Augmentation and Facilitation of Transmitter Release

A Quantitative Description at the Frog Neuromuscular Junction

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ABSTRACT Endplate potentials were recorded from frog and toad sartorius neuromuscular junctions under conditions of greatly reduced quantal contents. The magnitude of augmentation increased with the duration and frequency of stimulation, often increasing at an accelerating rate during 10–20-s conditioning trains. The magnitudes of the first and second components of facilitation also increased, but reached apparent steady state values within the first few seconds of stimulation. These observations could be accounted for by assuming (*a*) that augmentation and the first and second components of facilitation arise from underlying factors in the nerve terminal that act to increase transmitter release; (*b*) that each nerve impulse adds an increment to each of the underlying factors; (*c*) that the magnitude of the increment typically increases during the train for augmentation but remains constant for the components of facilitation; and (*d*) that the underlying factors decay with first-order kinetics with time constants of ~7 s for augmentation and 60 and 500 ms for the first and second components of facilitation, respectively. The increments of facilitation added by each impulse were about twice as large in the toad as in the frog. Facilitation was described better by assuming a power relationship between the underlying factor and the observed facilitation than by assuming a linear relationship. Augmentation was described by assuming either a linear or power relationship.

INTRODUCTION

The amount of transmitter released from a synapse by each nerve impulse varies as a function of previous synaptic activity (Feng, 1941; del Castillo and Katz, 1954*b*; Liley, 1956; Curtis and Eccles, 1960; Hubbard, 1963). This and the following paper (Magleby and Zengel, 1982) conclude a series of studies

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directed at developing a quantitative description of stimulation-induced changes in evoked transmitter release at the frog neuromuscular junction under conditions of low quantal contents.

Previous studies of this type have suggested that stimulation-induced changes in transmitter release can be separated into at least four components at the neuromuscular junction; the first and second components of facilitation, which decay with time constants of ~60 and 400 ms, respectively (Mallart and Martin, 1967; Magleby, 1973*a*; Younkin, 1974); augmentation, which decays with a time constant of ~7 s (Magleby and Zengel, 1976*a*, *b*; Erulkar and Rahamimoff, 1978); and potentiation, which decays with a time constant that ranges from tens of seconds to minutes (Hubbard, 1963; Gage and Hubbard, 1966; Rosenthal, 1969; Magleby, 1973*b*; Magleby and Zengel, 1975*a*, *b*). Some marked differences in the kinetic properties and ionic specificities of the components suggest that they are separable and that they can act somewhat independently of one another (Hubbard, 1963; Mallart and Martin, 1967; Landau et al., 1973; Magleby, 1973*a*, *b*; Magleby and Zengel, 1976*b*, *c*; Erulkar and Rahamimoff, 1978; Zengel and Magleby, 1980, 1981).

Although the kinetic properties of potentiation have been described (Magleby and Zengel, 1975*a*, *b*), those of augmentation have not, and there is still disagreement concerning the kinetic properties of facilitation (cf. Barrett and Stevens, 1972; Balnave and Gage, 1977). In addition, although it appears that potentiation has a multiplicative effect on facilitation (Landau et al., 1973; Magleby, 1973*b*), the relationship of augmentation to facilitation and potentiation has not been established. Finally, it is not known whether there are other components of increased transmitter release at the neuromuscular junction in addition to potentiation, augmentation, and the two components of facilitation.

In this paper we develop a model to describe the kinetic properties of augmentation and examine further the properties of the two components of facilitation. In the following paper (Magleby and Zengel, 1982), we formulate a quantitative description of stimulation-induced changes in transmitter release and show that the combined properties of potentiation, augmentation, and the two components of facilitation are sufficient to account quantitatively for the changes in endplate potential (EPP) amplitude that occur during and after repetitive stimulation under conditions of low quantal content. Preliminary reports of some of these results have appeared (Magleby and Zengel, 1977; Zengel and Magleby, 1979).

METHODS

Experimental Procedure

For most experiments, recordings of endplate potentials (EPPs) were obtained with a surface electrode from endplate regions of the frog (*Rana pipiens*) sartorius nerve–muscle preparation (details in Magleby, 1973*a* and Magleby and Zengel, 1976*a*). In addition, a few experiments (specifically indicated in the text) were performed on the local toad *Bufo marinus*. Under the conditions of low quantal content used in these experiments (see below), changes in surface-recorded EPP amplitudes give a good measure of

changes in transmitter release (Mallart and Martin, 1967; Magleby, 1973*a*; Magleby and Zengel, 1976*a*). Surface recording has the advantage over intracellular recording of summing the response from many endplates, reducing the number of conditioning–testing trials required to obtain estimates of the average response.

Experiments with intracellular recording indicated that EPP amplitudes were typically <0.1–1 mV in the absence of repetitive stimulation and seldom exceeded 10–20 mV during the conditioning trains. Thus, a correction for nonlinear summation of unit potentials has not been applied to the data because recent experiments of McLachlan and Martin (1981) suggest that no correction is needed if the EPP amplitudes are <10 mV and that the correction is <10% for EPP amplitudes as large as 20 mV.

The standard bathing solution had the composition (mM): 115 NaCl; 2 KCl; 1.8 $CaCl_2$; 2.16 Na_2HPO_4; 0.85 NaH_2PO_4; 5 glucose; 0.03 choline. This solution was modified by reducing Ca^{2+} to 0.4–0.6 mM and adding 5 mM Mg^{2+} to greatly decrease transmitter release. Osmolarity was maintained by reducing NaCl. The pH was adjusted to 7.2–7.4. Experiments were done at 20°C.

For experiments in which the intervals between EPPs were ≥30 ms, EPPs essentially decayed to the baseline between nerve impulses, and EPP amplitudes were measured, stored, and analyzed by computer as described previously (Magleby and Zengel, 1976*a*). For experiments with high stimulation rates during the trains or brief conditioning–testing intervals, the entire conditioning train plus the testing EPP was sampled at a rate of 10 points/ms and averaged during the experiment with data from previous trials of the same pattern. At the conclusion of these experiments, EPP amplitudes were measured as the vertical distance from the peak of the EPP to the projected tails of the previous EPP. Projected tails were obtained directly from the decaying phases of EPPs of the appropriate amplitude recorded in separate trials. An example of computer-sampled EPPs is presented in Fig. 4.

Depending on the experiment, 2–20 different types of conditioning–testing trials, which differed in the duration and frequency of the conditioning stimulation or in the interval between the end of the train and the testing impulse(s), were applied. The order of the trials was varied to control for possible long-term drift that can occur in a preparation with time (Magleby and Zengel, 1976*b*). Because of the low levels of transmitter release in these experiments, the quantal fluctuation (del Castillo and Katz, 1954*a*) in the data, even with surface recording, was usually too great to estimate the average response from single trials. Consequently, data from 5–50 trials of each type were averaged for analysis. Since data were only averaged from single preparations, it was often necessary to collect data for many hours from a single preparation. If the properties of the preparation changed significantly during an experiment, the data were divided into two or more groups of consecutive trials that were then analyzed separately.

Since the data analysis in this study involved comparisons between different types of trials within the same experiment, it was essential that the different types of trials be collected under the same average conditions. This requirement was considered to be met for experiments in which the stimulation rate was held constant and the duration of stimulation varied if the EPP amplitudes during the conditioning trains superimposed, as shown in Fig. 3A for the three trains of different duration at each frequency. Data collected at different frequencies were considered comparable if the control EPP amplitudes of the averaged trials for each frequency were the same.

Data are presented as mean ± SD.

The findings presented in this and the following paper summarize the results of more than 300 experiments performed over a 5-year period.

Analysis of Data

The first component of facilitation, F_1, the second component of facilitation, F_2, augmentation, A, and potentiation, P, are all defined in a similar manner: each is given by the fractional increase in a test EPP amplitude over a control when the other processes equal zero (see Zengel and Magleby, 1980). Experimental separation of these four components of increased transmitter release relies on the fact that they have distinct and widely separated time constants characterizing their decays. After conditioning stimulation, F_1 decays to insignificant levels in 200 ms, F_2 in 2 s, A in 20 s, and P in several minutes. To measure these decays, we began by expressing the change in testing EPP amplitudes after the conditioning train as V, the fractional increase in a testing EPP amplitude over the control, such that:

$$V = (\text{EPP}/\text{EPP}_0) - 1 \tag{1}$$

where EPP is testing EPP amplitude and EPP_0 is the control amplitude in the absence of repetitive stimulation. Notice that the right-hand term of Eq. 1 is simply the definition of F_1, F_2, A or P under the conditions where all the components except the one under consideration equal zero.

To estimate potentiation, V was plotted semilogarithmically against time, and the decay of potentiation was obtained by a least-squares fit to the data points between about 35 and 175 s after the end of the train when facilitation and augmentation had decayed to insignificant levels. (Not all the decay points are plotted in the figures.) The intercept of the line describing the decay of potentiation with the ordinate at 0 time gave the magnitude of potentiation, $P(T)$, at the end of a conditioning train of duration T. The time required for potentiation to fall to 1/e of this value gave the time constant of decay of potentiation. Since augmentation and the two components of facilitation fall on one or more slower decaying components, estimates of the faster decaying components can only be obtained in terms of a model for transmitter release that defines the manner in which the components interact (see Zengel and Magleby, 1980). The data were analyzed in this paper by assuming that facilitation, augmentation, and potentiation have a multiplicative relationship such that

$$\text{EPP}/\text{EPP}_0 = (F + 1)\ (A + 1)\ (P + 1) \tag{2}$$

where F is total facilitation as defined by Eqs. 12–14 in the Results. Support for Eq. 2 comes from the observation that facilitation does appear to have a multiplicative relationship to potentiation (Landau et al., 1973; Magleby, 1973*b*) and augmentation (Magleby and Zengel, 1982), and that the relationship described by this equation can account for stimulation-induced changes in transmitter release (Magleby and Zengel, 1982).

Combining Eqs. 1 and 2 gives:

$$V = (F + 1)\ (A + 1)\ (P + 1) - 1. \tag{3}$$

Rearranging Eq. 3 to solve for augmentation under conditions when facilitation is 0 (>2 s after a train) gives:

$$A = [(V + 1)/(P + 1)] - 1. \tag{4}$$

Values of A, calculated with Eq. 4 using observed values of V and the values of P obtained from the least-squares line that describes the decay of potentiation, were then plotted semilogarithmically against time. The magnitude and time constant of decay of augmentation were estimated from a least-squares line through the plotted

points falling between ~1.5 and 10–15 s after the train. Since the decay of augmentation can deviate from a simple exponential, decaying slightly faster immediately after the conditioning train (Magleby and Zengel, 1976*b*), the time constant of decay for augmentation determined in this manner for each plot of data was typically somewhat faster than if the least-squares line were drawn through data points defining a greater percentage of the decay.

Estimates of A and P were then used together with the observed values of V to estimate total facilitation, F, using

$$F = \{(V + 1)/[(A + 1)(P + 1)]\} - 1 \quad (5)$$

obtained from Eq. 3.

Estimates of F_1^* and F_2^*, the fractional changes in the underlying factor(s) that give rise to F_1 and F_2 for the three facilitation models described in the Results, were then obtained from total facilitation with Eqs. 12–14. F_2^* was estimated first, the data were corrected for F_2^*, and F_1^* was estimated. Detailed examples of estimating all four components of increased transmitter release for the power facilitation model (Eq. 14) incorporated into Eq. 3 are presented in Zengel and Magleby (1980, 1981).

RESULTS

The Four Components of Increased Transmitter Release

Facilitation has traditionally been examined after short conditioning trains and potentiation after longer conditioning trains. To determine whether all four components of increased evoked transmitter release (potentiation, augmentation, and the two components of facilitation) can be present after a conditioning train of fixed duration, we examined the decay of EPP amplitudes after a 300-impulse train delivered at 20 impulses/s. Fig. 1A presents a composite plot of EPP amplitudes against time for a series of conditioning–testing trials. For each trial, the nerve was first stimulated once every 5 s to establish a control response. A 300-impulse conditioning train was then delivered, and the effect of the conditioning stimulation was followed with testing impulses placed at various intervals after the train in the different trials. EPP amplitudes increased about 14 times during the train and decayed back to the control level after the train with an apparent multiexponential time course. This increase in EPP amplitudes is caused by an increase in the number of quanta of transmitter released from the nerve terminal, as quantal size and postsynaptic sensitivity remain constant under the low quantal content conditions of these experiments (del Castillo and Katz, 1954*b*; Liley, 1956; Magleby and Zengel, 1976*a*).

Fig. 1B presents a semilogarithmic plot of the decay of the fractional increase in EPP amplitudes after the conditioning train. The continuous line represents the exponential decay of potentiation, which had a time constant of 51 s. The magnitude of this component at the end of the conditioning train, P(T), was 0.77. Fig. 1C replots the data after correcting for the contribution of potentiation (see Methods). The continuous line in this figure represents the decay of augmentation, which had a time constant of 6.5 s. The magnitude of this component at the end of the conditioning train, A(T), was 2.3.

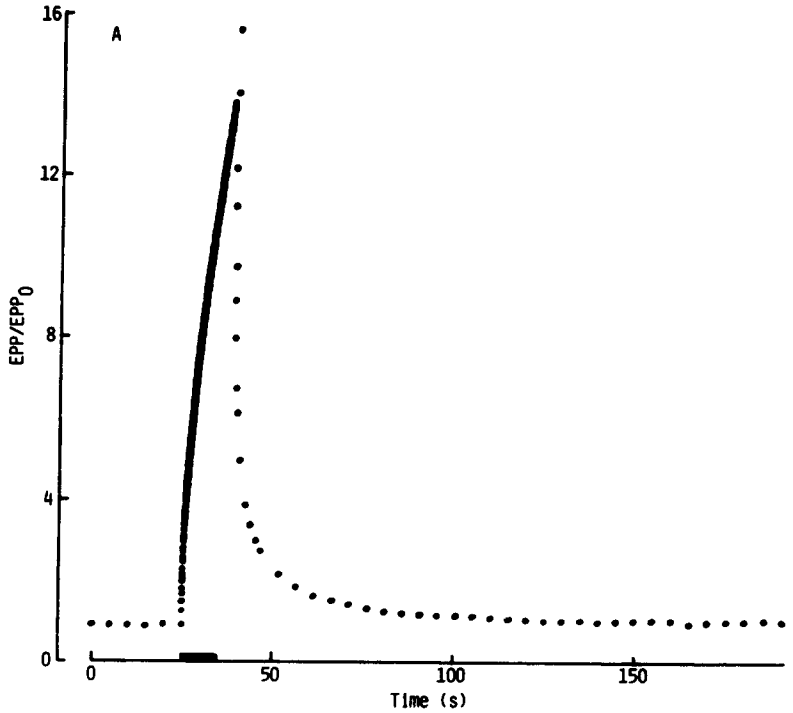


FIGURE 1. Facilitation, augmentation, and potentiation after a 300-impulse conditioning train in the frog. (A) Composite record of EPP amplitudes obtained during different conditioning-testing trials (data from 71 trials). For each trial the nerve was first stimulated once every 5 s for six impulses to establish a control response. The nerve was then conditioned with 300 impulses delivered at a rate of 20 impulses/s (horizontal bar on abscissa) and tested in the recovery period after the train with a single impulse at an interval ranging from 40 to 700 ms after the train, followed by six testing impulses at a rate of one every 1.5 s and 42 testing impulses at a rate of one every 5 s. The few additional testing impulses immediately after the train had little effect on augmentation and potentiation (Magleby and Zengel, 1976*a*). (B) Decay of V, the fractional increase in EPP amplitudes after the conditioning trains. The continuous line indicates the decay of potentiation: $P(T) = 0.77$, $\tau_P = 51$ s. (C) Decay of V after correcting for potentiation with Eq. 3. The continuous line indicates the decay of augmentation: $A(T) = 2.3$, $\tau_{A'} = 6.5$ s. (D) Decay of the fractional increase in the underlying factors in the nerve terminal responsible for facilitation determined for the power facilitation model (Eq. 14) with $n = 3$ after correcting V for augmentation and potentiation with Eq. 3. The continuous line indicates the decay of F_2^*. (E) Decay of F_1^* obtained by correcting the data in D for F_2^* with Eq. 14.

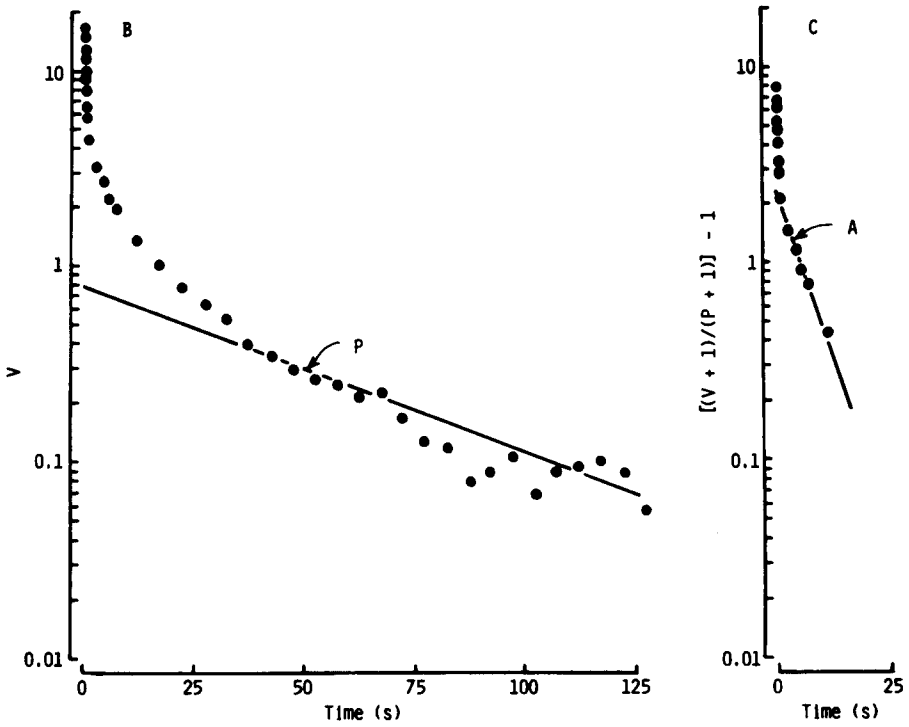
Correcting and replotting the data twice more revealed the decays of the underlying factors responsible for the second (Fig. 1D) and first (Fig. 1E) components of facilitation, with time constants of 513 and 61 ms, respectively.

The four-component decay of testing EPP amplitudes after a conditioning

train is thus similar to the four-component decay of miniature EPP frequency after repetitive stimulation (Zengel and Magleby, 1981), and suggests that all four components of increased transmitter release can be present after a conditioning train of fixed duration.

A Kinetic Model for Augmentation

The magnitude of augmentation increases with the duration of stimulation, often at an accelerating rate, whereas its time constant of decay remains relatively unchanged (Magleby and Zengel, 1976*a*, *b*). In this section, we develop a model to describe the kinetic properties of augmentation.

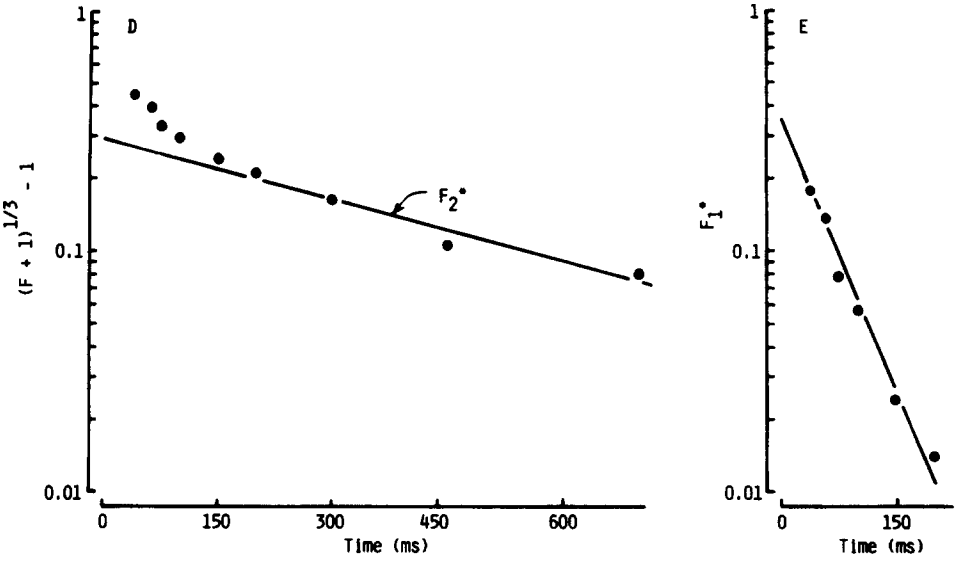


FIGS. 1B & C

An example of an experiment in which the magnitude of augmentation increased at an accelerating rate with repetitive stimulation is shown in Fig. 2A. The inserts in this figure plot EPP amplitude against time for conditioning–testing trials in which the conditioning trains were 50, 100, 200, and 400 impulses delivered at 20 impulses/s. The faster- and slower-decaying components of the EPP amplitudes after the trains reflect the decay of augmentation and potentiation, respectively, since facilitation would have decayed to insignificant levels in this experiment at the time of the first testing impulses 2 s after the trains. The increase in the amplitude of the faster-decaying compo-

nent with the duration of stimulation reflects an underlying increase in augmentation.

The magnitude of augmentation was estimated from the faster-decaying component after each train, as shown in Fig. 1, and plotted against the duration of stimulation in Fig. 2A as filled circles. These symbols indicate the rise of augmentation during 20 s of stimulation. In contrast to the accelerating increase in the magnitude of augmentation during the conditioning stimulation shown in this figure, the time constant of decay of augmentation after the conditioning trains remained relatively unchanged with values of 5.6, 6.3, 5.8, and 4.4 s after the 50-, 100-, 200-, and 400-impulse trains, respectively.



FIGS. 1D & E

Although it appears that the expression of augmentation may require external Ca (Erulkar and Rahamimoff, 1978), not enough is known about how Ca might be acting to formulate quantitatively a specific molecular mechanism for augmentation. Therefore, it seems most useful at this time to develop a general kinetic model for augmentation that can be used to study and test more specific mechanisms.

Augmentation will be assumed to arise from a change in some factor in the nerve terminal that affects transmitter release. This factor may represent Ca, a Ca-activated factor, or some other stimulation-induced change in the nerve terminal. If augmentation is related linearly to this factor, then

$$A = A^* \tag{6}$$

where A is the observed magnitude of augmentation and A^* is the fractional change in the underlying factor responsible for augmentation.

If there is a fourth-power relationship between augmentation and the factor responsible for augmentation, then

$$A = (A^* + 1)^4 - 1. \tag{7}$$

Relationships similar to that expressed by Eq. 7 have been used previously to explore the possibility that stimulation-induced changes in transmitter release are related to the fourth power of residual Ca accumulating in the nerve terminal during repetitive stimulation (Barrett and Stevens, 1972; Younkin, 1974; Magleby and Zengel, 1975*b*).

Fig. 2 shows that augmentation increases during and decays after repetitive stimulation. Consequently, A^*, the underlying factor that gives rise to augmentation, must also increase and decay. If each nerve impulse adds an incremental increase to A^*, then the rate of change of A^* with repetitive stimulation would be given by

$$dA^*/dt = J(t)a^* - k_{A^*} A^*, \qquad J(t) = \begin{cases} 1 \text{ at the time of each} \\ \quad \text{nerve impulse} \\ 0 \text{ at all other times} \end{cases} \tag{8}$$

where k_{A^*} is the rate constant for the decay of A^*, $J(t)$ represents a train of unit impulses (delta functions) occurring at an interval of 1/(stimulation rate), and a^* is the incremental increase in A^* with each nerve impulse. Since $J(t)$ is 1 at the time of each nerve impulse and is 0 at all other times, the increment a^* is added instantaneously to A^* at the instant of each nerve impulse. This leads to a step increase in A^* of magnitude a^*. This step increase with each impulse is shown in Fig. 5E. Notice from Eq. 8 that A^* is a function of time and stimulation rate.

The simultaneous Eqs. 6 and 8 describe a linear model of the type used to investigate facilitation and potentiation of transmitter release (Mallart and Martin, 1967; Magleby, 1973*a*; Magleby and Zengel, 1975*a*, *b*). Eqs. 7 and 8 describe a power model of the type used by Barrett and Stevens (1972) and Younkin (1974) to investigate facilitation of transmitter release. These two sets of simultaneous equations were solved by numerical methods to determine whether the models described by them could describe the rise of augmentation shown in Fig. 2A. In solving the equations, the rate constant k_{A^*} was determined from $1/\tau_{A^*}$, where τ_{A^*} was the mean value of the time constant of decay of A^* for the preparation. An example of the decay of A^* for the linear model, which is the same as the decay of augmentation, is shown by the continuous line in Fig. 1C. The decay of A^* used to determine k_{A^*} for the power model was determined from the observed decay of augmentation by solving Eq. 7 for A^*. Neither the linear model (dotted line, Fig. 2A) nor the power model (dotted and dashed line) could describe the effect of repetitive stimulation on augmentation. Thus, it appears that augmentation has kinetic properties that are not accounted for by models of the type that have been used to describe facilitation and potentiation.

In solving the equations, the assumption was made that a^*, the increment of A^* added by each nerve impulse, remained constant during repetitive stimulation. If, instead, a^* increased progressively during the conditioning

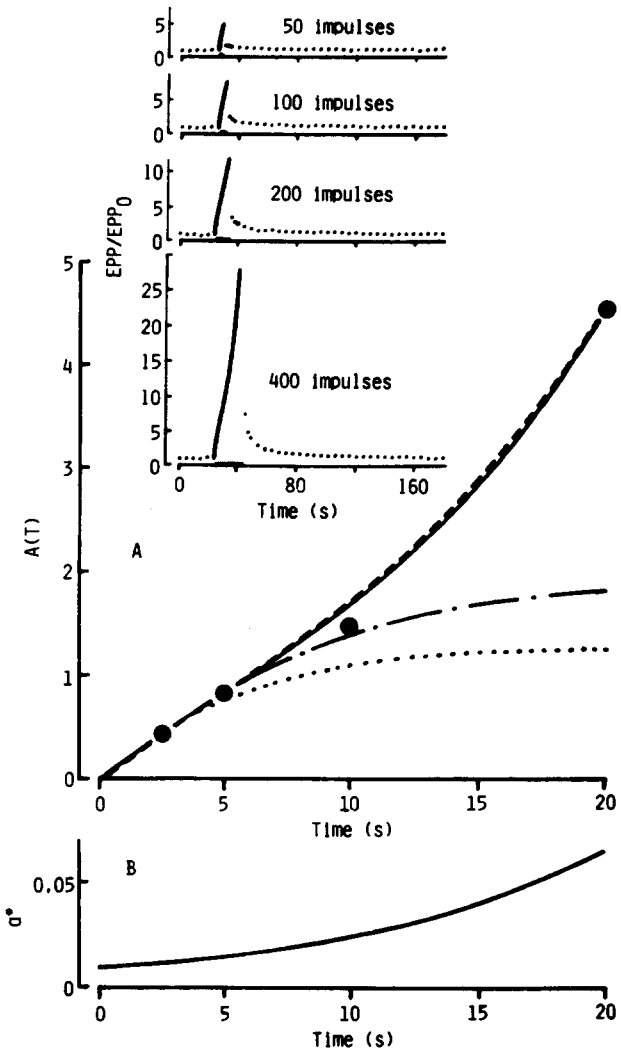


FIGURE 2. Effect of the duration of stimulation on augmentation. (A) Increase in the magnitude of augmentation during 20 s of repetitive stimulation at 20 impulses/s. Inserts: plots of EPP amplitudes against time for conditioning-testing trials in which 50, 100, 200, or 400 conditioning impulses were delivered at a rate of 20 impulses/s during the periods indicated by the bars on the abscissa; control and testing impulses were delivered once every 5 s except for the three impulses immediately after the trains, which were delivered at a rate of once every 2 s. Filled circles: estimates of $A(T)$ obtained from the data in the inserts are plotted against the duration of stimulation. Dotted line: augmentation calculated with the linear model assuming a^* remains constant using Eqs. 6 and 8 with $a^* = 0.012$ and $\tau_{A^*} = 5.5$ s. Dotted and dashed line: augmentation calculated with the power model assuming a^* remains constant using Eqs. 7 and 8 with $a^* = 0.0024$ and $\tau_{A^*} = 6.5$ s. Continuous line: augmentation calculated with the linear model assuming a^* increases during the train using Eqs. 6,

train (Magleby and Zengel, 1976*c*), then this might be expected to produce an accelerating increase in the magnitude of augmentation like that in Fig. 2A.

A mathematical expression that can give an accelerating increase in a^* with repetitive stimulation is

$$a^* = a_0^* Z^{JT} \tag{9}$$

where a^* is the incremental increase in A^* added by an impulse at time T during repetitive stimulation, a_0^* is the incremental increase in A^* added by a single impulse in the absence of repetitive stimulation, J is the stimulation rate such that JT is the number of impulses, and Z is a constant that determines the rate of increase in a^* with each impulse. Note that if $Z = 1$ there is no increase. For values of $Z > 1$, Eq. 9 predicts an unbounded increase in a^* if the number of conditioning impulses is sufficient. Although some form of limiting or saturation term would have to be added to Eq. 9 to prevent this, we have not added such a term, as we could not determine its form; there was no indication that saturation was occurring under the limited conditions of our experiments. In the application of Eq. 9, the return of a^* to the control level was assumed to be sufficiently slow (time constant of ~90 s, see Magleby and Zengel, 1976*c*) that there was no decay of a^* between nerve impulses during the conditioning trains. Sufficient time did elapse between trains (7–10 min), however, so that a^* would return to its initial value, a_0^*, by the start of the next conditioning train.

The continuous line in Fig. 2A, which was obtained by numerical solution of the simultaneous Eqs. 6, 8, and 9, shows that a linear model in which the increment of augmentation added by each impulse increases with repetitive stimulation can account for the experimental data. The calculated increase in a^* during the conditioning train with this model is shown in Fig. 2B. The increment of augmentation added by the first impulse in the train increased transmitter release ~1% of the control level ($a_0^* = 0.0095$); this increment increased to 6.4% of the control level by the 400th impulse. The dashed line in Fig. 2A (solution of Eqs. 7–9) shows that a power model with a progressive increase in a^* with repetitive stimulation can also account for the data. For the power model, a^* increased from 0.002 to 0.0057 during the conditioning train (not shown).

The kinetic models for augmentation described by Eqs. 6, 8, and 9 (linear model) and Eqs. 7–9 (power model) could also describe the effect of repetitive stimulation on augmentation in preparations in which the increase in augmentation was less pronounced than that shown in Fig. 2. In these experiments,

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8, and 9 with $a_0^* = 0.0095$, $Z = 1.0048$, and $\tau_{A^*} = 5.5$ s. Dashed line: augmentation calculated with the power model assuming a^* increases during the train using Eqs. 7–9 with $a_0^* = 0.002$, $Z = 1.0026$, and $\tau_{A^*} = 6.5$ s. k_{A^*} in Eq. 8 is given by $1/\tau_{A^*}$. (B) Calculated increase in the increment a^* during the conditioning train for the predicted increase in augmentation in A described by the continuous line (linear model with increasing a^*).

the value of Z used in the equations was either 1 (no increase in a^* during the train) or slightly >1. An example of such an experiment is shown in Fig. 3A in the following paper (Magleby and Zengel, 1982).

In 35 of 40 additional experiments with conditioning trains of up to 600 impulses delivered at 5–20 impulses/s, both the linear and power models adequately described the effect of the duration of stimulation on the magnitude of augmentation (predicted values deviated <5–15% from the observed). In the remaining five experiments, the data were described less well, but no consistent pattern was observed in the deviation.

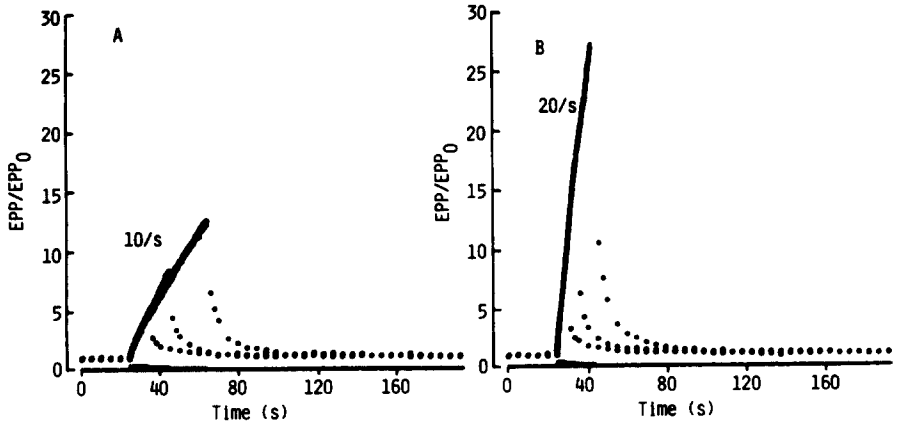


FIGURE 3. Predicting the effect of changes in the frequency and duration of stimulation on augmentation. (A and B) Superimposed records of EPP amplitudes obtained during conditioning-testing in which the nerve was conditioned with 100, 200, or 400 impulses delivered at 10 (A) and 20 (B) impulses/s. The durations of the various trains are indicated by the horizontal bars on the abcissa. Control and testing impulses as in Fig. 2. (C) Plots of the magnitude of augmentation against time obtained from the data in A and B. Filled circles: observed rise of augmentation during 20 s of stimulation at 20/s. Open circles: observed rise and decay of augmentation during and after 40 s of stimulation at 10/s. Continuous line: augmentation calculated with the linear model (Eqs. 6, 8, and 9) with $a_0^* = 0.02$, $Z = 1.0032$, and $\tau_{A^*} = 7.9$ s. Dashed line: augmentation calculated with the power model (Eqs. 7–9) with $a_0^* = 0.0035$, $Z = 1.00042$, and $\tau_{A^*} = 10.2$ s. For each model the equations were first solved to describe the 20/s data. Predicted augmentation for the 10/s data was then calculated without free parameters using the parameters obtained from the 20/s data.

Predicting the Effect of Stimulation Rate on Augmentation

In terms of the augmentation models developed in the previous section, delivering the same number of impulses at a higher stimulation rate should increase the magnitude of augmentation because there would be less time between impulses for augmentation to decay away. To test this possibility, the nerve was stimulated with 100-, 200-, and 400-impulse trains delivered at 10 and 20 impulses/s. Figs. 3A and B present superimposed plots of EPP amplitudes against time for the conditioning–testing trials. Estimates of the

rise of augmentation during these trains are plotted as circles in Fig. 3C. In addition, the decay of augmentation after the 400-impulse train delivered at 10 impulses/s is also plotted. Augmentation increased more rapidly and reached a higher level during the 20/s stimulation than during the 10/s stimulation. The linear model (Eqs. 6, 8, and 9) described the rise and decay of augmentation (continuous line) during and after the 10/s train using values of $a\bar{q}$, Z, and k_A, obtained from the 20/s data. The 10/s data were thus predicted without free parameters.

In 11 additional experiments of this type, the linear model typically described the effect of changes in frequency and duration of stimulation on

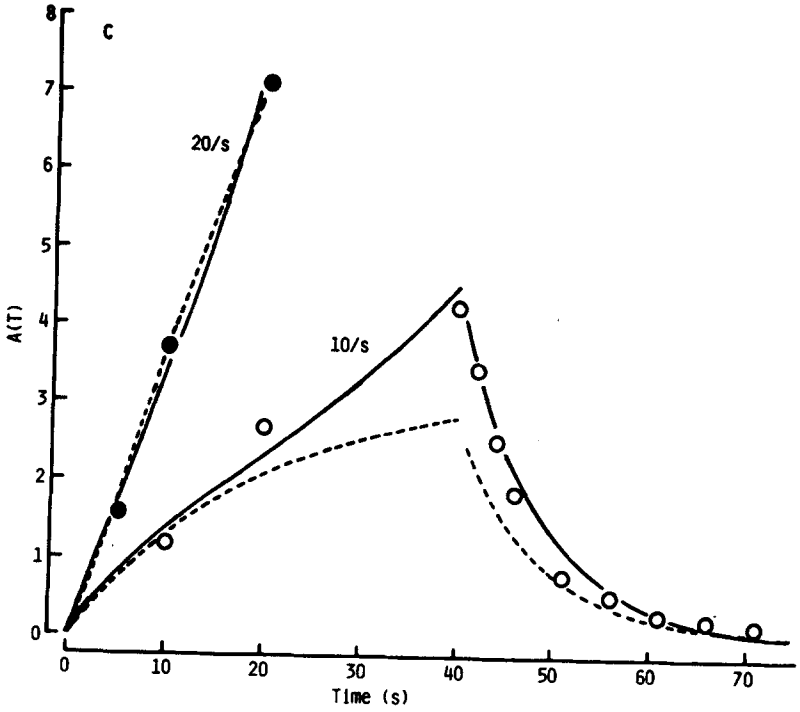


FIG. 3C

augmentation. In most cases the description was as good or better than that shown in Fig. 3, but in some experiments predicted results deviated 20–35% from the observed. In about half of the 11 additional experiments, the power model (Eqs. 7–9) underpredicted the 10/s data as shown in Fig. 3; in the others, the power model described the data about as well as the linear model. Although the linear model predicts a simple exponential decay of augmentation, the power model predicts that the decay of augmentation should deviate slightly from a simple exponential, decaying somewhat faster at greater magnitudes. The decay of augmentation does deviate from a simple exponential in a manner that can be accounted for by the power model (Magleby and Zengel, 1976*b*).

Parameters Used in the Models for Augmentation

The mean ± SD of the augmentation parameters for >30 preparations for the linear model (Eqs. 6, 8, and 9) were: $a_0^* = 0.012 \pm 0.008$; $Z = 1.002 \pm 0.002$; $\tau_{A^*} = 7.5 \pm 1.7$ s; and for the power model (Eqs. 7–9) were: $a_0^* = 0.0022 \pm 0.0012$; $Z = 1.001 \pm 0.001$; $\tau_{A^*} = 9.3 \pm 1.7$ s. The value of k_{A^*} in Eq. 8 is given by $1/\tau_{A^*}$.

Changes in a_0^* and Z with Successive Trials

The magnitude of augmentation at the end of identical conditioning trains typically increases during the course of prolonged experiments even though the decay rate of augmentation remains relatively unchanged or becomes faster (Magleby and Zengel, 1976*b*). It was therefore of interest to determine whether this increase results from an increase in one or both of the parameters, a_0^* and Z, in Eq. 9 that determine the incremental increase in A^* added by each impulse.

Estimates of a_0^* and Z were obtained for data collected early in seven selected experiments and many hours later in the same experiments after the magnitude of augmentation at the end of the conditioning trains had increased about four to five times over that at the start of the experiment. Z increased in these experiments from 1.0004 ± 0.0007 at the beginning of the experiment to 1.0031 ± 0.002 at the end, and a_0^* increased from 0.008 ± 0.003 to 0.025 ± 0.01. In terms of the kinetic analysis, it is the increase in both Z and a_0^* with successive trials and time that gives rise to the observed increase in the magnitude of augmentation that occurred in these experiments with successive trials and time. Increases in Z and a_0^* might also be the cause of the pronounced increase in augmentation (expression factor) that is observed when a second conditioning train is placed 40–170 s after the first (Magleby and Zengel, 1976*c*).

Although this section has discussed changes in Z and a_0^* that can occur over extended periods of time, it should be mentioned that over the shorter periods of time used to collect the data for experiments like those presented in Figs. 1–3, these parameters remained relatively stable.

Kinetic Models for Facilitation

In this and the following sections we examine several kinetic models for facilitation to determine which best describes the data when the contribution of augmentation and potentiation to transmitter release is accounted for.

The fractional change in the factor(s) that gives rise to the first and second components of facilitation will be designated F_1^* and F_2^*. F_1^* and F_2^* may represent Ca (see Katz and Miledi, 1968), Ca-activated factors, or some other stimulation-induced change in the nerve terminal. If each nerve impulse adds an incremental increase to F_1^* and F_2^* and if F_1^* and F_2^* return to the control level with first-order kinetics, as suggested by studies of miniature EPP frequency (Zengel and Magleby, 1981), then the rate of change in F_1^* and F_2^*

with repetitive stimulation would be given by

$$dF_1^*/dt = J(t)f_1^* - k_{F_1^*}F_1^*, \qquad J(t) = \begin{cases} 1 \text{ at the time of each} \\ \quad \text{nerve impulse} \\ 0 \text{ at all other times} \end{cases} \tag{10}$$

$$dF_2^*/dt = J(t)f_2^* - k_{F_2^*}F_2^*, \qquad J(t) = \begin{cases} 1 \text{ at the time of each} \\ \quad \text{nerve impulse} \\ 0 \text{ at all other times} \end{cases} \tag{11}$$

where $k_{F_1^*}$ and $k_{F_2^*}$ are the rate constants for the decay of F_1^* and F_2^*, $J(t)$ represents a train of unit impulses (delta functions) occurring at an interval of 1/(stimulation rate), and f_1^* and f_2^* are the incremental increases in F_1^* and F_2^* with each impulse. Since $J(t)$ is 1 at the time of each nerve impulse and is 0 at all other times, the increments f_1^* and f_2^* are added instantaneously to F_1^* and F_2^*, respectively, at the instant of each nerve impulse. This leads to step increases in F_1^* and F_2^* of magnitudes f_1^* and f_2^*. These step increases with each impulse are shown in Figs. 5B and C. Notice from Eqs. 10 and 11 that F_1^* and F_2^* are functions of time and stimulation rate.

Linear Facilitation Model

If there is a linear relationship between the underlying factors in the nerve terminal that give rise to the two components of facilitation and the observed facilitation, and if these factors sum linearly, then

$$F = F_1^* + F_2^* \tag{12}$$

where F is the total observed facilitation.

The linear facilitation model described by the simultaneous Eqs. 10–12 is similar to the linear facilitation models formulated by Mallart and Martin (1967) and Magleby (1973*a*), the major difference being the assumption by Mallart and Martin that the second component of facilitation comes on with a delay of ~120 ms after each impulse.

Multiplicative Facilitation Model

If F_1^* and F_2^* represent two separate factors whose joint action determines facilitation of release (see Magleby and Zengel, 1982), then

$$F = (F_1^* + 1)(F_2^* + 1) - 1. \tag{13}$$

Power Facilitation Model

If there is a power (n) relationship between the factor(s) that gives rise to the two components of facilitation and the observed facilitation, then

$$F = (F_1^* + F_2^* + 1)^n - 1. \tag{14}$$

When $n = 4$, the power facilitation model described by Eqs. 10, 11, and 14 is similar to the fourth-power residual Ca facilitation models examined by

Barrett and Stevens (1972), Linder (1973), Younkin (1974), and Zucker (1974*b*).

Estimates of f_1^*, f_2^*, $\tau_{F_1^*}$, and $\tau_{F_2^*}$ from the Decay of Facilitation

One of the assumptions typically used in previous studies on facilitation is that the increments f_1^* and f_2^* added by each impulse are the same for each

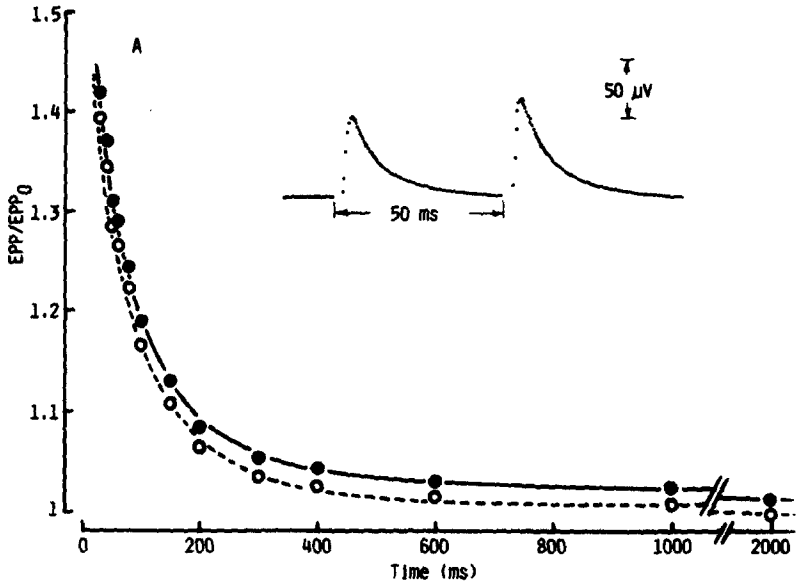
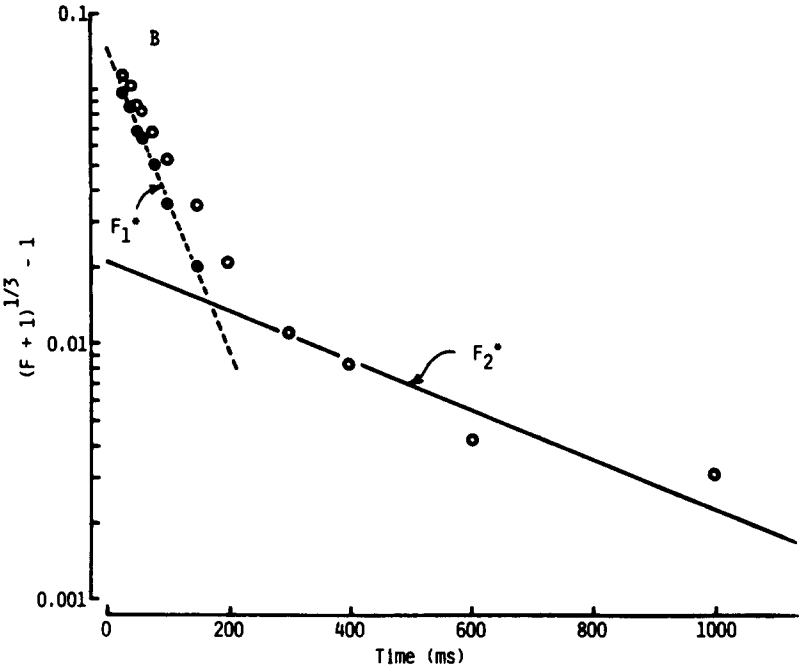


FIGURE 4. Decay of EPP amplitude and facilitation after a single nerve impulse. (A) Filled circles: decay of V, the fractional increase in testing EPP amplitude after the conditioning impulse. Each data point represents the average response of >50 conditioning-testing trials in which a single testing impulse was applied at an interval of 30 ms to 2 s after the conditioning impulse; 20 s elapsed between each trial. Insert: computer-sampled EPPs (surface recording) from a trial in which the conditioning-testing intervals was 50 ms. Open circles: decay of facilitation after correcting for augmentation and potentiation with Eq. 5 with $a_0^* = 0.01$, $\tau_{A^*} = 7$ s, $p^* = 0.009$, $\tau_{P_0} = 30$ s (see Magleby and Zengel, 1982). Facilitation and augmentation parameters were selected from within the range of observed values to account for the EPP amplitude at 2 s when facilitation would have decayed to insignificant levels. (B) Open circles: decay of the fractional increase in the underlying factors in the nerve terminal responsible for the two components of facilitation assuming the power facilitation model (Eq. 14) with $n = 3$. The continuous line indicates the decay of F_2^*. The filled circles represent the decay of F_1^* after correcting for F_2^*.

impulse in a train. Although this assumption appears to be valid for short (10-impulse) trains (Mallart and Martin, 1967), it is not clear whether this assumption can be extended to longer trains. In the previous sections it was found that the increment of augmentation a^* added by each impulse typically increased during long conditioning trains; f_1^* and f_2^* might also be expected

to increase during long trains if facilitation and augmentation have a common basis, such as residual Ca in the nerve terminal. To determine whether f_1^* and f_2^* increased with repetitive stimulation, we compared estimates of these parameters obtained from the decay of facilitation after conditioning trains of different durations.

FACILITATION AFTER A SINGLE IMPULSE The filled circles in Fig. 4A present a composite plot of the decay of testing EPP amplitudes after a single conditioning impulse. The insert presents computer sampled EPPs recorded during a trial in which the conditioning–testing interval was 50 ms. Augmentation and potentiation are too small after one impulse in the frog to measure



accurately, but it is possible to calculate the expected contributions of these components to EPP amplitudes after a single impulse using results obtained from the first part of this paper and previous studies (Magleby and Zengel, 1975*b*). The open circles in Fig. 4A plot facilitation of EPP amplitudes after correcting for the calculated augmentation and potentiation with Eq. 2. The apparent two-component decay of facilitation as described by Mallart and Martin (1967) is present.

Estimates of the fractional change, f_1^* and f_2^*, in the underlying factor(s) responsible for the first (faster-decaying) and second (slower-decaying) components of facilitation after a single impulse, are plotted in Fig. 4B for the power facilitation model with $n = 3$. Results from 12 experiments of this type are summarized in Table I.

FACILITATION AFTER LONG TRAINS The measured facilitation after long conditioning trains (Figs. 1D and E) is greater than after a single impulse because facilitation builds up during the train. Estimates of the increments f_1^* and f_2^* added by each impulse can be obtained from the measured facilitation after long conditioning trains from

$$f_1^* = F_1^*(T)(1 - e^{-\Delta t/\tau_{F1}}) \quad (15)$$

$$f_2^* = F_2^*(T)(1 - e^{-\Delta t/\tau_{F2}}) \quad (16)$$

where $F_1^*(T)$ and $F_2^*(T)$ are the magnitudes of F_1^* and F_2^* at the end of the conditioning train, τ_{F_1} and τ_{F_2} are the time constants of decay of F_1^* and F_2^*, Δt is the interval between impulses during the train, and T is the duration of

TABLE I

FACILITATION PARAMETERS FOR THE THIRD-POWER FACILITATION MODEL

Number of impulses	n	f_1^*	τ_{F1}, ms	f_2^*	τ_{F2}, ms
1*	12	0.16±0.02	56±10	0.029±0.006	437±101
10‡	26	0.15±0.04	62±13	0.025±0.005	383±70
100*	3	0.18±0.05	59±15	0.026±0.004	468±79
200*	4	0.17±0.05	60±11	0.026±0.004	539±90
300*	4	0.18±0.05	63±7	0.029±0.004	515±37
100–300§	40	0.16±0.03	62±13	0.027±0.005	507±84
Mean	89	0.17±0.01	60±3	0.027±0.002	475±58

* Values of f_1^* and f_2^* were determined from the decay of EPP amplitudes after conditioning stimulation as shown in Fig. 1 for one impulse and Fig. 4 with Eqs. 14 and 15 for 100–300-impulse trains.

‡ Value of f_1^* and f_2^* were obtained from numerical solutions of Eqs. 10 and 11 to describe the observed values of $F_1^*(T)$ and $F_2^*(T)$ at the end of the conditioning trains.

§ Values from numerical solutions to the rise of EPP amplitudes at the start of the conditioning trains using the power facilitation model incorporated into Eq. 2.

n is the number of experiments. Data presented as mean ± SD. Mean is the unweighted mean ± SD of the mean values of the facilitation parameters for the six sets of data.

stimulation (Mallart and Martin, 1967). T must be sufficiently long for the components of facilitation to reach a steady state level; this would take ~300 ms for the first component and 2 s for the second. For durations of stimulation less than those required to reach steady state levels, estimates of f_1^* and f_2^* can be obtained by numerical solution of Eqs. 10 and 11 to obtain the observed values of F_1^* and F_2^* at the end of the trains.

Values of f_1^* and f_2^* calculated with Eqs. 15 and 16 for the power facilitation model from the decay of facilitation after 100-, 200-, and 300-impulse trains are presented in Table I. These values are similar to those obtained directly after one impulse, which suggests that the increments f_1^* and f_2^* added by each impulse remain constant during repetitive stimulation. Notice also from Table I that the time constants of decay of the two components of facilitation

were independent of the duration of stimulation and hence of the magnitude of facilitation.

Predicting Facilitation during Short Trains of Stimulation

In testing the facilitation models, we took into account the effects that augmentation and potentiation have on transmitter release by using Eq. 2 together with the appropriate model of facilitation and kinetic models of augmentation and potentiation to calculate transmitter release. Details on predicting EPP amplitude with this transmitter release model are presented in the following paper (Magleby and Zengel, 1982).

The filled circles in Fig. 5A plot the observed rise of EPP amplitudes during a 10-impulse conditioning train delivered at 20 impulses/s. The continuous line, which describes accurately the experimental data, is the predicted rise calculated with a third-power facilitation model incorporated into Eq. 2. The constants used for the prediction were obtained from the decay of EPP amplitudes after the conditioning train; the response during the train was thus predicted without free parameters. Figs. 5B–F present the calculated increases in F_1^*, F_2^*, facilitation, augmentation, and potentiation that underlie the observed increase in EPP amplitude. Although the rapid increase in EPP amplitude at the start of the train was caused mainly by the two components of facilitation, the contributions of augmentation and potentiation became increasingly significant after the first few impulses; the predicted increase in EPP amplitudes that would result from facilitation alone (crosses, Fig. 5A) underpredicted release 24% by the 10th impulse.

A good description of the data was also obtained with the multiplicative facilitation model (dashed line, Fig. 5A). The linear facilitation model did not describe the data as well (dotted line).

What Power?

When we started this study on facilitation, we used a third power to be consistent with the findings of Bennett et al. (1975). The third-power facilitation model described our facilitation data and we have carried it through this series of studies. Our data could be equally well described by a fourth-power facilitation model, in which case f_1^* and f_2^* would be ~25% smaller. A second-power facilitation model also gave reasonable descriptions of the data, but did not describe the data as well as the third- or fourth-power models.

Predicting the Effect of Stimulation Rate on Facilitation

The power and multiplicative facilitation models incorporated into Eq. 2 also described accurately the increase in EPP amplitudes during short (six-impulse) trains of impulses delivered at stimulation rates of 20, 40, and 100 impulses/s using parameters obtained from the decay of EPP amplitudes after the 100/s stimulation. (For examples of experimental data see Mallart and Martin, 1967; Magleby, 1973*a*; Younkin, 1974). The linear facilitation model did not describe the data as well, overpredicting EPP amplitudes at the start of the trains similar to that shown in Fig. 5.

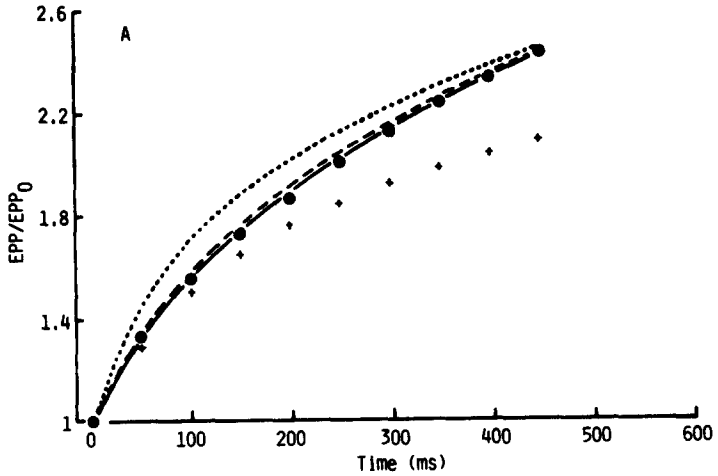
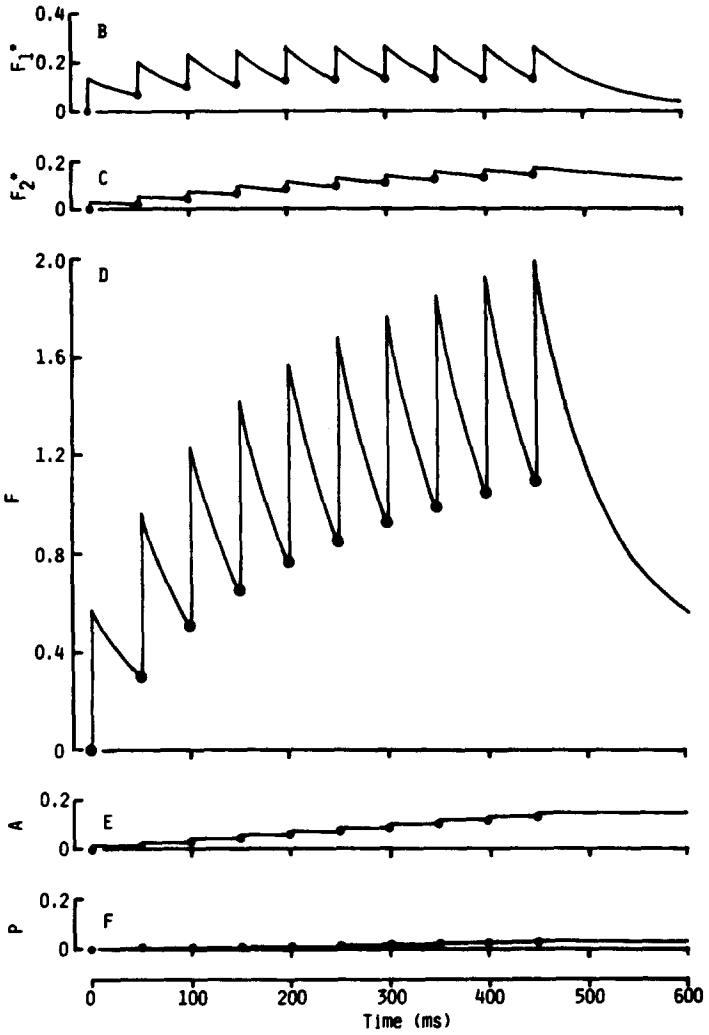


FIGURE 5. Predicting the rise of EPP amplitudes during 10-impulse conditioning trains in the frog. (A) Filled circles: observed rise of EPP amplitudes during 20/s stimulation. Average response from >500 trials in which a single testing impulse was applied from 40 ms to 20 s after the train; 30 s elapsed between trials. Only EPP amplitudes during the train are plotted; the EPP amplitudes after the train were used to obtain the parameters used to reconstruct the response during the train. Lines: predicted rise of EPP amplitudes calculated with the power (continuous line), multiplicative (dashed line), and linear (dotted line) facilitation models incorporated into Eq. 2 to account for augmentation and potentiation. Eqs. 4–9 in Magleby and Zengel (1982) were used in Eq. 2 to calculate the contribution of augmentation and potentiation to EPP amplitude. The crosses plot the rise of facilitation alone (power facilitation model). Predicted values only have meaning at the time of the EPPs because of the fluctuating response between impulses shown in B–F. (B–F) Calculated increases in the factors underlying the increase in EPP amplitude for the power facilitation model incorporated into Eq. 2. Facilitation, F, augmentation, A, potentiation, P, and the factors F_1^* and F_2^*, which give rise to each component of facilitation, are plotted. The filled circles represent the magnitudes of the various factors at the time of the nerve impulses. The abrupt rise in each factor just after each nerve impulse indicates the incremental increase contributed by the nerve impulse. The parameters used in the calculations were: power facilitation model: $n = 3$, $f_1^* = 0.135$, $\tau_{F_1} = 73$ ms, $f_2^* = 0.026$, $\tau_{F_2} = 467$ ms; multiplicative facilitation model: $f_1^* = 0.45$, $\tau_{F_1} = 71$ ms, $f_2^* = 0.086$, $\tau_{F_2} = 450$ ms; linear facilitation model: $f_1^* = 0.69$, $\tau_{F_1} = 69$ ms, $f_2^* = 0.086$, $\tau_{F_2} = 450$ ms; and for augmentation and potentiation: $p^* = 0.003$, $\tau_{P_0} = 30$ s, $B = 2$; $G = 2$; $a_0^* = 0.015$, $\tau_A = 7$ s; $Z = 1$.

Estimating f_1^*, f_2^*, τ_{F_1}, and τ_{F_2} from Numerical Solutions of Equations for Transmitter Release

In the previous sections, the parameters used to calculate facilitation were determined from the observed magnitudes and time constants of decay of facilitation after the conditioning trains. An alternative method of estimating the facilitation parameters would be to obtain them through numerical

solutions of equations that can describe transmitter release during repetitive stimulation. Such equations are developed in the following paper (Magleby and Zengel, 1982). In using these equations, the constants required to calculate augmentation and potentiation are determined from the decay of these components after conditioning trains of several durations (Fig. 2; Magleby



FIGS. 5B–F

and Zengel, 1975*a*, *b*). The equations are then solved for values of f_1^*, f_2^*, τ_{F_1}, and τ_{F_2} that describe the increase in EPP amplitudes during the first 2 s of the conditioning train, a time during which both components of facilitation increase rapidly to steady state levels. Examples of such experiments are presented in Fig. 3 in the following paper (Magleby and Zengel, 1982). Values obtained in this manner from 40 preparations (100–300-impulse trains) for

the power facilitation model incorporated into the transmitter release model described by Eq. 2 are included in Table I. These values are similar to those obtained by the more direct methods. This agreement suggests that the numerical solution technique can be used to obtain estimates of the facilitation parameters. The advantage of this method is that once the parameters used to calculate augmentation and potentiation are determined, the facilitation parameters can be calculated from the rise of EPP amplitudes during a conditioning train, instead of from the 10–20 different conditioning–testing patterns required to test directly for the decay of facilitation.

Facilitation in the Toad

Balnave and Gage (1977) have examined the effect of high-frequency (100/s) stimulation on transmitter release at the toad neuromuscular junction. They assumed that all changes in transmitter release during short trains of impulses arose only from the first component of facilitation, and found that a linear model would not describe their data, but that a two-step kinetic model would.

A comparison of their data to those obtained from the frog suggests that there is a greater increase in transmitter release during 100/s stimulation at the toad neuromuscular junction than at the frog neuromuscular junction (compare Fig. 1 of Balnave and Gage [1977] with Fig. 5 of Mallart and Martin [1967]). We examined facilitation in the toad and frog and found this to be the case. During short trains of stimulation, the increases in EPP amplitudes in the toad were about twice those observed in the frog. Estimates of f_1^* and f_2^* in the toad were also found to be about twice those observed in the frog, and this difference was sufficient to account for the greater increase in EPP amplitudes observed in the toad. The decay rates of analogous components of facilitation were about the same in the two preparations. As was the case in the frog, the power ($n = 3$ or 4) and multiplicative facilitation models could account for facilitation in the toad. In agreement with Balnave and Gage (1977), we found that the linear facilitation model did not describe facilitation in the toad.

DISCUSSION

Potentiation, augmentation, and the first and second components of facilitation contribute to stimulation-induced changes in transmitter release (Zengel and Magleby, 1980, 1981). This paper examines the kinetic properties of augmentation and re-evaluates several models for facilitation, taking into account the contributions to transmitter release from the other components.

The effect of repetitive stimulation on augmentation and the first and second components of facilitation could be accounted for by assuming: (*a*) that each component results from a change in some underlying factor (A^*, F_1^*, F_2^*) in the nerve terminal that increases transmitter release; (*b*) that each nerve impulse adds an increment (a^*, f_1^*, f_2^*) to the underlying factors; (*c*) that the magnitude of the increment added by each impulse typically increases during repetitive stimulation for augmentation, but remains constant for the first and second components of facilitation; and (*d*) that A^*, F_1^*, and F_2^* decay

with first-order kinetics with time constants of about 7 s, 60 ms, and 400 ms, respectively.

In terms of these models, each component builds up during repetitive stimulation because there is not sufficient time for the increment added by each nerve impulse to decay between nerve impulses, and also in the case of augmentation, because the increment a^* of augmentation added by each impulse increases with successive impulses. It is the accelerating increase in the increment a^* (Fig. 2B) that leads to the accelerating increase in the magnitude of augmentation that can occur during repetitive stimulation (Fig. 2A).

For augmentation, excellent descriptions of the experimental data were obtained by assuming a linear relationship between the change in the underlying factor A^* and the observed increase in transmitter release. The data were also described reasonably well in many cases, assuming a fourth-power relationship.

For facilitation, excellent descriptions of the data were obtained when a third- or fourth-power relationship was assumed between the change in the underlying factors F_1^* and F_2^* and the observed increase in transmitter release. An assumed linear relationship of the type considered by Mallart and Martin (1967) and Magleby (1973*a*) did not describe the data as well, although the predicted difference in results between the linear and power models was often small. Our results are thus consistent with those of Younkin (1974) and Barrett and Stevens (1972), who found that power facilitation models can describe the properties of facilitation.

When testing the facilitation models the assumption was made, as was done previously (Mallart and Martin, 1967; Barrett and Stevens, 1972), that the increments of facilitation added by each impulse, f_1^* and f_2^*, remained constant for each impulse in the train. When the data were analyzed with the power facilitation model and Eq. 2, this was found to be the case, even during prolonged (300-impulse) trains (Table I). If more direct experiments indicate that f_1^* and f_2^* actually increase during repetitive stimulation, then models other than a power model, such as a linear model in which the increments increase during the first second of stimulation, might be more appropriate.

Mechanisms

ACTION POTENTIAL Neither facilitation, augmentation, nor potentiation requires changes in the size or shape of the nerve action potential for expression (Martin and Pilar, 1964; Zucker, 1974*a*; Zucker and Lara-Estrella, 1979); these processes are still present after conditioning trains, retaining their kinetic properties and ionic sensitivity in the absence of nerve impulses, as indicated by studies of stimulation-induced changes in miniature EPP frequency (Erulkar and Rahamimoff, 1978; Zengel and Magleby, 1978, 1981). Thus, although each action potential produces an incremental increase in each component, the underlying changes in the nerve terminal that give rise to these increases do not require action potentials for their expression.

RESIDUAL CALCIUM It is the entry of Ca into the nerve terminal with

each nerve impulse that leads to evoked transmitter release (Katz, 1969; Llinas et al., 1981), and it appears that residual Ca in the nerve terminal can lead to stimulation-induced increases in transmitter release (Katz and Miledi, 1968; Rosenthal, 1969; Weinreich, 1971; Erulkar and Rahamimoff, 1978; Lev-Tov and Rahamimoff, 1980; Charlton et al., 1982). If augmentation and the two components of facilitation result from the direct action of residual Ca on the release sites, then the rise and fall of A^*, F_1^*, and F_2^* during and after repetitive stimulation would represent the increase and removal of this residual Ca. Some form of multiple compartment sequestration or removal system would have to be involved to give rise to the different time courses of the different components. In terms of this model, a^*, f_1^*, and f_2^* would represent the incremental increase in residual Ca in each apparent compartment with each impulse. Erulkar and Rahamimoff (1978) have suggested the augmentation may result from an increased Ca permeability of the nerve terminal. In this case, the kinetics of augmentation may reflect the general changes in permeability in addition to the kinetics of Ca in various compartments.

A progressive increase in the increment a^* with repetitive stimulation that gives rise to the accelerating increase in augmentation could result if there were a progressive increase in Ca entry with each nerve impulse or if there were a progressive decrease in Ca buffering capacity in the nerve terminal with repetitive stimulation.

The first possibility seems less likely since the amount of Ca influx per impulse into molluscan neurons or squid nerve terminals does not appear to increase during short trains of repetitive stimulation (Smith and Zucker, 1980; Charlton et al., 1982). An increased Ca entry as an explanation for the increase in a^* cannot be excluded, however, until it is known whether Ca entry into frog nerve terminals increases during prolonged (20–600-impulse) conditioning trains of the type used in our experiments.

The second possibility, a progressive decrease in the Ca buffering capacity with each impulse to account for the increase in a^*, could lead to a greater fraction of the entering Ca being available to interact with the transmitter releasing machinery, leading to an increase in a^* with each impulse. Consistent with this idea, the curves describing the increases in the increment a^* with repetitive stimulation (Fig. 2B) are similar to titration curves that can be obtained by plotting either the free ion concentration or the incremental change in free ion concentration against the amount of ion added to a buffer solution for that ion (see Woodbury, 1965). Further support for the possibility that the increase in a^* may reflect a decrease in buffering capacity comes from our observation that a^* typically increased significantly during a train only after several hours of experimentation when the buffering capacity of the nerve terminal might be expected to be reduced. The finding of Baker et al. (1971), that the Ca-activated light response from aequorin in squid axons is greatly increased during repetitive stimulation after prolonged experimentation, supports the possibility of decreased buffering capacity under these conditions.

Although the residual-Ca hypothesis is attractive because of its simplicity,

there are some observations that are difficult to account for with this hypothesis. First, if both augmentation and facilitation (and potentiation) result from residual Ca, then the apparent compartments that give rise to the components of increased transmitter release would have to have properties such that the increment a^* of augmentation added by each impulse would appear to increase during repetitive stimulation, whereas the increments f_1^* and f_2^* of the two components of facilitation (and p^* of potentiation) would not. Second, although residual Ca models can account for facilitation of EPP amplitudes, they have difficulty in their simplest form accounting for the relationship between stimulation-induced changes in EPP amplitude and miniature EPP frequency (Zengel and Magleby, 1981). These observations are not necessarily in conflict with experiments that suggest a power relationship between internal Ca and transmitter release (Dodge and Rahamimoff, 1967; Charlton et al., 1982); they merely suggest that simple fourth-power residual Ca models of the type tested here do not account for stimulation-induced changes in EPP amplitude and miniature EPP frequency under the conditions of our experiments. For example, Mg in the bathing solution, which was present in our experiments, can lead to greater release than expected from fourth-power Ca models at low quantal contents (Andreu and Barrett, 1980). Stimulation-induced changes in Na and Mg (and other metabolites) in the nerve terminal might also be expected to affect or contribute to stimulation-induced changes in transmitter release (Atwood et al., 1975; Birks and Cohen, 1968; Hurlbut et al., 1971; Charlton and Atwood, 1977; Lev-Tov and Rahamimoff, 1980; Kharasch et al., 1981). Comprehensive molecular models will most likely have to account for effects from other ions and factors in addition to Ca.

CALCIUM-ACTIVATED FACTOR Augmentation and facilitation may not simply reflect the direct action of Ca on the transmitter release sites, but may result from an increase in Ca-activated factors that affect transmitter release, such as perhaps an increase in the effective number of release sites or a change in the position of the synaptic vesicles. In this case, A^*, F_1^*, and F_2^* would represent the overall increases in selected factors and a^*, f_1^*, and f_2^* would represent the incremental increase with each impulse. The decay of each component would then reflect the decay of the Ca-activated factors to their control level; the decay could occur with kinetics similar to or slower than the removal of Ca from the activating sites. For example, there may be a Ca-activated phosphorylation that affects transmitter release (Castellucci et al., 1980; DeLorenzo, 1981). On the basis of this model, the apparent residue that leads to facilitation and/or augmentation would reflect changes in factors in addition to residual Ca, and consequently, the residue may not combine with the entering Ca as expected on the basis of the simplest versions of the residual Ca hypothesis.

Crayfish Neuromuscular Facilitation

Facilitation at the crayfish neuromuscular junction appears to be more complex than in the frog. Zucker (1974*b*) found that neither a fixed power model nor a multiplicative model could account for changes in transmitter

release during six-impulse conditioning trains, and Linder (1974) found that the facilitatory properties depend on the junction, tending to accumulate linearly at distal junctions and exponentially at more central ones. Neither of these studies corrected for possible changes in transmitter release that might occur from more slowly decaying components, and this may account for some of the differences in facilitation when compared with the frog. Another possibility is that facilitation is more complex than suggested by the simple models considered in our paper; since facilitation tends to reach considerably greater magnitudes at the crayfish neuromuscular junction than in the frog, this complexity may become expressed in the crayfish so that simple models are not sufficient to account for the observed facilitation.

Other Models

We did not test whether the two-step kinetic model of Balnave and Gage (1977) could account for our data, as there was no practical method to determine the six to seven parameters in the two-step model except by trial and error. In addition, their two-step model would at most be able to account for only the first component of facilitation, as it predicts a single exponential decay of EPP amplitudes with a time constant of ~40 ms after a conditioning train.

Maeno (1969) has proposed a model based on mobilization and demobilization of transmitter to account for "frequency facilitation." His model, like that of Balnave and Gage (1977), also predicts a single exponential decay after a train, but in contrast to the fast decay predicted by Balnave and Gage, his model predicts a decay with a time constant of ~14 s. Clearly, both the models of Balnave and Gage and Maeno would have to be expanded extensively to account for the multicomponent decay of EPP amplitudes that becomes evident (Mallart and Martin, 1967; Younkin, 1974; and Figs. 1 and 4) when the stimulation and testing parameters are extended over a wider range than those used by Balnave and Gage and Maeno.

Parnas and Segal (1980) have proposed a saturating facilitation model. Although their model can account for some aspects of facilitation, one of its major assumptions, that there is saturation in removal of residual Ca, is not consistent with the observation that the decay rates of the two components of facilitation are relatively independent of their magnitudes or the duration of stimulation (Figs. 1 and 4, Table I, and Mallart and Martin, 1967; Linder, 1974; Zucker, 1974*b*). A significant saturation in removal would require that the decay rate of facilitation be related to its magnitude if residual Ca does, in fact, directly determine the magnitude and time course of facilitation.

Following Paper

In the following paper (Magleby and Zengel, 1982) we show that the kinetic properties of augmentation and facilitation described in this paper are sufficient when combined with those of potentiation to account for stimulation-induced changes in transmitter release during prolonged stimulation.

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