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ENHANCEMENT OF LONG-TERM POTENTIATION IN THE RAT DENTATE GYRUS BY POST-TRIAL STIMULATION OF THE RETICULAR FORMATION

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SUMMARY

1. The possibility that post-trial stimulation of the mesencephalic reticular formation (m.r.f.) may modulate long-term potentiation (l.t.p.) at the perforant path to dentate granule cell synapses was studied in freely moving rats.

2. Extracellular potentials evoked in the dentate gyrus by test pulses to the perforant path were recorded before and at various delays after a series of high-frequency stimulus trains to the perforant path (ten trains of eight pulses at 400 Hz, delivered at 5 min intervals).

3. We have compared the magnitude and duration of l.t.p. of the population spike in this control condition with that observed when a low-intensity m.r.f. stimulation was delivered 10 s after each train to the perforant path.

4. Post-event m.r.f. stimulation enhanced the amount of l.t.p. induced by the series of high-frequency stimulus trains and prolonged its duration for several days.

5. The size of the population spike was unaffected by repeated m.r.f. stimulation in the absence of perforant path high-frequency stimulation, or when this failed to induce significant l.t.p.

6. The temporal gradient of efficacy of m.r.f. stimulation was investigated. M.r.f. stimulation delivered 10 s after a single high-frequency stimulation of perforant path fibres resulted in an enhanced l.t.p. of both the population excitatory post-synaptic potential (e.p.s.p.) and population spike. L.t.p. was unaffected by m.r.f. stimulation given either before, or 120 s after perforant path high-frequency stimulation.

7. These results show that low-intensity m.r.f. stimulation enhances lasting changes in synaptic function in the dentate gyrus when delivered during a critical period following high-frequency activation of perforant path fibres.

8. These results are discussed in the light of our previous findings on the effects of post-event m.r.f. stimulation on memory and on the development of associative changes in hippocampal multiunit activity during conditioning. It is hypothesized that l.t.p.-like mechanisms may be involved in the stabilization of neural networks by experience and that this process might be reinforced by diffuse m.r.f. activation.

INTRODUCTION

Post-excitatory facilitation is a form of synaptic plasticity which refers to an enhancement of synaptic efficiency following activation of presynaptic fibres. This form of enduring increase in synaptic strength resulting from use, first discovered in the peripheral system (see Hughes, 1958, for review), has long been considered as a hypothetical model of the physiological mechanisms subserving mnemonic processes. This idea was further strengthened when a longer lasting post-excitatory facilitation was discovered in forebrain pathways (Bliss & Lømo, 1973). This phenomenon, referred to as long-term potentiation (l.t.p.), was found on synaptic elements of granular cell dendrites in the dentate gyrus which receive input from the entorhinal cortex via perforant path axons and has been shown to persist for days or even weeks after brief triggering trains of stimuli (Bliss & Gardner-Medwin, 1973; Douglas & Goddard, 1975). Because of its time course and the small number of stimuli required and because it appears to be correlated with biochemical (Browning, Dunwiddie, Bennett, Gispen & Lynch, 1979; Bär, Schotman, Gispen, Tielen & Lopes da Silva, 1980) as well as structural (Van Harreveld & Fifkova, 1975; Lee, Schottler, Oliver & Lynch, 1980) modifications of synaptic elements, it has been increasingly proposed as a potential substrate of memory. If, however, l.t.p. is to be viewed as a mechanism modelling physiological events involved in the strengthening of synaptic connexions within memory networks, then a number of basic requirements can be identified if that model is to be compatible with behavioural data (Bloch & Laroche, 1984).

Behavioural studies have provided strong evidence that a mild post-trial stimulation of the mesencephalic reticular formation (m.r.f.) facilitates learning in a variety of experimental situations (Bloch, 1970; Deweer, 1976). The present experiments were thus designed to see whether m.r.f. stimulation influences l.t.p. in a manner which parallels its effect at the behavioural level.

Several lines of experimental evidence suggest the existence of anatomical (Nauta & Kuypers, 1958) and functional relationships between the m.r.f. and the hippocampal formation. First, synchronization of hippocampal activity (theta rhythm) can be triggered by stimulation of widespread regions in the mid-brain tegmentum, including the region of the dorso-lateral tegmentum in which, in our animals, stimulating electrodes were implanted (see Robinson & Vanderwolf, 1978; Vertes, 1982, for reviews). Secondly, reticular stimulation has been shown to evoke post-synaptic responses in hippocampal neurones (Grantyn & Grantyn, 1973). Moreover, m.r.f. stimulation enhances hippocampal unit responses to sensory stimuli (Vinogradova, 1975) and exerts a facilitatory influence on transmission from the entorhinal cortex to the dentate gyrus and the hippocampus proper (Bloch & Laroche, 1981). Thirdly, to these data is added a post-event effect in classical conditioning experiments (see Laroche, Falcou & Bloch, 1983): post-trial m.r.f. stimulation facilitates the development of associative changes in hippocampal and dentate multiunit activity in a manner which parallels its facilitating effect in behavioural learning tasks. It also results in a long-term enhancement of the responsiveness of hippocampal cells to a conditioned stimulus. Furthermore, this stimulation has no effect in the entorhinal cortex where no multiunit response to the conditioned stimulus was found, showing that the facilitatory effects of post-trial m.r.f. stimulation are specific to regions

displaying learning-induced changes in multiunit activity, at least within the neuronal circuit under study.

Taken together, these data suggest that the m.r.f. may exert a facilitatory influence on l.t.p. at the perforant path to dentate granule cell synapses. Accordingly, we examined the effect of m.r.f. stimulation given after perforant path high-frequency stimulation, on the development and duration of l.t.p. in freely moving rats. Since m.r.f. stimulation has been shown to facilitate learning when delivered during the first 90 s of the post-training period, we examined the temporal gradient of efficacy of m.r.f. stimulation on l.t.p.

Preliminary aspects of this work have appeared elsewhere (Laroche & Bloch, 1982).

METHODS

Subjects and implantation procedure

Twenty-two male Sprague–Dawley rats, weighing between 300 and 350 g at the time of surgery were used as subjects. Animals were anaesthetized with sodium pentobarbitone (60 mg/kg, I.P.) which was supplemented as necessary during implantation. They were stereotaxically implanted with a recording electrode in the dentate gyrus and two stimulating electrodes in the perforant path and the ipsilateral m.r.f., respectively. The recording electrode was made of either one or two 62 μm diameter nichrome wires, insulated except at the tips, ground to about 10 μm in diameter at the lower third of their length and inserted into a stainless-steel microtube (300 μm external diameter) whose non-insulated extremity was used for differential recordings. Nichrome wires were extended approximately 1·5 mm from the base of the microtube. The electrode carrier was positioned on the skull 4 mm in front of lambda and 2 mm lateral to the mid line.

Cortical electrodes, made of small silver balls, were inserted between the skull and the dura above frontal and contralateral parieto-occipital cortices.

Concentric bipolar electrodes (300–350 μm interelectrode separation) were used to stimulate the perforant path and the m.r.f. The stimulating electrode aimed at the perforant path was positioned on the skull 0 to −0·5 mm from lambda and 4·4 mm lateral to mid line. Coordinates for the electrode tip in the dorso-lateral m.r.f. (at a level dorsal to the posterior portion of the area cuneiformis, adjacent to the central grey) were 1·5 mm anterior, 1·5 mm lateral and 3 mm above interaural line.

The recording electrode was slowly lowered into the hippocampal formation while continuously recording multiunit activity which, after amplification and filtering, drove an audio-monitor and was displayed on a storage oscilloscope. The perforant path stimulating electrode was then lowered towards the angular bundle. Single pulses were delivered through the stimulating electrode and evoked responses displayed on a storage oscilloscope as the electrode was advanced into the brain. Adjustments of both recording and stimulating electrodes were then performed until maximal positive evoked potentials were encountered. Criteria for acceptance of position for both electrode tips were based on analysis of wave shapes and main characteristics of evoked potentials as described by others (Lømo, 1971; McNaughton & Barnes, 1977). All electrodes were connected to three multichannel miniature sockets, fixed to the skull with dental acrylic cement.

After completion of the experiment, animals were perfused under deep sodium pentobarbitone anaesthesia with 0·9 % saline and 10 % formalin solution (intracardiac perfusion). The brains were removed, placed in 10 % formalin solution for 15 days, then frozen, sliced at 50 μm and stained according to Nissl or Kluver and Barrera methods. Data reported here result from rats with correct tip location for both recording and stimulating electrodes.

Recording and stimulation

Evoked potentials elicited by perforant path pulses (square wave, 100 μs duration, delivered through a photically isolated constant current unit) were recorded through field-effect transistors placed on the animal's head. Signals were fed to a Grass pre-amplifier and bandpass filtered from 0·1 Hz to 3 kHz. Evoked potentials were displayed on a digital storage oscilloscope for on-line analysis and recorded on magnetic tape. Cortical and hippocampal electroencephalograms (e.e.g.) were continuously monitored on polygraph paper throughout the experiment.

The m.r.f. stimulation consisted of a 300 Hz sine-wave current, delivered through a photically isolated constant current unit. The total duration was 90 s, divided into 6 s periods of continuous stimulation, separated by 3 s intervals. Intensity was determined for each individual rat by preliminary measurement of behavioural arousal threshold using 2 s duration stimulation delivered to awake and immobile animals. Usually, the first observable modification of behaviour at the threshold value was an acceleration of the movements of the vibrissae which acccmpany respiration. The intensity used during the experiment was 10% below threshold values (group mean intensity was 5·3 μA). No overt behavioural effect could be detected with these latter values. Moreover, we must emphasize that, when delivered during slow-wave sleep, stimulation at this intensity did not awake the animals, or induce changes in the cortical or hippocampal e.e.g.

Experimental procedures

After a recovery period of 7–15 days, animals were habituated to the experimental chamber (50 × 24 × 24 cm, with a transparent front wall) and cable connexions for 3 days. Recording and stimulating counterbalanced cables were connected to the animals and relayed at the top of the experimental chamber through a multichannel rotating connector. Evoked responses to perforant path single shocks were then tested and, at this stage of the experiment, one animal with unrepresentative evoked potentials was discarded.

The basic design of the experiment included three phases (see Fig. 1*B*). First, a 5 day control period during which the amplitude of dentate-evoked potentials (population spike) elicited by single shocks delivered to the perforant path was recorded. Each day, animals were given several series of ten single shocks, one shock every 15 s, with varying current intensities. Intervals between series were varied from 2 to 10 min and the intensity was determined in a pseudo-random way in order to avoid non-specific sequential effects. The amplitude of the population spike was taken, separately for each response, between the first peak of the positive wave and the peak of the negative wave. At the end of this 5 day control period, the mean amplitudes of evoked responses were plotted as a function of stimulus intensity in order to define, for each animal, the intensity of perforant path stimulation to be used in the subsequent phases of the experiment. Individual intensity used was that producing a response approximately half of its maximum in amplitude. Secondly, on the sixth day (day E in Fig. 1*B*), a series of ten single shocks was given to each rat and then l.t.p. was induced by a series of ten high-frequency stimulus trains (400 Hz, 20 ms total duration, eight pulses of 100 μs each, per train), delivered to the perforant path with 5 min intertrain intervals. Post-excitatory facilitation was assessed 2 min after each high-frequency stimulation with a series of ten single shocks given at a rate of one every 15 s. Further test series (thirty single shocks) were then given at 15, 30, 60 and 360 min after the last high-frequency stimulation. Thirdly, l.t.p. was tested using a daily series of thirty perforant path single shocks until the amplitude of the evoked response recovered its base-line (pre-potentiation) level.

The basic design described above forms the control condition (Fig. 1*B*). The experimental condition was essentially the same except that this time, rats received m.r.f. stimulation 10 s after perforant path high-frequency stimulation. Each rat served as its own control and for some ($n = 6$), post-event m.r.f. stimulation was given first, while others ($n = 5$) were trained first in the control condition with no m.r.f. stimulation. The two conditions were separated by a 15 day rest period after l.t.p. had lapsed.

Three additional animals were submitted to the same procedure as that described for the experimental condition except that they did not receive the series of ten perforant path high-frequency trains (Fig. 1*B*).

In order to assess the time dependency of the m.r.f. stimulation effect, the strategy used in the following experiment was to monitor the response to constant perforant path single shocks before and after a single high-frequency stimulus train of the perforant path, either preceded or followed by m.r.f. stimulation. The amplitude of both population spike and population excitatory post-synaptic potential (e.p.s.p.), measured at a fixed latency after stimulus onset, were examined in this study. Five series of four perforant path single shocks (15 s intervals) were applied in the control period. L.t.p. was induced by a single perforant path high-frequency train 4 min after the end of the control period. Beginning 4 min after the train, the amount of l.t.p. was assessed by delivering test shocks at 15 s intervals for 5 min (test period). Each animal ($n = 7$) was submitted to five different conditions: no m.r.f. stimulation (C0), pre-event m.r.f. stimulation with offset either 120 s (C1) or 10 s (C2) before perforant path high-frequency stimulation and post-event m.r.f. stimulation

beginning either 10 s (C3) or 120 s (C4) after induction of l.t.p. Experimental conditions C1, C2, C3 and C4 were given in a pseudo-random order with one C0 condition given first and then between each experimental condition. Thus, each animal served as its own control and all conditions were given 48 h apart, an interval which preliminary work had shown to be sufficient to avoid residual l.t.p. with the parameters used.

Permanent cortical and hippocampal e.e.g. monitoring throughout this set of experiments allowed us to monitor the level of arousal and the degree of seizure activity. All stimulation was given during wakefulness in the absence of continuous theta activity.

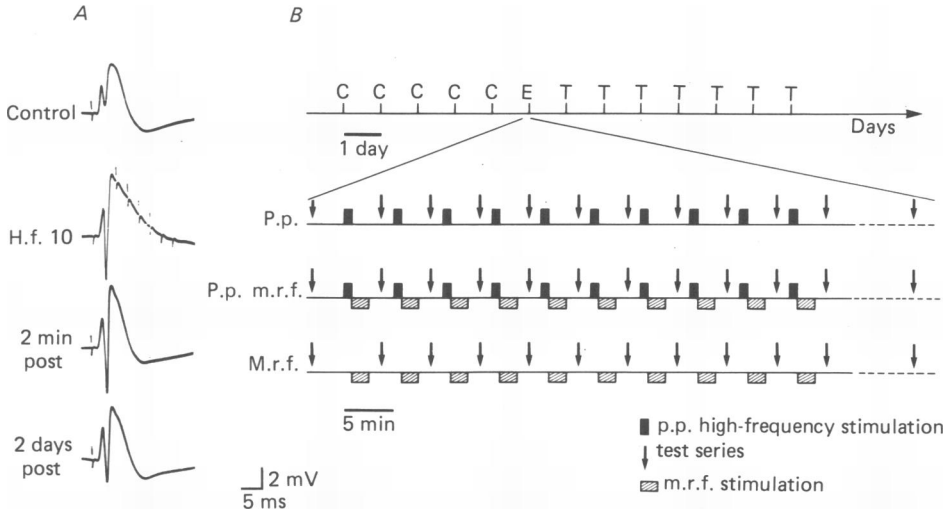

Fig. 1. *A*, examples of extracellular responses to perforant path single pulses recorded in the dentate gyrus before (control) and after (2 min post and 2 days post) the delivery of a series of high-frequency stimulations. The response during the tenth eight-pulse high-frequency stimulation is illustrated (h.f. 10). *B*, experimental design of the l.t.p. experiment. Upper trace, series of perforant path single pulses were delivered daily during the 5 day control period (C) and during the test period (T) following the establishment of l.t.p. (E). Lower traces indicate the training contingencies used in each condition. P.p., control condition with ten perforant path high-frequency stimulations. P.p. m.r.f., experimental condition in which m.r.f. stimulation was delivered 10 s after each perforant path high-frequency stimulation. The last trace shows the condition in which repeated m.r.f. stimulations were delivered without preceding high-frequency stimulation of perforant path fibres. Arrows indicate series of single pulses delivered to the perforant path after each high-frequency stimulation as well as 15, 30, 60 and 360 min following the establishment of l.t.p.

RESULTS

Effect of post-event m.r.f. stimulation on the magnitude of l.t.p.

Ten of the eleven animals submitted to high-frequency stimulation of the perforant path showed a reliable potentiation of the population spike. Data obtained from the eleventh animal in which l.t.p. did not reach statistical significance will be presented separately. Typical responses evoked by perforant path stimulation over the course of the experiment are presented in Fig. 1 *A*. Comparison of records in Fig. 1 *A* shows a considerable growth of the amplitude of the population spike following the delivery of ten high-frequency stimulus trains. In this case, potentiation was still prominent 2 days after the training session. Although not quantitatively studied in this first

experiment, an increase in slope of the e.p.s.p. also occurred. The response evoked by the tenth high-frequency stimulation is illustrated in Fig. 1*A*. As noted by others (Douglas, 1977), only one population spike occurred. The amplitude of the population spike is much greater than that of the control response since substantial potentiation took place during the nine preceding high-frequency trains. After-discharges were never observed with the parameters of stimulation used in this study. It was also noted that neither single shocks to the perforant path nor high-frequency stimulation were accompanied by any observable modification of behaviour.

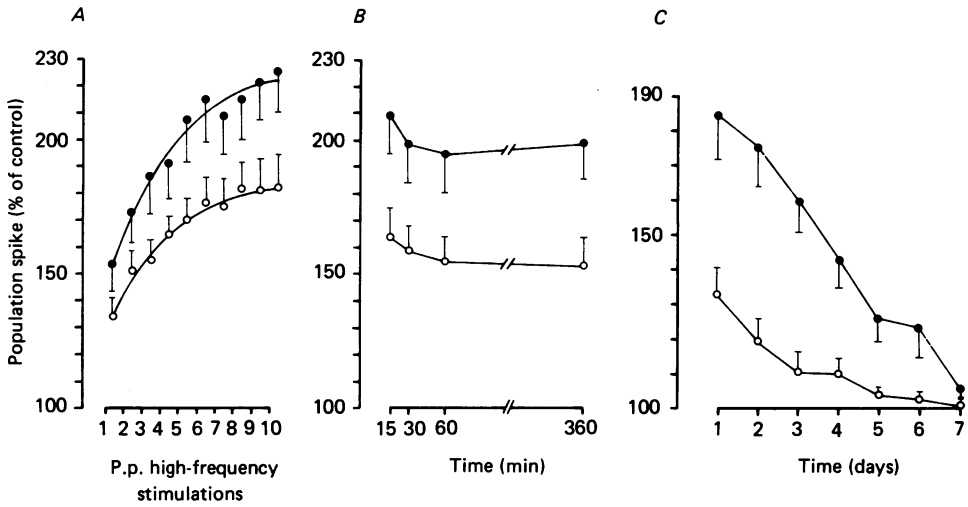

Fig. 2. Time course of l.t.p. of the population spike in the control (○) and experimental condition with post-event m.r.f. stimulation (●). *A*, mean repeated measurements of the population spike, expressed as a percentage of averaged control values, following each perforant path (p.p.) high-frequency stimulation. Vertical bars represent standard error. *B*, mean amount of l.t.p. plotted at indicated intervals after the last high-frequency stimulation. *C*, effect of post-event m.r.f. stimulation on the duration of l.t.p. The graph shows the progressive decay of l.t.p. in each condition during the days following its establishment.

The amount of post-excitatory facilitation achieved after each perforant path high-frequency train is reported in Fig. 2*A* for group data in the control condition (○). The increase in amplitude of the population spike from base-line level was statistically significant from the first high-frequency train (Student's t test for matched samples, $t = 3{\cdot}08$; $P < 0{\cdot}01$). The magnitude of this effect continued to grow with subsequent trains and the amplitude of the population spike remained significantly ($P < 0{\cdot}005$) above base-line level thereafter. Thus, the amount of potentiation appeared to be a function of the number of high-frequency stimulus trains delivered to the perforant path. Curvilinear regression revealed, however, that the magnitude of potentation had reached an asymptotic level at the end of the series of high-frequency trains.

In the experimental condition where animals received post-event m.r.f. stimulation, the amount of potentiation was greatly enhanced (Fig. 2*A*). Student's t test revealed that the amplitude of the population spike was significantly ($P < 0{\cdot}005$) above base-line level after every trial.

The data were analysed by a $2 \times 10 \times 2$ analysis of variance (repeated measures) with the sequential order of training condition as one factor, trial number as the second factor and treatment as the third. This analysis revealed a significant trial effect (F (9, 72) = 11·22; $P < 0·001$) and no significant effect of the training condition order ($F < 1$). Potentiation was significantly enhanced in the experimental condition with m.r.f. stimulation (F (1, 8) = 6·25; $P < 0·05$). None of the interactions between factors reached statistical significance. Subsequent t tests revealed the main effect of m.r.f. stimulation to be significant ($0·01 < P < 0·05$) after every perforant path high-frequency train.

Effect of post-event m.r.f. stimulation on the duration of l.t.p.

Data obtained from test series presented 15, 30, 60 and 360 min following the training phase showed that, after a small decrement, the amount of potentiation remained at a stable level in both conditions (Fig. 2*B*). Student's t tests for matched samples revealed that the amplitude of the population spike was significantly ($0·001 < P < 0·005$) above base-line level at the four delays tested, in the control as well as in the experimental condition. A $2 \times 4 \times 2$ analysis of variance was performed on these data as before. The amount of potentiation was found to be significantly greater in the experimental than in the control condition (F (1, 8) = 6·37; $P < 0·05$), while neither the delay effect (F (3, 24) = 2·36), nor the order of training condition effect (F (1, 8) = 0·24) were significant. None of the interactions between factors was found to be statistically significant.

We examined the long-term effects of high-frequency stimulation by presenting a daily series of single perforant path shocks. The amount of potentiation observed each day is presented in Fig. 2*C* for both conditions. 24 h after the last high-frequency stimulation, the amplitude of the population spike in the control condition was still significantly increased above base-line level. While l.t.p. decayed in both conditions, it decreased to a negligible level by the third day when animals were trained in the control condition, whereas it was considerably protracted in the experimental condition. A $2 \times 7 \times 2$ analysis of variance revealed a significant difference between conditions (F (1, 8) = 9·05; $P < 0·05$), a significant delay effect (F (6, 48) = 12·61; $P < 0·001$) and no significant effect of the order of training condition ($F < 1$). The treatment × delay interaction was found to be the only one to be significant (F (6, 48) = 4·05; $P < 0·005$). Subsequent t tests revealed that the amplitude of the population spike was significantly ($0·005 < P < 0·05$) above base-line level on days 1 and 2 in the control condition, while the difference remained significant over five consecutive days following training with m.r.f. stimulation. Moreover, a day-by-day analysis of between-conditions differences indicated that the amount of potentiation was significantly ($0·001 < P < 0·05$) greater in the experimental than in the control condition from day 1 to day 5.

Examination of individual results showed that, though the prolongation in time with post-event m.r.f. stimulation was a common feature, there was a large variability in the duration of l.t.p. among animals. For each individual rat, Student's t tests for matched samples were therefore computed to determine on each day whether or not the amplitude of the population spike was significantly ($P < 0·05$) above base-line level. It was thus possible to plot the number of animals per training condition with

significant potentiation of the population spike, as a function of time elapsed since perforant path high-frequency stimulation (Fig. 3). It is clear from Fig. 3 that, in the control condition, l.t.p. in 60% of the animals decayed rapidly in about 2 days while, when the same animals were trained with post-event m.r.f. stimulation, 50% of them were still significantly potentiated 5 days after perforant path high-frequency stimulations.

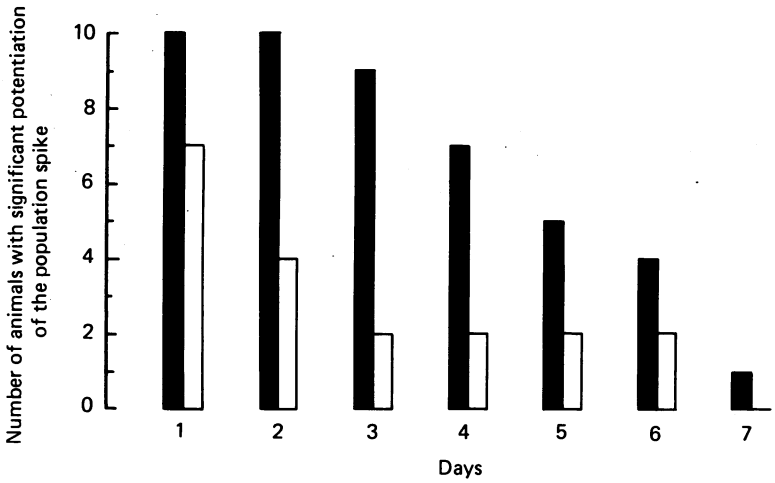

Fig. 3. Number of animals with statistically significant l.t.p. of the population spike as a function of time (days) elapsed since the delivery of a series of high-frequency stimulus trains to the perforant path. ■, with m.r.f. stimulation; □, without m.r.f. stimulation.

Thus, it appeared that post-event m.r.f. stimulation not only accelerated the rate at which l.t.p. could be established and enhanced its amplitude, but also protracted its duration by several days. We have mentioned that none of the statistical analysis revealed any significant effect of the order of training conditions. It was moreover verified that the input–output curves plotted at the end of the 5 day control periods preceding each training condition were not different. Thus, for a given rat, the intensity of perforant path stimulation was identical in both training conditions, and the base-line levels related to each training condition were very close to one another.

Effect of m.r.f. stimulation in absence of l.t.p.

Three additional animals served as controls for possible after-effects of m.r.f. stimulation on dentate-evoked potentials. They were trained in the same way as experimental animals except that perforant path high-frequency stimulation was not delivered (Fig. 1*B*). Neither the first m.r.f. stimulation, nor subsequent ones, induced any significant change in the amplitude of the population spike as compared to the base-line level (Fig. 4*A*). Moreover, no significant changes could be observed in the minutes or days following the series of m.r.f. stimulation. Finally, it was later confirmed that l.t.p. of the population spike could be induced in these animals provided that perforant path high-frequency stimulation was given.

Another control for the absence of effect of m.r.f. stimulation in non-potentiated animals came from an experimental rat in which perforant path high-frequency

stimulation failed to induce reliable l.t.p. (Fig. 4*B*). In this animal, the amplitude of the population spike observed immediately after each high-frequency train, or on the following minutes and days, was not significantly different from base-line level, either in the control, or in the experimental condition. Moreover, no statistically significant differences could be found between training conditions.

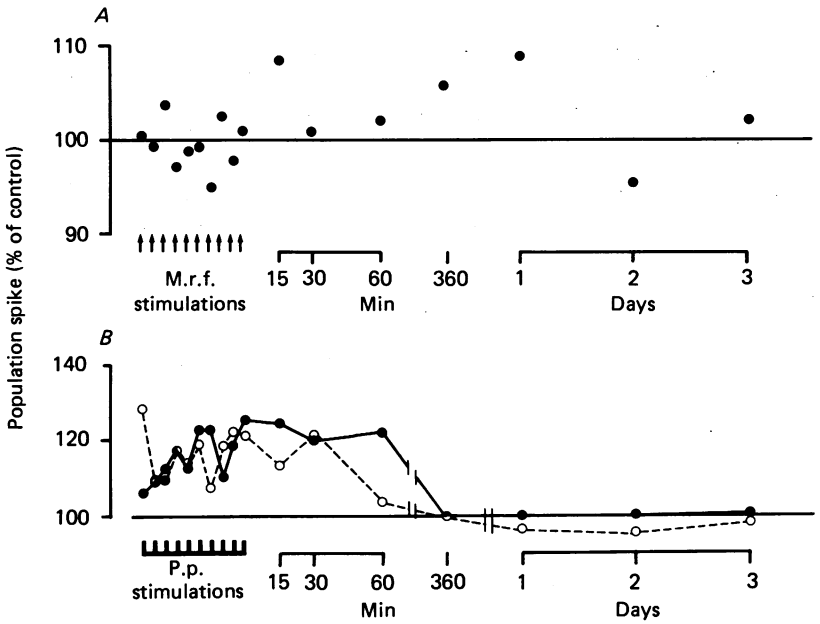

Fig. 4. Controls for the effect of m.r.f. stimulation in absence of l.t.p. *A*, mean percentage changes in the size of the population spike in the condition where m.r.f. stimulation was delivered without preceding high-frequency stimulation of perforant path fibres. *B*, individual case in which perforant path (p.p.) high-frequency stimulation failed to induce significant l.t.p. of the population spike. For this animal, the experimental condition with post-event m.r.f. stimulation (●) was given 2 weeks after day 3 in the control condition (○).

Time dependency of the m.r.f. stimulation effect

Seven animals received a single perforant path high-frequency train every 48 h, with or without m.r.f. stimulation. M.r.f. stimulation was given either before or after the perforant path high-frequency stimulation, at different delays, as previously mentioned. The amplitudes of the spike and e.p.s.p. components of evoked responses observed during the five series in the control (pre-l.t.p.) period were averaged to determine a base line for each individual rat. The means of each of the five series in the test (post-l.t.p.) period were then expressed as a percentage of this base-line level.

Reliable increases in amplitude for both population spike and population e.p.s.p. were observed as a result of high-frequency stimulation in all animals tested under every condition. In the control conditions (C0) with no m.r.f. stimulation, the mean amplitudes of the population spike and e.p.s.p. were 196 and 115 % of their respective base-line levels. Both progressively decreased over the 5 min test period to 154 and 111 %, respectively. The analysis of variance revealed no significant differences

between the four C0 curves, obtained 96 h apart, in the enhancement of population spike or population e.p.s.p. amplitudes. Data from these conditions have therefore been pooled and are shown as the curve labelled C0 in Fig. 5*A* and *B*.

As can be seen in Fig. 5*A*, potentiation of the population spike remained at a high level at the end of the test period, while progressively decreasing over that period in all conditions. The amount of potentiation as well as its decay function were comparable in all conditions except in condition C3. In this condition, m.r.f.

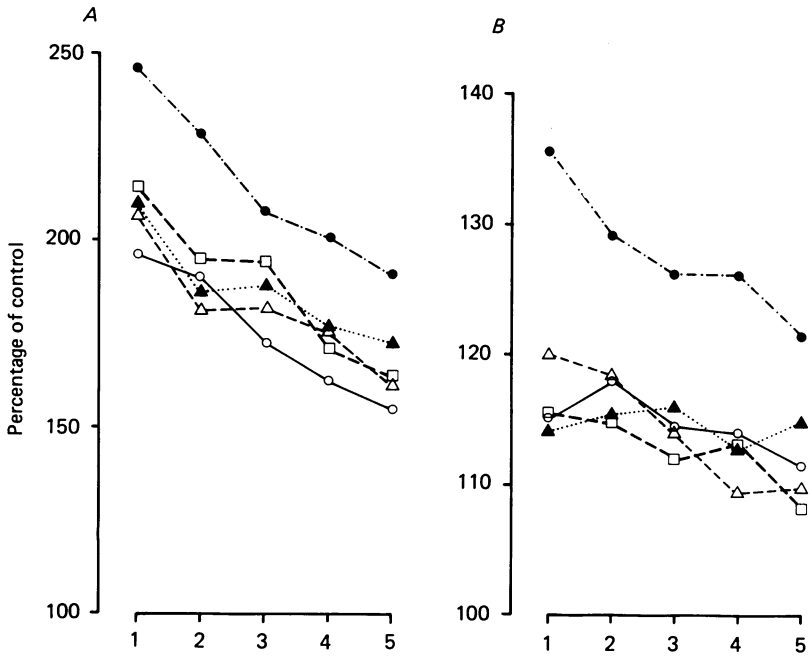

Fig. 5. Averaged increase in population spike (*A*) and population e.p.s.p. (*B*) following a single high-frequency stimulation of the perforant path. Ordinates indicate mean percentage of control over the series of single pulses in the test period, starting 4 min after high-frequency stimulation, and their average across animals. C0 (○), mean curve obtained in repeated control conditions with no m.r.f. stimulation. Conditions C1–C4 correspond to various contingencies between m.r.f. stimulation and perforant path high-frequency stimulation. In pre-event conditions, m.r.f. stimulation was delivered either 120 s (C1; ▲) or 10 s (C2; △) before high-frequency stimulation. In post-event conditions, m.r.f. stimulation was delivered 10 s (C3; ●) or 120 s (C4; □) after high-frequency stimulation.

stimulation given 10 s after perforant path high-frequency stimulation induced a marked increase in the amount of potentiation. Global analysis of variance revealed a significant series effect (F (4, 24) = 21·18; $P < 0·001$), a significant difference among conditions (F (4, 24) = 4·23; $P < 0·01$) and no significant interaction. Subsequent analysis showed that there was no significant difference between conditions C0, C1, C2 and C4 ($F < 1$), while the C3 condition differed significantly ($P < 0·001$) from all other conditions.

The analysis of the differences between conditions in the enhancement of the population e.p.s.p. showed essentially the same picture. The series effect as well as

the condition effect were significant (F (4, 24) = 4·72; $P < 0·01$ and F (4, 24) = 5·24; $P < 0·005$, respectively), while the interaction was not. Here again, conditions C0, C1, C2 and C4 did not differ significantly ($F < 1$), while condition C3 differed significantly ($P < 0·001$) from all other conditions.

Test stimuli were delivered to the perforant path 1 h after the high-frequency train in each of the experimental conditions (C1–C4). Measurements of the evoked responses showed that the increased amount of l.t.p. observed in condition C3 persisted over this 1 h interval. The population spike and e.p.s.p. were 207 and 113 % of their respective base-line levels when m.r.f. stimulation was given 10 s after the high-frequency stimulus train, while mean amplitudes in other conditions were 161 % (C1: 187·5 %, C2: 155·9 %, C4: 140·8 %; with no significant difference ($F < 1$) between these conditions) and 107 % (C1: 108·3 %, C2: 110 %, C4: 101·9 %; with no significant difference ($F < 1$) between these conditions), for the population spike and e.p.s.p., respectively. Statistical analysis indicated that the C3 condition differed significantly from other conditions for the spike (F (1, 47) = 6·27; $P < 0·05$) and e.p.s.p. (F (1, 47) = 7·03; $P < 0·05$) components of the evoked response.

Thus, analysis of either the population spike or of the population e.p.s.p., showed that the only condition resulting in a significant enhancement of l.t.p. was that with m.r.f. stimulation given 10 s after perforant path high-frequency stimulation. M.r.f. stimulation was ineffective in enhancing l.t.p. when given either 120 or 10 s before, or 120 s after perforant path high-frequency stimulation.

DISCUSSION

High-frequency stimulation of the perforant path results in an enduring increase in the magnitude of the population e.p.s.p., which reflects the synaptic current at the activated synapses (Lømo, 1971), and of the population spike formed by the nearly synchronous discharges of granular cells (Andersen, Bliss & Skrede, 1971). In the present experiment, potentiation of both components of the population extracellular field potential has been shown to occur as a result of brief high-frequency stimulation of the perforant path in awake animals. The results show that, with the same parameters as those used in behavioural experiments, post-event m.r.f. stimulation enhances the potentiation of both population e.p.s.p. and population spike while having no effect, by itself, on these components.

Enhancement of l.t.p. by post-event m.r.f. stimulation

Unspecific effects caused by repetition of the experiment in our situation where each rat served as its own control cannot account for the present results since statistical analysis as well as control procedures showed that l.t.p. after either perforant path high-frequency stimulation alone, or after its combination with m.r.f. stimulation, was followed by a return of the evoked responses to pre-l.t.p. levels, with no consequences for further l.t.p. production. Moreover, this corroborates earlier indications that, with such brief trains of high-frequency stimulation, l.t.p. fades out over time (Barnes, 1979).

In the experiment designed to assess the time dependency of the m.r.f. stimulation effect, a single perforant path high-frequency stimulus train was delivered, and

evoked responses were recorded during the following 9 min period. Post-tetanic potentiation may thus have contributed to the observed changes. However, post-tetanic potentiation in the dentate gyrus, which accompanies l.t.p., but has been attributed to a process distinct from it, shows a fast decay time constant (McNaughton, 1982). In our experimental conditions, significant increases in both population e.p.s.p. and population spike were seen at the end of the test period, i.e. at a time when short-term effects have been reported to be greatly attenuated (Racine & Milgram, 1983). Moreover, records taken after the last run of the experiment showed that, though small in amplitude, lasting effects were detectable 1 h after the high-frequency stimulus train (see also the observations with unanaesthetized rats in: Douglas, Goddard & Riives, 1982; Racine, Milgram & Hafner, 1983). In fact, the intensity used was well above population spike threshold and was thus likely to have provided simultaneous high-frequency activation of a sufficient number of input fibres for l.t.p. to occur (McNaughton, Douglas & Goddard, 1978). Thus, if we cannot totally rule out the possibility that resting post-tetanic potentiation, superimposed on l.t.p., may benefit from m.r.f. stimulation as well, these observations, together with the long-lasting effect seen in the first experiment, may best be interpreted as a modulation of the l.t.p. process itself.

Post-event m.r.f. stimulation not only increased the amount of l.t.p. but also had a slowing effect on its decay function. Although the amount and duration of l.t.p. appeared closely related, it was of interest to examine whether post-event m.r.f. stimulation differentially affects the amplitude and duration of l.t.p. An analysis of covariance indicated that post-event m.r.f. stimulation significantly protracted l.t.p. of the population spike though this effect was not entirely predictable from the magnitude of potentiation achieved. This analysis gives, however, no direct evidence as to whether m.r.f. stimulation affects different mechanisms which could be involved in the control of magnitude and duration of l.t.p. or if this differential modulation merely reflects non-linearity between the two variables. One aspect of non-linearity may rest on the fact that l.t.p. of the population spike can be greater than expected from the increased population e.p.s.p. (Bliss & Lømo, 1973; Andersen, Sundberg, Sveen, Swann & Wigström, 1980; Wilson, 1981; Wilson, Levy & Steward, 1981).

Temporal gradient of efficacy of m.r.f. stimulation and mechanisms involved

One of the main conclusions of the present experiment is that m.r.f. stimulation is only effective when delivered during a short period of time which follows perforant path high-frequency stimulation. The absence of effect of m.r.f. stimulation given prior to perforant path high-frequency stimulation strongly argues against proactive effects. The mechanism of this post-event strengthening effect is unknown, but it is suggested that m.r.f. stimulation exerts its effect on a process triggered by, and outlasting high-frequency activation of perforant path fibres. Relevant to this assumption is the finding that, in contrast to short-term phenomena such as augmentation or post-tetanic potentiation, l.t.p. does not reach its maximal value immediately after high-frequency stimulation but rather develops progressively while short-term effects are decaying (McNaughton, 1978, 1983). It is furthermore noteworthy that the critical period within which m.r.f. stimulation is effective falls within the rise time of l.t.p. reported by McNaughton in anaesthetized rats.

Whatever the mechanism involved in the genesis and maintenance of l.t.p. and its presynaptic and/or post-synaptic location (see Bliss & Dolphin, 1982, for review), it is possible to think that, after the triggering high-frequency stimulation, an ensemble of co-operative (McNaughton *et al.* 1978; Levy & Steward, 1979) synapses are set into an active state which may directly or indirectly benefit from widespread m.r.f. activation.

Other, biochemical (Baudry, Oliver, Creager, Wieraszko & Lynch, 1980; Dolphin, Errington & Bliss, 1982) or structural (Fifkova, Anderson, Young & Van Harreveld, 1982) modifications which accompany l.t.p. are also presumed to develop within relatively short periods after high-frequency stimulation. Our hypothesis would predict such changes to be augmented and/or strengthened in correlation with the enhancement of l.t.p. by m.r.f. stimulation.

The question of anatomical projections involved in the modulation of l.t.p. by m.r.f. cannot be resolved at present. As we have already shown (Bloch & Laroche, 1981), m.r.f. stimulation exerts a facilitating influence on transmission from the entorhinal cortex to the dentate gyrus. Similar effects have also been reported from more caudal loci in the pontine and medullary reticular formation (Winson, 1981). All these effects could be mediated through the medial septal nucleus, the stimulation of which has been shown to facilitate synaptic transmission in the dentate gyrus (Alvarez-Leefmans & Gardner-Medwin, 1975; Fantie & Goddard, 1982). Indeed, enhancement of l.t.p. can also be achieved by concurrent high-frequency activation of perforant path and septo-dentate fibres (Robinson & Racine, 1982).

The present effects could also be mediated through descending tracts towards the locus coeruleus or raphe nuclei (see Edwards & de Olmos, 1976) and then to the hippocampal formation. Facilitation of perforant path–granule cell synapses has been reported following stimulation of the locus coeruleus (Bliss & Wendlandt, 1977; Assaf, Mason & Miller, 1979) or raphe nuclei (Assaf & Miller, 1978; Winson, 1980). Moreover, reduction of l.t.p. has been shown to occur following depletion of either 5-hydroxytryptamine or, to a lesser extent, noradrenaline (Bliss, Goddard & Riives, 1983).

In conclusion, the available data do not provide unequivocal support for one or the other of these mediating pathways (or other possible ascending tracts through hypothalamus and anterior thalamic nuclei) and it always remains possible that the m.r.f. stimulation effect involves still longer polysynaptic pathways through cortical areas via non-specific thalamic nuclei (Steriade, Ropert, Kitsikis & Oakson, 1980).

Conclusions

In previous work (see Bloch & Laroche, 1981; Laroche, Falcou & Bloch, 1983), we have shown that post-event m.r.f. stimulation has similar facilitating effects on the development of associative changes in hippocampal and dentate multiunit activity during classical conditioning as those previously demonstrated in behavioural learning. In the context of perseveration theory (Müller & Pilzecker, 1900), these results were taken to lend support to the notion that a perseverative process, whatever its nature, was taking place in the circuits under study and that this process could help long-lasting modifications to occur at the synaptic level. The demonstration that the same low-intensity m.r.f. stimulation facilitates l.t.p. with a temporal

gradient of efficacy compatible with behavioural findings provides additional support for this hypothesis. Moreover, other treatments known to facilitate or disrupt memory have also been shown to produce enhancing or deleterious influence on l.t.p., respectively (Hesse & Teyler, 1976; Delanoy, Tucci & Gold, 1983). This in turn provides some support for the involvement of l.t.p.-like mechanisms in mnemonic processes. Indeed, correlations between l.t.p. duration and performances in a spatial discrimination task have been established (Barnes & McNaughton, 1980). When high-frequency stimulation of the perforant path is used as a conditioned stimulus, the amplitude of the resulting l.t.p. has been shown to be correlated with the acquisition of avoidance behaviour (Ott, Rüthrich, Reymann, Lindenau & Matthies, 1982). Moreover, we have recently reported an increased l.t.p. of the perforant path after the development of associative changes in dentate multiunit activity and the enhancement of this effect when conditioning had been facilitated by post-trial m.r.f. stimulation (Laroche, Bergis & Bloch, 1983). Finally, it has also been recently shown that classical conditioning produces an increase in glutamate receptor binding in the hippocampus (Thompson, Manounas, Lynch & Baudry, 1983), a process which has been hypothesized to accompany l.t.p. (Baudry *et al.* 1980).

It seems, therefore, reasonable to admit that l.t.p.-like mechanisms might occur in the brain following learning during the period referred to as the phase of memory consolidation. Such mechanisms would take place at the synapses activated in the network whose spatio-temporal pattern has been determined by the initial learning situation (Bloch & Laroche, 1984). If the topology of this activation represents a coding in short-term memory, l.t.p.-like mechanisms could then be viewed as an intermediate process upon which depends a more permanent marking of the network connectivity through biochemical and structural changes. In this framework, promnesic agents such as m.r.f. stimulation might exert their facilitatory effect on a perseverative process engaged in the stabilization of the relevant network after its state becomes inactive.

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