POSTSYNAPTIC POTENTIALS OF LIMITED DURATION IN VISUAL NEURONES OF A LOCUST

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SUMMARY

Large, second-order neurones of locust ocelli ('L-neurones') make both excitatory and inhibitory connections amongst each other. A single L-neurone can be presynaptic at both types of connection. At the excitatory connections, transmission can be maintained for long periods without decrement. In contrast, inhibitory postsynaptic potentials (IPSPs) never last for more than 15–35 ms. This paper examines mechanisms which could limit the duration of these IPSPs.

1. An IPSP begins 4·5 ms after a presynaptic neurone starts to depolarize from its resting potential, and the time-to-peak is 7 ms.

2. The amplitude of an IPSP depends both upon the amplitude of the peak presynaptic potential and upon the potential at which a presynaptic neurone is held before it is depolarized.

3. The rate at which a postsynaptic neurone hyperpolarizes to produce an IPSP is proportional to the rate at which the presynaptic neurone depolarizes, independent of the potential from which the presynaptic depolarization starts. A maximum rate of postsynaptic hyperpolarization is reached when the presynaptic neurone depolarizes at $10\,\mathrm{mV\,ms^{-1}}$.

4. Once an IPSP has occurred, both the amplitudes and the rates of hyperpolarization of subsequent IPSPs are depressed. The connection recovers its full ability to transmit over a period of 1·5 s. Larger IPSPs are followed by initially greater depression than smaller IPSPs.

5. A connection can begin to recover from depression while the presynaptic neurone is held depolarized from resting.

6. Transmission fails when a presynaptic neurone is depolarized by pulses shorter than 2 ms.

7. The most likely reason why the duration of the IPSPs is limited is that calcium channels in the presynaptic terminal inactivate within 7 ms of first opening.

INTRODUCTION

Large, second-order neurones of locust ocelli ('L-neurones') provide useful subjects for investigations on the transmission of signals both within a single neurone, and across synapses between neurones. Pairs of microelectrodes can be inserted into single

L-neurones to study the origin and transmission of signals within them (Wilson, 1978b), or, combined with a further microelectrode in a postsynaptic neurone, to study the operating characteristics of output connections from an L-neurone (Simmons, 1981, 1982a). Signals decrement very little throughout the length of an L-neurone (Wilson, 1978b; Simmons, 1982a), and L-neurones have very simple geometries (C. S. Goodman, 1976; L. J. Goodman, Patterson & Mobbs, 1975; Simmons, 1982a). This means that an electrode placed in the axon of an L-neurone will record fairly accurately the amplitudes and waveforms of potential changes which occur at pre- or postsynaptic sites.

The output connections which L-neurones make are of particular interest because two types of them, which have contrasting properties, have been found. A single L-neurone can make both types of output connection. L-neurones make excitatory connections with some large brain neurones which descend in the nerve cord (Simmons, 1981), and make both excitatory and inhibitory connections laterally amongst each other (Simmons, 1982a). At the excitatory connections, small, slowly changing variations in presynaptic potential are transmitted to the postsynaptic neurone, as long as the presynaptic neurone is depolarized from a threshold potential. Because the normal resting potential of an L-neurone is depolarized above the threshold for release of transmitter at the excitatory connections, small hyperpolarizations, such as those that occur when ocellar illumination is increased, are communicated across these connections (Simmons, 1981, 1982a). Transmission at the excitatory connections can be sustained for long periods without decrement. In contrast, at the inhibitory connections which L-neurones make amongst each other, the postsynaptic potential (PSP) has a duration limited to less than 35 ms, no matter for how long the presynaptic terminal is held depolarized (Simmons, 1982a). Two of the possible mechanisms which would limit the duration of these PSPs are that presynaptic transmitter becomes depleted, or that postsynaptic receptors desensitize. If either of these processes occurred rapidly, then slow changes in presynaptic potential would not be transmitted across the inhibitory connections, and fast depolarizing potentials, such as spikes, would be required to effect transmission. L-neurones produce single spikes, or, rarely, short bursts of spikes when illumination is reduced (Chappell & Dowling, 1972; Patterson & Goodman, 1974; Wilson, 1978a) or when a pulse of hyperpolarizing current injected into an L-neurone ends (Wilson, 1978b). The amplitude of a spike in an L-neurone depends on both the amplitude and duration of the preceding hyperpolarization (Simmons, 1982a) and L-neurones do not produce trains of spikes when depolarized. In this paper it is shown that neither transmitter depletion, nor desensitization of postsynaptic receptors is likely to occur at the inhibitory connections among L-neurones. Instead, transmission may be limited in duration by properties of calcium channels in the presynaptic membrane. The importance of rapid depolarization, such as a spike provides, in effective transmission at these connections is demonstrated.

METHODS

Experiments were performed on *Schistocerca gregaria* (Forskål), taken from culture at least 4 days after their final moult. Mouthparts were removed, the wounds were

sealed with wax, and the locust was fixed upright on a plasticene block. The lateral ocellar nerves and the dorsal surface of the brain were exposed. The head was stabilized against movement by inserting pins through the genae into the plasticene. A constant supply of saline (Eibl, 1978) bathed the brain. The tracheae of the brain were not disturbed, and ventilation was provided by the animal's own continual pumping movements. The laboratory was darkened as much as possible during an experiment. The temperature of the preparation was 18–22 °C.

In most preparations, to ease the passage of electrodes through the connective tissue sheath of the ocellar nerves and brain, a 1 % solution of protease in saline was applied for 2 min. Routinely, up to three independent glass microelectrodes were employed. They were filled with $2 \, mol \, l^{-1}$ potassium acetate and had d.c. resistances of 40–80 MΩ. Each electrode was connected to a d.c. amplifier which incorporated a bridge circuit, allowing current to be injected through the electrode. Current was measured by using a virtual ground amplifier, which measured current flowing along the chloridized silver wire which was employed as the indifferent electrode. This electrode lay along the inside of the thorax, with its free end placed as close as possible to the brain. Results were recorded with an FM tape recorder and were analysed later with the aid of a North Star Horizon microcomputer, fitted with a Comart 7A + D analogue/digital converting card. Pre-triggered displays on the oscilloscope screen and signal averaging were achieved by use of programmes written in Pascal M/T (Simmons, 1985). The figures in this paper were drawn by an X-Y plotter, driven by the microcomputer.

L-neurones were identified as such by the waveform of their responses to light, delivered from a green light emitting diode (for justification of this method see Simmons, 1982a). Particular care was taken to ensure that the neurones were not damaged by penetration with microelectrodes. A number of observations were used to check for damage. The most direct was to observe any change in membrane potential recorded by one electrode as a second was inserted into the same neurone. A second method was to monitor the responses of L-neurones to pulses of light, delivered at frequent intervals to the ocellus from a green light emitting diode. In the dim light in which the experiments were conducted, undamaged L-neurones showed a constant barrage of distinct hyperpolarizing potentials, at least some of which probably corresponded to the capture of single photons by photoreceptors (Wilson, 1978c). Changes in the amplitude of these proved to be a sensitive and convenient measure of the health of a neurone. In most experiments, at least one pair of L-neurones connected by an inhibitory connection was found. Observations of IPSPs have been made in over 150 locusts, and in about half of these, two electrodes were inserted into the presynaptic neurone while a third recorded postsynaptic potential. Measurements were made from about one in three of these latter experiments. In the remaining experiments, measurements were not made for reasons which included: damage to neurones, small amplitudes of IPSPs relative to other postsynaptic events, and unacceptable coupling artefacts between electrodes. Where graphs were constructed, measurements were made from single sweeps and were later averaged. All the observations reported in this paper were made in at least three different preparations, with the exception of that in Fig. 6D, which was repeated twice.
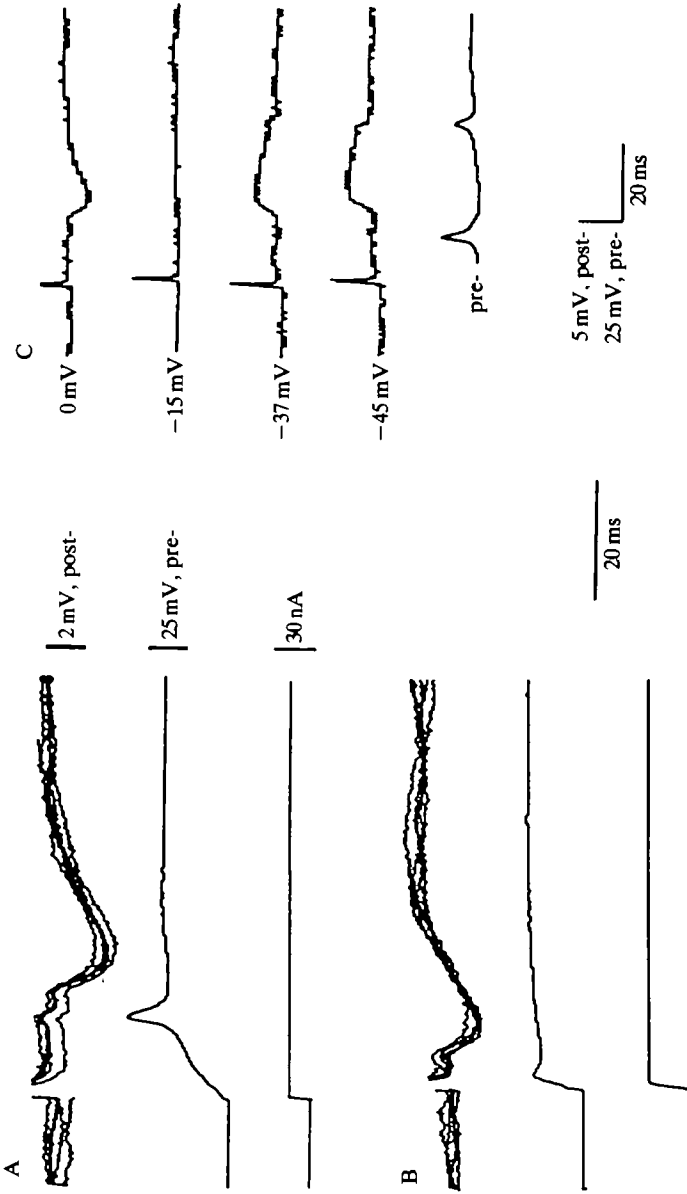
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Fig. 1. (A,B) IPSPs mediated by spikes and by longer depolarizing potentials in a presynaptic neurone. Traces are: top, five superimposed sweeps showing IPSPs; second, presynaptic potential; bottom, current injected into the presynaptic neurone through a separate electrode. In A, the spike was triggered at the end of a pulse of hyperpolarizing current, 300 ms long, injected into the presynaptic neurone. In B, the pulse of current depolarized the presynaptic neurone from its normal resting potential. The apparent depolarization in postsynaptic membrane potential, when the ends of these traces are compared with the beginnings, is due to d.c. coupling between microelectrodes, the extent of which varied from preparation to preparation. In addition, a transient coupling artefact always accompanied a change in current injection. (C) Two electrodes were inserted into the postsynaptic neurone, one to inject hyperpolarizing current and the other to record potential. A single electrode in the presynaptic neurone was used both to inject hyperpolarizing current pulses and to record spikes. When the postsynaptic neurone was hyperpolarized to 15 mV from resting, no IPSP was recorded, and the IPSP became depolarizing when the postsynaptic neurone was hyperpolarized further. The potential of the postsynaptic neurone relative to resting is indicated with each trace (the traces are not aligned precisely with these holding potentials).

The time course of the inhibitory postsynaptic potential

The inhibitory postsynaptic potential (IPSP) at a connection between two L-neurones was examined by producing in the presynaptic neurone either a spike (Fig. 1A) or a more long-lasting depolarization (Fig. 1B). A spike was produced by switching off a hyperpolarizing current and lasted 4 ms, measured between departure from and return to resting potential (Fig. 1A). Resting potential was -30 to -35 mV (Simmons, 1981). To produce an IPSP by long depolarization, the presynaptic neurone was first held hyperpolarized by 4 mV, before a depolarization lasting 200 ms (Fig. 1B)

Latency was measured from the time the presynaptic neurone depolarized from resting potential, and was always 4·5 ms. Time-to-peak was 7 ms. Duration was 10–35 ms, varying with the amplitude of the IPSP, probably as a function of the membrane time constant.

Pulses of current injected to depolarize an L-neurone from resting were never observed to produce discrete spikes. However, such pulses did appear to activate regenerative channels in L-neurones, as the rate of potential change of an L-neurone was greater when the neurone was depolarized from resting than when it was repolarized back to resting potential. In addition, following the initial peak depolarization, there was usually a slight hyperpolarization in the membrane potential of an L-neurone, before it climbed again to a more sustained plateau depolarization, e.g. Fig. 1B. In 20 experiments, the potential was recorded at two different locations of an L-neurone while current was injected through a third electrode. The response recorded in the ocellar neuropile had a very similar waveform to that recorded in the axon in the brain. Measurements from five of these experiments gave values for the space constant of an L-neurone of 2·5 to 3·5 times the length of one of the lateral L-neurones (Goodman, 1976; Simmons, 1982*a*). In contrast to the statement in the summary of Wilson's (1978*b*) paper, spikes were conducted with little attenuation along L-neurone axons (some of Wilson's paired recordings may have been from two L-neurones linked by an excitatory connection rather than from a single axon; see also Simmons, 1982*a*). All recordings in this paper were made from L-neurone axons within 200 μm of the junction of the ocellar nerve with the brain. Because of the simple geometry (C.S. Goodman, 1976; L. J. Goodman *et al.* 1975; Simmons, 1982*b*) and the large space constants of L-neurones, the potentials recorded from this location were probably at least 70 % of the value of the potentials occurring at any other location in an L-neurone, except for the cell body and its neurite. In ultrastructural studies, synapses have been found between large axons in ocellar nerves near to the brain (Goodman, Mobbs & Guy, 1977; Koontz & Edwards, 1984), although a detailed description of the distribution of synapses made by an L-neurone has not been made.

An IPSP could be reduced in amplitude, and then reversed in polarity, by injecting progressively greater amounts of hyperpolarizing current into the postsynaptic neurone (Fig. 1C). This is to be expected if the IPSP is generated by an increase in postsynaptic conductance to one or more species of ion. There was no indication of any late excitatory PSP (EPSP), which might be invisible at the normal postsynaptic
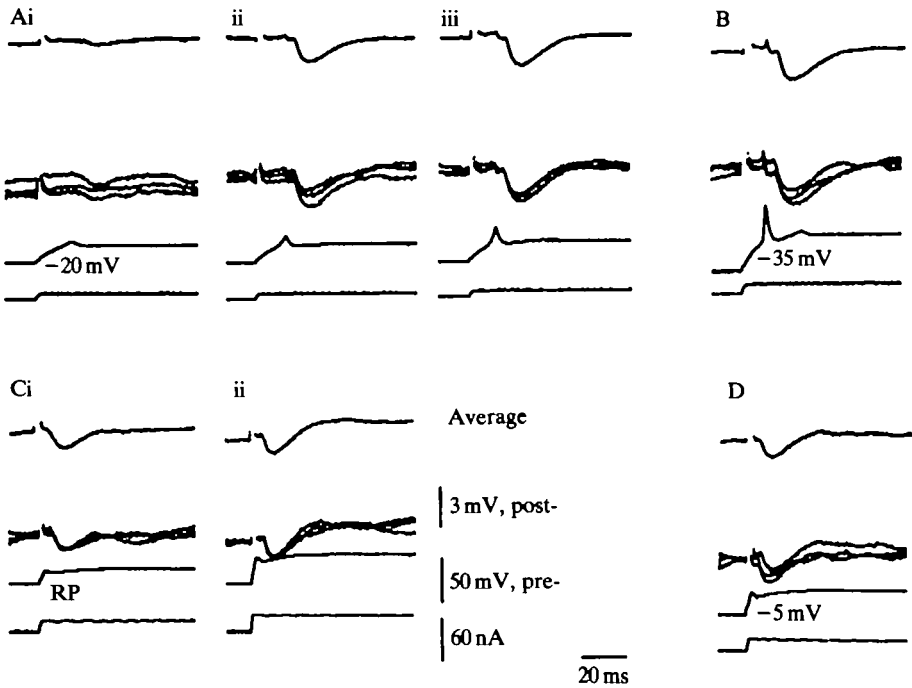
Fig. 2

resting potential, but which could terminate an IPSP and limit its duration. No sign of such an EPSP was seen, whether the IPSP was mediated by a presynaptic spike or by a more prolonged presynaptic depolarization. The time taken for an IPSP to reach its peak amplitude was unaffected by the membrane potential at which the postsynaptic membrane was held, but as the neurone was hyperpolarized by progressively greater amounts, the total duration of the IPSP increased. This was probably due to

the increase in PSP amplitude and to an increase in membrane resistance (Wilson, 1978*b*). The decay of an IPSP had a similar waveform and duration to the decay in potential at the end of a small pulse of hyperpolarizing current injected through an electrode into an L-neurone. In six experiments, the conductance of a postsynaptic neurone was measured both before and 40 ms following the start of an IPSP by injecting a 5 ms current pulse into the neurone. No conductance change following an IPSP was found.

The relationship between pre- and postsynaptic potentials

The amplitude of the IPSP was influenced by the potential at which the presynaptic neurone was held before depolarizing current was injected into it as well as by the peak of the presynaptic depolarizing potential (Fig. 2). In Fig. 2A, spikes were initiated in the presynaptic neurone by terminating pulses of hyperpolarizing current. These pulses hyperpolarized the neurone by 20 mV from its resting potential, and spikes of different amplitudes were produced by varying the duration of the hyperpolarizing pulses (Simmons, 1982*a*). For spikes reaching between 5 and 10 mV above resting potential, each 1-mV increase in spike amplitude caused an increment of 0·2 mV in IPSP amplitude (open circles, Fig. 2E). For larger spikes, the proportionate increase in IPSP amplitude fell off until, for spikes of 25 mV and greater, the IPSP reached its maximum amplitude of 2 mV. In Fig. 2C depolarizing pulses of current were injected to depolarize the presynaptic neurone from its resting potential. These presynaptic potentials (filled circles, Fig. 2E) produced smaller IPSPs than did presynaptic spikes reaching equivalent potentials from resting (open circles, Fig. 2E). For the depolarizations from resting, each 1-mV increase in peak depolarization of the presynaptic neurone caused an increment of 0·1 mV in IPSP amplitude, between presynaptic potentials of 10 and 20 mV. Fig. 2D and the open squares in Fig. 2E show IPSPs produced when the presynaptic neurone was hyperpolarized 5 mV from resting potential between depolarizing pulses. These IPSPs were intermediate in amplitude between those produced by spikes and those produced when the presynaptic potential returned to resting between pulses.

There was considerable variation in the maximum amplitudes of IPSPs which could be elicited in different preparations, although a detailed statistical analysis was not made. A few IPSPs reached 10 mV in amplitude, but most studies were made of IPSPs between 2 and 3 mV high. Often smaller IPSPs were recorded,

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Fig. 2. The relationship between pre- and postsynaptic potentials. In A–D the traces are: top, the average from ten repetitions of an IPSP; second, the first three IPSPs from which the average was derived are superimposed; third, presynaptic potential; bottom, current that was injected presynaptically through a separate electrode. In A, each spike was initiated at the end of a pulse of hyperpolarizing current. Each current pulse hyperpolarized the presynaptic neurone by 20 mV, and spike amplitude was altered by varying the duration of the hyperpolarizing pulses. The lengths of the hyperpolarizing pulses had been: (i) 40 ms, (ii) 60 ms and (iii) 150 ms. In B, each current pulse (300 ms long) hyperpolarized the presynaptic neurone by 35 mV, and produced a spike which rose 35 mV from resting potential. In C, the presynaptic neurone was initially at its resting potential, and pulses of current were injected to depolarize it from this. D shows a pulse which depolarized the presynaptic neurone from an initial potential of − 5 mV. In E, the initial peak presynaptic potential caused by each depolarizing pulse of current is plotted against IPSP amplitude. The presynaptic potential is measured relative to resting potential (RP). Each point is plotted from ten repetitions. Starting potential: ○, − 20 mV; □, − 5 mV; ●, resting potential.

Ai

ii   Post-

                                                        |2 mV

                                                        |25 mV

                                      Pre-

                                      ___
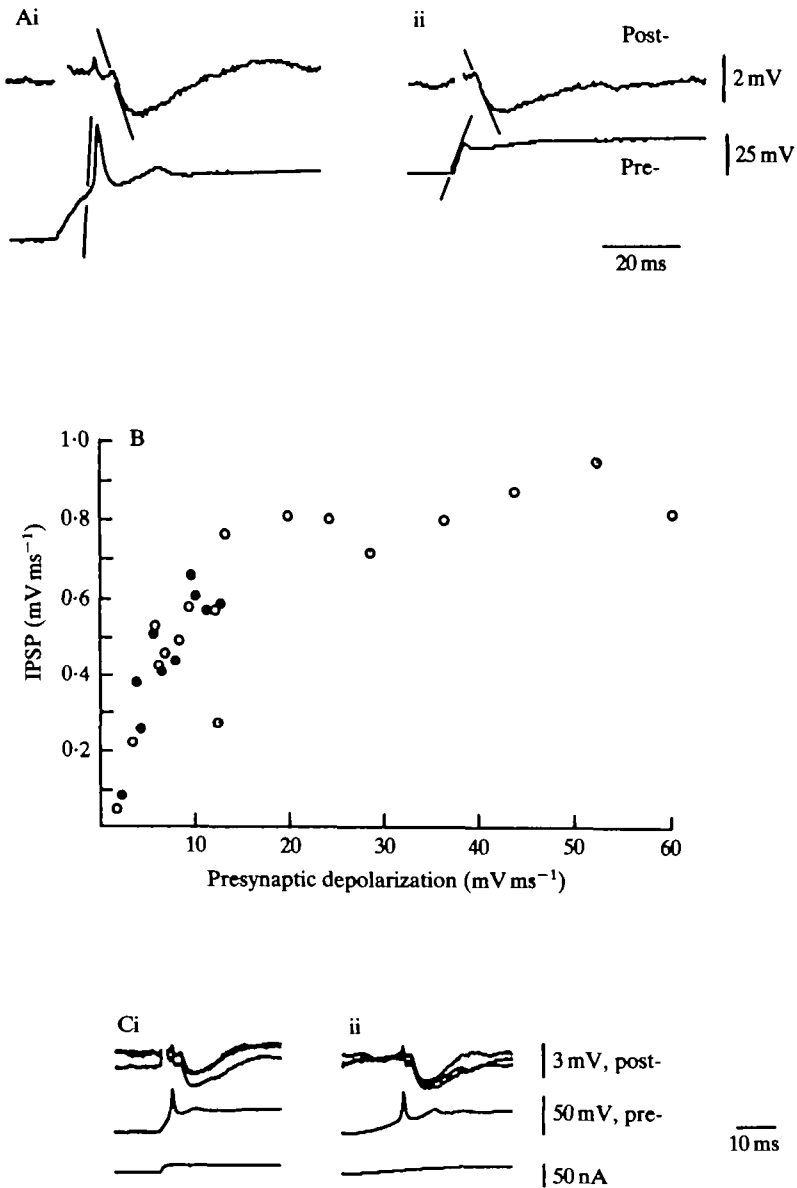   20 ms

B

Fig. 3. Relationship between the rates of change in presynaptic and in postsynaptic potentials. In Ai, the spike and the IPSP it mediated are from Fig. 2A, and in Fig. Aii, the presynaptic depolarizing pulse and the IPSP it mediated, are from Fig. 2C. The lines drawn on each trace show the gradients that were measured. The small initial rapid phase of depolarization is probably an artefact due to coupling between electrodes. (B) A plot of the rate of presynaptic depolarization against rate of postsynaptic hyperpolarization, taken from the same experiment; O, spikes; ●, depolarizing pulses. In C, which was from a different experiment, the presynaptic neurone was initially hyperpolarized 12 mV from its resting potential, and a ramp of current depolarized it above resting. The rates of current change were different in i and ii. In each, the presynaptic neurone spiked, and spikes mediated IPSPs. Three examples of the IPSPs are superimposed in each record.

(Figure B: scatter plot with y-axis "IPSP (mV ms⁻¹)" marked 0·2, 0·4, 0·6, 0·8, 1·0 and x-axis "Presynaptic depolarization (mV ms⁻¹)" marked 10, 20, 30, 40, 50, 60)

Ci   ii

                        |3 mV, post-

                        |50 mV, pre-
                                      ___
                                      10 ms
                        |50 nA

but measurements on these were difficult as they are about the same amplitude as fluctuations in potential caused by the random arrival of photons at photoreceptors (Wilson, 1978*c*).

The relationship between rates of change in pre-and postsynaptic potentials

The reason why the starting potential of the presynaptic neurone influences IPSP amplitude became clear when the rates of change of pre- and postsynaptic potentials were measured. For the experiment shown in Fig. 2, the rates of potential change in the two neurones were plotted (Fig. 3A), and the rates of presynaptic depolarization were plotted against rates of postsynaptic hyperpolarization both for presynaptic spikes and for more long lasting depolarizations (Fig. 3B). The rate of postsynaptic hyperpolarization, causing an IPSP, depended on the rate of presynaptic depolarization, and was independent of the initial potential of the presynaptic neurone. When a pulse of depolarizing current was injected into an L-neurone, the neurone depolarized more rapidly if it was initially hyperpolarized than if it was initially at resting potential. In the plot of Fig. 3B, for each $1\,mV\,ms^{-1}$ increase in the rate of presynaptic depolarization up to a rate of $10\,mV\,ms^{-1}$, the rate of postsynaptic hyperpolarization increased by $0.08\,mV\,ms^{-1}$. For more rapid presynaptic depolarizations, which were achieved only by spikes, there was little or no increase in the rate of postsynaptic hyperpolarization beyond a maximum of $0.8\,mV\,ms^{-1}$. The maximum recorded rate of hyperpolarization during an IPSP was $3\,mV\,ms^{-1}$, and the amplitude of an IPSP was linearly proportional to its rate of rise. In a total of five experiments of this type, similar results were obtained. The rate of presynaptic depolarization at which the maximum rate of postsynaptic hyperpolarization was achieved was always within $2\,mV\,ms^{-1}$ of $10\,mV\,ms^{-1}$. The rate of postsynaptic hyperpolarization, as well as the maximum amplitude of an IPSP, varied from experiment to experiment.

When gradual ramps of current were injected into a presynaptic neurone to depolarize it from an initially hyperpolarized potential, the neurone usually spiked and the spike mediated an IPSP (Fig. 3C). Judged from the inflection in the rate of depolarization, spikes were always initiated as the membrane potential was depolarized to resting, no matter how rapid the depolarization. Attempts to determine the minimum rate of presynaptic depolarization that would mediate an IPSP were thwarted by the tendency of L-neurones to spike. Ramps of current which produced depolarizations at less than $1\,mV\,ms^{-1}$ produced neither spikes nor detectable IPSPs.

Recovery of the ability of inhibitory connections to transmit

When one IPSP had been produced, the ability of an inhibitory connection to transmit was depressed, and full recovery took about $1.5\,s$. Larger spikes caused a more pronounced depression (Fig. 4A) than did smaller spikes (Fig. 4B). Separate pulse generators and current sources were used to trigger the two spikes of a pair in order to ensure that the two spikes were of equal amplitude. Similar results were obtained in two experiments where pulses of depolarizing current were injected into the presynaptic neurone (not shown). Depression could be expressed as the amplitude of a second IPSP relative to the first (Fig. 4C) and the results were essentially similar to those obtained by plotting reduction in the rate of hyperpolarization which produced an IPSP (not shown).
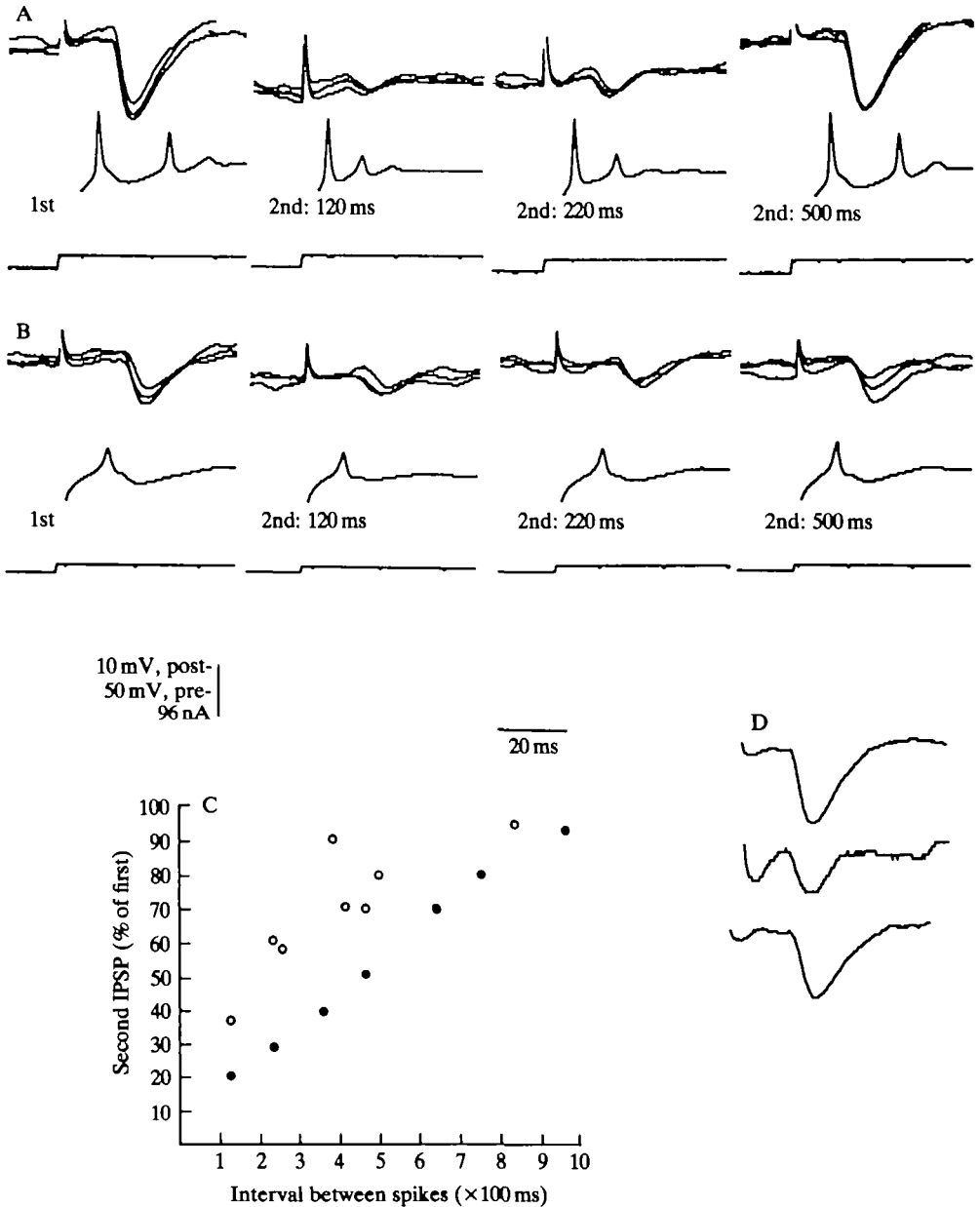
Fig. 4

Both the latency of an IPSP, and the time taken for it to reach its peak hyper-polarization, were the same for first or second IPSPs of a pair, or for IPSPs following large or small spikes. This is illustrated in Fig. 4D, where the traces are aligned with each other according to the times of the presynaptic spikes.

A connection could recover most of its ability to transmit while the presynaptic neurone was held depolarized from resting potential (Fig. 5). In Fig. 5A, pairs of spikes, separated by 100 ms, were produced by delivering pairs of hyperpolarizing

current pulses to the presynaptic neurone. The first spike of each pair produced an IPSP 2·2 mV in amplitude, and the second spike produced an IPSP of 0·8 mV, or 36 % of the amplitude of the first IPSP. In Fig. 5B, pairs of depolarizing pulses were injected into the presynaptic neurone. In each pair, the first pulse lasted for 48 ms, and the start of the second pulse occurred 100 ms after the start of the first pulse. The IPSP caused by each first pulse was 1·9 mV in amplitude, and the amplitude of the IPSP caused by each second pulse was 0·7 mV, or 37 % of the amplitude of the first IPSP. Therefore, the connection was recovering some of its ability to transmit before the presynaptic potential returned to resting at the end of the first pulse. The depolarizing pulses caused no more long-lasting depression than did single spikes. Similar results were obtained with much longer lasting pulses of depolarizing current injected into the presynaptic neurone (not shown). However, in three experiments, there was a relatively short interval between the end of the first pulse and the start of the second, and the second IPSP was usually reduced or absent. The reduction in IPSP amplitude was accompanied by a change in the shape of the initial depolarizing potential in the presynaptic neurone, which indicates that the short interval between pulses probably provided insufficient time for the conductance of the presynaptic membrane to return to its resting value. For example, in Fig. 5C, the interval between the start of the first and second pulses of each pair was 450 ms. The first pulse of each pair (Fig. 5Ci) produced an IPSP 1·4 mV in amplitude. IPSPs produced by the second pulses, when the duration of the first pulse was 390 ms, had amplitudes of 1·1 mV, or 79 % of the amplitudes of the first IPSPs (Fig. 5Cii). When the first pulse was lengthened to 410 ms, no IPSP was detected following each second pulse (Fig. 5Ciii). Here, the rate of depolarization of the presynaptic neurone at the start of the second pulse was reduced, as was the amplitude of the hyperpolarization which followed the initial peak depolarizing potential.

PSPs following short depolarizing pulses in presynaptic neurones

As pulses of current injected presynaptically were reduced to a length of 4 ms and less, both the IPSPs and EPSPs they mediated became smaller (Fig. 6). The pulse was 50 ms long in Fig. 6Ai, raising the presynaptic potential by 4 mV, and mediated and IPSP of 2·5mV. In Fig. 6Aii, the presynaptic pulse reached an amplitude of 4 mV

Fig. 4. Following an IPSP, the ability of a connection to transmit is depressed and gradually recovers. Single microelectrodes were inserted into each neurone here. Spikes were initiated in the presynaptic neurone when pulses of hyperpolarizing current ended. In each recording in A and B, three superimposed IPSPs are shown for each spike. Pairs of spikes were produced, and an example of the first spike of a pair, together with IPSPs it mediated, is shown on the left of each sequence of recordings. The remaining recordings in each line show second spikes, and examples of the IPSPs they mediated. The interval between the first and second spikes of a pair is indicated. Three seconds separated the second spike of one pair and the first spike of the next pair. In A, the spikes peaked at 50 mV from resting potential, and in B the spikes peaked at 17 mV from resting potential. In C the amplitude of the second IPSP, expressed as a percentage of the amplitude of the first IPSP of a pair, is plotted against the interval between the two spikes which mediated the IPSPs. Each point is plotted as the average of ten repetitions of each pair; ○, smaller spikes; ●, larger spikes. In D, three IPSPs, at various voltage gains, are shown in order to illustrate latency and time to reach peak. The IPSPs are aligned according to the times of the spikes that mediated them (not shown in D). The top IPSP was produced by a large 1st spike, from A; the middle IPSP is also from A, and is the 2nd IPSP of a pair, mediated by large spikes separated by 120 ms; and the bottom IPSP is the 1st IPSP mediated by a smaller spike, from B.
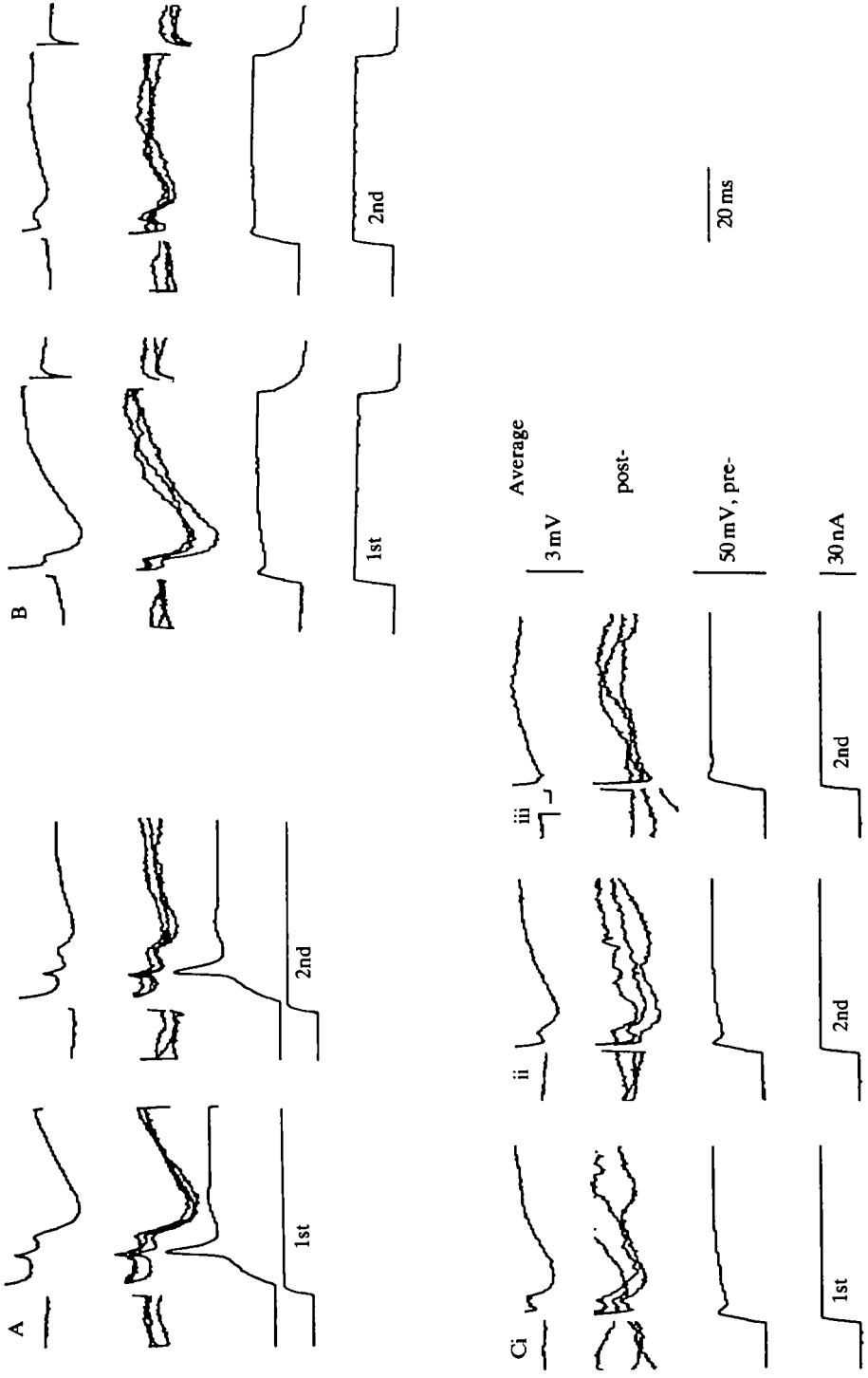
Fig. 5

for 3 ms, and mediated an IPSP of 1·5 mV. With a shorter pulse (Fig. 6Aiii) the presynaptic neurone was depolarized to a maximum of 2 mV for 0·7 ms. There was no detectable IPSP following this depolarization.

Further evidence that presynaptic depolarizations shorter than 4 ms are less effective than longer pulses at mediating transmission was provided by examining the recovery of connections from depression. When two presynaptic stimuli, each longer than 4 ms, were separated by 100 ms, the amplitude of the second IPSP was reduced to 20–35 % of its value in a non-depressed connection (Fig. 4C). The reduction could be expected to be less if transmission during the first IPSP were to be reduced. This was examined by following each of the presynaptic pulses in Fig. 6A, after 100 ms, by a 50-ms test pulse (Fig. 6C). The amplitudes of the IPSPs elicited by these test pulses were compared with those of IPSPs elicited by single presynaptic pulses of the same amplitude and duration (Fig. 6B). The presynaptic pulses in Fig. 6B reached an amplitude of 12 mV, and elicited IPSPs of amplitude 4·5 mV. The ability of the electrode to depolarize the neurone declined during the last part of the experiment (Fig. 6Ciii), but this did not affect the results adversely. Following a first pulse 50 ms long (Fig. 6Ai), the test IPSP (Fig. 6Ci) had an amplitude of 2·5 mV, or 55 % of the amplitude of the IPSP in Fig. 6B. As the length of the first pulse decreased (Fig. 6Aii, iii) the amplitudes of the test IPSPs (Fig. 6Cii, iii) increased to 90 % and 100 % of the amplitude of the control IPSP in Fig. 6B. The IPSPs which followed presynaptic pulses 5 ms long had the same amplitude as those which followed longer presynaptic pulses, and 5-ms presynaptic pulses caused the same decrement in test IPSPs as did longer pulses.

Reduction in efficiency of transmission as presynaptic pulses were reduced in length was also observed at excitatory connections between L-neurones. In all three recordings of Fig. 6D current pulses depolarized the presynaptic neurone to 15 mV from resting. In Fig. 6D the pulse lasted for 50 ms, and the postsynaptic neurone depolarized to 2 mV above resting. The peak presynaptic depolarization of 15 mV lasted for 2·5 ms in Fig. 6D, and the postsynaptic potential reached 1 mV above resting. For a shorter depolarizing pulse, where the presynaptic membrane returned towards resting as soon as the peak depolarization was arrived at, the postsynaptic potential had an amplitude of 0·4 mV (Fig. 6Diii).

Fig. 5. Comparison of depression caused by spikes with depression following more long-lasting presynaptic depolarizing potentials. A and B are from one experiment, and C is from a different one. In each recording the traces are: top, an averaged IPSP, derived from ten repetitions; second, the first three IPSPs used in deriving the average are superimposed; third, presynaptic potential; bottom, current that was injected presynaptically through a separate electrode. (A) Recordings when a pair of presynaptic spikes were separated by 100 ms. (B) The presynaptic neurone was initially at its resting potential, and the IPSPs were produced by pulses of depolarizing current injected presynaptically. The starts of the 1st and 2nd depolarizing pulses were separated by 100 ms. Although the 1st pulse lasted for 48 ms, the 2nd IPSP was no smaller than the 2nd IPSP in Fig. 5A. (C) 1st and 2nd IPSPs were separated by 450 ms. 1st IPSPs are shown in Ci, and 2nd IPSPs in Cii and Ciii. For Cii, the length of the 1st presynaptic stimulus had been 390 ms; and for Ciii, the length of the presynaptic stimulus had been 410 ms. Note that the rate of presynaptic depolarization was less in Ciii than in Cii, and that the brief hyperpolarization which followed the initial peak was also reduced.
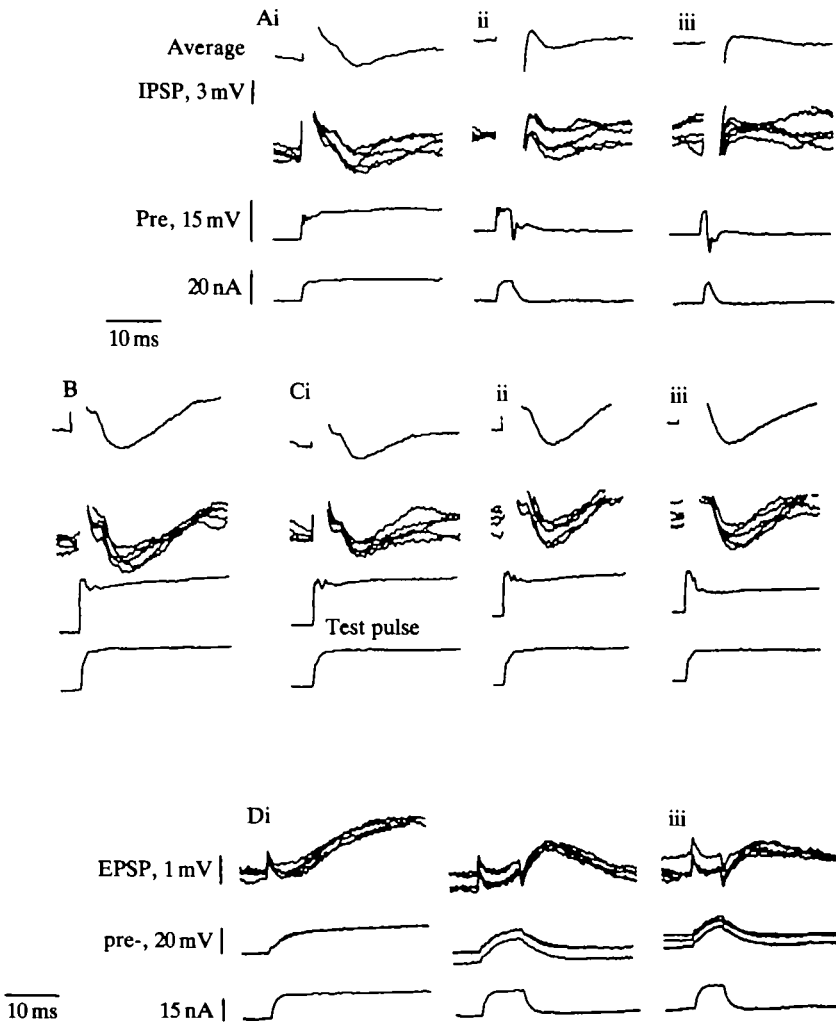
Fig. 6. IPSPs and EPSPs mediated by short depolarizing pulses injected into the presynaptic neurone. Separate electrodes injected current into and recorded potential from the presynaptic neurone. An inhibitory connection is shown in Fig. 6A–C. The traces are: top, IPSP averaged from ten sweeps; second, five single IPSPs superimposed; third, presynaptic potential; bottom, current which was injected presynaptically. Coupling artefacts on the IPSP recordings are particularly marked in these recordings. The presynaptic neurone was hyperpolarized by 7 mV from resting between depolarizing pulses, in order to produce a reasonably rapid rate of depolarization, and so to increase IPSP size. (A) Progressively shorter pulses of depolarizing current were injected into the presynaptic neurone, as described in the text. The size of a presynaptic potential was ultimately limited by the time constants of the membrane and electrode. (B) 50-ms depolarizing pulses were injected into the presynaptic neurone, and depolarized it by 12 mV from resting potential. Similar pulses were injected presynaptically in C in order to test whether the pulses in A had caused any depression, as described in the text. The start of a test pulse (Ci, ii, iii) followed the start of the corresponding first pulse (Ai, ii, iii) by 100 ms. (D) Progressively shorter stimuli were delivered to the presynaptic neurone at an excitatory connection. Traces are: top, five EPSPs superimposed; middle, presynaptic potential; bottom, current injected presynaptically. The presynaptic neurone was at its resting potential between stimuli.

DISCUSSION

Possible mechanisms for limiting the duration of a PSP are: (1) that stores of transmitter in the presynaptic neurone become depleted; (2) that postsynaptic receptors for the transmitter desensitize; (3) that the PSP is followed by a second PSP of opposite sign, curtailing it; and (4) that prolonged entry of calcium ions into the presynaptic sites is impossible, for reasons discussed below. At the inhibitory connections between L-neurones the first three mechanisms can be discounted. It is unlikely that stores of transmitter become depleted at presynaptic sites of L-neurones because if a presynaptic L-neurone remains depolarized for some time following an IPSP, the connection starts to recover its ability to transmit during this depolarization (Fig. 5). If transmitter stores did become rapidly depleted, the connection would not start to recover its ability to transmit until the end of the depolarizing pulse. Transmitter depletion has been demonstrated at a synapse between large neurones in the hatchet-fish (Highstein & Bennett, 1975), when fairly intense trains of spikes are elicited presynaptically, and depletion may occur in some circumstances at synapses between giant neurones in the squid (Charlton, Smith & Zucker, 1982). However, there is no instance where depletion has been demonstrated to occur when naturally-occurring bursts of spikes invade presynaptic sites, and it has not been shown to occur at any synapse where the presynaptic neurone operates without producing trains of spikes. In some synapses, a spike is thought to cause the release of a single vesicle of transmitter from a presynaptic bouton (Korn, Mallet, Triller & Faber, 1982; Neale *et al.* 1983), and when such small stores are held ready for release at presynaptic terminals, depletion could play a role in shaping PSPs. The experiments to study the recovery from depression following long presynaptic depolarizing pulses (Fig. 5) also show that desensitization of postsynaptic receptors does not play an important role in limiting the duration of these IPSPs. If desensitization were important, transmitter would have to be released throughout the duration of a presynaptic depolarization, and the receptors would not begin to resensitize until the pulse ended. The possibility that the postsynaptic receptors would desensitize if exposed to transmitter for some time cannot be excluded, since the presynaptic neurone may not be able to release transmitter continually. A way to test for densitization would be to apply the transmitter directly to the postsynaptic membrane, as has been done at a neuromuscular junction in a frog (Katz & Thesleff, 1957), and with some neurones in *Aplysia* (Gardner & Kandel, 1977). The time courses both of desensitization and of resensitization in these two examples are considerably slower than the time courses of decrement and recovery of transmission at the inhibitory connections between L-neurones.

No evidence for a PSP which follows and curtails an IPSP has been found in the connections between L-neurones. Such a PSP would be expected to have a time course to match the recovery of a connection from depression, which occurs over a period of several hundred milliseconds (Fig. 4). A late PSP could have a reversal potential around the resting potential of the neurone, and curtail the IPSP by short-circuiting the membrane. Neither hyperpolarizing of the postsynaptic membrane (Fig. 1C) nor depolarization ever revealed a PSP which followed an IPSP. Also, no PSP was revealed by measurements of L-neurone conductance following an IPSP, and it has been shown previously (Simmons, 1982*a*) that an IPSP mediated by one

connection does not influence the amplitude of an IPSP mediated by a different connection onto the same postsynaptic L-neurone. Mixed synapses, where one type of PSP is followed by a second, both PSPs mediated by the same presynaptic neurone, have been reported for nudibranch molluscs (e.g. Wachtel & Kandel, 1967; Getting, 1981).

One mechanism which could reduce or eliminate prolonged entry of calcium ions into a presynaptic neurone is closure of the calcium channels by hyperpolarization of the membrane. This hyperpolarization could be caused by an increase in potassium conductance triggered by a depolarizing potential, or by synaptic feedback onto the L-neurone. Following a rapid depolarization, an L-neurone does produce a hyper-polarizing potential (Fig. 1A,B). However, this hyperpolarization is relatively small throughout the length of an L-neurone, and it is much less prolonged than the depression in the ability of an inhibitory connection to transmit following the produc-tion of an IPSP (Fig. 4). After a spike, changes in L-neurone conductance can persist for several tens of milliseconds, and for this reason care was taken to ensure that the two spikes were of equal amplitude, when pairs of spikes were produced in studying the recovery of the ability of a connection to transmit (Fig. 4). No difference in the duration of two spikes of such pairs was found, as would be expected if a long-lasting increase in potassium conductance was reducing transmitter release caused by the second spike. Because a good correlation between rates of pre- and postsynaptic potential change during transmission has been observed (Fig. 3B), and because changes in the shape of a presynaptic depolarization can be correlated with changes in IPSP amplitude (Fig. 5C), it is likely that any important hyperpolarizing potential that did occur at the presynaptic sites would have been recorded. Synaptic feedback onto the presynaptic neurone is unlikely because, if it occurred, a connection would not start to recover its ability to transmit until the end of a long depolarizing pulse applied to the presynaptic neurone. These inhibitory connections do start to recover their ability to transmit while the presynaptic neurone is depolarized from resting (Fig. 5).

The most likely explanation for the limited duration of the IPSPs described here is that calcium channels at the presynaptic sites inactivate after a few milliseconds, limiting the time for which transmitter can be released. At a synapse between two giant neurones in the stellate ganglion of the squid, it has been shown that the release of transmitter from the presynaptic neurone is proportional to the calcium current which enters it during a spike (Llinás, Steinberg & Walton, 1981; Llinás, Sugimori & Simon, 1982). When long depolarizing currents are injected into presynaptic termi-nals of the squid preparation, no reduction in calcium current is recorded (Llinás et al. 1981). In contrast, in a skin sensory neurone of Aplysia, repeated spikes cause progressive reduction in the calcium current which enters the neurone during each spike, and this reduction in calcium current is also found in isolated cell bodies when potassium and sodium currents have been blocked (Klein & Kandel, 1980). Patch clamping of single calcium channels has been performed in some snail neurones (Lux & Nagy, 1981; Lux & Brown, 1984a,b) and some of these channels inactivate (Lux & Brown, 1984b), but it is unknown whether these are the same calcium channels which mediate release of transmitter from these neurones. It is, therefore, possible that the calcium channels in some presynaptic sites inactivate, in a similar way to the

inactivation of voltage-gated sodium channels in the membrane of squid giant axons (Hodgkin & Huxley, 1952*a,b*), and at other presynaptic sites calcium channels do not inactivate, behaving more like potassium channels which cause the hyperpolarizing phase of the action potential in the squid giant axon (Hodgkin & Huxley, 1952*a,b*).

L-neurones possibly have at least two types of calcium channel: inactivating calcium channels, which mediate transmitter release at inhibitory connections to other L-neurones; and non-inactivating calcium channels, which mediate the release of transmitter at excitatory connections to other L-neurones (Simmons, 1982*a*) or to large, descending brain neurones (Simmons, 1981). In the leech, different output synapses of a single neurone have been found to facilitate at different rates, and the most likely explanation for this is that the neurone contains two different types of calcium channel (Muller & Nicholls, 1974). A number of non-spiking neurones make connections at which transmission can apparently be maintained for long periods without decrement (for example – vertebrate retina: Dowling & Ripps, 1973; Toyoda & Kujiraoka, 1982; arthropod central ganglia: Burrows & Siegler, 1978; Siegler, 1981; Blight & Llinás, 1980) and the presynaptic membrane of these neurones probably contains calcium channels which do not inactivate. Vertebrate photoreceptors have been shown to possess a non-inactivating calcium conductance (Bader, Bertrand & Schwartz, 1982), in contrast to horizontal cells maintained in culture, which produce a calcium current that inactivates after about 2 s (Tachibana, 1983). At some other synapses where the presynaptic neurone normally does not spike, a long pulse of depolarizing current injected presynaptically produces a PSP which has an initial peak amplitude, followed by a more sustained, lower plateau level of potential (spiny lobster stomatogastric ganglion, Graubard, 1978; dragonfly ocellar photoreceptors to L-neurones, Simmons, 1982*b*). At these synapses, the presynaptic neurones may contain an intermediate type of calcium channel, or else a mixture of calcium channels, some of which do and some of which do not inactivate.

If the hypothesis that the calcium channels inactivate at presynaptic sites of the inhibitory connections between L-neurones is correct, the operation of these connections is probably as follows. The rate at which transmitter is released depends upon the rate at which the calcium channels open. This is determined by the rate of depolarization of the presynaptic membrane. The calcium channels in the presynaptic membrane inactivate within 7 ms of opening, and the amplitude of an IPSP is limited to the potential reached in this time. The calcium channels recover from inactivation over a period of 1·5 s. The effect of inactivation is to reduce the rate at which transmitter can be released from a presynaptic site. There is probably a threshold potential for transmitter release, but it was not possible to produce suitable presynaptic potentials to measure this. Excitatory connections made by L-neurones have presynaptic threshold potentials 10–15 mV hyperpolarized from resting (Simmons, 1981, 1982*a*).

Two lines of experiment can be envisaged to test the hypothesis that calcium channels in the presynaptic membrane at the inhibitory connections inactivate. One involves the injection of calcium ions into presynaptic neurones directly. If IPSPs are short-lived because of inactivation of the channels through which calcium ions normally enter the presynaptic membrane, direct injection of calcium should cause unusually prolonged IPSPs. To perform this experiment, the calcium injection must be made precisely at a presynaptic site, and the locations of these must first be

established. This experiment also relies on the commonly accepted assumption that all neurotransmitter release is mediated by calcium entry, although some neurones, when isolated from the vertebrate retina, can release a neurotransmitter when depolarized in the absence of the entry of calcium ions (Schwartz, 1982). The second line of experiments is to measure the flow of ionic currents across the membrane of an L-neurone. The eventual aim would be to measure the entry of calcium into a presynaptic neurone, and correlate this with the production of PSPs. The following predictions about this calcium current can be made. First, its rate will be determined by the rate at which the presynaptic membrane depolarizes, and the current will reach a maximum value when the membrane depolarizes at $10\,mV\,ms^{-1}$. Second, it will inactivate within 7 ms, and recover from this over about 1·5 s. It will recover even if the presynaptic neurone is held depolarized.

A further prediction is that the presynaptic calcium channels will open relatively slowly. At the synapse between giant neurones in the squid, most of the synaptic delay (0·7 ms out of slightly less than 1 ms) is due to the time taken for the calcium channels in the presynaptic membrane to open (Llinás *et al.* 1981). The latency for transmission at output connections made by L-neurones is four times greater than that at the squid giant synapse. One possible explanation is that the connections are polysynaptic, but this is unlikely from the following considerations of structure and physiology. In ultrastructural studies, synapses between large neurones in the ocellar nerve and in the ocellus have been seen (Goodman *et al.* 1977; Goodman, Mobbs & Kirkham, 1979; Koontz & Edwards, 1984 make the same observation in a cricket). The only large axons in the ocellar nerves belong to L-neurones, as judged from sections and from cobalt back-fills from the ocellus (Goodman, 1976; Wilson, 1978a; Goodman *et al.* 1975). A synapse between a pair of L-neurones stained by intracellular injection of horseradish peroxidase has been found (A. H. D. Watson & P. J. Simmons, unpublished observations). In considering the following physiological observations, it should be borne in mind that physiological criteria which have been established previously (Berry & Pentreath, 1976; Getting, 1981) apply to presynaptic neurones that operate with trains of spikes. The first observation is from the experiments where presynaptic neurones were depolarized by short pulses of current (Fig. 6), and these lend strong support to the suggestion that presynaptic calcium channels open slowly. Many connections in the locust central nervous system have latencies of about 1 ms. If the connections between L-neurones were polysynaptic, involving a chain of connections which transmit with a latency of 1 ms, then presynaptic pulses 2 ms in duration should produce PSPs just as large as those produced by longer presynaptic depolarizations. Second, the smooth relationships between pre- and postsynaptic potential at excitatory connections (Simmons, 1981, 1982a), or between the rates of change in pre- and postsynaptic potential (Fig. 3), would probably not be found if additional neurones were interposed between the connected L-neurones. Finally, no suitable intermediary neurones have been found in over 100 recordings from pairs of interconnected neurones in the ocellar nerves of locusts. In a number of other animals, connections have been reported which are believed to be monosynaptic, but which have latencies of several milliseconds, even allowing for delay in propagation between the synaptic sites and recording sites. These include connections made by leech neurones in culture, where there is no doubt the connections are monosynaptic

(Fuchs, Henderson & Nicholls, 1982; Henderson, Kuffler, Nicholls & Zhang, 1983), synapses in the stomatogastric ganglion of the spiny lobster (Graubard, 1978), synapses in *Tritonia* (Getting, 1981) and synapses between photoreceptors and L-neurones in dragonfly ocelli (Chappell & Dowling, 1972; Dowling & Chappell, 1972; Simmons, 1982*b*). In their patch clamp study on single calcium channels in the cell body of a snail neurone, Lux & Brown (1984*b*) found that there could be a waiting time of 4–5 ms between the onset of a depolarization applied across the membrane and the opening of a calcium channel. Calcium channels in presynaptic membranes may well vary in the time they take to open, as well as in whether or not they inactivate.

One function of the spike in L-neurones is to ensure that these neurones can produce the rapid depolarizations that are required for transmission at the inhibitory connections between L-neurones. The ionic basis of this spike remains to be investigated. Similar spikes in second-order neurones of barnacle ocelli are mediated by calcium ions (Stuart & Oertel, 1978). In locust L-neurones, the channels which produce the rising phase of a spike are probably not the same channels through which calcium ions enter the presynaptic membrane to mediate transmitter release. The reasons for this suggestion are: first, there is a relatively long latency, of 4·5 ms, between the start of a spike and the start of a PSP; and second, when a pair of spikes, separated by a few 100 ms, are produced in the presynaptic neurone, the rate of hyperpolarization during the second IPSP is less than the rate during the first IPSP, although the rates of depolarization during the two spikes are the same (Fig. 4). In a crab, the flow of currents across the membrane of a non-spiking stretch receptor have been studied (Mirolli, 1983). Here, there is a fast initial transient to depolarizing potentials, which is mediated by sodium ions and is sensitive to tetrodotoxin, in segments of the neurone that are isolated from presynaptic membrane. In presynaptic regions of these neurones, fast transient depolarizing potentials may also be produced by the entry of calcium ions (Blight & Llinás, 1980; Mirolli, 1983). Fast depolarizing transients may well be necessary for transmission at other synapses, besides that described in this paper, which produce PSPs of limited duration. The reason why such PSPs have been found between at connections between L-neurones is that it is feasible to record pre- and postsynaptic potentials simultaneously. Possibly PSPs of limited duration are quite common in nervous systems, but the experiments needed to reveal them might rarely be feasible.

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