Title

Post-tetanic decay of evoked and spontaneous transmitter release and a residual-calcium model of synaptic facilitation at crayfish neuromuscular junctions.

Journal

Journal of General Physiology, 81(3)

ISSN

0022-1295

Authors

ZUCKER, Robert S.
Lara-Estrella, L

Publication Date

1983-03-01

DOI

10.1085/jgp.81.3.355

Peer reviewed

Post-Tetanic Decay of Evoked and Spontaneous Transmitter Release and a Residual-Calcium Model of Synaptic Facilitation at Crayfish Neuromuscular Junctions

ROBERT S. ZUCKER and LUIS O. LARA-ESTRELLA

From the Department of Physiology-Anatomy, University of California, Berkeley, California 94720

ABSTRACT The post-tetanic decay in miniature excitatory junction potential (MEJP) frequency and in facilitation of excitatory junction potentials (EJPs) was measured at crayfish neuromuscular junctions. A 2-s tetanus at 20 Hz caused the MEJP frequency to increase an average of 40 times and the EJP amplitude to increase an average of 13 times. Both MEJP frequency and EJP facilitation decayed with two time constants. The fast component of MEJP frequency decay was 47 ms, and that of EJP facilitation was 130 ms. The slow component of MEJP frequency decay was 0.57 s, and that of EJP facilitation was ~1 s. These results were consistent with the predictions of a residual calcium model, with a nonlinear relationship between presynaptic calcium concentration and transmitter release.

INTRODUCTION

When an action potential invades a nerve terminal, the voltage-dependent calcium channels in the terminal open briefly to admit calcium, leading to the phasic release of neurotransmitter and the subsequent postsynaptic potential (PSP) (Llinás et al., 1981). If transmitter release is kept at a low level to reduce depression and depletion of transmitter stores, then for a period after one or more action potentials, a presynaptic impulse may release more transmitter than a single impulse in isolation, eliciting a larger than normal PSP. This process is variously referred to as synaptic facilitation, augmentation, potentiation, or post-tetanic potentiation, depending on its duration and the number of presynaptic action potentials needed to elicit the effect (Magleby, 1979).

The physiological mechanism of synaptic facilitation has been studied most

Address reprint requests to Dr. Robert Zucker, Dept. of Physiology-Anatomy, University of California, Berkeley, CA 94720. Dr. Lara-Estrella's present address is Departamento de Tecnologia de Procesos Biologicos y Bioquimicos, Universidad Simón Bolivar, Apartado 80659, Caracas 1081, Venezuela.

J. GEN. PHYSIOL. © The Rockefeller University Press · 0022-1295/81/03/0355/18 $1.00 355
Volume 81 March 1983 355-372

extensively at neuromuscular junctions and at the squid giant synapse. At the giant synapse, a brief facilitation is caused neither by changes in the presynaptic action potential nor by presynaptic afterpotentials (Charlton and Bittner, 1978). Instead, it is probably due to the action of a residual intracellular calcium or calcium complex, called active calcium (Charlton et al., 1982). The idea is that a lingering small increase in active calcium, called residual calcium, leads to an increase in transmitter release evoked by the sudden, brief, and constant increment in active calcium accompanying a nerve impulse. This calcium increment releases more transmitter in the presence of residual calcium because of a nonlinear, power-law relationship between transmitter release and active calcium. A similar hypothesis is supported by somewhat less direct evidence at neuromuscular junctions (Katz and Miledi, 1968; Rahamimoff, 1968; Zucker, 1977).

If there is a prolonged increase in active calcium after presynaptic activity, this should be reflected not only in facilitation of transmitter release evoked by a nerve impulse, but also by an increase in the spontaneously occurring tonic release of neurotransmitter quanta (Miledi and Thies, 1971; Rahamimoff and Yaari, 1973). Indeed, stimulation of motor neurons is followed by an increased frequency of miniature endplate potentials (MEPPs), lasting about as long as the various phases of facilitation, augmentation, and potentiation (Barrett and Stevens, 1972; Erulkar and Rahamimoff, 1978; Lev-Tov and Rahamimoff, 1980; Zengel and Magleby, 1981).

Recently, Zengel and Magleby (1981) attempted to use a simple but precisely formulated residual calcium model to relate the tetanic and post-tetanic time courses of endplate potential (EPP) facilitation and increased MEPP frequency. In this model, both phasic and tonic transmitter release are controlled in a highly nonlinear fashion by the instantaneous level of active calcium at presynaptic release sites. This active calcium has three components: (*a*) a resting or steady state level, Ca_S, present in the absence of nerve activity and contributing to the resting frequency of MEPPs; (*b*) an increment in active calcium entering from the external medium during an action potential, Ca_E, and causing the phasic release of transmitter; and (*c*) a residual calcium, Ca_R, after nerve activity, representing that extra active calcium remaining from previous activity in the region of release sites. The post-tetanic increase in MEPP frequency is due directly to Ca_R, whereas facilitation of spike-evoked release is due to Ca_E added to Ca_R.

In this model, MEPP frequency is more sensitive than phasic release to residual calcium, and so the former will show a greater increase after a tetanus. Moreover, as residual calcium decays, MEPP frequency should decline to the resting level faster than EPP amplitude. Although the first prediction was confirmed qualitatively in measurements of EPPs and MEPPs, the second was not. The increase in MEPP frequency was greater than the increase in EPP magnitude (although less so than predicted), but they declined at similar rates, contrary to prediction.

Zengel and Magleby (1981) pointed out that in the early part of a train of stimuli, the predicted relation between the development of EPP facilitation

and MEPP frequency was more closely followed. They suggested that the early components of the increase in transmitter release, namely facilitation, might follow their predictions better than the slower components, augmentation and potentiation.

We decided to test the residual-calcium theory of the relation between EPP amplitude and MEPP frequency under conditions where facilitation is the dominant effect of stimulation. We chose to study the relation between post-tetanic excitatory junctional potential (EJP) facilitation and miniature EJP (MEJP) frequency at neuromuscular junctions in the crayfish opener muscle. These synapses display two prominent components of EJP facilitation (Zucker, 1974) similar to those seen at frog neuromuscular junctions (Magleby, 1973*a*; Younkin, 1974), but there is little sign of augmentation and potentiation. Only with very long trains, lasting ≥10 min, does a slow component termed long-term facilitation, kinetically similar to potentiation, become evident at these synapses (Sherman and Atwood, 1971). Crayfish synapses have the further advantage that fibers can be found that display no sign of synaptic depression, even at normal levels of transmitter release. We report here that at crayfish neuromuscular junctions the residual-calcium hypothesis gives a reasonable description of the observed changes in post-tetanic MEJP frequency and EJP amplitude.

METHODS

EJPs and MEJPs were recorded intracellularly from abductor muscles of the dactyl in the first or second walking leg of the crayfish *Procambarus clarkii*. Small animals, ~8 cm long, were preferred, as these had smaller muscle fibers and consequently larger MEJPs (Katz and Thesleff, 1957). The dissection, mounting, and illumination of the muscle and motor nerves are described in Dudel and Kuffler (1961). The excitor nerve to the opener muscle was stimulated using platinum wire electrodes, with 0.05-ms, suprathreshold isolated pulses. Muscle potentials were recorded with 2–4-MΩ electrodes pulled from thin-walled tubing, with tip diameters ~1 μm and filled with 4 M potassium acetate. These were connected through a KCl bridge and an Ag-AgCl pellet to a low-noise DC amplifier (P-18; Grass Instrument Co., Quincy, MA). The reference electrode was an Ag-AgCl pellet in the bath.

Special care was taken to keep noise in the recording system as low as possible, because MEJP amplitudes were only 0.1–0.75 mV. High-frequency noise was reduced with a single-pole, low-pass filter with a cutoff frequency of 1,500 Hz. The large DC potential caused by summation of EJPs during a tetanus was eliminated by connecting the amplifier output to a Butterworth two-pole, high-pass active filter with a cutoff frequency of 1 Hz. These filters had no significant effect on EJP or MEJP amplitudes. The DC membrane potential was also continuously monitored by a digital voltmeter connected before the high-pass filter. Healthy fibers maintained a membrane potential of −60 to −80 mV for the duration of the experiment.

The physiological solution was composed of (mM): 195 NaCl, 5.4 KCl, 13.5 $CaCl_2$, 2.6 $MgCl_2$, 10 HEPES buffer, adjusted to pH 7.3 with NaOH. The temperature was kept at 15°C with Peltier thermoelectric units and constant superfusion with aerated Ringer.

In these experiments, only one stimulus paradigm was used. The excitor nerve was stimulated for 2 s at 20 Hz. Tetani were separated by at least 30 s, which is sufficient

for return of the EJP amplitude and MEJP frequency to baseline levels between tetani. Because of variability between fibers, we collected sufficient data from each synapse to measure the full time course of EJP and MEJP changes after a tetanus. Experiments usually lasted 5–6 hr.

The decay of EJP facilitation after a tetanus was measured using an averaging computer (Neurolog NL-750; Digitimer Ltd., Welwyn Garden City, Hertfordshire, England) to generate averaged EJPs at various intervals after a tetanus. Facilitation was measured at closely spaced intervals during its early rapid decay and less frequently as transmission leveled off. Each tetanus was followed by a single test EJP at one of the following intervals (ms): 20, 40, 60, 80, 100, 150, 200, 250, 300, 400, 500, 600, 800, 1,000, 1,500, or 2,000. Eight EJPs at each interval were averaged, displayed on an oscilloscope screen, and photographed. The order of measuring intervals was randomized, and repeated checks at the same interval revealed a stationary decay of facilitation over the hour required to collect this data. The first EJP of eight trains was also averaged and measured as an unfacilitated EJP. The film was later projected onto a grid and the amplitudes were tabulated by hand. EJP facilitation at time t after a tetanus was calculated from

$$F_e(t) = v(t)/v_o - 1, \quad (1)$$

where $v(t)$ is the average EJP amplitude at time t after the peak of the last EJP in the conditioning train and v_o is the average unfacilitated EJP amplitude.

MEJP frequency was measured using two recording paradigms, one for determining resting or control MEJP frequency and obtaining a low-resolution measurement of MEJP frequency for 20 s after a tetanus, and the other for obtaining a high-resolution measurement of MEJP frequency for 2 s after a tetanus.

Each set of data consisted of one low-resolution measurement followed by 10 high-resolution measurements. For the low-resolution measurements, MEJPs were displayed continuously on an oscilloscope sweeping at 0.5 s/div, and successive sweeps were recorded on film moving vertically at 1 mm/s. The control MEJP frequency was estimated from the number of MEJPs observed during 50 s of rest before a tetanus. During the tetanus, MEJPs could not be discerned clearly on the large facilitated EJPs, which were often 20 times the amplitude of MEJPs. After the last EJP in the tetanus, time was divided into 0.5-s intervals for the first 5 s, and 1-s bins for the next 15 s. In this way bin size was changed according to the rate of change of MEJP frequency. Small bin sizes were used when MEJP frequency was high and changing rapidly, and large bin sizes were used when MEJP was low and changing slowly. For each train, the resting MEJP frequency was estimated from the recordings preceding the train, while the numbers of MEJPs in the various intervals after the train were tabulated.

After a set of low-resolution measurements, data were collected to determine the detailed time course of decay of MEJP frequency during the first 2 s after the tetanus. For this purpose, the oscilloscope was triggered on the last stimulus of the tetanus, and subsequent MEJPs were displayed continuously on an oscilloscope sweeping at 20 ms/div and photographed on film moving vertically at 20 cm/s. Post-tetanic time was now divided into 20-ms intervals for the first 200 ms after the last EJP, then 50-ms intervals for the next 800 ms, and finally 100-ms intervals for the next second. The number of MEJPs in each post-tetanic interval was tabulated for 10 such tetani repeated every 30 s to generate a set of high-resolution measurements of MEJP frequency.

The entire sequence of 10 measurements of post-tetanic MEJP frequency at high resolution, plus one measurement of resting and post-tetanic MEJP frequency at low

resolution, was repeated 10–16 times in each experiment, yielding high-resolution data from 100 to 160 tetani at a single synapse. These data could later be combined and averaged, or grouped into sections corresponding to different times during the experiment to check for nonstationarity in the data. Data from synapses whose behavior changed with time are not included in this analysis.

Programs were written in BASIC (*a*) to store onto floppy discs the numbers of MEJPs in different time bins and the data on EJP amplitude, (*b*) to convert the MEJP numbers to frequencies, and (*c*) to calculate facilitation of EJP amplitude and MEJP frequency. Facilitation of MEJP frequency at time t following a tetanus was calculated from

$$F(t) = f(t)/f_0 - 1, \tag{2}$$

where $f(t)$ is the MEJP frequency at time t after the peak of the last EJP in the conditioning train and f_0 is the control MEJP frequency.

RESULTS

Post-Tetanic Decay of MEJP Frequency and EJP Facilitation

After stimulation for 2 s at 20 Hz of the excitor nerve, the MEJP frequency recorded from opener fibers increased from an average prestimulus frequency of 1.29 ± 0.62 (SD) to 47.30 ± 24.49/s ($N = 8$) at the end of the tetanus. The MEJP frequency increased on the average to 39.55 times the resting level, or the MEJP frequency showed an average facilitation at the end of the tetanus, F, of 38.55 ± 20.76. During the same tetani, the EJPs increased from an average unfacilitated amplitude of 0.25 ± 0.21 to 2.57 ± 1.34 mV at the end of the tetanus, for an average facilitation, F_e, of 12.23 ± 5.32. So, as predicted by the residual-calcium model (Zengel and Magleby, 1981, and see below), MEJP facilitation was substantially greater than EJP facilitation. This was observed in every synapse studied.

As indicated by the large standard deviations, the quantitative performance of individual synapses was quite variable. In particular, the unfacilitated EJP amplitude varied from almost zero in synapses in which the first stimulus in a train frequently released no quanta, to more typical synapses in which the unfacilitated EJP was about the same average amplitude as the average MEJP size, to those synapses in which the unfacilitated EJP usually consisted of more than one quantum. The degree of EJP facilitation was also quite variable, ranging from 4.41 to 17.75 at eight synapses (cf. Zucker, 1974; Bittner and Sewell, 1976). The resting and post-tetanic MEJP frequencies were somewhat less variable, increasing usually from just over 1/s to ~50/s. MEJP amplitudes were also variable, ranging between 0.1 and 0.75 mV in different fibers and often showing a twofold variability within single fibers. Much of the latter variability is due to the multiterminal innervation of crustacean muscles, so that a microelectrode will be located at different electrotonic distances from the various synaptic contacts from the excitor motor neuron (Fatt and Katz, 1953).

Because of this wide variability in preparations, we decided to collect sufficient data from individual synapses to be able to characterize accurately the effects of tetani on EJP amplitude and MEJP frequency without lumping

together data from several synapses. A rough estimate of the effect of a tetanus on MEJP frequency was available from the low-resolution measurement of MEJP frequency before the tetanus and for 20 s afterwards. Fig. 1 shows these data from one experiment. Since only 12 trials are averaged, the data points fluctuate about the presumed decay path. Nevertheless, it is evident that MEJP frequency had decayed to near control levels after a few seconds. In most fibers, the decay was nearly complete in ~2 s. Fig. 2 shows the decay of MEJP frequency for the first 2 s after the tetanus at higher resolution and accuracy. In this figure, data from 120 trials have been averaged. The dotted line indicates the resting MEJP frequency, 1.20/s, in this fiber. This frequency was estimated from 600 s of prestimulus records. The decay of EJP facilitation in this fiber after the same tetani is shown in Fig. 3. Each point is the

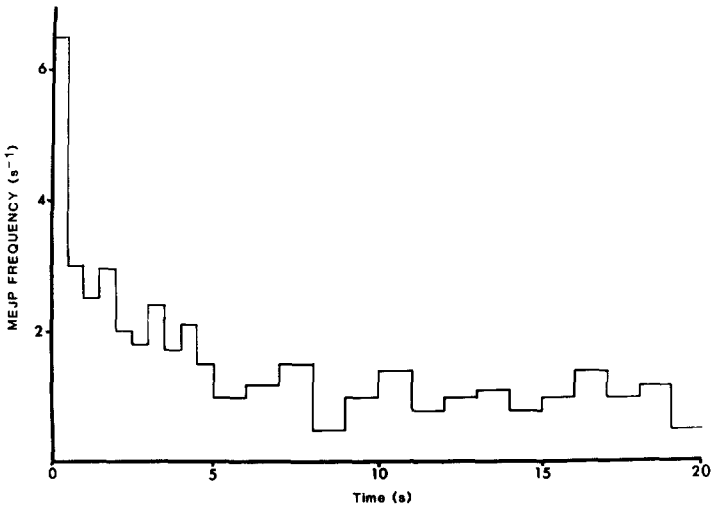

FIGURE 1. Low-resolution measurement of the decay of increased MEJP frequency for 20 s following a tetanus of 40 action potentials at 20 Hz in the excitor motor neuron to the crayfish walking leg abductor muscle. The MEJPs were recorded intracellularly from a muscle fiber. Average of 12 trials. Temperature, 15°C.

amplitude of eight computer-averaged EJPs (see Methods). The dotted line shows the amplitude of the unfacilitated EJP as 0.30 mV. From Figs. 1–3, it is evident that MEJP frequency declined faster than EJP facilitation, as predicted by the residual-calcium model (Zengel and Magleby, 1981, and see below). Similar results were obtained in all eight experiments that were analyzed.

To compare decays of EJP amplitude and MEJP frequency of diverse magnitudes in different fibers, we plotted the normalized decays in the post-tetanic facilitation of MEJP frequencies and EJP amplitudes, expressing these as percentages of the maximum values seen immediately after the tetanus. Figs. 4 and 5 show this data for three additional preparations different from the one illustrated in Figs. 1–3. These four fibers, and the other four we

studied, were remarkably uniform in their rapid decays of MEJP frequency and slower decays of EJP facilitation.

Figs. 4 and 5 suggest that the post-tetanic decay of both tonic and phasic release can be described as the sum of two exponentials. To estimate the time constants of these components, the measurements were plotted on semilogarithmic coordinates after subtracting the control values, as shown in Fig. 6 for the data of Figs. 2 and 3. A least-squares line was fitted to the late part of the decay. These points appear to lie on a straight line. This line was extrapolated to zero and subtracted from the early points lying above it. A least-squares

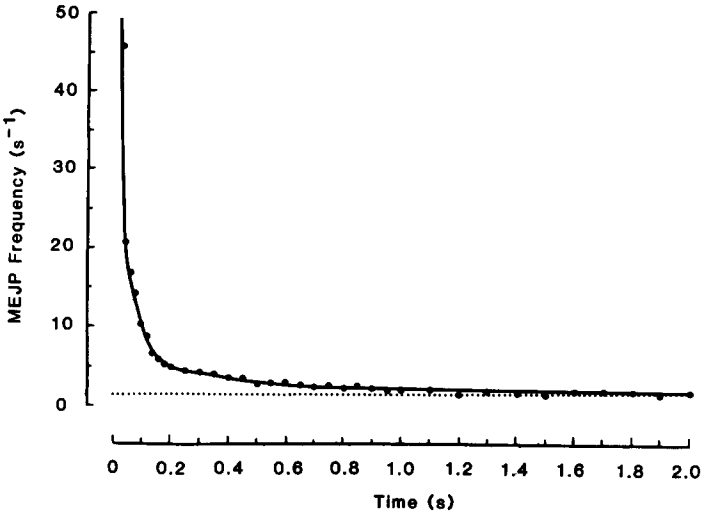

FIGURE 2. High-resolution measurement of the MEJP frequency for 2 s following a tetanus of 40 presynaptic impulses at 20 Hz. The points are averages of 120 trials, from the same fiber as in Fig. 1. The dotted line marks the prestimulus level of MEJP frequency, averaged from 10 min of records. The solid line shows the behavior of a residual calcium model fitted to this data, represented by Eqs. 4 and 7, with the following parameter values: K = 1.2 quanta·s^{-1}·(active calcium concentration)$^{-n}$, Ca_S = 1 unit of calcium concentration, n = 5, A_1 = 1.078 units of calcium concentration, A_2 = 0.425 unit of calcium concentration, τ_1 = 50.6 ms, τ_2 = 0.563 s.

line was fitted to the difference. Finally, the two components extracted by this method were recombined to form a composite curve that can be compared with the original measurements. This comparison reveals that the double-exponential analysis provides a good description of the decay of both EJP amplitude and MEJP frequency.

Both components of MEJP frequency decay were consistently faster than the two components of EJP facilitation decay. The time constant of the fast component of MEJP decay rate was 47.1 ± 5.5 ms, whereas that of the EJP amplitudes was 129.5 ± 35.5 ms. The time constant of the slow component of MEJP frequency decay was 0.57 ± 0.18 s, and that of EJP facilitation decay

was 1.01 ± 0.25 s. The slow component of the EJP decay was 53 ± 5% as large as the fast component in six of the eight synapses studied, but in the remaining two synapses no distinct slow component was evident. The slow component of the MEJP frequency was only 11 ± 3% as large as the fast component. Thus, the slow MEJP component was difficult to measure accurately, and the differences in average slow decay rate of MEJP frequency and EJP facilitation can be estimated only roughly from the data.

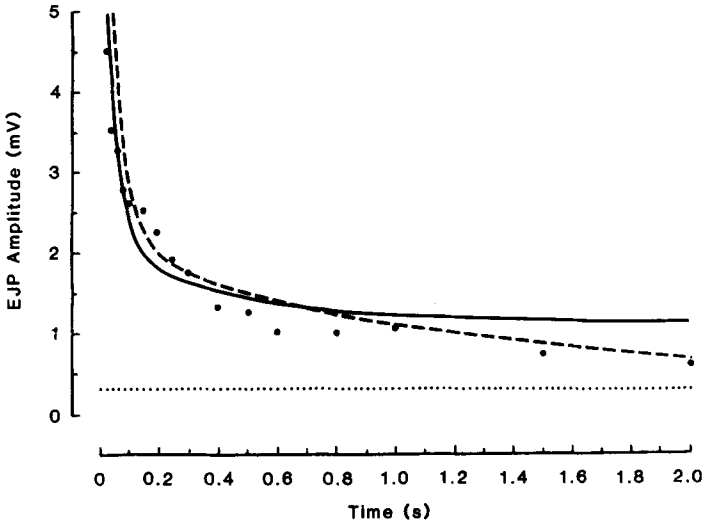

FIGURE 3. Post-tetanic decay of EJP amplitude, measured with single test EJPs at various intervals after 2-s trains of action potentials at 20 Hz. The points are averages of eight trials, from the same fiber as in Figs. 1 and 2. The dotted line marks the amplitude of unfacilitated EJPs. The solid line is the prediction of the residual-calcium model assuming that the resting MEJP frequency is determined by steady state active calcium and represented by Eqs. 6 and 7 with the following parameter values: $Q = 0.59$ mV/quantum, $T = 4$ ms, $Ca_E = 2.279$ units of calcium concentration; other values are the same as in the Fig. 2 legend. The dashed line is the prediction of the residual-calcium model assuming that the resting MEJP frequency is calcium independent, using Eq. 6 modified as described in the text, and with the following parameter values: $Ca_S = 0, f_I = f_o = 1.2\ s^{-1}$, $Ca_E = 2.410$, $A_1 = 1.044$, $A_2 = 1.296$, $\tau_1 = 66.6$ ms, $\tau_2 = 2.35$ s; other values are as before.

Our measurements of the decay rate of EJP facilitation are somewhat slower than those reported earlier (Zucker, 1974; Bittner and Sewell, 1976). The difference is probably due to the lower temperature used in the present study. Our preliminary observations confirm that facilitation is prolonged at low temperature.

Residual-Calcium Model of Facilitation

We wanted to know whether a simple model of presynaptic residual active calcium could account quantitatively for our measurements of the post-tetanic

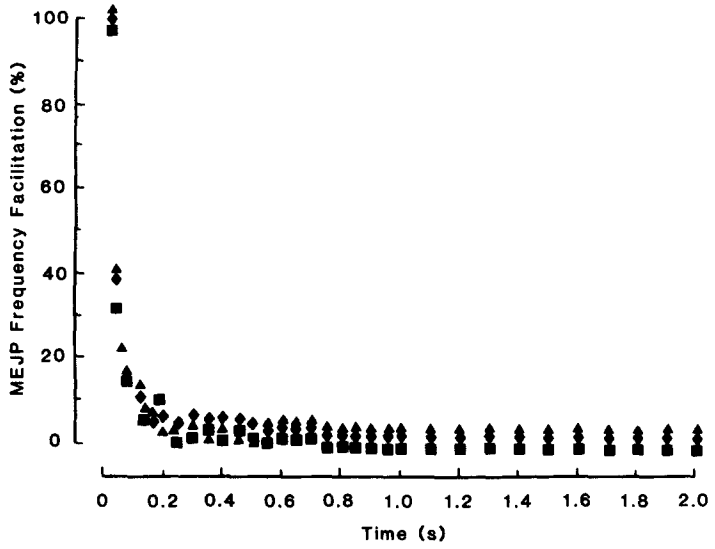

FIGURE 4. Normalized post-tetanic decay of increased MEJP frequency for 2 s after 2-s trains of presynaptic impulses at 20 Hz. Results from three different experiments are shown. MEJP frequencies were converted to MEJP facilitation values using Eq. 1, and these were plotted as percentages of the maximum post-tetanic values. The peak and resting MEJP frequencies in the three fibers were 48.0 and 1.05, 37.6 and 1.21, and 56.9 and 2.21 per second. Overlapping symbols have been displaced vertically slightly for clarity.

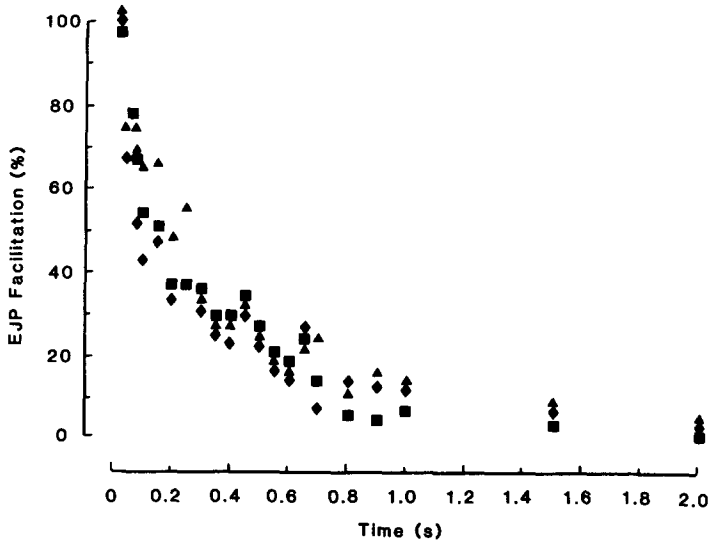

FIGURE 5. Normalized post-tetanic decay of EJP facilitation in the same fibers as Fig. 4. EJPs were converted to facilitation values using Eq. 2, and these were plotted as percentages of the maximum post-tetanic values. The peak and resting EJP amplitudes were 0.7 and 0.04, 2.0 and 0.10, and 1.8 and 0.18 mV.

decay of MEJP frequency and EJP amplitude. For this purpose, we used the formulation proposed by Miledi and Thies (1971) and developed by Zengel and Magleby (1981) to predict the relation between the decays of MEJP frequency and EJP amplitude. In this model, transmitter release, $R(t)$, is determined instantaneously by its power-law dependence on the three components of active calcium: Ca_S, the steady state concentration of active calcium that controls resting MEJP frequency, Ca_E, the active calcium that enters during an action potential and leads to an EJP, and $Ca_R(t)$, the residual extra active calcium remaining in the nerve terminal at time t after a tetanus.

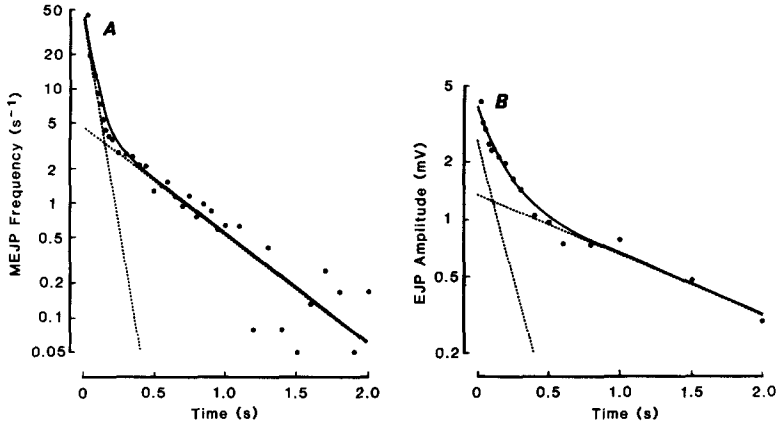

FIGURE 6. Dissection of the post-tetanic decay of MEJP frequency and EJP amplitude into two exponential components by regression analysis. (A) The logarithm of MEJP frequency minus the prestimulus frequency of 1.2/s is plotted vs. time from the end of the tetanus. (B) The logarithm of EJP amplitude minus the unfacilitated EJP amplitude (0.3 mV) is plotted vs. time from the end of the tetanus. Same data as in Figs. 2 and 3. The dotted lines represent the two exponential components of MEJP frequency decay or EJP facilitation decay fitted to the data as described in the text, and the solid line shows the time courses reconstructed from these components. The magnitudes and time constants of the fast and slow components of MEJP frequency decay were 39.36 and 4.67 s^{-1}, and 59 and 463 ms. Those for EJP amplitude decay were 2.53 and 1.35 mV, and 153 and 1,400 ms.

Transmitter release is given by

$$R(t) = K[Ca_S + Ca_E + Ca_R(t)]^n, \tag{3}$$

where R is in units of quanta per second.

The post-tetanic rate of spontaneous transmitter release, $f(t)$, is governed by Ca_S and $Ca_R(t)$ in the absence of Ca_E,

$$f(t) = K[Ca_S + Ca_R(t)]^n, \tag{4}$$

whereas the resting MEJP frequency, f_o, depends only on Ca_S,

$$f_o = K(Ca_S)^n. \tag{4a}$$

The phasic release of transmitter evoked by an action potential can be regarded as a transient but intense increase in the rate of MEJPs (Liley, 1956), lasting for a brief period, T, after an action potential. In that case, the amplitude of an EJP, v, will be given by the product of the rate of transmitter release during the evoked burst, R, the duration of phasic transmitter release, T, and the postsynaptic effectiveness of a quantum of transmitter, Q:

$$v = QTR. \tag{5}$$

The post-tetanic EJP amplitude will then be given by

$$v(t) = QTK[Ca_S + Ca_E + Ca_R(t)]^n, \tag{6}$$

whereas the unfacilitated EJP, v_o, will be generated by Ca_S and Ca_E in the absence of Ca_R:

$$v_o = QTK(Ca_S + Ca_E)^n. \tag{6a}$$

Since both MEJP frequency and EJP facilitation decay with fast and slow components after a tetanus, and both are supposed to be due to the decline of Ca_R, we imagine that $Ca_R(t)$ also declines with both fast and slow components that can be approximated as exponential functions,

$$Ca_R(t) = A_1 e^{-t/\tau_1} + A_2 e^{-t/\tau_2}. \tag{7}$$

Eqs. 4 and 6 describe the decay of MEJP frequency and EJP amplitude after a tetanus, caused by residual calcium, $Ca_R(t)$, decaying according to Eq. 7. Ca_R can be envisaged as the residual tail of Ca_E, the calcium that enters during action potentials, which accumulates during a tetanus and decays afterwards, as diagrammed in Fig. 7. However, the predicted relation between MEJP frequency, $f(t)$, and EJP amplitude, $v(t)$, should be valid regardless of the source of Ca_R or the factors controlling it.

To test the ability of this formulation to account for our results, we need to assign values to the parameters. We have no direct measure of the presynaptic concentration of active calcium, so Ca_S, Ca_E, and Ca_R must be expressed in arbitrary units. We use Ca_S as the basic concentration unit to which active calcium is scaled and assign it a value of unity. From Eq. 4*a* with $Ca_S = 1$, we have $K = f_o$, so K can be estimated from the resting MEJP frequency.

The exponent n can be related to the average number of calcium ions that must react at presynaptic sites to release a quantum of transmitter (Dodge and Rahamimoff, 1967). To estimate n, we note that Eq. 4 can be rewritten as

$$[f(t)/K]^{1/n} - Ca_S = Ca_R(t), \tag{8}$$

and Eq. 6 can be rewritten as

$$[v(t)/QTK]^{1/n} - Ca_S = Ca_R(t) + Ca_E. \tag{9}$$

With $Ca_S = 1$ and the correct choice of n, a plot of the left sides of Eqs. 8 and 9 vs. time should yield two parallel straight lines, with Eq. 9 displaced vertically above Eq. 8 by an amount corresponding to Ca_E. We tried values of

n from 2 to 9 and found that MEJP data plotted according to Eq. 8 and EJP data plotted according to Eq. 9 fell most closely on parallel straight lines with $n = 5$. This method also provided an estimate of Ca_E, after estimating Q and T as discussed below. It is noteworthy that 5 is similar to the value of n that provided the best, albeit an imperfect, fit to the growth of EJP facilitation in a tetanus in an earlier simulation of a residual-calcium model (Zucker, 1974).

With n and Ca_S determined as above, we used Eq. 8 to estimate the decay of $Ca_R(t)$ from the recovery of post-tetanic MEJP frequency. We then used regression methods (see above) to fit two exponentials to $Ca_R(t)$ and estimate A_1, A_2, τ_1, and τ_2. We found that $Ca_R(t)$ could be described adequately by Eq. 7, as shown in Fig. 8A, and that the description of $Ca_R(t)$ provided a good fit, as would be expected, to the MEJP data from which it was derived (Fig. 2).

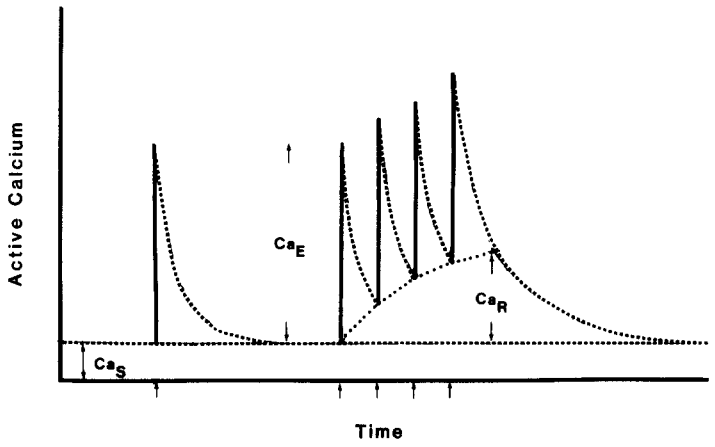

FIGURE 7. Hypothetical diagram of the relationships between steady state active calcium, Ca_S, active calcium entering during an action potential, Ca_E, and residual active calcium, Ca_R, after one presynaptic impulse and in a tetanus.

Ca_E could also be estimated from Eq. 6*a* in a somewhat less cumbersome way than the method described above. With $Ca_S = 1$, Eq. 6*a* can be rewritten as

$$Ca_E = (v_o/QTK)^{1/n} - 1. \tag{10}$$

Q was estimated from the average amplitude of MEJPs. At crayfish neuromuscular junctions, phasic transmitter release lasts ~8 ms at 3°C and 1 ms at 23°C (Zucker, 1973, and unpublished observations). We used $T = 3$ or 4 ms for 15°C. Then Eq. 10 could be used to determine Ca_E from the unfacilitated EJP amplitude, v_o, or Ca_E could be estimated by comparing Eqs. 8 and 9 as described above.

These procedures provided us with estimates for all the parameters in Eqs. 4, 6, and 7 that were appropriate to any one set of data. Except for n and Ca_E, these parameters and the decay of residual calcium were estimated entirely from the post-tetanic decay of MEJP frequency. These equations were then

used to predict the post-tetanic decays of EJP amplitude. One set of model predictions is shown as the solid line in Fig. 3. This simple residual-calcium model provided a qualitative, but not quantitative, prediction of post-tetanic EJP facilitation. Several points of similarity between the predicted and observed behaviors are evident. MEJP facilitation was much greater and decayed more rapidly than EJP facilitation, as predicted. However, the time course of EJP facilitation was not exactly that predicted by the model. In particular, a smaller value of Ca_E would be required to fit the late part of the EJP facilitation decay than that required for the early part.

DISCUSSION

Assumptions and Limitations in Our Model

To understand the probable reasons why the residual calcium model did not predict perfectly the relation between MEJP frequency and EJP amplitude, we need to explore two assumptions implicit in our formulation of the model: (*a*) spontaneous and evoked transmitter release, both resting and post-tetanic, are determined entirely by the concentration of active calcium at presynaptic release sites; (*b*) the relationship between transmitter release and active calcium can be represented by a simple power-law equation over the full range of active calcium concentration and transmitter release.

Regarding the first assumption, the temperature dependence (Barrett et al., 1978) and effects of low calcium concentration (Andreu and Barrett, 1980) on resting and post-tetanic EJP amplitude and MEJP frequency have led to the suggestion that tonic and phasic transmitter release are not controlled by exactly the same processes. In particular, it was suggested that a component of the resting MEJP frequency may be largely calcium independent.

If resting MEJP frequency is partly calcium independent, we should modify Eqs. 4 and 4*a* by adding a calcium-independent term, f_I, to their right sides and allowing Ca_S to vary. This results in Eq. 8 being modified also, with $f(t) - f_I$ replacing $f(t)$. Similarly, Eqs. 6 and 6*a* should be modified to include the term QTf_I on their right sides. The effect of adding this additional parameter is to make K an arbitrary scaling constant for the units of active calcium. We can set K to the same value as we determined by assuming that resting MEJP frequency was due entirely to Ca_S, with Ca_S set equal to one. Solving Eq. 4*a* for Ca_S yields $Ca_S = [(f_o - f_I)/K]^{1/n}$, and our estimate of Ca_S will be reduced. This, in turn, affects our estimates of Ca_E and Ca_R: both will be increased (see Eqs. 8 and 10).

To explore the full effects of the assumption that resting MEJP frequency is calcium dependent, we considered the extreme alternative that resting MEJP frequency is entirely independent of active calcium. This is equivalent to setting $f_I = f_o$, or $Ca_S = 0$. Using the modified version of Eq. 8 mentioned above, $Ca_R(t)$ was estimated anew from the decay of MEJP frequency, $f(t)$ (see Fig. 8B), and Eq. 6 modified as described was used to predict EJP facilitation (dashed line in Fig. 3). This version of the model described the post-tetanic decay of MEJP frequency just as well as the first version. It was

somewhat more successful in predicting the relation between the post-tetanic decays of MEJP frequency and EJP amplitude than the version that assumed a calcium-dependent resting MEJP frequency. Thus, the resting rate of transmitter release may well be determined largely by factors other than steady-state active calcium, as suggested by Barrett et al. (1978) and Andreu and Barrett (1980).

It is unlikely that resting MEJP frequency is entirely independent of intracellular calcium. Stimulation of the motor nerve in a zero-calcium solution, where the electrochemical gradient for calcium across the nerve

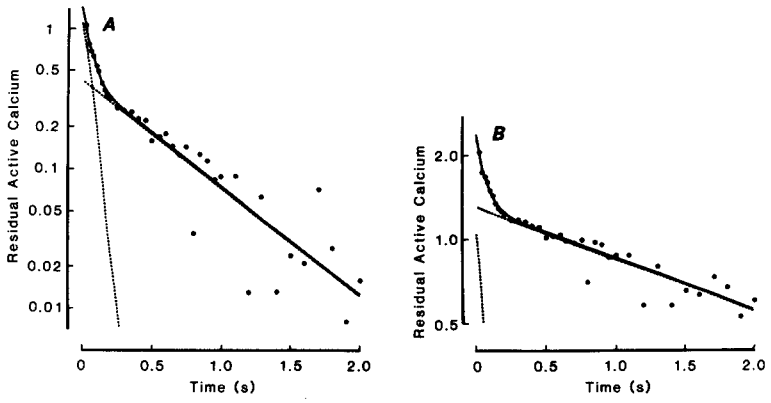

FIGURE 8. Calculation of residual active calcium, $Ca_R(t)$, from the post-tetanic decay of MEJP frequency, using the data of Fig. 2. (A) These points were calculated assuming that resting MEJP frequency is determined by the steady state level of active calcium, and Ca_S was set equal to one. The points were computed using Eq. 8, and two exponential components were fitted to the points, as represented by Eq. 7, with the following parameters: $A_1 = 1.078$, $A_2 = 0.425$, $\tau_1 = 50.6$ ms, $\tau_2 = 0.563$ s. The solid line is the sum of these two components. (B) MEJP is now assumed to be calcium independent, and Ca_S was set to zero. The points were computed using the modified form of Eq. 8 as described in the text, and these were fitted with the following two exponential components: $A_1 = 1.044$, $A_2 = 1.296$, $\tau_1 = 66.6$ ms, $\tau_2 = 2.35$ s. The solid line is the sum of these components. These calculations of residual calcium were used to generate the predictions of post-tetanic EJP facilitation plotted in Fig. 3.

membrane is reversed, should lead to an efflux of calcium and a reduction in intracellular calcium concentration. This treatment results in a reduction in resting MEJP frequency (Erulkar et al., 1978), which suggests that resting MEJP frequency is at least partially dependent on intracellular calcium. Thus, our best prediction of the decay of EJP facilitation would lie somewhere between the dashed and solid lines of Fig. 3.

In regard to the second assumption implicit in our model, it has often been reported that the relation between external calcium concentration and transmitter release is linear in crayfish (Bracho and Orkand, 1970; Ortiz and Bracho, 1972; Zucker, 1974; Staggs et al., 1980), although it has been pointed

out that saturation of calcium entry could mask a nonlinearity between active calcium and transmitter release (Parnas and Segel, 1981). Recently, more careful measurements of the relation between transmitter release and external calcium indicate that the relation is nonlinear (Dudel, 1981) and that it is in fact consistent with a fourth- or higher-power dependence of transmitter release on intracellular active calcium (Parnas et al., 1982). Nevertheless, it is not clear that Eq. 3 provides an accurate description of the calcium dependence of transmitter release, and a saturating Michaelis-Menten-type relationship has been proposed on both theoretical and experimental grounds (Dodge and Rahamimoff, 1967; Zucker, 1974; Parnas et al., 1982). This clearly would change the details of the expected relationship between MEJP frequency and EJP amplitude.

Another oversimplification in our use of Eq. 3 is that we tried only integral values of n in fitting our observations to a residual-calcium model. This is appropriate if we imagine that exactly n calcium ions must combine in a reaction leading to transmitter release. However, it is more likely that varying numbers of calcium ions participate in reactions leading to the release of a quantum of transmitter, entailing a much more flexible relationship between calcium and release (Hubbard et al., 1968). This would permit a closer fit between the model and our data.

Considering these limitations in our formulation of the residual-calcium model, it is surprising that the model describes the data as well as it does. Although the residual-calcium model is undoubtably oversimplified in the form we have presented it, we feel that the residual-calcium hypothesis remains the best explanation in general terms of MEJP and EJP facilitation.

Objections to the Residual-Calcium Hypothesis

A number of objections have been raised to the residual-calcium model of facilitation. For crayfish neuromuscular junctions, the most serious problem had been the apparent linear dependence of transmitter release on external calcium (Zucker, 1974, 1977). As mentioned above, recent measurements and theoretical considerations (Dudel, 1981; Parnas et al., 1982) have removed this objection.

Another problem, arising from work on frog neuromuscular junctions, is that EJP facilitation was reported to summate linearly (Mallart and Martin, 1967; Magleby, 1973*a*) in a way inconsistent with a simple residual-calcium model (Bittner and Schatz, 1981). However, recent and more careful measurements of the accumulation of facilitation in frogs suggests that facilitation does not summate linearly, but rather in a fashion consistent with a residual-calcium model and power-law relation between transmitter release and active calcium (Zengel and Magleby, 1982; see also Younkin, 1974). Similarly, in crayfish, the accumulation of facilitation in a train was described better, although still imperfectly, by a power-law residual-calcium model than by a linear-summation model (Zucker, 1974; Bittner and Sewell, 1976; Parnas et al., 1982).

Magleby (1973*b*) and Zengel and Magleby (1980) have suggested that the

kinetic behavior of the various components of increased transmitter release at frog neuromuscular junctions (slow and fast facilitation, augmentation, and potentiation) is difficult to reconcile with a simple residual-calcium hypothesis. This is particularly true because the different components appear to behave independently in their accumulation in long tetani and to interact in a complex fashion rather than simply summate, and because facilitation is specifically enhanced by strontium and augmentation by barium.

We do not regard these properties as fundamentally inconsistent with a residual-calcium hypothesis of facilitated transmitter release. The different kinetic components may reflect different mechanisms for the removal of residual active calcium from release sites (diffusion, active extrusion, uptake) or different sources for the residual calcium (calcium influx in facilitation and augmentation, release from intracellular stores in potentiation). Even one process alone, such as diffusion, can lead to a multi-exponential decay (Zucker and Stockbridge, 1983). The different ionic sensitivities of facilitation, augmentation, and potentiation may reflect differential effects of strontium and barium ions on several processes responsible for the decay of residual calcium. The nonadditive interaction of potentiation with facilitation and augmentation (Magleby, 1973*b*; Magleby and Zengel, 1982) does suggest that potentiation reflects more than simply the effect of residual calcium summating with entering calcium in eliciting transmitter release. Nevertheless, this interaction does not contradict the idea that residual calcium is the major process responsible for fast and slow phases of facilitation (and augmentation), and a process contributing to potentiation.

The recent results of Zengel and Magleby (1981), in which a residual-calcium model failed to account for the relation between post-tetanic MEJP frequency and EJP facilitation, certainly indicate that a simple residual-calcium hypothesis, where residual calcium sums with entering calcium to release transmitter in a power-law fashion, cannot account for all the effects of repetitive stimulation on enhanced transmitter release. Possible additional effects are (*a*) a change in calcium influx in late tetanic and post-tetanic action potentials, especially since changes in nerve terminal potentials have been observed in long tetani (Lev-Tov and Rahamimoff, 1980); (*b*) fatigue or exhaustion of the release process, possibly but not necessarily caused by depletion of transmitter stores, and affecting phasic and tonic release differently; (*c*) differential effects of accumulated presynaptic intracellular sodium or magnesium on phasic and tonic release; and (*d*) a secondary effect of residual calcium on neurosecretion, beyond simple summation with entering calcium in releasing transmitter. These processes would not be expected to play a significant role in short-term facilitation, which may be why, when facilitation dominates as it does in our experiments and those of Parnas et al. (1982) on crayfish, as well as those of Barrett and Stevens (1972) on frogs, a residual-calcium model is more successful in explaining the results.

We are indebted to Dr. K. L. Magleby for thoughtful suggestions and criticisms of an earlier draft of the manuscript. This work was supported by National Institutes of Health grant NS

15114. L.O.L.-E. was supported by grants from CONICIT of Venezuela and the Universidad Simón Bolivar in Caracas.

Received for publication 22 August 1982 and in revised form 4 October 1982.

REFERENCES

Andreu, R., and E. F. Barrett. 1980. Calcium dependence of evoked transmitter release at very low quantal contents at the frog neuromuscular junction. *J. Physiol. (Lond.)*. 308:79–97.

Barrett, E. F., J. N. Barrett, D. Botz, D. B. Chang, and D. Mahaffey. 1978. Temperature-sensitive aspects of evoked and spontaneous transmitter release at the frog neuromuscular junction. *J. Physiol. (Lond.)*. 279:253–273.

Barrett, E. F., and C. F. Stevens. 1972. The kinetics of transmitter release at the frog neuromuscular junction. *J. Physiol. (Lond.)*. 227:691–708.

Bittner, G. D., and V. L. Sewell. 1976. Facilitation at crayfish neuromuscular junctions. *J. Comp. Physiol.* 109:287–308.

Bittner, G. D., and R. A. Schatz. 1981. An examination of the residual calcium theory for facilitation of transmitter release. *Brain Res.* 210:431–436.

Bracho, H., and R. K. Orkand. 1970. Effect of calcium on excitatory neuromuscular transmission in the crayfish. *J. Physiol. (Lond.)*. 206:61–71.

Charlton, M. P., and G. D. Bittner. 1978. Presynaptic potentials and facilitation of transmitter release in the squid giant synapse. *J. Gen. Physiol.* 72:487–511.

Charlton, M. P., S. J. Smith, and R. S. Zucker. 1982. Role of presynaptic calcium ions and channels in synaptic facilitation and depression at the squid giant synapse. *J. Physiol. (Lond.)*. 323:173–193.

Dodge, F. A., Jr., and R. Rahamimoff. 1967. Co-operative action of calcium ions in transmitter release at the neuromuscular junction. *J. Physiol. (Lond.)*. 193:419–432.

Dudel, J. 1981. The effect of reduced calcium on quantal unit current and release at the crayfish neuromuscular junction. *Pflügers Arch. Eur. J. Physiol.* 391:35–40.

Dudel, J., and S. W. Kuffler. 1961. The quantal nature of transmission and spontaneous miniature potentials at the crayfish neuromuscular junction. *J. Physiol. (Lond.)*. 155:514–529.

Erulkar, S. D., and R. Rahamimoff, 1978. The role of calcium ions in tetanic and post-tetanic increase of miniature end-plate potential frequency. *J. Physiol. (Lond.)*. 278:501–511.

Erulkar, S. D., R. Rahamimoff, and S. Rotshenker. 1978. Quelling of spontaneous transmitter release by nerve impulses in low extracellular calcium solutions. *J. Physiol. (Lond.)*. 278:491–500.

Fatt, P., and B. Katz. 1953. Distributed 'end-plate potentials' of crustacean muscle fibres. *J. Exp. Biol.* 30:433–439.

Hubbard, J. I., S. F. Jones, and E. M. Landau. 1968. On the mechanism by which calcium and magnesium affect the release of transmitter by nerve impulses. *J. Physiol. (Lond.)*. 196:75–86.

Katz, B., and R. Miledi. 1968. The role of calcium in neuromuscular facilitation. *J. Physiol. (Lond.)*. 195:481–492.

Katz, B., and S. Thesleff. 1957. On the factors which determine the amplitude of the 'minature end-plate potential.' *J. Physiol. (Lond.)*. 137:267–278.

Lev-Tov, A., and R. Rahamimoff. 1980. A study of tetanic and post-tetanic potentiation of miniature end-plate potentials at the frog neuromuscular junction. *J. Physiol. (Lond.)*. 309:247–273.

Liley, A. W. 1956. The effects of presynaptic polarization on the spontaneous activity at the mammalian neuromuscular junction. *J. Physiol. (Lond.)*. 134:427–443.

Llinás, R., I. Z. Steinberg, and K. Walton. 1981. Relationship between presynaptic calcium current and postsynaptic potential in squid giant synapse. *Biophys. J.* 33:323–352.

Magleby, K. L. 1973*a*. The effect of repetitive stimulation on facilitation of transmitter release at the frog neuromuscular junction. *J. Physiol. (Lond.)*. 234:327–352.

Magleby, K. L. 1973*b*. The effect of tetanic and post-tetanic potentiation on facilitation of transmitter release at the frog neuromuscular junction. *J. Physiol. (Lond.)*. 234:353–371.

Magleby, K. L. 1979. Facilitation, augmentation, and potentiation of transmitter release. *Prog. Brain Res.* 49:175–182.

Magleby, K. L., and J. E. Zengel. 1982. A quantitative description of stimulation-induced changes in transmitter release at the frog neuromuscular junction. *J. Gen. Physiol.* 80:613–638.

Mallart, A., and A. R. Martin. 1967. An analysis of facilitation of transmitter release at the neuromuscular junction of the frog. *J. Physiol. (Lond.)*. 193:679–694.

Miledi, R., and R. Thies. 1971. Tetanic and post-tetanic rise in frequency of miniature end-plate potentials in low-calcium solutions. *J. Physiol. (Lond.)*. 212:245–257.

Ortiz, C. L., and H. Bracho. 1972. Effect of reduced calcium on excitatory transmitter release at the crayfish neuromuscular junction. *Comp. Biochem. Physiol.* 41:805–812.

Parnas, H., J. Dudel, and I. Parnas. 1982. Neurotransmitter release and its facilitation in crayfish. I. Saturation kinetics of release, and of entry and removal of calcium. *Pflügers Arch. Eur. J. Physiol.* 393:1–14.

Parnas, H., and L. A. Segel. 1981. A theoretical study of calcium entry in nerve terminals, with application to neurotransmitter release. *J. Theor. Biol.* 91:125–169.

Rahamimoff, R. 1968. A dual effect of calcium on neuromuscular facilitation. *J. Physiol. (Lond.)*. 195:471–480.

Rahamimoff, R., and Y. Yaari. 1973. Delayed release of transmitter at the frog neuromuscular junction. *J. Physiol. (Lond.)*. 228:241–257.

Sherman, R. G., and H. L. Atwood. 1971. Synaptic facilitation: long-term neuromuscular facilitation in crustaceans. *Science (Wash. DC)*. 171:1248–1250.

Staggs, D. R., E. Pofcher, R. L'Heureux, C. L. Ortiz, and R. K. Orkand. 1980. Excitatory neuromuscular transmission in crayfish: calcium dependence is unaffected by picrotoxin. *J. Neurobiol.* 11:629–632.

Younkin, S. G. 1974. An analysis of the role of calcium in facilitation at the frog neuromuscular junction. *J. Physiol. (Lond.)*. 237:1–14.

Zengel, J. E., and K. L. Magleby. 1980. Differential effects of Ba^{2+}, Sr^{2+}, and Ca^{2+} on stimulation-induced changes in transmitter release at the frog neuromuscular junction. *J. Gen. Physiol.* 76:175–211.

Zengel, J. E., and K. L. Magleby. 1981. Changes in miniature endplate potential frequency during repetitive nerve stimulation in the presence of Ca^{2+}, Ba^{2+}, and Sr^{2+} at the frog neuromuscular junction. *J. Gen. Physiol.* 77:503–529.

Zengel, J. E., and K. L. Magleby. 1982. Augmentation and facilitation of transmitter release: a quantitative description at the frog neuromuscular junction. *J. Gen. Physiol.* 80:583–611.

Zucker, R. S. 1973. Changes in the statistics of transmitter release during facilitation. *J. Physiol. (Lond.)*. 229:787–810.

Zucker, R. S. 1974. Characteristics of crayfish neuromuscular facilitation and their calcium dependence. *J. Physiol. (Lond.)*. 241:91–110.

Zucker, R. S. 1977. Synaptic plasticity at crayfish neuromuscular junctions. *In* Identified Neurons and Behavior of Arthropods. G. Hoyle, editor. Plenum Publishing Corp., New York. 49–65.

Zucker, R. S., and N. Stockbridge. 1983. Presynaptic calcium diffusion and the time courses of transmitter release and synaptic facilitation at the squid giant synapse. *J. Neurosci.* In press.