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THE KINETICS OF TRANSMITTER RELEASE AT THE FROG NEUROMUSCULAR JUNCTION

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SUMMARY

1. Fluctuations in the latency of focally recorded end-plate currents were analysed to determine the time course of the probabilistic presynaptic process underlying quantal release evoked after single nerve stimuli at the frog neuromuscular junction.

2. The early falling phase of the presynaptic probability function can be fitted by a single exponential over two orders of magnitude of quantal release rate. The time constant of the early falling phase is about 0·5 msec at 11° C, and increases with decreasing temperature with a Q_{10} of at least 4 over the range 1–12° C.

3. After this early exponential fall, quantal release probability returns to control levels with a much slower time course.

4. Conditioning nerve stimuli increase the magnitude and slightly prolong the early time course of release evoked by a test stimulus. When facilitation is calculated for matched time intervals following the conditioning and testing stimuli, it is found that the magnitude of the small, late residual tail of release is facilitated by a greater percentage than the magnitude of larger, early portions of release.

5. These results are discussed in terms of the hypothesis (Katz & Miledi, 1968) that evoked release and facilitation are mediated by a common presynaptic factor which activates release in a non-linear manner.

INTRODUCTION

At the neuromuscular junction transmitter quanta are released with a variable delay following an action potential in the motor nerve terminal; the major portion of this delay, as well as its variability, originates presynaptically (Katz & Miledi, 1965*b*, *c*). Analysis demonstrates that the fluctuations in evoked quantal latency during steady-state stimulation

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reflect a unique probabilistic process, uniform in magnitude and time course after each stimulus presentation (Barrett & Stevens, 1972). The uniqueness of this process makes it possible to use a histogram of quantal latencies measured after many stimulus presentations to calculate the time course of the probabilistic process that underlies release on each single trial. The work presented here uses quantal latency fluctuations to measure presynaptic release kinetics at the frog neuromuscular junction, and to determine how temperature and conditioning nerve stimulation alter these kinetics.

METHODS

The experimental preparation, bathing solutions and focal extracellular recording techniques are described in the preceding paper (Barrett & Stevens, 1972). Alternating single and paired suprathreshold pulses were delivered to the motor nerve at 1–4 sec intervals, or as noted. Data series with average evoked quantal contents between 0·5 and 3 were selected for analysis.

Quantal latency, the interval between a pulse triggering nerve stimulation and the onset of the quantal current, was measured from a computer display of the tape-recorded voltage records. The latency of the first quantum released on a trial was measured to within $\pm 0{\cdot}05$ or $\pm 0{\cdot}10$ msec, depending on the sampling interval used. Latency thus includes both a fixed stimulation-nerve conduction time and a variable interval between nerve terminal depolarization and quantal release (the synaptic delay, Katz & Miledi, 1965*a*, *b*, *c*). The analysis of release kinetics described below requires only the relative magnitude of the latency fluctuations, not the absolute magnitude of each synaptic delay.

For each data series a post-stimulus early release period (ERP) was defined, which began with the first distinguishable increase in quantal release rate and extended until the probability of a first quantal release (see below) became very low. The ERPs defined following paired conditioning and testing stimuli were of equal duration. ERPs ranged between 3 msec (at 12° C) and 20 msec (at 1° C).

Stability criteria

The work described here assumes that analysis of quantal latency fluctuations collected from a *large* series of trials can yield accurate information concerning the probabilistic presynaptic process underlying release on each *single* trial. This assumption is valid if post-stimulus release probability is uniform in magnitude and time course from trial to trial. Two criteria were used to confirm the invariance of the presynaptic probability function: (1) each series of end-plate currents was checked for stability in time of mean quantal content (estimated from the ratio of total trials to failures) and median first quantal latency. Data blocks showing substantial drift were rejected; (2) stable data blocks were tested for quantal independence and for uniqueness of the post-stimulus release function as described in Barrett & Stevens (1972). Only the early portions of evoked release were tested. Later post-stimulus release rates always increased noticeably during the 15–30 min recording session.

Estimating release kinetics

Katz & Miledi (1965*b*, *c*) used a histogram of quantal latencies measured after many stimulus presentations to approximate the early portion of the presynaptic probability of release function $\alpha(t)$. If quanta are released independently of each other, this method of obtaining $\alpha(t)$ is theoretically sound, but demands accurate

measurement of *each* quantal latency. Failure to distinguish simultaneously released quanta will lead to an underestimate of peak release rates (Katz & Miledi, 1965*b*). In addition, even when quantal releases are not synchronous it is often difficult to measure the exact latencies of later quanta. Because of the likelihood of latency measurement errors during multiquantal responses, Katz & Miledi's histogram method measures early release kinetics most accurately when the average quantal content of evoked release is low ($m \leqslant 0{\cdot}5$).

To avoid errors due to quantal overlap and thus to extend the range of quantal contents over which release kinetics can be accurately estimated, we calculated $\alpha(t)$ from a histogram of *first* quantal latencies as follows (see also Stevens, 1968): let $s(t)$ represent the probability of a *first* quantal latency occurring in an interval about t, and let $S(t)$ denote the probability of a first latency occurring between t_1, the beginning of the ERP, and t:

$$S(t) = \int_{t_1}^{t} s(\tau)\,\mathrm{d}\tau.$$

Then $(1-S(t))$ gives the probability of a first latency longer than t, that is, the probability that no release has occurred up to time t. Let $\alpha(t)$ represent the probability of *any* quantal release occurring in an interval about t ($\alpha(t)$ is assumed to be a unique function, invariant from trial to trial). From these definitions it follows that

$$s(t) = (1-S(t))\,\alpha(t)$$

which on arrangement yields

$$\alpha(t) = \frac{s(t)}{1-S(t)}. \tag{1}$$

Both $s(t)$ and $S(t)$ were obtained directly from a histogram of first quantal latencies (see Fig. 1*A*).

Data series in which an average of 0·5–3 quanta were released during the ERP were most amenable to kinetic analysis using eqn. (1). When the average quantal content was lower than 0·5 it was difficult to collect enough stable first latency data, and when the average quantal content exceeded 3 the denominator of eqn. (1) became too small toward the end of the ERP. Calculations of $\alpha(t)$ were based on 130–490 (average 315) first quantal latencies. Calculation intervals during peak release were 0·1–0·3 msec (the shorter intervals were used at higher temperatures). Intervals were lengthened at late times as release rates fell.

During the later phases of evoked release, when release rates were low (minimizing the occurrence of quantal overlap) and slowly changing (reducing the required accuracy of latency measurements), release kinetics were estimated from a histogram of all quantal latencies.

In cases of apparent interaction among quantal releases (e.g. Fig. 6*B* of Barrett & Stevens, 1972) the probability of evoked quantal release will not be uniform on every trial, but will change after each quantal release. The first quantal latencies used in calculating $\alpha(t)$ are not affected by intratrial interactions or depletion, so in cases of quantal non-independence equation (1) estimates the probability function underlying release on each trial up to the time that the first quantum is released. Thus $\alpha(t)$ can be used as a 'base line' release curve in calculating the effect of various simulated quantal interactions (Appendix II, Barrett & Stevens, 1972). When the number of releasable quanta (n) is small, eqn. (1) can still be used to estimate the probability function acting on a single quantum ($\alpha_1(t)$), since $\alpha(t)$ equals $n\alpha_1(t)$. It is important to note that, for small n, in contrast to the method based on eqn. (1), the technique of estimating $\alpha(t)$ from all quantal release times (Katz & Miledi, 1965*b*, *c*) provides differing results depending on the number and distribution of the releases because n varies significantly over the release period.

RESULTS

Early release

Sample end-plate currents are illustrated in Fig. 2 of Barrett & Stevens (1972).

The shaded portion of Fig. 1*A* plots the early time course of the probability of a *first* quantal release for an extracellular synaptic region cooled to 1° C. The continuous lines depict the presynaptic probability of release function $\alpha(t)$ calculated from this first quantal latency data according to eqn. (1). The calculated release function shows the same general features described earlier by Katz & Miledi (1965*b*, *c*): a fairly rapid rise to peak release rate, followed by a slower return to control levels of release. The spontaneous release rate, estimated during intervals just prior to motor nerve stimulation, was about 0·1/sec, too low to affect the shape of the early evoked release function significantly.

Exponential decay

In Fig. 1*B*, $\alpha(t)$ is replotted on semilogarithmic coordinates. The falling phase of the curve is reasonably linear over the two orders of magnitude of release rate illustrated here. Point scatter can be attributed to the probabilistic nature of quantal release. That the falling phase of a semilogarithmic plot of $\alpha(t)$ can be fitted by a straight line suggests that a single exponential process underlies the early falling phase of post-stimulus release probability.

Probability of release curves similar to that of Fig. 1*A* were calculated using eqn. (1) for a total of twenty-six series of end-plate currents, recorded at temperatures between 1 and 12° C. At some synaptic regions up to six series were collected as temperature and/or stimulation parameters were varied. The early falling phase of evoked release probability did not show 'humps' at regular intervals suggestive of the release cycling postulated by Martin & Veale (1967). In twenty-three of the twenty-six series semilogarithmic plots of $\alpha(t)$ showed early falling phases with point scatter small enough to allow time constant estimation. In cases where data from the same synaptic region were analysed in two parts because of latency drifts, the time constant of the early falling phase for the first half of the data was usually slightly smaller than the time constant for the data collected later, although the difference in time constants was always within measurement error.

Temperature dependence

As reported by Katz & Miledi (1965*c*), release kinetics show a steep dependence on temperature. Semilogarithmic plots of $\alpha(t)$ calculated for

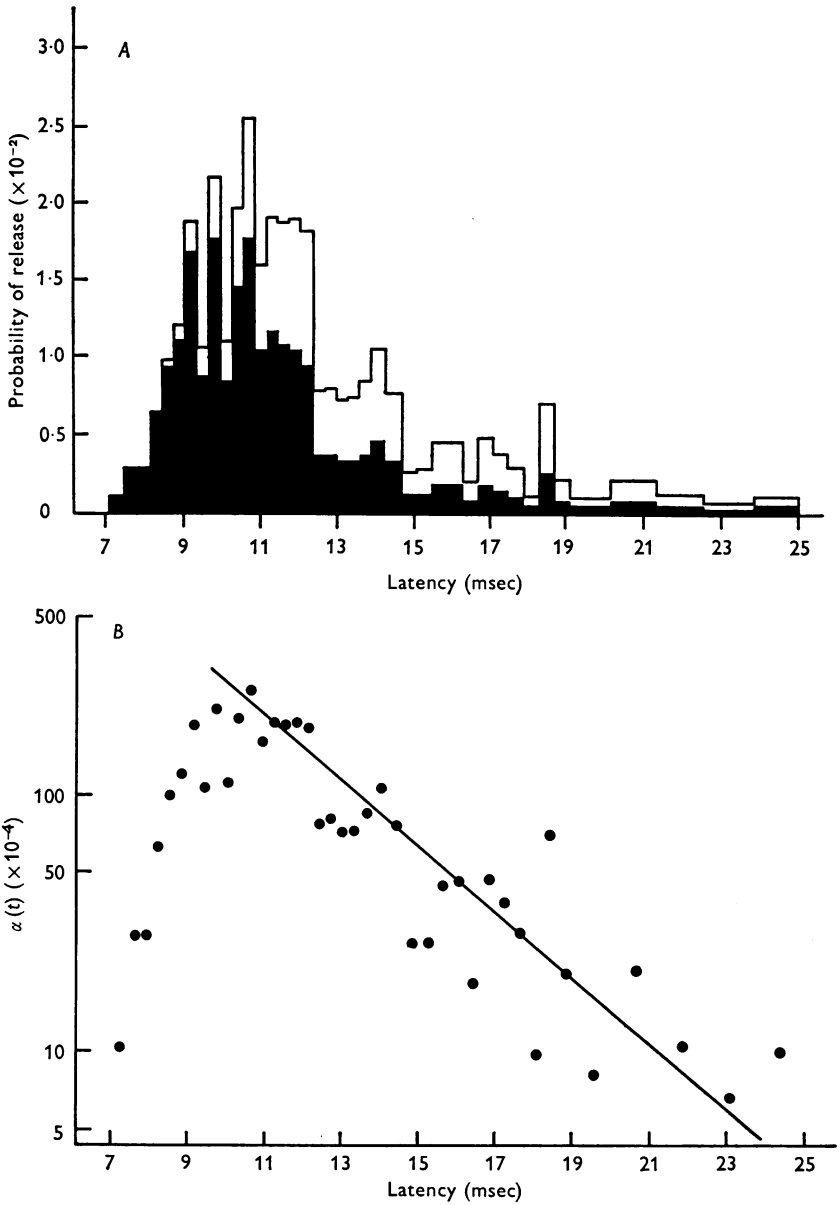

Fig. 1. *A*, shaded histogram indicates the probability of a *first* quantal release as a function of time after motor nerve stimulation for a series of 482 first quantal latencies recorded in 712 total trials (230 response failures) at 1° C. Continuous lines plot $\alpha(t)$ calculated for these data using eqn. (1). Ordinate is release probability per 0·1 msec. Stimulation rate 0·25/sec. *B*, semilogarithmic plot of $\alpha(t)$ from Fig. 1*A*. Line was fitted by eye to points after peak release, giving an estimated time constant of 3·5 msec for the early falling phase.

end-plate currents recorded at four different temperatures (Fig. 2) demonstrate that the early falling phase remains exponential over the illustrated 10° C range, and that the decay constant decreases with increasing temperature. It was difficult to collect sufficient stable data from a single extracellular synaptic region at more than two temperatures, but data from both the same and different regions (circles, Fig. 3) suggest a Q_{10} of at least 4 for the decay constant of early release.

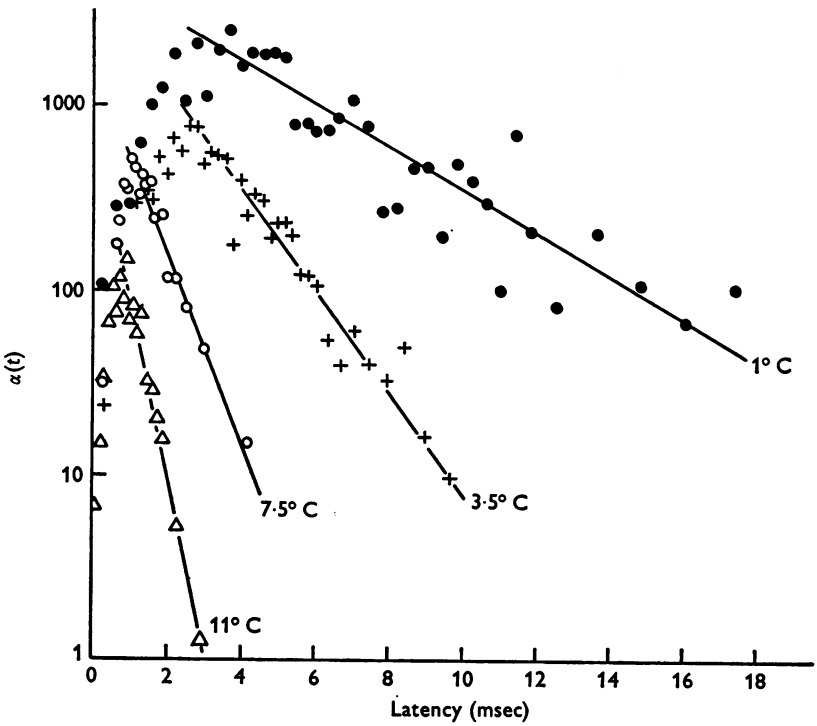

Fig. 2. Semilogarithmic plot of $\alpha(t)$ for early release in four series of end-plate currents recorded at different myoneural junctions at the indicated temperatures. $\alpha(t)$ was calculated from histograms of first quantal latencies using eqn. (1). The early release period (ERP) for each series begins at zero time. The ordinate is relative: data series were shifted vertically for better visibility. Lines were fitted by eye to points after peak release. Filled circles from data of Fig. 1, 1° C; crosses, 3·5° C; open circles, 7·5° C; triangles, 11° C. Estimated time constants for the early falling phase of $\alpha(t)$ were (in msec) 3·5 (1° C), 1·8 (3·5° C), 0·90 (7·5° C) and 0·45 (11° C).

Probability of release functions obtained from histograms of all quantal latencies (see Methods) are presented in Katz & Miledi (1965*b*, *c*; 1967*a*), Miledi (1966) and Betz (1969). When the more detailed histograms from these sources are replotted on semilogarithmic coordinates, their falling

phases are also linear. Time constants estimated from these semilogarithmic plots are denoted by crosses in Fig. 3.

The rising phase of $\alpha(t)$ is also faster at higher temperatures (Fig. 2), but was not analysed further because, for all but the lowest temperatures, it is rapid compared to the accuracy of the latency measurements.

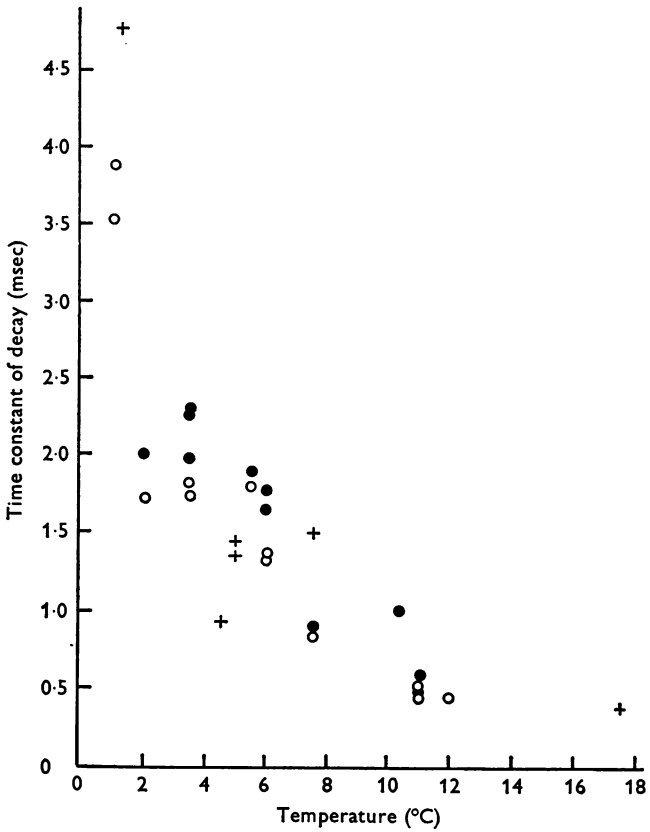

Fig. 3. Temperature dependence of the time constant of the early falling phase of $\alpha(t)$, calculated from first latencies using eqn. (1). Open circles indicate decay constants following conditioning (control) pulses; filled circles, decay constants following test (facilitated) pulses. Circles at the same temperature were obtained from the same synaptic region. In addition, time constants at 2, 7·5 and 12° C were obtained from one region; those at 5·5 and 10·3° C from another region. Crosses show decay constants estimated from latency histograms published in Betz (1969, Fig. 30, 1·2° C), Katz & Miledi (1965*b*, Fig. 4, 17·5° C; 1965*c*, Fig. 3, 7·5° C; 1967*a*, Fig. 8, 4·5° C) and Miledi (1966, Fig. 2*a*, *b*, 5° C). Decay constants seem to show wide variation at very low temperatures. Data recorded at 2·5° C by Katz & Miledi (1965*c*, Fig. 3) and at 0·5° C by Betz (1969, Fig. 28) had time constants of about 8·4 and 8·3 msec, respectively.

Effect of conditioning stimuli

In several experiments paired stimuli separated by intervals of 30–140 msec were applied to the motor nerve to assess the effect of conditioning stimulation on early release kinetics. The conditioning stimulus increased the overall magnitude of release evoked by the test stimulus (facilitation). Semilogarithmic plots of the early falling phase of $\alpha(t)$ calculated for both

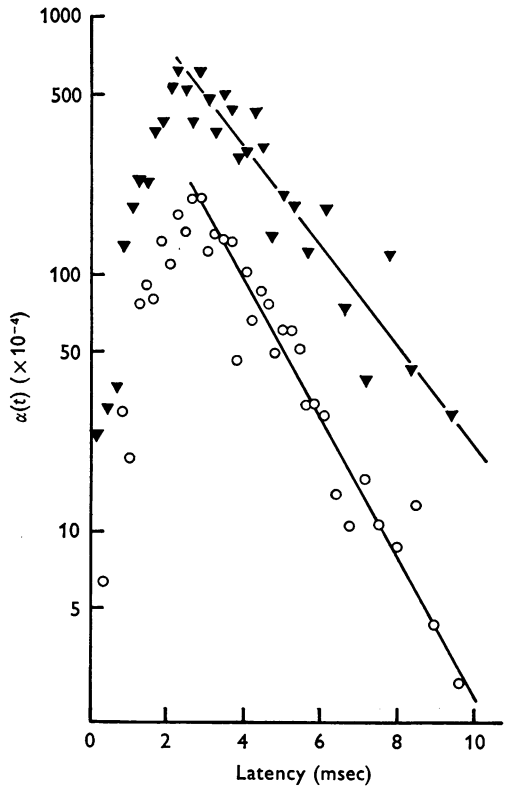

Fig. 4. Semilogarithmic plot of $\alpha(t)$ (eqn. (1)) calculated for the first (circles) and fourth (triangles) stimuli in a train. Lines were fitted to falling phases by eye; estimated decay constants were 1·8 msec (first pulse) and 2·3 msec (fourth pulse). Average quantal contents were 0·54 for the first pulse; 2·0 for the fourth pulse. Nerve stimulated at 4 sec intervals with a train of 4 pulses at 10/sec. Ordinate is log release probability per 0·1 msec. Latency (msec) is measured from the start of the 10 msec ERP. 3·5° C.

control and test release were linear. Fig. 3 shows that at any given temperature the decay constant for test release (filled circles) was slightly longer than that for control release (open circles), but the difference was small, considering the error involved in time constant estimation.

In one preparation in which the nerve terminal action currents were large the minimal synaptic delay measured for both control and test release was 1·4 msec (11° C, 70 msec conditioning-testing interval).

To investigate the effect of several conditioning stimuli on early release kinetics a four-pulse stimulus train was applied to the motor nerve at 4 sec intervals. When the intratrain stimulus frequency was 5/sec the time constant of the early falling phase of release was 1·75 msec for the first (control) stimulus, 2·0 msec for the fourth. When the intratrain stimulus frequency was increased to 10/sec the early decay constant was 1·8 msec after the first (control) stimulus and 2·3 msec after the fourth (Fig. 4). In both cases the facilitated decay constant was slightly greater than the control decay constant.

Late release

Quantal release rates remain above control levels for a period extending far beyond the early exponential decay discussed above. Quantitative analysis of the late decay of release after a single nerve stimulus is difficult because late release rates are orders of magnitude lower than early release rates, and because late release decays slowly. At the lower temperatures employed here, repetitive stimulation at rates as low as 0·25/sec for 15–20 min consistently brings cumulative facilitation of late release, even if the magnitude and time course of early release show no significant change.

Fig. 5*A* is a semilogarithmic plot of the late decay of release probability at the synaptic region whose early release kinetics are illustrated in Fig. 1. The early decay constant for this region was about 3·5 msec (Fig. 1*B*). The later phases of release shown in Fig. 5*A* could be fitted well by the sum of two exponentials with time constants of 15–20 msec and 230 msec. Although the late evoked release rate became indistinguishable from the background release rate after 400 msec, the effects of nerve stimulation persisted for a much longer time, as evidenced by a cumulative rise in the background release rate (estimated in a 35 msec interval preceding nerve stimulation) from 0·07/sec in the first half of the experiment to 0·17/sec in the second half.

Data from other synaptic regions confirmed the existence of a late, slow falling phase of release probability after the early exponential decay, but few of these later falling phases were well fitted by exponentials. In all cases, however, the rate of decay of release probability became progressively slower with time, as in Fig. 5*A*.

Differential facilitation of late release

Conditioning stimulation that increases the magnitude of early release to a test stimulus (e.g. Fig. 4) also increases the magnitude of the later phases of test release. In fact, if facilitation is defined as the ratio of release

rates in matched intervals following the test and control stimuli, conditioning stimulation facilitates late release more than early release. The later the release interval considered, the greater is the observed facilitation. For

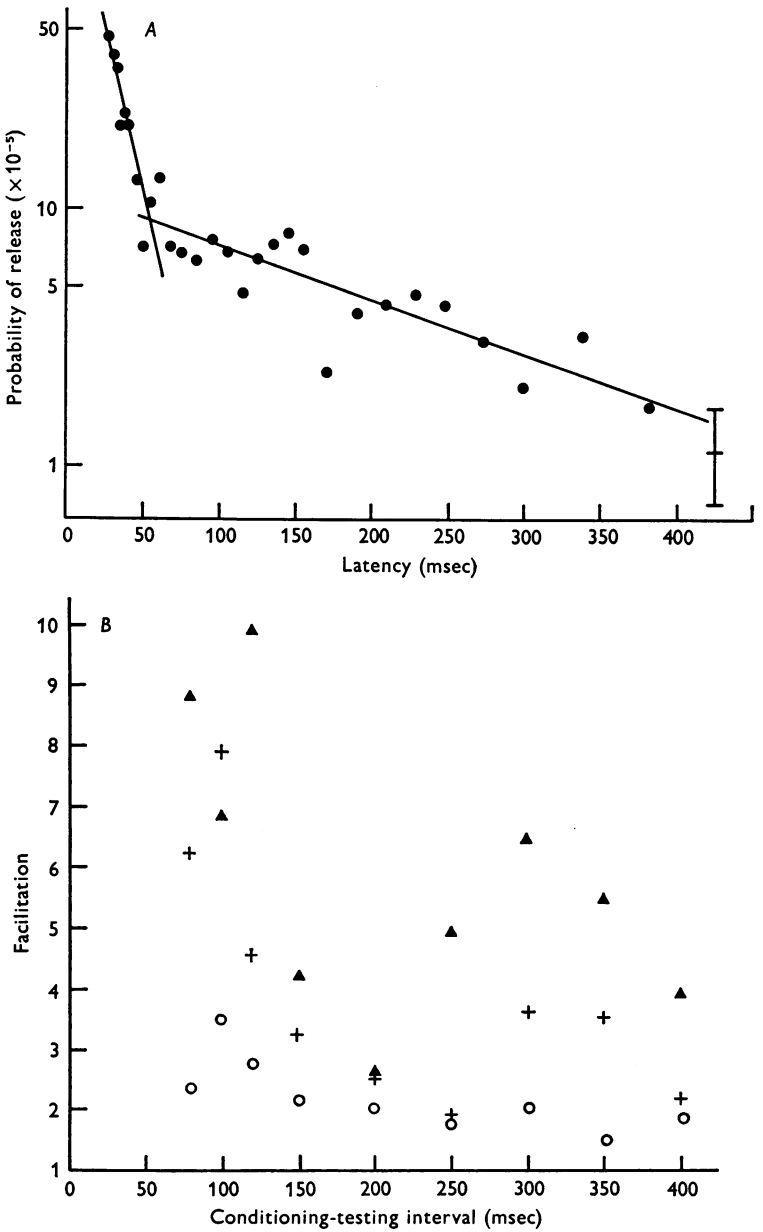

Fig. 5. For legend see facing page.

example, in one experiment (2° C, 140 msec conditioning-testing interval), the facilitation of release occurring during the 9·5 msec ERP was 1·9, but rose to 4·3 and 6·0 during two subsequent matched 12·5 msec intervals. Another example of this differential facilitation of late release is illustrated in Fig. 5*B* for the synaptic region whose early and late release kinetics are plotted in Figs. 1 and 5*A*, respectively. At a given interval between the conditioning and testing stimuli, the later release occurring 25–75 msec after stimulation (denoted by crosses and triangles) shows significantly more facilitation than the early release occurring within the 20 msec ERP (open circles).

Since late release rates are so low, it was not possible to determine the effect of conditioning stimulation on the time course of late release.

DISCUSSION

Possible mechanisms for the multiphasic decay of evoked release probability

For purposes of discussion the post-stimulus probability of release function can be divided into early and late portions. The early portion, consisting of the rising phase, peak and early exponential fall, accounts for the bulk of the end-plate current and is essentially identical after each nerve stimulus (Barrett & Stevens, 1972). The late, small, slowly-decaying portion is probably analogous to the late 'tails' of elevated quantal release rate mentioned by previous investigators (frog neuromuscular junction,

Fig. 5. Time courses of late release and facilitation at the synaptic region whose early release kinetics are illustrated in Fig. 1 (1° C). *A*, semilogarithmic plot of probability of quantal release (per 0·1 msec) between 25 and 400 msec after nerve stimulation. Each point was obtained by counting all the quanta released in a given time interval following at least 900 nerve stimuli. Lines fitted by eye had time constants of about 15–20 msec and 230 msec. Bar at right indicates the range of background release rates (see text). *B*, facilitation of release to a test stimulus introduced at intervals of 80–400 msec after a control stimulus. Facilitation of early release (circles) was measured as the ratio of the average summed amplitudes of all quanta released in 20 msec ERPs following the test and control stimuli. Facilitation of late release was measured as the ratio of quanta counted in subsequent 15 msec (crosses) and 35 msec (triangles) intervals following the test and control stimuli. Each point represents at least 60 (usually 90–100) conditioning-testing response pairs. Conditioning-testing intervals were presented in the following order (msec): 200, 150, 250, 300, 350, 120, 400, 100, 80. The wide scatter in late facilitation measurements is probably due to (1) random statistical fluctuations, significant at low release rates, and (2) cumulative effects. Intervals presented earlier in the experiment (150, 200, 250) showed less late facilitation than intervals presented later (100, 120, 300, 350). The nerve terminal may have been partially refractory at the 80 msec interval (Katz & Miledi, 1968).

Katz & Miledi (1965*b*, 1967*a*), Miledi (1966), Betz (1969); rat neuromuscular junction, Hubbard (1963); group Ia afferent synapses on cat motoneurones, Kuno (1964)). We detected elevated quantal release rates as late as 400 msec after a single stimulus to the nerve at 1° C, but it is likely that residual release persists even longer, because both late evoked release and 'background' release showed cumulative facilitation at stimulation rates as low as 0·25–1/sec. The early and late portions of evoked release probability discussed here share features of the early phasic and late residual phases of 'releasing factor' formation postulated by Miledi & Thies (1971).

One possible explanation for the multiphasic decay of post-stimulus release probability is that early and late release are mediated by different presynaptic mechanisms. If, as suggested by Miledi & Thies (1971), the increase in the frequency of miniature end-plate potentials observed during repetitive nerve stimulation results from a cumulation of residual post-stimulus tails, then it appears that early and late release are differentially sensitive to the external concentrations of Ca, Mg and Sr ions. Early release is strongly dependent on the external Ca concentration and is inhibited by Mg (del Castillo & Engbaek, 1954; Katz & Miledi, 1967*b*; Dodge & Rahamimoff, 1967), but the increase in miniature end-plate potential frequency during repetitive stimulation persists in low-Ca solutions and is not inhibited by Mg (Miledi & Thies, 1971; Hurlbut, Longenecker & Mauro, 1971). Sr activates early release less effectively than Ca but increases the late tails of release (Miledi, 1966; Dodge, Miledi & Rahamimoff, 1969; Meiri & Rahamimoff, 1971). However, since so little is known about the concentration of these cations at the presynaptic releasing sites, it is difficult to assess whether these different sensitivities to external divalent ions indicate fundamentally different presynaptic mechanisms for early and late release. For the present it seems more profitable to attempt to explain early and late phases of post-stimulus release in terms of a common presynaptic mechanism.

Several lines of evidence suggest that this 'common presynaptic mechanism' involves Ca ion (reviewed in Katz, 1969, pp. 33–35). According to the calcium hypothesis formulated by Katz & Miledi (1968, 1970), depolarization increases the calcium conductance of the nerve terminal membranes, and the incoming Ca ions contribute to as-yet unknown presynaptic reactions which transiently increase the probability of quantal release. Data reported by Dodge & Rahamimoff (1967) indicate that the relationship between release and the external concentration of Ca ion is non-linear. These investigators suggested that release is proportional to the fourth power of the concentration of some presynaptic Ca-receptor complex (CaX).

If both early and late phases of depolarization-evoked release are mediated by CaX, then the monotonic, multiphasic decay of release probability described here could reflect (1) the declining calcium conductance of the presynaptic nerve terminal, (2) inactivation of CaX and/or (3) dissociation of the CaX complex as ionized Ca is bound or sequestered. The first alternative seems unlikely in view of Katz & Miledi's (1967*c*) demonstration that a localized, iontophoretic pulse of Ca activates release only if it precedes presynaptic depolarization, suggesting that external Ca is normally utilized *before* the first detectable increase in quantal release probability. Furthermore, the entire decay of post-stimulus release probability cannot be due to inactivation of CaX because of the facilitation observed when a test stimulus is applied during the falling phase (Fig. 5*A*, *B*; see also Fig. 2 of Katz & Miledi, 1968). Thus it seems most probable that the decay of release probability reflects removal of Ca (or of some other presynaptic releasing factor). The high Q_{10} of the early exponential decay of release probability (Fig. 3) suggests that this removal is not diffusion-limited.

The very different time courses of the decay of early and late release (compare Figs. 1*B*, 5*A*) argue that the removal of releasing factor follows non-linear kinetics and/or that there are at least two removal mechanisms which operate at different rates. Sample calculations demonstrated that models with only one linear sequestering mechanism could yield a multiphasic decay of release probability if release were proportional to a higher power of the concentration of the active releasing factor (e.g. the fourth-power model of Dodge & Rahamimoff, 1967), but the ratio of early and late decay constants could not exceed that power, and the examples in Figs. 1*B* and 5*A* show early and late decay constants differing by a factor exceeding 50. Perhaps the early fall of release probability reflects rapid, reversible binding of Ca by presynaptic Ca buffers, while the late decay mirrors the slower uptake of Ca into internal stores or extrusion of Ca from the terminal (see Baker, Hodgkin & Ridgway, 1971, p. 747).

Are facilitation and late release related?

Katz & Miledi (1968) have demonstrated that the facilitating effect of a conditioning depolarization is very sensitive to the availability of external Ca during the stimulus, and they have suggested that 'a residue of the "active calcium" which enters the terminal axon membrane during the nerve impulse is responsible for short-term facilitation' (p. 481). On this hypothesis one might expect that the late 'tail' of elevated release probability following stimulation should extend into the intervals during which short-term facilitation can be demonstrated, although such tails might be very small if the relationship between 'active calcium' and release were

very non-linear. Fig. 5 in this study and Fig. 1 in Hubbard (1963) show that the time courses of late release and primary facilitation do overlap to some extent. However, a more critical test of this residual calcium model of facilitation would be to use the late tail of release following the conditioning nerve stimulus to predict the facilitation of release to a test stimulus.

In Appendix I an equation relating the residual release rate and facilitation is derived using a version of the power model postulated by Dodge

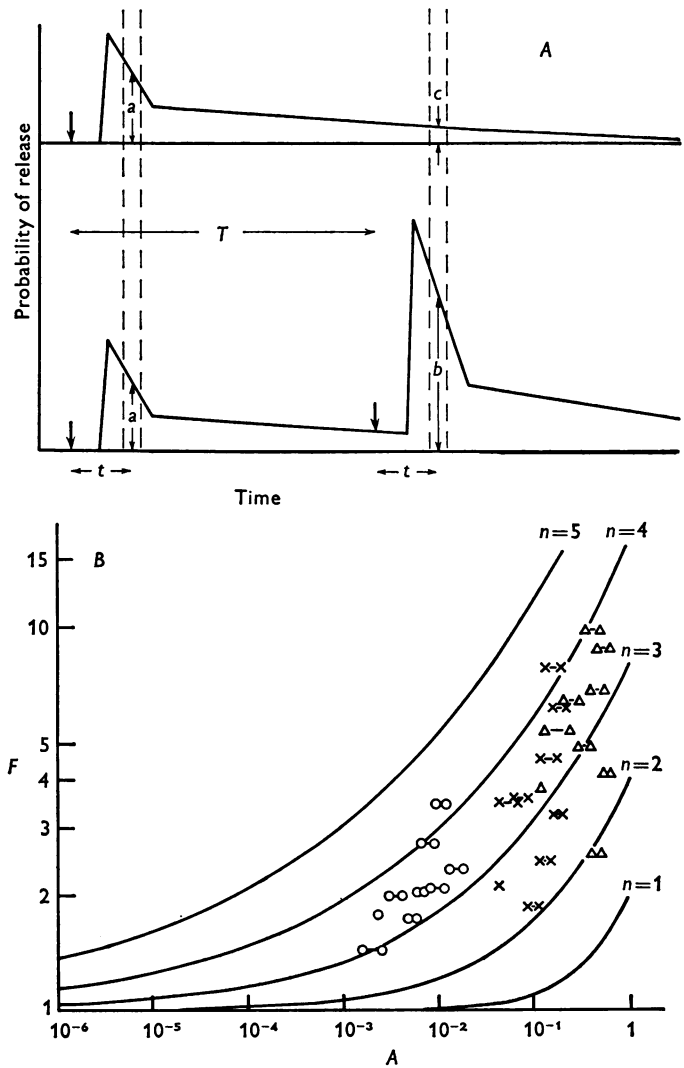

Fig. 6. For legend see facing page.

& Rahamimoff (1967). The predicted facilitation (F) depends on the ratio of residual release rate to control release rate (A) and on the power n. The solid lines in Fig. 6*B* plot the predicted relationships between F and A calculated for the indicated values of n using eqn. (8). The symbols in Fig. 6*B* represent data from one synaptic region. It is evident that the experimental relationship between F and A is extremely non-linear. The experimental points tend to lie between the curves calculated for $n = 3$ and $n = 4$.

According to Dodge & Rahamimoff's (1967) fourth-power model, the data points should cluster about the $n = 4$ line, but the discrepancy between data and predictions could easily be the result of inaccuracies in measuring low residual release rates. The assumptions used in deriving eqn. (8) are another possible source of error. For example, in view of the cumulative rise in background release rate (see p. 699) it is unlikely that the concentration of presynaptic releasing factor Y is actually zero prior to the conditioning stimulus. When the estimated background release rate (vertical bar, Fig. 5*A*) is subtracted from the residual release rates used to calculate the ratio A, the data points in Fig. 6*B* shift closer to the $n = 4$ line. Another questionable assumption is linear addition of $[Y]$.

Considering the many sources of error, it seems to us significant that most data points suggest at least a third-power relationship between release and $[Y]$. The power model certainly accounts for the observation (Fig. 5*B*) that the small release rates in later post-stimulus intervals are facilitated by a greater percentage than the larger release rates in earlier post-stimulus intervals. Rahamimoff (1968) noted that the facilitation measured at a given conditioning-testing interval increased as the quantal

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Fig. 6. *A*, schematic time course of quantal release probability following a single nerve stimulus (arrow, upper trace) and paired nerve stimuli separated by an interval T (arrows, lower trace). During the post-stimulus interval t denoted by the dashed lines, the average release probabilities following the control and test stimuli are a and b, respectively. The residuum of release to the control stimulus is c. Facilitation is defined as (b/a); A (as defined in Appendix I) equals (c/a). In the terminology of Appendix I, $a = R_1(t)$, $b = R_2(t)$, $c = R_1(t+T)$. Time and probability scales are arbitrary. *B*, double logarithmic plot of facilitation (F) as a function of A, the ratio of residual to control release probabilities (see Fig. 6*A*). Continuous lines are calculated using eqn. (8) (Appendix I) for the indicated values of n. Symbols represent data from the synaptic region of Figs. 1 and 5. Facilitation was evaluated for various conditioning-testing intervals T (see Fig. 5*B*) and for three consecutive post-stimulus intervals t: the earliest lasting 20 msec (circles), the next 15 msec (crosses), the latest 35 msec (triangles). Horizontal lines connect data points plotted with (left) and without (right) correction for the background release rate (see text).

content of release evoked by the conditioning stimulus decreased (see also Mallart & Martin, 1968), and indicated that the fourth-power calcium model would also explain this finding.

Application of eqn. (8) to successive small post-stimulus intervals predicts that the decay of facilitated release probability should be slower than the decay following the conditioning stimulus, an effect evident in the data of Figs. 3 and 4. However, available data did not allow a quantitative comparison of the predicted and experimental time courses of facilitated release probability.

The preceding analysis suggests a quantitative method for testing the hypothesis that facilitation is mediated by a residuum of presynaptic releasing factor. The data of Fig. 6*B* are certainly consistent with this residuum hypothesis, although they are not adequate to prove the specific fourth-power model outlined in Appendix I. If future experiments indicate that this model does adequately describe two-pulse facilitation, additional assumptions will almost certainly be necessary to reconcile the model with the linear addition of facilitation observed for short trains of conditioning stimulation by Mallart & Martin (1967) and for longer trains by Magleby (1970). It is also difficult to explain the existence of pronounced facilitation at crustacean neuromuscular junctions, where release appears to be linearly related to the external Ca concentration (Bracho & Orkand, 1970; Ortiz & Bracho, 1972).

APPENDIX I

Prediciting facilitation from late release

This section uses Dodge & Rahamimoff's (1967) power model to derive an equation which predicts facilitation as a function of the late residual release to the conditioning stimulus.

Let t designate the post-stimulus interval during which facilitation (F) is measured, and let T equal the interval between the conditioning and testing stimuli (see schematic diagram, Fig. 6*A*). It is evident from Fig. 5*B* that F is a function of both t and T. Let $R_1(t)$ and $R_2(t)$ indicate the average probability of release in the interval about t following the conditioning and testing stimuli, respectively, and let $R_1(t+T)$ represent the probability of residual release when no test stimulus is given. By definition

$$F(t, T) = R_2(t)/R_1(t). \tag{2}$$

Assume that R_1 and R_2 are related by the same proportionality constant k to the nth power of the concentration of some presynaptic releasing factor Y (in Dodge & Rahamimoff's (1967) model $n = 4$ and Y is a complex involving Ca). Let Y_1 and Y_2 indicate the releasing factor produced by the conditioning and testing stimuli, respectively, and assume (1) that no Y is

present before the conditioning stimulus, (2) that both stimuli produce Y at the same rate and (3) that concentrations of Y add linearly. $[Y_1(t)]$ indicates the concentration of Y in the interval t after the first stimulus, $[Y_1(t+T)]$ the concentration of Y remaining from the first stimulus in the interval during which test release is evaluated, and $[Y_2(t)]$ the concentration of Y from the second stimulus during the interval t after the second stimulus. The following relationships emerge from these assumptions:

$$R_1(t) = k[Y_1(t)]^n \quad \text{or} \quad [Y_1(t)] = \sqrt[n]{[R_1(t)/k]}, \tag{3}$$

$$R_1(t+T) = k[Y_1(t+T)]^n \quad \text{or} \quad [Y_1(t+T)] = \sqrt[n]{[R_1(t+T)/k]}, \tag{4}$$

$$R_2(t) = k[Y_1(t+T)+Y_2(t)]^n. \tag{5}$$

Since both stimuli produce Y at the same rate, $[Y_2(t)] = [Y_1(t)]$, and eqn. (5) can be simplified to give:

$$R_2(t) = k[Y_1(t+T)+Y_1(t)]^n. \tag{6}$$

Values for $[Y_1(t)]$ and $[Y_1(t+T)]$ obtained from eqns. (3) and (4), respectively, can be substituted into eqn. (6) yielding

$$R_2(t) = k(\sqrt[n]{[R_1(t)/k]}+\sqrt[n]{[R_1(t+T)/k]})^n. \tag{7}$$

Eqns. (7) and (2) combine to define F in terms of $R_1(t)$ and $R_1(t+T)$:

$$F = (\sqrt[n]{[R_1(t)/k]}+\sqrt[n]{[R_1(t+T)/k]})^n/kR_1(t)$$

which simplifies to

$$F = (1+\sqrt[n]{A})^n, \tag{8}$$

where $A = (R_1(t+T)/R_1(t))$ (note that the definition of facilitation used here exceeds Mallart & Martin's (1967) definition by 1).

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