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# LONG TERM CHANGES IN AUGMENTATION, POTENTIATION, AND DEPRESSION OF TRANSMITTER RELEASE AS A FUNCTION OF REPEATED SYNAPTIC ACTIVITY AT THE FROG NEUROMUSCULAR JUNCTION

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SUMMARY

1. End-plate potentials (e.p.p.s) were recorded from frog neuromuscular junctions under conditions of low quantal content to study the long-term effects of repeated synaptic activity on transmitter release.

2. The nerve terminal was presented with 30–100 successive conditioning-testing trials applied once every 7–10 min over a 4–16 hr period. Each conditioning-testing trial consisted of a 200–600 impulse conditioning train followed by a series of testing impulses. The magnitudes and time constants of decay of augmentation and potentiation following each successive conditioning train were determined by measuring the e.p.p. amplitudes resulting from the testing impulses.

3. The magnitude of augmentation immediately following the conditioning trains increased an average of 3·4 times (range 1–20) with successive trials.

4. As the magnitude of augmentation increased with successive trials the decay of augmentation deviated from a simple exponential, decaying faster immediately after the conditioning train. This faster decay led to a 20% decrease with successive trials in estimates of the time constant obtained from the first 10 or 20 sec of the decay of augmentation. The deviation of the decay of augmentation from a simple exponential could be accounted for if augmentation is related to the 4th power of some substance which decays with a simple exponential time course. Some alternative explanations for the non-exponential decay of augmentation are also discussed.

5. The magnitude of potentiation increased or decreased about 25% with successive trials.

6. The time constant characterizing the decay of potentiation increased an average of 1·5 times (range 0·8–5 times) with successive trials.

7. The increase in the magnitude of augmentation with successive trials was accompanied by a similar increase in the magnitude of the e.p.p. amplitudes during the conditioning trains, suggesting that augmentation develops during the conditioning train. In some preparations augmentation appeared to be the major factor acting to increase e.p.p. amplitudes during the conditioning train, having a greater effect than facilitation or potentiation.

8. If a sufficiently large number of successive trials were applied, a depression of e.p.p. amplitudes developed during the conditioning trains and estimates of the magnitude of potentiation following the depressed conditioning trains were reduced.

9. In contrast to potentiation, the magnitude of augmentation continued to increase for a few successive trials after the onset of depression even though the amount of depression during the conditioning train was also increasing with successive trials. This observation that the magnitude of augmentation could increase at the same time that the magnitude of depression was increasing suggests that augmentation and depression do not arise from inverse changes in a common process.

10. The differential effects of successive trials on augmentation and potentiation suggest that at least some of the factors involved in increasing transmitter release by these processes are different for the two processes.

## INTRODUCTION

The preceding paper (Magleby & Zengel, 1976) demonstrated that augmentation (a process which acts to increase transmitter release with a time course intermediate in duration between facilitation and potentiation) increased with the duration of the conditioning stimulation. This increase was typically greater after several hours exposure to successive conditioning trains, suggesting that there are long-term changes in the properties of the nerve terminals of isolated nerve-muscle preparations. Understanding these long-term changes might very well provide further insight into the operation of the nerve terminal. The purpose of this paper is to characterize systematically the effects of the long-term changes in the properties of the nerve terminals on augmentation, potentiation, and depression of transmitter release. It is found that the long-term changes have a differential effect on augmentation and potentiation: the magnitude of augmentation is greatly increased with a decrease in its time constant of decay, while the magnitude of potentiation is either slightly increased or decreased with an increase in its time constant of decay. It is suggested

that at least some of the factors involved in increasing transmitter release by augmentation and potentiation are different. It is also found that the magnitude of augmentation and depression can increase at the same time, leading to the suggestion that augmentation and depression do not arise from inverse changes in a common process.

## METHODS

The frog sartorius nerve-muscle preparation, bathing solutions, and methods used to record e.p.p.s in this paper are the same as those described in the preceding paper (Magleby & Zengel, 1976). Experiments were done at 20° C in Ringer solution with increased Mg and decreased Ca in order to decrease transmitter release.

In some preparations (probably due to an unusually dense localization of surface end-plates in a small area) the quantal fluctuations in surface recorded e.p.p. amplitudes were sufficiently small that reliable estimates of the parameters characterizing augmentation and potentiation could be made from a single trial making it unnecessary to average trials. Data from such a preparation are shown in Figs. 2 and 4.

Augmentation and potentiation are defined as the fractional increase of a test e.p.p. amplitude over a control such that

$$A(t) = \frac{v(t)}{v_0} - 1 \quad \begin{cases} F(t) = 0 \\ P(t) = 0 \end{cases} \tag{1}$$

$$P(t) = \frac{v(t)}{v_0} - 1 \quad \begin{cases} F(t) = 0 \\ A(t) = 0, \end{cases} \tag{2}$$

where $A(t)$ is augmentation, $P(t)$ is potentiation, $F(t)$ is facilitation, $v(t)$ is the e.p.p. amplitude at time $t$, and $v_0$ is the control e.p.p. amplitude. The experimental method used to determine these processes is shown in Fig. 1.

$V(t)$ is defined as the fractional increase of a test e.p.p. amplitude over a control e.p.p. such that

$$V(t) = \frac{v(t)}{v_0} - 1, \tag{3}$$

where $v(t)$ is the e.p.p. amplitude at time $t$ and $v_0$ is the control e.p.p. amplitude before the conditioning train.

Depression is defined as the fractional decrease in e.p.p. amplitude over a control such that

$$D(t) = 1 - \frac{v(t)}{v_0} \quad \begin{cases} F(t) = 0 \\ A(t) = 0 \\ P(t) = 0, \end{cases} \tag{4}$$

where $D(t)$ is depression, $v(t)$ is the e.p.p. amplitude at time $t$, and $v_0$ is the control e.p.p. amplitude.

S.D. refers to the standard deviation of the observations.

Time is indicated in two ways. For example, $A(T)$ refers specifically to the magnitude of augmentation at the end of a conditioning train of $T$ sec duration, while $A(t)$ refers to the decay of augmentation where $t$ is time after the end of the conditioning train.

## RESULTS

### *A(T) and $\tau_P$ increase with successive trials*

The effect of successive trials on augmentation and potentiation is shown in Fig. 1 where the preparation was presented with seventy-five successive conditioning-testing trials in which the conditioning stimulation

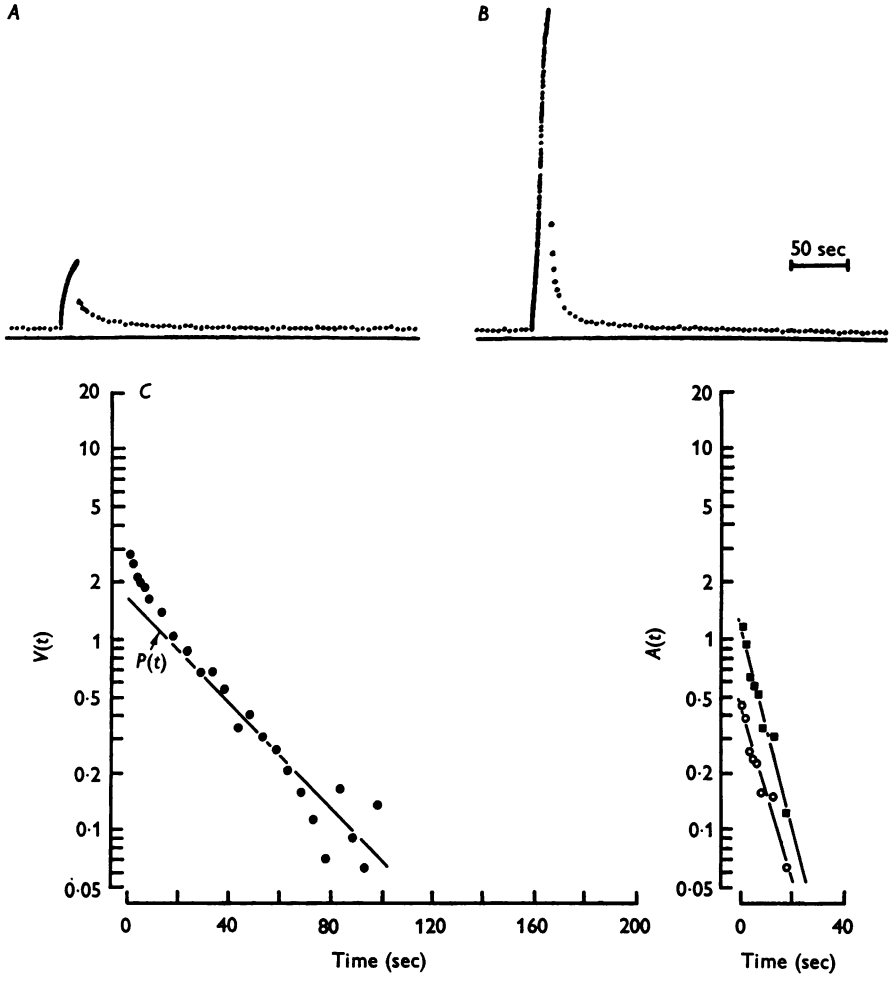


Fig. 1. Changes in augmentation and potentiation of transmitter release with successive trials. The preparation was presented with seventy-five consecutive conditioning-testing trials in which the conditioning stimulation was 300 impulses at 20/sec. Before each conditioning train the nerve was first stimulated once every 5 sec to establish a control response. Following

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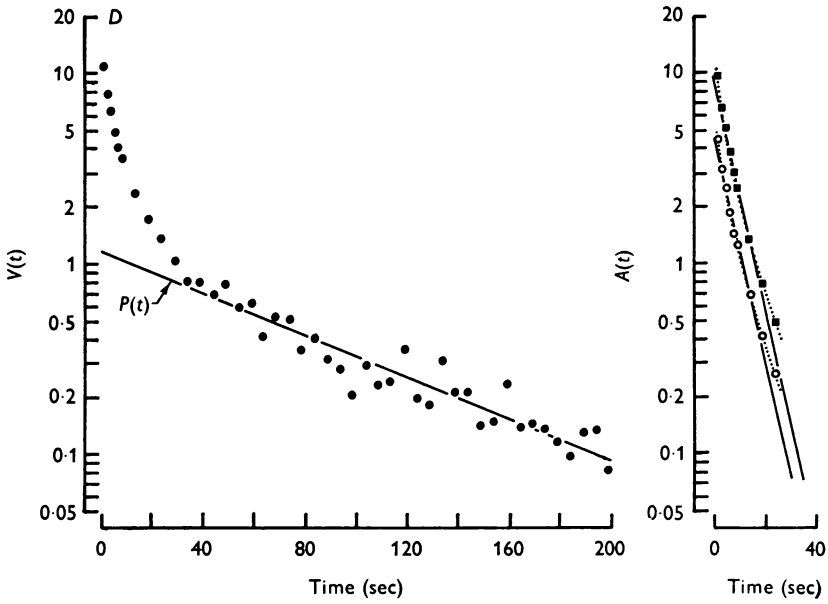



the conditioning train the nerve was tested once every 1·5 sec for 6 impulses and then once every 5 sec for 59 impulses. *A*, *B*, surface recorded e.p.p. amplitudes before, during and following the conditioning stimulation. *A*, mean response of trials 25–29 from early in the experiment. *B*, mean response of trials 69–73 collected about 5 hr later in the experiment. The data are scaled so that the control responses for both trains are the same amplitude. Control amplitude: *A* 29 $\mu$V, *B* 14 $\mu$V. *C*, *D*, semilogarithmic plots of the decay of potentiation, $P(t)$, and augmentation, $A(t)$, following conditioning stimulation. *C*, from data shown in *A* collected early in the experiment. *D*, from the data shown in *B* collected about 5 hr later. The filled circles represent the decay of $V(t)$, the fractional increase in e.p.p. amplitude (eqn. (3)); the lines through the filled circles, determined by least-squares fits to the data points beyond 30 sec, represent the exponential decay of $P(t)$, which had a time constant, $\tau_P$, that increased from 31 sec in *C* to 79 sec in *D*. $P(T)$, the initial magnitude of potentiation immediately following the conditioning train, is given by the intercept of these lines with the ordinate at 0 time and was 1·64 in *C* and 1·17 in *D*. Estimates of augmentation, $A(t)$, were obtained assuming a multiplicative (open circles) or additive (filled squares) relationship with $P(t)$ by dividing or subtracting off the effect of $P(t)$ from $V(t)$ (see eqns. (5) and (6) in Magleby & Zengel, 1976). The lines through the open circles and filled squares represent least-squares fits to the first 20 sec of data. $A(T)$, the initial magnitude of augmentation immediately following the conditioning train, is given by the intercept of these lines with the ordinate at 0 time. $A(T)$ increased from 0·47 (multiplicative) or 1·2 (additive) in *C* to 4·5 (multiplicative) or 9·8 (additive) in *D*. The corresponding time constants for the decay of augmentation, $\tau_A$, decreased from 9·9 sec (multiplicative) or 8·5 sec (additive) in *C* to 7·5 sec (multiplicative) or 7·2 sec (additive) in *D*. Notice that the decay of augmentation deviates from a simple exponential in *D*, as indicated by the difference between the dotted lines which are drawn through the data points and the continuous lines which represent exponential decays.

was 300 impulses at 20/sec. Fig. 1*A* shows a plot of e.p.p. amplitudes against time for the mean response of trials 25–29 from early in the experiment while Fig. 1*B* shows a similar plot for trials 69–73 recorded about 5 hr later. The two plots are scaled so that the control e.p.p. amplitudes are the same. It can be seen that the relative increase in e.p.p. amplitudes during and immediately following identical conditioning trains was much greater later in the experiment. Especially obvious is the marked increase in the first five testing e.p.p. amplitudes delivered immediately after the conditioning train, suggesting a large increase in the magnitude of augmentation later in the experiment. This increase is clearly shown in Fig. 1*C* and *D* where the decays of e.p.p. amplitudes following the conditioning trains for the data in Fig. 1*A* and *B* are plotted semilogarithmically against time as filled circles. The continuous lines through the filled circles represent the decay of potentiation, $P(t)$ (Magleby & Zengel, 1975*a*). The open circles represent the decay of augmentation, $A(t)$, assuming that potentiation has a multiplicative effect on augmentation, and the filled squares represent the decay of augmentation assuming that potentiation and augmentation add (see eqns. (5) and (6) in Magleby & Zengel, 1976). From Fig. 1 it can be seen that $A(T)$, the magnitude of augmentation immediately following the conditioning train, increased from 0·47 early in the experiment (Fig. 1*C*) to 4·5 near the end of the experiment (Fig. 1*D*) assuming a multiplicative relationship between $A(t)$ and $P(t)$. The corresponding time constant for the decay of augmentation, $\tau_A$, decreased from 9·9 to 7·5 sec. In direct contrast to the increased magnitude of augmentation, the magnitude of potentiation, $P(T)$, fell from 1·6 early in the experiment to 1·2 later in the experiment. The corresponding time constants for the decay of potentiation, $\tau_P$, more than doubled, increasing from 31 to 79 sec. Thus it appears that the long-term changes in the properties of the nerve terminal that occur during the course of an experiment can have differential effects on augmentation and potentiation.

The decay of augmentation deviated slightly from a simple exponential decay later in the experiment when the magnitude of augmentation was large. The nature of this deviation is shown by the dotted lines in Fig. 1*D*. It can be seen that augmentation decays first faster and then slower than the simple exponential decays determined by the method of least squares (continuous line). Consequently, estimates of the time constant of decay of augmentation should be viewed as indicating the average rate of decay for the period of time over which the time constant was estimated. Notice also that successive trials had approximately the same effect on augmentation whether $A(t)$ was determined by assuming an additive or a multiplicative relationship to $P(t)$. It is not known which relationship best

describes the true relationship, but a multiplicative relationship will be assumed for the rest of this paper.

*The time course of changes in $A(T)$, $\tau_A$, $P(T)$, $\tau_P$, and $V(T)$ with successive trials*

Experiments were performed to determine the time course of the progressive changes in augmentation and potentiation with successive trials suggested by the data in Fig. 1. Data from such an experiment are shown in Fig. 2. In this experiment four different conditioning trains of 300 or 600 impulses delivered at 10 or 20/sec were presented in a variable order for ninety-five successive trials. Eight to 10 min elapsed between each trial, and over 14 hr were required to collect the data. In Fig. 2*A* and *B* estimates of the magnitudes and time constants for the decay of the augmentation and potentiation immediately following the 600 impulse conditioning trains delivered at 20/sec are plotted against trial number. Each estimate was determined by the method shown in Fig. 1, and examples of the decays of e.p.p. amplitudes used to derive these estimates are shown in Fig. 4*A* which should be examined in conjunction with Fig. 2. From these Figures it can be seen that $A(T)$, the magnitude of augmentation, first increased and then decreased with successive trials. The increase occurred gradually at first with $A(T)$ changing from 0·4 to 2 during the first fifty-six trials, and then the increase occurred more rapidly with $A(T)$ increasing to almost 8 during the next twenty trials for a nineteenfold increase in magnitude (filled circles, Fig. 2*A*). In contrast, $P(T)$, the magnitude of potentiation, increased only 1·6 times from 1·4 to 2·2 during the first sixty-four consecutive trials before it then decreased (filled circles, Fig. 2*B*).

Successive trials also had a differential effect on the decay of augmentation and potentiation. The time constant for the decay of augmentation, $\tau_A$, decreased 35% from about 8·5 sec during the first fifty-six trials to about 5·5 sec during the last thirty trials (open circles, Fig. 2*A*). In contrast, the time constant for the decay of potentiation, $\tau_P$, increased about 5 times from 40 to 200 sec during the entire ninety successive trials (open circles, Fig. 2*B*). The estimates of $\tau_A$ plotted in Fig. 2*A* were made from the first 10 sec of the decay of augmentation after the conditioning train. If the estimates were made from the first 20 or 30 sec of decay, estimates of $\tau_A$ did not decrease as much with successive trials because of the nature of the non-exponential decay of augmentation (see Discussion).

If, as seems most likely, augmentation develops during the conditioning train, then the e.p.p. amplitudes at the end of the conditioning train should be greater in those trials where $A(T)$, the magnitude of augmentation immediately following the conditioning train, is also greater. This is

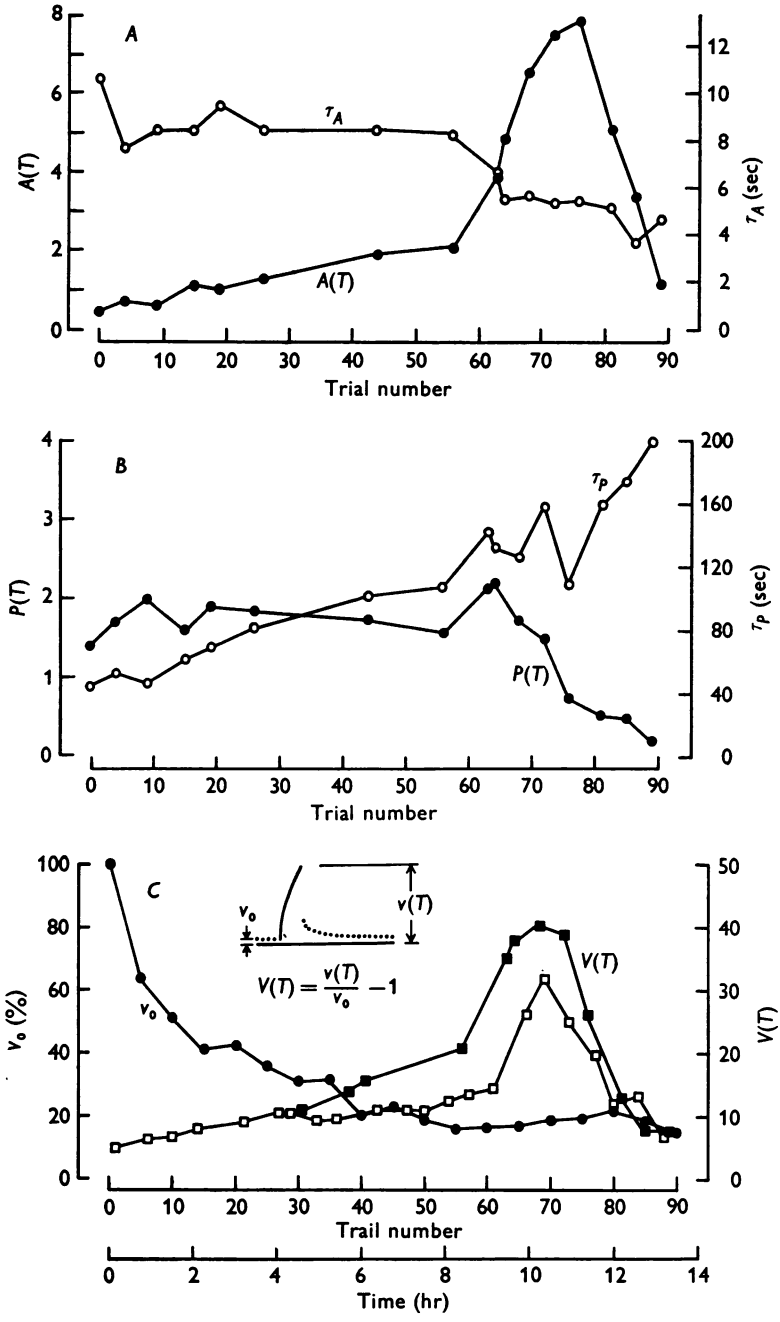



Fig. 2. For legend see facing page.

found to be the case. The filled squares in Fig. 2*C* show $V(T)$, the normalized e.p.p. amplitude at the end of the 600 impulse conditioning trains, plotted against trial number. The open squares are values of $V(T)$ at the end of the 300 impulse trains. It can be seen that $V(T)$ increased with successive trials with a time course similar to the increase in $A(T)$. The magnitude of $V(T)$ was smaller with shorter trains as were the magnitudes of $A(T)$ and $P(T)$. This correlation between the increase in $A(T)$ and $V(T)$ suggests that the augmentation following the conditioning train develops during the conditioning train. Estimates of $V(T)$ following the 600 impulse conditioning trains delivered at 20/sec were not plotted in Fig. 2*C* for the first thirty trials because of a recording artifact due to slight twitching of the muscle at the end of the conditioning trains. After the first thirty trials, the e.p.p. amplitudes were never large enough to cause muscle twitching but they most likely became large enough near the end of the conditioning trains for non-linear summation of unit potentials to occur (Martin, 1955). Consequently, $V(T)$ is probably underestimated in this experiment.

*Changes in $v_0$, the control level of transmitter release, with successive trials*

The estimates of $A(T)$, $P(T)$, and $V(T)$ plotted in Fig. 2 are expressed in terms of the control e.p.p. amplitude for each trial (eqns. (1), (2), and (3)) and give no information about absolute levels of transmitter release. To show this information, $v_0$, the control e.p.p. amplitude for each trial

Fig. 2. Time course of the changes in augmentation, potentiation, and transmitter release with successive trials. In this experiment four different conditioning trains of 300 or 600 impulses delivered at 10/sec or 20/sec were presented in a variable order for ninety-five successive trials, with 8–10 min elapsing between each train. The nerve was stimulated 10 times once every 5 sec before each conditioning train and 6 times once every 1·5 sec and then 80 times (300 impulse trains) or 100 times (600 impulse trains) at once every 5 sec after each conditioning train. Estimates of $A(T)$ and $P(T)$, the initial magnitudes of augmentation and potentiation, and of $\tau_A$ and $\tau_P$, the time constants for the decay of augmentation and potentiation, were determined by the method shown in Fig. 1 and plotted against trial number. Values for augmentation were derived assuming a multiplicative relationship with potentiation. *A*, estimates of $A(T)$ (filled circles) and $\tau_A$ (open circles) following the 600 impulse conditioning trains delivered at 20/sec. *B*, estimates of $P(T)$ (filled circles) and $\tau_P$ (open circles) for the same trials analysed in *A*. *C*, estimates of $V(T)$ (filled squares), the e.p.p. amplitude at the end of the conditioning trains expressed as a fractional increase over control, for the same trials analysed in *A*, and estimates of $v_0$ (filled circles), the control level of transmitter release expressed as a percentage of the initial value at the start of the experiment, plotted for every 5th trial. The open squares are estimates of $V(T)$ at the end of the 300 impulse conditioning trains delivered at 20/sec. The initial (100 %) value of $v_0$ was 53 $\mu$V as measured by surface recording.

(see insert Fig. 2*C*), is plotted against trial number in Fig. 2*C* as filled circles. It can be seen that $v_0$, the control e.p.p. amplitude, decreased to about 20% of its initial level during the first forty successive trials and then stayed at this level.

The question arises then whether the observed increase in $A(T)$, the magnitude of augmentation, with successive trials arises perhaps by a decrease in the control level of some process, as reflected by the decrease in $v_0$, the control e.p.p. amplitudes, rather than from an actual increase in whatever determines $A(T)$. Two lines of evidence argue against the first possibility. Firstly, the magnitude of augmentation increases only slowly with successive trials at the start of the experiment when the control e.p.p. amplitude is decreasing rapidly. Secondly, the rapid increase in the magnitude of augmentation observed from trials 56–76 occurs at a time when the control e.p.p. amplitude is not changing with successive trials so that the observed upward inflexion reflects an absolute increase in transmitter release. Thus, the rapid upward inflexion in the magnitude of augmentation most likely represents a change in some process or substance which acts to increase transmitter release, and does not result simply from a decrease in the control level of transmitter release.

The reason for the rapid upward inflexion in the magnitude of augmentation with successive trials is not known, but it is probably not due to, for example, a decrease in the rate of removal of the substance that is directly responsible for the observed decay of augmentation. The time constant for the decay of augmentation, $\tau_A$, decreased (open circles, Fig. 2*A*) at the time of the upward inflexion instead of increasing as would be expected if the upward inflexion resulted from a decrease in the rate of removal of such a substance.

*Summary of the effect of successive trials on* $A(T)$, $\tau_A$, $P(T)$, $\tau_P$, $V(T)$, *and* $v_0$

Qualitatively similar results to those shown in Figs. 1 and 2 were obtained for thirty-two experiments that were analysed in an analogous manner. Most of the experiments summarized below were analysed using averaged trials, as in Fig. 1, so that the changes in the estimated parameters were less than those shown in Fig. 2 which were obtained from individual trials. The results were as follows.

(1) The magnitude of augmentation first increased with successive trials. ($A(T)$ increased in thirty-one of thirty-two preparations for a mean increase of $3{\cdot}4 \pm 2$ (S.D.) times with a range of 1–20 times. This increase was statistically significant, $P < 0{\cdot}001$, $t$ test, paired data.) A rapid upward inflexion in the magnitude of augmentation with successive trials as shown in Fig. 2*A* was not always seen nor was it always so pronounced. Not all experiments were continued as long as the one shown in Fig. 2,

however, and an upward inflexion might have occurred if the experiment were continued. It was also not always necessary to present twenty or thirty successive trials before obtaining large magnitudes of augmentation, for in some experiments magnitudes of $A(T)$ of 2–3 (300–600 impulses at 20/sec) were observed in the first few trials. If the experiments were continued long enough the magnitude of augmentation eventually decreased with successive trials.

(2) The time constant characterizing the initial 10 or 20 sec of the decay of augmentation decreased by 20% with successive trials (decreases occurred in thirty of thirty-two preparations with $\tau_A$ changing from $8{\cdot}4 \pm 2{\cdot}1$ sec to $6{\cdot}7 \pm 1{\cdot}6$ sec (mean $\pm$ S.D.). This decrease was statistically significant $P < 0{\cdot}001$, $t$ test, paired data).

(3) In about 40% of the experiments the magnitude of potentiation first increased by about 25% with successive trials; in the rest of the experiments the magnitude of potentiation gradually decreased by about 25% with successive trials. If the experiments were continued long enough, however, the magnitude of potentiation would eventually decrease to insignificant values.

(4) The time constant for the decay of potentiation increased an average of 1·5 times (range 0·8–5 times) with successive trials. (Increases occurred in twenty-three of thirty-two preparations with $\tau_P$ changing from $61 \pm 21$ to $89 \pm 28$ sec (mean $\pm$ S.D.). This increase was statistically significant, $P < 0{\cdot}01$, $t$ test, paired data.)

(5) The control e.p.p. amplitude in the absence of repetitive stimulation, $v_0$, usually decreased with successive trials. However, the pattern and magnitude of this decrease was quite variable. In about 70% of the preparations the decrease was similar to that shown in Fig. 2*C*, although the plateau level could vary from 20 to 70% of the initial value and the number of trials that preceded the plateau ranged from 2 to 50. In some of the remaining preparations $v_0$, the control e.p.p. amplitude, would sometimes decrease in an approximately linear manner throughout the experiment. In other preparations $v_0$ would decrease, then increase, often above the initial value, and then decrease again. If the experiment were continued long enough, however, there was always a sudden decrease in $v_0$ with successive trials (this occurred just after trial 92 for the data in Fig. 2) which was accompanied by rapid decreases in $A(T)$, $P(T)$, and $V(T)$ to insignificant levels. Data from this period were not used in these experiments.

(6) The magnitude of $V(T)$, the e.p.p. amplitude at the end of the conditioning train when compared to the control, increased and then decreased with successive trials as would be expected from the changes in augmentation and potentiation with successive trials.

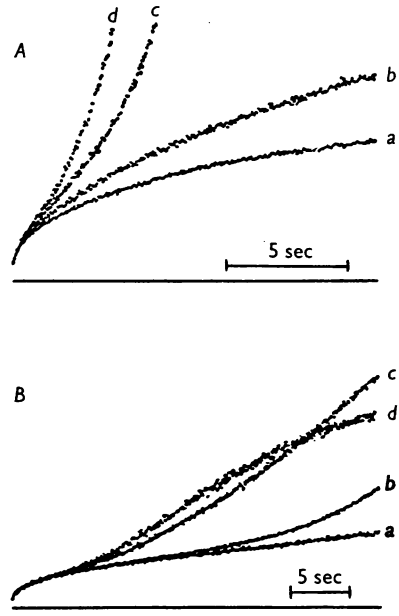
*The effect of successive trials on transmitter release during the conditioning train*

The correlation between the increase in $A(T)$ and $V(T)$ with successive trials (Fig. 2) suggests that augmentation develops during the conditioning train. This hypothesis was tested by making a detailed examination of the rise of e.p.p. amplitudes during the conditioning trains. Fig. 3*A* presents plots of e.p.p. amplitudes against time for a series of conditioning trains obtained at different periods of time during an experiment in which the conditioning trains were 300 impulses at 20/sec. The data are scaled in terms of the control e.p.p. amplitude which is given by the first point in the train. Conditioning train *a* was obtained early in the experiment, and trains *b*, *c*, *d* were obtained after an increasing number of successive trials (trains *c* and *d* extend out of the Figure). Over 6 hr and fifty trials elapsed between trains *a* and *d*. A similar plot of the rise of e.p.p. amplitudes during a series of conditioning trains from another preparation is shown in Fig. 3*B*. In this experiment the conditioning stimulation was 600 impulses at 20/sec. What is immediately apparent from Fig. 3*A* and *B* is that e.p.p. amplitudes increase during the conditioning train and that the amount of this increase at the end of the conditioning train, $V(T)$, becomes greater with successive trials. Facilitation (Mallart & Martin, 1967), presumably augmentation (Fig. 2), and potentiation (Magleby, 1973*b*) can all act to increase e.p.p. amplitudes during the conditioning train. An increase in any one of these processes with successive trials, then, could account for the greater increase in e.p.p. amplitudes during each conditioning train that occurred with successive trials. The increase shown in Fig. 3 is not due to an increase in potentiation, however. $P(T)$ decreased 40% with successive trials for the series shown in Fig. 3*A*, and $P(T)$ remained constant at about 1·8 following conditioning trains *a*–*c*, and then decreased 22% from this level to 1·4 following conditioning train *d*, for the series shown in Fig. 3*B*. The increase in e.p.p. amplitudes with successive trials is also probably not due to an increase in facilitation unless the defined properties of facilitation change drastically with successive trials. Facilitation is thought to increase rapidly during the first several hundred msec of repetitive stimulation, approaching a steady-state level after 1–2 sec (Mallart & Martin, 1967; Magleby, 1973*a*; Younkin, 1974). Notice that the first 7 e.p.p. amplitudes (300 msec) in Fig. 3*A* and the first 100 e.p.p. amplitudes (5 sec) in Fig. 3*B* of the different conditioning trains superimpose. This suggests that facilitation (which is the main factor increasing e.p.p. amplitudes at the start of a train) remains relatively unchanged with successive trials. It appears, then, that an increase in augmentation with successive trials is the major

factor responsible for the greater increase in e.p.p. amplitudes during the conditioning trains that occurred with successive trials. This conclusion is supported by the approximate parallel increase in $A(T)$ and $V(T)$ with successive trials shown in Fig. 2. If this conclusion is correct the differences between the conditioning trains in Fig. 3 would reflect the changes in the properties of augmentation that occur with successive trials. Notice that the conditioning trains tend to superimpose at their start and then inflect upward sooner and steeper with successive trials. From this description it might appear as if some compartment that is involved in inactivating or storing the substance that is directly responsible for augmentation fills up progressively sooner with successive trials, perhaps because it is less effective in emptying between conditioning trains later in the experiment. A model this simple seems unlikely though because $\tau_A$, the time constant characterizing the decay of augmentation, typically becomes shorter with successive trials (Fig. 2*A*), instead of longer, as would be predicted by this model.

*Onset of obvious depression with successive trials*

The decline in the rate of growth of e.p.p.s in Fig. 3*B* curve *d* is interpreted as indicating the onset of depression, which is assumed to result at least in part from a depletion of the store of transmitter immediately available for release (Thies, 1965; Betz, 1970). A similar but more pronounced decline was largely responsible for the decrease in estimates of $V(T)$, the normalized e.p.p. amplitudes at the end of the conditioning trains, shown in Fig. 2. In this experiment and in the one presented in Fig. 3*B*, $A(T)$ continued to increase for several successive trials after the onset of obvious depression. For the experiment shown in Fig. 3*B*, $A(T)$ after train *c*, during which there was no obvious depression, was 4·1. $A(T)$ after train *d*, which was obtained 12 trials later, was 27 % greater at 5·2 even though there was obvious depression of the e.p.p. amplitudes during the conditioning train. In contrast to the increase in augmentation from train *c* to *d*, $P(T)$ decreased from 1·8 to 1·4. These changes in $A(T)$ and $P(T)$ with the onset of depression are clearly shown in Fig. 3*C* which plots the decays of the e.p.p. amplitudes after train *c* (open circles) and train *d* (filled circles). A possible interpretation of these observations is that estimates of $P(T)$ decreased from train *c* to *d* due to depression that developed during train *d*, while estimates of $A(T)$ increased from train *c* to *d* because the magnitude of augmentation was increasing so rapidly with successive trials that depression was not yet sufficient to obscure this increase. After conditioning train *d*, however, estimates of $A(T)$ did decrease with successive trials, presumably because of a still greater increase in depression.



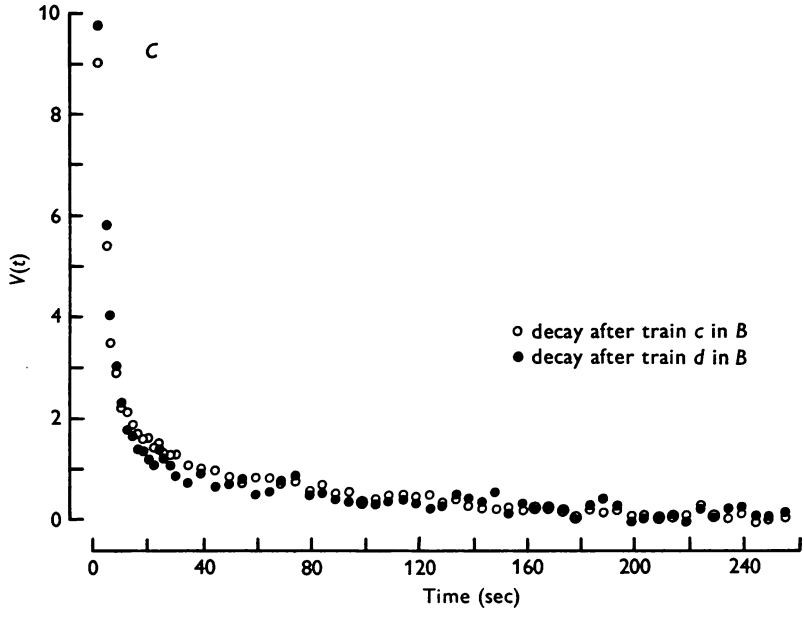




Fig. 3. For legend see facing page.

This sequence of changes in augmentation, potentiation, and depression with successive trials is shown in detail in Fig. 4*A* which presents data from the same experiment as the one shown in Fig. 2. An increase in the magnitude of augmentation with successive trial and a decrease only after the onset of obvious depression (which was present during the conditioning train of trial 72) are readily apparent. A decrease in the magnitude of potentiation at the onset of depression can also be seen. Notice that potentiation develops after a delay in trial 89, analogous to the delayed onset of potentiation (PTP) under conditions of higher levels of transmitter release (Rosenthal, 1969; Magleby, 1973*b*).

The question arises whether the marked change in augmentation and potentiation that occurs after the onset of obvious depression could simply result from depression or whether some other factor is also involved. The recovery from depression appears to follow a simple exponential time course (Takeuchi, 1958; Betz, 1970; Lass, Halevi, Landau, & Gitter, 1973). If the recovery from depression is exponential in our experiments and if depression has a multiplicative effect on transmitter release, then observed e.p.p. amplitudes can be corrected for depression by

$$V'(t)+1 = (V(t)+1)(1-De^{-t/\tau_D}), \tag{5}$$

where $V'(t)$ and $V(t)$ are the corrected and observed fractional increases in e.p.p. amplitudes at time $t$ respectively, $D$ is the magnitude of depression immediately following the conditioning train as defined by eqn. (4), and $\tau_D$ is the time constant for the recovery from depression. E.p.p. amplitudes

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Fig. 3. Effect of successive trials on the rise of e.p.p. amplitudes during the conditioning train. The data are scaled in terms of the control e.p.p. amplitude which is given by the first point in the train. *A*, rise of e.p.p. amplitudes during conditioning trains of 300 impulses at 20/sec. Mean response from: *a*, trials 25–29; *b*, trials 57–61; *c*, trials 69–73; *d*, trials 73–77. Trains *c* and *d* extend out of the Figure. Trains *a* and *c* are the conditioning trains from the trials shown in Fig. 1*A* and *B*, respectively. *B*, rise of e.p.p. amplitudes during conditioning trains of 600 impulses at 20/sec from a different preparation. Mean response from: *a*, trials 11–15; *b*, trials 21–29; *c*, trials 38–43; *d*, trials 51–53. Notice that the conditioning trains superimpose for the first seven e.p.p. amplitudes in *A* and for the first 100 e.p.p. amplitudes in *B*, and then inflect upward sooner and steeper with successive trials. The difference between the curves is thought to arise mainly from increases in augmentation with successive trials. *C*, effect of depression during the conditioning train on the decay of e.p.p. amplitudes following the conditioning train. Open circles: decay of e.p.p. amplitudes following conditioning train *c* from *B*. Filled circles: decay of e.p.p. amplitudes following conditioning train *d* from *B* in which there was obvious depression. Notice that augmentation can increase after the appearance of obvious depression during the conditioning train while potentiation appears to decrease.

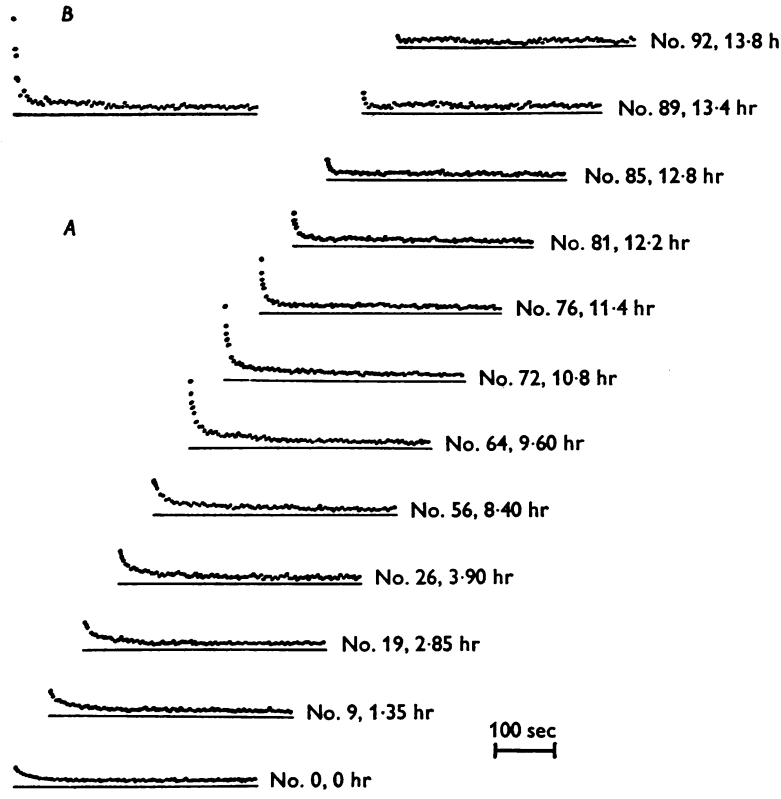


Fig. 4. Effect of depression on e.p.p. amplitudes following conditioning trains of 600 impulses at 20/sec. Same experiment as the one shown in Fig. 2. *A*, decay of e.p.p. amplitudes following single trials presented at various times during the experiment. Notice that the magnitude of potentiation, as indicated by the e.p.p. amplitudes between about 30 and 150 sec, remained relatively constant with successive trials and then decreased with the onset of obvious depression which occurred in trial 72. The magnitude of augmentation, as indicated by the first 30 sec of the curves, increased with successive trials, decreasing only after the onset of obvious depression. Trial numbers and time during the experiment are indicated. *B*, decay of e.p.p. amplitudes from trial 89 corrected for depression using eqn. (5). The magnitude (0·8) and time constant (70 sec) for the recovery from depression used in the correction were selected to make the corrected decay of potentiation similar to that before the onset of obvious depression. Notice that the magnitude of augmentation is greater for the corrected trial 89 than for the uncorrected trial 72 taken just at the onset of obvious depression. The data in *A* and *B* are scaled so the plotted control e.p.p. amplitudes are the same. The control amplitudes before normalization are shown in Fig. 2*C*.

from trial 89 in Fig. 4*A* were corrected for depression using eqn. (5) and were replotted in Fig. 4*B*. It can be seen that to a first approximation depression could account for the form of the observed decrease in the magnitude of augmentation and potentiation in trial 89. The magnitude (0·8) and time constant (70 sec) for the recovery from depression used in the correction were selected to make the corrected potentiation after the onset of obvious depression approximately similar to that before the onset of obvious depression. If this form of correction is valid, then it can be seen from Fig. 4*A* and *B* that the magnitude of augmentation is greater for the corrected trial 89 than for the uncorrected trial 72 taken just at the onset of obvious depression. Similar results were found for the other trials after 72 following correction for depression, suggesting that the magnitude of augmentation continues to increase with successive trials and that the observed decrease in the magnitude of augmentation results from depression.

### *Effect of time*

The question arises whether the changes in augmentation, potentation, and depression of transmitter release shown in Fig. 4 are functions of time or repeated synaptic activity. After 2–15 hr of repeated synaptic activity, a 15–30 min rest between two successive conditioning trains (instead of the usual 7–10 min) would usually reverse the increases in the magnitude of augmentation (and depression when present) for the following trial. With the application of additional trials the magnitude of augmentation (and depression when present) would then once again increase. This reversibility suggests that the effects shown in Fig. 4 are mainly functions of repeated synaptic activity. However, the magnitude of augmentation and the time constant for the decay of potentiation usually also increased slowly with time in the absence of synaptic activity (determined with paired muscles). suggesting that some of the changes in transmitter release shown in Fig. 4 may be partly due to time, as well as synaptic activity.

## DISCUSSION

Perhaps one of the most obvious and important conclusions to be drawn from this study is that long-term changes in the properties of the *in vitro* nerve terminal can and usually do occur with successive conditioning-testing trials and/or time. The amount of change that we observed was quite variable depending on the preparation. Some preparations would remain relatively stable (as determined by estimates of augmentation, potentiation, and the control level of transmitter release) for hours while others never quite stabilized. The mechanisms of these long term changes in the nerve terminal are not known, but it is important to point out that

the 7–10 min allowed between successive conditioning trains should have been more than sufficient time for augmentation and potentiation to decay to insignificant levels between trials. Thus, the long-term changes in augmentation and potentiation shown in Figs. 1–4 are perhaps best viewed as reflecting changes in secondary factors in the nerve terminal which then affect the primary factors which directly determined the observed augmentation and potentiation. It is not known what the secondary factors are, but they may involve changes in the concentration of ions ($Ca^{2+}$, $Na^{+}$, $K^{+}$, $Mg^{2+}$) and metabolites (ATP) inside various compartments of the nerve terminal, changes in the numbers and positions of the synaptic vesicles, and/or changes in the numbers and properties of the presumed transmitter release sites (see reviews by Hubbard, 1970, 1973, for a discussion of the factors involved in transmitter release). It is interesting to note that the time course of decay of augmentation and the effects of repetitive stimulation and time on the magnitude of augmentation are similar to the time course of decay and the effects of repetitive stimulation and time on changes in the ionized $[Ca^{2+}]$ in squid giant axons (Baker, Hodgkin & Ridgway, 1971).

It has previously been suggested that the rise of e.p.p. amplitudes after the first second of repetitive stimulation is mainly due to potentiation (Magleby, 1973*a*, *b*). It now appears that this statement only applies to some preparations, for we have demonstrated in this paper that augmentation can be a major factor in increasing transmitter release during repetitive stimulation, often having a greater effect than potentiation. In fact, the rate of increase in augmentation sometimes accelerated with the duration of the conditioning train, increasing most rapidly after the time (20 sec) when it might be assumed that augmentation would be approaching a steady-state level because of its 7 sec time constant. Facilitation still appears to be the major factor acting to increase transmitter release during the first few impulses of a conditioning train because augmentation and potentiation only reach significant magnitudes after a number of impulses.

The experiments reported in this paper were done under conditions of low quantal content to decrease transmitter release to reduce the possibility of depression due (at least in part) to a depletion of transmitter available for release (Thies, 1965; Betz, 1970). However, even with a control quantal content of as low as 0·2, the amount of transmitter released during a 600 impulse conditioning train can be significant. If the average increase in e.p.p. amplitude during the conditioning train is 10 times the control, then (0·2)(10)(600) = 1200 quanta will be released during the conditioning train, which is equal to or greater then estimates of the readily releasable store of transmitter (Martin, 1966; Wernig, 1975).

This calculation showing that a large number of quanta can be released

during a conditioning train even when the control quantal content is low, and the appearance of an obvious depression of e.p.p. amplitudes if an experiment were continued long enough raise the possibility that a masked depression may be present in some experiments. If the factors acting to increase transmitter release during repetitive stimulation are increasing faster than the onset of depression, then depression (as indicated by a fall in e.p.p. amplitudes during the conditioning train) will not be obvious even though it is present. A masked depression might account for the apparent decrease in potentiation that occurred in 60 % of the preparations long before the onset of obvious depression. A masked depression could also contribute to the observed increase in the time constant for the decay of potentiation that occurs with successive trials, but it seems unlikely that depression is the major factor responsible for the increase. The decay of potentiation was usually well described by a single exponential during the first part of the experiment even though the time constant for the decay of potentiation was increasing with successive trials. It seems unlikely that depression and potentiation would interact to give what appears as a single time constant for the decay of potentiation if depression were very significant. Supporting this conclusion is the observation that later in the experiments following the onset of obvious depression, the decay of potentiation could seldom be described by a single exponential. As a tentative conclusion then, it appears that there is an actual increase in the time constant for the decay of potentiation that occurs with successive trials. This increase could arise if successive synaptic activity led to the accumulation (or depletion) of some substance in the nerve terminal that determines the rate constant for the removal of the substance directly responsible for potentiation. We have previously suggested that a mechanism of this type may be responsible for the observation that increasing the duration of the conditioning train leads to an increase in the time constant for the decay of potentiation (Magleby & Zengel, 1975*a*; and see Fig. 2*F* in the previous paper, Magleby & Zengel, 1976).

The observation that augmentation can increase transmitter release at the same time that depression is decreasing transmitter release (trials 72–76 in Figs. 2 and 4 and trial *d* in Fig. 3*B* and *C*) suggests that augmentation and depression do not arise from inverse changes in a common process. For example, if depression is a decrease in $n$, the number of quanta immediately available for release, then augmentation cannot be an increase in this same $n$.

A significant observation in this study was that the decay of augmentation became faster, deviating slightly from a simple exponential decay as the magnitude of augmentation increased with successive trials (Figs. 1 and 2*A*). Some possible explanations for these changes in the decay of

augmentation are summarized in Fig. 5. If it is assumed that the observed augmentation, $A(t)$, arises from the accumulation of some substance, $A^*(t)$, in the nerve terminal, then the observed augmentation, $A(t)$, could decay in the non-exponential manner shown in Fig. 1*D* (1) if the rate constant, $k$, decreases with the amount of the residual substance, $A^*(t)$, (2)i $A(t)$, the observed augmentation, is related by some power function $q$ to $A^*(t)$, the residual substance, or (3) if some factor, $E$, in addition to $A^*(t)$, the residual substance, also acts to determine $A(t)$, the observed augmentation. Our data are not sufficient to decide between these possibilities, but possibly (2) is especially interesting since transmitter release is related to the 4th power of $Ca^{2+}$ outside the nerve terminal (Dodge & Rahamimoff, 1967) and facilitation may have 3rd or 4th power properties (Younkin, 1974; Bennett, Florin, & Hall 1975). To test whether a power relationship between $A^*(t)$ and $A(t)$ is sufficient to account for the non-exponential decay of augmentation observed when the magnitude of augmentation

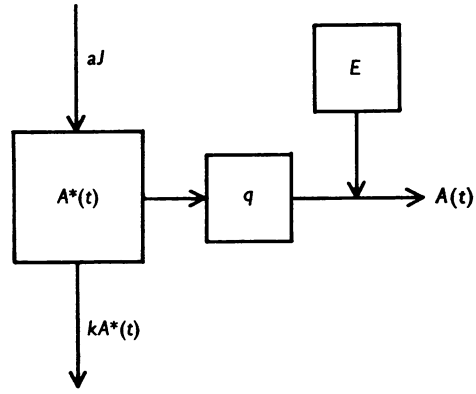


Fig. 5. Working hypothesis for the mechanism of augmentation. The observed augmentation, $A(t)$, is assumed to arise from the accumulation (or depletion) of some substance, $A^*(t)$, in the nerve terminal and it is further assumed that the rate at which $A^*(t)$ changes, $dA^*(t)/dt$, is given by $aJ-kA^*(t)$, where $a$ is the incremental change in $A^*(t)$ resulting from each impulse, $J$ is the stimulation rate, and $k$ is the rate constant for the removal of $A^*(t)$ from its site of action. From this hypothesis the deviation of the decay of $A(t)$ from a simple exponential (Fig. 1) could arise (1) if the rate constant $k$ is a function of $A^*(t)$, decreasing as $A^*(t)$ decreases, (2) if there is a power relationship ($q$) between $A(t)$, the observed augmentation, and $A^*(t)$, the residual substance, or (3) if some additional factor ($E$ for expression factor) also acts to determine $A(t)$. The observed increase in $A(t)$ with successive trials shown in Fig. 2*A* could be explained on the basis of this working hypothesis (1) if there is an increase with successive trials in $a$, the incremental change in $A^*(t)$ with each impulse, or (2) if there is an increase with successive trials in an expression factor, $E$, which acts to determine the observed $A(t)$.

increases, we analysed our data based on this assumption. Estimates were made of the decay of $A^*(t)$, the residual substance responsible for augmentation, by assuming a 4th power relationship between $A^*(t)$ and $A(t)$, such that

$$A^*(t) = \sqrt[4]{(A(t)+1)-1}, \tag{6}$$

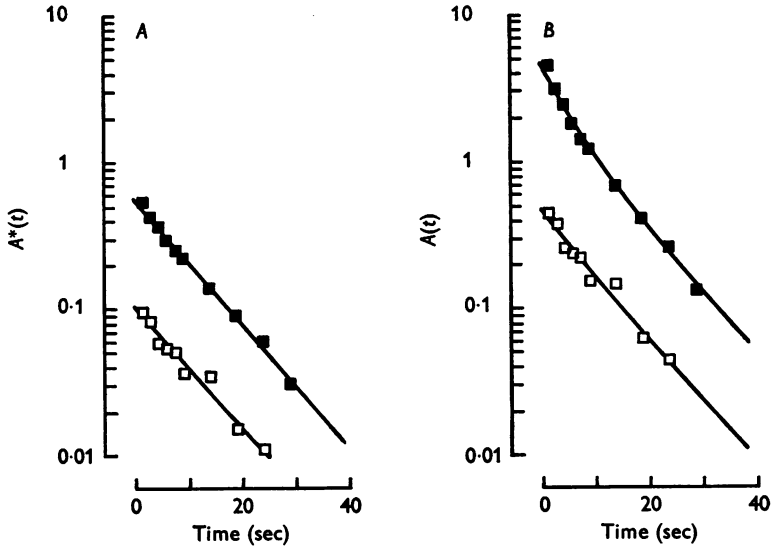


Fig. 6. Test of the possibility that a 4th power relationship between $A(t)$, the observed augmentation, and $A^*(t)$, the residual substance responsible for augmentation, can describe the non-exponential decay of augmentation. *A*, estimates of the decay of $A^*(t)$ derived with eqn. 6 from the decay of $A(t)$. Open squares: estimates derived from data (indicated by the open circles in Fig. 1*C*) collected early in an experiment. Filled squares: estimates derived from data (indicated by the open circles in Fig. 1*D*) collected about forty trials (5 hr) later in the same experiment when the magnitude of augmentation had increased 10 times. Straight lines: estimates of the decay of $A^*(t)$ obtained by the method of least squares. The time constants for the decays of $A^*(t)$ were similar for data collected early and later in the experiment (10·6 and 10·0 sec, respectively). Estimates of $A^*(t)$, the initial magnitude of the residual substance immediately following the conditioning trains, were 0·103 and 0·56 for early and later in the experiment. *B*, comparison of the observed and predicted decay of augmentation. Open squares: observed decay of $A(t)$ for the data collected early in the experiment. Filled squares: observed decay of $A(t)$ for the data collected later in the experiment. Continuous lines: predicted decays of $A(t)$ using eqn. (6) and assuming that $A^*(t)$ decays exponentially with the time constant of 10·6 sec obtained from the data in *A* that was collected early in the experiment. The initial magnitudes of $A^*(T)$ used in the calculations were 0·103 and 0·56. Notice that to a first approximation a 4th power relationship described by eqn. (6) can account for the deviation of the decay of augmentation from a simple exponential.

where $A(t)$ is the magnitude of augmentation at time $t$ following the conditioning train. This relationship has previously been derived in detail in a number of different forms (Barrett & Stevens, 1972; Linder, 1973; Younkin, 1974; Magleby & Zengel, 1975*b*). Estimates of $A^*(t)$ derived with the use of eqn. (6) are shown in Fig. 6*A* for the data from Fig. 1 where the conditioning stimulation was 300 impulses at 20/sec. The open squares represent estimates of $A^*(t)$ derived from the data obtained early in the experiment while the filled squares represent estimates of $A^*(t)$ derived from the data obtained later in the experiment when the magnitude of augmentation had increased about 10 times. The straight lines are least squares fits to the estimates of $A^*(t)$ and were used to determine the time constant for the decay of $A^*(t)$. It can be seen that $A^*(t)$, the assumed substance responsible for augmentation, decays exponentially and that the time constant characterizing this decay appears relatively independent of the magnitude of augmentation in this preparation, being 10·6 and 10·0 sec for the data collected early and later in the experiment, respectively. Once the time constant for the decay of $A^*(t)$ is known, it is possible to test whether the power relationship expressed by eqn. (6) is sufficient to account for the observed non-exponential decay of augmentation. Using the time constant of 10·6 sec for the decay of $A^*(t)$ obtained early in the experiment, it was possible to predict the non-exponential decay of augmentation observed later in the experiment as shown in Fig. 6*B*, where the filled squares represent the observed decay of augmentation, $A(t)$, and the continuous line passing through these squares represents the calculated decay. The open squares and the continuous line through these squares represent the observed and predicted decay of $A(t)$ for data obtained early in the experiment. Notice in Fig. 6*B* that the predicted and observed decays of $A(t)$ appear exponential when the magnitude of augmentation is small. When the magnitude of augmentation is large, however, the predicted and observed decays no longer decay exponentially, but decay fastest immediately after the conditioning train and then decay slower with time as the magnitude of augmentation falls, eventually approaching the time constant for the decay of $A^*(t)$. In the twelve preparations that were analysed in the manner shown in Fig. 6 the decay of augmentation could be approximated by assuming that the observed augmentation is related to the 4th power of a residual substance that decays exponentially. The mean time constant for the decay of $A^*(t)$, the residual substance, in these experiments was $8{\cdot}7 \pm 1{\cdot}3$ (S.D.) sec. In the previous paper (Magleby & Zengel, 1976) it was found that the mean time constant characterizing the decay of augmentation remained relatively unchanged as the magnitude of augmentation increased with the duration of stimulation, instead of decreasing slightly as predicted by

eqn. (6). In individual preparations in which the change in the magnitude of augmentation was large a slight decrease in the time constant of augmentation was usually observed, however, and the power relationship expressed by eqn. (6) was sufficient to account for this decrease, but variability in the estimates of the time constants was often large compared to the expected change. More experiments will be needed to test critically the applicability of eqn. (6) under these conditions of increasing the magnitude of augmentation by increasing the duration of conditioning stimulation. It should be mentioned once again that the observed non-exponential decay of augmentation could arise from a number of factors so that the power relationship described by eqn. (6) must be considered as only one possible explanation for this observation.

The differential effects of successive trials on the decay of augmentation and potentiation and the typical 10 times difference in the time constants characterizing the decay of these two processes suggests that different mechanisms are responsible for the observed decays of these two processes. The differential effect of successive trials on the magnitudes of augmentation and potentiation also suggests that at least some of the factors involved in determining the magnitudes of augmentation and potentiation are different for the two processes.

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