*J. Physiol.* (1982), **324**, *pp.* 249–262
*With 6 text-figures*
*Printed in Great Britain*



# LONG-TERM SYNAPTIC ENHANCEMENT AND SHORT-TERM POTENTIATION IN RAT FASCIA DENTATA ACT THROUGH DIFFERENT MECHANISMS

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(*Received 13 November 1980*)

SUMMARY

1. The component processes contributing to post-activation change in synaptic efficacy in the perforant pathway to the fascia dentata were studied in rats under sodium pentobarbitone anaesthesia.

2. With low stimulus strength, which activated only a relatively small number of perforant path fibres, repetitive stimulation led to effects which had very similar characteristics to those observed at neuromuscular synapses under similar conditions. Paired shocks resulted in a short ($\sim$ 100 ms) facilitation superimposed on a depression, possibly due to depletion of available transmitter, which recovered more slowly ($\sim$ 4 s). Short trains of stimuli at 125–250 Hz led to a longer lasting increase in synaptic strength which decayed to control levels with a double exponential time course. The two exponential components behaved like augmentation and potentiation at neuromuscular synapses, with time constants at 33 °C of about 5 s and about 90 s respectively.

3. High-intensity stimulus trains of identical frequency and duration led to an enhancement of synaptic strength which lasted for longer than 30 min.

4. The paired shock depletion effect was increased in direct proportion to the amount of augmentation and potentiation present following low-intensity stimulus trains. Following high-intensity trains the paired shock depletion effect was increased by the same amount, and recovered with the same time course as following low-intensity stimulus trains, even though there remained a significant enhancement of the synaptic response.

5. The results are interpreted as indicating that augmentation and potentiation are due to an increase in the probability of transmitter release whereas long-term enhancement acts through some other, as yet undetermined, mechanism. Following high-intensity stimulation all three processes are activated.

## INTRODUCTION

Activation of perforant path synapses in the fascia dentata may result in marked alterations in their efficacy on subsequent trials (Andersen, Holmqvist & Voorhoeve, 1966, Bliss & Gardner-Medwin, 1973; Bliss & Lømo, 1973; Douglas & Goddard, 1975;

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Gloor, Vera & Sperti, 1964; Lømo, 1966, 1971; McNaughton, Douglas & Goddard, 1978). The sign, magnitude, and time course of these alterations are functions of both the temporal pattern of the preceding activity, and of the number of fibres which were co-active. Of particular interest has been the long-term increase in synaptic transmission which occurs when a sufficient number of afferent fibres are simultaneously active at high frequency. This change has been referred to as long-term or long-lasting potentiation. The data of McNaughton *et al.* (1978), however, suggested that the generation of the long-term effect followed different rules from the generation of potentiation seen at most other synaptic types (Larabee & Bronk, 1947; Lloyd, 1949; Liley & North, 1953; Liley, 1956; Magleby, 1973; Martin & Pilar, 1964; Rosenthal, 1969; Weinrich, 1971). Unlike other systems, in which potentiation could be elicited by high-frequency activation of a single afferent fibre, the long-term change in the fascia dentata required the concurrent activity of a reasonably large number of input fibres. For this reason, McNaughton *et al.* (1978) suggested the use of the alternate term *enhancement* to refer to the long-lasting effect, since the conditions for its generation were different from neuromuscular *potentiation*. Nevertheless, in spite of the differences in mode of generation, it remained possible that enhancement in the fascia dentata resulted from an increased probability of transmitter release in the same way as is believed to occur for neuromuscular potentiation (see below). The purpose of the present report is to show that short-term changes in synaptic efficacy, behaving identically to those described in neuromuscular systems, also occur at perforant path terminals, and in particular that enhancement does not have the same mechanism as potentiation.

At least two different processes have been recognized for some time to result in increased transmitter release in neuromuscular and other central and peripheral synapses following repetitive electrical stimulation of the afferent fibres. These were originally referred to as *facilitation* and *post-tetanic potentiation*. More recently, Magleby & Zengel (1975, 1976*a*, *b*) have shown that post-tetanic potentiation, which was earlier thought to be a single process, is better described as the sum of two exponential components which they defined as *augmentation* and *potentiation* respectively. Facilitation, augmentation, and potentiation were each defined as the fractional increase of a test response over control level in the absence of the other two processes. The three processes decayed in an exponential manner with time constants of about 100 ms, 7 s, and 2–3 min respectively in frog neuromuscular junction at 20 °C. Facilitation is thought to differ from potentiation because the two phenomena approach a multiplicative relationship as the maximal quantal release on any impulse approaches a negligible fraction of the available quanta (Landau, Smolinsky & Lass, 1973). At higher levels of quantal release, however, the time course of facilitation is confounded by an apparent *depletion* of available quanta, an effect which itself recovers exponentially over several seconds (Betz, 1970; Christensen & Martin, 1970; del Castillo & Katz, 1954; Lundberg & Quilisch, 1952*a*, *b*; Otsuka, Endo & Nonomura, 1962; Takeuchi, 1968). This depletion process results in an apparent reduction in relative facilitation in the presence of potentiation (Landau *et al.* 1973; Magleby, 1973), a fact which provides the major evidence that potentiation is itself due to an increase in the probability of release.

It has recently been shown that the effect of paired shocks to the perforant path

on the field e.p.s.p. is a composite of a depletion phase on which is superimposed a shorter lasting facilitation (McNaughton, 1980). The kinetics of these components are very similar to those seen in neuromuscular synapses discussed above. In particular, the depletion phase is reduced in proportion to the magnitude of the initial response as the external calcium concentration is reduced. The experiments to be described here first establish that perforant path synapses show short-term changes which are quantitatively similar to augmentation and potentiation as defiend by Magleby & Zengel (1976*a*). It will then be shown that the depletion component of the paired shock effect is increased in proportion to the combined magnitudes of these components, but is unaffected by the presence of long-term enhancement.

An abstract of some of the present results has been published (McNaughton, 1977).

## METHODS

The experiments to be described were carried out on twenty-eight male Long-Evans rats of the Charles River strain weighing between 350 and 550 g. Stimulation sites were confined to the lateral perforant pathway as defined electrophysiologically and by anterograde degeneration by McNaughton & Barnes (1977) and McNaughton (1980). The details of electrode verification can be found in those reports. The lateral pathway was chosen both to reduce experimental variation due to mixed pathway responses, and because the short-term effects of repetitive stimulation are considerably larger than in the medial pathway (McNaughton, 1980), thus making their resolution more accurate. Animals were maintained under sodium pentobarbitone anaesthesia by continuous low rate intraperitoneal infusion.

Electrodes for extracellular stimulation and recording consisted of 114 $\mu$m (coated diameter) teflon-insulated stainless-steel wire (Medwire Corp.). The recording electrode was placed in the hilus fascia dentatae by monitoring multiple unit activity density (McNaughton, 1980) at 3·5 mm posterior and 1·8 mm lateral to bregma with the skull surface horizontal. The stimulation electrode was located approximately 8·0 mm posterior and 4·5 mm lateral to bregma, fine adjustments being made until low-intensity shocks elicited field e.p.s.p.s with rise times of 2·3 ms or greater. Lesions in these locations were previously shown to result in terminal degeneration in the lateral perforant path termination field in the outer third of the molecular layer (McNaughton & Barnes, 1977).

Primary signal amplification was by means of a Grass P51B a.c. amplifier with 1/2 amplitude filters set at 10 Hz and 3 kHz. These signals were sampled on-line by a PDP 11/34 computer at a rate of 10 kHz. Stimulation consisted of photically isolated constant-current diphasic square waves ranging in amplitude from 30 to 150 $\mu$A (each way) and in duration from 30 to 250 $\mu$s (each half cycle). In all experiments described here, the stimulus intensity was set at 30 $\mu$s times whatever current was necessary to elicit a positive extracellular e.p.s.p. of between 0·5 and 2·0 mV. This was always well below the threshold for granule cell discharge. The initial stimulus intensity was held constant throughout any given experiment with the exception that, where it was desired to elicit long-term synaptic enhancement, stimulus intensity was increased during the high-frequency train by increasing pulse width. The stimulus intensity was always returned immediately thereafter to the initial control level for testing the effects of the high-frequency train.

The effect of any experimental treatment on the amplitude of the field e.p.s.p. was expressed as a percent change of the response at time $t$ ($V_t$) relative to the baseline control level ($V_0$), i.e.

$$\% = 100 \times (V_t - V_0)/V_0$$

The components defined as augmentation and potentiation were analysed by successive removal of exponentials from semi-logarithmic plots of percent increase versus time assuming the relation

$$\text{total percent change} = A_0 e^{-t/\tau_A} + P_0 e^{-t/\tau_P}$$

where $A_0$ and $P_0$ are the initial values of augmentation and potentiation, and $\tau_A$ and $\tau_P$ are the corresponding decay time constants. For experiments involving paired stimuli, the percent difference between responses within pairs is denoted by $f$.

## RESULTS

### *Short-term augmentation and potentiation can be elicited repeatedly*

As shown by McNaughton *et al.* (1978), provided that the stimulus intensity was kept below a level sufficient to elicit a field e.p.s.p. of less than (on average) 5·6 mV, tetanization of perforant path fibres failed to elicit long-term synaptic enhancement. Only short-term changes were observed. McNaughton (1980) showed that these short-term changes had decay kinetics resembling augmentation and potentiation at neuromuscular synapses. The following experiments were performed to illustrate the decay kinetics, and to demonstrate that the short-term effects can be elicited repeatedly, without change in their magnitudes or decay rates, provided sufficient time is allowed for the recovery of baseline e.p.s.p. amplitudes between tetani.

In one experiment ten trials were given, each being separated by 10 min. Each trial consisted of twenty-five low-intensity shocks delivered at 3 s intervals to establish the baseline e.p.s.p. amplitude, followed by a single seventy-five pulse tetanus at 250 Hz. The effect of the tetanus on the e.p.s.p. was then tested for a further seventy-four shocks at 3 s intervals. Fig. 1*A* shows these results plotted as percent change relative to baseline. The average percent change is shown in linear co-ordinates in Fig. 1*B* and in semilogarithmic co-ordinates in Fig. 1*C*. The two component decay function can be readily observed in the latter plot. The exponential parameters for the data obtained by linear regression on the semilogarithmic plots between 45 and 150 s following the tetanus (potentiation) and on the residuals after subtraction of potentiation, between 6 and 24 s (augmentation) are given in Table 1. There was no significant trend over trials in any of the derived exponential parameters.

A second experiment was carried out essentially as just described, with the exception that twenty trials were given and the frequency of test stimulation was alternated between trials from 1/3 Hz to 1/15 Hz. Again, there was no trend over trials in the exponential parameters of augmentation and potentiation at a given frequency of test stimulation. However, the decay of potentiation was significantly slower in the 1/15 Hz test condition. A more detailed study of the effect of test frequency is in preparation.

### *Effects of tetanus duration on augmentation and potentiation*

Magleby & Zengel (1975, 1976*a*) examined the effects of increasing stimulus train duration on the exponential parameters of augmentation and potentiation. Their data showed that the initial magnitudes of both components increased as the train length increased. However, the variance in the data was too large to determine whether the two parameters increased differentially. Nevertheless, they did show that there was an approximately linear positive relation between the initial magnitude of potentiation ($P_0$) and its decay time constant ($\tau_P$) whereas the decay time constant of augmentation ($\tau_A$) remained constant over a wide range of the initial magnitude of augmentation ($A_0$). The following experiment examined these effects in the lateral perforant path.

Four experiments were carried out, one on each hemisphere of each of two animals. The animals' body temperatures were lowered to 27 °C in order to reduce response

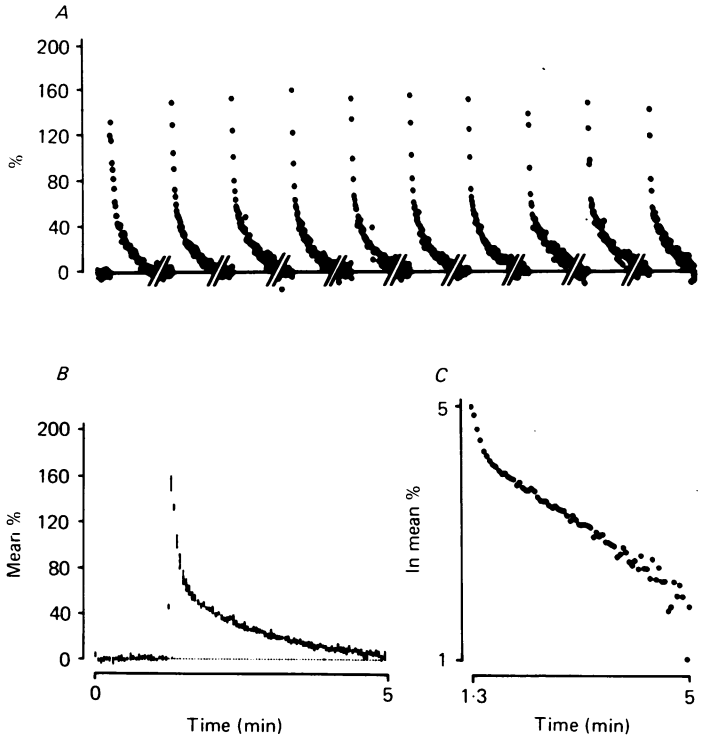


Fig. 1. *A*, the percent increase in e.p.s.p. response following a train of seventy-five impulses at 250 Hz using low stimulus intensity is shown as a function of time for ten consecutive trials. Each trial was separated by 10 min and consisted of 100 sweeps at 1/3 Hz with the train delivered at sweep 26 of each series. The gaps in the record represent 5 min of non-stimulated recovery period. The mean percent change for the ten trials is shown in linear co-ordinates in *B* and semilogarithmic co-ordinates in *C*. The latter plot clearly shows a rapidly decaying component (augmentation) superimposed on a more slowly decaying component (potentiation). The logarithmic regression parameters for these data are given in Table 1.

TABLE 1. Logarithmic regression analysis of the relation between the percent increase in field e.p.s.p. and time following high-frequency stimulation (same data as Fig. 1*C*)

| | Potentiation | | Augmentation |
|---|---|---|---|
| $P_0$ | 69·41 % | $A_0$ | 242·76 % |
| $\tau_P$ | 87·01 s | $\tau_A$ | 4·73 s |
| $r^2$ | 0·984 | $r^2$ | 0·997 |
| $n$ | 36 | $n$ | 6 |

variability and to provide greater resolution of the decay parameters, which were prolonged at reduced temperature. This also permitted a calculation of the $Q_{10}$ for the decay time constant of augmentation which was reported as 3·8 by Magleby & Zengel (1976*a*).

The stimulation procedures for both animals consisted of 100 test stimuli at 0·5 Hz followed by a 250 Hz train which, in turn, was followed by a further 155 test

stimuli at 0·5 Hz. These trials were repeated at 13 min intervals with the number of pulses in the tetanus being varied from trial to trial. In one animal, trains of twelve, twenty-five, fifty, seventy-five, 100, 125, and 150 pulses were delivered in ascending order over trials. This series was repeated six times for a total of forty-two trials. In the other animal, forty trials were carried out in which the number of stimuli in the trains was varied from three to 112. The order of presentation was drawn at random from a set which was weighted towards lower values.

The e.p.s.p. amplitudes following the tetani were expressed as percent of baseline and the augmentation and potentiation components were determined by linear regression on the logarithms as described above. While both augmentation and potentiation increased with increasing stimulus train duration, potentiation appeared to reach an asymptote which attained half its maximum value following about twenty-five pulses. Augmentation, on the other hand, increased more steeply than potentiation in an apparently linear fashion. In agreement with the neuromuscular data of Magleby & Zengel (1976*a*), the time constant of potentiation showed a significant positive correlation with the initial magnitude of potentiation whereas the time constant of augmentation was unchanged over the range of initial magnitudes observed. An example of these data is shown in Fig. 2 and the statistical summary is presented in Table 2.

In these experiments, the over-all mean decay time constant for augmentation at 27 °C was 10 s. In the experiment above describing the effects of repeated tetani at 32·6 °C the time constant was 4·73 s. Thus the $Q_{10}$ for the decay of augmentation in perforant path synapses is 3·78, which is almost identical to the value in neuromuscular synapses.

In general then, these data show that augmentation and potentiation in perforant path synapses are remarkably similar to the corresponding processes in the neuromuscular junction. There can be little doubt that common mechanisms are involved.

*Interactions with paired shock facilitation and depletion*

In order to examine the effects of augmentation and potentiation on the response of perforant path synapses to paired shocks, six experiments were carried out in which stimulus pairs separated by 30 ms were used as test stimuli rather than the single shocks employed above. The dependent variable in these studies was thus the percent change of the second e.p.s.p. relative to the first in each pair. The short interval was chosen so as to make the change in response due to the decay of augmentation and potentiation negligable (the response decrement due to the decay of augmentation during this interval is less than 1 %). Following a period of baseline testing, seventy-five shocks at 250 Hz were given, after which paired-pulse testing was resumed until augmentation and potentiation had decayed. The test pairs were delivered every three seconds. In all cases, there was a reduction in the relation between the first and second responses in each pair in almost direct proportion to the combined magnitude of augmentation and potentiation at any given time. The baseline relation between the paired responses recovered as augmentation and potentiation decayed. An example of these data is shown in Fig. 3 and the over-all regression parameters for the six experiments summarized in Table 3. These data strongly suggest that, as with neuromuscular synapses, there is an increased depletion of transmitter following

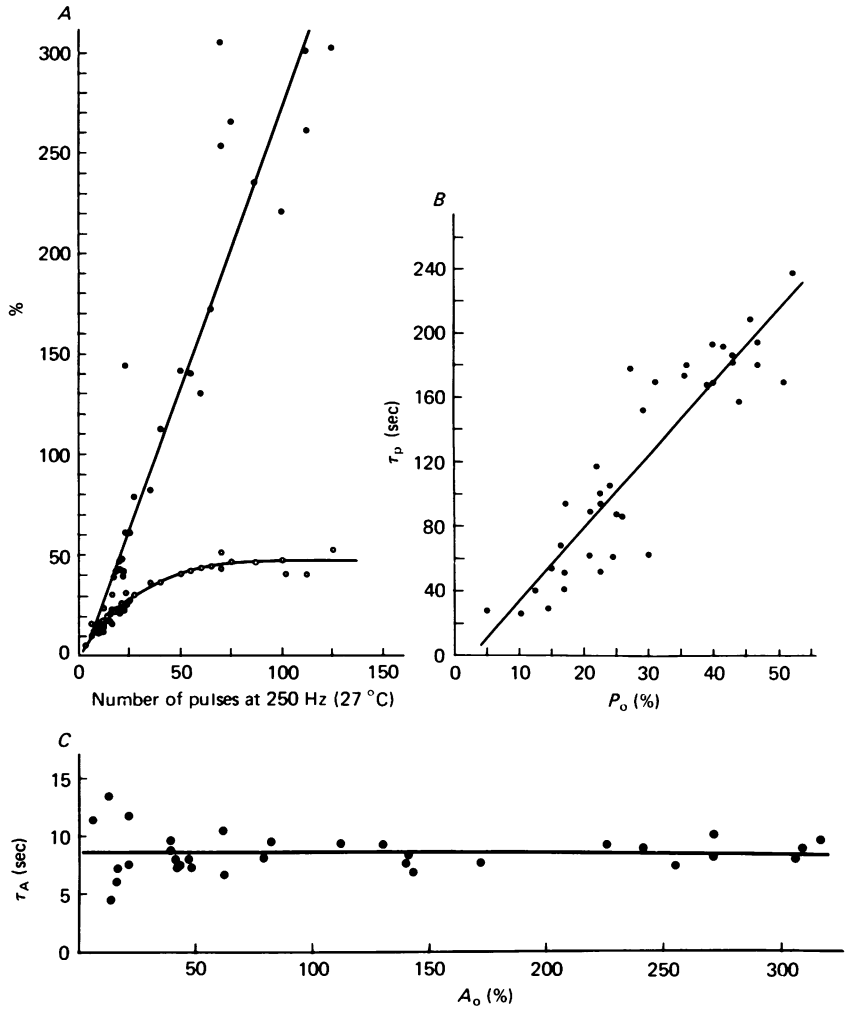


Fig. 2. *A*, example of the effect of varying the number of pulses in a 250 Hz low-intensity stimulus train on the initial magnitudes of augmentation ($A_0$; ●) and potentiation ($P_0$; ○) derived as described in the text. In four such experiments $A_0$ increased in an approximately linear fashion as the number of stimulus pulses was increased, whereas $P_0$ clearly tended towards an asymptotic value. $P_0$ reached one-half of its maximum value within about twenty-five impulses. *B*, plot of the decay time constant of potentiation ($\tau_P$) versus $P_0$. As with neuromuscular potentiation, $\tau_P$ showed a significant linear relationship with $P_0$. *C*, the relation between the time constant for augmentation ($\tau_A$) and $A_0$. Also as with neuromuscular data, there was no significant regression for $\tau_A$ *vs.* $A_0$. The statistical summary and average regression parameters for the four experiments is given in Table 2.

single shocks in the presence of augmentation and potentiation due to the elevated probability of release during these processes.

As demonstrated previously (McNaughton *et al.* 1978), long-term enhancement of perforant path synapses could be reliably elicited using the same stimulus frequency characteristics as in the above experiments merely by increasing the stimulus

intensity during the tetani so as to activate a larger number of afferent fibres. An example of this effect is shown in Fig. 4. The following experiment was carried out in order to determine whether the increased depletion of transmitter release inferred from the previous study would also be manifested in the presence of long-term enhancement.

TABLE 2. Linear regression analysis on exponential parameters of augmentation and potentiation

Initial value of augmentation *vs.* number of stimuli

| | Mean | S.E. of mean |
|---|---|---|
| Slope | 1·564 | 0·384 |
| Intercept | 5·914 | 5·093 |
| $r^2$ | 0·864 | 0·092 |

$P < 0·05$ in four of four cases

Decay time-constant of potentiation *vs.* initial value of potentiation

| | Mean | S.E. of mean |
|---|---|---|
| Slope | 3·514 | 0·470 |
| Intercept | 26·557 | 13·937 |
| $r^2$ | 0·754 | 0·080 |

$P < 0·05$ in four of four cases

Decay time-constant of augmentation *vs.* initial value of augmentation

| | Mean | S.E. of mean |
|---|---|---|
| Slope | 0·001 | 0·004 |
| Intercept | 9·930 | 0·523 |
| $r^2$ | 0·083 | 0·068 |

$P < 0·05$ in none of four cases

Note: no regression analysis was carried out for the initial value of potentiation *vs.* number of stimuli as this relation was clearly asymptotic (see example Fig. 3(*a*). Data from four experiments.

Pairs of low-intensity test stimuli with a 50 ms inter-pulse interval were delivered at a rate of one pair every 5 s over a total period of 33·3 min with the exception that the 76th and 226th test pairs were substituted with stimulus trains of fifty shocks at 125 Hz. The first train was given at the same low intensity as the test shocks. During the second train the stimulus intensity was raised to a level which on single shocks was sufficient to evoke a population spike. Nine such experiments were carried out, the results of which are summarized in Table 4.

The first stimulus train elicited augmentation and potentiation which decayed back to baseline in a similar manner to the experiments described above. By 10 min following the tetanus, the average change in the magnitude of the first response in each pair relative to control was not significantly different from zero. As in the experiment described above, the relation between the first and second responses in each pair was reduced in direct proportion to the combined magnitudes of augmentation and potentiation present at any given time following the high-frequency train. By 10 min after the train, the relation had recovered to a level not significantly different from baseline.

The second, high-intensity, stimulus train evoked an increase in the first e.p.s.p. in each pair which did not recover baseline during the course of the experiment. At 10 min following this train, the response was elevated on average 20·2 %. The relation between responses within pairs was reduced during the early part of the post-train

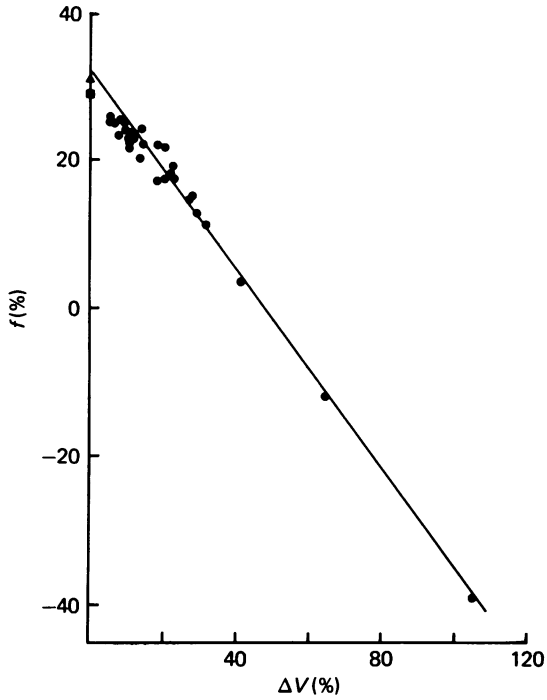


Fig. 3. The relationship between the relative change in e.p.s.p. amplitude ($f$) within pairs (inter-stimulus-interval 30 ms) and the absolute increase in the amplitude of the first e.p.s.p. in each pair due to the presence of augmentation and potentiation following a low-intensity stimulus train (●). The pretetanus value of $f$ is given by ▲ and the value measured 10 min after the train (after augmentation and potentiation had decayed to negligible levels) by ■. The statistics for six such experiments were given in Table 3.

TABLE 3. Mean parameters for regression of relation between within-pair responses ($f$) on combined magnitude of augmentation and potentiation. (See example Fig. 3).

| | Base-line $f$ | Intercept | Slope | $r$ |
|---|---|---|---|---|
| Mean | 26·0 % | 22·3 % | −0·63 | −0·985 |
| S.E. of mean | 3·0 | 2·5 | 0·03 | 0·002 |

($n = 6$).

time-course, but recovered its control level by 10 min. The recovery time courses for the relation between responses within pairs was compared between the low-and high-intensity trains by fitting exponential curves to the slow (potentiation) component of the recovery. The average recovery time constants for the two conditions corresponded to within 2 % of each other (see Table 4). Results qualitatively similar to those just described were obtained in twelve other experiments in which the test

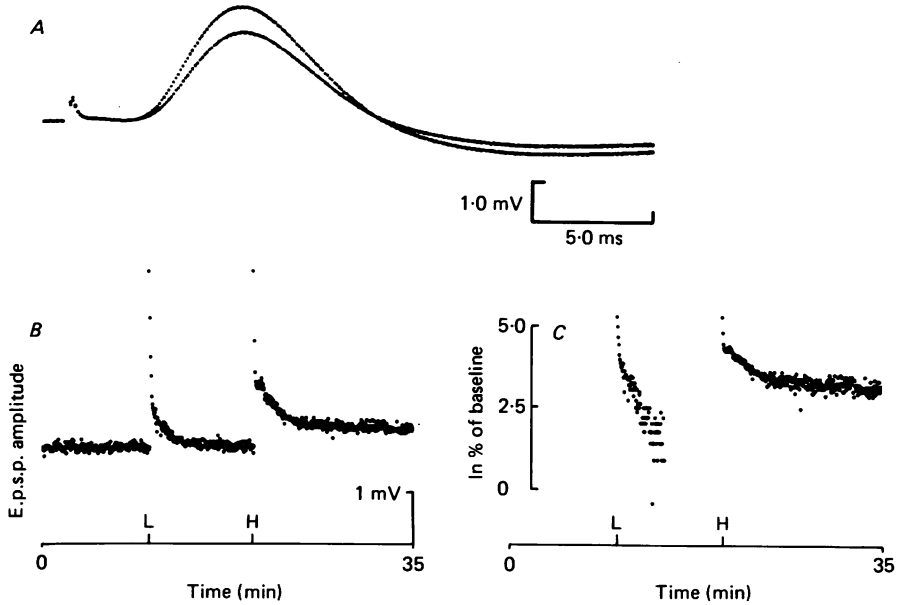


Fig. 4. *A*, representative example of the extracellular e.p.s.p. recorded in the hilus fascia dentatae following moderately low-intensity stimulation (30 $\mu$S × 50 $\mu$A) of the lateral perforant pathway. The smaller amplitude and larger amplitude responses were elicited, respectively, 15 min before and 15 min after a single 250 Hz stimulus train of seventy-five pulses at 200 $\mu$S × 50 $\mu$A. *B* and *C* show, in linear and semilogarithmic co-ordinates respectively, the effects on e.p.s.p. amplitude of low- (L) and high- (H) intensity stimulus trains. The same low-intensity stimulation was used throughout for testing. The low-intensity tetanus produced only augmentation and potentiation, whereas high-intensity tetanization appears to have introduced a third component (enhancement) whose decay was orders of magnitude slower than potentiation.

TABLE 4. Effects of low- and high-intensity stimulation on e.p.s.p. amplitude and relation between within-pair responses (*f*)

| | Mean | S.E. of mean |
|---|---|---|
| Base-line *f* | 26·7 % | 4·5 |
| Parameters following low-intensity tetani | | |
| Change in e.p.s.p. 10 min after tetanus | −0·5 % | 1·4 |
| Magnitude of *f* 10 min after tetanus | 26·1 % | 4·8 |
| Time constant for recovery of *f* after tetanus (slow component) | 202·3 s | 11·1 |
| Intercept of slow component of recovery of *f* after tetanus | −19·1 % | 1·6 |
| Parameters following high-intensity tetani | | |
| Change in e.p.s.p. 10 min after tetanus | 20·2 % | 3·6 |
| Magnitude of *f* 10 min after tetanus | 25·8 % | 4·0 |
| Time constant for recovery of *f* after tetanus (slow component) | 204·6 s | 20·5 |
| Intercept of slow component of recovery of *f* after tetanus | −17·4 % | 2·8 |

($n$ = 9).

and tetanization parameters were varied from case to case. An example of the data from one of these is shown in Fig. 5.

A third experimental procedure was carried out in one animal to examine whether the relation between response pairs was altered in the presence of long-term enhancement at any interval in the range of 30 ms to 1·6 s. Seven intervals were

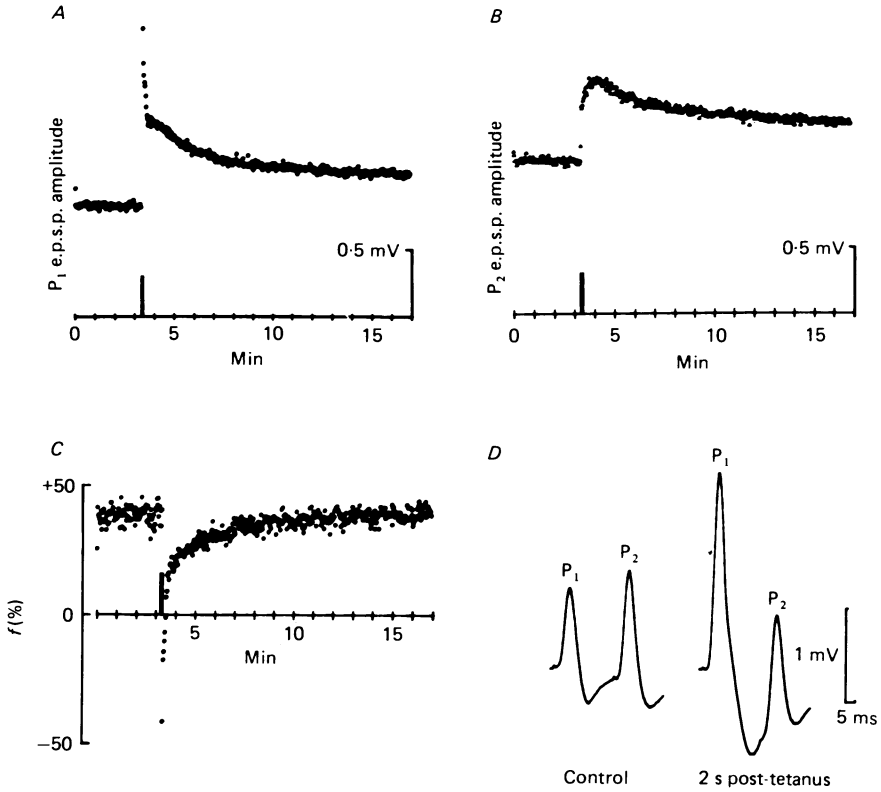


Fig. 5. An example of the interaction of paired-pulse facilitation/depletion with augmentation and potentiation and the lack of interaction with enhancement. Low-intensity pulse pairs separated by 25 ms were delivered at 0·5 Hz. After 100 baseline pulse pairs, a single 250 Hz, seventy-five pulse train (vertical bar) was delivered using high-intensity (200 $\mu$S) stimulation. The amplitudes of the first ($P_1$) and second ($P_2$) responses in each pair are plotted in *A* and *B* respectively. The percent difference between $P_1$ and $P_2$ is shown in *C*. This shows that the facilitation/depletion balance is shifted towards depletion in the presence of augmentation and potentiation, but recovers to control level even though the response itself remains elevated by 30 % due to enhancement. Examples of response pairs during the control period and two seconds following the high-frequency train are shown in *D*. Similar results were obtained in all of the twenty other cases studied in this way. A quantitative summary of nine of these experiments, in which the stimulus parameters were held constant from experiment to experiment, is given in Table 4.

tested: 30, 50, 100, 200, 400, 800, and 1600 ms. These were presented in an ascending series with 10 s between the last stimulus in one pair and the first stimulus of the next. The entire series was repeated twenty times and the corresponding within-pair percent differences in e.p.s.p. amplitude were averaged. A single high-intensity tetanus of 100 pulses at 250 Hz was then delivered. This resulted in an average

enhancement of the e.p.s.p. of 46% measured between 11 and 18 min following the tetanus. During this time, the paired shock procedure was repeated. There were no significant differences in the relation between responses within pairs at any interval tested. These data are shown in Fig. 6.

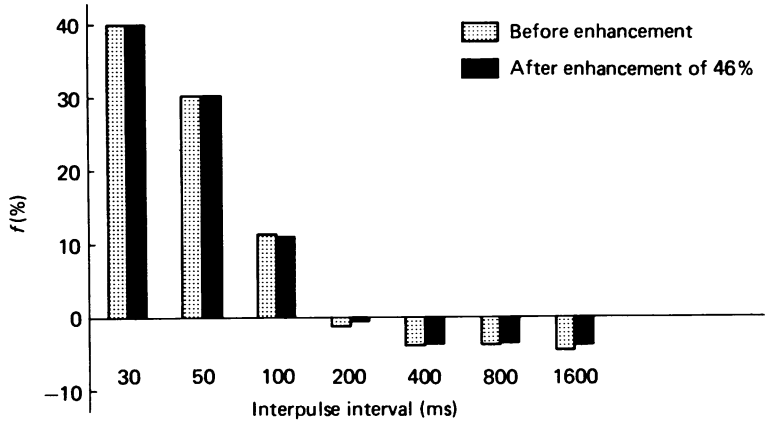


Fig. 6. The time course of the paired-pulse effect (30 ms to 1·6 s) is shown prior to and following enhancement of 46%. The post-enhancement data were taken between 11 and 18 min following the enhancing stimulus (i.e. after the complete decay of augmentation and potentiation). All values represent the mean of twenty observations. There were no statistically significant ($p < 0{\cdot}05$) differences at any of the interpulse intervals.

## DISCUSSION

The time course of the paired shock effect at perforant path synapses has previously been shown to bear a quantitative similarity to the convolution of facilitation and depletion seen at neuromuscular synapses (McNaughton, 1980). In particular, the magnitude of depletion was proportional to the magnitude of the initial response in each pair under conditions in which the probability of transmitter release was manipulated by altering the external calcium ion concentration. In the present study, the magnitude of the first response in each pair was increased by the generation of augmentation and potentiation. This resulted in a directly proportional reduction in the relation between the first and second responses in each pair. These results thus strongly suggest that both augmentation and potentiation at perforant path synapses act to increase the probability of transmitter release. Furthermore, the quantitative similarities in the kinetics of augmentation and potentiation in the present system to those described by Magleby & Zengel (1975, 1976*a*) for neuromuscular synapses make it very likely that the effects in the two systems result from common mechanisms.

Long-term synaptic enhancement, which was induced in perforant path only when the stimulus intensity was raised above a certain level, had no effect on the time-course of the paired shock response when tested at a time after the normal decay of augmentation and potentiation. Thus enhancement appears not to involve an increase in the probability of transmitter release, and hence differs in mechanism from augmentation and potentiation. In the period immediately following the high-intensity stimulus train, however, depletion was increased by the same amount, and recovered

with the same time-course as following low-intensity stimulus trains which elicited only augmentation and potentiation. These data indicate that following high-intensity stimulation augmentation and potentiation are superimposed on enhancement.

The results presented here appear to limit the possible *presynaptic* mechanisms for enhancement to either an increase in quantal size or to an increase in the statistical parameter *n*. The latter might occur through an increase in the number of quanta available for release, an increase in the number of release sites, or the formation of new synaptic connexions. In addition to these presynaptic mechanisms, a variety of *post-synaptic* candidate mechanisms have yet to be ruled out. In either case, the results clearly show that enhancement is not merely a long-term form of potentiation.

I am grateful to Dr C. A. Barnes for assistance and to Drs G. V. Goddard and J. O'Keefe for helpful discussions and use of facilities. This work was supported by postgraduate and postdoctoral fellowships from the Natural Sciences and Engineering Council of Canada.

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