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Further Evidence for the Dynamic Formation of Transmitter Quanta at the Neuromuscular Junction

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Fatt and Katz (*Nature* 166:597–598, 1950; *J Physiol* 117:109–128, 1952) attributed miniature endplate potentials (MEPPs) to the action of a standard quantity of transmitter, the quantum (Del Castillo and Katz, *J Physiol* 124:560–573, 1954). Quantal packets of transmitter were proposed to be preformed (Del Castillo and Katz, In CNRS Paris (Ed): "Microphysiologie comparée des éléments excitables" 67:245–258, 1957) and stored in large numbers in the motor nerve terminal. Statistical analyses of intervals between MEPPs and numbers of quanta composing small endplate potentials indicated that quantal release was a random process and that release sites functioned independently of each other. With the discovery of synaptic vesicles it was proposed that each contained one quantum of transmitter. The quantal-vesicular hypothesis (Del Castillo and Katz, as cited above) fails, however, to explain amplitude distributions of MEPPs that are skewed and/or that show multiple peaks (Kriebel et al., *Brain Res Review* 15:167–178, 1990). The drop formation process (Shaw, "The Dripping Faucet as a Model Chaotic System," Santa Cruz, CA: Aerial Press, Inc., 1984) was shown to generate amplitude classes of drops that were similar to classes of MEPPs which suggested that rapid changes in quantal size and ratios of skew- to bell-MEPPs could be explained with a simple dynamic process which determines quantal size at the moment of release (Kriebel et al., as cited above, 1990). Further similarities between miniature endplate currents (MEPCs) and the formation of drops are reported here. We found that rapid changes in MEPC amplitudes and time courses, which accompany an increase in frequency, mimic changes in drop sizes that accompany increases in flow rate. MEPC intervals have a minimum and their distributions are comparable to those of drop intervals. During an increased rate of transmitter release, MEPP amplitudes and intervals were positively correlated. The results suggest that spontaneously released transmitter "packets" are formed at the moment of release and that transmitter supply to the process that forms packets is continuous. © 1992 Wiley-Liss, Inc.

Key words: spontaneous transmitter release, quantal release

INTRODUCTION

A miniature endplate potential (MEPP) represents the release of a single packet of transmitter by the presynaptic terminal (Del Castillo and Katz, 1954, 1957; Boyd and Martin, 1956; Liley, 1956) and the study of MEPPs has provided much information about the release process. The time course of MEPPs is attributed to the postsynaptic response of an almost instantaneous release of a standard amount of transmitter (Wathey et al., 1979; Dwyer, 1981; Head, 1983). MEPPs were formerly described as having a uniform time course and forming a bell-shaped amplitude distribution with "no secondary peaks" (Fatt and Katz, 1952). For these reasons, transmitter release was said to be a quantal process (Del Castillo and Katz, 1954; 1957), but at that time, the origin for the quantum was not proposed. Fatt and Katz (1952) found that large numbers of quanta could either be evoked during tetanic stimulation or released at high frequency during hypertonic challenges with no apparent change in size. These observations suggested that large numbers of transmitter packets of standard size were preformed in the presynaptic terminal. The presence of numerous synaptic vesicles in presynaptic terminals (Birks et al., 1960) and images of vesicles fused with the plasma membrane (Couteaux and Pécot-Dechavassine, 1970; Hauser et al., 1979; Dunant, 1986) indicated that exocytosis of vesicle contents could represent the release process (see Terakawa et al., 1991, about non-synaptic

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release of transmitter). The amount of transmitter required to generate a quantal response (Kuffler and Yoshikami, 1975) could fit inside a vesicle (Miledi et al., 1980) which suggested that the internal volume of the vesicle determines quantal size.

Recordings with low noise levels revealed the presence of a class of MEPPs with a smaller mode and a skewed amplitude distribution (termed skew-MEPPs to distinguish them from the standard MEPPs forming a bell-shaped amplitude distribution (termed bell-MEPPs; see Kriebel et al., 1990 for review). In addition, MEPP and unitary EPP histograms showed multiple, integral peaks (Matteson et al., 1981; Vautrin, 1986; and Kriebel, 1988 for review). Autocorrelation or harmonic analyses of histograms demonstrated that the peaks found in amplitude distributions were significant and not the result of random variations in sample size (Magleby and Miller, 1981; Csicsaky et al., 1985; Vautrin, 1986). A class structure in MEPP amplitude distributions has no explanation within the quantal and vesicular theories unless MEPPs are composed of subunits, in which case the notion of a quantum of transmitter is shifted to a smaller size (Kriebel and Gross, 1974; Wernig and Stirner, 1977).

The incidence of skew-MEPPs has been rapidly changed by many experimental conditions (see Kriebel et al., 1990 for review). Examples of gradual and rapid alterations in the shape of the distribution (from bell-shaped to skewed or the reverse) have been observed during acute treatments (Kriebal and Florey, 1983; Vautrin and Kriebel, 1991a; Vautrin, 1991), spontaneously during synaptogenesis (Muniak et al., 1982) and with ageing (Vautrin and Kriebel, unpublished). Changes in the shape of amplitude distributions from bell-shaped to skewed were accompanied by a decrease of the mean amplitude and did not require the release of a large number of quanta (Kriebel et al., 1976; Carlson et al., 1982; Kriebel and Pappas, 1987). Rapid changes in quantal size are not easily explained with the hypothesis of preformed quanta. Skew-MEPPs were not generated by a different, secondary process since they appeared to be composed of the same subunits as the bell-MEPPs (Matteson et al., 1981; Erxleben and Kriebel, 1988a,b; Vautrin and Kriebel, 1991, 1992) and the overall shape of the amplitude distribution was readily changed.

We introduced a new model for transmitter packet formation (Kriebel et al., 1990) based on the similarities in the dynamics of MEPP generation and drop formation (Shaw, 1984). Drop formation is a process that spontaneously makes packets without the requirement of a container for prepackaging. A dripping faucet generates preferred drop intervals which depend on the flow rate (Shaw, 1984). Distributions of drop sizes may be bell-shaped, skewed or both and preferred drop sizes may form multiple peaks in size distributions (Kriebel et al., 1990). The relationship of drop intervals and volumes depends on a constant flow rate and complex oscillations in the process of drop formation (Shaw, 1984; Kriebel et al., 1990). We report similarities in the patterns of change in MEPC and drop amplitudes and time courses when their frequencies were increased. We found patterns in MEPC intervals which suggest the presence of a process that forms quanta from a continuous supply of transmitter in a manner analogous to that of water drop formation.

METHODS

Drops were recorded with a photoresistor and a light source (as described by Kriebel et al., 1990). We adjusted the width of the slit placed in front of the photoresistor and the distance between nozzle and recording apparatus so that the peak of a drop signal was proportional to the volume of the drop. MEPCs are known to be much longer than the release of transmitter packets. In order to match these relative timings a 0.5KHz low pass filter was used to increase the rise time of drop signals.

MEPCs were recorded with a two-electrode voltage clamp amplifier (Dagan) from frog cutaneous pectoris and sartorius muscles maintained at 25°C. Bath saline contained (in mM) NaCl (125), KCl (3-5), $CaCl_2$ and Tris buffer adjusted to pH 7.4. Because MEPC frequencies at rest, in normal saline, were too low (less than 1Hz) for analyses, frequencies were increased (to a few Hz) with addition of KCl (up to 5mM). Rapid increases of MEPC frequencies were obtained by addition of NaCl (200-500mOsm) or ethanol (2-8%). The synaptic signal was filtered at 3KHz which did not significantly change the MEPC time course.

Drop and MEPC signals were digitized at 30-50KHz from magnetic tape and analyzed on a microcomputer using the SCAN program from Strathclyde University (Glasgow, UK). All events were inspected and artifacts and noisy signals were discarded. The peak amplitude was obtained by subtracting the mean value of 10 points before the event and 10 points composing the maximal amplitude. Rise times were measured between 10% and 90% of the peak amplitude using the SCAN program. All signals showing a constant, positive slope were analyzed. Of course, if two events occurred within an interval shorter than their rise time, they generated one signal (see Vautrin and Kriebel, 1991; Vautrin, 1992). Signals with transient negative slopes on their rising phase were rejected. This selection criterion excludes long rise time and small amplitude signals from the graphs. In order to optimize the interval measurements, MEPC and drop signals were shortened by differentiation (MEPC durations were less than 2 msec).

Signals were digitized at 5Hz and intervals were measured using a modified version of PAT program from Strathclyde University.

RESULTS

Effect of Frequency on Drop and MEPC Sizes

During relatively low flow rates, an increase in the flow rate raised the drop frequency without a significant change in drop size. There was a critical flow rate above which the distribution of drop sizes became highly sensitive to the flow rate. Physical characteristics of the nozzle such as orifice diameter and wall thickness affected the value of the critical flow rate. We selected a nozzle which increased the incidence of smaller and larger drops after an increase of drop frequency of 20% (Fig. 1A,B).

When MEPC frequencies were increased from 0.1 to 10Hz, there was usually no change in MEPC amplitude distributions; but substantial alterations in amplitude distributions were observed at frequencies greater than 100Hz. MEPC frequencies were increased to 100-300Hz by adding 200-500mOsM NaCl to the saline. This increase in osmotic pressure significantly increased the input impedance (during the challenge and after returning in normal medium), but MEPC signals (voltage clamp) provided an estimate of the size of the transmitter packet because synaptic currents are independent of the input impedance of the muscle fiber. Furthermore, MEPCs are much faster than MEPPs (a MEPP results from a MEPC through the impedance of the fiber) so MEPCs provided a better resolution of the time course of transmitter release. The amplitude distribution of MEPCs was broadened with high frequencies (Fig. 1C,D). Similar effects have been obtained with other treatments which increase MEPC frequency such as ionophores (Carlson et al., 1982), or ammonium chloride (Vautrin, 1992). The increases in frequencies of MEPCs and drops were accompanied by an increase in the percentage of small and large MEPCs and drops although the increase in large MEPCs was greater than that of large drops.

Effect of Frequency on Drop and MEPC Rise Times

When the rate of flow was increased such that the amplitude distribution of drops was altered, drop rise times were also changed (Fig. 2). At low drop frequencies (Fig. 2A), most drop signals showed the same short rise times. A few longer rise times were observed which suggests coincident, or nearly so, drops. Intervals of less than 20ms produced unitary signals that were greater in amplitude and had longer rise times than most (Fig. 3A). Assuming that drops were randomly distributed in time, the number of expected coincidences was calculated. Thus, 380 random events at 9.9Hz ($P = 0.198$) would yield 70 intervals of under 20 msec. We found only four large signals in 380 drops suggesting coincident events. This low value is expected because drops are not randomly distributed. The four signals with long rise times in Figure 2A are too small to be produced by coincidences of normal sized drops. When the critical flow rate was reached, drop sizes and frequencies became unstable and highly sensitive to further increases in flow rate. At 12Hz (Fig. 2B, same data set as for Fig. 1B) the percentage of events with long rise times was significantly increased but all of these signals are too small to correspond to chance coincidences of two normal sized drops. The long rise time signals were generated by coincidence of smaller drops (a spray of smaller drops) (Fig. 3). Drop intervals and amplitudes are linked because drops are formed from a constant flow. At higher flow rates, only small drops were formed.

At low frequencies, and despite a shorter time course, more MEPCs than drops coincide (Fig. 2A,C). For example, assuming MEPCs were randomly distributed in time, at 8.2Hz, the probability to observe a MEPC in a 1-msec time frame is 0.0082, so that 5-6 coincidences would be expected in 700 1-msec time frames. The number of MEPCs with larger and slightly longer rise times (Fig. 2C) was close to this value and could be explained by random coincidences of MEPCs. At higher MEPC frequencies more coincidences are expected and a higher incidence of long rise times was observed (Fig. 2D). Indeed, most of the MEPCs over 6nA had long rise times. Many rise times were 2-4 times longer than the shortest rise times suggesting that they were produced by multiple coincidences (Fig. 3B). Moreover, high frequencies induced small MEPCs with long rise times which could not be produced by coincidences of MEPCs of standard size (Fig. 2C,D). In other words, smaller MEPCs showed a higher tendency to cluster in time.

Amplitude and Interval Relationship

Shaw (1984) has demonstrated that intervals between drops are not random and we also found preferred drop intervals (Fig. 5A,B). When the rate of flow was increased, the drop frequency increased and there was a higher percentage of smaller intervals. The interval distribution became somewhat skewed and showed distinct peaks (Fig. 4B).

The overall profiles of MEPP interval distributions were exponential (see Fatt and Katz, 1952). A pattern of MEPP intervals was strongly suggested with appropriate conditions, even though the number of MEPPs observed in a given time frame could fit a Poisson distribution (Fig. 4, cf. Kriebel and Stolper, 1975). MEPC intervals were individually measured with high resolution (see Methods), and they show a distinct mode out of the noise (Fig. 5C). There is a significant lack of small values of

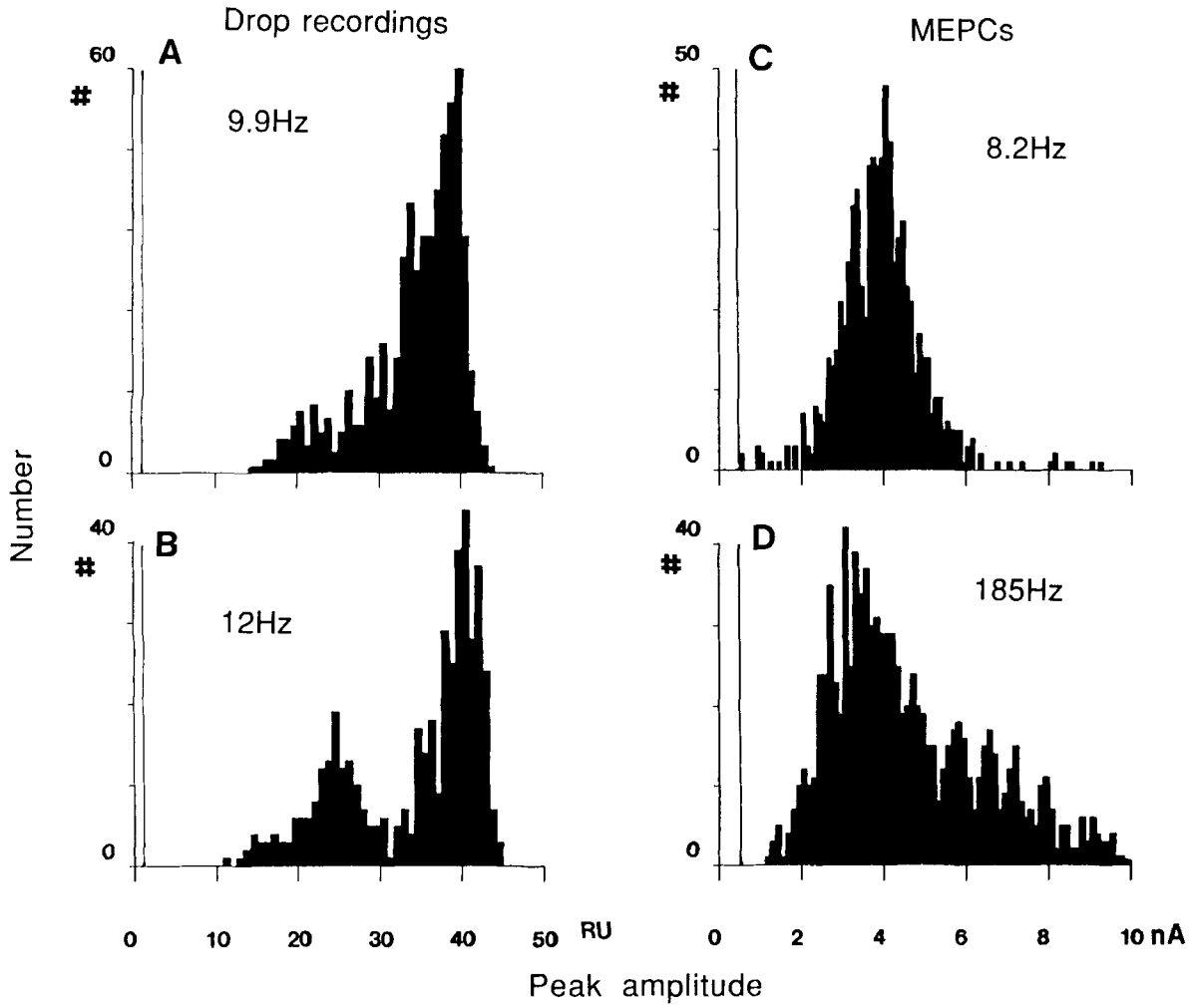

Fig. 1. Amplitude distributions of drop signals (A & B) and MEPCs (C & D). The line on the left corresponds to the threshold level of the automatic detection of events and it indicates the resolving power. **A:** 380 drops at 9.9Hz. Horizontal: amplitude, relative units (RU); vertical: number of observations. **B:** 380 drops at 12.4Hz. **C:** 700 MEPCs at 8.2Hz. Horizontal: amplitude in nA; vertical number of observations. **D:** 700 MEPCs at 185Hz (same junction as in C). Osmotic pressure was increased (+300 mOsm) with addition of NaCl. Note that high frequencies alter peaks and shapes of both drop and MEPC amplitude distributions by increasing the percentage of large and small amplitude events.

intervals which would be expected with an exponential distribution. Peaks were observed in MEPP and MEPC interval distributions and serial correlation tests detected regular spacing between peaks in the histograms (cf. Magleby and Miller, 1981) which demonstrates preferred intervals between MEPCs (see Fig. 5D). Preferred intervals varied from experiment to experiment and were sometimes near the 60 cycle frequency of the power supply (Fig. 5D).

During constant flow rates we found alternating regimes of large and small drops with respective long and short intervals (Kriebel et al., 1990). We have already reported that smaller MEPCs are often preceded by short intervals (see also Vautrin and Kriebel, 1991) which suggested a relationship between MEPC intervals and amplitudes. Alternating regimes of large and small amplitudes are observed at frequencies of 100-200Hz (Fig. 6A). For these series, plots of MEPP amplitude versus the following interval (n = 3) showed a lack of short intervals following large amplitudes and a high proportion of small intervals following small amplitudes. There was a positive, statistically significant correlation between amplitudes and intervals (Fig. 6B).

DISCUSSION

Analyses of MEPCs

Measurements of MEPC amplitudes with osmotic challenges were more reliable than voltage recordings to study the effect of frequency on quantal size during os-

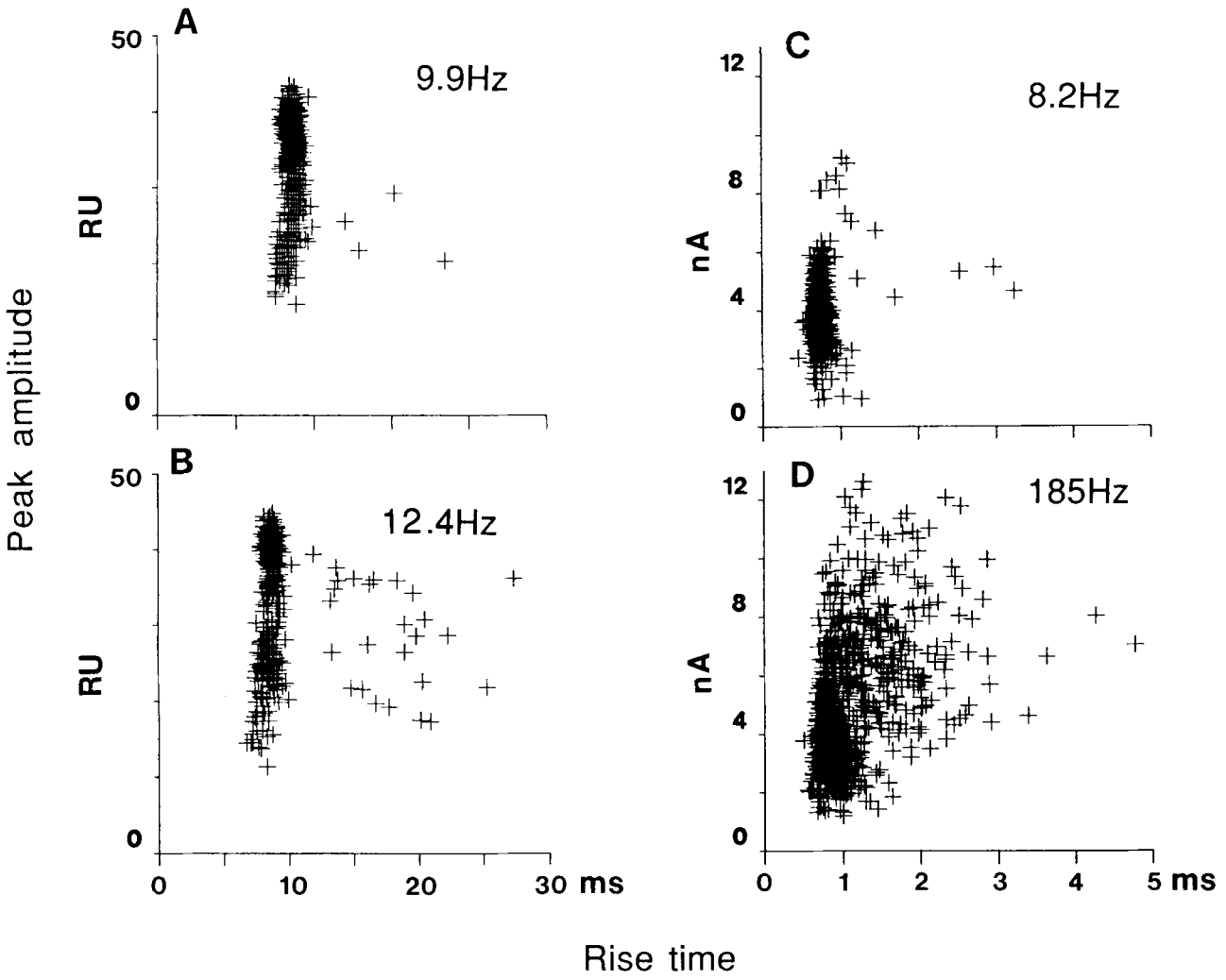

Fig. 2. Peak amplitude vs rise time for drop sizes (A & B) and MEPCs (C & D). Same signals as in Figure 1. **A:** Drops at 9.9Hz. Horizontal: rise time, milliseconds; vertical: amplitude, relative size units (RU). **B:** Drops at 12.4Hz. The percentage of smaller and slower events increased with the increase in frequency. Slow events may represent clusters of small drops. **C:** MEPCs at 8.2Hz. Horizontal: rise time: milliseconds; vertical: amplitude, nA. MEPC characteristics are tightly grouped. **D:** MEPCs at 185Hz. The percentage of smaller and slower MEPCs has increased. Many of the large MEPCs (6-12nA) are slow MEPCs (rise time > 1ms). Note that small, slow MEPCs (1-4nA, rise time > 1ms) were absent at lower frequency.

motic treatment because the impedance increased during treatment and remained high (+20%) after returning to normal saline (increases of resistance up to 50 times were obtained with hypertonic saline). In addition, rise times are better resolved when synaptic events were recorded as current. Larger and smaller MEPCs were observed during or after hyperosmotic treatment and most of the larger MEPCs had a longer than normal rise time. Long rise time events induced by acute treatments are not due to remote transmitter action for the following reasons: (1) long rise times may be recorded with focal extracellular electrodes (Cooke and Quastel, 1973; Augustine and Levitan, 1983; Vautrin and Kriebel, 1992); (2) the amplitude and rise time relationship of signals generated at remote sites would be negatively correlated, whereas the data showed a positive correlation (Fig. 2D; Erxleben and Kriebel, 1986b; Vautrin and Kriebel, 1991a); and (3) many signals with long rise times had breaks (Vautrin and Kriebel, 1992). Spontaneous events are known to clump (Cohen et al., 1973; Kriebel and Stolper, 1975; Vautrin and Kriebel, 1991). Thus, if intervals between transmitter packets become shorter than the individual rise times, the postsynaptic responses fuse into single events with relatively longer rise times. Since postsynaptic responses usually show a constant rise time (Dwyer, 1981) and a constant amplitude (Fatt and Katz,

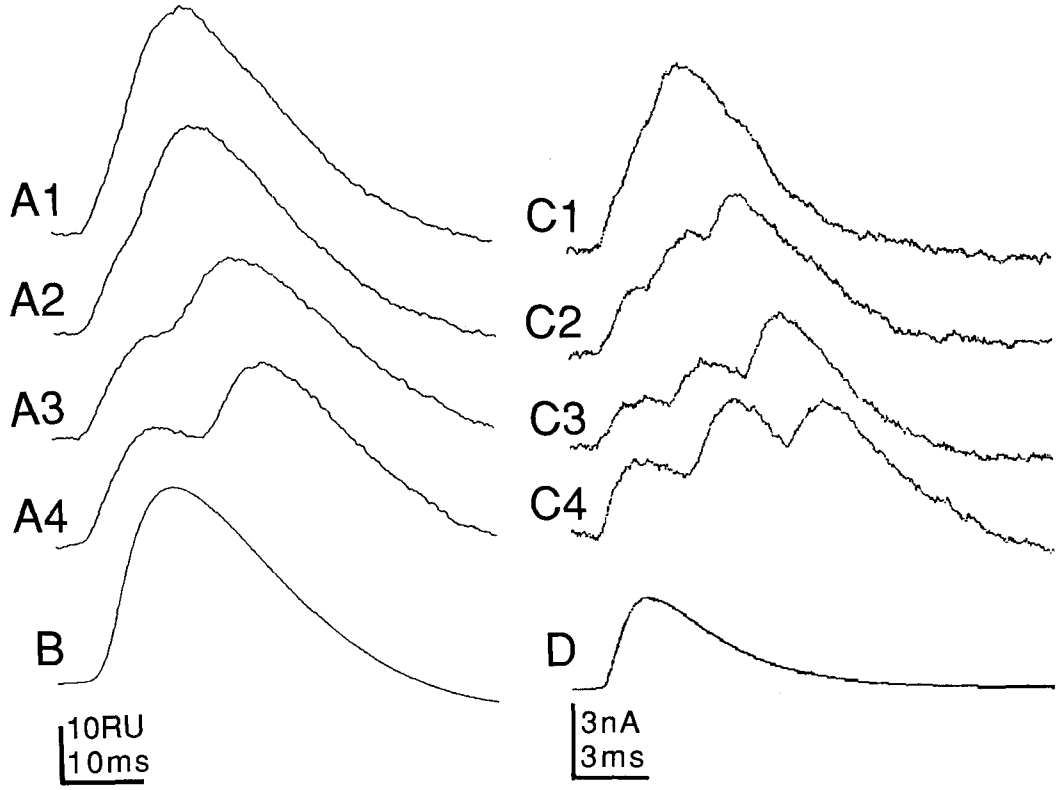

Fig. 3. Clusters of drops and MEPCs. These are selected signals to show signal summation. A1 to A4: Graded series of summated drop signals separated by greater and greater intervals which have been selected from the same data set. A4 & A3 were discarded from further analyses because they show a transient negative slope on the rising phase (see Methods). B: Mean signal of all the signals which were selected for analysis of data series. C1 to C4: Graded series of summated MEPCs with different intervals which were selected from the same data set. C2 to C4 were discarded from further analyses because they show transient negative slopes on their rising phases. D: Mean signal of all the signals which were selected for analyses in that series. Note that clustering events (drops and MEPCs) are smaller than the average event.

1952) a quantum of transmitter has been defined as a constant amount of transmitter which is instantaneously released. However, MEPC amplitudes may vary widely with appropriate conditions (Kriebel et al., 1990; Van der Kloot, 1991 for reviews) so the rise time would be a better criterion than amplitude to identify individual packets of transmitter and to evaluate the larger synaptic events observed in hypertonic medium (Fig. 1; see also Van der Kloot, 1987; Yu and Van der Kloot, 1991). Since these larger events have long rise times (Fig. 2), we propose that they are generated by multiple packets of transmitter.

Skew- and Bell-Classes of Synaptic Events

Bell and smaller skew-MEPCs can have the same rise time characteristics Erxleben and Kriebel, 1989). Vautrin and Kriebel have shown with conditions that increase the percentage of skew-MEPCs that rise times are correlated with amplitudes (Vautrin and Kriebel, 1991; Vautrin, 1992). Moreover, many larger events are probably composite events. In contrast, the rise time of bell-MEPCs is relatively independent of amplitude. There is no obvious boundary between small and large skew-MEPCs. Therefore, large skew-MEPCs represent currents many times greater than that of bell-MEPCs. The continuum of shapes of large skew-MEPCs that are obvious composite events to those with a smooth rising phase reveals the repetitive activity of the release process. A release process which can function repetitively explains rapid changes in MEPC amplitudes and increases in the percentage and numbers of the skew class.

Transmitter Packets and Water Drops

Preferred sizes of drops do not result from the coalescence of sub-drops but reflect the dynamics of a nonlinear system (Shaw, 1984). The intrinsic properties (gravity, surface tension, geometry of the nozzle, and

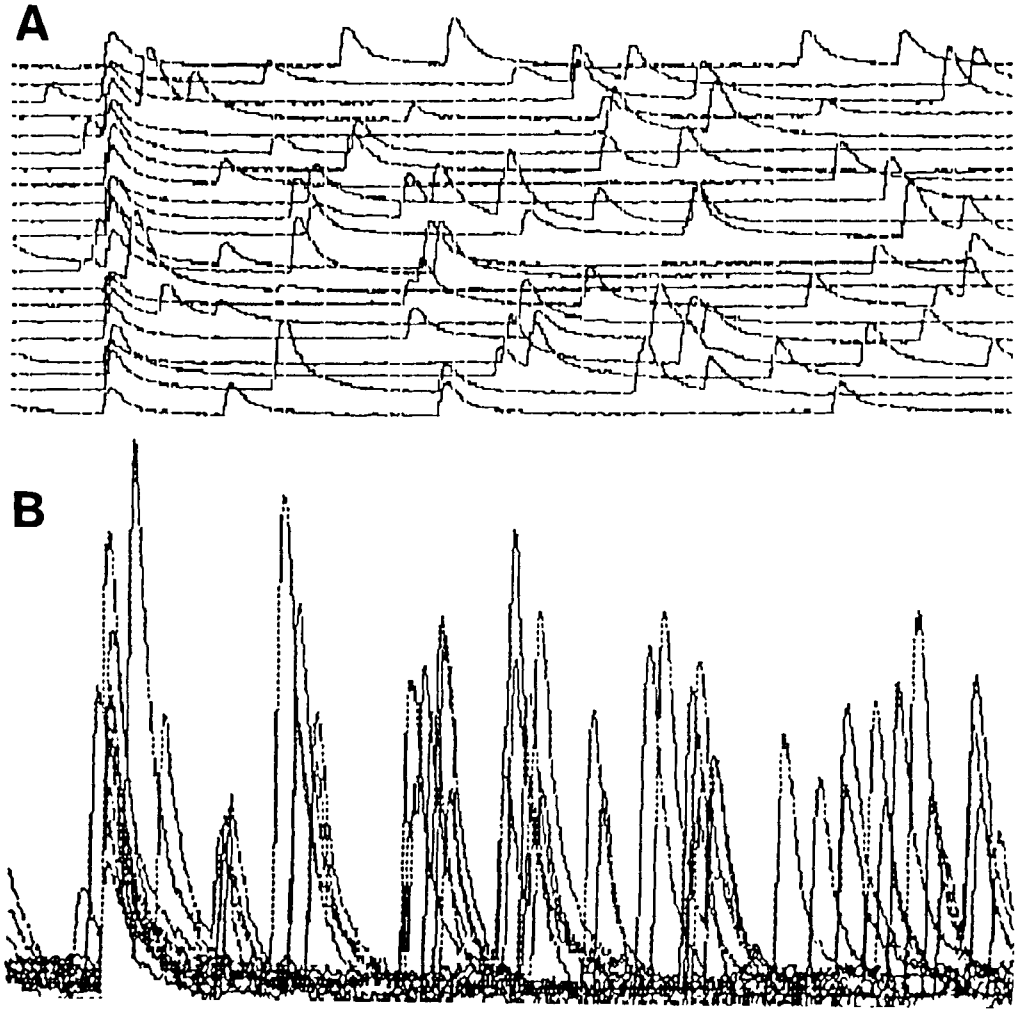

Fig. 4. Distribution in time of MEPPs. Frequency was increased with 5% ethanol. A: Twenty-one successive recordings of MEPPs are aligned with a MEPP used to trigger the oscilloscope. B: The same recordings are superimposed at a greater gain. Note the preferred intervals, although the number of events observed in 64 time bins (each 50ms) fits Poisson's statistics:

Number	0	1	2	3	4
Observed	20	24	15	3	1
Predicted	21.7	23.1	12.3	4.3	1.1

flow rate) of the system determine sequential drop sizes (noise could contribute to the variance of the classes).

The model of drop formation indicates that packets with complex amplitude distributions and random-like occurrences may result from the interaction of a driving force and an energy barrier. The observations that MEPCs and drops can rapidly change in size indicate that the kinetics of the release process may be similar to those of water drops. A process for transmitter packet formation with dynamic properties could explain the reduction in amplitude of the single events at high frequency and the small amplitude variance of the MEPP subclasses (cf. Katz, 1978; Matteson et al., 1981; Vautrin, 1986). The variance of each subclass would be the same and not an integral multiple of the sub-unit variance.

One or Two Processes of Release

It has been suggested that the shew-class observed during high frequencies of release could be generated by a different, limited process in addition to the process responsible for bell-MEPPs. This is not likely since bell-MEPCs and skew-MEPCs show the same subunit (Matteson et al., 1981; Erxleben and Kriebel, 1988b, Vautrin and Kriebel, 1991) and the overall distribution of MEPPs may be turned rapidly or progressively from the bell class to the skew class (Vautrin and Kriebel, 1991; Vautrin, 1992; see Kriebel, 1988, for review). Moreover, only skew MEPPs are observed at low frequencies, during synaptogenesis (Kriebel and Gross, 1974; Muniak et al., 1982) and during ageing (Vautrin and Kriebel, unpublished). We propose that the changes in skew to

bell ratios which result from a wide range of agents, simply reflect a non-specific influence on the process which forms transmitter packets.

Morphological Correlate of the Release Process

Even though we selected small junctions, the organization we detected between amplitudes and intervals suggests that only a few zones are active at a given time and/or that they are interdependent such that activity of a given release zone may influence neighboring zones. The relationship of a dynamic release process to the active zones (Couteaux and Pécot-Dechavassine, 1970) is at this time not known. The high frequency of transmitter release which produces MEPC amplitude and rise time alterations may reflect a critical rate of release which alters the release process.

A Dynamical Process of Quantal Release

Because interval and drop size are linked by a constant flow, drops show preferred intervals (Fig. 4A and Shaw, 1984) and preferred sizes (Kriebel et al., 1990). MEPPs (MEPCs) do not always appear randomly distributed in time. The number of events in a given time may fit Poisson's law, even though a series of events can show preferred intervals. The basic organization of MEPP intervals (see also Vautrin and Mambrini, 1981) is comparable with the interval structure of drops because at high rates of release a minimal interval was observed between MEPCs. This may be attributed to the fact that transmitter packets have a minimal amplitude, which is that of the subunit (Erxleben and Kriebel, 1988b; Matteson et al., 1981). The minimal interval could define the time necessary to mobilize transmitter for the next packet. The occurrence of small MEPCs after short intervals suggests that the formation of a packet temporarily depletes the immediately releasable store of transmitter.

Two hypotheses have been proposed for the origin of transmitter packets. Quanta either represent exocytosis of the contents of synaptic vesicles (Heuser et al., 1979; Hurlbut, 1989; Biscoe, 1989; Van der Kloot, 1991) or are formed by a membrane process from a cytoplasmic pool of transmitter (Tauc, 1982; Israël and Manaranche, 1984; Dunant, 1986). The dynamic properties of transmitter release described here may be incorporated into either model. Synaptic vesicles may contain a subunit of transmitter so that complex interdependencies between multiple vesicle exocytoses would explain the interactions reported here (Kriebel and Gross, 1974;

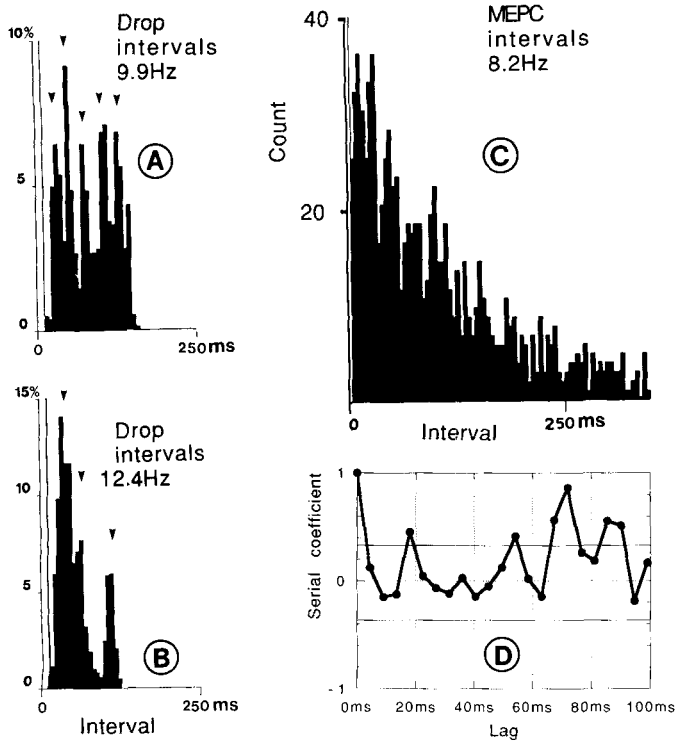

Fig. 5. Frequency distribution of intervals between drops (A & B) and MEPCs (C). **A:** Drop intervals. Same data as in Figs. 1A and 2A (n=380). As shown by Shaw (1984), drops may exhibit preferred intervals (arrowheads). **B:** Drop intervals from the same system at a greater rate of flow. Same data as in Figs. 1B & 2B (n=380). At the higher drop frequency the percentage of small intervals is increased and the interval distribution shows three peaks. Note that the mode of the distribution does not correspond to the first histobar which demonstrates that there is a minimal interval between drops. **C:** MEPC intervals. Same data set as in Figs. 1C & 2C (n=898). The general form of the distribution is a decreasing exponential: Count = $40.4 \times e^{-.0099ms}$, r=0.934. The resolving power exceeds the bin size (4.5ms). The first bin was discarded because it reflects the resolving power of the method (open histobar at left). The first significant histobar has 26 counts but 37.9 were expected according to an exponential fit. The X^2 calculated on bin two and the rest of the counts ($\nu=1$) is 3.86. Thus, the number of observations in bin two is unexpectedly low for a Poisson distribution ($0.05<P<0.025$) and is thus unlikely to result from random variations in the sample size. **D:** Serial analysis of the MEPC interval distribution displayed in C). The serial correlation coefficient, $R_h=(\Sigma x_i x_{i+h}-x\Sigma\, x_i)/\Sigma(x-x_i)^2$, was calculated on the series of differences between the counts of the 40 first histobars and the corresponding expected values for a Poisson distribution. The successive values of R_h reveal a positive correlation for 17-18ms intervals ("lag" on the graph; the horizontal lines indicate the significance limit for $\alpha_2=0.02$). This demonstrates that the multiple peaks in the interval histogram are not the result of random variation in the sample. However, the intervals are near that of 60Hz (16.6 msec) which may indicate in this case that the release process may be driven.

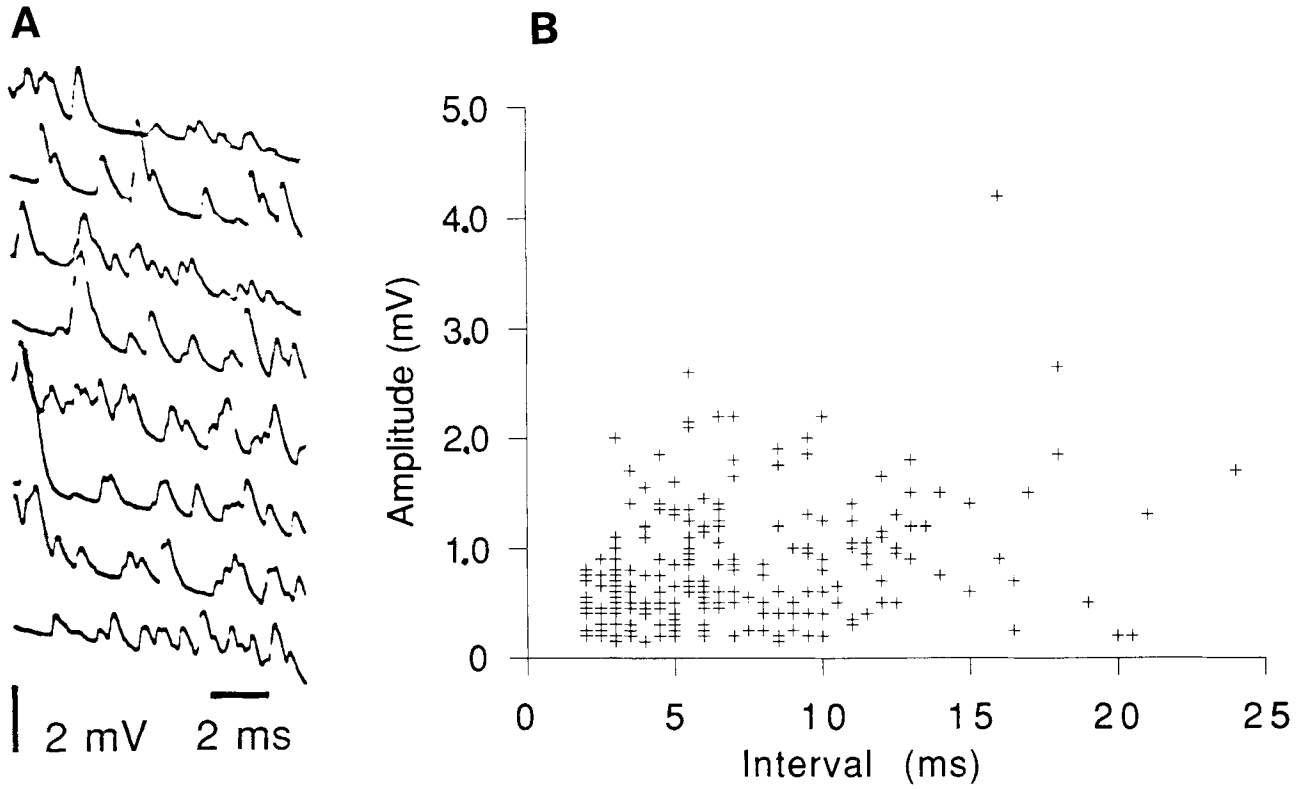

Fig. 6. Relationship betwen amplitude and interval of MEPPs. **A:** Oscilloscope recordings of MEPPs obtained with conventional intracellular recording from a frog neuromuscular junction. Frequency was increased to 128Hz by increasing the osmotic pressure with NaCl. This continuous sequence of traces has been selected because it shows a large range of MEPP amplitudes and it appears that larger MEPPs are further apart than smaller MEPPs. **B:** Plot of peak amplitude vs preceding interval of the series displayed in A. The correlation coefficient, r, is positive and has a value of 0.277 (n=230). The critical value of r is 0.230 ($P<0.001$, Student's test) which indicates that the hypothesis that there is no correlation between amplitude and interval should be rejected.

Wernig and Stirner, 1977; see also Birk, 1960; Gray, 1976; Alvarez de Toledo and Fernandez, 1990). We propose that the dynamic formation of transmitter packets results from diffusion of transmitter through a restricted passage (Israël and Morel, 1990).

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