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

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SUMMARY

1. Potential changes evoked by stimulation of the perforant path have been recorded in the dentate area of the hippocampal formation in chronically prepared unanaesthetized rabbits.

2. Components attributed to excitatory synaptic current flow and to action potentials in the granule cell population were distinguishable, with characteristics largely the same as in anaesthetized rabbits.

3. Stimulation at 15/sec for several seconds usually led to the granule cells being more effectively activated by the individual stimuli of the train ('frequency potentiation'). Single stimuli then commonly produced multiple discharges in the granule cell population.

4. After single periods of stimulation at 15/sec for 15–20 sec there was on 26% of the occasions (41% of those on which there was good frequency potentiation) a long-lasting potentiation of the responses to subsequent stimuli, lasting from 1 hr to 3 days.

5. After a further 20% of the periods of repetitive stimulation there was a shorter lasting potentiation, and after 8% there was a short lasting depression.

6. The potentiation, when present, was characterized by some or all of the following changes: increases in the amplitudes of the synaptic wave and population spike, reduction in the latency of the population spike, and reductions in the variability of the characteristics of the population spike.

7. During the long-lasting potentiation there was an increase in the excitability of the post-synaptic cells and, on some but not all occasions, an increase in the extracellular current flow produced directly by synaptic action.

INTRODUCTION

In previous work on the synaptic activation of the granule cells in the dentate area of anaesthetized rabbits, it has been shown that brief periods of high-frequency stimulation of the perforant pathway can enhance the effects of subsequent stimulation for periods of several hours (Lømo, 1966; Bliss & Lømo, 1970, 1973). In the present experiments the same phenomenon has been investigated without anaesthesia and under the more stable conditions which can be obtained by using chronically prepared animals. Since an increase in the effectiveness of synapses may be a process underlying some forms of memory, it was considered important to establish whether long-lasting synaptic changes could be produced in animals in an approximately normal physiological condition.

Brief accounts of this work have been published (Bliss & Gardner-Medwin, 1971*a*; Bliss, Gardner-Medwin & Lømo, 1973).

METHODS

The experiments were performed on 2–3 kg rabbits after recovery from an operation to implant electrodes at fixed sites in the brain. The electrodes were positioned under Nembutal anaesthesia (*ca.* 40 mg/kg, I.V.). The rabbit's head was held in a non-traumatic head-holder, and its skull was exposed and scraped clean. The upper surface of the hippocampal formation on one side was exposed through an opening in the bone approximately 10 mm square, by carefully sucking away the overlying neocortex. The lesion was made just large enough to allow the convex surface of the hippocampus and the transverse groove which marks the posterior parts of the ventricle to be identified. The transverse groove, which the superficial commissural fibres known as the 'angular bundle', provided the main landmark for positioning the electrodes. Bleeding was controlled by lining the sides of the lesion with haemostatic cellulose net (Surgicel, Ethicon Ltd.). The surface of the hippocampus was kept moist with 0·9 % saline.

The recording electrodes consisted of single enamelled stainless-steel wires (100 μm diam.) cut off at their tips at an angle of about 45°. The stimulating electrodes were pairs of enamelled steel or platinum wires glued together along their length and cut at their tips so that one led the other by about 0·4 mm. Monopolar recording was used, with the animals earthed through a soft stainless-steel wire looped through holes drilled in the frontal bone.

The procedure for positioning the electrodes was based upon the description of the relation between the responses and the anatomy of the hippocampal formation given by Lømo (1971*a*).

A recording electrode was first lowered to about 2 mm beneath the convex hippocampal surface, into the dentate area. It was then possible by stimulating the angular bundle to obtain disynaptic responses, characterized by a latency greater than 5 msec and severe depression of the response to a second shock. The recording electrode was moved up and down to check that the evoked potential over a range of electrode positions less than about 200 μm is the crucial evidence that the potential is generated locally in the dentate area. The point of reversal has been shown (Lømo, 1971*a*) to be within the molecular layer of the dentate area, about one third of the way out along the dendrites from the granule cell bodies.

With the recording electrode in position about 0·5 mm below the point of reversal, the bipolar stimulating electrode was moved to produce monosynaptic responses from direct stimulation of the perforant path. The optimum position was found by trial and error, usually at a site about 1–2 mm in front of the angular bundle at a depth of 1–2 mm. The criterion of a satisfactory placement was that it produced a response with a latency less than 3 msec showing a clear reversal when the recording electrode was moved up and down, and that the response to a second shock after 22 msec showed a clear, potentiated, synaptic wave. With the recording electrode in a medial position in the hippocampus, two stimulating electrode pairs could be placed more than 2 mm apart in such a way that both gave good responses at the one recording electrode.

When the recording and stimulating electrodes (sometimes two of each) had all been placed satisfactorily, they were cemented in position with dental acrylic cement (Simplex). As much fluid and blood was removed from the cavity in the brain as possible, and it was filled with acrylic. Sometimes there was an epileptic discharge during the setting of the acrylic and a subsequent depression, but this was nearly always followed by a complete recovery. A sudden or gradual deterioration of the responses was occasionally seen, and was attributed to movement of the brain, perhaps due to bleeding. When none of the combinations of recording and stimulating electrodes gave responses which had remained substantially unaltered, either a new set of electrodes was tried, or the animal was rejected.

After the electrodes had been cemented in position, the block of acrylic was attached rigidly to the skull by extending it to envelop stainless-steel screws secured in the bone. The electrodes were soldered to a Cannon Micro-K (seven-way) socket, which was then also embedded in acrylic. The skin wound was sewn up as much as possible. The animal was given penicillin (0·5 ml. Penidural, I.M., repeated daily for 2 days), and allowed to recover.

Two animals (JA, WG) developed an infection within a fortnight and were killed. Five others survived for periods of several months, continuing to give satisfactory responses with at least some electrodes.

During recording sessions the animals were in a large deep box, without a lid. They had access to food and water *ad lib*. The implants were connected to the apparatus by two multicore screened cables (one for recording; one for stimulating) about 3 m long, suspended on elastic thread. The animals needed no attention for long periods, and recording sessions often went on all night with automatic recording equipment.

Stimuli were derived from 0·05 msec pulses from Devices isolated stimulators, with the bipolar stimulating electrodes often connected through one or two 0·01 μF capacitors to reduce the stimulus artifacts.

A Linc-8 computer was used for on-line analysis and recording. The main functions of the computer were to collect and average responses, to measure various parameters of the responses, and to store and plot the information on a Calcomp incremental plotter or on digital magnetic tape. In addition it also provided a visual display in the experimental room of various recent responses, and plots of recent values of selected parameters. The program was switched into different modes by pulses from two Devices Digitimers, which controlled the timing in the experiments. Off-line programmes enabled plots of responses and parameters to be made from the information stored on magnetic tape. A short account has been published, giving a common sequence of the operations of the computer (Bliss & Gardner-Medwin, 1971*b*).

Various parameters were required from the responses (Fig. 1*b*). The height from the base line (taken as the last sampled point before the stimulus) was calculated at a fixed time after the stimulus, during the rising phase of the synaptic wave. The heights and times of the maxima and minima corresponding to the onset and peak

of the population spike were obtained, and the difference between the two heights (called the 'amplitude of the population spike') was calculated. The points taken as maxima and minima were the first points, within a restricted range of times, which were higher or lower than points on either side of them. No smoothing was employed before deriving the stationary points, since even the individual responses were very smooth and free from noise. When the population spike was too small to produce a maximum and minimum on the response, its amplitude was counted as zero and the times to its onset and peak were registered as unmeasurable. All the points used by the computer for deriving parameters were marked on the visual display of the responses, so that a check could be kept that spurious points were not being chosen for any reason. This was in fact never any problem.

During a period of high-frequency stimulation the computer was not used for recording. The responses were filmed directly from the oscilloscope.

RESULTS

Responses to single and double stimuli. The responses after the animals had recovered from the anaesthetic were generally similar to the responses at the time of implantation. When there was a substantial change the animal was rejected.

Examples of the responses recorded after recovery are shown in Fig. 1(*a*) Two equal shocks were given 22 msec apart at each of the indicated strengths. The response to the first shock at low strengths was simply a smooth positive wave (the 'synaptic wave' or 'population e.p.s.p.' (Lømo, 1971*a*)). As the strength was increased this positive wave increased in size and acquired a superimposed negative wave. This negative wave (the 'population spike') increased in amplitude and decreased in latency with increasing strength, and from one shock to another was often extremely variable. These features of the first response reflect closely the description given by Lømo (1971*a*).

The response to the second shock at low stimulus strengths was also a smooth synaptic wave, which was often larger than the first response. This potentiation was not as marked as at the time of the operation, or as has been described by Lømo (1971*b*) in acute preparations. In some animals potentiation was not present at all after recovery. In all the animals the potentiation (if any) at low shock strengths was replaced at high strengths by a reduction of the synaptic wave produced by the second shock (Fig. 1(*a*), at 10 V).

When a high stimulus strength was used there was never a short latency population spike in the second response. This can be attributed to recurrent post-synaptic inhibition (Andersen, Holmqvist & Voorhoeve, 1966). Because of the lack of a population spike, the second response varied less than the first response from one pair of shocks to another. This made it possible to observe some of the after-effects of a conditioning train of stimuli more clearly.

Responses to stimuli at 15/sec. Trains of stimuli at 15/sec for 15–20 sec were used in these experiments for attempting to induce long-lasting after-effects. A variety of changes took place in the responses during the course of these trains. These short term changes will be considered here before the after-effects are described.

Fig. 2 shows some stretches of a film of the consecutive responses during a train of stimuli at 15/sec. There were three main phases of the responses which were seen during trains of stimuli in each animal, though they

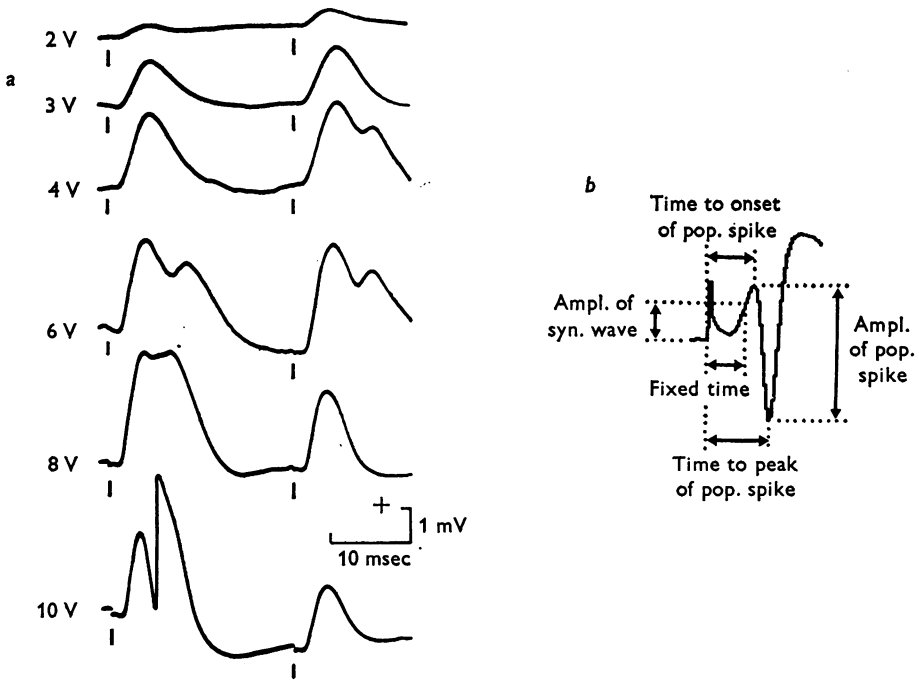

Fig. 1. *a*, Responses to two equal stimuli at each of the indicated strengths. Inter-stimulus interval, 22 msec. Animal SD. *b*, a typical average response recorded using the computer, with the derived parameters labelled on it. Average of sixteen responses.

varied in their relative durations. The initial phase, often following a depression of the second or second and third responses, was a period with a clear synaptic wave in each response but only a small or absent population spike. Frequently a population spike would be present and absent on alternate responses, as in Fig. 2 after about 4·5 sec. The second phase was a period during which the whole response became severely depressed, as in Fig. 2 after about 9·5 sec. This was followed by the third phase, in which several population spikes appeared in each response, often 3 or

4 mV in amplitude, and sometimes as many as four in each response. This third phase with enhanced activation of the granule cells can be seen developing over the period 11·5–15·5 sec in Fig. 2. Its mechanism is not completely understood, but has been given the name *frequency potentiation* (Andersen *et al.* 1966). During prolonged trains of stimuli there was sometimes a final fourth phase of depression, in which the multiple population spikes fell in number and amplitude.

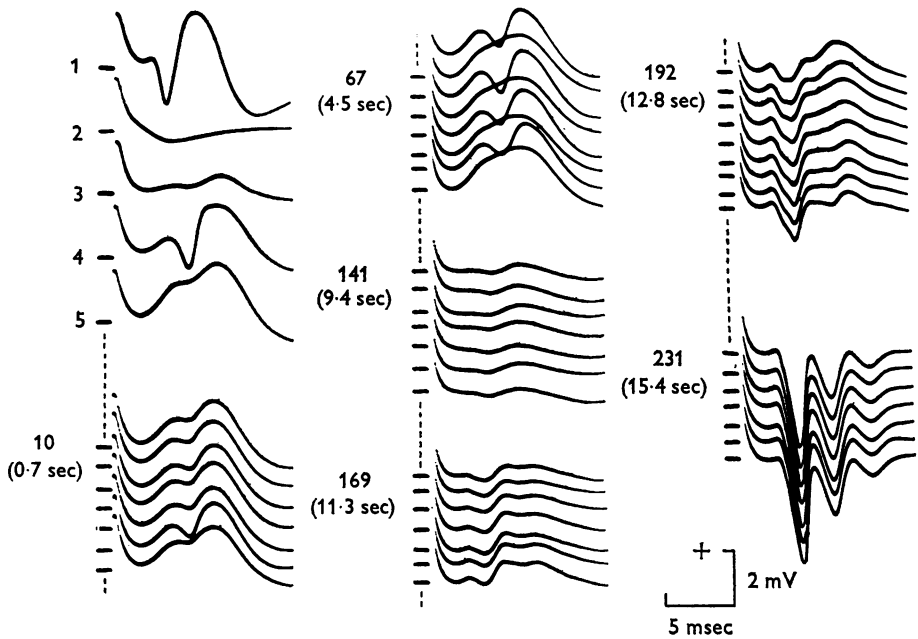

Fig. 2. Responses during a train of stimuli (30 V) at 15/sec. Short samples of the sequence of successive responses are shown, with the sequential number of individual stimuli and their approximate times during the train indicated. Animal HE.

The durations of the various phases varied from animal to animal, and with the strength of stimulation. With low stimulus strengths the responses did not get beyond the first phase, with a synaptic wave and a depressed or absent spike.

In two animals (JA, SD) stimuli which were capable of producing large population spikes when presented singly sometimes failed to produce frequency potentiation during a 15/sec, 15 sec train. The reason for this was not clear.

After-effects using the same electrodes for conditioning and testing. When a pair of electrodes was used for delivering a conditioning train of

stimuli we always recorded responses to stimuli given at a low rate at the same electrodes before and after the conditioning train, in order to test for after-effects. In some experiments, described in the next section, we could also use a separate pair of electrodes for testing.

In the following sections the word *train* will be used to refer to a train of stimuli given at 15/sec.

In all, 121 trains were given to seven animals. Nearly always a gap of at least ½ hr was allowed between trains, and more when an after-effect was apparent. Thirty-two trains (26%) produced clear potentiation of

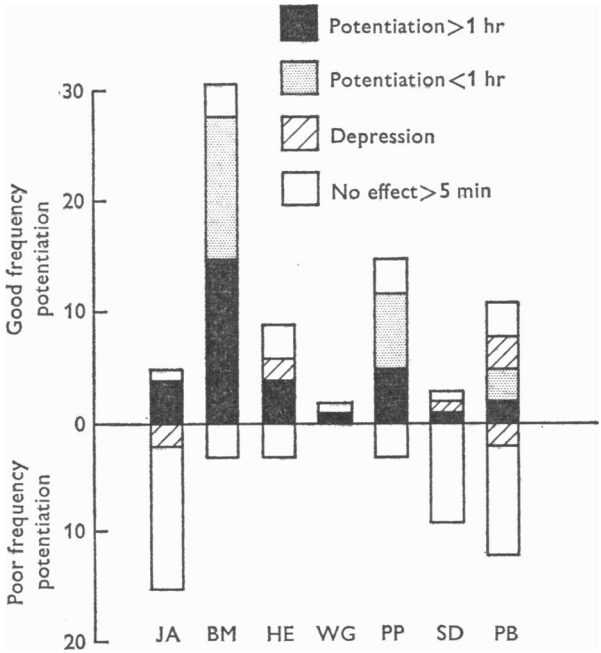

Fig. 3. The distribution of the types of after-effects seen after trains of stimuli at 15/sec in the different animals. Trains which produced good frequency potentiation (two or more large population spikes in the responses at some time during the train) are separated from those which produced little or no frequency potentiation by the horizontal line.

subsequent responses for 1 hr or more. Twenty-four trains (20%) produced potentiation lasting less than 1 hr. Ten trains (8%) produced a depression, lasting generally for about ½ hr. The other trains (46%) were without any detectable effect lasting more than about 5 min. The individual results for the separate animals are shown in Fig. 3.

Those trains which produced a long-lasting potentiation had all produced frequency potentiation during the stimulus train, with two or more

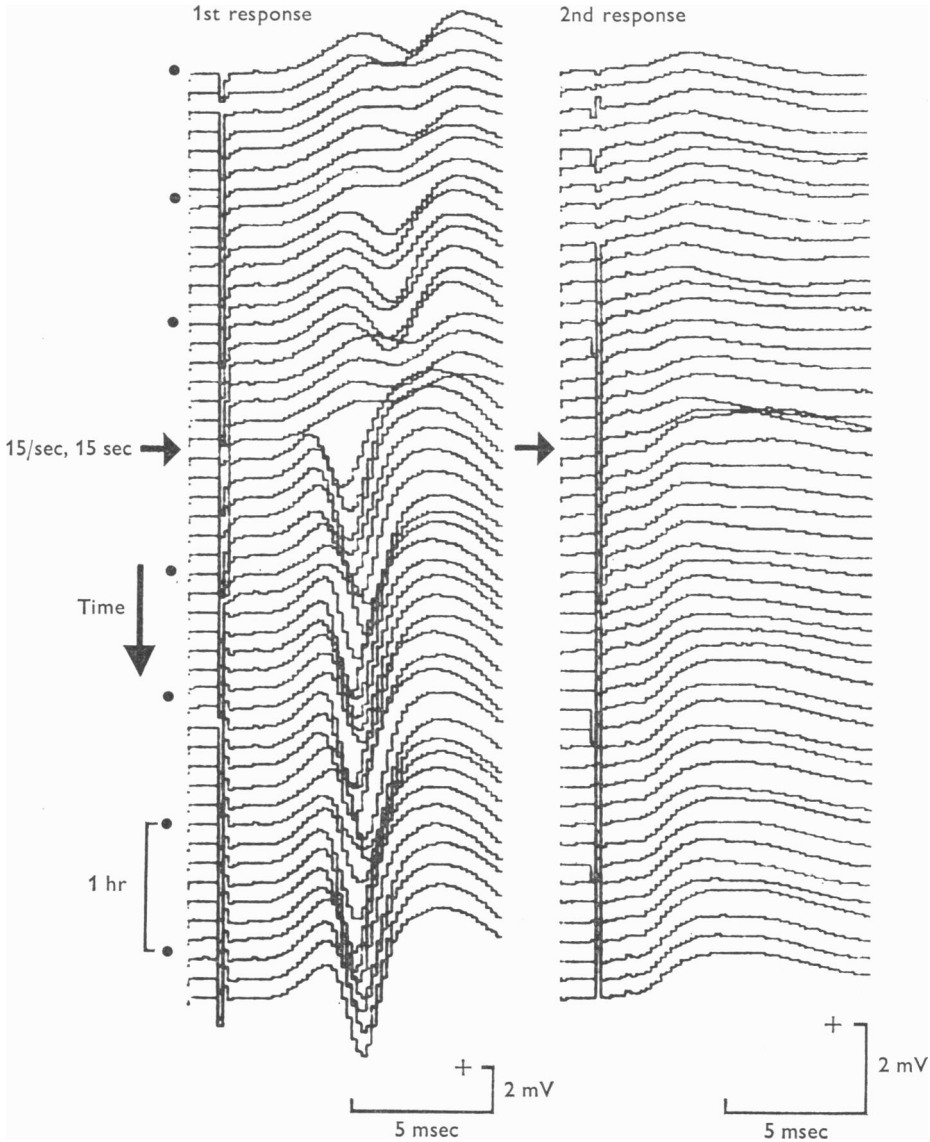

Fig. 4. Average responses recorded every 9 min and plotted in sequence down the page. Averages (of 16) are shown for both the first and second responses to pairs of equal stimuli (30 V, 22 msec apart). A train of stimuli (30 V, 15/sec, 15 sec) was given at the time indicated by an arrow. Animal WG.

large population spikes in each response for several seconds. Sixty-three per cent of the trains produced marked frequency potentiation defined in this way, so the trains which produced long-lasting potentiation (longer than 1 hr) constituted 41 % of those which produced marked frequency potentiation.

In Fig. 4, successive average responses before and after a train are reproduced in sequence, drawn by the computer. Two stimuli were delivered 22 msec apart every 30 sec, so that an average of 16 was obtained and plotted for both the first and second responses every 9 min. Each new average is drawn beneath the one before. After about 3 hr of control recording, the sequence was interrupted for a minute or so for a 15 sec period with 15/sec stimulation. The effect on the subsequent responses to the test stimuli is clear. The slope of the initial positive-going phase (the synaptic wave) of both the first and the second responses was increased. The amplitude of the population spike in the first response was increased, and its latency was decreased.

In the experiment of Fig. 5 stimuli were presented at a higher rate (two stimuli, 22 msec apart, every 6 sec). The averages of parameters measured from individual responses are plotted against time for the period before and after the train, and for short periods during the next 2 days. Sample average responses from before and after the train are shown. Each of the measured parameters can be seen to have changed as a result of the conditioning train.

In Fig. 5 there is a clear reduction in the variability of the amplitude of the population spike. This was seen many times, though it was seldom as marked as here. The population spike also increased gradually over about $\frac{1}{2}$ hr, though the other changes reached their maximum in a few minutes. A change of the population spike taking only a few minutes was more common.

Figs. 4 and 5 are chosen because they illustrate within single experiments all the main changes seen during potentiation. An increase in spike amplitude was seen most commonly (26 times) during the 32 periods of potentiation which lasted more than 1 hr. An increase in synaptic wave amplitude was seen 19 times, and a reduction in spike latency 10 times.

On two occasions a reduction of spike latency provided the only sign of potentiation. Each of these was the first occasion on which the respective animals (JA and SD) had shown long-lasting potentiation. On one of them (animal JA) there was actually a decrease in spike amplitude, although the over-all effect (which lasted for 2 hr) was nevertheless classed as long-lasting potentiation. On no other occasion did any two of the various signs of potentiation point in contrary directions.

When the parameters of the test responses were plotted as functions of the stimulus voltage (Fig. 6), it was evident that there was no change

in the threshold for a synaptic wave after a train, and that the population spike was generally earlier or larger even when compared to responses with an equal-sized synaptic wave before the train.

The duration of the potentiation produced by single trains varied. The measured parameters returned gradually towards control levels over periods of up to 3 days. On eleven occasions the duration was more than

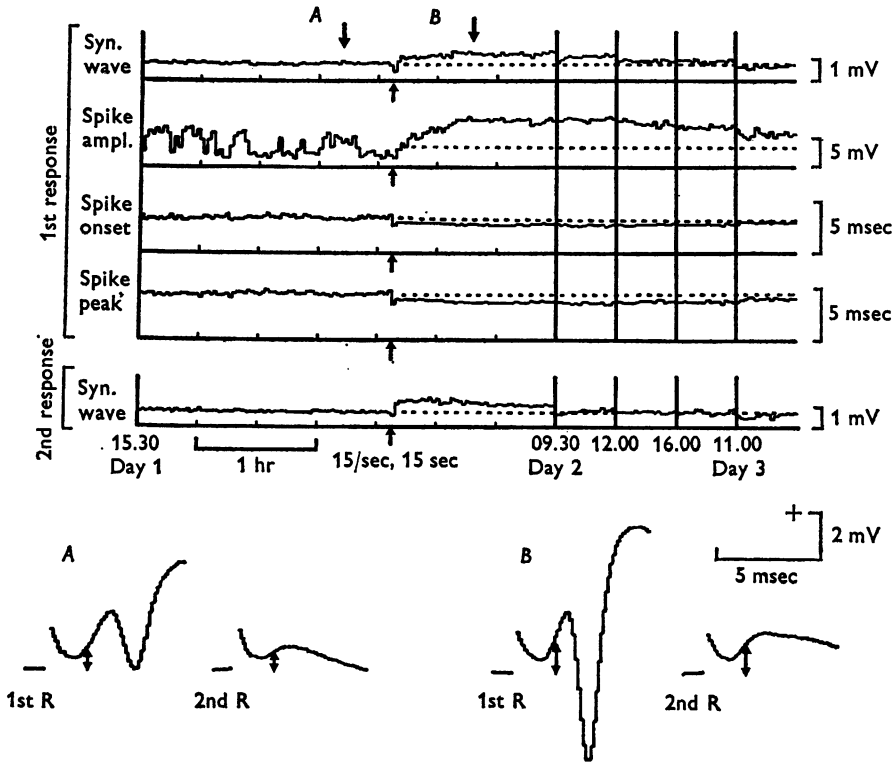

Fig. 5. Average parameters of the responses to pairs of stimuli (50 V, 22 msec apart, averaged in groups of 16), plotted over the period before and after stimulation at 15/sec (50 V) for 15 sec, and for short periods during the next 2 days. Dotted lines indicate the approximate average values before the conditioning train. Average responses recorded at the times marked *A* and *B* are shown, with the measurements of the synaptic wave amplitudes indicated on them by arrows. Animal HE.

6 hr. Sometimes there was a gradual drift downwards of the control responses during the recording session, in the opposite direction to potentiation. This made it difficult to know how long the longer periods of potentiation really did last (see, for example, the after-effect illustrated later in Fig. 9). The downward drifts were reduced by connecting the stimula-

ing electrodes through capacitors (see Methods), and were probably due to a cumulative effect of the current pulses at the stimulation site. Upward drifts of the control responses were not seen.

We cannot conclude from our data whether or not previous trains given to an animal increased or decreased the likelihood that a particular train would produce long-lasting potentiation. In five animals the first train which gave marked frequency potentiation also produced long-lasting

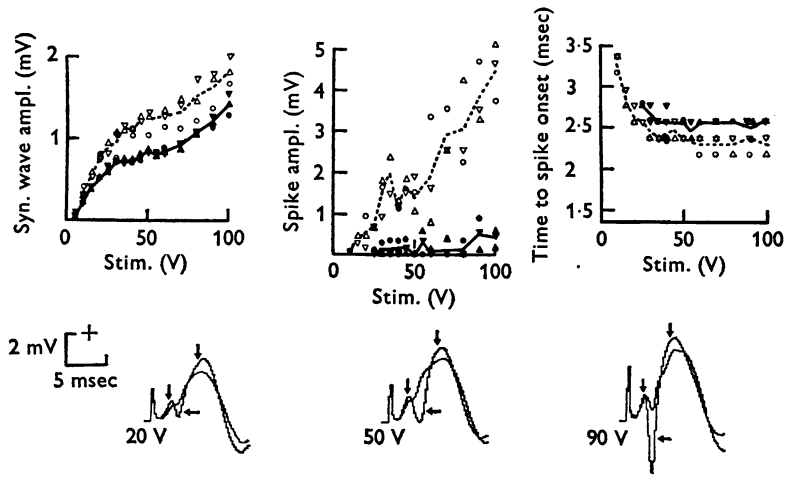

Fig. 6. Graphs of three parameters measured from the average responses to single stimuli, plotted against stimulus strength at various times before (filled symbols) and after (open symbols) a period of stimulation at 15/sec (50 V) for 15 sec. Times in hours relative to the time of the conditioning train: ▼, −4·2; ▲, −2·5; ●, −1; ○, +1; △, +2·3; ▽, +5·2. Lines are drawn through the average values before and after the train. Superimposed average responses at three strengths are shown from 1 hr before and 1 hr after the train: the later response is indicated by arrows. Averages: 16 responses. Animal PB.

potentiation, and in two of these animals this was the very first train. In one animal (PB) we gave thirteen trains (at least three of them producing marked frequency potentiation) before we produced a clear after-effect, which was then a substantial one (illustrated later in Fig. 8).

Frequently a train with a high stimulus strength was effective at producing potentiation when one at a lower strength had failed, even when the lower strength shocks were equal to the test shocks. This is illustrated in Fig. 7, in which test responses were produced with 30 V shocks, while trains were given at strengths of 10, 30 and 60 V. Only the 60 V trains produced subsequent potentiation (an increase of the spike amplitude only), although both the 30 and 60 V trains produced marked frequency potentiation. After a series of nine trains at these strengths (three each),

the spike amplitude remained above control levels during recordings over 16 weeks. This may indicate that an exceedingly long after-effect was produced, though it is hard to be certain that the spike amplitude might not have increased gradually and spontaneously over such a long period.

During periods of up to 3 days with continuous or repeated recording and without any trains, there were no spontaneous changes in the responses resembling the effects of a train. Periods of a few minutes at a time

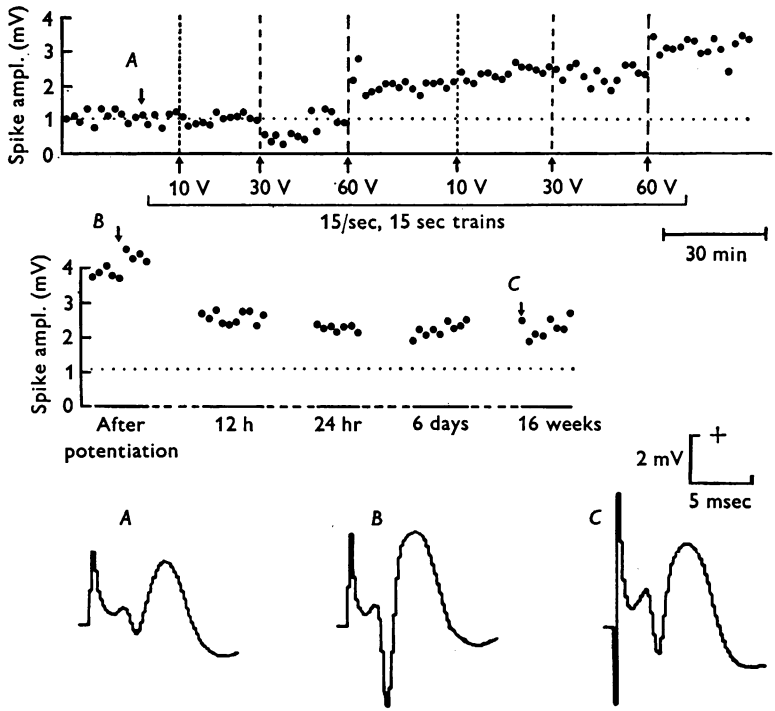

Fig. 7. Measurements of spike amplitude (from averages of sixteen responses to 30 V stimuli) plotted as several trains of stimuli (15/sec, 15 sec) were given at the indicated strengths, and at various indicated times afterwards. The approximate mean spike amplitude before the conditioning trains is shown by the dotted lines. Average responses at the times marked *A*, *B* and *C* are shown beneath. Animal HE.

occurred, during which the average population spike amplitudes were higher or lower than normal (see, for example, Figs. 4 and 5). These periods were short, and were not associated with any obvious changes in the behaviour of the animals, or with whether they appeared to be asleep or awake. It is possible that more careful study could reveal such associations, however.

After-effects detected with a different stimulating electrode. In three animals (BM, PP, PB) we had two stimulating electrodes producing satisfactory responses in the hippocampus on the same side of the brain. In these animals we could look for transfer of the after-effects of repetitive stimulation. There were fifteen occasions in these animals on which we found long-lasting potentiation of the responses obtained from the conditioning site while we were also testing the responses to stimulation at a second site. On nine of these occasions (BM 5, PP 2, PB 2) we saw a similar long-lasting potentiation of the responses to stimulation at the other site. On six

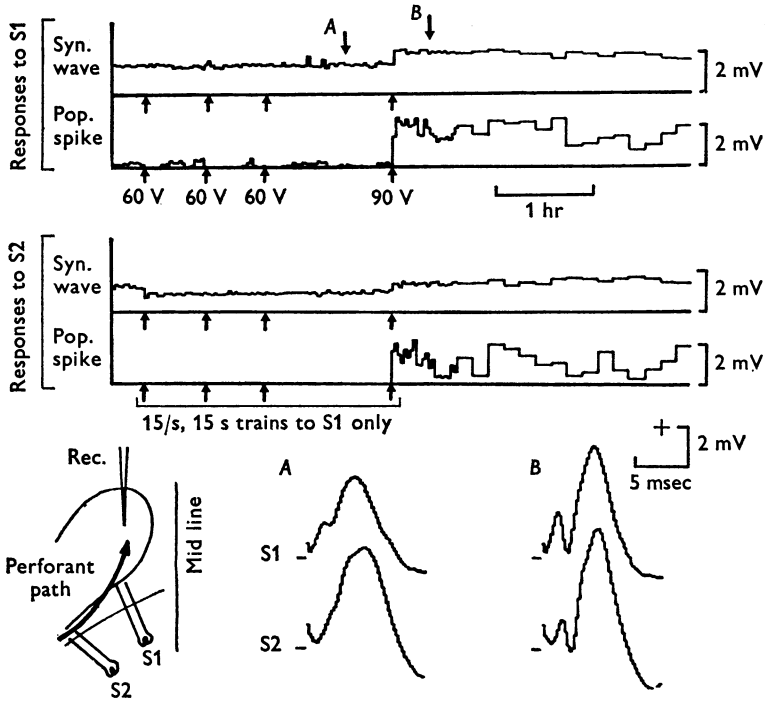

Fig. 8. The synaptic wave and population spike amplitudes of responses at a single recording site to 60 V stimuli given alternately at two stimulation sites 3 mm apart. Trains of stimulation at 15/sec for 15 sec were given at the indicated strengths and times to S 1 alone. Sample average responses are shown below, from the times marked *A* and *B*. Animal PB.

occasions (BM 5 and PP 1) we saw no effect, or some depression, tested with the separate stimulus. In each case we were using the same electrode for recording the responses to both stimuli. Examples of the presence and absence of transfer are shown in Fig. 8 and Fig. 9.

There is little evidence which helps to explain why in two of the animals

there was sometimes transfer and sometimes not. The two results were sometimes obtained with identical trains; and transfer did not appear to become either more or less likely as successive trains were given to the same animal.

Behavioural observations. Stimulation had no marked effect on the behaviour of the animals. Single shocks given at low rates did not seem to affect behaviour at all. In four animals there was sometimes evidence

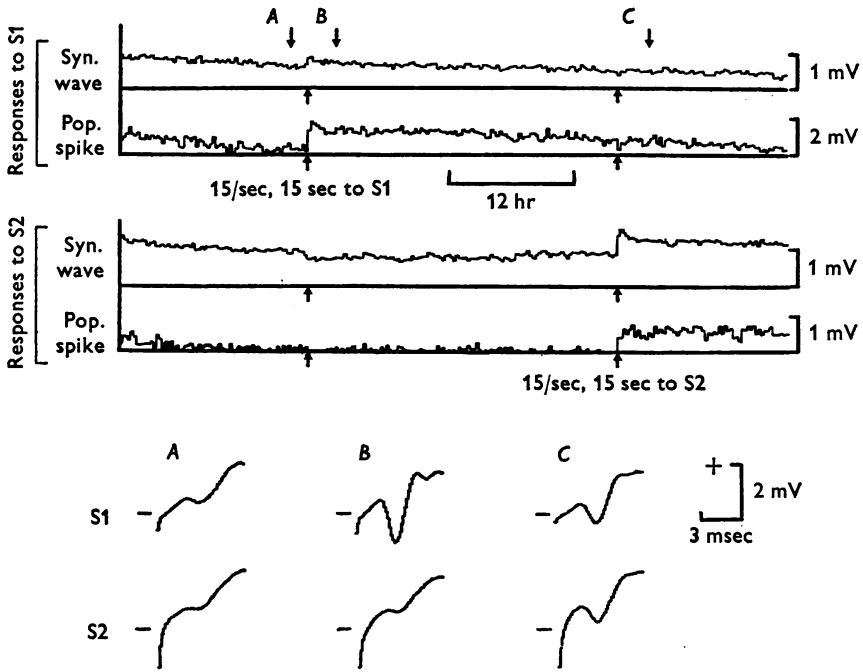

Fig. 9. The synaptic wave and population spike amplitudes of responses at a single recording site to stimulation at two sites 2 mm apart. Test stimuli: S 1, 70 V; S 2, 50 V. Trains of stimuli at 15/sec for 15 sec were given at S 1 (70 V) and at S 2 (100 V) at the indicated times. Average responses to the test stimuli are shown below, from the times marked *A*, *B* and *C*. Animal BM.

of restlessness during a conditioning train, characterized by rapid eating and chewing movements, and by circling in the box. One of these animals (PB) kicked the floor vigorously with its hind legs during and immediately after some of the trains. In no case did the observed behavioural effects outlast a train by more than 2–3 min.

DISCUSSION

The first, and most important, conclusion which can be drawn from this work is that long-lasting potentiation in the dentate area can be produced even in healthy, active, unanaesthetized animals. It is thus unlikely that the effect requires the synapses or cells to be initially in a depressed unphysiological state.

The second main conclusion is that the after-effects of single periods of repetitive stimulation may last for more than 24 hr, but do not last indefinitely. The potentiation which followed just a single train was studied most commonly in these experiments, since it was found that a single train was often sufficient to produce a large after-effect. The duration of the after-effects of these single trains varied widely, but was never longer than 3 days. It is possible that the effects of repeated trains may last for longer. This was suggested by one experiment in which there was an increased spike amplitude for several weeks after a series of trains at different strengths (Fig. 7).

The responses to single and double shocks. The characteristics of the responses were largely similar to those in acute anaesthetized preparations (Lømo, 1971*a*, *b*).

Even in the unanaesthetized preparations single perforant path volleys were unable to activate more than a small proportion of the granule cells, since the population spike produced by single volleys was much smaller than that produced during repetitive stimulation (when there is *frequency potentiation*). The population spike produced by single volleys was also extremely variable, so that some shocks produced a normal-sized synaptic wave with practically no population spike. The variability was often markedly reduced after a train which led to long-lasting potentiation. Both this change and the increase in excitability of the granule cells which was generally seen during potentiation can be explained if there was a reduction of some varying inhibitory influence on the cells following a conditioning train.

There was either no potentiation or only slight potentiation of the synaptic wave produced by a second stimulus at intervals of about 20 msec, even with low stimulus strengths. With high stimulus strengths the synaptic wave in the second responses was depressed. These characteristics of the second response are unusual in acute anaesthetized preparations, although in recent work (A. R. Gardner-Medwin & T. Lømo unpublished) acute preparations which closely resembled the chronic preparations have sometimes been found. It seems likely that the short-lasting potentiation of the synaptic wave described by Lømo (1971*b*) is due to an increase in the effectiveness of individual activated synapses,

and that the magnitude of this intrinsic property of the synapses varies between preparations. The depression of the synaptic wave after large volleys in the acute preparations has been found in the cell body layer and not in the synaptic layer, and thus is probably associated with a change in the relative conductance of different parts of the granule cell membranes.

The conditions for producing long-lasting potentiation. In some animals we were able to demonstrate long-lasting potentiation with test stimulating electrodes more than 2 mm from the site of conditioning. This, together with the absence of any demonstrable threshold change at the conditioning electrodes, rules out the possibility that the phenomenon was due to a local change at the conditioning site.

When after-effects were studied by using test shocks of one strength, it was often found that conditioning trains at the same strength were ineffective at producing potentiation while conditioning trains at higher strengths were effective. This suggests that the phenomenon was not simply a result of the frequent activation of the synapses, since the synapses activated with the weak shocks were presumably activated during conditioning in just the same way whether weak shocks or strong shocks were used during a train. Thus the effect on the post-synaptic cells during conditioning seems likely to be important in determining whether or not potentiation takes place. The fact that potentiation was only seen in this study on occasions when there had been marked frequency potentiation strengthens this suggestion, though on three occasions in the study with anaesthetized rabbits long-lasting potentiation was seen without frequency potentiation (Bliss & Lømo, 1973).

Long-lasting potentiation was not produced on all occasions when there was frequency potentiation, and on some occasions there was no transfer to the responses produced by stimulating with separate test electrodes. This variability makes the phenomenon difficult to work with, and shows that there must be hidden factors which are important in the conditions for producing potentiation.

The nature of the changes underlying the potentiation. The main possibilities for the basis of the potentiation are a change in the number of fibres stimulated, a change in the number of synaptic boutons to which activity in any one fibre can propagate, a change in the effectiveness of the synapses, a change in the intrinsic properties of the granule cells, and a change in the tonic excitatory or inhibitory influences which the granule cells may be subject to.

An increase in the number of fibres activated is unlikely since there was no detectable decrease in the electrical threshold for producing a response, and since the potentiation could not be mimicked by recruiting extra fibres with a larger stimulus before conditioning.

The fibres of the perforant path make numerous synapses *en passage* on to the granule cell dendrites (Blackstad, 1958). So a failure in transmission of an action potential somewhere along the axon could result in fewer synapses being activated. If such failure became less likely after a conditioning train, the effects would not have been distinguishable from changes due to an increase in the effectiveness of individual synapses.

The increase in the synaptic wave which was sometimes (but not always) seen during potentiation suggests that there was an increase in the effectiveness of individual synapses or in the number of synapses activated. The synaptic wave is a measure of the current which flows into the cells in the synaptic region of the molecular layer and out of the cells primarily in the cell body layer; it starts before the cells undergo an action potential (Lømo, 1971*a*). The increase could be produced either by an increase in the number or effectiveness of the activated synapses, or by a change in the distribution of conductance along the cell membranes. It is not possible to be certain on present evidence which of these factors is responsible.

Increases in amplitude of the population spike and decreases in its latency have sometimes been seen with no change in the synaptic wave. Such combinations were seen also in the acute anaesthetized preparations (Bliss & Lømo, 1973). It seems likely therefore that at least a component of the potentiation must be due to an increase in post-synaptic excitability. This could be due to a change of the tonic influences acting on the granule cells, or to a change in their inherent excitability.

The significance of these results lies in the ability of the perforant path synapses, after a moderate conditioning regime, to activate the granule cells more effectively for periods of many hours or days. The mechanism of the effect remains uncertain. Since the phenomenon is present in healthy unanaesthetized animals it is at least possible that its mechanism could underly some form of plasticity under normal conditions in the hippocampus.

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