J. Physiol. (1973), **232**, *pp.* 331–356
With 12 *text-figures*
Printed in Great Britain

LONG-LASTING POTENTIATION OF SYNAPTIC TRANSMISSION IN THE DENTATE AREA OF THE ANAESTHETIZED RABBIT FOLLOWING STIMULATION OF THE PERFORANT PATH

By T. V. P. BLISS and T. LØMO

From the National Institute for Medical Research, Mill Hill, London NW7 1AA and the Institute of Neurophysiology, University of Oslo, Norway

(*Received 12 February 1973*)

SUMMARY

1. The after-effects of repetitive stimulation of the perforant path fibres to the dentate area of the hippocampal formation have been examined with extracellular micro-electrodes in rabbits anaesthetized with urethane.

2. In fifteen out of eighteen rabbits the population response recorded from granule cells in the dentate area to single perforant path volleys was potentiated for periods ranging from 30 min to 10 hr after one or more conditioning trains at 10–20/sec for 10–15 sec, or 100/sec for 3–4 sec.

3. The population response was analysed in terms of three parameters: the amplitude of the population excitatory post-synaptic potential (e.p.s.p.), signalling the depolarization of the granule cells, and the amplitude and latency of the population spike, signalling the discharge of the granule cells.

4. All three parameters were potentiated in 29% of the experiments; in other experiments in which long term changes occurred, potentiation was confined to one or two of the three parameters. A reduction in the latency of the population spike was the commonest sign of potentiation, occurring in 57% of all experiments. The amplitude of the population e.p.s.p. was increased in 43%, and of the population spike in 40%, of all experiments.

5. During conditioning at 10–20/sec there was massive potentiation of the population spike ('frequency potentiation'). The spike was suppressed during stimulation at 100/sec. Both frequencies produced long-term potentiation.

6. The results suggest that two independent mechanisms are responsible

for long-lasting potentiation: (*a*) an increase in the efficiency of synaptic transmission at the perforant path synapses; (*b*) an increase in the excitability of the granule cell population.

INTRODUCTION

These experiments arose from an observation made during a study of the phenomenon of frequency potentiation in the dentate area of the hippocampal formation (Lømo, 1966). It was noticed that the response evoked in the dentate area by single test shocks to the afferent perforant pathway often remained potentiated for a considerable time after short periods of stimulation at 10–20/sec. In this paper we describe the effect in the anaesthetized rabbit. In the following paper (Bliss & Gardner-Medwin, 1973) it is shown to be present also in the unrestrained and unanaesthetized animal. Preliminary reports have been published (Bliss & Lømo, 1970; Bliss & Gardner-Medwin, 1971; Bliss, Gardner-Medwin & Lømo, 1973).

METHODS

Preparation

The experiments were performed on eighteen adult rabbits. Both sexes were used. Anaesthesia was induced with a mixture of urethane (0·75 g/kg) and chloralose (40 mg/kg) given I.V., and maintained with urethane alone. The dorsal hippocampus was exposed bilaterally by removing the overlying cortex with a suction pipette. A small silver plate was sewn into the neck muscles to act as the indifferent electrode.

Electrodes and equipment

Conventional NaCl-filled glass micro-electrodes with resistances of 1–3 MΩ were used for recording. Stimulating electrodes were constructed from electrolytically sharpened tungsten wire insulated with several coats of varnish. They were used in a monopolar tip-negative configuration to give constant voltage pulses with a duration of 0·1 msec and an amplitude of up to 100 V. The circuit was completed via a silver ball electrode positioned on a neighbouring cut muscle surface. The recording electrodes were connected through cathode followers to a.c. preamplifiers with 3 dB cut-off points at 4 Hz and 1 kHz. Evoked responses were displayed on an oscilloscope, either directly or after averaging, and photographed.

Anatomy

The hippocampal formation contains two curved interdigitating layers of cells, the pyramidal cells of regions CA_1 and CA_3, and the granule cells of the dentate area (Fig. 1*A*). It is with the perforant path input to the latter group of cells in the dorsal hippocampal formation that this paper is concerned. The thinly myelinated fibres of the perforant path arise from cells in the medial entorhinal area (Nafstad, 1967; Hjorth-Simonsen & Jeune, 1972) and ascend in the angular bundle in a rostromedial direction before fanning out to cross the subiculum and the hippocampal fissure. At this point some or all of the fibres bifurcate, one branch supplying the upper limb and the other the lower limb of the dentate fascia. The fibres run in the molecular layer, which contains the apical dendrites of the granule cells (Fig. 1*B*). Here they make

synaptic contacts of the *en passage* type with dendritic spines (Blackstad, 1958), each fibre making contact with many granule cells. The synapses are restricted to the middle third of the molecular layer, and account for nearly 40 % of the total synaptic population in that region (Nafstad, 1967); the origin of the remainder is not known.

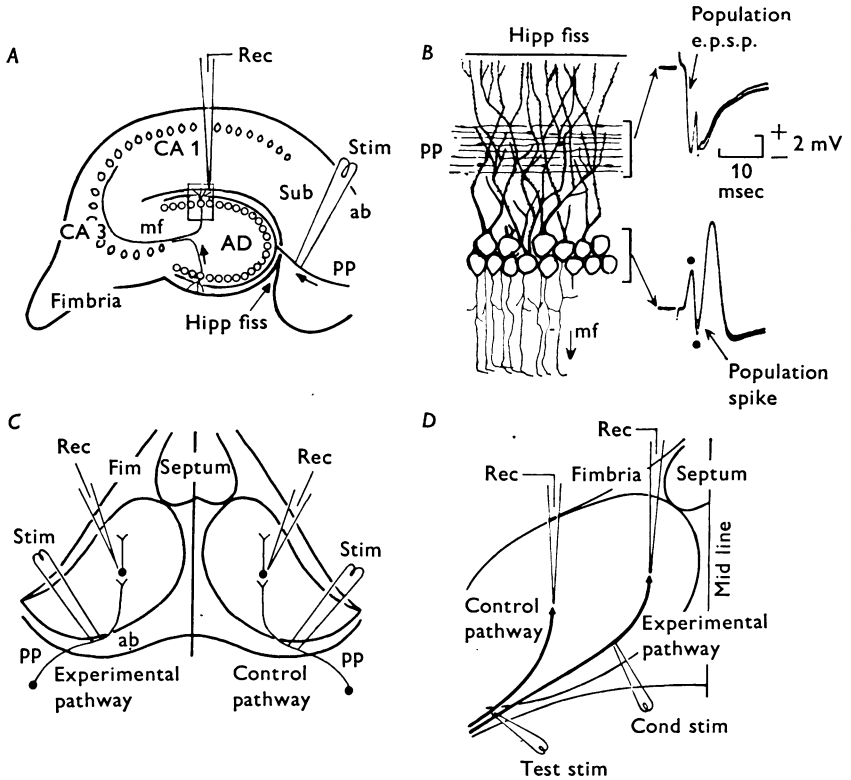

Fig 1. *A*, diagrammatic parasagittal section through the hippocampal formation, showing a stimulating electrode placed beneath the angular bundle (ab) to activate perforant path fibres (pp), and a recording microelectrode in the molecular layer of the dentate area (AD). *B*, the region enclosed in the rectangle in *A* enlarged to show the apical dendritic field of the granule cells, with the perforant path fibres confined to the central one third of the field. The population responses evoked by a strong perforant path volley in the synaptic layer (upper trace) and in the cell body layer (lower trace) are displayed on the right. The spots (lower trace) mark the peaks between which the amplitude of the population spike was measured. *C*, arrangement of electrodes for experiments in which the control pathway was situated in the contralateral hippocampus. *D*, electrode arrangement for experiments in which both test and control pathways were on the same side.

Abbreviations: ab, angular bundle; AD, dentate area; CA_1, CA_3, pyramidal fields CA_1 and CA_3; Fim, fimbria; hipp fiss, hippocampal fissure; mf, mossy fibres; pp, perforant path.

Population potentials

Fig. 1*B* shows the population potentials recorded at two levels in the dentate area, following a strong perforant path volley. The upper trace was obtained with the recording electrode in the region of the perforant path synapses, i.e. in the middle third of the molecular layer. The two components of interest are the initial negative deflexion and the superimposed positive-going spike. If the recording electrode is advanced 150 μm into the cell body layer, all components of the potential become reversed (lower trace). This sequence of deflexions, and their reversal with depth, is explained in the following way. Activation of the perforant path synapses causes depolarization of the subjacent dendritic membrane, and extracellular current flows from the cell body layer towards the dendrites, resulting in a negative potential in the synaptic region, and a positive potential in the cell body region, relative to a distant electrode. This early synaptic potential is called the 'population e.p.s.p.' or, where there is no risk of confusion, simply the e.p.s.p., by analogy with the corresponding intracellular potential (Lømo, 1971*a*). The second component of the response, the 'population spike', is correlated in time and magnitude with unit discharges (Lømo, 1971*a*; Andersen, Bliss & Skrede, 1971*a*). It reflects the number of granule cells discharged, as well as the synchrony with which they fire, and is thus a measure of the over-all excitability of the granule cell population. The population spike is maximally negative in the cell body layer, where the current 'sink' of the spike generating mechanism is greatest.

The synaptic and spike potentials both reverse polarity at a depth intermediate between the granule cell bodies and the perforant path synapses and it is in this region that the potential profile is steepest. In contrast, the potential recorded in the cell body layer changes little, if at all, as the recording electrode is advanced into the hilus. This has an important practical application, since it means that potentials recorded in the hilus do not vary appreciably with slight brain movement.

Experimental arrangement

The arrangement adopted at first is shown in Fig. 1*C*. The hippocampal formation was exposed bilaterally. A long sagittal cut was sometimes made to one side of the mid line to prevent commissural interaction between the two sides. Stimulating and recording electrodes, held in micromanipulators, were positioned in the perforant path and dentate area respectively, one pair on either side of the mid line.

The position and depth of each electrode was carefully adjusted so that maximal responses were obtained. Usually only one recording electrode was available on each side, and we preferred to place it just below the cell body layer, where the evoked potential was less affected by slight brain movements, rather than in the synaptic layer. The procedure followed was to move the recording electrode down to the reversal point for the synaptic potential, about 50 μm below the depth for maximal negativity. It was then advanced a further 150 μm from this easily identifiable reference point. The tip of the electrode was then 50 μm or so below the lower edge of the cell body layer.

Once the electrode positions were optimized on both sides, the routine testing of the excitability of each pathway began. Single shocks at a fixed strength, repeated at intervals of 2–3 sec, were given through each stimulating electrode. Superimposed single responses were photographed at regular intervals (usually every minute) together with averaged responses based on 20 or 30 consecutive single responses. A sequence of conditioning trains, each at intervals of 30 min or more, was given to one side only (the experimental pathway), the other side acting as a control for generalized excitability changes and gross movements of the brain. Throughout the

course of an experiment frequent checks were made to ensure that the responses remained maximal, and appropriate minor adjustments of the electrodes were made if they were not. Adjustments of this sort were more frequently necessary when recording from the synaptic layer, but even there stable recordings for periods of an hour or more were not uncommon.

With the above arrangement the same stimulating electrode was used for both testing and conditioning, and the possibility of local changes at the site of stimulation leading to a larger number of fibres being stimulated by the standard test volley could not be excluded. In order to control for this contingency, we adopted in later experiments a rather different design. This exploited the fact that the perforant path fibres, as they ascend laterally from the entorhinal area, lie close together beneath the angular bundle, before fanning out to invest the dentate area of the dorsal hippocampal formation. A stimulating electrode placed in this lateral position will therefore monosynaptically excite granule cells over a wide area. Conversely, an electrode placed more medially and rostrally will excite only a narrow beam of cells (Lømo, 1971*a*; Andersen, Bliss & Skrede, 1971*b*), as indicated in Fig. 1*D*. Recording electrodes were placed in the dentate area at two points a few mm apart along the long axis of the hippocampus. Roughly equal responses could be obtained from both recording sites with the lateral stimulating electrode (Figs. 3*C* and 4*A*). A second stimulating electrode was then placed close to the more medial recording electrode and its position adjusted so that, with a suitable stimulus strength, a response was obtained at the medial, but not at the lateral recording site. Standard test shocks were given regularly throughout the experiment via the lateral stimulating electrode, while the medial electrode was used only to deliver the conditioning trains. With this ipsilateral arrangement, the effects of any generalized change in hippocampal excitability, or of gross brain movements, could be monitored with the control pathway, while any local changes at the site of the conditioning electrode would be unlikely to affect the distant test electrode.

Parameters of the evoked response

Three parameters of the evoked response were selected for analysis.

1. The amplitude of the population e.p.s.p., measured either as a negative potential in the synaptic layer, or as a positive potential in the cell body layer. Measurements were made at an arbitrarily chosen latency, fixed for each experiment and usually not more than 1 msec after onset to avoid distortion by the subsequent population spike.

2. The peak-to-peak amplitude of the population spike, recorded in the cell body layer, and measured between the initial peak positivity and peak negativity (spots, Fig. 1*B*, lower trace).

3. The latency of the population spike, taken as the time from the stimulus artifact to the initial peak positivity.

RESULTS

Effects of repetitive stimulation

Stimulation of the perforant path at 10–15/sec for several seconds normally resulted in the following sequence of events.

1. A rapid build-up of the population spike during the conditioning train ('frequency potentiation'; Andersen, Holmqvist & Voorhoeve, 1966; Lømo, 1966).

2. A brief (1–2 sec) period of spike potentiation immediately after the conditioning train.

3. A phase of spike depression lasting from a few seconds to more than a minute.

4. A phase of potentiation, sometimes lasting for several hours, and in many cases involving synaptic as well as spike components of the response.

Although there was considerable variation in the degree and duration of these four phases in different preparations, the sequence as a whole was a

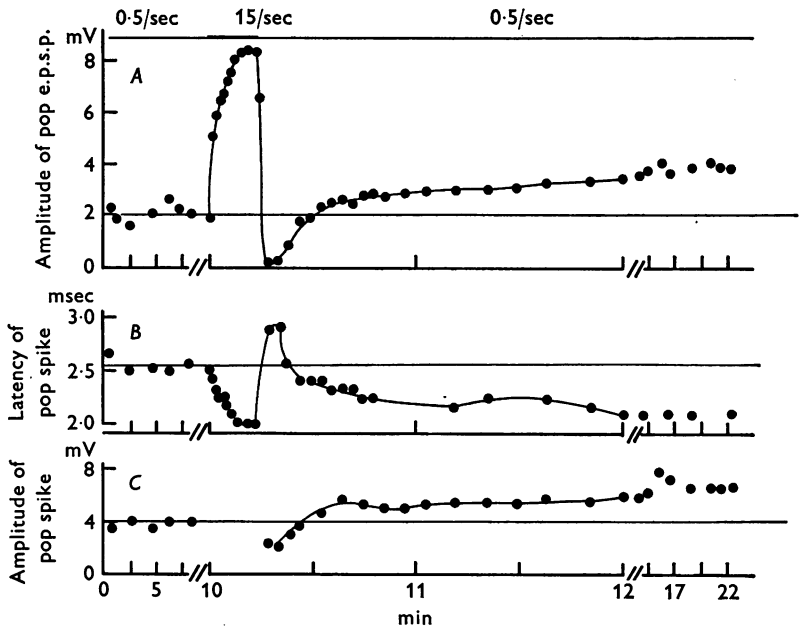

Fig. 2. Frequency potentiation and the immediate after-effects of a conditioning train. The graphs show the changes produced in three parameters of the evoked response by increasing the rate at which the perforant path was stimulated from 0·5/sec to 15/sec for 15 sec. The points obtained during the conditioning train and the following 1 min 45 sec are shown on an expanded time scale. Note immediately after the train the brief period of spike potentiation followed by depression, and the subsequent maintained potentiation. The value of the population e.p.s.p. during the train could not be accurately measured from the film record and is not plotted.

characteristic feature of the experiments. An example is given in Fig. 2, which shows the effect of a conditioning train (15/sec for 15 sec) on the three parameters of the evoked response. During the train the spike amplitude increased greatly (Fig. 2*A*) and there was a corresponding decrease in latency (Fig. 2*B*). It was impossible to measure the population e.p.s.p. accurately during the conditioning train because of the distortion

of the traces caused by the continuously moving film, and these values are therefore not plotted (Fig. 2*C*). There is, nevertheless, no doubt that the population e.p.s.p. was severely depressed during the latter part of the train, presumably owing to the profound depolarization of the granule cells which is associated with frequency potentiation (T. Lømo, unpublished observations). On this occasion the amplitude of the population spike began to rise steeply as soon as the conditioning train was initiated, reaching a maximal value after 10 sec. In other cases, the growth of the spike was delayed for several seconds, and was sometimes preceded by a brief period of depression. Within 1–2 sec after the end of the train, a marked depression of the spike occurred and its latency increased. The population e.p.s.p., on the other hand, began to recover from its depressed state almost immediately, and after about 15 sec passed into a supernormal or potentiated phase. The increase in the e.p.s.p. was associated with a gradual recovery and subsequent potentiation of the spike, and a concomitant fall in its latency. The potentiated values achieved after conditioning are plotted on a reduced time scale in Fig. 2. These levels were maintained for the rest of the experiment (1½ hr). Second and subsequent conditioning trains produced no further potentiation, in contrast to the steplike increases seen in some experiments (Figs. 4, 9).

The form of the evoked potentials during each of the four phases listed above can be seen in Fig. 3, taken from a different experiment with electrode positions as in Fig. 3*A*. Fig. 3*B* shows superimposed responses from the cell body layer of the experimental pathway, recorded at the times indicated during a 15/sec train of stimuli delivered through the conditioning electrode. There is clear frequency potentiation, with an increase in spike amplitude, reduction in spike latency and development of a second spike. Before and after the conditioning train, test stimuli at 0·5/sec were delivered through the more laterally placed electrode. The responses evoked by these stimuli in the synaptic and cell body layers of the experimental pathway are shown in the first two columns of Fig. 3*C* and *D* (*C* before, and *D* after the conditioning train). The records in the third column are the responses evoked in the synaptic layer of the control pathway by the same test stimulus. In the 70 min control period preceding the first conditioning train, the size of the population spike evoked in the experimental pathway slowly declined (Fig. 3*C*). Immediately after the train it was further depressed (Fig. 3*D* upper trace), but within 2 min, marked potentiation had developed, which was still clearly present 45 min after the train (Fig. 3*D*, experimental pathway, lower row). Again, potentiation took the form of an increase in the population spike, reduction in spike latency and a steeper rise of the population e.p.s.p. To show this more clearly, the responses immediately before (stippled trace)

and 45 min after the train have been superimposed in Fig. 3*E*. The increase in the population e.p.s.p. was present not only in the synaptic layer where the active current sink is located, but was equally pronounced in the region of the passive source in the cell body layer. No similar potentiation occurred in the control pathway, where the slope of the early part of the population e.p.s.p. was the same after conditioning as it was before.

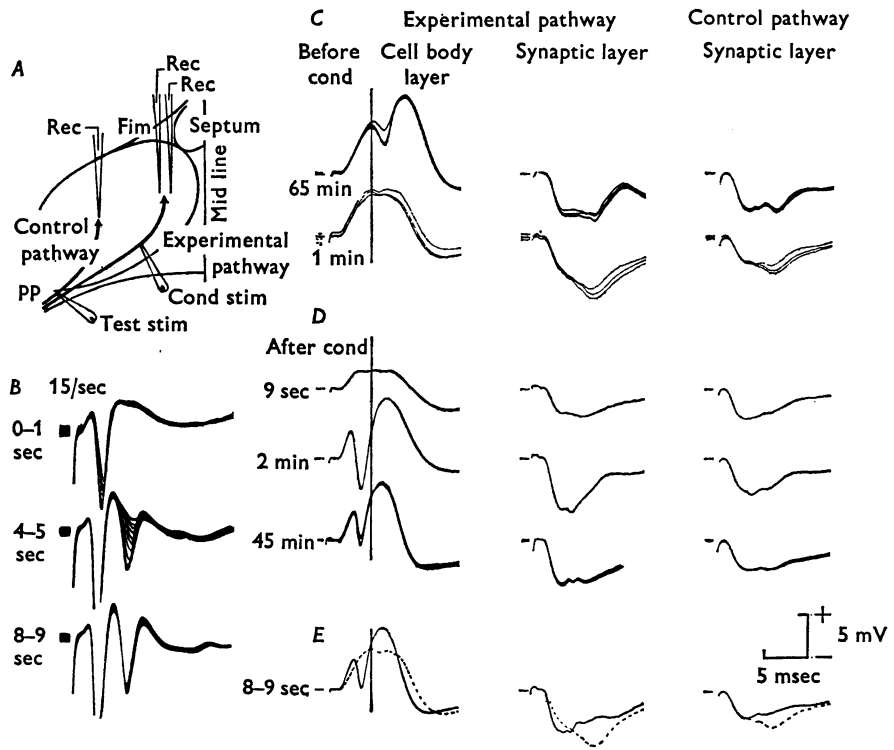

Fig. 3. The effects of a conditioning train on the shape of the evoked response. *A*, arrangement of electrodes. Note the two recording electrodes in the experimental pathway, one in the synaptic layer, the other in the cell body layer. *B*, development of frequency potentiation recorded in the cell body layer during a conditioning train at 15/sec. The time from the beginning of the train is given on the left of each set of eight–twelve superimposed consecutive responses. *C*, *D*, evoked responses obtained at various times before (C) and after (D) the 15 sec conditioning train illustrated in *B*. Three potentials are shown in each case: the responses recorded in the cell body (left) and synaptic (centre) layers of the experimental pathway, and in the synaptic layer of the control pathway (right). For comparison, single responses obtained 1 min (stippled trace) and 45 min after conditioning have been superimposed in *E*. The vertical line in the left-hand column marks the pre-conditioning value of the latency of the population spike.

In this experiment there was a decrease in the size of the late components of the response in both experimental and control pathways; a change in these later, presumably polysynaptic, components of the response was seen in several experiments (Fig. 4*B*).

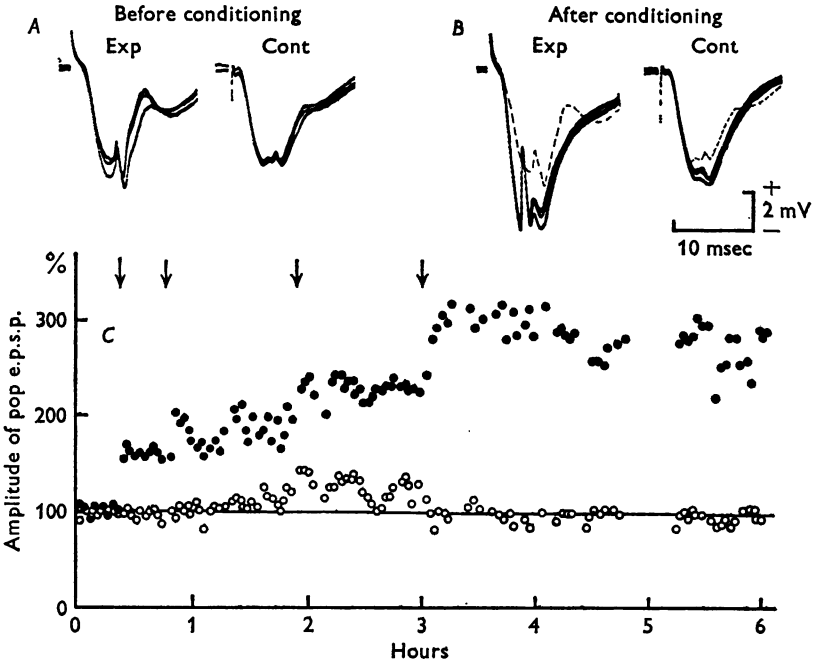

Fig. 4. An experiment in which all three standard parameters of the evoked response were potentiated. Three superimposed responses obtained in the synaptic layer for both the experimental and control pathways are shown in *A* (before conditioning) and in *B* (2·5 hr after the fourth conditioning train). *C*, graph showing the amplitude of the population e.p.s.p. for the experimental pathway (filled circles) and the ipsilateral control pathway (open circles) as a function of time. Each point was obtained from the computed average of thirty responses by measuring the amplitude of the negative wave 1 msec after its onset. The values are plotted as percentages of the mean pre-conditioning value. Conditioning trains (15/sec for 10 sec) were given through a medially placed conditioning electrode at the times indicated by the arrows.

Long lasting potentiation

The potentiation which followed a conditioning train varied considerably in both duration and degree from experiment to experiment. If long-lasting potentiation is arbitrarily defined as potentiation lasting 30 min or more, then a positive result was obtained in fifteen out of eighteen animals. In most animals more than one pathway was conditioned. A total of

twenty-four out of the thirty-five conditioned pathways showed long-lasting potentiation. Potentiation of all three parameters was observed in nine of the thirty-five conditioned pathways, although in some cases more than one conditioning train was needed to achieve this result. Considering the response parameters separately, a decrease in spike latency was the commonest form of facilitation (twenty pathways). The population e.p.s.p. was potentiated in fifteen pathways and the spike in fourteen pathways. A lack of correlation between e.p.s.p. and spike potentiation was a notable feature of several experiments and is discussed more fully below.

Potentiation of all parameters

An example of an experiment which involved potentiation of all three parameters is seen in Figs. 4 and 5. Four conditioning trains, each at 15/sec for 10 sec, were given at the times indicated by arrows. The amplitude of the e.p.s.p. for both the experimental (filled circles) and control pathway (open circles) is plotted in Fig. 4, while the amplitude and latency of the population spike for the experimental pathway are shown in Fig. 5. Each symbol in Fig. 4*C* represents thirty responses averaged over a period of 1·5 min. The first half minute after each train has not been included, so the initial post-activation depression is not seen. Each of the four conditioning trains resulted in a sudden increase in the population e.p.s.p., which reached a maximal level two to three minutes after each train and which then remained at that level until the next conditioning train was delivered. In several experiments the e.p.s.p. showed a gradual decline towards the preconditioned value over a period of one or more hours. In some of these cases a second conditioning train brought the e.p.s.p. size abruptly back to a new and higher value from which the decline was slower than before (see, for example, Fig. 9*C*). The experiment in Fig. 4 was continued beyond the 6 hr period illustrated. Six hr and 7·25 hr after the beginning of the experiment, fifth and sixth conditioning trains were given. These later trains had little further potentiating effect on the e.p.s.p., which started to decline about 8 hr after the beginning of the experiment. The three superimposed single responses shown in Fig. 4*A* (before conditioning) and in *B* (after conditioning, with the preconditioning responses indicated by stippled traces) illustrate the potentiation in the experimental pathway and the lack of a similar effect on the early part of the response in the control pathway. It can safely be concluded that the effect on the population e.p.s.p. is restricted to the experimental pathway and is a direct result of the brief conditioning trains limited to this pathway. Potentiation of the population spike was marked by a fall in mean latency after each conditioning train and an increase in amplitude after the fourth train. In

Fig. 5*A*, far right, the potentiated response obtained after 6 hr may be compared with that obtained before conditioning (stippled trace). Again (as in Figs. 3, 7 and 11), potentiation of the population e.p.s.p. can be seen not only in the synaptic layer (Fig. 4*A* and *B*) but also in the cell body layer, as an increase in the slope of the initial positive wave. In *B* the

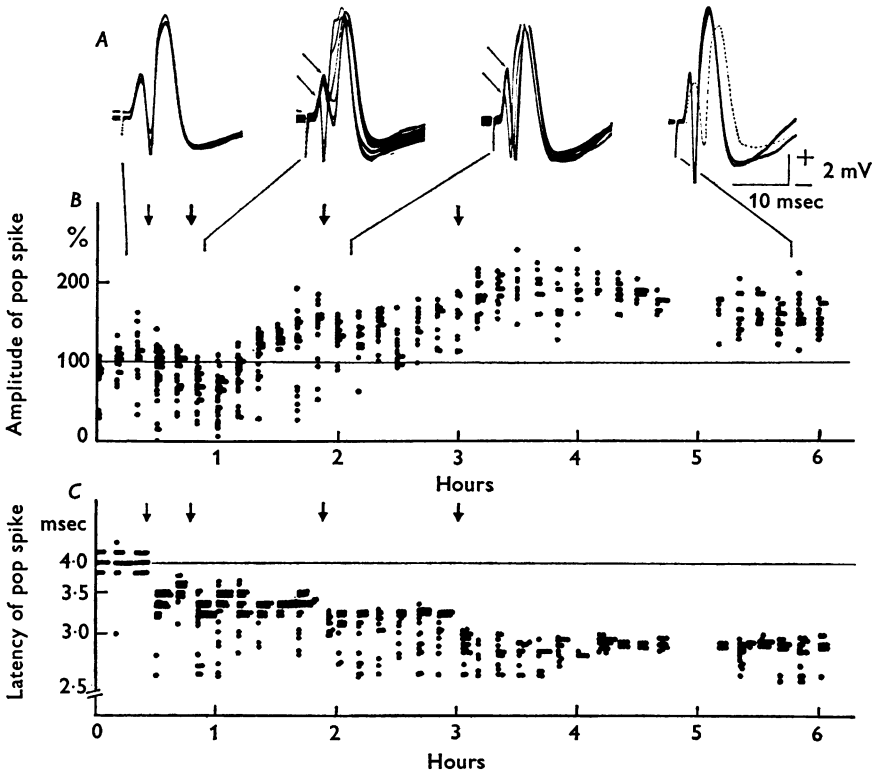

Fig. 5. Potentiation of the population spike parameters in the experiment of Fig. 4. The Figure also illustrates the variability of the population spike. *A*, superimposed responses to several consecutive perforant path volleys, obtained at the four different times shown. Arrows indicate the two preferred latencies adopted by the population spike in the period between the second and fourth conditioning trains. The amplitude and latency of the population spike are plotted in *B* and *C* respectively, as functions of time. Each point was obtained from a single response, and each group of points represents a number (six to thirty) of consecutive responses.

amplitude of a large number of individually measured population spikes, grouped at 10 min intervals, is displayed in order to show the considerable variability of this parameter. The mean amplitude of the population spike actually decreased after the first and the second conditioning train,

in spite of the increase in the population e.p.s.p. (Fig. 4*C*). It was not until after the fourth train that the spike became clearly larger than it had been before conditioning.

The latency of the spike also varied, as can be seen both from the superimposed records of Fig. 5*A* and from the plot of individual latency measurements in Fig. 5*C*. Two separate phenomena underlie the latency changes observed in this and other experiments. One is the sudden reduction in latency which occurred after each conditioning train; the other is the tendency of the spike to jump, from one stimulus to the next, between an early and a late response as indicated by the arrows in Fig. 5*A*. These latency jumps were not associated with any corresponding change in the slope of the synaptic wave. Latency fluctuations were most marked after the first three trains in this experiment, but also occurred both before conditioning and after the fourth train when the spike as well as the population e.p.s.p. were maximally potentiated (Fig. 5*C*). The mean delay between the onset of the population e.p.s.p. and the early and late population spikes was 1·4 msec and 2·1 msec, respectively, before conditioning. After the fourth train these values had fallen to 0·9 msec and 1·3 msec. Thus, the onset of both types of spike was advanced with successive trains, while the time difference between them was reduced.

Stimulus-response relationships

Additional information can be obtained by studying the changes which occurred in the stimulus–response curves after conditioning. In Fig. 6*A* and *B*, the amplitudes of the population e.p.s.p. and population spike in the experimental pathway have been plotted against the strength of the test stimulus for the same experiment as in Figs. 4 and 5. Filled circles show the relation before the first conditioning train, while the open circles give the same relation about 10 hr later (3 hr after the sixth and last conditioning train). The response was potentiated over the whole range of stimulus strengths, except near threshold. By this time (10 hr) the amplitude of the population e.p.s.p. evoked by a given stimulus in the control pathway was smaller than its preconditioning value (Fig. 6*C*), reflecting the gradual decline which set in after the eighth hour of the experiment; stimulus-response curves obtained at 7 hr in the control pathway were virtually identical to those obtained at the beginning of the experiment. A similar gradual decline of the e.p.s.p. occurred in the experimental pathway after the eighth hour. In spite of this the e.p.s.p. was still considerably potentiated at 10 hr (Fig. 6*A*) and remained potentiated for another 2 hr. In some experiments, as here, a small increase in the voltage threshold for the e.p.s.p. was seen; in no case was there a decrease in threshold after conditioning.

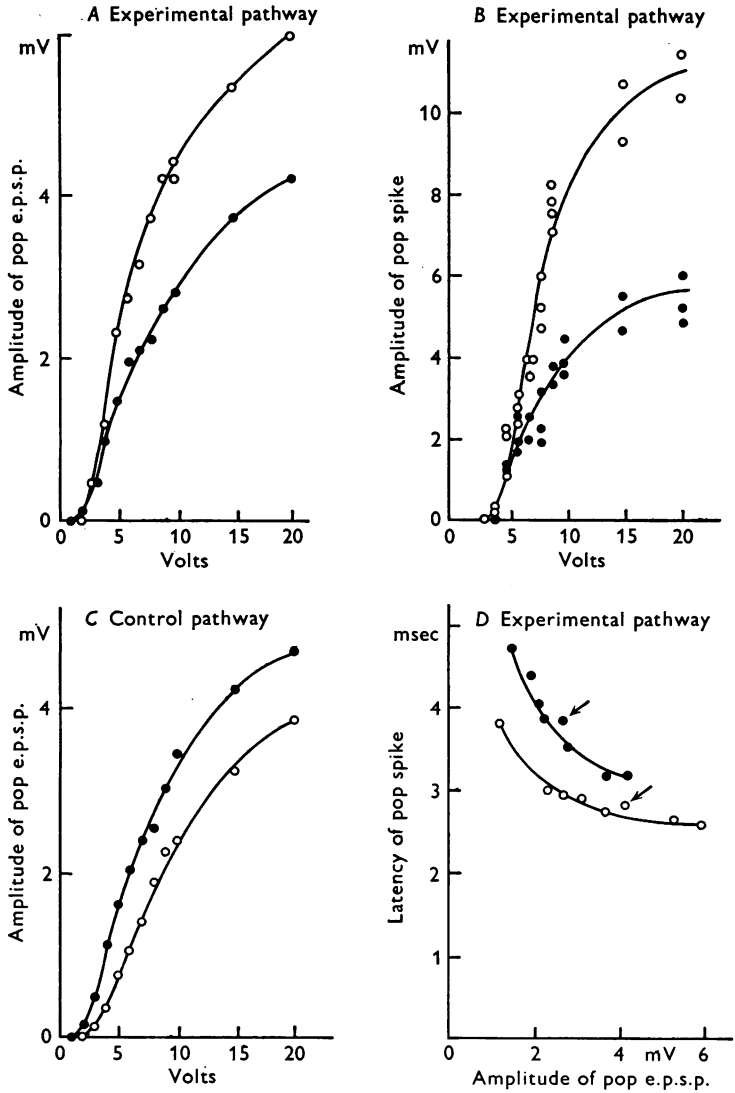

Fig. 6. Effect of conditioning on stimulus–response curves. Same experiment as in Figs. 4 and 5. The two sets of points in *A* give the values of the population e.p.s.p. for the experimental pathway, as a function of stimulus strength before conditioning (filled circles), and almost 10 hr later (open circles) which was 3 hr after the last of six conditioning trains. Similar pairs of curves are plotted in *B* for the amplitude of the population spike (experimental pathway), and in *C* for the amplitude of the population e.p.s.p. (control pathway). In *D* the latency of the population spike has been plotted as a function of the amplitude of the population e.p.s.p. at the same times before (filled circles) and after (open circles) conditioning as in *A*–*C*. The arrows indicate the points obtained at the stimulus strength used for the standard test shocks given throughout the experiment.

In the majority of cases for which we have adequate data, the potentiation of the spike parameters could not be explained wholly in terms of potentiation of the e.p.s.p. In Fig. 6*D* the data from *A* and *B* have been replotted to show the latency of the population spike as a function of the e.p.s.p. amplitude, before and after conditioning. The two arrows point to values obtained at the normal test strength of 9 V. Before conditioning, an e.p.s.p. of 2·7 mV was associated with a spike latency of 3·9 msec, while

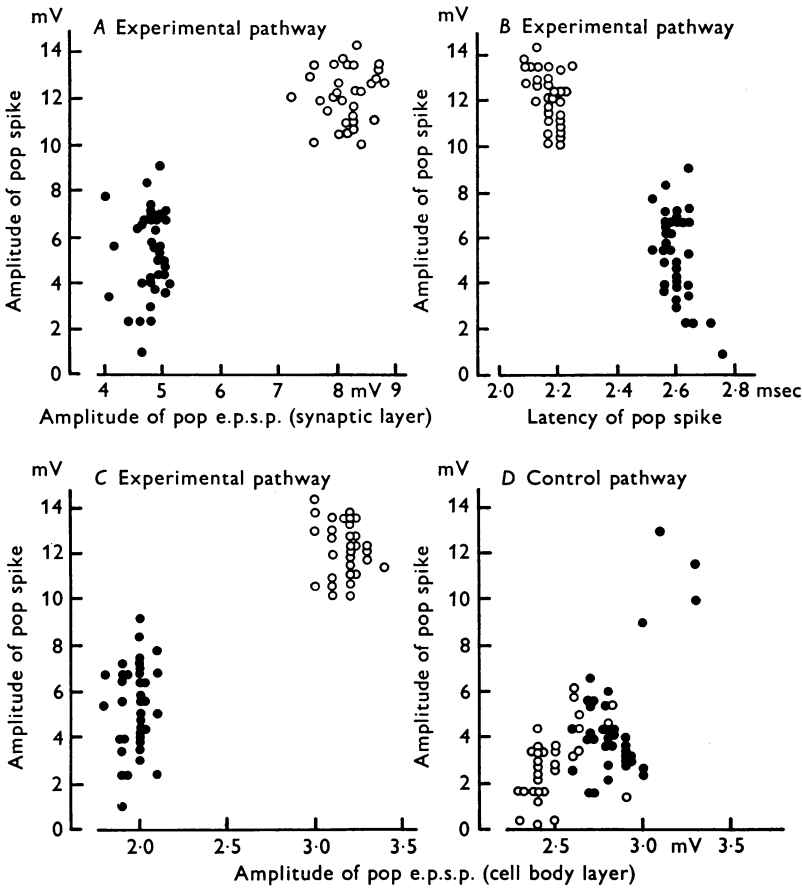

Fig. 7. Effect of conditioning on the variability of the population spike. *A–C*, experimental pathway. The amplitude of the population spike of over thirty consecutive responses obtained before (filled circles) and after (open circles) conditioning, is plotted as a function of the amplitude of the population e.p.s.p. (synaptic layer in *A* and cell body layer in *C*), and of the latency of the population spike (*B*). A similar plot of the amplitude of the population spike as a function of the population e.p.s.p. (measured in the cell body layer) is shown in *D* for the control pathway.

after conditioning the same e.p.s.p. (obtained with a weaker stimulus) produced a spike with a latency of only 2·9 msec. There was thus a component of spike facilitation which could not be explained by e.p.s.p. potentiation alone.

Variability of the population spike

The experiment displayed in Figs. 7 and 8 was chosen to illustrate the common observation that the population spike evoked by a constant test stimulus could vary markedly in amplitude, often from one response to the next (see also Fig. 5*B*). As with the variations in latency already described, these variations in amplitude were not accompanied by similar changes in the e.p.s.p. The variability tended to diminish as spike potentiation developed after conditioning. In Fig. 7, the spike amplitudes of over 30 consecutive responses obtained before and after conditioning (filled and open circles respectively) have been plotted against the amplitude of the population e.p.s.p. for each response (abscissa, Fig. 7*A*, *C* and *D*) and against spike latency (*B*). The increase in spike amplitude after conditioning

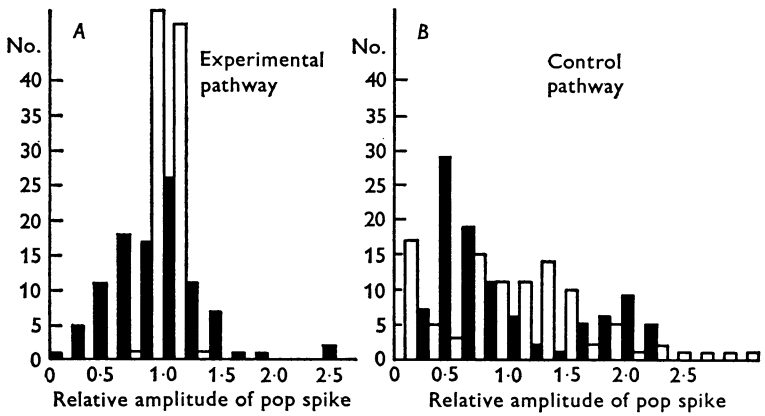

Fig. 8. Histograms showing the amplitude distributions, expressed relative to the mean, of two sets of 100 consecutive population spikes, obtained before (filled bars) and 20–40 min after (open bars) a conditioning train to the experimental pathway. *A*, experimental pathway. *B*, control pathway. Same experiment as in Fig. 7.

was associated with an increase in the amplitude of the e.p.s.p., both in the synaptic layer (*A*) and in the cell body layer (*C*). The reduction in variability of spike amplitude, which occurred despite a slight increase in the range of e.p.s.p. values (*A*), is clearly brought out by this method of displaying individual responses. The range of values for spike amplitude in *A* was 1·0–9·0 mV before conditioning, and 10·0–14·5 mV after condi-

tioning. Expressed in terms of the dimensionless index, standard deviation/mean, the variability fell from 0·35 to 0·09. In the control pathway (*D*), the changes were restricted to a general reduction of both e.p.s.p. and spike amplitudes, with the disappearance after conditioning of the unusually large responses which in this experiment occasionally occurred, for unknown reasons, before conditioning.

The reduction in variability brought about by conditioning can also be seen in Fig. 8, taken from the same experiment. Two samples of one hundred consecutive responses, collected before and 20–40 min after a conditioning train, have been classified according to spike amplitude, expressed relative to the mean amplitude for each sample. The filled bars show the amplitude distribution before conditioning, for both experimental (*A*) and control (*B*) pathways. After conditioning, the amplitude distribution of the experimental pathway was much more sharply peaked (*A*, open bars), while that of the control pathway, although different in detail, was no less broad.

Potentiation of spike parameters without corresponding changes in the population e.p.s.p.

In the experiments described so far, conditioning resulted in a larger e.p.s.p. and a larger and earlier population spike. In some cases the spike facilitation was more than could be accounted for directly by the increase in the e.p.s.p. (Fig. 6*D*). Figs. 9 and 10 show that the spike was sometimes potentiated by conditioning trains which had little or no effect on the population e.p.s.p. In Fig. 9*C* the population spike of both the experimental (filled circles) and the contralateral control pathways (open circles) gradually declined in the 45 min before the first train was delivered to the experimental side. The first two trains were followed by a powerful but transitory potentiation of the population spike on the experimental side. With further trains, the time course of potentiation became progressively longer, until by the fourth train a stable plateau had been reached. No similar effects were seen on the control side. Averaged responses obtained before and after conditioning on the two sides are shown in Fig. 9*A*. In contrast to the steplike increase in spike size after each conditioning train (*C*), spike latency was not further reduced after the first train (*D*). The population e.p.s.p., measured in the cell body layer, increased slightly during the course of the experiment (*B*), but this increase was not clearly related to any of the conditioning trains.

A similar lack of correspondence between the population spike and population e.p.s.p. is seen in Fig. 10. In this case there was a sharp fall in latency, first on the left side (from 2·9 to 2·4 msec) and then on the right side, as each side received its first conditioning train. These shifts in latency can be

seen in the superimposed averaged records shown in *B*. Conditioning had no obvious effect on the population e.p.s.p. (*B* and *E*) or the population spike (*B* and *F*), on either side. It is worth noting that a later experiment on a more lateral and previously unstimulated segment of the dentate area in the same animal produced potentiation of all three parameters (Fig. 2).

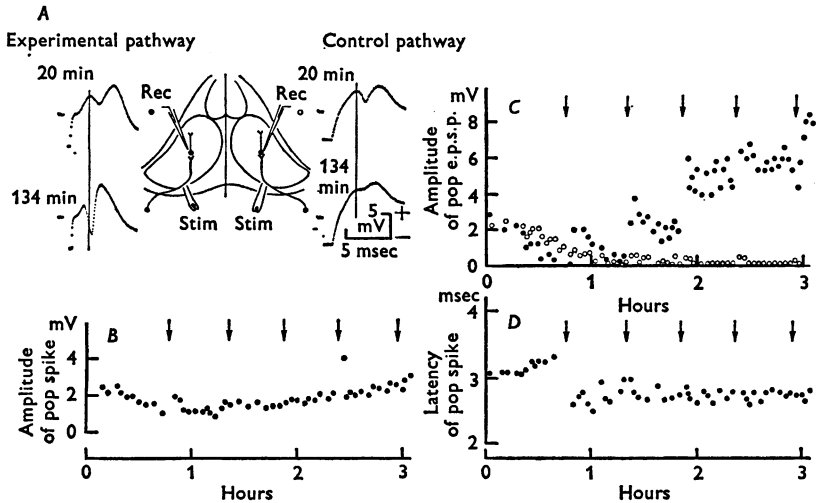

Fig. 9. Potentiation of the population spike without potentiation of the population e.p.s.p. *A*, electrode arrangement. Sample average potentials for both experimental and control pathways before and after conditioning are shown. *B–D*, plots of the three standard parameters of the evoked response on the experimental side as functions of time. Conditioning trains (20/sec for 20 sec) were given at the times marked by arrows. In *C* the amplitude of the population spike is plotted for both experimental and control pathways (filled and open circles respectively).

Conditioning with high frequency trains

A question of some interest is whether long term potentiation depends on the ability of the conditioning train to discharge the granule cells. In order to reduce or eliminate firing of the granule cells during a conditioning train, the frequency was increased to 100/sec in three experiments. The duration of the train was reduced to 3–4 sec so that approximately the same number of stimuli was given. From field potential and single unit recordings it appears that granule cell discharges are usually abolished after the first shock in a train of stimuli at 100/sec. Furthermore, in two of the three experiments of this type, a weak stimulus was used so that no population spike was evoked before or during the conditioning train. In spite of the absence of synchronous granule cell discharges, potentiation of the e.p.s.p. lasting an hour or more was obtained in all three experiments.

The result of one of these experiments is shown in Fig. 11. The increase in slope and in the peak amplitude of the population e.p.s.p. after conditioning is apparent in the superimposed records in *A*, obtained from the synaptic and cell body layers just before and 45 min after the first of the three trains indicated in *B*. As in other experiments the e.p.s.p. potentiation was present both in the cell body and synaptic layers. The time course of the e.p.s.p. potentiation is plotted in *B*. Each train had an immediate effect which fell off within 10–20 min to the potentiated level set

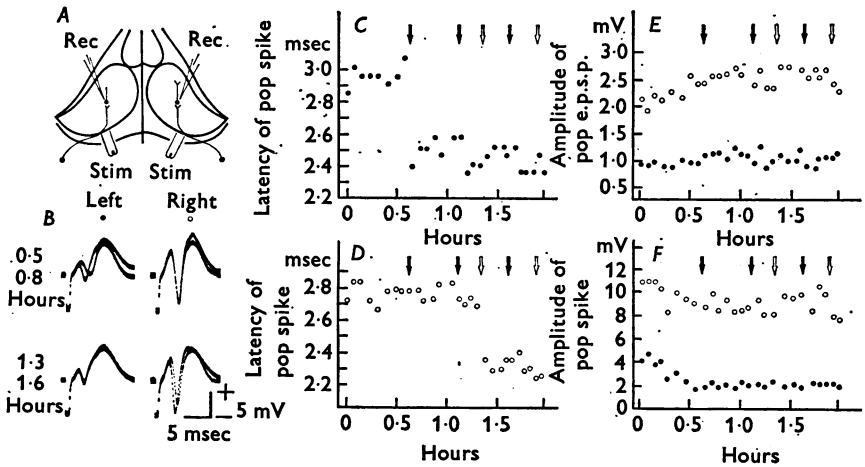

Fig. 10. Decrease in the latency of the population spike, without change in the amplitude of the population spike or e.p.s.p. *A*, electrode arrangement. *B*, superimposed averages obtained before and after conditioning first the left-hand pathway (upper row) and later the right-hand pathway (lower row), showing that only the conditioned pathway was affected in each case. The times at which the averages were obtained is given on the left. *C–F*, plots of the time courses of the three standard parameters for both pathways. Conditioning trains (15/sec for 10 sec) were given either to the left side (filled arrows) or to the right side (open arrows) at the times indicated. *C*, *D*, latency of the population spike for the left-hand pathway (*C*) and the right-hand pathway (*D*). *E*, *F*, plots of the amplitude of the population e.p.s.p. measured in the cell body layer (*E*) and the amplitude of the population spike (*F*) for both left- and right-hand pathways (filled and open circles respectively).

by the first conditioning train. Fig. 11*C* shows that no synchronous discharges of the granule cells occurred during the 100/sec train. Although extensive single unit recordings would be required to exclude the possibility of asynchronous spike discharges during the train, these results suggest that firing of the granule cells is not a requirement for long lasting potentiation of the e.p.s.p.

Duration of the after-effect

The longest after-effects we observed are illustrated in Fig. 12. The complete time course of the increase in amplitude of the population spike, for the experiment on Figs. 4–6, is shown in Fig. 12*A*. By the sixth conditioning train it was clear that no further increase could be induced. Thereafter, the spike remained undiminished until its sudden collapse nearly 7 hr later. By then the control responses had also declined markedly, suggesting that the over-all condition of the preparation had deteriorated.

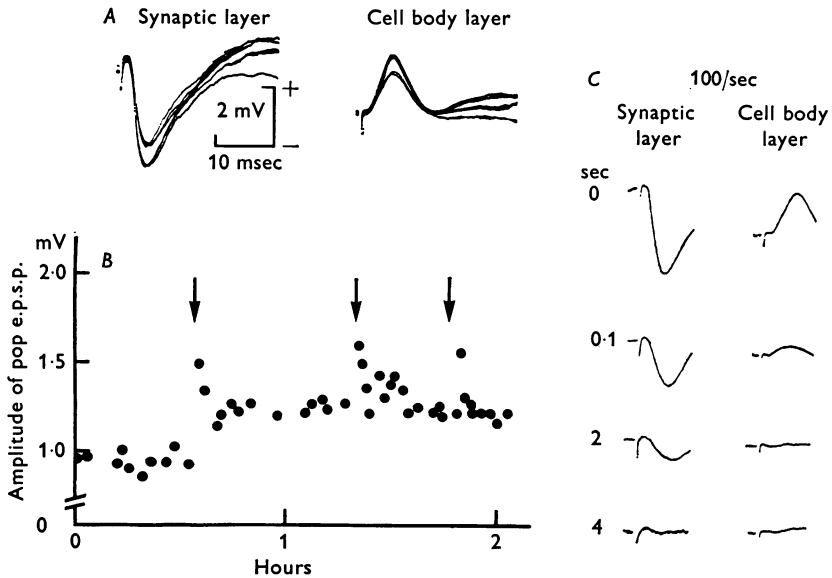

Fig. 11. Long-lasting potentiation after conditioning at 100/sec. Synaptic potentials recorded in both the synaptic layer and in the cell body layer before conditioning and 45 min after the first conditioning train are superimposed in *A*. The time course of potentiation is plotted in *B*. The amplitude of the e.p.s.p. was measured 1·5 msec after the onset of the negativity in the synaptic layer. Conditioning trains at 100/sec for 4 sec were given at the times indicated. The absence of any population spike during the conditioning train can be seen from the sample responses in *C*, taken from both synaptic and cell body layers at the indicated times after the start of the conditioning train.

In another experiment (Fig. 12*B*), almost maximal potentiation of the population e.p.s.p. was reached after the second of four trains given alternately at 15/sec and 100/sec, the two later trains producing only transient

further increases. Six to eight hr after the last train the amplitude began to fall off gradually, but was still well above its pre-conditioning value 10 hr after the last train.

DISCUSSION

The amplitude of an evoked population potential depends on a number of factors, and it will be helpful to review these before considering the possible mechanisms which might be responsible for long-lasting potentiation.

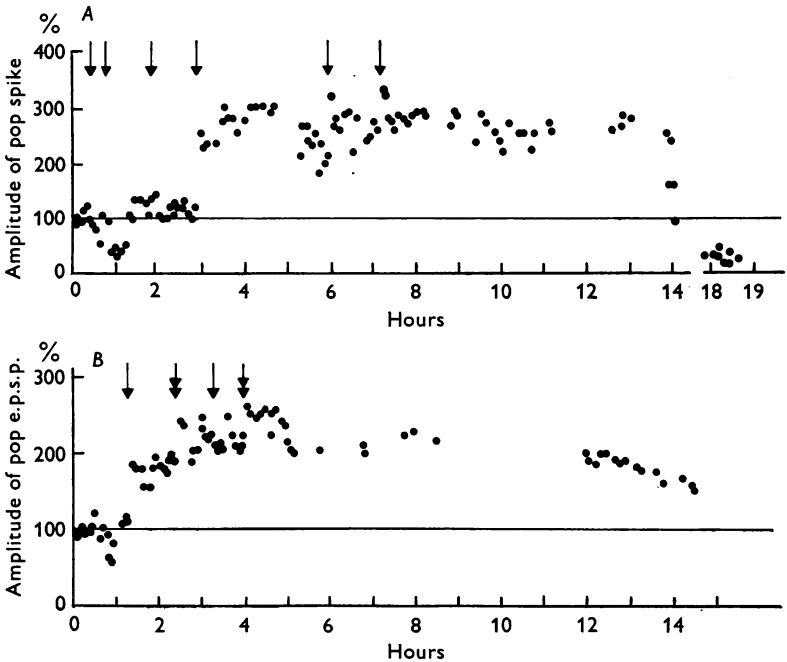

Fig. 12. Two examples of potentiation lasting for many hours. *A*, the complete time course of the potentiation of the population spike for the experiment presented in Figs. 4–6. A total of six conditioning trains, each at 15/sec for 10 sec was given. *B*, the time course of e.p.s.p. potentiation in another experiment in which conditioning trains at 15/sec for 15 sec, and 100/sec for 3 sec were given alternately (single and double headed arrows respectively).

As Rall & Shepherd (1968) have emphasized, the extracellular current which results from synchronous activation of a laminated cell population flows in a direction parallel to the long axes of the cells. From the point of view of the distribution of potential, whether actively or passively propagated, the situation resembles that of a nerve trunk in oil, and cable theory can be applied to extra- as well as intracellular potentials. The

amplitude of the extracellular potential, V_e, is related to the evoked membrane depolarization, ΔV_m, by the expression

$$V_e = -\frac{r_e}{r_i + r_e}\Delta V_m + K, \quad (1)$$

where r_i and r_e are the resistances per unit length of the internal and external media respectively. K is a term containing constants of integration which need not be considered further in this context, providing, as was always the case, the amplitude of the potential was measured at the same depth and latency throughout the experiment.

The magnitude of the depolarization in the region of the perforant path synapses, $(\Delta V_m)pp$, will depend on the extent to which the conductance change $(\Delta g)pp$, evoked by the perforant path volley, short-circuits the membrane. There are two factors to consider – the magnitude of the evoked conductance change itself, and the effective membrane conductance, G_m, in the absence of perforant path activity. If there is no other afferent activity we may use the lumped, resistive equivalent circuit introduced by Fatt & Katz (1953) for the end-plate to write

$$(\Delta V_m)pp = \frac{(\Delta g)pp}{(\Delta g)pp + G_m} E, \quad (2)$$

where E is the difference between the resting membrane potential and the equilibrium potential for the perforant path transmitter. In cases where other afferent activity is present, eqn. (2) is no longer strictly applicable, but it remains true that any increase in G_m due to additional shunting conductances will result in a reduction in the evoked depolarization $(\Delta V_m)pp$ (see Ginsborg, 1967, p. 307 for a quantitative discussion of this point).

Thus, it is the magnitude of the evoked conductance change, relative to the effective conductance of the membrane, that determines the magnitude of the evoked synaptic potential, and both these factors must be taken into account when considering the mechanisms underlying potentiation.

The most likely causes of an increase in the amplitude of the synaptic response may now be summarized:

(1) an increase in the number of perforant path fibres activated by the test shock;

(2) an increase in the efficacy of the conditioned synapses;

(3) a decrease in the level of tonic afferent activity.

The first two possibilities would work by increasing $(\Delta g)pp$ in eqn. (2); the third by decreasing G_m.

The amplitude of the population spike reflects the number and synchrony of granule cell discharges (Andersen *et al.* 1971*a*). For a given synaptic input, the number of granule cells discharged will depend on the

excitability of the population, and this could be controlled either by intrinsic factors, such as those which determine threshold, or by the extrinsic modulation of tonic excitatory and inhibitory afferent activity. Again, either mechanism might be available for long term modification. We now examine our results in the light of these various possibilities.

Evidence against an increase in the size of the perforant path volley

We were not able to detect a presynaptic compound action potential, and thus to obtain a direct measure of the size of the presynaptic volley. It is, however, unlikely that the number of perforant path fibres excited by the test volley increased significantly after conditioning, since there was no reduction in the threshold for evoking an e.p.s.p. after conditioning, and since the potentiated response could not be mimicked by increasing the size of the afferent volley with stronger shocks before conditioning. It seems evident from Fig. 6*A*, for example, that no pre-conditioning shock, however powerful, would have evoked an e.p.s.p. as large as those obtained with strong shocks after conditioning. Furthermore, after conditioning the magnitude of the spike parameters corresponding to a given synaptic input (i.e. to a given size of e.p.s.p.), were usually different from the values observed before conditioning: potentiation is thus not simply a question of moving up an 'input–output' curve (Fig. 6*D*), as would be expected under the hypothesis of a larger presynaptic volley. Finally, the evidence is conclusive that in some experiments potentiation of the population spike could not have been due to an increased perforant path volley, because there was in these cases no increase in the e.p.s.p. (Figs. 9 and 10).

Increased synaptic efficacy

The potentiation of the synaptic wave can in our view be most simply accounted for in terms of facilitated synaptic transmission. A similar conclusion was reached by Lømo (1971*b*) in his account of the brief facilitation of the e.p.s.p. which follows a single conditioning volley to the perforant path. Facilitation could take the form of an increase in the number of terminals invaded by the constant test volley, an increase in the amount of transmitter released per synapse, an increase in the sensitivity of the post-synaptic junctional membrane, or a reduction in the resistance of the narrow stem by which spines are attached to the parent dendrite (Rall, 1970). We have no evidence which could distinguish between these various possibilities.

Another possible mechanism for e.p.s.p. potentiation is a change in the level of tonic activity at other inputs. Such a change would affect the membrane conductance, which is one of the factors controlling the extracellular synaptic potential (eq. (2)). If, as a result of conditioning, there were a decline in tonic inhibitory activity to the same

region of dendrites as that occupied by the perforant path synapses, the reduction in local shunting would lead to a larger e.p.s.p., both in the synaptic layer and at other levels. Furthermore, the reduction in inhibitory tone would lead to an over-all increase in excitability which would be reflected in the degree of spike potentiation. It seems unlikely, however, that the tonic inhibitory input to the region of the perforant path synapses which this scheme requires could be so powerful that its suppression would result in the doubling or even trebling of the e.p.s.p. seen in some experiments. The available physiological evidence suggests that inhibitory afferents to the granule cells terminate predominantly on the cell bodies and proximal dendrites (Andersen *et al.* 1966; Lømo, 1968). This conclusion is supported by recent anatomical observations on the distribution of axonal terminals with spheroidal and flattened vesicles in the dentate area (Gottlieb & Cowan, 1972).

Increase in excitability of the granule cell population

The likelihood that the granule cell population is subject to continuous fluctuations in excitability was strongly suggested in several experiments by the observation that large variations in the size and latency of the population spike could occur from one stimulus to the next, without any change in the e.p.s.p. (Figs. 5 and 7). Variations in the level of tonic afferent activity is the most probable explanation for these fluctuations.

The effect of conditioning seems to have been twofold. On the one hand, the variability of the spike, and hence of the presumed fluctuations in tonic activity, was frequently reduced (see p. 345); on the other, the mean level of tonic activity was shifted so that over-all the cell population became more excitable. We have no evidence for which pathways, whether intrinsic or extrahippocampal, might be responsible for this effect.

It is possible to find another explanation for experiments in which spike potentiation was greater than allowed for by e.p.s.p. potentiation alone. If the not unreasonable assumption is made that a substantial number of granule cells are so weakly excited by the unpotentiated perforant path input that they do not fire however strong a perforant path volley is given (see Lømo, 1971*a*), then the cells for which this were true would yield a population e.p.s.p. but no population spike. If the efficiency of the synapses increased above threshold as a result of conditioning then an increase in the population spike would be produced which could not have been mimicked before conditioning by an increase in stimulus strength. Whether or not a mechanism of this sort plays a role in spike potentiation is undecidable on the present evidence; it cannot, however, explain those experiments in which spike potentiation occurred in the absence of e.p.s.p. potentiation.

Changes in synchrony of activation

The extent to which increased synchrony of discharge is responsible for the increase in spike amplitude is difficult to estimate. Generally it seemed to be the case that where spike variation, or potentiation, was superimposed on a stable e.p.s.p., there was little or no sign of increased synchrony, as estimated by the width of the spike at its base. In cases where there was substantial e.p.s.p. potentiation the correspondingly potentiated

spike was usually narrower at its base, suggesting that the increase in amplitude in these cases was at least partly due to increased synchrony of discharge, resulting, presumably, from a more rapid approach to threshold (see, for example, Fig. 5*A*).

Variability of the effect

A conspicuous feature of these experiments was the great variation in the degree of potentiation, both in different animals and in the same animal at different times and with different electrode positions (cf. Figs. 2 and 9 which are from the same animal). In three animals, no after-effect at all was seen, despite repeated conditioning trains. These differences cannot be explained as the result of differences in the perforant path input, since the monosynaptic e.p.s.p. was larger in some animals with little or no long-lasting potentiation than in others showing a marked effect.

Frequency potentiation

Frequency potentiation was present in the great majority of experiments. It is not, however, a sufficient condition for potentiation, as shown by the three animals in which we failed to produce any after-effect; in each case, frequency potentiation was well developed. Nor is it a necessary condition; in three cases (two animals) there was clear-cut potentiation after conditioning trains at 10–14/sec in which frequency potentiation failed to develop. In another three cases (two animals) e.p.s.p. potentiation was recorded after conditioning at 100/sec (Fig. 11). These experiments show that the synaptic component of potentiation, at least, is not dependent on the massive synchronous firing of granule cells which takes place during frequency potentiation. Whether or not the same is true of the excitability component of spike potentiation is a question of some interest, but one which we are unable to answer on the available evidence.

The critical parameters for conditioning

Potentiation occurred at all frequencies used for conditioning, i.e. 10–20/sec and 100/sec. The lower bound for producing an effect was greater than 0·5/sec, the rate used for testing. The number of stimuli during a train was normally 150–400. In one experiment a train at 5/sec for 4 sec produced an effect lasting 20 min, but we have not investigated systematically the minimum requirements for long-lasting potentiation, nor the relation between the size and duration of the after-effect and the number and frequency of the conditioning stimuli.

Significance of the effect

The interest of these results derives both from the prolonged duration of the effect, and from the fact that an identifiable cortical pathway is involved. The perforant path is one of the main extrinsic inputs to the hippocampal formation, a region of the brain which has been much discussed in connexion with learning and memory (Douglas, 1967; Olds, 1972). Our experiments show that there exists at least one group of synapses in the hippocampus whose efficiency is influenced by activity which may have occurred several hours previously – a time scale long enough to be potentially useful for information storage. Whether or not the intact animal makes use in real life of a property which has been revealed by synchronous, repetitive volleys to a population of fibres the normal rate and pattern of activity along which are unknown, is another matter.

REFERENCES

ANDERSEN, P., BLISS, T. V. P. & SKREDE, K. K. (1971*a*). Unit analysis of hippocampal population spikes. *Expl Brain Res.* **13**, 208–221.

ANDERSEN, P., BLISS, T. V. P. & SKREDE, K. K. (1971*b*). Lamellar organization of hippocampal excitatory pathways. *Expl Brain Res.* **13**, 222–238.

ANDERSEN, P., HOLMQVIST, B. & VOORHOEVE, P. E. (1966). Entorhinal activation of dentate granule cells. *Acta physiol. scand.* **66**, 448–460.

BLACKSTAD, T. W. (1958). On the termination of some afferents to the hippocampus and fascia dentata. *Acta anat.* **35**, 202–214.

BLISS, T. V. P. & GARDNER-MEDWIN, A. R. (1971). Long-lasting increases of synaptic influence in the unanaesthetized hippocampus. *J. Physiol.* **216**, 32–33*P*.

BLISS, T. V. P. & GARDNER-MEDWIN, A. R. (1973). Long lasting potentiation of synaptic transmission in the dentate area of the unanaesthetized rabbit following stimulation of the perforant path. *J. Physiol.* **232**, 357–374.

BLISS, T. V. P. & LØMO, T. (1970). Plasticity in a monosynaptic cortical pathway. *J. Physiol.* **207**, 61*P*.

BLISS, T. V. P., GARDNER-MEDWIN, A. R. & LØMO, T. (1973). Synaptic plasticity in the hippocampus. In *Macromolecules and Behaviour*, ed. ANSELL, G. B. & BRADLEY, P. B., pp. 193–203. London: Macmillan.

DOUGLAS, R. J. (1967). The hippocampus and behaviour. *Psychol. Bull.* **67**, 416–442.

FATT, P. & KATZ, B. (1953). The effect of inhibitory nerve impulses on a crustacean muscle fibre. *J. Physiol.* **121**, 374–389.

GINSBORG, B. L. (1967). Ion movements in junctional transmission. *Pharmac. Rev.* **19**, 289–316.

GOTTLIEB, D. I. & COWAN, W. M. (1972). On the distribution of axonal terminals containing spheroidal and flattened vesicles in the hippocampus and dentate gyrus of the rat and cat. *Z. Zellforsch. mikrosk. Anat.* **129**, 413–429.

HJORTH-SIMONSEN, A. & JEUNE, B. (1972). Origin and termination of the hippocampal perforant path in the rat studied by silver impregnation. *J. comp. Neurol.* **144**, 215–232.

LØMO, T. (1966). Frequency potentiation of excitatory synaptic activity in the dentate area of the hippocampal formation. *Acta physiol. scand.* **68**, suppl. 277, 128.

LØMO, T. (1968). Nature and distribution of inhibition in a simple cortex (dentate area). *Acta physiol. scand.* **74**, 8–9*A*.

LØMO, T. (1971*a*). Patterns of activation in a monosynaptic cortical pathway: The perforant path input to the dentate area of the hippocampal formation. *Expl Brain Res.* **12**, 18–45.

LØMO, T. (1971*b*). Potentiation of monosynaptic EPSPs in the perforant path – granule cell synapse. *Expl Brain Res.* **12**, 46–63.

NAFSTAD, P. H. J. (1967). An electron microscope study on the termination of the perforant path fibres in the hippocampus and the fascia dentata. *Z. Zellforsch. mikrosk. Anat.* **76**, 532–542.

OLDS, J. (1972). Learning and the hippocampus. *Rev. Can. Biol.* **31** (suppl.), 215–238.

RALL, W. (1970). Cable properties of dendrites and effects of synaptic location. In *Excitatory Synaptic Mechanisms*, ed. ANDERSEN, P. & JANSEN, J. K. S., pp. 175–187. Oslo: Universitetsforlaget.

RALL, W. & SHEPHERD, G. M. (1968). Theoretical reconstruction of field potentials and dendrodendritic synaptic interactions in olfactory bulb. *J. Neurophysiol.* **21**, 884–915.