, 1964). The discovery of quantal transmission not only established important new insights into the nature of transmitter release from the terminals but also provided a method for analysing the relative contribution to synaptic transmission of changes in presynaptic and postsynaptic mechanisms. Del Castillo & Katz (1954a, b), Liley (1956a, b) and subsequently others, analysed alterations in synaptic effectiveness in terms of quantal transmission and found that both homosynaptic depression and post-tetanic potentiation represented a presynaptic alteration in the number of transmitter quanta released per impulse. The sensitivity of the receptor seemed not to be affected.

This work was extended in a major direction in 1961 when, following the earlier suggestion of Frank & Fuortes (1957), Dudel and Kuffler described the first clear instance of a heterosynaptic interaction: presynaptic inhibition at the crayfish nerve-muscle synapse. Dudel & Kuffler (1961a) found that in the crayfish the inhibitory axon to muscle has a double function: (1) it produces an inhibitory postsynaptic potential in the muscle, and (2) it depresses the excitatory postsynaptic potential produced by the excitatory axon. By applying a quantal analysis Dudel & Kuffler (1961b) found that presynaptic inhibition reduces the number of transmitter quanta released by the excitatory axon without affecting the sensitivity of the receptor molecules. These experiments provided the first evidence that the membrane of the presynaptic terminals contains receptors to transmitter molecules, and that these receptors can control transmitter release. Subsequently, Kandel & Tauc (1964), Epstein & Tauc (1970), and Castellucci and his colleagues (1970) presented suggestive evidence for presynaptic facilitation of transmitter release in the synapses of *Aplysia*. Recently

again based upon a quantal analysis, Castellucci & Kandel (1976) provided direct evidence for a presynaptic mechanism.

In 1967 Katz and Miledi advanced the study of synaptic plasticity still one important step further by finding that transmitter release is dependent on the influx of Ca^{2+} that occurs with each action potential. They proposed that Ca^{2+} allows the synaptic vesicles, the subcellular organelles that store transmitter (and which are thought to represent individual quanta), to bind to release sites. In addition, Katz & Miledi (1967*a, b*) found that the presynaptic terminal contained a high density of voltage-gated Ca^{2+} channels.

These findings suggested to Katz & Miledi (1968) and to Rahamimoff (1968) that changes in the intracellular level of free Ca^{2+} might be important for short-term synaptic plasticity. Rahamimoff (1968; Alnaes & Rahamimoff, 1975) proposed that the intracellular concentration of Ca^{2+} is controlled by intracellular organelles that buffer Ca^{2+} – the *mitochondria and endoplasmic reticulum* – and that aspects of synaptic plasticity might depend upon this control. In support of this idea, Rahamimoff found that short-term homosynaptic facilitation seems to be due to *residual* Ca^{2+}, the Ca^{2+} that remains in the terminal following a series of action potentials, and that is taken up slowly by the buffering organelles. A similar mechanism also seems to be operative in *Aplysia* (Kretz, Shapiro & Kandel, 1980).

Another factor that controls the free Ca^{2+} levels in the terminals is the Ca^{2+} influx. It seemed to us (as it had earlier to Zucker (1974) and to Stinnakre & Tauc (1973)) that influx might not be constant but might be modulated. In the past, however, this idea has proven difficult to examine in central neurones showing plastic changes, since the most direct test of the hypothesis would require recording of Ca^{2+} current in presynaptic terminals simultaneous with monitoring of transmitter release. This problem can be overcome, to a certain degree, in *Aplysia* neurones. First, the membrane of the cell body of *Aplysia* neurones contains Ca^{2+} channels whose properties seem to resemble those of the terminal membrane (Geduldig & Junge, 1968; Geduldig & Gruener, 1970; Stinnakre & Tauc, 1973; Llinas, Steinberg & Walton, 1976). Moreover, in certain cases changes in the calcium current of the cell body parallel the changes in transmitter release at the terminals (Klein & Kandel, 1978). In addition, in some of these neurones the presynaptic terminals controlling transmitter release appear to be sufficiently close to the cell body electrically so that it is possible to modify transmitter release from the terminals by injecting current into the cell body (Shimahara & Tauc, 1975; Shimahara & Peretz, 1978; Shapiro, Castellucci & Kandel, 1980*a*). The observations suggested to us that we might be able to examine the relationships between transmitter release and specific ionic currents of the presynaptic membrane. To this end we have combined analysis of ionic currents of the cell body of the presynaptic neurone, using voltage-clamp analysis and pharmacological blockade of specific ion channels, with assay of transmitter release from the presynaptic cell, using intracellular recordings of the synaptic potential in the postsynaptic cell (Fig. 1). These combined techniques provide a powerful method for studying changes in specific ionic conductances associated with various presynaptic mechanisms for synaptic plasticity.

We have applied these techniques to two identified synaptic connexions in the abdominal ganglion of *Aplysia*: (1) the connexions made between the multi-action
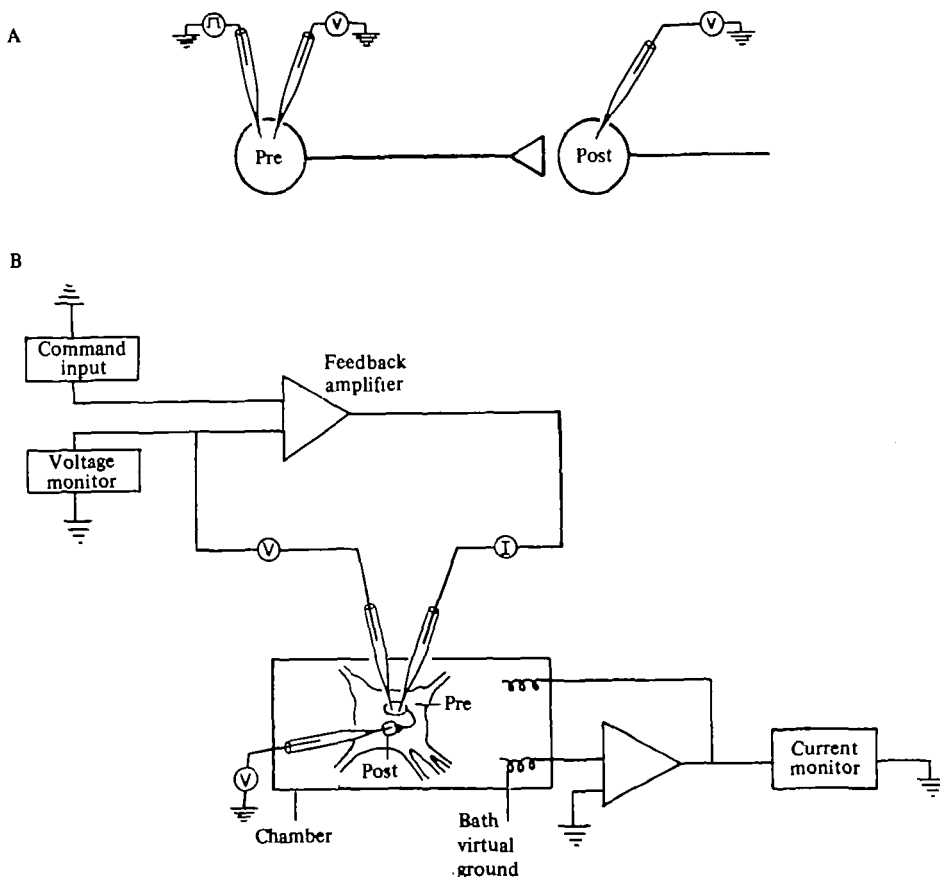
Fig. 1. Diagram of experimental arrangement for analysing both pre- and postsynaptic aspects of the four types of synaptic plasticity examined in this review. (A) Voltage clamp of the presynaptic neurone allows analysis of the ionic currents controlling transmitter release. Simultaneous recording of synaptic potentials from the postsynaptic cell allows examination of the consequences of the presynaptic currents for synaptic transmission. (B) Voltage clamp used for sensory neurone experiments was a Dagan Instruments 8500 pre-amp-voltage clamp. The voltage-clamp circuit described in Byrne et al. 1979, was used for L10 experiments. Virtual ground and current monitor were Ag-Agcl wires in the bath. Two independent intracellular electrodes of 1–3 MΩ resistance for cell L10 and 5–10 MΩ resistance for sensory neurones were used for voltage recording and current passing. The electrodes were filled with 2 M-K citrate.

cholinergic cell L10 and its identified follower cells, and (2) the connexions between the mechanoreceptor sensory neurones of the gill-withdrawal reflex and their follower cells.

The situation was particularly favourable for L10 and its synapses. When we blocked the Na^+ channels with tetrodotoxin and axotomized L10 we found that graded depolarizing commands applied to the membrane of the presynaptic cell body under the voltage clamp caused graded transmitter release from the terminals, as determined by intracellular recording from postsynaptic cells (Figs. 2A, 2B, and 3A). We obtained further evidence that release sites were being controlled by the clamp currents in the cell body by finding that transmitter release is also a graded function of the
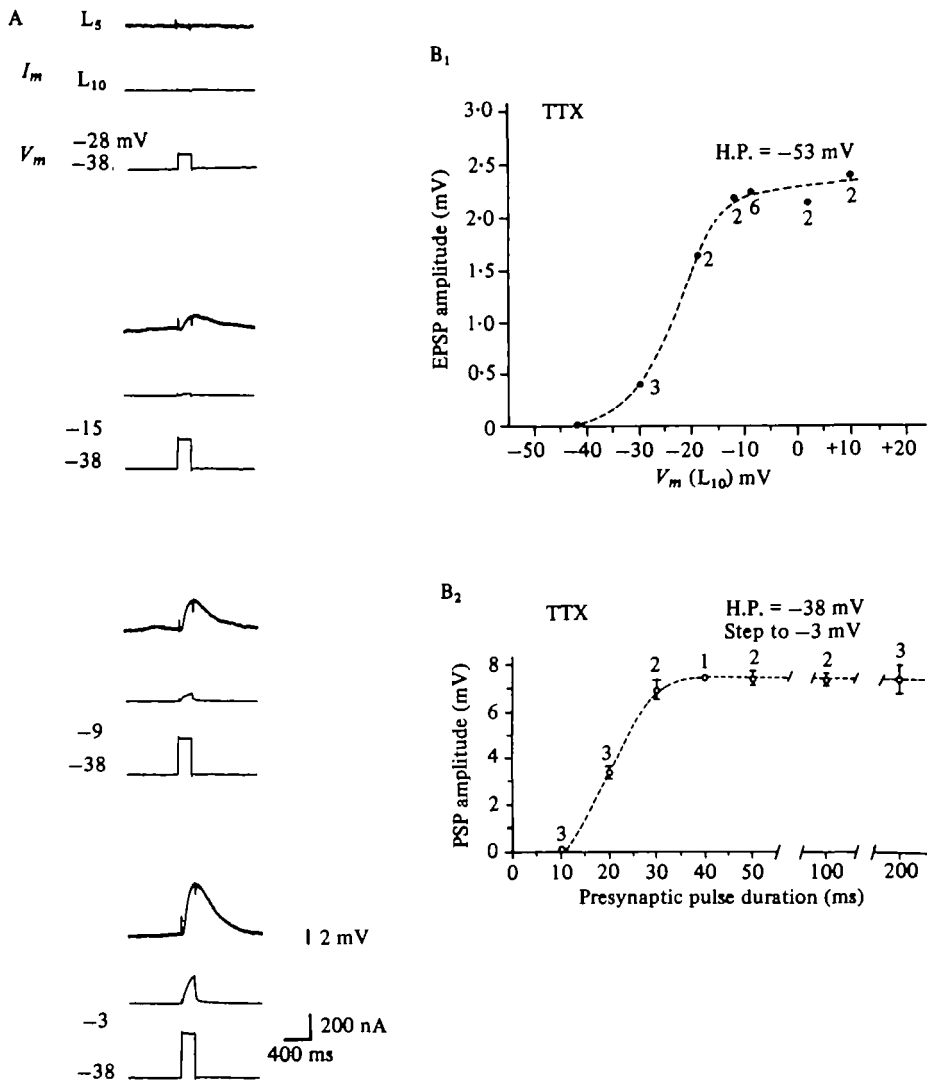
Fig. 2. (A) Selected records of presynaptic currents under voltage clamp and postsynaptic potentials produced by graded increases in command steps to the presynaptic neurone. Cell L10 was voltage clamped at a holding potential of -38 mV in high-divalent cation sea water (60 mM-Ca^{2+}, 140 mM-Mg^{2+}, 265 mM-Na^+) containing TTX. Depolarizing pulses of 200 ms duration were stepped to various membrane potentials once every 30 s and the size of the IPSP (reversed by hyperpolarizing the postsynaptic cell) elicited was monitored. PSP size is a graded function of step depolarization. (B) Voltage-clamp depolarizations of presynaptic neurone L10 elicit graded release of transmitter. (B_1) EPSP amplitude (RB cell) is a graded function of size of presynaptic cell (L10) depolarization. Cell L10 was voltage clamped at a holding potential of -53 mV in normal sea water containing TTX. Depolarizing pulses of 1000 ms duration were stepped to various membrane potentials once every 1 min and the size of the EPSP elicited was plotted against the size of the step depolarization. The range of membrane potentials in which the PSP size is increasing is similar to that in which voltage-dependent Ca^{2+} current is increasing. (B_2) IPSP amplitude (L5) is a graded function of duration of presynaptic (cell L10) depolarization. Depolarizing voltage clamp steps to -3 mV from a holding potential of -38 mV were delivered once every 30 s. Duration of this pulse was varied. From durations of 10 ms to about 40 ms the size of the PSP is a graded function of the presynaptic depolarization. Number of PSPs averaged for each duration is given above each point. Same experiment as Fig. 2A. (From Shapiro *et al.* 1980*a*.)
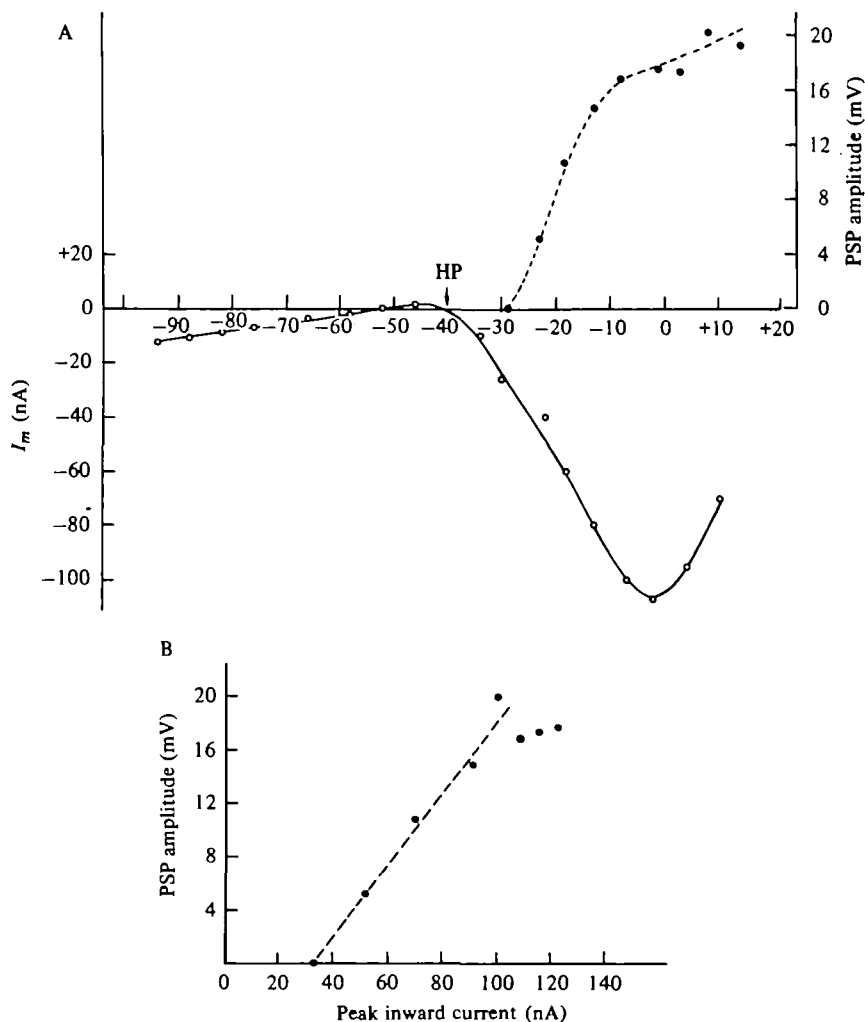
Fig. 3. Transmitter release and presynaptic Ca^{2+} current.   (A) Cell L10 was voltage clamped at a holding potential of -40 mV in a sea water solution containing high-divalent cation. Transmitter release was evoked by 200 ms duration depolarizing clamp steps. The size of the PSP recorded in cell L5 was plotted against step depolarization. The preparation was then treated with 4-AP (10 mM), TEA^+ (25 mM) and extracellular Ca^{2+} was replaced by Ba^{2+} ions, to block K^+ currents. The peak inward current evoked by 200 ms step depolarizations from a holding potential (HP) of -40 mV to various voltages is plotted in the lower part of the figure. (B) Plot of the relationship between peak inward current (corrected for leakage) and PSP amplitude for this cell.

duration of the depolarizing step (Fig. 2 B_2). Voltage control can be further improved by adding pharmacological agents that block each of the three known outward K^+ currents: tetraethylammonium ions (TEA^+), which block the delayed K^+ channel; 4-aminopyridine (4-AP), which blocks the early K^+ channel; and substitution of Ba^{2+} ions for Ca^{2+}, which block the Ca^{2+}-dependent K^+ channel (see Adams, Smith & Thompson, 1980; Shapiro, Castellucci & Kandel, 1980a, b). Blocking these K^+ channels also lengthens the effective space constant of the neurone.

Using these K^+ channel-blocking agents we could now examine the relationship

etween transmitter release and presynaptic Ca^{2+} current (Fig. 3 A). We found that in the range of depolarizing voltages in which the Ca^{2+} current was increasing, transmitter release increased in a linear fashion (Fig. 3 B). These results suggest that in this voltage range (from about -40 to zero mV) we could control transmitter release from the terminals with voltage clamp of L10's cell body.

Although in many cases (particularly in the studies of the connexions made by sensory neurones), we lacked ideal voltage control of the terminals, these procedures nonetheless allowed sufficient control to study transmitter release while at the same time examining ionic currents in the soma of the presynaptic neurone. Moreover, we repeated all experiments except those involving presynaptic inhibition (where the transmitter is not known) in mechanically isolated presynaptic cell bodies where optimal space clamp control can be achieved.

Using this approach, we have examined four types of synaptic plasticity, including two directly involved in simple nonassociative forms of learning: (1) the control of transmitter release in spike generating neurones by the membrane potential of the presynaptic neurones; (2) presynaptic inhibition; (3) homosynaptic depression (the mechanism underlying short-term habituation); and (4) presynaptic facilitation (the mechanism of behavioural sensitization). We have found that each of these involves modulation of the Ca^{2+} current, although the details of the mechanisms differ in each case.

The control of transmitter release by the membrane potential of the presynaptic cell

In 1975 Shimahara and Tauc described a simple form of synaptic plasticity whereby the membrane potential of the presynaptic neurone exerts a powerful influence over the effectiveness of the connexions made by that neurone. Hyperpolarizing the presynaptic cell decreased the synaptic potential elicited by the presynaptic action potential whereas depolarizing the presynaptic cell enhanced the synaptic action. Although this effect was opposite to that described in the giant synapse of the squid (Hagiwara & Tasaki, 1958; Takeuchi & Takeuchi, 1962) and at first seemed paradoxical, similar potential dependent control of transmitter release by membrane potential has now been described in the leech (Nicholls & Wallace, 1978a). A form of this plasticity occurs between the identified multiaction cholinergic cell L10 and its follower cells (Waziri, 1977; Shapiro, Castellucci & Kandel, 1980a).

Intracellular stimulation of neurone L10 produces EPSPs in some follower cells (R15 and RB cells) and IPSPs in others (L1 to L6, LB_{VC}, LD_{HI}, etc.; see Kandel *et al.* 1967; Wachtel & Kandel, 1967, Kehoe, 1972; Koester *et al.* 1974; Koester & Kandel, 1977). When the membrane potential of L10 is progressively increased, its ability to release transmitter at its various branches is reduced (Fig. 4). Even a slight increase in membrane potential of 5 mV produces significant depression of transmitter release. With larger hyperpolarizing changes of 20–40 mV, transmitter release can be completely blocked (Fig. 4A). Conversely, depolarizing the cell's membrane potential increases transmitter release. The changes in membrane potential affect the duration and height of the presynaptic action potential: spikes become narrower and shorter with hyperpolarization and broader and taller with depolarization (Fig. 4B). th of these potential induced alterations are likely to alter transmitter release because ey alter the Ca^{2+} current (Horn & Miller, 1977; Klein & Kandel, 1978). However.
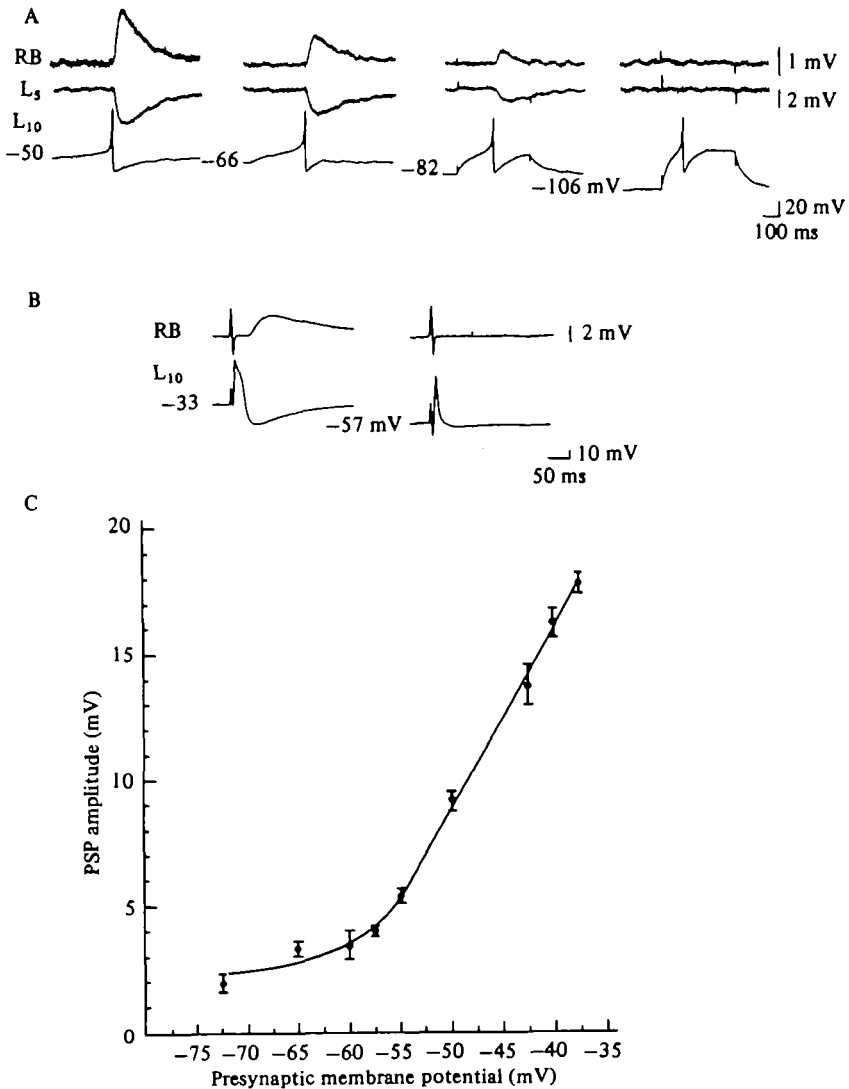
Fig. 4. Effect of presynaptic membrane potential on presynaptic spike and on transmitter release. (A) Spikes are elicted in interneurone L10 by intracellular injection of depolarizing current while maintaining the cell at various holding potentials (indicated at the lower left of each set of traces). At −50 mV L10 produces a large EPSP and IPSP in two of its follower cells. As the holding potential is increased to −106 mV the ability of the action potential in L10 to release transmitter is reduced progressively. (B) Effect of resting membrane potential on the duration and amplitude of the spikes in L10. The spikes elicited by brief (2–5 ms) intracellular current injection are broader and larger from more depolarized membrane potentials than from more hyperpolarized levels. The duration of the spike parallels the effectiveness of synaptic transmission between L10 and its RB follower cells. (C) Size of the EPSP in RB cells as a function of presynaptic (L10) holding potential. Experimental protocol as in A, different preparation. Each point on the graph is the mean of 10 determinations (±s.e.m.). A solution containing a high concentration of divalent cations was used in all these experiments. (From Shapiro et al. 1980a.)

we shall discuss below, these alterations account for only part of the effects of membrane voltage on release. Part of the effect of membrane voltage is independent of changes in spike height and duration.

Release of transmitter from the terminals can be controlled from the cell body in a graded manner

The ability to alter transmitter release from the terminals by injecting current into the cell body implies that at least some release sites are electrically close to the soma. If the axon of the cell is cut close to the ganglion and the preparation is treated with TTX, graded depolarizing steps under voltage clamp lead to graded release of transmitter (Figs. 2, 3 and 5). The sigmoid function relating transmitter release to presynaptic depolarization is similar to that reported at the squid giant synapse (Katz & Miledi, 1969; Llinas *et al.* 1976). As is the case with the squid giant synapse the function relating transmitter release to presynaptic depolarization overlaps the voltage sensitivity of the presynaptic Ca^{2+} current (Fig. 3). However, in contrast with results in squid, hyperpolarizing the presynaptic cell decreases the size of the synaptic potential elicited by step depolarization to a given level (Fig. 5B).

Outward currents are decreased by depolarization

At depolarized holding potentials, currents elicited by step depolarizations under voltage clamp are less outward than at hyperpolarized holding potentials (Fig. 5A and Connor & Stevens, 1971b; Adams *et al.* 1980). This difference in net current presumably accounts for the difference in the configuration of the action potential at the two holding potentials in unclamped cells (Fig. 4B). In addition, the finding that the synaptic potentials are graded with the *duration* of the command pulse (Fig. 2B₂) provides further evidence that the K^+ currents, which control spike duration and amplitude, could modulate transmitter release. However, the ability of the membrane potential to modulate transmitter release under voltage-clamp conditions, in which the duration and amplitude of the presynaptic command pulse is held constant, suggested that mechanisms other than modulation of the K^+ current contribute to this form of plasticity. One possible additional mechanism is a change in transient Ca^{2+} current. To test this idea we blocked the several outward K^+ currents which are responsible for alterations in the shape of the spike to see whether the membrane potential was still capable of modulating the transmitter release caused by a depolarizing command, and whether this modulation was due to a direct action on the transient Ca^{2+} current.

Transient Ca^{2+} current is not increased by depolarization

Three pharmacologically separable K^+ conductances have been described in molluscan somata (Thompson, 1977; Adams *et al.* 1980). Two of these are voltage-dependent; an early, rapidly inactivating K^+ current sensitive to 4-AP (Connor & Stevens, 1971b; Byrne *et al.* 1979; Thompson, 1977), and a delayed K^+ current sensitive to TEA⁺ (Connor & Stevens, 1971a; Thompson, 1977; Byrne *et al.* 1979). A third K^+ conductance is thought to be not dependent on voltage but on intracellular Ca^{2+} concentration, and can be blocked by agents which block the Ca^{2+} current (e.g. Ca^{2+}, EGTA) and by substituting Ba^{2+} ions for Ca^{2+} ions (Meech, 1972; Meech & Standen, 1975; Eckert & Lux, 1976; Thompson, 1977; Adams *et al.* 1980; Shapiro
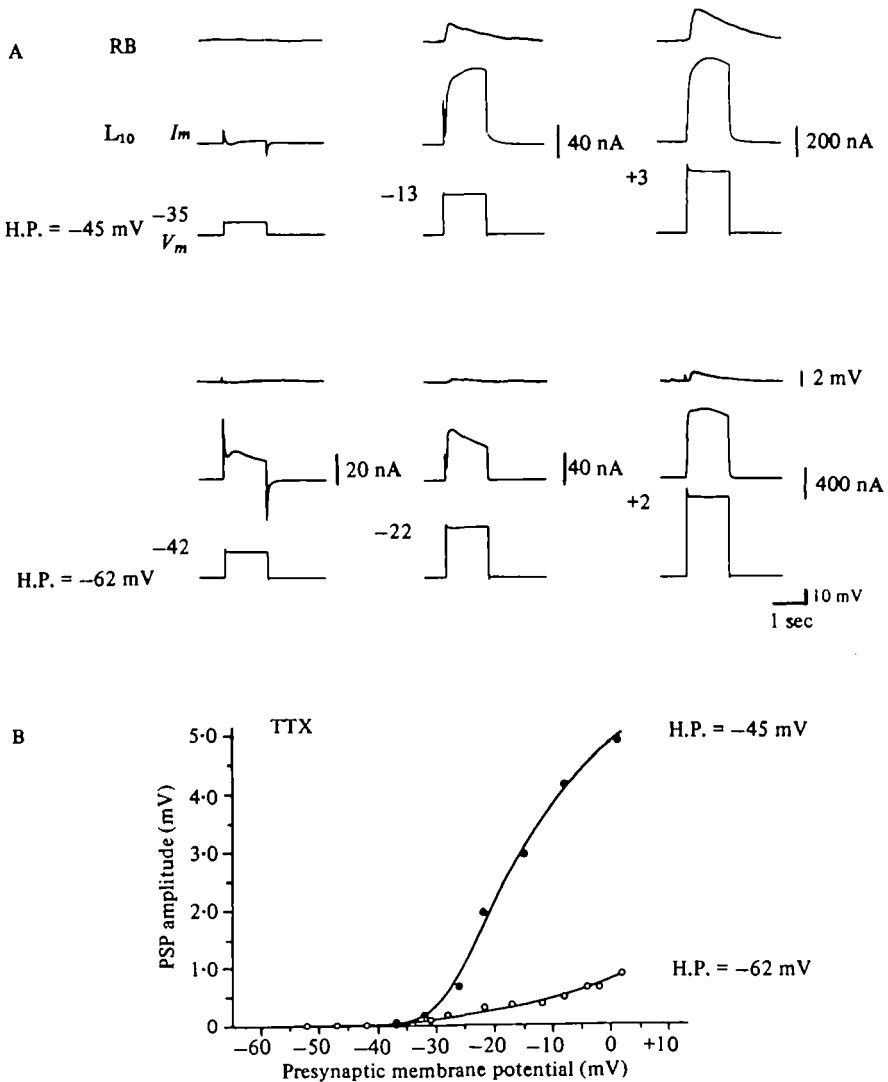
Fig. 5. Synaptic potential is modulated by presynaptic membrane potential. (A) Presynaptic cell L10 is voltage clamped at a holding potential of -45 mV (top set of traces) and -62 mV (bottom set of traces) in an artificial sea water solution containing 30 μM-TTX. Step depolarizations of 1·0 s from each holding potential (interpulse interval = 1 min) evoke EPSPs in the follower cell (top trace in each set of traces). The middle trace is the monitored current from L10 and the bottom trace of each set is the voltage record. From a holding potential of -45 mV the step depolarizations beyond -37 mV elicit PSPs. As the size of the depolarizations increases, clamp current becomes more outward and PSP size increases. From a holding potential of -62 mV, step depolarization elicit smaller PSPs. Each clamp current is more outward than for steps from -45 mV: For example, compare steps to $+3$ and $+2$ mV, noting change in current calibration. (B) Graph of the results of the experiment illustrated in Part A. At the two different holding potentials, -45 mV (●) and -62 mV (○), the threshold of evoked release is similar. (From Shapiro et al. 1980a.)
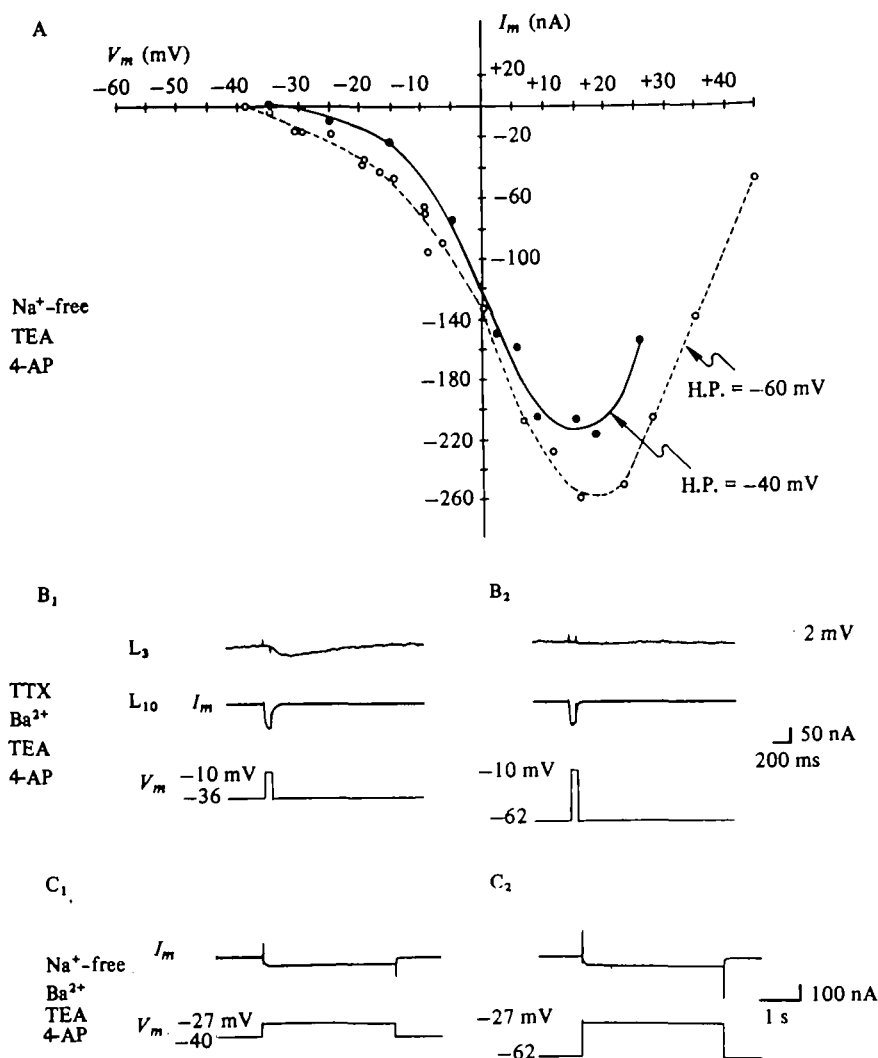
Fig. 6. (A) Transient calcium currents are independent of holding potential. The current-voltage relationship for Ca^{2+} current of the presynaptic neurone L_{10} from two holding potentials (-60 (O) and -40 (●) mV). The experiment was carried out in a Na^+-free solution containing 265 mM-TEA^+ and 5 mM-4-AP. The peak transient inward current is not increased when elicited from depolarized holding potentials. It may be slightly decreased, as is the case here, perhaps due to steady-state inactivation or build-up of intracellular calcium. The graphs are corrected for leakage by subtracting the extrapolated outward leakage current elicited by small hyperpolarizing steps from -60 mV.

(B) Amplitude of PSP is still modulated by holding potential after blockage of most K^+ currents. Presynaptic neurone L_{10} is voltage clamped from two holding potentials in a Ca^{2+}-free solution containing 60 mM-Ba^{2+}, 30 μM-TTX, 25 mM-TEA^+ and 10 mM-4-AP. A 100 ms step depolarization to -10 mV from holding potential of -36 elicits a large transient inward current and a large IPSP. A 100 ms step depolarization to -10 mV from a holding potential of -60 mV elicits a transient inward current as large from -36 mV but only a small IPSP. The difference in the inward tail currents at the two holding potentials may be due to the slower inactivation of the Ca^{2+} channels at the depolarized holding potential. (Parts A and B from Shapiro *et al.* 1980*a*.)

(C) Steady-state current through calcium channels is relatively unchanged when neurone L_{10} is stepped from a depolarized (-40 mV) or hyperpolarized (-62 mV) holding potential to the same level (-27 mV). Records were not corrected for leakage. Leakage correction makes the inward current elicited from -62 mV larger than the elicited from -40 mV (see part A of this figure).

et al. 1980*a*). Blocking the voltage-dependent K^+ channels caused peak transient inward currents to appear relatively unchanged from different holding potentials (Fig. 6A). With all K^+ channels blocked pharmacologically (so that there is no voltage-sensitive outward current with depolarizing command) and in a solution that was free of both Na^+ and Ca^{2+} (265 mM-TEA^+, 60 mM-Ba^{2+}, 10 mM-4-AP) the inward current through the Ca^{2+} channels, now carried by Ba^{2+} ions, can be observed directly. When this inward current was activated with a step depolarization it was not increased by changing the holding potential from −62 to −40 mV (Fig. 6C). At the same time, the synaptic potential elicited by depolarization from the two levels was still affected by membrane potential (Fig. 6B). When high concentrations of TEA^+ and 4-AP were utilized together, the peak inward Ca^{2+} current even decreased slightly when evoked from more depolarized holding potentials (Fig. 6A), perhaps as a result of steady-state inactivation, or because of an increase in the intracellular concentration of Ca^{2+} at depolarized holding potentials.

These results indicate that differences in the transient currents observed with steps from different holding potentials (Fig. 4A) are due to changes in K^+ conductances and not to changes in Ca^{2+} current. The holding potential does not control transmitter release by regulating *directly* the *transient* activation of the Ca^{2+} channel (Fig. 6).

Depolarization activates a steady-state Ca^{2+} current

Although membrane potential regulation of transmitter release does not result from direct modulation of the transient Ca^{2+} current, changes in membrane potential could lead to changes in the steady-state activation of the Ca^{2+} channels (Fig. 6C). Steady-state activation – the contribution of Ca^{2+} current to the resting current – might be greater at depolarized levels. Such steady-state activation of Ca^{2+} channels has been described in molluscan somata by Eckert & Lux (1976) and by Akaike, Lee & Brown (1978).

A steady-state activation of Ca^{2+} could lead to an increased concentration of intracellular Ca^{2+} at more depolarized holding potentials, which would add to the Ca^{2+} influx during the action potential, resulting in a higher total intracellular concentration of Ca^{2+} available for transmitter release. In addition, increased intracellular Ca^{2+} could cause relative saturation of intracellular Ca^{2+} buffering systems that are thought to compete with the release process (Alnaes & Rahamimoff, 1975; Rahamimoff, 1968). A third possibility is that increased intracellular Ca^{2+} concentration causes changes in screening of surface membrane proteins by changing internal membrane surface charges, and transmitter release efficacy (Bass & Moore, 1966; Van der Kloot & Kita, 1973). In each of these possibilities invasion of the terminal by an action potential would provide a more release-effective Ca^{2+} concentration.

To obtain evidence for steady-state Ca^{2+}-channel activation, we examined the steady-state membrane conductance of L_{10} at different holding potentials in the range of membrane voltages where modulation of release was occurring. We found that the steady-state conductance showed a region of reduced positive slope that is due to the steady-state Ca^{2+} channel. The steady-state Ca^{2+} current in turn activates a Ca^{2+}-dependent K^+ conductance that is blocked by substituting Ba^{2+} ions for ext
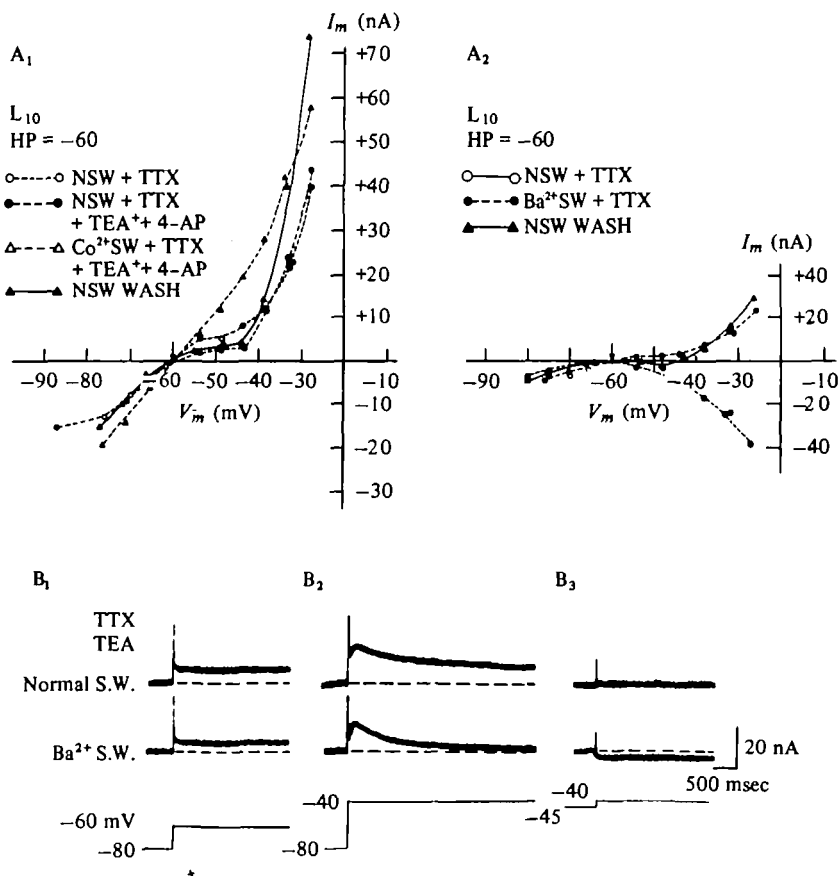
Fig. 7. Steady-state activation of Ca²⁺ and Ca²⁺-dependent K⁺ currents. (A) Steady-state current–voltage curves of L_{10}. In graphs A_1 and A_2, L_{10} was voltage clamped to a holding potential of -60 mV and 5 s clamp steps were delivered every 30 s to various hyperpolarized and depolarized membrane potentials. The current at the end of each 5 s voltage step was plotted against membrane voltage. In normal sea water and TTX the steady-state I–V curve shows a pronounced region of reduced positive slope in the range of the normal resting potential of L_{10} (-60 to -40 mV). In A_1 application of TEA⁺ and 4-AP have little effect on the steady-state I–V curve since transient K⁺ channels normally inactivate during the prolonged (5 s) voltage steps. Replacement of extracellular Ca²⁺ with 10 mM-Co²⁺ ions, however, linearizes the I–V curve by blocking a steady state inward Ca²⁺ current. When normal sea water is washed back the normal steady-state I–V curve is restored. When, in A_2, Ba²⁺ ions are substituted for Ca²⁺ ions, the normally observed area of steady-state reduced positive slope is converted into a region of inward-going rectification. Ba²⁺ carries the steady-state inward current through the Ca²⁺ channel, but does not activate an opposing Ca²⁺-dependent current.

(B) Ba²⁺ substitution. Presynaptic neurone L_{10} voltage clamped in artificial sea water containing 30 μM-TTX and 50 mM-TEA (upper traces) and solution containing 30 μM-TTX and 50 mM-TEA with Ba²⁺ substituted for Ca²⁺ (middle traces). The current elicited by a step from -80 mV to -60 mV is only slightly reduced by substitution of Ba²⁺ for Ca²⁺, but the steady-state current elicited by steps from -80 to -40 mV is more inward in presence of Ba²⁺, indicating the presence of a steady-state inward current at -40 mV, and blockage of the normally present Ca²⁺-dependent K⁺. The steady-state activation of net inward current can be observed directly with smaller step from -45 to -40 mV, which avoids recruiting the unchanged outward leakage current seen to be elicited by steps from -80 mV to -60 mV. (From Shapiro *et al.* 1980*a*.)
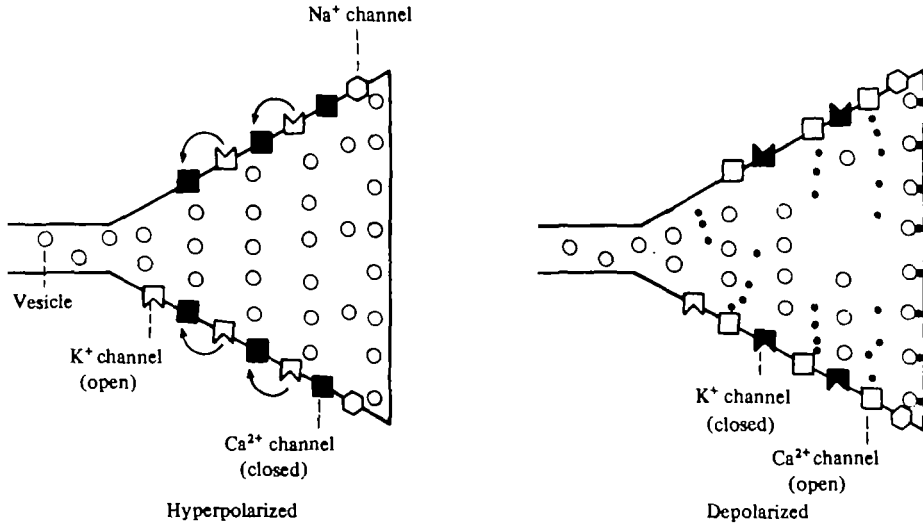
Fig. 8. Diagrammatic summary of the effects of resting membrane potential on presynaptic currents and transmitter release. When the neurone is held at a hyperpolarized resting level two currents are altered. One, the steady-state calcium current is reduced and consequently the resting intracellular calcium concentration is reduced. As a result the influx of calcium that occurs with an action potential may not by itself be sufficient to cause maximal transmitter release, and in the limit, to cause any release. Second, the voltage-sensitive potassium currents are activated to a greater degree when an action potential occurs from a hyperpolarized than from a depolarized membrane potential. The resulting spike is decreased in both duration and amplitude and therefore allows a smaller calcium influx to occur. Thus, at depolarized membrane potential, increased steady-state calcium currents as well as potassium current inactivation cause a larger amount of calcium to be available for transmitter release, resulting in increased synaptic transmission. Filled symbols represent closed channels and clear symbols represent open channels. Arrows from the K^+ channels to the Ca^{2+} channels represent the flow of hyperpolarizing current (through the potassium channels) which closes open Ca^{2+} channels and prevents additional Ca^{2+} channels from opening. The filled dots ($\bullet$) represent the calcium concentration, which is increased at depolarized membrane potentials due to increased number of open calcium channels.

cellular Ca^{2+}. Co^{2+} blocks this steady-state Ca^{2+} inward current as well as the Ca^{2+}-dependent K^+ current (Fig. 7A_1, A_2).

Ba^{2+} flows through Ca^{2+} channels without activating the K^+ conductance (see Figs. 6B, C and 7B and Adams *et al.* 1980). In the presence of Ba^{2+}, a voltage-dependent steady-state inward current flows through the Ca^{2+} channels at depolarized levels of holding potential (Fig. 7A_2, 7B); this inward current is not present at hyperpolarized levels (Fig. 7B). To activate this K^+ conductance the concentration of intracellular Ca^{2+} in the steady state must be significantly larger at more depolarized than at more hyperpolarized membrane potentials.

The role of steady-state Ca^{2+} current in the modulation of transmitter release by the membrane potential is supported by the observations of Nicholls & Wallace (1978*b*) that spontaneous quantal release of transmitter in leech neurones is increased at depolarized potentials. Our results indicate that an additional mechanism also operates in cell L10: a decrease of both voltage-dependent K^+ currents with depolarization. We have not as yet attempted to evaluate quantitatively the relative contributions of the changes in the steady-state Ca^{2+} and those in the transient K^+ current. In an unclamped cell, the decrease in the K^+ currents accounts for the increase in the

eight and duration of the spike. In this way the transient Ca^{2+} current can also be modulated, albeit indirectly. Our findings also suggest that despite a transient Ca^{2+} current that is not directly affected by changes in holding potential, powerful control over synaptic transmission – ranging from total block to enhanced effectiveness – can be achieved by variations in the steady-state Ca^{2+} current (Fig. 8).

These results support the idea first proposed by Shimahara & Tauc (1975) that EPSPs and IPSPs have a dual function. In addition to controlling the probability of firing an action potential, synaptic potentials also set the level for transmitter release. The results further suggest a possible mechanism for long-term regulation of synaptic output by metabolic or hormonal actions which affect resting potential (Thomas, 1972; Mayeri, Brownell & Branton, 1979; Mayeri *et al.* 1979).

Presynaptic inhibition

Presynaptic inhibition has been described in several vertebrate and invertebrate synapses and has been attributed to a depolarization of the synaptic terminals (reviewed in Burke & Rudomin, 1977; Ryall, 1978). This idea seemed consistent with the release properties described at the squid giant synapse where depolarization reduces transmitter release (Miledi & Slater, 1966). However, as described above, in *Aplysia* and in the leech, depolarization enhances rather than depresses transmitter release (Shimahara & Tauc, 1975; Nicholls & Wallace, 1978a; Shapiro *et al.* 1980a). At these synapses, a different mechanism must account for presynaptic inhibition.

In *Aplysia* the synapses of cell L10 that are modulated by membrane potential can also be modulated by presynaptic inhibition. Stimulation of one of the fibre pathways to the abdominal ganglion causes a depression of both the monosynaptic excitatory and the inhibitory connexions made by L10 (Fig. 9; Waziri, Kandel & Frazier, 1969; Shapiro *et al.* 1980b). Recently, Byrne (1980) has discovered a group of cells (the L32 cells) which, when stimulated, produce presynaptic inhibition without producing any conductance changes in the *postsynaptic* follower cells of L10.

Each of the ways of producing presynaptic inhibition tends to produce an IPSP in the presynaptic neurone, L10. Thus one change in the presynaptic neurone that clearly contributes to presynaptic inhibition is the hyperpolarization that occurs as a result of the IPSP. This hyperpolarization would reduce the output of transmitter by reducing the steady-state Ca^{2+} influx and by removing some of the inactivation of the K^+ channels. However, presynaptic inhibition outlasts the hyperpolarization and can also be observed in cases in which the cell does not hyperpolarize. To determine whether additional mechanisms are operative we controlled for changes in membrane potential by examining the presynaptic neurone under voltage-clamp conditions.

Presynaptic inhibition can be observed under voltage-clamp conditions

In a solution containing TTX and TEA^+, and with the cell held at a membrane potential which inactivates the early outward K^+ current, the inward current produced by a depolarizing clamp step is carried by Ca^{2+} ions, and the residual outward current is mainly due to Ca^{2+}-dependent K^+ current and the leakage current. Under these circumstances presynaptic inhibition is correlated with a decrease in transient inward current evoked by the depolarizing command (Fig. 10).

The decrease in inward current in voltage-clamp records based on net currents can
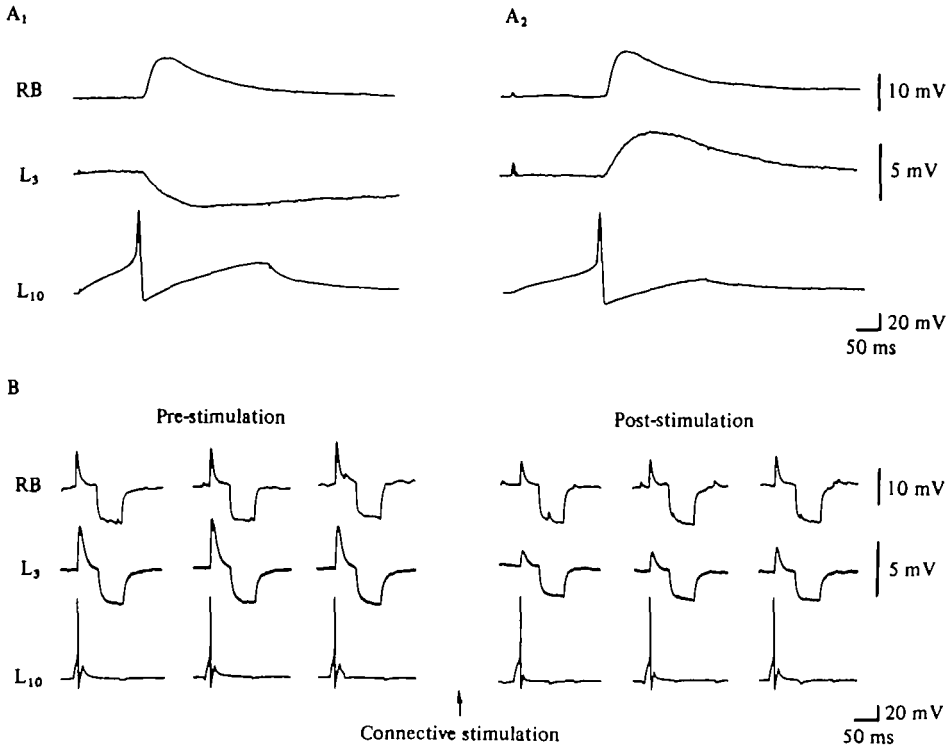
A₁   A₂

RB _____/‾‾‾_____   _____/‾‾_____ | 10 mV

L₃ ‾‾_____/‾‾‾   ‾‾|____/‾‾‾_____ | 5 mV

L₁₀ ‾|_____/‾‾   ____|_____

                                                    _| 20 mV
                                                     50 ms

B

          Pre-stimulation   Post-stimulation

RB _/_/‾ _/_/‾ _/_/‾   _/_/‾ _/_/‾ _/_/‾ | 10 mV

L₃ _/_/‾ _/_/‾ _/_/‾   _/_/‾ _/_/‾ _/_/‾ | 5 mV

L₁₀ _/___ _/___ _/___   _/___ _/___ _/___

                         ↑   _| 20 mV
              Connective stimulation   50 ms

Fig. 9. Presynaptic inhibition in *Aplysia*. (A₁) Action potentials in cell L10 elicit, through different branches, EPSPs in some cells (RB) and IPSP in others (L3). (A₂) Cell L3 can be hyperpolarized to reverse the IPSP. (B) Action potentials are produced in cell L10 every 2 s and the excitatory and inhibitory PSPs produced by two of its branches are monitored. The input resistance is monitored by intracellular injection of hyperpolarizing pulses. Stimulation of the connective for 5 s causes a decrease in PSP size not correlated with changes in post-synaptic input resistance. Solution containing 265 mM-Na²⁺, 60 mM-Ca²⁺, and 140 mM-Mg²⁺. (From Shapiro *et al.* 1980*b*.)
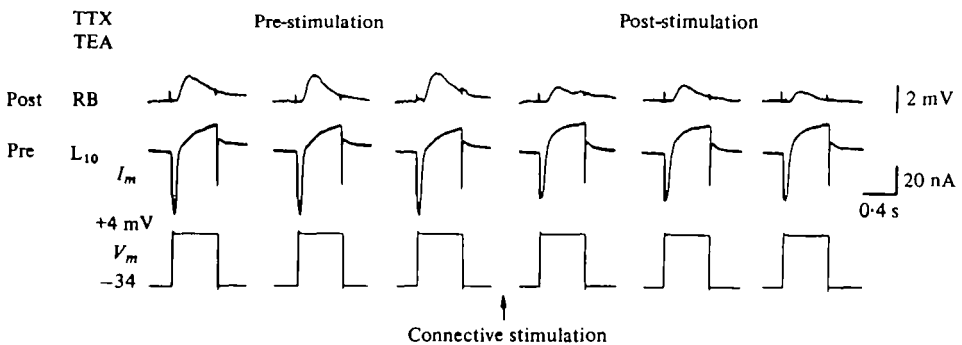
       TTX   Pre-stimulation   Post-stimulation
       TEA

Post RB _/‾_ _/‾_ _/‾_   _/‾_ _/‾_ _/‾_ | 2 mV

Pre   L₁₀
      Iₘ ‾|\|‾ ‾|\|‾ ‾|\|‾   ‾|\|‾ ‾|\|‾ ‾|\|‾   _| 20 nA
                                                             0·4 s
      +4 mV
      Vₘ _|‾|_ _|‾|_ _|‾|_   _|‾|_ _|‾|_ _|‾|_
      −34
                         ↑
              Connective stimulation

Fig. 10. Presynaptic inhibition with cell L10 under voltage-clamp control. In normal sea water containing TTX (30 μM) and TEA (50 mM) and with the cell held at −34 mV to inactivate the early K⁺ current, a step depolarization elicits in the presynaptic neurone an inward Ca²⁺ and an outward current due to Ca²⁺-dependent K⁺. The depolarizing step also elicits an EPSP in the RB follower cell. After stimulation of the presynaptic inhibitory pathway for 5 s the same step depolarization elicits a decreased inward Ca²⁺ current and a reduced PSP. (From Shapiro *et al.* 1980*b*.)
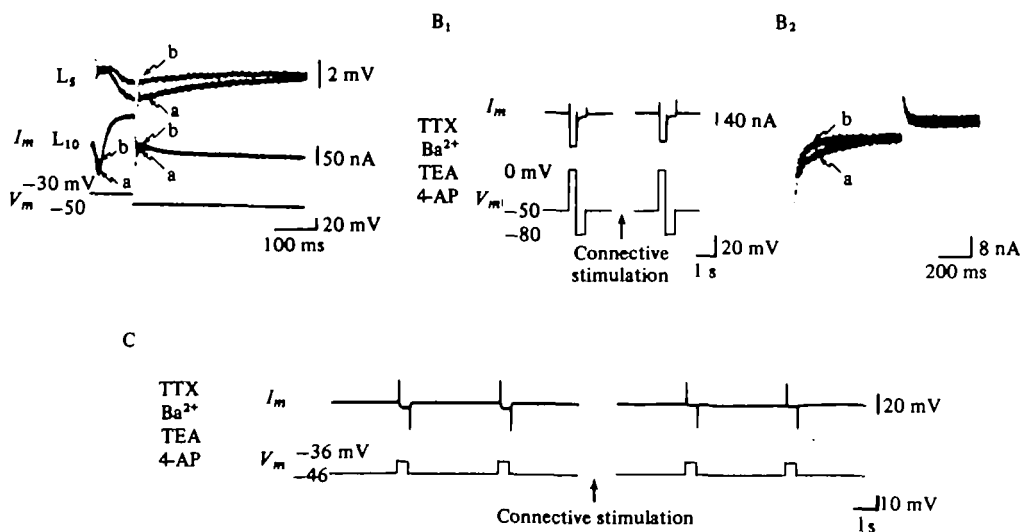
Fig. 11. Stimulation of presynaptic inhibitory pathway directly reduces current through the Ca^{2+} channel.

(A) Cell L10 voltage clamped in normal sea water containing TTX and TEA. The depolarizing command step is from -50 to -30 mV. Superimposed currents before (*a*) and after (*b*) connective stimulation. Presynaptic inhibition is correlated with reduced Ca^{2+} current and unchanged K^+ current. PSP was reduced by presynaptic inhibition from 2·2 to 1·2 mV. The tail-current upon repolarization of L10 to the holding potential is less inward after connective stimulation than prior to connective stimulation. (B₁) L10 voltage clamped in high-Ba^{2+} sea water containing TTX (30 μM), TEA (25 mM), and 4-AP (10 mM). A depolarizing step from -50 mV to zero mV elicits large inward current. When the cell is repolarized to -80 mV (the approximate level of E_K as determined by the reversal potential of the fast transient outward current) there is an inward tail current. Stimulation of the presynaptic inhibitory pathway (for 5 s) causes a reduction in the inward net current as well as in the inward tail current upon repolarization to -80 mV. The PSP was not monitored in this experiment.

(B₂) Same experiment as B₁ to show at higher gain and faster sweep speed the tail currents at E_K (-80 mV) superimposed before (*a*) and after (*b*) connective stimulation.

(C) Stimulation of presynaptic inhibitory pathway reduces steady-state inward currents. Cell L10 voltage clamped in high-Ba^{2+} sea water containing TTX (30 μM), TEA (25 mM), and 4-AP (10 mM). A small depolarizing voltage step produces a steady-state inward current. Stimulation of presynaptic inhibitory pathway (for 5 s) reduces this inward current. (From Shapiro *et al.* 1980*b*.)

be the result of an increase in small residual outward K^+ current, or a decrease in the inward Ca^{2+} current. The observation that the decrease in early inward current during presynaptic inhibition is larger than the increase in late outward and leakage currents (Fig. 10, Fig. 11 A) suggests that the Ca^{2+} current may be modulated directly. But to distinguish between these alternative possibilities directly we performed two types of experiments: pharmacological block of all the K^+ channels, and an examination of the voltage dependence of the presynaptic action.

The inward current decreases even in the presence of K^+ channel blockers

To test the involvement of Ca^{2+} channels in presynaptic inhibition we examined the divalent cation current in isolation after blocking the Na^+ channel with TTX and the three known K^+ channels with TEA and 4-AP, and with substitution of Ba^{2+} for Ca^{2+}. Under these circumstances we found that connective stimulation reduced the inward current (Fig. 11 B, 11 C). This reduction paralleled the presynaptic inhibition.
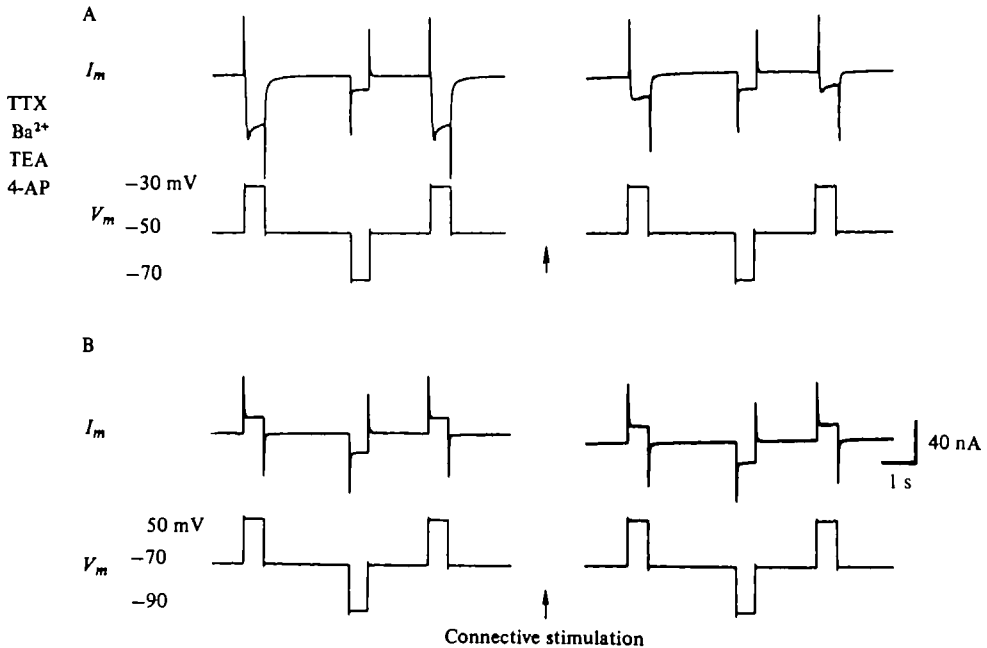
Fig. 12. The conductance changes caused by stimulation of presynaptic inhibitory pathway are voltage sensitives Cell L10 is voltage clamped in sea water containing Ba^{2+}, TTX (30 μM), TEA (25 mM), and 4-AP (10 mM). Under these pharmacologic conditions 30 min intervals were used between runs. (A) From a holding potential of -50 mV, alternating 20 mV depolarizing and hyperpolarizing voltage steps elicit inward currents. Connective stimulation markedly reduces inward Ba^{2+} current through the Ca^{2+} channel of the depolarizing step, and only slightly increase leakage current of the hyperpolarizing step. (B) From a holding potential of -70 mV, alternating 20 mV depolarizing and hyperpolarizing voltage steps again elicit inward current with hyperpolarizing step but elicit primarily outward leakage currents with depolarizing steps. In this voltage range depolarizing command pulses do not significantly activate inward currents. Only small leakage currents are produced by both hyperpolarizing and depolarizing steps. Connective stimulation does not affect these currents. (From Shapiro et al. 1980b.)

In addition, changes in the steady-state inward current also occurred with connective stimulation (Fig. 11C). These results imply that the decrease in inward current reflects a decrease in the transient and in the steady-state Ca^{2+} current during presynaptic inhibition.

Presynaptic actions occur only in the voltage range where the calcium current is activated

Since the Ca^{2+} current is voltage dependent, one would expect that the synaptic transmitter that mediates presynaptic inhibition would have no effect on ionic currents at hyperpolarized levels of membrane potential where the Ca^{2+} current is not activated. We therefore examined the effects of presynaptic inhibition on currents in the voltage range where the Ca^{2+} channel was activated and in the range where it was not activated. When cell L10 was held in the voltage range in which the Ca^{2+} channel was activated, connective stimulation caused decreases in inward current (Fig. 12A). When the cell was held in the voltage range in which the Ca^{2+} channel was not activated, no conductance change was observed after connective stimulation (Fig. 12 These data support the idea that presynaptic inhibition is due to a direct action on the
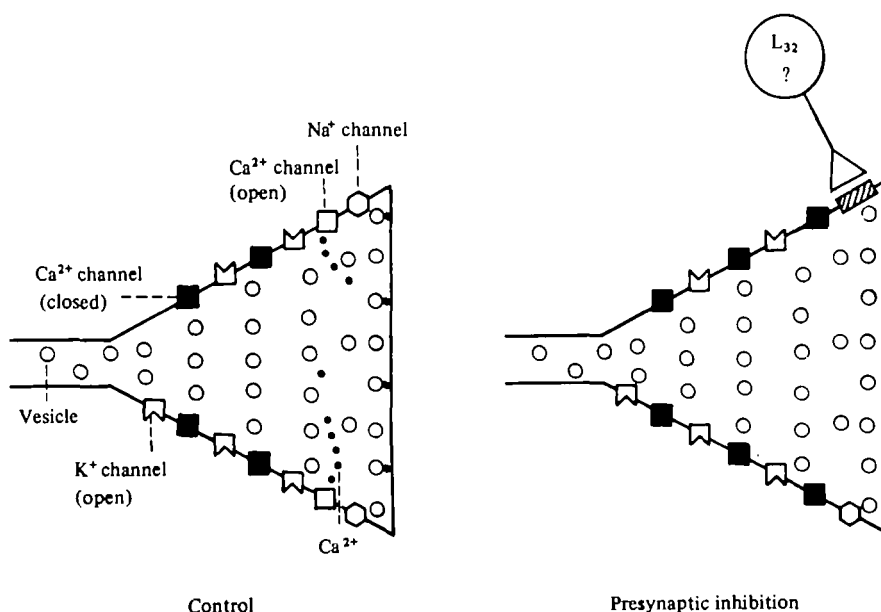
Fig. 13. Diagram of mechanism of presynaptic inhibition. An unidentified presynaptic inhibitory transmitter directly reduces both steady-state and transient calcium currents, making less calcium available for transmitter release A specific cell (L32) has recently been found to mediate this presynaptic inhibition (Byrne, 1980). (See legend Fig. 8 for key.)

Ca^{2+} channel (Fig. 13). A similar mechanism for presynaptic inhibition has been discovered in dissociated dorsal root ganglion cells by Dunlap & Fischbach (1978) and by Mudge, Leeman & Fischbach (1979).

In vertebrate heart muscle Giles & Noble (1976) have found that ACh, acting on muscarinic receptors in the heart, decreases the Ca^{2+} current in the heart muscle. This action of ACh is similar to the mechanism of presynaptic inhibition in *Aplysia* and in dissociated dorsal root ganglion cells. Reuter (1979; Reuter & Scholz, 1977) has suggested that cGMP, or the ratio of cAMP/cGMP in heart muscle, may control the maximum calcium conductance of the muscle. An alternative possibility (Fig. 27) is that the modulatory transmitter closes a Ca^{2+} channel-receptor complex directly without the mediation of an intracellular second messenger.

In mammals, presynaptic inhibition has been correlated with presynaptic depolarization and with increased excitability in afferent fibres. The observed conductance change at some of these synapses follows the chloride Nernst potential (reviewed in Burke & Rudomin, 1977). However, as indicated above, results similar to those reported here have been obtained by Fischbach and his colleagues (Dunlap & Fischbach, 1978; Mudge *et al.* 1979) on chick dorsal root ganglia. Whether the presynaptic depolarization and the increased Cl^- conductance in vertebrates are epiphenomena, that are not directly related to the mechanism of presynaptic inhibition, and are due only to the artificial modes of activation, or whether there are several distinct mechanisms for presynaptic inhibition, needs now to be determined (for a critical review the studies of presynaptic inhibition in the mammalian brain, see Ryall, 1978).
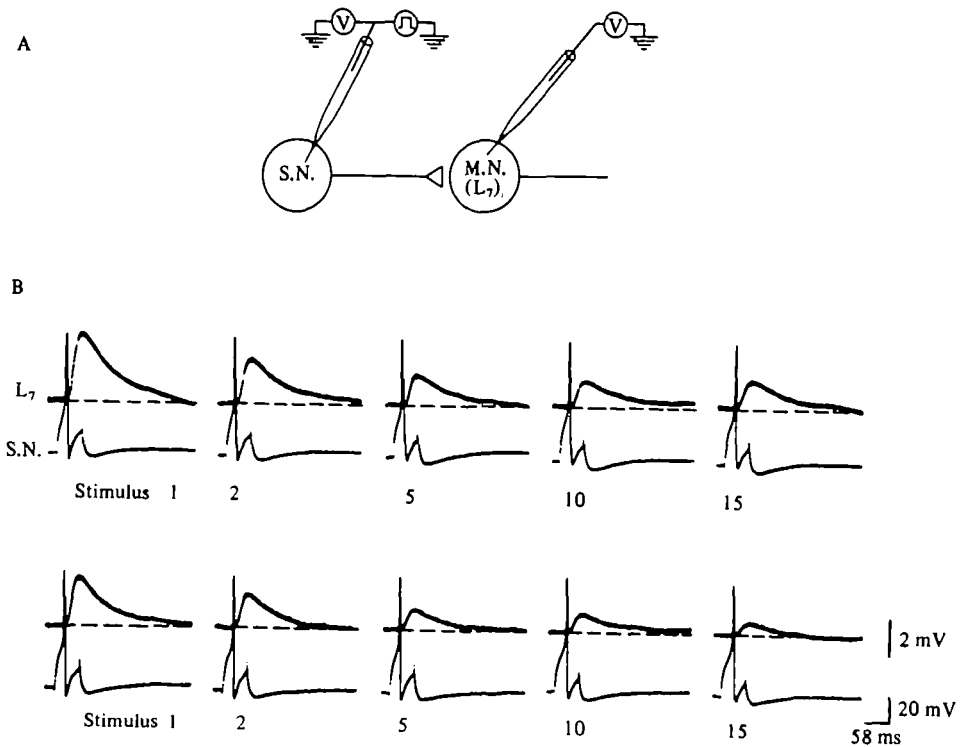
Fig. 14. Homosynaptic depression accompanying habituation in the gill-withdrawal reflex. (A) Diagram of arrangement for recording monosynaptic EPSP from sensory neurone to gill motor neurone in abdominal ganglion of *Aplysia*. (B) Homosynaptic depression of the monosynaptic sensory-to-motor EPSP. Action potentials evoked in a sensory neurone give rise to an EPSP which undergoes depression when elicited repeatedly every 10 s (upper part). Lower part shows partial recovery after rest, and a further decrease in EPSP size with continued stimulation. (From Castellucci & Kandel, 1974.)

Homosynaptic depression

Homosynaptic depression – a self-induced depression in excitatory transmission at the synapses made by the sensory neurones on their central target cells – is the mechanism for short-term habituation of the siphon and gill-withdrawal reflexes in *Aplysia* (Fig. 14; Castellucci *et al.* 1970). This depression, which is very profound and rapid at these sensory neurone synapses, is due to a progressive decrease in the amount of transmitter released by each action potential (Castellucci & Kandel, 1974).

The depression is correlated with a decrease in the calcium component of the action potential in TEA

As a first step in examining homosynaptic depression, we analysed the Ca^{2+} contribution to the action potential in the sensory neurone. Under normal circumstances, the action potential is rapidly terminated by the repolarizing action of large outward potassium currents, masking the small contribution of the calcium current to the action potential. We therefore reduced the voltage-dependent potassium current substantially by bathing the ganglion in TEA^+. This caused the peak amplitude of t

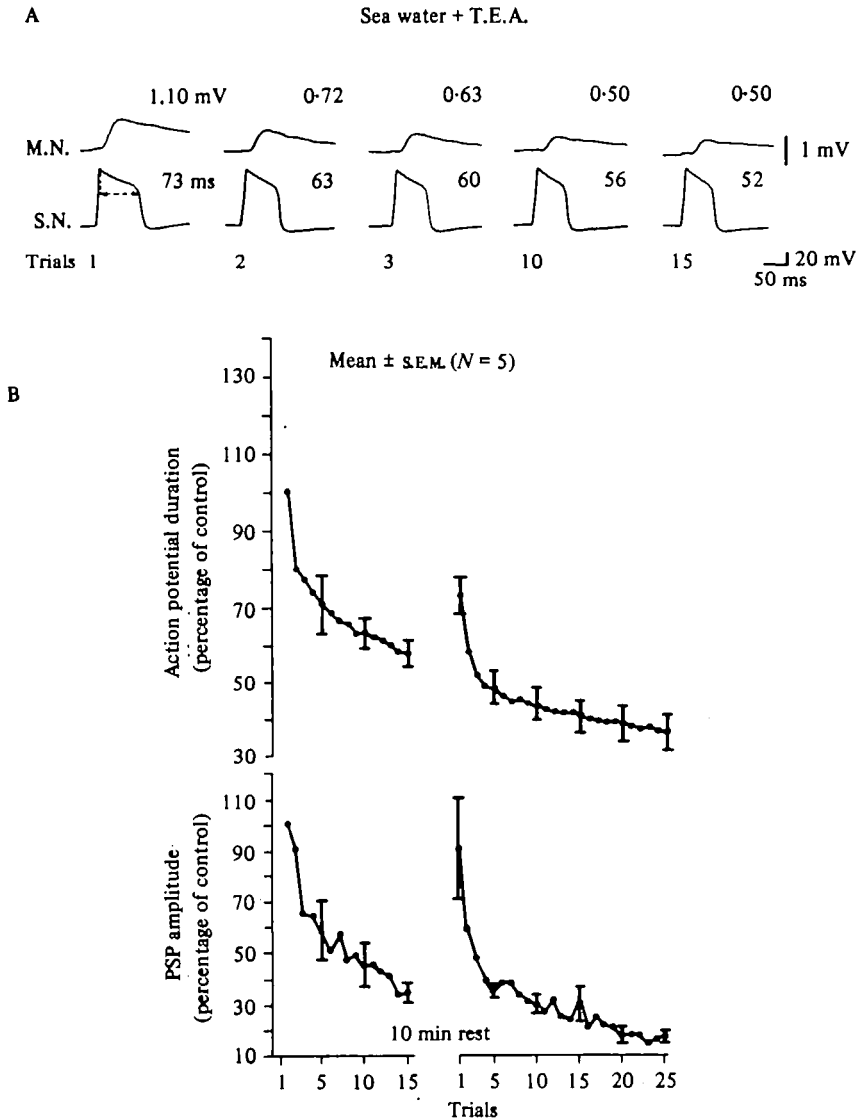A   Sea water + T.E.A.

Fig. 15. Change in Ca^{2+} current during homosynaptic depression. (A) Correlation between sensory neurone action potential in sea water containing 100 mM-TEA and monosynaptic EPSP with repeated stimulation. Action potentials fired at 0·1 HZ. (B) Average action potential duration and EPSP amplitude (based on 5 preparations) during a first habituation run, after a 10 min rest, and during a second run. Spikes evoked at 0·1 Hz in sea water containing TEA.

action potential to increase and a plateau to develop on the descending limb, thereby increasing the width of the action potential at half-maximal amplitude by about 50-fold. During this plateau the inward current, which is carried by Ca^{2+}, is unmasked sufficiently to produce a self-sustaining depolarization of the membrane. That the plateau is due to calcium current is supported by the finding that it occurs in the absence of sodium, and that the amplitude of the plateau follows the external calcium concentration as predicted by the Nernst equation. Furthermore, the plateau can

be abolished with cobalt or nickel ions (Klein & Kandel, 1978), which block the calcium channels of other neurones (Adams *et al.* 1980).

The duration of the action potential in TEA is a sensitive measure of the calcium current because it is determined by the balance between the inward calcium current and the outward potassium and leakage currents. As long as the calcium current is at least equal to the outward currents (the leakage and the K^+ currents) the plateau is maintained; when the outward currents outweigh the inward current the membrane repolarizes. The total calcium influx during the plateau phase can therefore be regulated both by intrinsic changes in the calcium channels as well as by changes in potassium or leakage currents. Hence, changes in the duration of the TEA spike are a good indication of changes in total Ca^{2+} influx, but they do not indicate whether the changes result from a direct action on the calcium channel or whether the influx is affected indirectly by a potassium conductance not blocked by TEA. In addition, some part of the plateau also represents firing of other parts of the neurone outside the cell body. Calcium spikes in the axon and terminal regions can contribute to the duration of the TEA action potential recorded in the cell body, except, of course, when the cell body is isolated from its processes by tying it off, for example.

We next examined transmitter release from the terminals of the sensory neurones, as measured by the size of the synaptic potential in postsynaptic neurones, and the simultaneous change in calcium current, as measured by changes in the duration of the action potential (Fig. 15). Repeated stimulation of the sensory neurone at rates that produce habituation led to a progressive decrease in the PSP (with kinetics similar to what is observed in normal sea water) together with a reduction in the duration of the TEA action potential, indicating a progressive decrease in the calcium current of the action potential. Spontaneous recovery of synaptic transmission with rest was accompanied by an increase in the calcium current.

Depression parallels the decrease in the Ca^{2+} current

To determine whether the decrease in Ca^{2+} current was due to a direct action on the Ca^{2+} current or an action on a K^+ current, we voltage clamped the cell body of the sensory neurone. In normal sea water, depolarizing commands elicit EPSPs in the follower cells and an inward current in the sensory neurone which is due largely to Na^+ and a lesser degree to Ca^{2+}, followed by an outward current (Fig. 16A). With repeated depolarizing commands repeated at every 10 sec the EPSPs decrease in amplitude but there is no change in the presynaptic current. This is consistent with the lack of a change in the action potential in normal sea water in the absence of Na^+ or K^+ channel-blocking agents.

We therefore next blocked the Na^+ channels with TTX and the delayed and early K^+ channel with a very high concentration of TEA (Fig. 16B). With the larger Na^+ current blocked, this inward current is due to Ca^{2+}. Depolarizing commands now still produced EPSPs in the motor cell and these declined with repetition. Fig. 17 shows averaged data on the depression of the EPSP and of the inward current from three sensory neurones where 15 EPSPs were elicited at 10 sec intervals in two consecutive training sessions separated by 5 min of rest. The changes in the EPSP parallel quite closely the changes in the peak inward current. However, in these experiments some portion of the current results from uncontrolled active responses in other parts of the
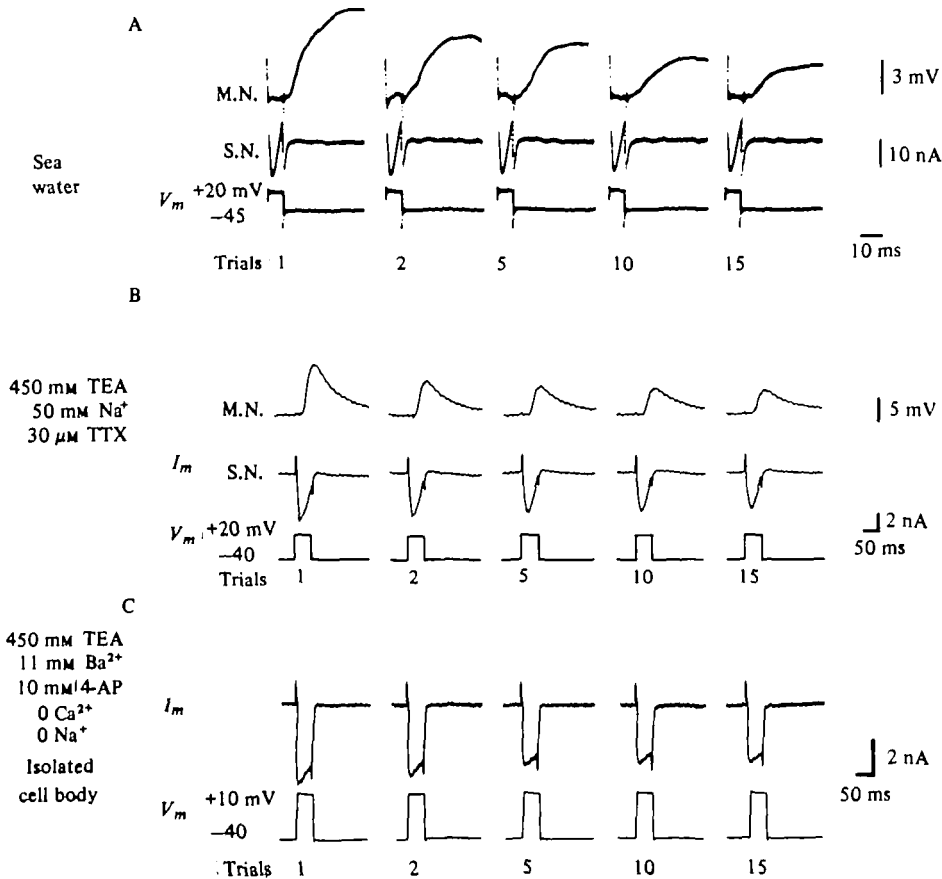
Fig. 16. Homosynaptic depression and decline in calcium current under voltage clamp. (A) In normal sea water the inward current is due to sodium and calcium. The presynaptic voltage clamp pulses elicit EPSPs that undergo depression with repetition of the depolarizing command. The decrease in EPSP is not accompanied by any change in either the outward K⁺ or the inward Na⁺ current. (B) With the sodium current blocked with TTX and a large part of the potassium currents blocked with TEA presynaptic voltage-clamp pulses elicit EPSPs that undergo depression with repetition of the depolarizing command. The decrease in EPSP is paralleled by a decrease in the inward current. (C) In the absence of Na⁺ ions with virtually all K⁺ currents blocked (by bathing in a high concentration of 4-AP in addition to TEA and Ba²⁺) and with voltage-clamp control maximized by ligating the sensory neurone cell body, inward current decreases with 0·1 Hz stimulation, indicating that current moving through the calcium channels undergoes a progressive decline independent of changes in Na⁺ or K⁺ currents.

neurone, including the fine terminal processes of the neurone where transmitter release occurs. As a result, the interpretation of the decline in inward current is not unambiguous. It is possible, for example, that the threshold for firing calcium spikes in fine processes increases during habituation, resulting in transmitter release from fewer terminals. Alternatively (or in addition), the decline in calcium current could occur in a graded manner throughout all parts of the neurone, although perhaps not equally everywhere, so that progressively less transmitter is released from each terminal during habituation. The experiments do show, however, that drastic reduction of the K⁺ currents by TEA does not affect the ability of repeated activity to depress
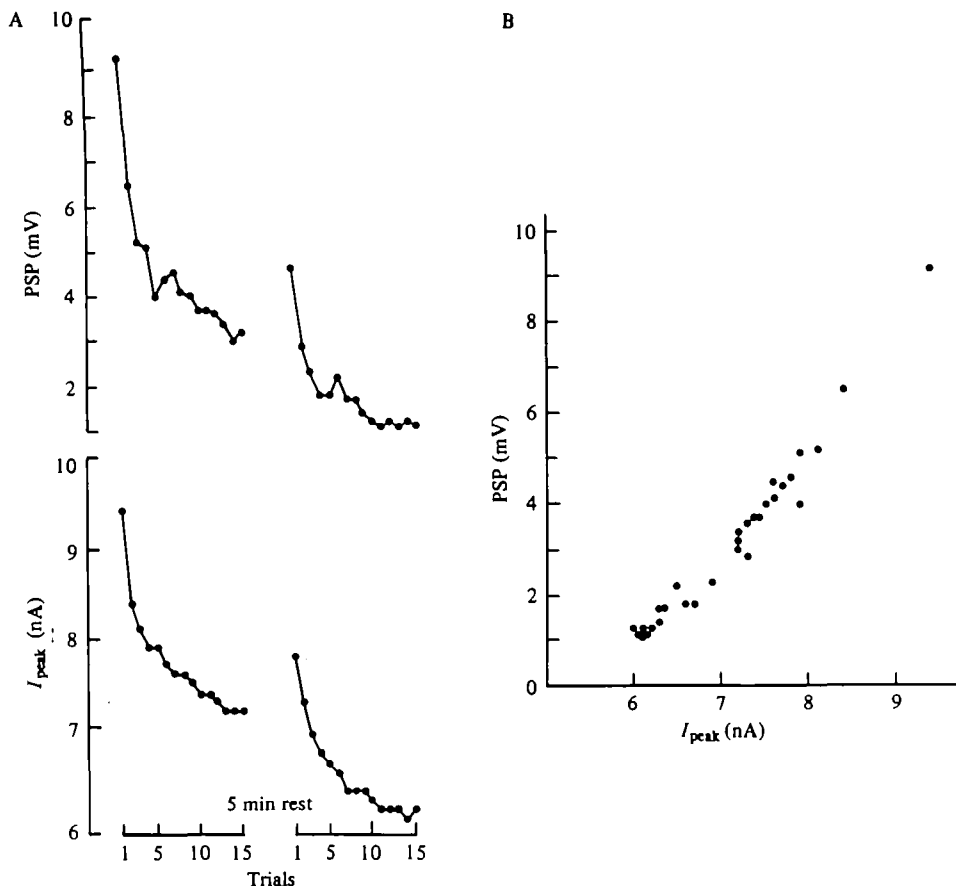
Fig. 17. Correlation of the amplitude of the monosynaptic EPSP produced in the follower cell by the sensory neurone and presynaptic inward current. (A) Two consecutive habituation training sessions were separated by a 5 min period of rest. The sensory neurones were voltage clamped in artificial sea water containing Na^+ and K^+ current blockers, and EPSPs were elicited by 50 ms depolarizing steps every 10 s. (B) The amplitude of the EPSP in the follower cell is plotted as a function of the peak inward Ca^{2+} current.

the EPSP. The parallel decline of the PSP and of the inward current therefore appears to result from an intrinsic change in the calcium current.

To examine the inward current in a situation where the cell body is optimally space-clamped so that uncontrolled responses are eliminated we isolated the cell body of the sensory neurone by tying it off. We next blocked all the K^+ currents by substituting Ba^{2+} for Ca^{2+} and using 4-AP in addition to high concentrations of TEA. Under these circumstances we still found a progressive decrease in the inward currents with repeated depolarizing commands (Fig. 16C).

We do not know whether this graded decline in inward current is quantitatively the same as that which occurs in the intact neurone, and whether the depression of the inward current with repeated stimulation is the only factor that accounts for habituation. However, habituation clearly does not involve an increase in K^+ current. In fact, in normal sea water with long depolarizing commands the K^+ current actua
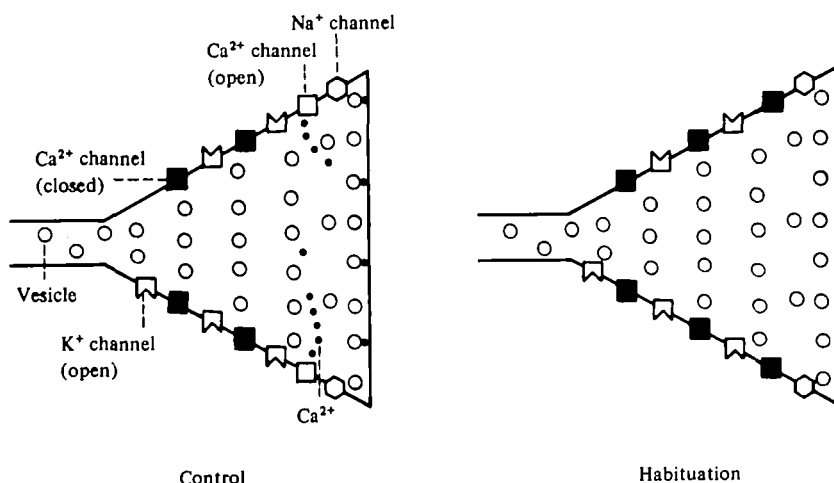
Fig. 18. A schematic model of habituation. With repeated activation, calcium channels undergo inactivation of calcium inflow, either as a direct effect of depolarization, or as a result of some other process. Arrows from potassium to calcium channels represent the flow of hyperpolarizing current (through the potassium channels) which repolarizes the action potential and thereby closes open calcium channels and prevents new ones from opening (see legend of Fig. 8 for key).

decreases with repeated depolarization, whereas the depression of the PSP is unaffected (see, for example, Fig. 22B, left half of figure).

The term 'inactivation' has been applied to a decrease in an ionic current elicited at a constant membrane voltage that is not due to an increase in an opposing current or to a change in driving force. We have provided evidence that the decrease in inward current is not the result of the activation of a residual K^+ current (Fig. 16C). It is also unlikely that the reduction in inward current is due to a change in driving force (resulting from an accumulation of divalent cations in the cell or near the inner surface of the membrane) since we have found that while the transient inward current decreases with repeated depolarizing command pulses of 60 to 70 mV (designed to simulate the action potential), the steady-state current through the Ca^{2+} channel (elicited with small voltage-clamp steps) does not decrease. A change in driving force should affect both transient and steady-state components of the current. Thus, the homosynaptic depression that underlies habituation is correlated with a prolonged inactivation in the transient inward current resulting from its repeated activation (Fig. 18).

Tillotson & Horn (1978) have described the tendency of the transient Ca^{2+} current to undergo prolonged inactivation in cells R2 and R15 of *Aplysia*. This inactivation differs from Na^+ inactivation in that the inactivation of the Ca^{2+} channel is not voltage dependent, but may depend on the intracellular calcium concentration (see Brehm & Eckert, 1978; Tillotson, 1979). The inactivation of the Ca^{2+} current underlying habituation in the sensory neurones, however, seems to differ in certain respects from that studied by Tillotson in cells R2 and R15 (Tillotson, 1979; Tillotson & Horn, 1978). First, in R2 and R15 the Ca^{2+} channels do not inactivate when Ba^{2+} carries the inward current, whereas in the sensory neurones the channels do inactivate with Ba^{2+}
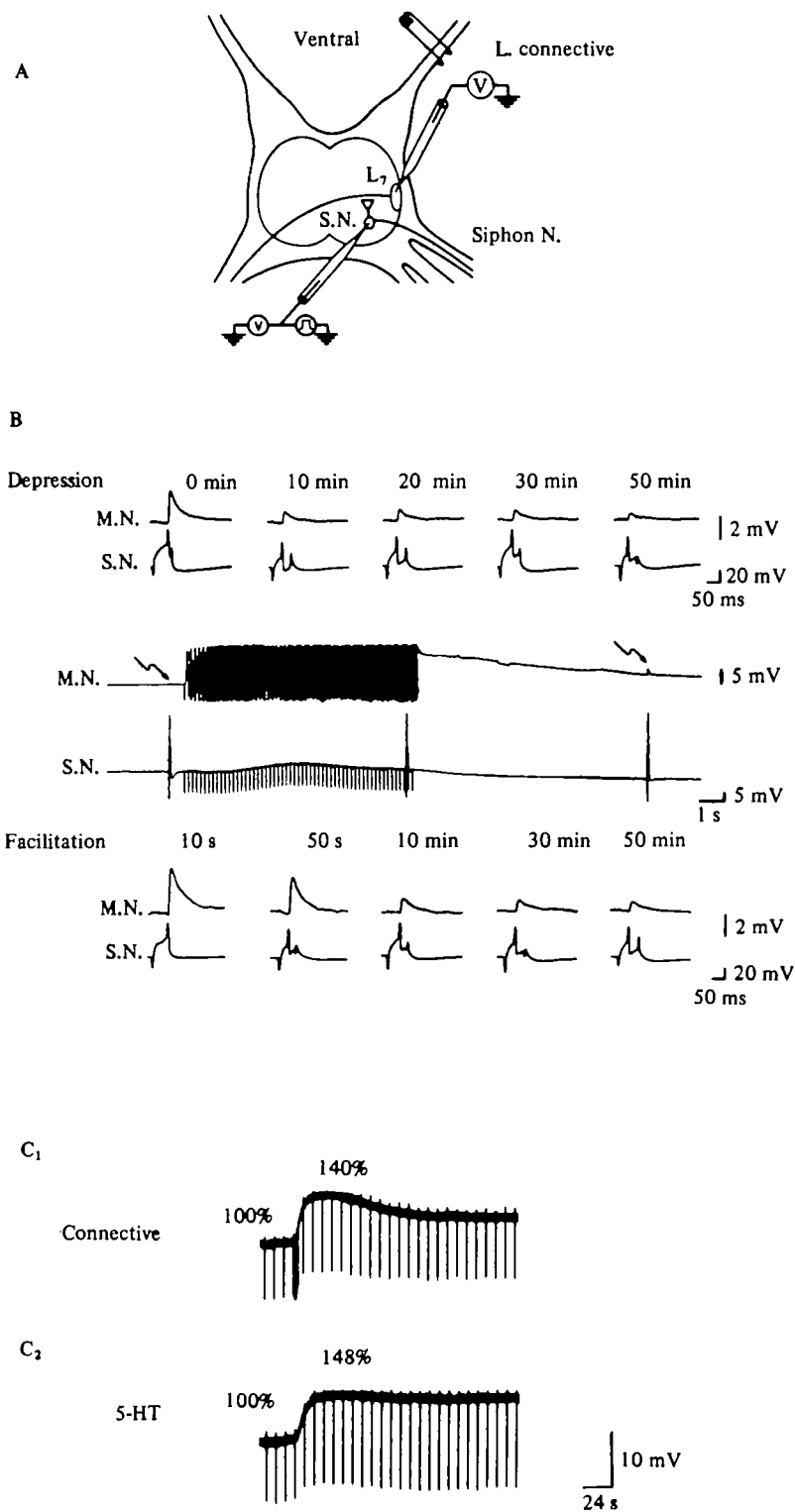
s the charge carrier. In addition, the time course of recovery from inactivation is much slower in the sensory neurones than in cells R2 and R15. It would therefore be of interest to explore the specific mechanisms that underlie the prolonged Ca^{2+} current inactivation in the sensory neurones.

Presynaptic facilitation

As is often the case with behavioural habituation, reflexes that illustrate this form of learning also show an opposite learning process: sensitization. Presynaptic facilitation was first encountered while trying to develop a cellular analogue for reflex sensitization, a simple form of learning which involves the enhancement of a reflex response by a strong stimulus (Kandel & Tauc, 1965a, b). This process was subsequently shown to be actually utilized in behavioural sensitization of the gill-withdrawal reflex (Kupfermann et al. 1970).

As we have seen, repeated sensory stimulation at rates that produce habituation in the intact animal produces a depression in the monosynaptic excitatory connexions between sensory neurones and their followers due to a decrease in the number of transmitter quanta released per impulse (Castellucci & Kandel, 1974). On the other hand, stimulation of a different pathway for a few seconds causes an increase in transmitter release from the sensory neurones (Castellucci & Kandel, 1976 and Fig. 19). This presynaptic facilitation is simulated by cyclic AMP and by serotonin, but not by a number of other candidate transmitter substances that were examined. A sensitizing stimulus produces two actions on the sensory neurones: (1) a slow EPSP that lasts up to half an hour (Klein & Kandel, 1978), and (2) a similarly long facilitation of synaptic transmission at the sensory-to-motor synapses (Fig. 19; Castellucci et al. 1970). To determine what was responsible for the increase in transmitter release, we examined the slow EPSP and other changes in the membrane properties of the sensory neurone cell body that accompany presynaptic facilitation.

The slow EPSP is due to a decrease in the conductance to K^+

We first examined the changes in the membrane conductance produced in the presynaptic neurone by the slow EPSP. Using electrotonic potentials produced by

Fig. 19. Synaptic facilitation at the synapse between mechanoreceptor neurones and motor neurones. (A) Ventral aspect of the abdominal ganglion of *Aplysia*, illustrating simultaneous recording from gill motor neurone L7 and a mechanoreceptor sensory neurone (SN). Stimulation of the left connective (LC), which carries information from the head to the abdominal ganglion, is used as the facilitating stimulus. (B) Depression and subsequent facilitation of the monosynaptic EPSP after a strong stimulus. Top two series of traces illustrate selected records of depression of the EPSP in the motor cell (MN) during a series of 50 min of consecutive intracellular stimuli to the sensory neurone (SN) (every 10 s) prior to the presentation of the facilitating stimulus. Middle two traces illustrate the effects of the facilitating stimulus (a train of shocks to the LC at 6 Hz for 10 s). Arrows indicate the last EPSP before the facilitating stimulus and the first EPSP after the stimulus. Bottom series of two traces illustrate the gradual decline of the facilitation over the subsequent 50 min, during which the sensory neurone was again stimulated once every 10 s. The middle two traces are at lower gain and at a slower time scale. (From Castellucci & Kandel, 1976.) (C) Effects of connective stimulation and 5-HT on sensory neurone membrane potential and resistance. (C_1) Connective stimulation caused a depolarization and an increase (40 %) in membrane resistance as measured with electrotonic potentials elicited by constant-current pulses applied through the second barrel of a double-barrelled microelectrode. (C_2) Incubation with 5-HT also produces a long-lasting depolarization and increased membrane resistance. (From Klein & Kandel, 1978.)

constant current pulses in the sensory neurones, we found that the slow EPSP was accompanied by an apparent decrease in the conductance of the membrane and that this decrease was simulated by serotonin, the putative facilitating transmitter, by cyclic AMP, and by phosphodiesterase inhibitors (Klein & Kandel, 1978). This was confirmed with voltage-clamp experiments. Stimulating the facilitatory pathway or incubating the ganglion or the isolated cell body with serotonin under voltage clamp caused an inward shift in the holding current and a decrease in the 'leakage' currents elicited by small step hyperpolarizations. Moverover, the slow EPSP and the inward shift in the holding current produced by connective stimultion or by serotonin were blocked by K^+ channel-blocking agents (compare Fig. 22C_1 and C_2), and by replacing intracellular K^+ in the sensory neurones with Cs^+, an impermeant cation. These results imply that this EPSP is due to a decrease in K^+ conductance. The EPSP is voltage dependent. Current–voltage curves are highly non-linear. At levels of membrane potentials more depolarized than -50 mV the non-linearity is reduced by connective stimulation and serotonin but at more hyperpolarized potentials than -60 mV synaptic stimulation or serotonin gave rise to little or no synaptic current. By contrast, when the external K^+ concentration was doubled, the current reversed at about -50 mV. The association of a decrease in a voltage-sensitive K^+ conductance with presynaptic facilitation suggested that the facilitation might occur through the reduction of a presynaptic K^+ current that is activated by the action potential, thereby leading to an increase in the duration of the action potential and increased transmitter release. We therefore examined the presynaptic action potential and the currents activated by them under a variety of conditions to see whether or not a reduction in the K^+ current of the presynaptic action potential could account for presynaptic facilitation.

Presynaptic facilitation produces an increase in the calcium current of the action potential

When we bathed the abdominal ganglion in TEA and examined action potentials in the sensory neurones, we found that they were prolonged by stimulating the connective, indicating an increase in the Ca^{2+} current. As with homosynaptic depression, the changes in the duration of the action potential as a result of presynaptic facilitation paralleled the changes in transmitter release (Fig. 20). A similar prolongation of the action potential was also produced by serotonin, by cAMP, and by phosphodiesterase inhibitors (Klein & Kandel, 1978).

Calcium current prolongation is due to a depression of the K^+ current

The increase in Ca^{2+} current could be produced by a direct action on the Ca^{2+} channel or by an indirect action by means of a reduction in an opposing K^+ current. To distinguish between direct and indirect actions on the Ca^{2+} current, we voltage clamped the cell body of the sensory neurone and analysed the changes in ionic currents produced by connective stimulation and by serotonin, the presumed facilitating transmitter.

We again introduced depolarizing commands in the sensory neurones under voltage clamp and recorded both the presynaptic currents and PSPs in follower cells. In normal sea water stimulation of the connective produced facilitation of the EPSP and a decrease in the outward current (Fig. 21). This experiment confirms in the absenc
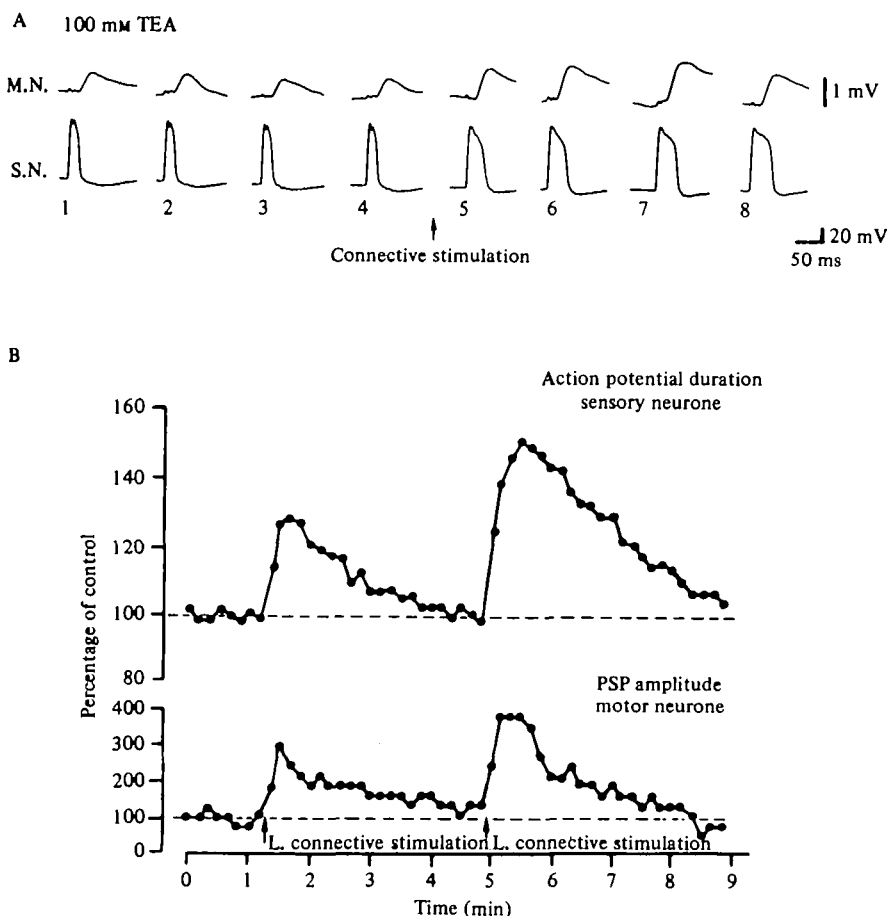
A   100 mM TEA

M.N. (waveform traces) | 1 mV

S.N. (waveform traces)

1   2   3   4   5   6   7   8

Connective stimulation

_| 20 mV
50 ms

B

Action potential duration
sensory neurone

Percentage of control

160
140
120
100
80

PSP amplitude
motor neurone

400
300
200
100
0

L. connective stimulation   L. connective stimulation

0   1   2   3   4   5   6   7   8   9

Time (min)

Fig. 20. Increase in Ca^{2+} current that parallels presynaptic facilitation. (A) Broadening of the action potential in TEA accompanying presynaptic facilitation of monosynaptic sensory-to-motor EPSP. (B) Graph of EPSP amplitude and presynaptic spike duration during an experiment in which the facilitating pathway was first stimulated weakly, and then more strongly. In both A and B spikes were evoked at 0·1 Hz in 100 mM-TEA sea water. (From Klein and Kandel, 1978.)

of drugs the finding made in TEA solution that connective stimulation can alter the configuration of the currents contributing to the action potential. To analyse this change in the presynaptic current, we isolated the cell body to maximize voltage-clamp control, using serotonin to simulate the presynaptic facilitation. In sea water containing no drugs, serotonin caused a decrease in transient outward currents elicited with depolarizing steps, as well as the decrease in resting conductance (Fig. 22B, C_1). However, when potassium currents were blocked with TEA and 4-AP, serotonin had no effect on any membrane conductance (Fig. 22A, C_2). The effects of serotonin were blocked with either Ca^{2+} or Ba^{2+} as the divalent cation that was carrying current through the Ca^{2+} channels.

To rule out the possibility that the K+ channel-blocking agents were interfering with the action of serotonin on the sensory neurone, we also eliminated the K+
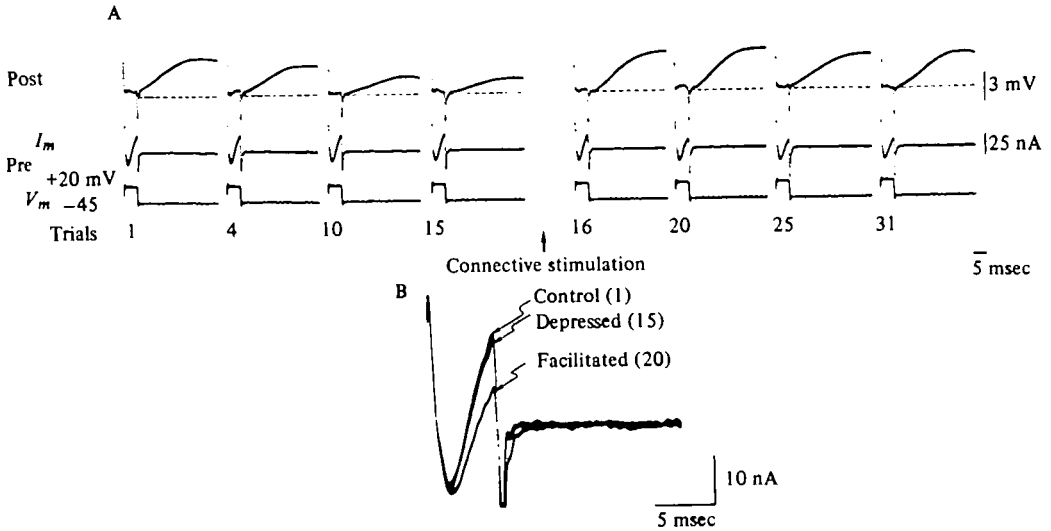
Fig. 21. Decrease in outward current parallels presynaptic facilitation. (A) In normal sea water depolarizing commands in the sensory neurones produce inward and outward currents in the sensory neurone and EPSPs in the motor cells. The cell was stimulated at one per 10 s to simulate habituation and the repeated commands led to a depression of the EPSP. Connective stimulation produced a decrease in the outward current and a parallel facilitation of the EPSP. (B) Three traces from Part A are superimposed to illustrate the lack of significant change in the outward currents with the repeated depolarizing commands designed to simulate habituation (traces 1 and 15) and the depression of the outward current following stimulation of the connective designed to simulate sensitization (trace 20).

current in a different way. We substituted the non-permeant cation Cs^+ for the intracellular K^+ using the antibiotic nystatin, which makes the membrane permeable to monovalent cations (Russell, Eaton & Brodwick, 1977). We then washed out the nystatin and recorded from the cell in K^+-free solution with electrodes filled with CsCl. Under these circumstances, serotonin produced no modulation of the current. By contrast, when a small amount of K^+ was reintroduced into the cell with a microelectrode filled with KCl, the net current became outward, and now was again affected by serotonin (Fig. 23). These experiments imply that serotonin, and presumably presynaptic facilitation, act on K^+ currents, and have no significant *direct* action on the calcium channel.

The finding that presynaptic facilitation works by means of the depression of K^+ currents implies that the increase in Ca^{2+} current is secondary to a change in the configuration of the action potential. This in turn leads to three predictions: (1) The action potential of the sensory neurones in normal solutions should be prolonged by connective stimulation and by serotonin; (2) Prolonging a depolarizing step under voltage clamp should produce similar enhancement of synaptic transmission; and (3) Facilitation should cause a characteristic change in the shape of the synaptic potential. We have tested each of these predictions experimentally and have found good agreement with the experimental results.
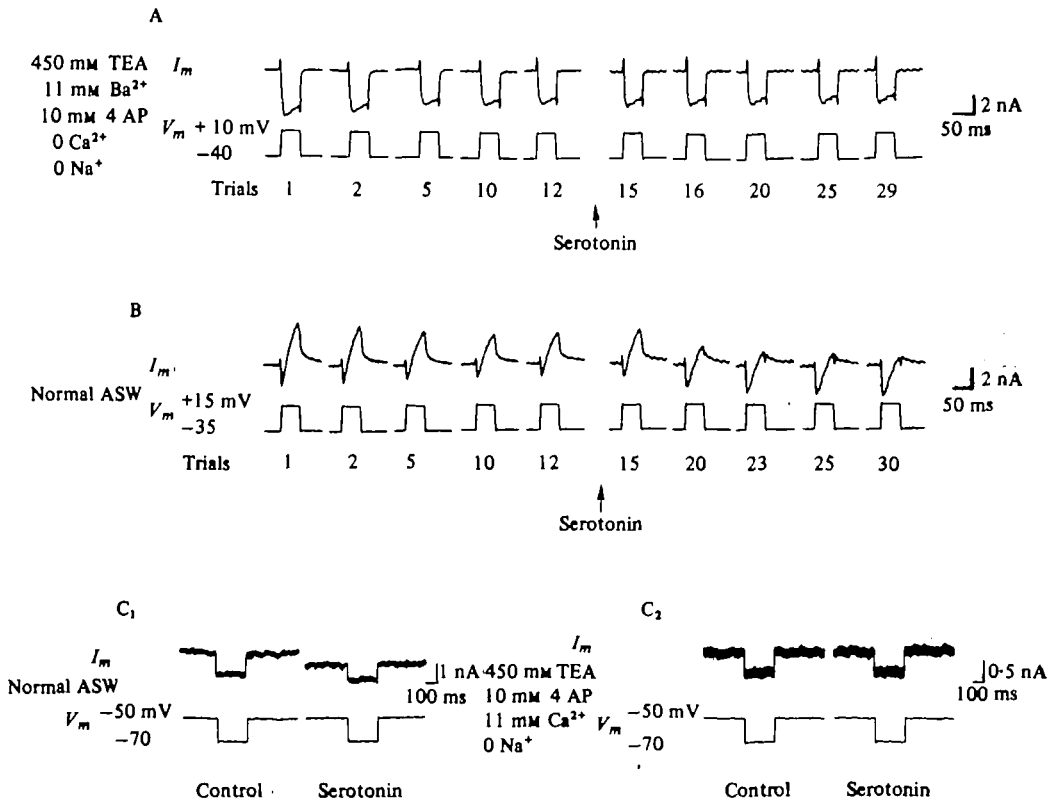
Fig. 22. Changes in presynaptic currents of the cell body of sensory neurones isolated by ligation, with stimuli that produce habituation and sensitization. Parts A and B are from the same cell examined first with all the K⁺ channels blocked and then in normal sea water. (A) With all K⁺ currents blocked, a step depolarization every 10 s (bottom records) still cause gradual inactivation of inward current (top records); however, 5-HT produces no effect. (B) When the same cell as in part A was placed in normal sea water after washing out the K⁺ channel blocking agents a step depolarization every 10 s (bottom records) again causes gradual inactivation of inward current (top records). But now 5-HT again produces an increase in the transient inward current and a depression of the outward current. In addition the holding current becomes more inward and the leakage current is reduced. (C) Holding and leakage current (produced by a small hyperpolarizing command) in normal sea water (C₁) and in K⁺ channel-blocking solution (C₂). C₁. In normal sea water, 5-HT causes the holding current to become more inward and the leakage to decrease (C₂). In sea water containing K⁺ blockers both effects of serotonin are abolished.

Presynaptic facilitation produces an increase in duration of the action potential

Connective stimulation (or serotonin) produces a broadening of the action potential. In the cell body the broadening is modest (Fig. 24 A). Since the density of the Ca²⁺ channels relative to the Na⁺ channels may be higher in the terminals than in the cell body, we increased the extracellular Ca²⁺ concentration and lowered the Na⁺ concentration, to simulate the action potential that may be present in the terminals. Under these circumstances, the increase in duration is greater and there is also an increase in the peak amplitude of the action potential (Fig. 24 B).
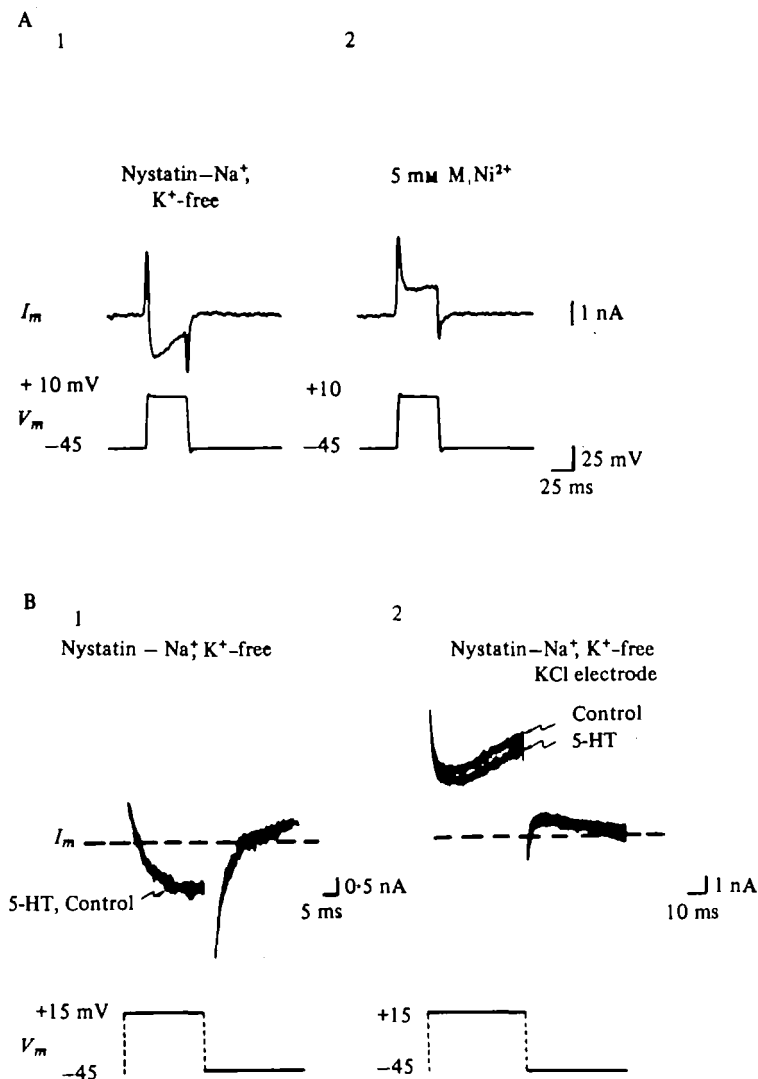
Fig. 23. Effect of serotonin on calcium current after exchange of intracellular K^+ for Cs^+. The ganglion was first bathed in a solution containing the antibiotic nystatin, which greatly increases membrane permeability for monovalent cations. K^+ and Na^+ ions were washed out and replaced with Cs^+ ions. After ion substitution, nystatin was washed out, and a test solution containing only Ca^{2+}, Mg^{2+}, Cl^- and TRIS ions was used. Cells were impaled with 3M-CsCl electrodes. (A) A_1. The inward current after nystatin ion exchange is due to Ca^{2+} and there are no significant outward currents. Calcium current after Cs^+ substitution. (A_2.) When nickel is added to the bath, the calcium current is blocked and the remaining record shows only leakage and no significant active currents. (B) Serotonin had no effect on isolated calcium current until K^+ was reintroduced into cell. (B_1) Failure of serotonin to modulate isolated $I_{Ca^{2+}}$. (B_2). A CsCl electrode was withdrawn from the cell and a KCl electrode was substituted for it. After a short period of iontophoresing K^+ into the neurone, an outward potassium current appeared. This current was reduced when serotonin was reintroduced into the bath.
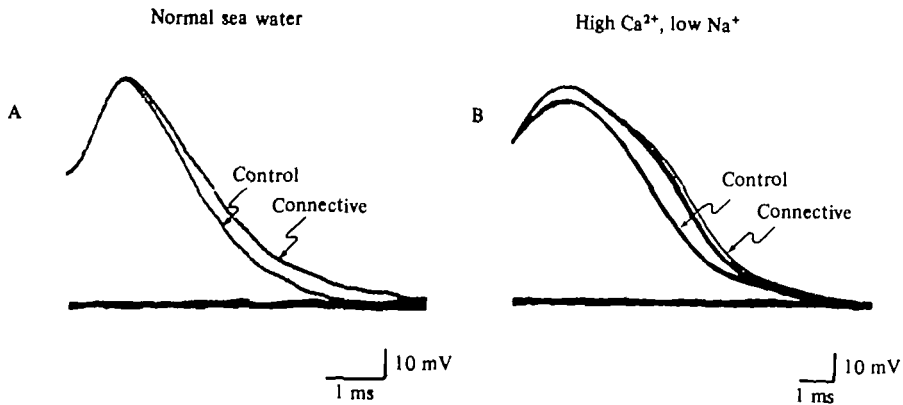
Normal sea water   High Ca²⁺, low Na⁺

Fig. 24. Presynaptic facilitation produces action potential broadening in solutions containing no K⁺ blocking agents. (A) Normal sea water. (B) Sea water containing 0·5 × sodium, 5·5 × calcium, 2 × magnesium.

Prolongation of the depolarizing command enhances transmitter release

To strengthen the conclusion that increased transmitter release during sensitization is caused by broadening of the action potential, we next increased the duration of the presynaptic depolarization under voltage clamp in an attempt to simulate the changes that occur in the action potential. We clamped a sensory neurone in the presence of Na⁺ and K⁺ channel-blocking agents and elicited an EPSP in a follower cell with a depolarizing command step. When we increased the duration of a 20 msec depolarizing step by 25%, the EPSP doubled in amplitude (Fig. 25). This occurs because the Ca²⁺ current has a very long rise time (approximately 10–30 msec) compared to the duration of the step, and, in this cell, is still increasing at the end of the 20 msec command. Lengthening the command by 5 msec increases appreciably the calcium influx (measured by the area under the current). If the depolarization were as short as an action potential (approximately 2 msec at half amplitude), an even smaller percentage increase in duration of the spike might increase the EPSP significantly. The observed changes in action potential duration *could* therefore cause the increase in transmitter release that underlies sensitization.

Presynaptic facilitation is accompanied by a change in the shape of the EPSP

If increased release were a result of broadening of the presynaptic action potential, the Ca²⁺ current would be activated for a longer time, and we might expect to see a corresponding change in the configuration of the EPSP. When we superimposed the two EPSPs of Fig. 25 produced by command pulses of different duration, adjusting for the amplitude difference, we found, not surprisingly, that the PSP elicited with the 25 msec depolarization reached its peak later than the PSP elicited with a 20 msec step (Fig. 25 C). Similarly, the PSPs produced by action potentials after a sensitizing stimulus show longer rise times compared to the PSPs elicited prior to sensitization. These changes in shape are qualitatively similar to those produced by increasing the duration of a voltage-clamp command (Fig. 26).

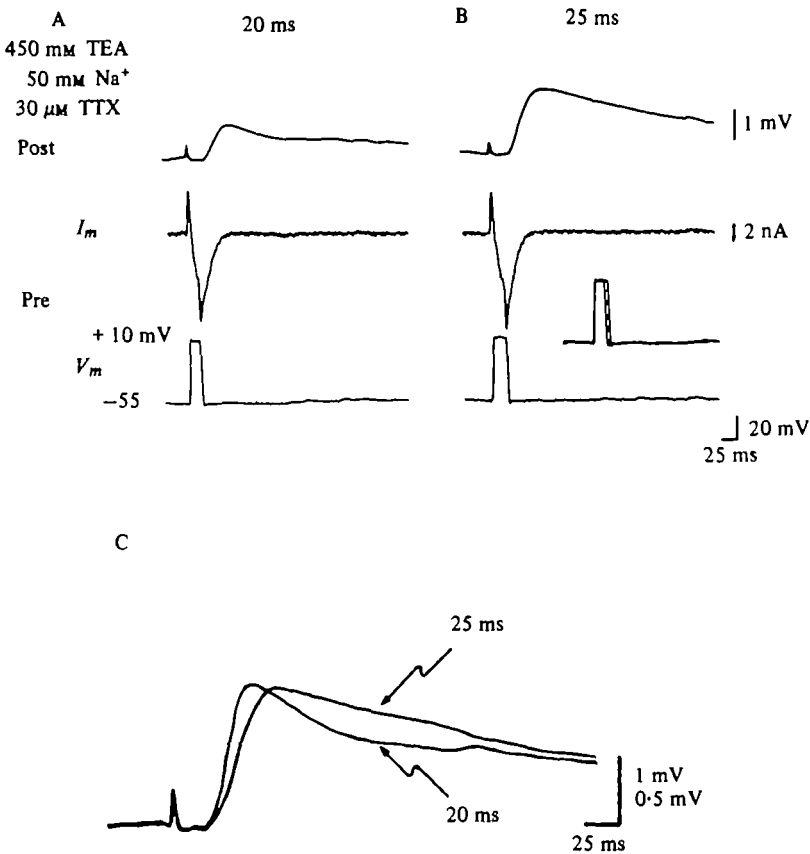This change in shape is not simply a consequence of the difference in size before

Fig. 25. Increasing the duration of the presynaptic command pulse increases the amplitude of the EPSP. (A) A 20 ms voltage-clamp pulse gave rise to an EPSP (top) approximately 1 mV in amplitude. (B) When the step was prolonged to 25 ms, the EPSP doubled in size. Inset shows superimposition of the voltage records. (C) Superimposition of the EPSPs in Parts A and B to illustrate the change in shape of the facilitated EPSPs. The EPSPs were scaled so as to match their peak amplitudes.

and after facilitation. The changes in PSP amplitude produced by habituation and spontaneous recovery from habituation do not affect the shape of the synaptic potential (Fig. 26). Similarly, changes in PSP amplitude produced by altering the calcium and magnesium concentrations do not produce shape changes (Castellucci & Kandel, 1974). On the other hand, when the PSP is increased by adding TEA to the bathing solution, blocking K^+ current, the increase in PSP amplitude is accompanied by a prolongation of its rise time, similar to that seen in presynaptic facilitation (Castellucci & Kandel, 1974). These findings strengthen our earlier conclusion that habituation is caused directly by an intrinsic decline in calcium current rather than increased potassium current, and that habituation and sensitization reflect different modes of calcium current modulation.

These several findings indicate that the increase in Ca^{2+} current that accompanies presynaptic facilitation is due to an indirect action on the Ca^{2+} current mediated by a depression of the opposing K^+ current. Turning off the K^+ current causes an increa
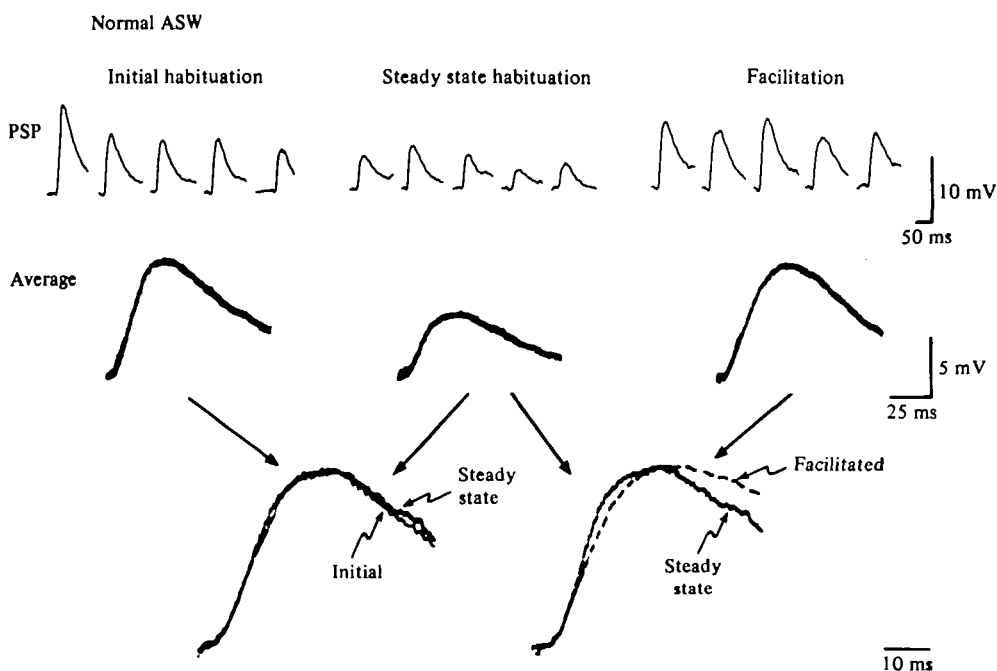
Normal ASW

Fig. 26. Comparison of EPSP shapes after habituation and sensitization. Monosynaptic EPSPs were evoked with sensory neurone action potentials fired once every 10 s in normal sea water. Blocks of five EPSPs at the beginning and end of the habituation run and after connective stimulation (uppermost traces) were averaged using a signal averager (middle traces). The averages were scaled so that their peak amplitudes were the same and were then superimposed in pairs (bottom traces). The traces at the bottom left show superimposition of the averages taken from the beginning and end of the habituation run. Note that despite the fact that the EPSP has decremented considerably during the run, its shape has remained constant. The traces on the bottom right show the superimposition of the averages of the habituated EPSP and the facilitated EPSP. The facilitated EPSP has a longer time to peak and a slower initial decline than the habituated EPSP. These data suggest that habituation occurs through a mechanism which is independent of presynaptic action potential duration, while in sensitization action potential duration is significantly increased.

in the duration of the action potential which increases the Ca²⁺ influx (Fig. 27). Thus, one of the interesting consequences of the slow modulatory synaptic action that produces sensitization is that it does not simply change the membrane potential. Instead, the synaptic action has a novel effect: it acts on voltage-dependent K⁺ channels to alter the ionic makeup and the configuration of the action potential, thereby altering transmitter release.

Our studies so far have indicated that presynaptic facilitation is due to a decrease in the K⁺ currents of the presynaptic neurone, but we have not delineated which of the K⁺ currents is critically involved. Using the various blocking agents we can now dissect out these currents and determine how much each current contributes to the modulation of the total outward current. Having done that, it might also be possible to specify what aspect of the current is altered (number of channels activated, channel selectivity, or rate constant of activation or inactivation).
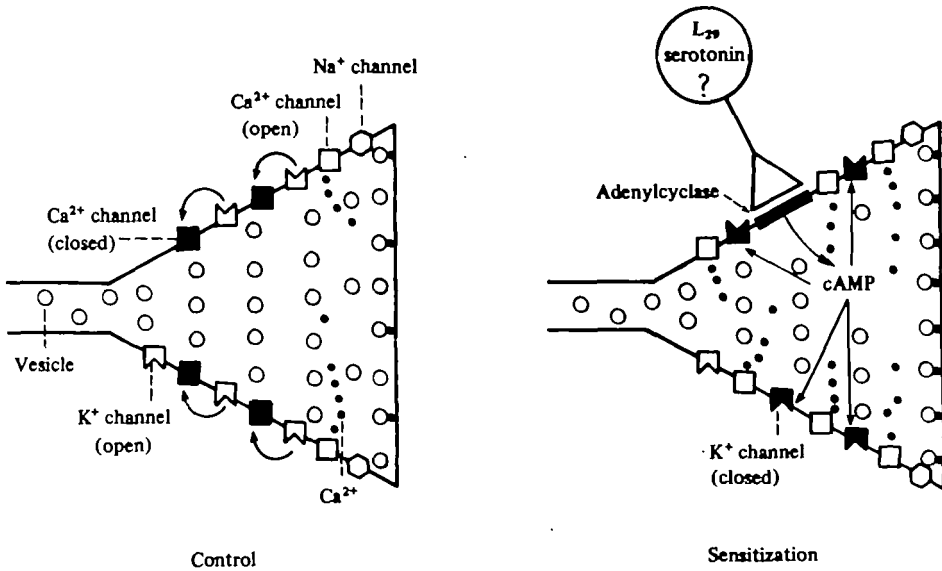
Fig. 27. Schematic model of presynaptic facilitation underlying sensitization. The presumptive presynaptic facilitating transmitter serotonin thought to be released by the facilitating neurone L29 acts on the terminals of the sensory neurones to increase the level of cAMP. The cAMP acts on K^+ channels causing a decreased K^+ current which in turn prolongs the Ca^{2+} influx into the terminal and thereby increases transmitter release. Symbols as in Fig. 8.

An overall view

The ability to analyse simultaneously the Ca^{2+} current of the presynaptic cell body and transmitter release from the terminals has allowed us to explore the alterations in Ca^{2+} current underlying a variety of plastic changes in transmitter release: the control of transmitter release by membrane potential, presynaptic inhibition, homosynaptic depression, and presynaptic facilitation. In this type of analysis most of the total voltage-clamp current is recorded across the soma membrane, which parallels the transmitter-releasing membrane area but probably does not directly contribute to release. Nevertheless, it is possible to demonstrate that these currents correlate with transmitter release. Moreover, the cell body currents examined in isolated somata where the clamp control is optimal have properties similar to the currents recorded in intact cells.

It has been known for more than a decade that the influx of Ca^{2+} into the terminals with each action potential is an essential step for transmitter release (Katz & Miledi, 1967a). Whether changes in Ca^{2+} influx are involved in the well-known capability of

Table 1

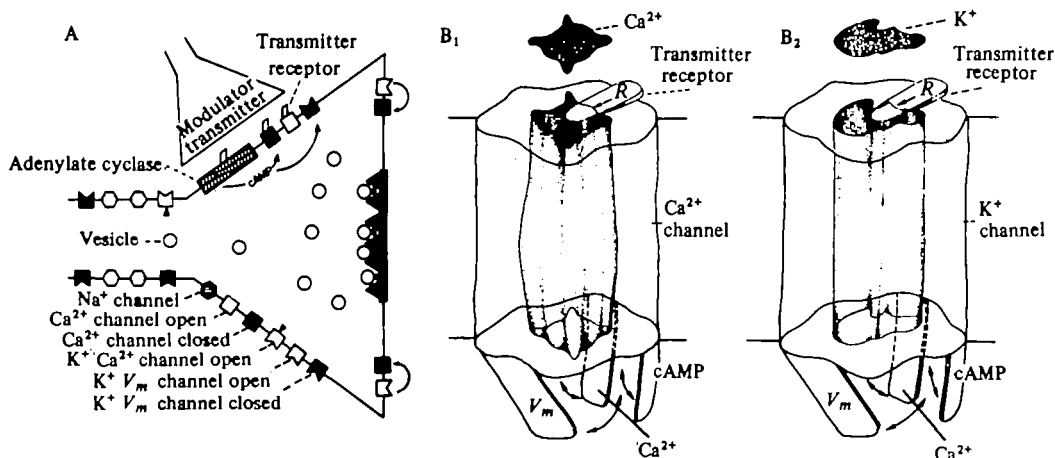| Plastic change	Ca^{2+} current	Direct (Ca^{2+} channel)	Indirect (K^+ channel)	Intrinsic	Synaptic
Presynaptic inhibition	↓	+	.	.	+
Homosynaptic depression (habituation)	↓	+	.	+	.
Heterosynaptic facilitation (sensitization)	↑	.	+	.	+
Depolarization dependent increase in transmitter release	↑	+	+	+	.

Fig. 28. Models of modulation of Ca^{2+} current.   (A) Schematic model of synaptic terminal re-
lease area, illustrating several intrinsic membrane proteins, the voltage-dependent Na^+ channels,
the voltage-dependent K^+ channels, and the Ca^{2+} channels, and the Ca^{2+}-dependent K^+ chan-
nels. Ca^{2+} current can be modulated indirectly by increases or decreases in K^+ currents, and
directly by an action directly on the Ca^{2+} channel protein (see B).   (B₁) General model of the
Ca^{2+} channel. The channel can be modulated by four regulatory signals: (i) membrane voltage,
(ii) intracellular Ca^{2+} concentration (Tillotson, 1979), (iii) second messenger (Reuter & Scholtz,
1977) and (iv) modulatory transmitters. The channel has a selectivity pore that may be a Ca^{2+}
recognition site.   (B₂) General model of the K^+ channels. Different potassium channels can be
regulated by membrane voltage and intracellular Ca^{2+} concentration, and can also be modula-
ted directly by neurotransmitters, perhaps through the action of a second messenger. (Modified
from Shapiro *et al.* 1980*b*.)

chemical synapses to undergo plastic changes in synaptic effectiveness was not known,
however. The experiments presented in this paper provide direct evidence that the
calcium influx into the terminals can be regulated in both directions, that the regulation
can be direct or indirect, and that this regulation can contribute to control of synaptic
efficacy lasting minutes to hours (Table 1). The indirect modulation of the Ca^{2+}
current is achieved through the regulation of K^+ currents that control the height and
duration of the action potential and thereby determine the time during which the
Ca^{2+} channels are activated. Direct modulation of the Ca^{2+} current is achieved, on
the other hand, perhaps through actions on the Ca^{2+} channel protein itself (Fig. 28).

Our data suggest that the ability to modulate the Ca^{2+} current *directly* derives
from an interesting characteristic of the Ca^{2+} channel protein: that it is endowed
with several regulatory sites. These regulatory sites are illustrated schematically
in Fig. 28B₁. Based on our data we can identify sites that are sensitive to at
least two different regulatory signals: (1) membrane voltage and (2) chemical trans-
mitters. Data of others studying properties of the Ca^{2+} channel in the vertebrate
heart, in paramecium, and in molluscan nerve cell bodies suggest the presence of
two other sites on the Ca^{2+} channel: those that respond to (3) cyclic nucleotide action
(Reuter & Scholtz, 1977) and those that are sensitive to (4) intracellular Ca^{2+} con-
centration (Brehm & Eckert, 1978; Tillotson, 1979). The ability to modulate the Ca^{2+}
current indirectly seems to derive from the existence of a similar set of regulatory
sites on the K^+ channel (Fig. 28B₂). In particular, both the K^+ and the Ca^{2+} channel

are capable of being affected by membrane voltage on the one hand and by chemical transmitters (and perhaps by one or more intracellular regulatory signals) on the other.

That certain ionic channels are sensitive to both transmitters and membrane voltage has now been confirmed in a number of systems. At certain synapses the voltage sensitivity of a chemically activated channel affects primarily the decay of the synaptic current; the voltage sensitivity does not alter the rate of rise or the peak current (Kordas, 1969; Anderson & Stevens, 1973). At other synapses the voltage dependence is more dramatic and can alter significantly the amplitude and the configuration of the PSP at various membrane potentials. (Pellmar & Wilson, 1977; Pellmar & Carpenter, 1979; Hartzell *et al.* 1977; Weiss *et al.* 1978). Our data suggest that voltage-gated molecules that are transmitter sensitive occur in or near the terminal region of the neurone. The sensitivity to transmitter actions of these voltage-dependent channels can lead to changes in the ionic makeup of the action potential that can exert a powerful control over transmitter release.

Thus, the existence on both the Ca^{2+} and K^+ channels of regulatory sites for chemical transmitters on the one hand and for membrane voltage on the other allows a mechanistic explanation for several different forms of long-term synaptic plasticity: presynaptic inhibition, presynaptic facilitation, homosynaptic depression, and the dependence of transmitter release on membrane potential. Although these forms of plasticity vary in their detailed mechanisms, they all share a common mode of expression – the modulation of the Ca^{2+} current. The finding of multiple controls of the Ca^{2+} current also makes the modulation of this current an attractive candidate mechanism for long-term synaptic control of the sort that might be involved in the development of synaptic connexions on the one hand and long-term learning on the other.

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