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# REDUCTION OF LONG-TERM POTENTIATION IN THE DENTATE GYRUS OF THE RAT FOLLOWING SELECTIVE DEPLETION OF MONOAMINES

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SUMMARY

1. Brief, high-frequency stimulation of the perforant path results in a long-term potentiation (l.t.p.) of the field response evoked in the dentate gyrus by single shocks to the perforant path. We have compared the magnitude and duration of l.t.p. in normal, anaesthetized rats with animals depleted of noradrenaline (NA), 5-hydroxytryptamine (5-HT), or both.

2. All animals were exposed to an identical sequence of eight high-frequency trains of increasing intensity given over a period of 140 min to the perforant path of one hemisphere. The potential evoked by test shocks to the perforant path was monitored in both hemispheres throughout this period and for a further 96 min after the last train.

3. Plots of the mean potentiation of the population e.p.s.p. as a function of time were computed for all animals in each group. L.t.p. in the NA-depleted group was about 50% of that in the non-depleted control group throughout the course of the experiment. L.t.p. in the 5-HT-depleted group was more severely affected; mean potentiation did not exceed 30% of that in the control group at any time.

4. The duration of l.t.p. was unaffected by NA depletion and reduced by 5-HT depletion.

5. The threshold for the intensity of high-frequency current pulses necessary to elicit l.t.p. was unaffected by NA depletion and raised by 5-HT depletion.

6. Short-term potentiation of the population e.p.s.p. was unaffected by either NA depletion or 5-HT depletion.

7. The effect of monoamine depletion on granule cell excitability was investigated. 5-HT depletion, but not NA depletion, induced an increase in the excitability of the granule cell population, in the sense that a population e.p.s.p. of a given size was associated with a larger population spike.

8. Long-term potentiation of granule cell excitability was not affected by NA depletion, but was reduced by 5-HT depletion.

9. These results show that monoamines can modulate long-term changes in synaptic function in the dentate gyrus, and suggest that 5-HT is more potent in this respect than NA.

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## INTRODUCTION

Brief, high-frequency stimulation of the perforant path, an excitatory fibre system originating in the entorhinal cortex and terminating on granule cells in the dentate gyrus, gives rise to a sustained increase in the amplitude of the field potential evoked by single shocks to the perforant path (Bliss & Lømo, 1973; Douglas & Goddard, 1975; McNaughton, Douglas & Goddard, 1978). Long-term potentiation (l.t.p.) of synaptically evoked responses has also been described in a number of other excitatory pathways in the hippocampus (see Bliss, 1979, for review). The extended time course of the effect, which is measurable in days (Bliss & Gardner-Medwin, 1973; Barnes, 1979) and its identification in the hippocampus, a structure implicated in memory processes in humans (Milner, 1972) has invited speculation that l.t.p. may provide a model for the physiological mechanisms underlying memory and learning.

In addition to the intrinsic and extrinsic pathways whose sharply delineated patterns of termination are a characteristic feature of the hippocampus, there are other afferent systems notably those arising from the monoaminergic nuclei of the brain stem, which possess more diffuse terminal fields. Noradrenergic fibres from the locus coeruleus, and serotonergic fibres from the raphe nuclei both project widely to the hippocampus, with the greatest density of terminals in the hilus of the dentate gyrus (Ungerstedt, 1971; Lindvall & Bjorklund, 1974; Moore & Halaris, 1975; Segal, 1975; Jones & Moore, 1977; Azmitia, 1978; Azmitia & Segal, 1978; Loy, Koziell, Lindsey & Moore, 1980). Stimulation of the ascending monoaminergic pathways can modulate both the rate of spontaneous unit activity in the hippocampus, and the amplitude of potentials evoked by stimulation of the main excitatory hippocampal pathways (Segal & Bloom, 1974; Bliss & Wendlandt, 1977; Assaf & Miller, 1978; Assaf, Mason & Miller, 1979; Winson, 1980). There is evidence that the ascending monoaminergic pathways are involved in the reinforcement of conditioned behaviour (Crow, 1968; Anlezark, Crow & Greenway, 1973; Kety, 1972; Fibiger, Roberts & Price, 1975; Ogren, Archer & Ross, 1980) and in the control of neural plasticity (Pettigrew & Kasamatsu, 1978).

Taken together, these observations suggest that the monoaminergic pathways may have the capacity to influence l.t.p. As a first step towards testing this hypothesis, we have examined the effects of depleting the forebrain of noradrenaline (NA), 5-hydroxytryptamine (5-HT), or both, on stimulus-induced changes in synaptic efficacy and cellular excitability in the hippocampus.

Preliminary aspects of this work have been reported elsewhere (Goddard, Bliss, Robertson & Sutherland, 1980; Bliss, Goddard, Robertson & Sutherland, 1981).

## METHODS

### *Electrodes and surgery*

Generally our procedures were as described by McNaughton *et al.* (1978). Adult male Long Evans rats were anaesthetized with sodium pentobarbitone (60 mg/kg) and maintained under anaesthesia with incremental doses or by continuous intraperitoneal infusion at a level which suppressed large-amplitude low-frequency spindling in the hippocampal e.e.g., but allowed minimal respiratory or abdominal reflexes to a tail pinch. Recording electrodes of Teflon-coated stainless steel wire (76 $\mu$m o.d.; Medwire Corp.), were stereotaxically guided into the hilus of each side at co-ordinates 4 mm posterior to bregma and 2·5 mm lateral to the mid line. Monopolar stimulating electrodes

made from nichrome wire, 127 $\mu$m o.d., and insulated with Diamel, were stereotaxically guided into the perforant path of each side at co-ordinates 8·0 mm posterior to bregma and 4·8 mm lateral to the mid line. The depth of each electrode was adjusted to maximize the amplitude of the positive-going synaptic field potential evoked in the hilus by weak shocks delivered to the perforant path.

*Electrical stimulation*

The design of the experiments called for a standard test shock to be given at regular intervals, interrupted at longer intervals by trains of high-frequency stimuli at increasing intensity. The standard test shock consisted of a diphasic constant-current pulse with a duration of 60 $\mu$s per half-wave, and an amplitude individually adjusted for each side so that the stimulus was slightly above threshold for evoking a population spike (see below). Once set (range: 100–400 $\mu$A per half-wave), the amplitude remained constant at each electrode site throughout the experiment. Variations in stimulus intensity required for the high-frequency conditioning trains were achieved by varying the stimulus *duration*, a parameter which was under computer control.

The experimental protocol was identical for each animal. A total of 512 test shocks was delivered to both sides at a rate of one every 30 s for a period of 4 h 16 min. High-frequency conditioning trains were given every 20 min to one side only, beginning on trial 41 with very weak trains and ending on trial 321 with very strong trains. Low-intensity conditioning trains do not produce l.t.p. in the perforant path (McNaughton *et al.* 1978), and the purpose of presenting this sequence of conditioning trains was to establish whether depleted animals differ from normals in their threshold for l.t.p. Thus, while the control side received only test pulses, the experimental side received both test pulses and eight sets of conditioning trains. Each set of conditioning trains consisted of five bursts of ten diphasic pulses at 400 Hz given 1 s apart. The duration of the pulses was increased stepwise with each set from 30 $\mu$s on trial 41, through the sequence 40, 50, 70, 90, 130, 180 to 250 $\mu$s on trial 321. This sequence of conditioning trains was under computer control and was identical for all animals.

*Components of the evoked response*

The potential recorded in the hilus consisted of an early positive wave on which, provided the stimulus was sufficiently effective, a negative-going spike was superimposed (Fig. 1*B*). These two components have been characterized as the population e.p.s.p. (or synaptic wave) and the population spike, the latter reflecting the synchronous discharge of granule cells (see Lømo, 1971, for a detailed description of this potential). Fig. 1*B* shows examples of the potentials evoked in the control and experimental sides of a normal animal before and after the series of potentiating high frequency trains. Arrows in Fig. 1*B* indicate the points of fixed latency at which the amplitudes of the synaptic wave were measured; the exact latency varied slightly between sides and between animals but was usually about 3 ms (near the mid point of the synaptic wave, but sufficiently early not to be contaminated by the population spike). All potentials were amplified conventionally (system half-amplitude band width: 3 Hz–3 kHz), and passed to a PDP 11/34 computer, sampling at 10 kHz with 10-bit resolution, for disk storage and on-line display of e.p.s.p. amplitude. The amplitude of the population spike, and other parameters of the evoked response such as its latency, were measured off-line, and the computer was also used to normalize the data from individual experiments and to compile group means and excitability curves, as described in the Results.

*Drug treatments*

*Reserpine-treated animals.* In one group of animals, hippocampal stores of 5-HT, dopamine and NA were depleted by giving an injection of reserpine (Sersapil, Ciba, 5 mg/kg I.P.) 15–18 h before testing for l.t.p., followed by a second dose (2·5 mg/kg) 2 h before testing.

*6-hydroxydopamine lesions.* Bilateral lesions to deplete the hippocampus of NA were placed in the dorsal noradrenergic bundle by intracerebral injection of 6-hydroxydopamine hydrobromide (6-OHDA) under sodium pentobarbitone anaesthesia (50 mg/kg, I.P.). The co-ordinates used, with the upper incisor bar positioned 3 mm below the interaural plane, were 2·6 mm anterior to stereotaxic zero, 1·1 mm lateral to the mid line, and 3·6 mm above the interaural plane.

At each site, 2 $\mu$l. of a freshly prepared solution of 6 $\mu$g of 6-OHDA in physiological saline containing *l*-ascorbic acid (0·2 mg/ml) was injected at a rate of 0·5 $\mu$l./min. using a motorized syringe pump. The 30 gauge injection needle was left in position for 5–10 min after injection. Control

animals received an identical injection of vehicle. Each rat was given an intramuscular injection of penicillin-G (60000 i.u.) at the end of the operation. Maximal levels of NA depletion were attained within 2 weeks of the injection of 6-OHDA. All animals were tested for l.t.p. at 14–20 days after treatment. A group of normal (untreated) animals was also tested for l.t.p. concurrently with the NA-depleted and vehicle control groups.

*5,7-dihydroxytryptamine lesions.* In order to obtain a selective bilateral depletion of 5-HT stores, another group of rats was treated with an intraventricular injection of 5,7-dihydroxytryptamine (5,7-DHT), prepared by dissolving 5,7-dihydroxytryptamine creatinine sulphate (150 $\mu$g free base) in 20 $\mu$l. of sterile saline containing 1 mg/ml. *l*-ascorbic acid. To protect noradrenergic terminals, desmethylimipramine (DMI, 20 mg/kg) in sterile $H_2O$ was injected I.P., 40–60 min prior to the intraventricular injection of 5,7-DHT (Breese & Cooper, 1975). Under nembutal anaesthesia, animals were mounted in a stereotaxic instrument with the incisor bar set 5 mm above the interaural plane. Injection of 5,7-DHT was made into the right ventricle in the anterior plane of bregma, 1·5 mm lateral to the mid line and 4·7 mm below the surface of the skull, approximately 15 min being allowed for the injection of 20 $\mu$l. Between 13 and 16 days were allowed to elapse before testing for l.t.p. 18–24 h before the measurement of l.t.p., all but five of the animals treated with 5,7-DHT were injected with *p*-chloramphetamine (*p*-CA) in sterile saline to release residual stores of 5-HT (Sanders-Bush, Bushing & Sulser, 1972); animals weighing over 350 g received a dose of 15 mg/kg while animals weighing less than 350 g received 10 mg/kg. This combination of drugs, particularly when followed by prolonged nembutal anaesthesia during the acute experiment to test for l.t.p., proved lethal in many cases and we obtained a complete set of data from little more than half the animals prepared in this way. Four groups of control animals were examined: a normal control group without treatment of any kind, a group which received DMI followed by 5,7-DHT, and two groups given either DMI alone or *p*-CA alone. All animals were tested for l.t.p. 13–16 days after treatment, except for those in the *p*-CA alone group which were tested 48 h later.

*Monoamine assays.* At the end of each experiment, the brain was removed, the hippocampus quickly dissected from each hemisphere, weighed, and stored at −20 °C to await assay for either NA or 5-HT. NA levels in the hippocampus were determined by the radio-enzymatic method of Coyle & Henry (1973), and 5-HT levels by the spectrophotofluorometric method of Curzon & Green (1970).

## RESULTS

### *Monoamine levels in depleted animals*

*Noradrenaline.* The average level of hippocampal NA in the pooled normal ($n = 12$) and vehicle ($n = 4$) control groups was 429 pg/mg. In all animals treated with reserpine ($n = 9$) and in all animals injected with intracerebral 6-OHDA and with a minimum of two weeks survival ($n = 11$), NA concentration was less than 32 pg/mg, the average sensitivity of the assay. Thus depletion was 92·5% or greater in all animals in the NA-depleted group.

*5-HT.* The mean depletion of hippocampal 5-HT was 72% in animals treated with a combination of DMI, 5,7-DHT and *p*-CA ($n = 13$), when compared with the mean level of 5-HT found in the pooled normal ($n = 7$) and vehicle injected ($n = 6$) controls. After *p*-CA alone ($n = 4$) the mean depletion was 65%; after DMI and 6,7-DHT ($n = 5$) it was only 32%. Treatment with DMI alone had no effect on hippocampal 5-HT levels.

### *Effect of monoamine depletion on the perforant path evoked response*

The field potentials evoked in the hilus of the dentate area by stimulation of the perforant path in monoamine-depleted rats had a similar wave form to those seen in normal animals. The threshold for the population spike, measured as the mean

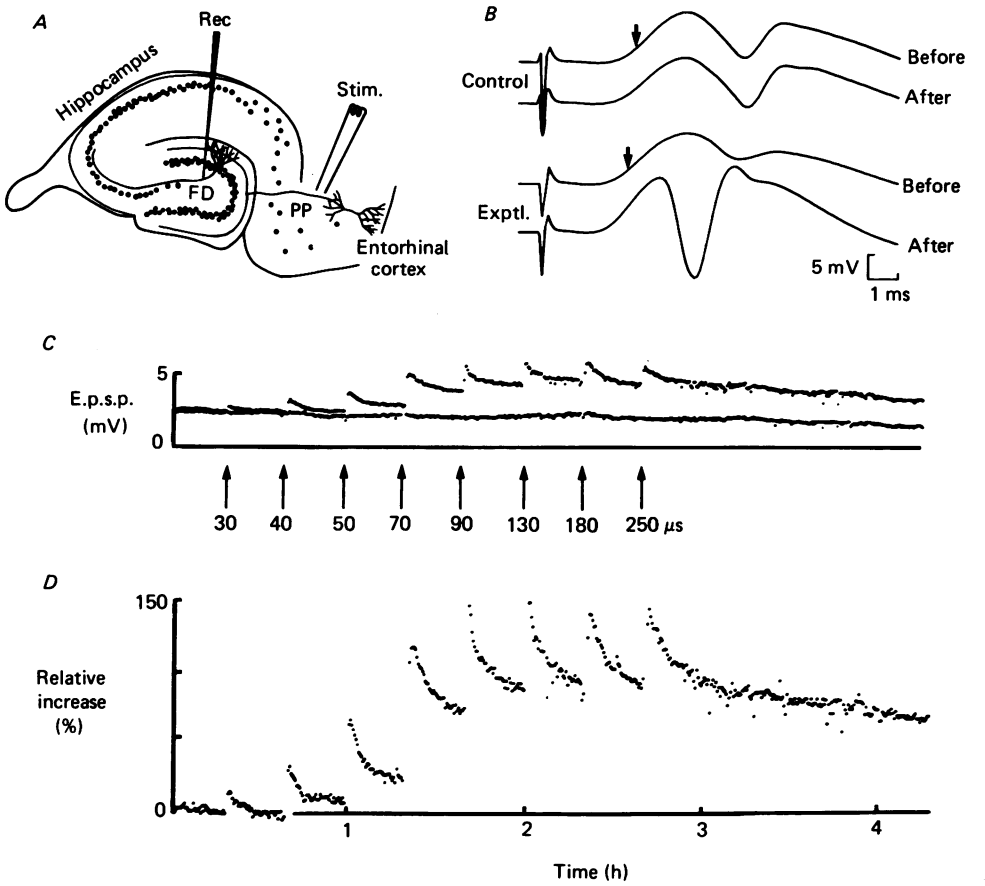


Fig. 1. *A*, schematic transverse section through the hippocampal formation showing the location of stimulating and recording electrodes. A second pair of electrodes was positioned symmetrically in the contralateral hippocampus. PP, perforant path; FD, fascia dentata. *B*, individual evoked responses recorded from the control and experimental sides of a normal animal in response to constant test pulses delivered before and after the delivery of a series of high-frequency conditioning trains to the experimental side. Arrows indicate points of fixed latency at which the e.p.s.p. amplitudes were measured. *C*, plot of the amplitudes of the population e.p.s.p.s evoked by 512 constant test pulses in the experimental (upper plot) and control (lower plot) sides of a normal rat. Arrows indicate the trials on which conditioning trains were delivered to the experimental side; numbers beneath the arrows indicate the duration, in μs, of the pulses within each train, *D*, amplitudes of the population e.p.s.p.s expressed as percentages of the preconditioning mean amplitude. Control side percentages were subtracted from the experimental side percentages, and the difference, denoting the relative increase in the population e.p.s.p., is displayed for each of the 512 test trials.

charge per half-wave necessary to elicit a just detectable spike, was not affected by monoamine depletion. There was evidence, however, that the granule cell population in 5-HT-depleted animals was more excitable than in normal animals, in the sense that a larger population spike was obtained for a given e.p.s.p. (see section on Excitability below).

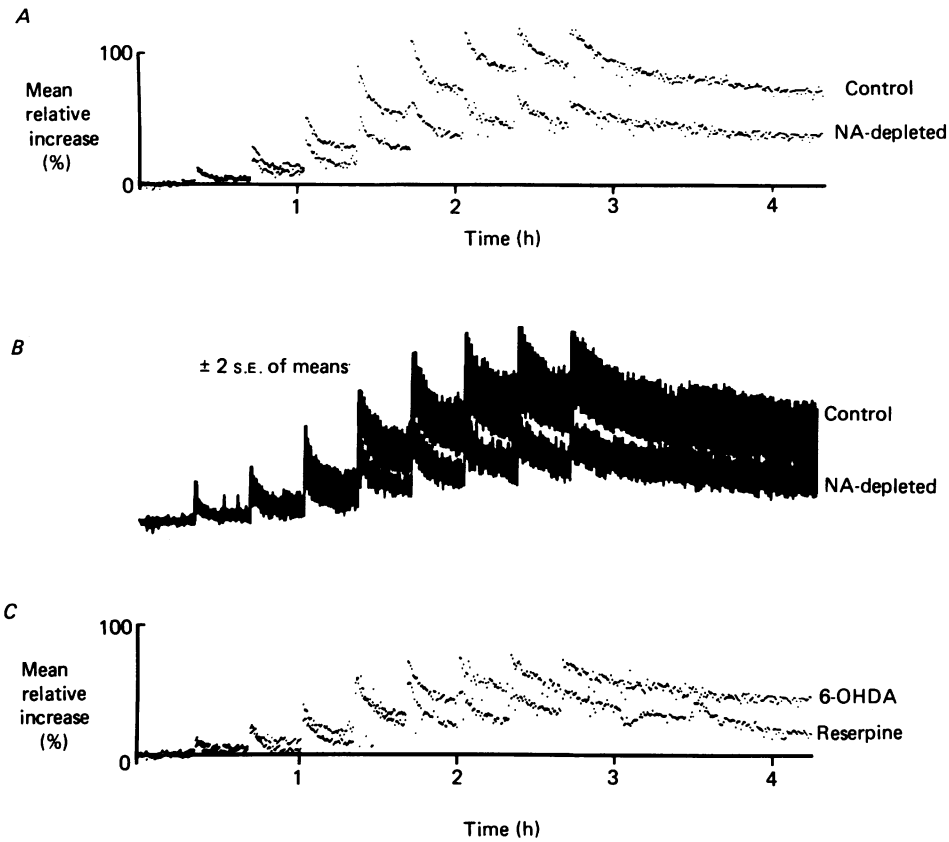


Fig. 2. Reduction of l.t.p. in NA-depleted rats. Test pulses and conditioning trains were delivered as in Fig. 1*C* and the relative increase in the population e.p.s.p. was calculated for each animal as in Fig. 1*D*. These values were then averaged across all the animals in each group to find the mean relative increase in population e.p.s.p. for each of the 512 test points. *A*, mean relative increase in population e.p.s.p. for animals with less than 7·5 % normal hippocampal NA content, following either 6-OHDA lesions ($n = 11$) or reserpine treatment ($n = 9$), compared to a control group consisting of twelve normal and four vehicle-injected rats. *B*, as in *A*, with a bar representing $\pm 2$ S.E. of means superimposed on each point. *C*, mean relative increases in population e.p.s.p. for the group of eleven animals treated with 6-OHDA (upper plot), compared to the group of nine animals treated with reserpine.

*Effect of monoamine depletion on l.t.p. of the population e.p.s.p.*

*Measurement of potentiation.* An example, from a normal animal, of the standardized experiment performed on all the animals in this study is shown in Fig. 1. The amplitude of the synaptic wave is plotted as a function of time for the control and conditioned sides in Fig. 1*C*. The thirty values obtained on each side between trials 10 and 39 were averaged to determine a base line. All values were then expressed as percentages of this base-line level. The percentage scores for each of the 512 values obtained on the control side were then subtracted from the equivalent percentage

scores on the experimental side in order to remove the effects of variations common to both sides. The resulting percentage increase on the experimental side, relative to the control side, is presented graphically in Fig. 1*D*; discontinuities correspond to the times at which high-frequency trains were given to the experimental side. Each conditioning train was followed by a sharp but relatively short-term increase in the amplitude of the population e.p.s.p., with a time course of about 15 min (McNaughton, 1977). Long-term potentiation was recruited after the second train, each succeeding train resulting in a further increase in the magnitude of l.t.p., until saturation was reached after the fifth train. There was some downward drift after the last train, but the relative increase in the amplitude of the e.p.s.p. on the conditioned side was still more than 50% when the experiment ended 96 min later.

*NA-depleted animals.* In order to assess the effect of depletion on l.t.p., group mean curves were computed for all animals receiving the same treatment. Curves giving the relative increase in the amplitude of the e.p.s.p., of the kind seen in Fig. 1*D*, were obtained for each animal in the group, and a group mean computed for each of the 512 points. When this was done for the normal and vehicle-injected animals which formed the control groups for NA-depleted animals, no difference between the two mean curves was found. Data from these animals has therefore been pooled, and is shown as the curve labelled 'control' in Fig. 2*A*. In the same Figure, data from animals treated either with reserpine or with 6-OHDA have been pooled to give the mean relative increase for the NA-depleted animals. Comparison of the two curves shows that while the threshold for l.t.p. was the same in the two groups (corresponding to the second conditioning train, with pulses of 40 $\mu$sec half-width), and the magnitude and duration of short-term potentiation was very similar, the magnitude of l.t.p. in the NA-depleted group was only about half that of the control group throughout the course of the experiment. The standard error ($\times 2$) associated with each point in Fig. 2*A* is displayed in Fig. 2*B* to demonstrate that the difference in the magnitude of l.t.p. was statistically significant after the fourth train, and continued to be so thereafter. (Any corresponding pair of points at which the lines representing $\pm 2$ S.E. of the mean do not overlap, represents a statistically significant difference, $P < 0{\cdot}05$, two-tailed *t*-test. Fig. 2*B* is thus a graphical representation of 512 *t*-tests conducted on the eight blocks of data represented by the eight conditioning trains. Where overlap does not occur on more than 5% of the test points, the two groups may be considered to be significantly different).

In Fig. 1*C*, the reserpine and 6-OHDA groups are compared. Although the mean amplitude of l.t.p. for the animals treated with reserpine was consistently lower than those given intracerebral 6-OHDA, the difference was not statistically significant. Both groups, however, differed significantly from the control group.

*5-HT-depleted animals.* No difference in the magnitude of l.t.p. was seen between the normal and vehicle-injected controls and the data for these two groups have been pooled to give the 'control' curve in Fig. 3*A*, which also contains the curve obtained from the group depleted of 5-HT with a combination of DMI, 5,7-DHT and *p*-CA. Comparison of Figs. 2 and 3 shows that l.t.p. was more severely affected in animals depleted of 5-HT by this treatment than it was in animals depleted of NA with 6-OHDA or reserpine. The mean level of l.t.p. in the 5-HT-depleted group never exceeded 35% of that in the corresponding control group (Fig. 3*A*), and the difference between

depleted and control groups was statistically significant from the second conditioning train onwards (Fig. 3*B*). (These experiments were done several months after the experiments on NA-depleted animals, and for unknown reasons the magnitude and between-animal variability of l.t.p. was smaller in the 5-HT control group than it was in the NA control groups (compare Figs. 2*A* and 3*A*)).

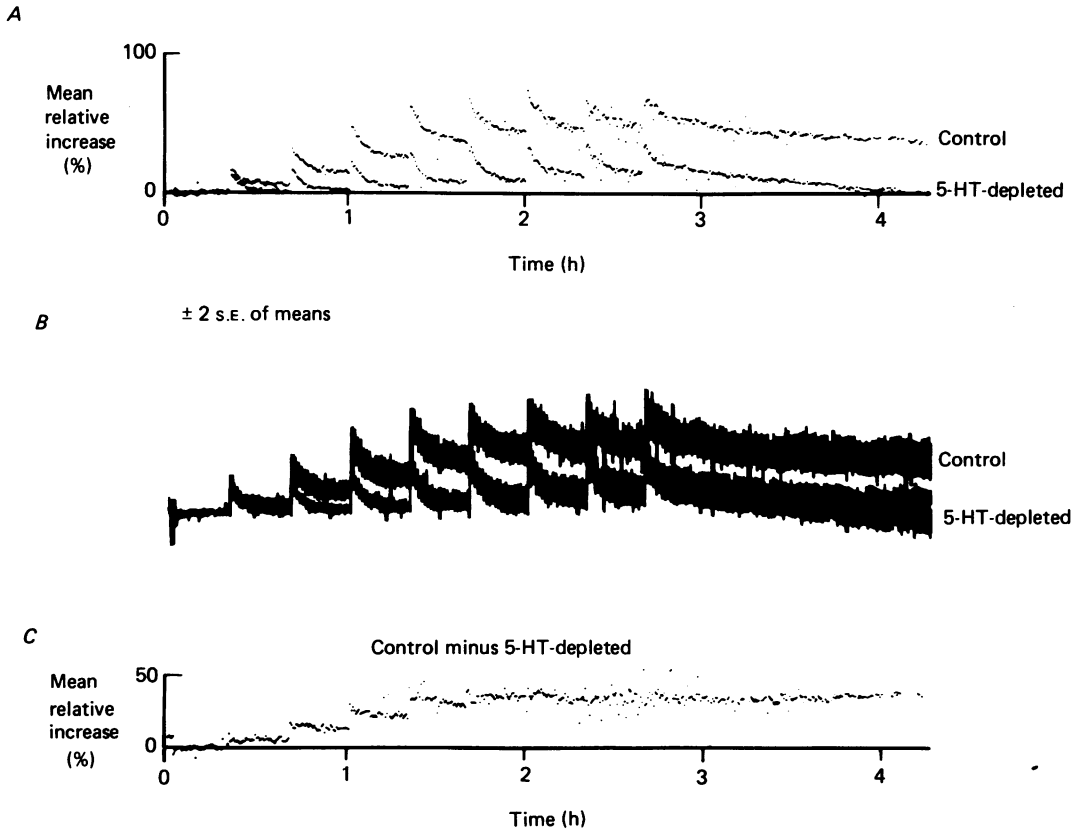


Fig. 3. Reduction of l.t.p. in 5-HT-depleted rats. *A*, average relative increase of the population e.p.s.p. in thirteen animals depleted of 5-HT with a regime of DMI, 5,7-DHT and *p*-CA, compared to a control group of thirteen concurrently tested normal or vehicle injected animals. The average residual level of hippocampal 5-HT in the treated groups was 28%. *B*, the two sets of means in *A* with bars representing ±2 S.E. of means superimposed on each point. *C*, the experimental plot in *A* subtracted from the control plot to show that there was no significant difference in the amplitude or duration of short-term potentiation between the normal and 5-HT-depleted groups.

By the end of the experiment, the mean amplitude of the e.p.s.p. in the 5-HT-depleted group had, on average, declined to its pre-conditioning level; a similar reduction in the duration of l.t.p. was not seen in the NA-depleted group. Furthermore, the threshold for l.t.p., unchanged in animals treated with 6-OHDA, was raised in 5-HT-depleted animals; in the control group, l.t.p. occurred after the first conditioning train (30 $\mu$s pulse half-width), while in the 5-HT-depleted group it was not indisputably present until after the third train (50 $\mu$s per half-wave).

The mean levels of l.t.p. in the other groups (DMI only, DMI and 5,7-DHT lesions without *p*-CA, and *p*-CA only) were all similar to each other and were close to the mean of the control group. Reduction of the magnitude of l.t.p. was not a simple matter of residual 5-HT levels. In three animals the combined method caused less than 60% depletion of 5-HT. The mean l.t.p. of these three animals was as low as the mean of six animals depleted more than 75% by the combined method. On the other hand, 65% depletion by *p*-CA alone failed to reduce l.t.p., and two animals which were depleted by 59 and 60% by 5,7-DHT lesions without *p*-CA also showed normal levels of l.t.p.

The pairs of curves in Figs. 2*A* and 3*A* suggest that depletion of hippocampal monoamines had little effect on the short-term component of potentiation. Fig. 3*C* shows the result of subtracting each point of the 5-HT-depleted curve in Fig. 3*A* from the corresponding point of the control curve; the virtual disappearance of short-term potentiation confirms that it has the much same magnitude and time course in the 5-HT-depleted animals as in control animals. A similar result was found for the NA-depleted animals.

*Changes in granule cell excitability*

*Measurement of excitability.* In an attempt to assess changes in the excitability of the granule cell population brought about by high-frequency trains, and the effect of monoamine depletion on any such changes, we compiled excitability curves before and after conditioning for thirty-two of the animals used in this study; these curves relate the population spike (representing the summed discharges of activated granule cells) to the population e.p.s.p. (representing the summed synaptic input). A total of 140 stimuli, varying in duration from 10 to 250 $\mu$s per half-wave, were delivered in an ordered increasing–decreasing sequence at a frequency of 0·2 Hz at the beginning of the experiment, and again at the end. A computer program (written by R. M. Douglas) was used to measure the slope of the population e.p.s.p. between two assigned points, and the amplitude of the population spike, as determined by the height between the negative-going peak of the population spike and a line drawn between the positive-going peaks which mark the onset and termination of the population spike. The slope of the e.p.s.p. was used in preference to its magnitude at a fixed latency to ensure that slight changes in the onset latency of the response at different stimulus strengths did not distort the measurements. Pre- and post-conditioning excitability curves were constructed for each animal by plotting the 140 values obtained for the slope of the e.p.s.p. against the corresponding values for the population spike. Finally, sets of mean excitability curves were calculated for each of the four main groups (NA-depleted and their controls; and 5-HT-depleted and their controls) by averaging, at each stimulus strength, the e.p.s.p. slope and the population spike for all animals in the same group.

*NA-depleted animals.* Pairs of excitability curves, obtained before and after the sequence of high-frequency conditioning trains, are shown in Fig. 4 for both conditioned and non-conditioned sides of NA-depleted animals and their controls; in each pair, the curve whose maximum extent is marked with an arrowhead was obtained before conditioning. The first question to be asked is whether loss of NA itself causes any change in the excitability of granule cells. Our evidence suggests not: if the curves from normal and NA-depleted animals obtained at the beginning of the

acute experiment are compared (for example, the arrowed curves in Fig. 4*A* and *C*), little difference can be seen between them.

High-frequency conditioning trains produced a substantial effect on the excitability of granule cells in the normal animal. Fig. 4*B* shows that, in addition to the expected increase in the maximum value of the e.p.s.p., there was a reduction in spike threshold, and throughout the range where such a comparison is possible, an increase

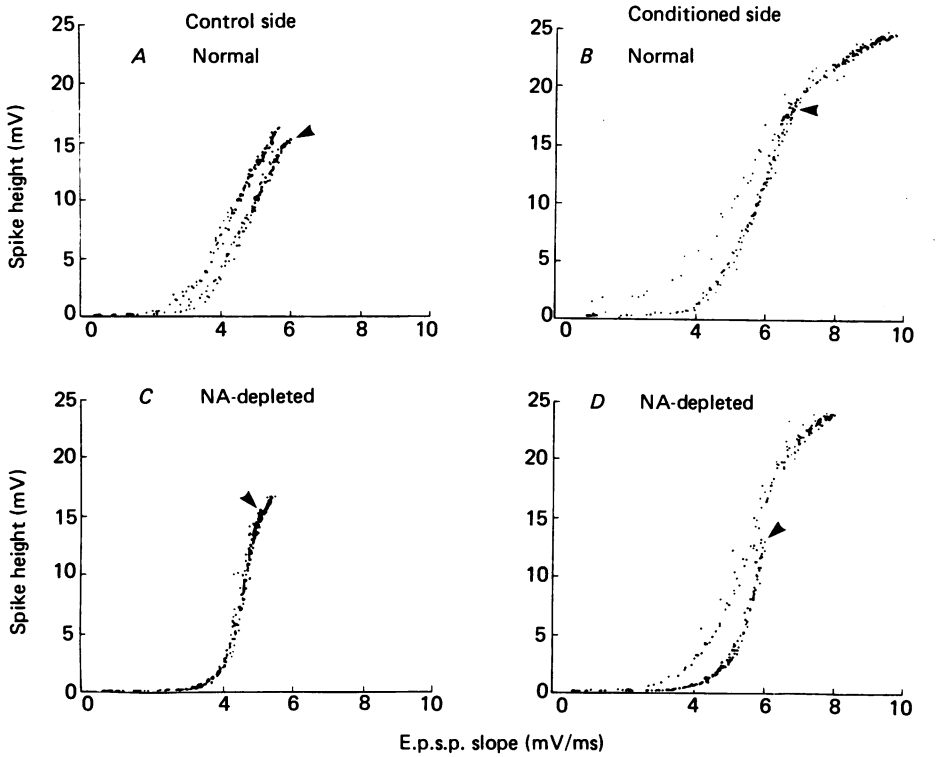


Fig. 4. Averaged excitability curves showing the amplitude of the population spike as a function of the slope of the e.p.s.p. in a group of ten normal animals and a group of seven concurrently tested NA-depleted rats (see text). Points are plotted separately for control and conditioned sides, with the curves obtained before and after conditioning superimposed; the arrow on each pair indicates the upper limit of the pre-conditioning curve. *A* and *B*, mean excitability curves obtained before and after conditioning for the control and conditioned sides in the group of normal animals. *C* and *D*, similar curves for the group of NA-depleted animals.

in excitability, in the sense that a given e.p.s.p. was associated with a larger population spike. It is convenient to refer informally to this change as a shift to the left in the excitability curve. (There was also a small but definite shift to the left in the excitability curve for the control side (Fig. 4*A*). This is a reflexion of an unexplained gradual upward drift in the amplitude of the population spike, without a corresponding change in the e.p.s.p., which often occurred on the control side in all groups of animals, and which was unrelated to the abrupt, conditioning-dependent changes in amplitude induced on the experimental side.)

Equivalent data are presented for the NA-depleted animals in Figs. 4*C* and *D*. The pre- and post-conditioning curves for the control side were almost superimposed in this case. If the effects of conditioning on the NA-depleted group (Fig. 4*D*) are compared with its effects on the non-depleted control group (Fig. 4*B*), it is evident that the shift to the left of the excitability curve was much the same in the two groups, suggesting that loss of NA does not affect the long-term increase in granule cell excitability which is a characteristic component of l.t.p. in normal animals.

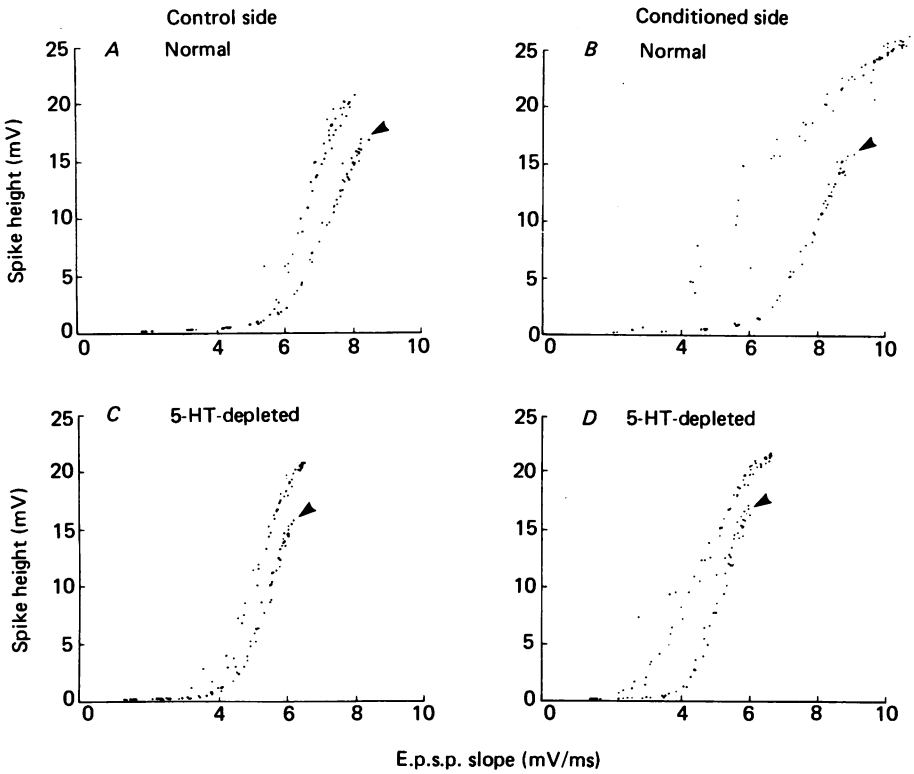


Fig. 5. Excitability curves obtained as in Fig. 4, before and after conditioning, for a group of normal rats ($n$ = 10), and a group of concurrently examined 5-HT-depleted rats ($n$ = 5).

*5-HT-depleted animals.* The data obtained from 5-HT-depleted animals and their controls, presented in Fig. 5, reveals a number of differences between the NA- and 5-HT-depleted groups. In the first place, loss of 5-HT, unlike loss of NA, itself induces a marked increase in excitability (compare the shift to the left of the arrowed curve in Fig. 5*C* with respect to the arrowed curve in Fig. 5*A*, and similarly for the arrowed curves in Fig. 5*B* and *D*). Secondly, although there was again a conditioning-induced increase in excitability in the non-depleted group, as evidenced by the far greater shift to the left of the excitability curve on the experimental side than on the control side (compare the 'before' and 'after' curves in Fig. 5*B* with the 'before' and 'after' curves in Fig. 5*A*), there was no such difference in the 5-HT-depleted group after

conditioning (Figs. 5*C* and *D*). Loss of 5-HT therefore not only reduces l.t.p. of the e.p.s.p., but also reduces the long-term enhancement of granule cell excitability.

## DISCUSSION

L.t.p. has two distinct and independent components; potentiation of the population e.p.s.p., and a potentiation of the population spike which is more than can be accounted for by potentiation of the synaptic wave alone (Bliss, Gardner-Medwin & Lømo, 1973; Andersen, Sundberg, Sveen, Swann & Wigstrom, 1980; Wilson, 1981; Wilson, Levy & Steward, 1981). This latter effect, which we refer to as a long-term potentiation of excitability, is revealed as an increase in slope, or shift to the left, of the excitability curve relating the amplitude of the population spike to the slope of the e.p.s.p. The main result of this study is that l.t.p. of the population e.p.s.p. depends for its full expression on the integrity of the forebrain serotonergic and noradrenergic projections. The excitability component of l.t.p., on the other hand, though depressed by 5-HT lesions, is not significantly affected by lesions of the noradrenergic projection.

### *Mechanisms of monoamine involvement in l.t.p.*

The present experiments give few clues to the possible mechanisms by which monoamines might affect l.t.p. We have measured monoamine levels only in the hippocampus, but our lesions will certainly have depleted NA and 5-HT levels throughout the cortex, and spinal and cerebellar projections are also likely to have been affected. Thus we cannot exclude the possibility that the reduction in l.t.p. in lesioned animals is due to a neural mechanism originating outside the hippocampus, or indeed to some generalized metabolic or circulatory effect of the lesions. Nevertheless, the most straightforward interpretation of our data is that the reduction in l.t.p. is a consequence of the loss of monoamine terminals in the hippocampus. It is therefore worth examining our results in the context of what is known about the action of NA and 5-HT on hippocampal neurones.

*Evidence from unit studies.* Both NA and 5-HT tend to suppress spontaneous or glutamate-induced firing of pyramidal cells (Segal & Bloom, 1974; Jahnsen, 1980) and granule cells (S. Y. Assaf, personal communication) in the hippocampus. There is a good deal of evidence from *in vitro* experiments that monoamines cause an increase in membrane conductance in both types of cell (Jahnsen, 1980; Segal, 1980, 1981; Langmoen, Segal & Andersen, 1981; Assaf, Crunelli & Kelly, 1981) and it is probable that the inhibition is a consequence of this effect. Changes in membrane potential have also been noted. NA hyperpolarizes granule cells (M. Segal, personal communication), but there are conflicting reports regarding the action of 5-HT on granule cells, Assaf *et al.* (1981) reporting a depolarization and M. Segal (personal communication) a hyperpolarization. In principle, changes in membrane potential or conductance could lead to alterations in both the synaptic and spike components of the evoked potential (Bliss & Lømo, 1973; Winson, 1980), but it is not easy to see how conductance changes alone could explain the shift to the left of the excitability curve in the 5-HT-depleted animals, or the reduced ability of both 5-HT- and NA-depleted animals to sustain l.t.p. A change in membrane potential offers a more promising possibility. If, as Segal (1981) suggests, the serotonergic input to granule

cells is hyperpolarizing, the loss of 5-HT terminals would result in a diminution of the synaptic current evoked by a perforant path volley because of the reduction in driving potential. The synaptic potential for a given stimulus would therefore be smaller, while the reduced threshold of the chronically depolarized cell would tend to prevent any concomitant decrease in the population spike. The shift to the left in the excitability curve of the 5-HT-depleted animals could be explained in this way, as could the reduction in l.t.p., since any non-linearity of the membrane would ensure that the more a cell were depolarized the smaller would be its response to a given increment of synaptic input (Ginsborg, 1967). This cannot be the whole story, however, since a change in membrane potential would be expected to affect all types of potentiated response in the same way, yet the magnitude of short-term potentiation was unaffected in both NA- and 5-HT-depleted animals.

*Synaptic modulation by monoamines.* In contrast to the actions of NA and 5-HT described above, the effects of monoamine depletion on l.t.p. are primarily, and in the case of NA, exclusively, on the synaptic wave. Although the mechanism of l.t.p. itself is still in doubt, potentiation of the synaptic wave is generally supposed to involve local mechanisms operating at the synapse (Bliss & Lømo, 1973; Bliss, 1979; Andersen *et al.* 1980; Dolphin, Errington & Bliss, 1982; but see Douglas, Goddard & Riives, 1982); if so, monoamines must ultimately express their action at the synaptic level, either presynaptically to affect transmitter release, or post-synaptically to modify the response of the cell to the perforant path transmitter. Evidence exists that monoamines can play such a role in other systems. Thus, monoamines modulate the release of transmitter from cholinergic (Vizi, Harsing & Zsilla, 1981; Vizi, Ronai, Harsing & Knoll, 1977) and glutaminergic (Dolphin, 1982) pathways in the mammalian nervous system; and a 5-HT-mediated presynaptic facilitation has been described in *Aplysia* (Brunelli, Castellucci & Kandel, 1976; Shimahara & Tauc, 1977). Long-lasting increases in synaptic efficacy, based on the action of monoamines and involving presynaptic (Kuba, Kato, Kumamoto, Koketsu & Hirai, 1981) or post-synaptic (Libet, Kobayashi & Tanaka, 1975; Kobayashi, Hashiguchi & Ushiyama, 1978) mechanisms have been proposed in vertebrate sympathetic ganglia.

Whether or not similar pre- or post-synaptic mechanisms can account for the depression of l.t.p. in the monoamine-depleted hippocampus remains for future work to resolve. A major problem is the fact that although there is a weak projection of both NA (Loy *et al.* 1980) and 5-HT fibres to the molecular layer of the dentate gyrus, the majority of fibres of both types terminate in the hilus, several hundred microns from the perforant path terminals. In this respect, it is interesting to note that a train of stimuli to the contra or ipsilateral hilus can, if delivered just before or during a conditioning train to the perforant path, prevent the induction of l.t.p. (Douglas, 1978; Douglas *et al.* 1982). The mechanism of this suppression is unknown, but it is presumably a consequence of activation of CA4 pyramidal cells, which are located in the hilus and have a bilateral projection to the dentate gyrus (Swanson, Sawchenko & Cowan, 1980). This raises the intriguing possibility that the reduction of l.t.p. in monoamine-depleted animals is due to the removal of a tonic monoaminergic inhibitory input to CA4 cells. Direct evidence for an inhibitory action of monoamines on CA4 cells has not so far been provided, but, as pointed out above, monamines are inhibitory elsewhere in the hippocampus.

*Conclusions*

Previous work has established that there are threshold values for both the frequency and intensity of the conditioning train to the perforant path which must be exceeded if l.t.p. is to be induced (Bliss & Lømo, 1973; McNaughton *et al.* 1978). To these two requirements must now be added a third: the presence of NA and 5-HT are necessary for the full elaboration of l.t.p. Residual levels of NA, and more particularly 5-HT, were present in all our animals, but even with our most successful lesions there was clearly some degree of l.t.p. In this sense monoamines are facilitatory rather than obligatory to the processes underlying l.t.p.

A question of some interest, and one which we cannot answer on the basis of the present experiments, is whether the induction of l.t.p. requires a specific temporal contingency between monoamine activity and the conditioning train. The point is relevant to the hypothesis that activity in the ascending noradrenergic system acts as a reinforcing signal to strengthen those synaptic connections associated with concurrent, biologically rewarding behaviour (Crow, 1968; Kety, 1972). If l.t.p. is to be an appropriate exemplification of this model of the synaptic processes underlying learning, then an increase in NA levels associated with the conditioning train should produce maximal l.t.p. Laroche & Bloch (1981) have recently found that stimulation of the reticular formation at the same time as a high-frequency train to the perforant path results in an increase in l.t.p. Similar experiments with stimulating electrodes placed in the locus coeruleus or median raphe nucleus should enable the prediction derived from Crow's theory to be tested.

These experiments emphasize the independent nature of the three types of potentiation commonly seen following high-frequency stimulation of the perforant path: short-term potentiation, long-term potentiation of the synaptic wave, and long-term potentiation of granule cell excitability. Our results confirm the finding of McNaughton *et al.* (1978) that short-term potentiation occurs with low intensity conditioning trains which are too weak to induce l.t.p., and show also that short-term potentiation, unlike l.t.p., is not affected by depletion of hippocampal monoamines. Finally, the observation that long-term changes in excitability are blocked by 5-HT-depletion but are relatively unaffected by NA depletion, whereas synaptic potentiation is diminished by both types of lesion, identifies a drug-induced dissociation between these two components of l.t.p.

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