Facilitation of Transmitter Release at Squid Synapses

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ABSTRACT   Facilitation is shown to decay as a compound exponential with two time constants (T_1, T_2) at both giant and non-giant synapses in squid stellate ganglia bathed in solutions having low extracellular calcium concentrations ($[Ca^{++}]_o$). Maximum values of facilitation (F_1) were significantly larger, and T_1 was significantly smaller in giant than non-giant synapses. Decreases in $[Ca^{++}]_o$ or increases in $[Mn^{++}]_o$ had variable effects on T_1 and F_1, whereas decreases in temperature increased $\bar{T}_1$ but had insignificant effects on $\bar{F}_1$. The growth of facilitation during short trains of equal interval stimuli was adequately predicted by the linear summation model developed by Mallart and Martin (1967. *J. Physiol. (Lond.).* **193:** 676–694) for frog neuromuscular junctions. This result suggests that the underlying mechanisms of facilitation are similar in squid and other synapses which release many transmitter quanta.

INTRODUCTION

Successive action potentials in a presynaptic axon terminal often evoke responses of increasing amplitude in a postsynaptic cell. This phenomenon, called facilitation, is of interest to physiologists for several reasons. First, facilitation is a common feature of chemical synapses and hence may represent a basic aspect of the mechanism of transmitter release (del Castillo and Katz, 1954; Dudel and Kuffler, 1961; Kuno, 1964; Charlton and Bittner, 1974). Knowledge of the mechanism of facilitation may enable greater understanding of the transmitter release process. Second, facilitation could play an important integrative role by affecting the relationship between presynaptic firing frequency and postsynaptic depolarization.

Facilitation at the squid giant synapse has been noted by other workers but has not been extensively quantified (Takeuchi and Takeuchi, 1962; Bloedel et al., 1966; Miledi and Slater, 1966; Katz and Miledi, 1967; Charlton and Bittner, 1974; Kusano and Landau, 1975). In fact, most of the available data (cf. Charlton and Bittner, 1974) indicate that the time-course of facilitation in squid synapses is quite different from that reported for other synapses such as frog neuromuscular junctions (Mallart and Martin, 1967). The disparity in the data might be explained by the fact that the experiments on frog synapses used solutions containing high magnesium and low calcium concentrations to reduce transmitter release and to enhance facilitation whereas the squid experiments

employed repetitive stimulation to depress transmitter release to subthreshold levels in normal $[CA^{++}]_o$. Thus, it is unclear whether Takeuchi and Takeuchi (1962) or Miledi and Slater (1966) studied the same kind of facilitation as was studied in the frog. In the present experiments, solutions of low calcium concentration were used to reduce transmitter release to subthreshold levels to enhance the detection of facilitation. We have shown that the magnitude, time-course of decay, and summation of facilitation are similar in squid and other animals. This result lends generality to our findings in the squid concerning the mechanism of facilitation discussed in this and the following paper (Charlton and Bittner, 1978).

MATERIALS AND METHODS

The squid *Loliguncula brevis* was obtained near the University of Texas Marine Science Institute, Port Aransas, Texas, where all experiments on this species were performed. These squid were found in large numbers all year and were caught by bottom trawl. Undamaged animals survived indefinitely when kept in running, clarified seawater, in a circular fiberglass tank (diameter, 2.5 m; depth, 0.8 m) and fed live or frozen fish or shrimp. The work on *Loligo pealei* was done at the Marine Biological Laboratory, Woods Hole, Massachusetts.

Stellate ganglia and nerves were isolated as described by Miledi (1967) and Arnold et al. (1974). Because decapitation often elicited sustained mantle contractions, only the tentacles and mouth parts were cut off during dissection. This procedure avoided repetitive firing of the presynaptic axons which synapse on the third order postsynaptic giant cells originating in the stellate ganglion (Young, 1939). Excised ganglia were bathed in flowing artificial seawater containing 3 mM sodium bicarbonate, 54 mM magnesium, 467.5 mM sodium, 10 mM calcium, and 10 mM potassium with chloride as the anion (pH 7.6). Salines were oxygenated by bubbling 99.5% O_2, 0.5% CO_2 before and during experiments. This gas mixture allowed adequate oxygenation and avoided the rise in pH that occurred when 100% O_2 was used. Saline flowed through a regulated Peltier-effect cooler before entering the experimental chamber where temperature was measured by a thermistor.

Once the synapses were visually identified, we bathed the preparation in salines having calcium concentrations of 2–6 mM, with appropriate modification of sodium concentration to keep osmotic pressure constant. Preparations kept in calcium concentrations <4 mM seemed prone to damage by microelectrodes and often lost excitability within 1 h. In order to obtain small postsynaptic potentials (<5 mV) and to ensure prolonged synaptic function, we kept the calcium concentration in most *L. pealei* experiments above 4 mM and added a small amount (<5 mM) of manganese to depress transmitter release (Katz and Miledi, 1969; Meiri and Rahamimoff, 1972; Balnave and Gage, 1973). Data were recorded after stable responses were obtained in new salines (20–90 min).

Synapses of giant, accessory giant, and non-giant presynaptic fibers were examined to determine whether different synapses in the same animal or homologous synapses in different species had similar facilitation properties. In this paper we have classified all but the second order presynaptic giant cell as presynaptic non-giant cells. Non-giant axons which form the proximal synapses (Young, 1939) were used when the presynaptic giant axon was non-functional. In some cases we were able to identify the presynaptic "accessory giant axon" (Miledi, 1967) as the active axon, but in others, we knew only that the active axon was not the presynaptic giant axon and was therefore one of at least three other axons which form synapses on the postsynaptic giant cell. All of these presynaptic

axons release sufficient transmitter to generate a suprathreshold potential in the postsynaptic cell at normal $[Ca^{++}]_o$.

Presynaptic axons were stimulated via extracellular wire electrodes applied to the preganglionic nerve or by intracellular microelectrodes. Extracellular stimulation of the presynaptic giant axon was confirmed by simultaneous intracellular recording of presynaptic potentials and postsynaptic potentials (PSP's). Synaptic input from non-giant presynaptic axons at proximal synapses was avoided by careful positioning of stimulating electrodes and by selectively pinching or crushing the preganglionic nerve in regions not containing the presynaptic giant axon. The postsynaptic potentials and synaptic delays were carefully monitored while the extracellular stimulus strength and polarity were varied to ensure that only one synapse was activated. Unless otherwise indicated, all PSP's were recorded from the third order giant cell which was located in the most caudal stellar nerve.

Pre- and postsynaptic giant cells were penetrated under visual control with microelectrodes ($5-10M\Omega$) containing 3 M KCL or 0.5 M K-citrate. These electrodes were always inserted within the zone of synaptic overlap of the pre- and postsynaptic giant axons. Presynaptic action potentials and PSP's were displayed using conventional electrophysiological techniques. 20 or 40 responses were stored and averaged in a computer of average transients (CAT 1000, Mnemotron Corp., Pearl River, N.Y.), and averaged responses were plotted on a chart recorder. The number of responses averaged was chosen to reduce the effect of variation in junction potential size while allowing the experiment to be completed in a reasonable length of time. We found that an interval of 10 s between recording sweeps was sufficient to allow the facilitation accumulated during a train to decay completely by the time the next train was delivered. PSP's rising from the falling phase of the previous potential were measured from the peak of the former to the projected tail of the latter and corrected for nonlinearity according to the procedure of Martin (1955).

Experiments were performed only on unfatigued preparations in which normal synaptic transmission could be demonstrated. Synapses usually regained their original transmission properties when placed in normal calcium saline at the end of an 8-h experiment in low calcium saline containing manganese.

RESULTS

Amount and Decay of Twin Pulse Facilitation

The second pulse of a stimulus pair was used to test for the amount of facilitation remaining from that contributed by the first, or conditioning pulse. The time-course of the decay of this facilitation was determined by varying the interval between the two pulses. (Each stimulus pulse caused a presynaptic action potential which resulted in a PSP.) Facilitation was defined as the ratio between the amplitudes of the second (V_1) and first (V_0) PSP's minus 1; equivalently,

$$f = \frac{V_1 - V_0}{V_0},\tag{1}$$

(Mallart and Martin, 1967; Magleby, 1973 *a*).

A typical output plot of a pair of PSP's from the averaging computer is shown in Fig. 1 (insert) in which the interval between stimuli was ~ 10 ms. The second PSP was clearly larger than the first, and, as the interval between the two stimuli

was increased, the value of f decreased. The relation between the interpulse interval (t) and f is shown in the lower part of Fig. 1. The points between 5 and 15 ms generally fall on a straight line when plotted on semilog coordinates. Owing to the refractory period of the presynaptic axons, stimulation could not be produced reliably at intervals of <5 ms. Therefore, the facilitation at zero time or the maximum facilitation (F_1) produced by the first impulse could only be estimated by extrapolating to the ordinate, a line passing through the points
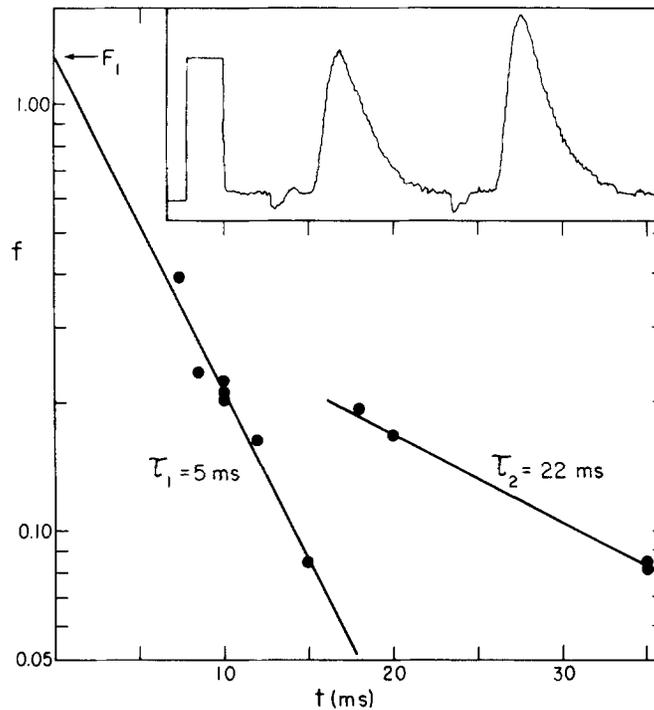

FIGURE 1.   Facilitation in a giant synapse of *L. brevis*. The facilitation (f) is plotted using semilogarithmic coordinates against the interval between conditioning and test stimuli (t). The first component of facilitation is described by $f = F_1 \exp (-t/T_1)$, where in this case F_1 (the intercept with the ordinate) = 1.3 and T_1 (the time constant of decay) = 5 ms. Insert shows a plot of a computer average of 20 postsynaptic potentials with an intrapulse interval of ~ 10 ms. Calibration pulse: 2mV, 2 ms. $[Ca^{++}]_o = 4$ mM, 18°C.

between 5 and 15 ms. This analysis assumes that F_1 decays exponentially from t = 0, although this may not be the case in other systems when artifical depolarizations are given at very short intervals (Katz and Miledi, 1968). It is also assumed that the second component of facilitation begins abruptly sometime after the action potential (Mallart and Martin, 1967).

From the graph of f vs. t, we derived a value for F_1 and a value for the time constant of decay (T_1) which was defined as the time required for f to decay to F_1/e. Inasmuch as the relation between f and t appears to be a straight line on a

semilog plot, it can be described by the exponential equation:

$$f = F_1 \exp(-t/T_1).\qquad(2)$$

Similar relations have been found in frog (Mallart and Martin, 1967; Magleby, 1973 a), toad (Balnave and Gage, 1974) and crayfish neuromuscular junctions (Linder, 1974; Zucker, 1974; Bittner and Sewell, 1976).

The slope of the decay curve of f almost always decreased at stimulus intervals between 10 and 30 ms after which f decayed at a rate slower (T_2) than T_1 (Figs. 1–4). In most preparations the decay of f was nonmonotonic, i.e., the change in the slope of the decay curve was accompanied by an increase (hump) in facilitation (Figs. 1, 2, and 4). A similar result has been found in the frog and crayfish neuromuscular junctions by Mallart and Martin (1967) and by Bittner and Sewell (1976). Facilitation during the second component of decay was quite small and difficult to quantify. Values of facilitation (F_2) at the peak of the hump (T_{max}) ranged from 0.15 to 0.2 while the second time constant of decay (T_2) had values between 22 and 63 ms.

In both species of squid, the average values of F_1 were significantly larger in presynaptic giant cells than in non-giant cells (Table I). Conversely, average values for T_1 were smaller for giant than for non-giant synapses (Table I). The presynaptic terminals of the giant synapses are much larger than those of the non-giant synapses (Martin, 1977; Young, 1939, 1973), but it is not at all obvious how this should affect the results. Synaptic contacts in both types of terminals are found on processes from the postsynaptic cell which penetrate the intervening sheath and end at the membrane of the presynaptic cell (Martin, 1977).

TABLE I

MEANS AND STANDARD DEVIATIONS OF FACILITATION PARAMETERS IN GIANT AND NON-GIANT SYNAPSES OF *L. brevis* AND *L. pealei*

Synapse	n	T	$\bar{F}_1 \pm$ (SD)	$\hat{T}_1 \pm$ (SD)	T_{max}
		°C		ms	ms
L. brevis					
Giant	9	20°	1.5 ± (1.2)	5.1 ± (1.9)	15–20
Non-giant	13	20°	0.9 ± (0.9)	10.6 ± (4.4)	15–20
		$\bar{F}_1$ giant > $\bar{F}_1$ non-giant significant at 0.05 level			
		$\hat{T}_1$ giant < $\hat{T}_1$ non-giant significant at 0.05 level			
L. pealei					
Giant	19	20°	1.2 ± (0.7)	6.5 ± (3.6)	10–15
	8	15°	0.9 ± (0.5)	9.5 ± (2.8)	20–25
	1	12°	1.6	11.9	—
Non-giant	3	20°	0.6 ± (0.1)	10.3 ± (2.9)	—
	2	15°	0.7 ± (0.8)	12.3 ± (0.6)	—
		$\bar{F}_1$ giant > $\bar{F}_1$ non-giant significant at 0.05 level			
		$\hat{T}_1$ giant < $\hat{T}_1$ non-giant significant at 0.05 level			
		$\hat{T}_1$ 20°C < $\hat{T}_1$ 15°C significant at 0.05 level			

Means determined from preparations bathed in $[Ca^{++}]_o$ from 2.5–6 mM and $[Mn^{++}]_o$ from 0–6 mM. Temperature ± 1°C. n = number of preparations tested. Significant differences determined by Student's t test for *L. brevis* and by Multiple Comparisons for nonbalanced two-way classification (Ferguson, 1967) for *L. pealei*. F_1, T_1 determined by regression analysis.

Effects of Repetitive Stimulation on Facilitation

Short trains of stimuli were delivered to giant or non-giant presynaptic axons and responses monitored in the largest postsynaptic giant cells. Facilitation was measured by comparing the size of successive PSP's (V_n) to the size of the first PSP of the train (V_0):

$$f_n = \frac{V_n - V_0}{V_0}. \tag{3}$$

The rate of growth of facilitation during a train and the maximum facilitation attained were both greater at higher frequencies of stimulation (Figs. 2, 3, 4).

Facilitation was found to accumulate during repetitive stimulation in a manner indicating that increases in facilitation become smaller and smaller. As an aid in describing the accumulation of facilitation, we compared our data to predictions of a linear summation model devised by Mallart and Martin (1967) and a similar model which assumes that transmitter release is a power function of the concentration of some substance, such as calcium, which accumulates during a train of impulses (Katz and Miledi, 1968; Rahamimoff, 1968; Miledi and Thies, 1971). The predictions (Figs. 2-4) of the various models were derived from equations presented in Mallart and Martin, 1967; Linder, 1973; Younkin, 1974; and Zucker, 1974, and utilize the parameters F_1 and T_1 determined using the twin-pulse paradigm.

In Figs. 2-4 the observed growth of facilitation during short trains of stimuli is plotted and compared to the predicted growth (solid line) calculated from the linear summation model. Predictions of the power models are mentioned in the figure legends. In Fig. 2 at a stimulus interval (Δt) of 5 ms, the linear model predicted $f = 1.5$ at $t = 35$ ms whereas the models with powers of 2, 3, or 4 predicted $f = 1.80, f = 2.07,$ and $f = 2.16$, respectively. The observed facilitation was 1.5. At a longer stimulus interval $(\Delta t = 10)$, the prediction of the linear model fell short of the observed facilitation at $t = 40$ ms, perhaps because the second component of facilitation added significantly to the summed first component of facilitation. Facilitation was accurately predicted by the linear model at $t = 10, 20, 30$ ms, but the power models predicted too much facilitation.

Fig. 3 shows an experiment on an *L. pealei* giant fiber in which the rise in facilitation predicted by the linear model was greater than the observed data at $\Delta t = 6$ ms. This deviation can be explained by the fact that the amount of facilitation detected in this preparation at short intervals (6 ms) was somewhat depressed (Fig. 3 A) and should therefore not sum to as high a level as predicted using F_1 and T_1 calculated from longer pulse intervals. It should be noted that the summation $\Delta t = 10$ ms was accurately predicted by the linear model, but the power models all predicted too much facilitation. Similarly in Fig. 4, the power models predicted more facilitation than was actually observed.

We ignored the delayed component of facilitation (F_2) and its subsequent slow decay (T_2) because it should contribute little to summation during short trains of stimuli which end about $t = T_{max}$. We could not determine F_2 and T_2 with the same accuracy as we could F_1 and T_1. Because of this and other technical

limitations, we were not able to determine whether F_2 accumulated in the same manner as F_1 or whether F_2 summed linearly with F_1.

Effects of Changes in Temperature on Facilitation

Changes in temperature could either increase or decrease F_1 in any one preparation (Fig. 3). However, the average value of F_1 ($\bar{F}_1$) obtained from several preparations at 20°C was not significantly different from that at 15°C.
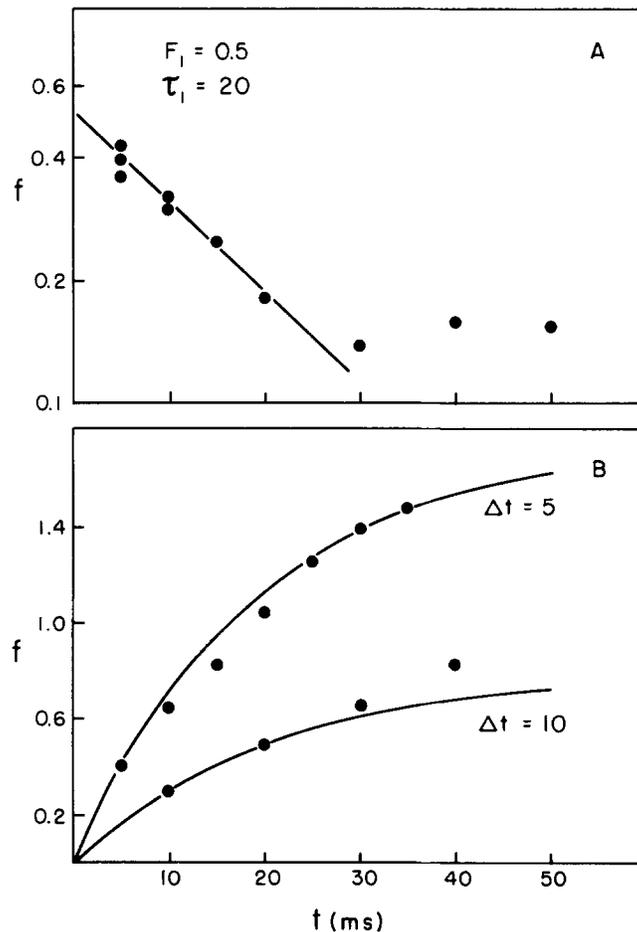

FIGURE 2. (A) Decay and (B) summation of facilitation in an *L. brevis* non-giant synapse. Solid lines in (B) are the predicted curves from the linear summation model using $F_1 = 0.51$, $T_1 = 20$ found in (A) for stimulus intervals of (lower) 10 ms and (upper) 5 ms. At $\Delta t = 5$ ms, $t = 35$ ms, power models predicted that f should equal 1.80, 2.07, and 2.16 for powers of 2, 3, and 4, respectively. At $\Delta t = 10$ ms, $t = 40$ ms, the predictions of the power models were 0.72, 0.75, and 0.76 for powers of 2, 3, and 4, respectively. At $t = 20$ ms, the predictions were 0.50, 0.51, and 0.52. (A) and (B) from the same synapse. $[Ca^{++}]_o = 3$ mM, 20°C.

Reduction of temperature always caused an increase in the time constant of decay of facilitation in L. pealei giant synapses. These results are summarized in Table I. Owing to the increase in T_1, the average facilitation at twin-pulse intervals of 10 ms was 33% greater at 15°C than at 20°C in L. pealei giant synapses
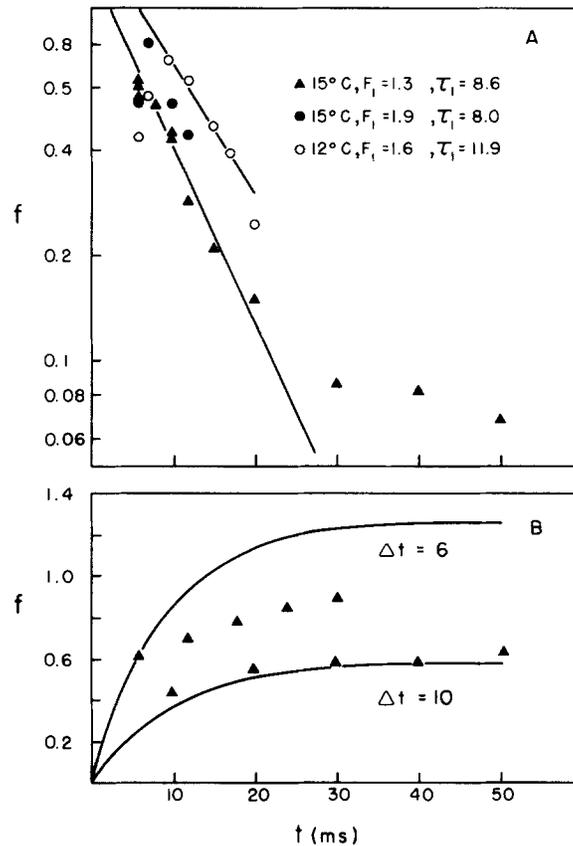

FIGURE 3.   (A) Decay of facilitation at 15°C (▲, ●) and 12°C (○) in a giant synapse of L. pealei. Experimental sequence was 15°C → 12° → 15° (●). (B) Summation of facilitation (▲) during trains of stimuli at intervals of 6 ms ($\Delta t = 6$) and 10 ms ($\Delta t = 10$) during the first 15°C experiment above. Solid lines represent growth of facilitation predicted by the linear summation model using $F_1 = 1.3$, T = 8.6 from (A). At $\Delta t = 6$ ms, $t = 30$ ms, the predictions for f using the power models were 1.46, 1.56, and 1.62 for powers of 2, 3, and 4, respectively. For $\Delta t = 10$ ms, $t = 40$ ms, the predictions for f were 0.62, 0.63, and 0.64 for powers of 2, 3, and 4, respectively. (A) and (B) from same synapse. $[Ca^{++}]_o = 5$ mM, $[Mn^{++}]_o = 4$ mM.

despite the fact that the average values of F_1 at 15°C and 20°C were not significantly different (Table I).

The changes in slope (hump) of the decay curve occurred earlier ($t = 10$–15 ms at 20°C) at warm temperatures than at colder temperatures ($t = 20$–25 ms at 15°C) in L. pealei giant synapses (Table I). In some preparations facilitation was reduced at short intervals at cold temperatures (Fig. 3).

Effect of Changes in Ionic Concentrations on Facilitation

An increase in $[Ca^{++}]_o$ or decrease in $[Mn^{++}]_o$ was always accompanied by large increases in PSP amplitude, but changes in facilitation parameters (F_1, T_1) under these conditions were not consistent between preparations. For example, in three experiments an increase in $[Ca^{++}]_o$ or decrease in $[Mn^{++}]_o$ led to an
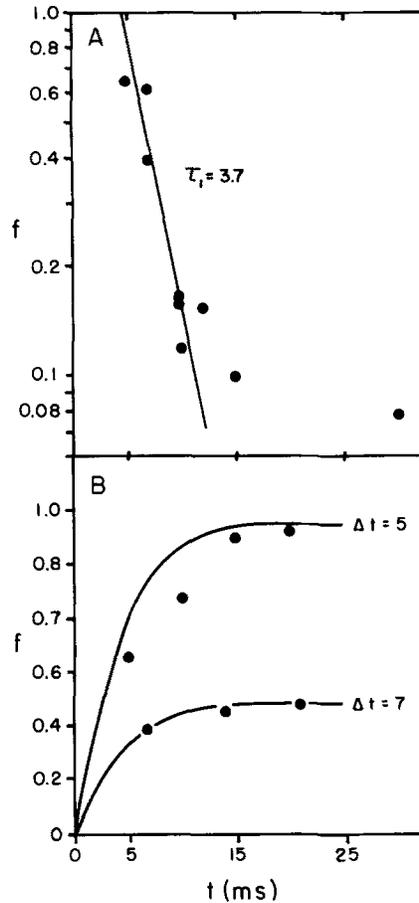

FIGURE 4. (A) Decay of facilitation in an *L. pealei* giant synapse. $F_1 = 2.7$, $T_1 = 3.7$. (B) Summation of facilitation in same synapse. Solid lines represent the linear summation prediction. For $\Delta t = 5$ ms, $t = 20$ ms, the predictions of the power models were 1.01, 1.04, and 1.06 for powers of 2, 3, and 4, respectively. For $\Delta t = 7$ ms, $t = 21$ ms, the predictions of the power models were 0.490, 0.495, and 0.498 for powers of 2, 3, and 4, respectively. (A) and (B) from the same synapse. $[Ca^{++}]_o = 6$ mM, $[Mn^{++}]_o = 2$ mM, 19.6°C.

increase in F_1 and a decrease in T_1; in one preparation there were a small increase in F_1 and little apparent change in T_1; yet another preparation showed a decrease in F_1 and an increase in T_1. Relatively few experiments involving change in ion concentration were attempted because the equilibration time for these changes was often as long as $1\frac{1}{2}$ h.

DISCUSSION

Facilitation after a Single Impulse

Our data indicate the facilitation produced during an action potential and its subsequent decay in squid giant and non-giant synapses are qualitatively and quantitatively similar to that found at many synapses in other organisms (Table II). The values for zero-time facilitation (F_1) in the squid are close to those found in frog neuromuscular junction. The decay of facilitation is somewhat

TABLE II

COMPARISON OF INITIAL FACILITATION PARAMETERS IN VARIOUS PREPARATIONS

Animal	Synapse	Temp.	F_1	T_1	Authors
		°C			
Frog	NMJ	22	1.27	35	Mallart and Martin, 1967
Frog	NMJ	20	0.8	50	Magleby, 1973 a
Frog*	NMJ	19–23	0.7–1.1	12	Rahamimoff, 1968
Toad	NMJ	21	2.0	34	Balnave and Gage, 1970
Toad	NMJ	19	2.7	45	Balnave and Gage, 1974
Guinea pig	SCG	37	0.4	200	McLachlan, 1975
Crab	NMJ-FA	8	2.9	8.5	Linder, 1973
Crab	NMJ-SA	8	2.9	12.4	Linder, 1973
Crayfish	NMJ-SC	20	3.3	19	Zucker, 1974
Crayfish	NMJ-SC	20	4.6	32	Bittner and Sewell, 1976
Crayfish	NMJ-SD	20	1.8	180	Bittner and Sewell, 1976
Crayfish	NMJ-SD	17	0.5–4.0	>160	Linder, 1974
Crayfish	NMJ-SC	17	2.7	20–30	Linder, 1974
Monkey	CMS	37	0.85	10	Muir and Porter, 1973
Squid	Non-giant	20	0.74	10.5	–average of both species
Squid	Giant	20	1.35	5.8	–average of both species

* Initial decay replotted on semilog coordinate gives approximate straight line.
Abbreviations: NMJ, neuromuscular junction; SCG, superior cervical ganglion; FA and SA, fast axon and slow axon; SD and SC, superficial distal and central muscle fibers; CMS, corticomotorneuronal synapses.

faster in the squid than in some other organisms, but is probably slower than the decay in crab neuromuscular junctions at comparable temperatures (Linder, 1973). The decay of facilitation followed a dual exponential time-course in the squid as it does in most of the preparations mentioned in Table II.

The decay of facilitation has been described as two components which begin immediately after an action potential (Magleby, 1973 a; Zucker, 1974; Linder, 1973, 1974) and can be represented by:

$$f = F_1 \exp(-t/T_1) + F_2 \exp(-t/T_2) \tag{4}$$

This description would be an oversimplification of much of our data (Figs. 1, 2, and 4) and that of Mallart and Martin (1967) or Bittner and Sewell (1976) who report that the second component of decay is often preceded by a slight additional facilitation so that the composite decay curve displays a hump. Although data from some other preparations do not always show this hump, it might be obscured in experiments where responses are recorded from a population of synapses or if the parameters F_1 and T_1 are calculated, using linear summation assumptions, from the incremental increases in PSP amplitude which accompany repetitive stimulation (Magleby, 1973 a). The delayed onset and the increase in facilitation associated with the second component suggests that the decay of facilitation after a single action potential is more complex than the smooth decay predicted by simple two compartment models.

The two phases of exponential decay found in our study are not apparent in the work reported by Takeuchi and Takeuchi (1962) or by Miledi and Slater (1966). This difference might result from the fact that we used low $[Ca^{++}]_o$ salines which enhance the possibility of detecting facilitation (Mallart and Martin, 1967; Kusano and Landau, 1975) whereas Takeuchi and Takeuchi (1962) and Miledi and Slater (1966) all employed normal $[Ca^{++}]_o$ salines and used repetitive stimulation to depress transmitter release to subthreshold levels, a procedure which might have depleted transmitter stores. Hence, the facilitation measured by Takeuchi and Takeuchi (1962) and Miledi and Slater (1966) may have been complicated by recovery from depression in the interval between twin pulse stimuli.

We should note that Kusano and Landau (1975) have also studied facilitation at the squid synapse in low $[Ca^{++}]_o$. These authors stimulated at fairly long intervals (20 ms) and reported facilitation values of only 0.14 after four stimuli which decayed exponentially with a half-decay time of 20 ms at 16–18°C (time constant of decay about 29 ms). These values agree well with our measurements for the second component of decay in *Loligo* giant synapses at 20°C, in which F_2 values are rather small, begin at $t = 10$–20 ms, and decay with $T_2 = 22$–63 ms. Kusano and Landau (1972) have also detected facilitation at these squid synapses bathed in normal calcium, thereby indicating that this facilitation is not an artifact of low $[Ca]_o^{++}$ solutions.

Growth and Facilitation during Repetitive Stimulation

Repetitive stimulation of presynaptic axons to the stellate ganglion usually produced the increases in facilitation predicted by the linear summation model (Figs. 2–4) devised by Mallart and Martin (1967) to describe facilitation in the frog neuromuscular junction. Our results are generally consistent with the hypothesis that (a) each action potential of a train produces an equal amount of facilitation which (b) decays in a manner similar to that after a single action potential and (c) adds linearly to that produced by previous action potentials. This simple model appears to apply quite well to squid, frog, (Mallart and Martin, 1967; Magleby, 1973 a), and monkey synapses (Muir and Porter, 1973), although some researchers (Younkin, 1974; Barrett and Stevens, 1972; Bennett

et al., 1975), using different paradigms, find that third- and fourth-power models can explain facilitation in frog and rat synapses. In our experiments, the power models usually predict too much facilitation, but the amount of deviation from the data depends on the stimulus frequency and the values of F_1 and T_1. Neither linear nor power models give adequate predictions in crayfish synapses (Zucker, 1974; Bittner and Sewell, 1976).

Depression cannot explain the lack of agreement of our data with the power models because there was no evidence of depression during long trains of stimuli at frequences similar to those used for testing the power models. Also, there was no evidence of recovery after the end of a train.

Finally, our experiments only examined the summation of facilitation due to the first component of facilitation (F_1, T_1). With longer duration of stimulation, our analysis would have been complicated by the second component of facilitation (F_2, T_2) and by the possible introduction of additional potentiating factors such as those reported by Mallart and Martin (1967) and Magleby (1973 a, b) at frog synapses.

Effect of Temperature on Facilitation

Although the absolute amount of facilitation at zero time (F_1) is, on the average, not affected by changes in temperature, the decay of facilitation proceeds at a slower rate as temperture is lowered (Fig. 3). The extrapolated Q_{10} of the average time constant $(\bar{T}_1)$ of the first component (between 20°C and 12°C) is about 2, a value which is approximately one-half that found by Balnave and Gage (1974) in the toad neuromuscular junction. Since most diffusion processes have a Q_{10} much less than 2 (Giese, 1962), it is unlikely that the decay of facilitation is brought about solely by free diffusion of an activating agent (such as calcium ions) away from its site of action. Although F_1 is not significantly different at 20°C and 15°C, the observed facilitation at 5–30 ms after the conditioning pulse is usually larger at the lower temperature because the time constant of decay is greater.

Transmitter release is generally reduced at low temperature (see Weight and Erulkar, 1976, and references therein) but the decay of facilitation is prolonged. Thus, during repetitive activity at low temperatures, facilitation could help to maintain transmitter release at a high level in some animals. However, the adaptative significance of facilitation in the squid giant fiber system is not entirely clear. Inasmuch as the giant synapse has a safety factor for transmission of action potentials of about 5 (Katz and Miledi, 1967) at normal $[Ca^{++}]_o$, transmitter release would have to be drastically reduced to cause failure of transmission. Furthermore, the cycling rate of mantle contractions (and presumably giant fiber action potentials) is ~ 1 Hz (Arnold et al., 1974), so the facilitation described here would not be expected to sum and affect transmitter release.

Effect of Other Factors on Facilitation

Because spontaneous miniature potentials are difficult to record in squid synapses (Takeuchi and Takeuchi, 1962; Miledi, 1967), we were not able to rule out completely an increase in receptor sensitivity or quantal size during

facilitation in this preparation. However, we suspect that facilitation has a presynaptic origin in squid synapses because of the many similarities between facilitation in squid synapses and facilitation in frog, toad, and crayfish synapses where postsynaptic mechanisms have been eliminated as the cause of facilitation (del Castillo and Katz, 1954; Dudel and Kuffler, 1961; Bittner, 1968; Bittner and Harrison, 1970; Balnave and Gage, 1974).

Facilitation appears to be dependent on the presence of extracellular calcium ions (Katz and Miledi, 1968; Younkin, 1974), and it has been proposed that some of the calcium which enters a nerve terminal during an action potential lingers in the terminal and thus increases the probability of transmitter release during a subsequent action potential (Katz and Miledi, 1968; Rahamimoff, 1968, 1973; Miledi and Thies, 1971; Rahamimoff and Yaari, 1973). (For a recent review of the residual calcium theory, see Atwood, 1976.) Some authors have indicated that this residual calcium theory predicts that increases in $[Ca^{++}]_o$ should be accompanied by decreases in facilitation. This result has been found in frog synapses at certain levels of transmitter release and at certain stimulus intervals (Rahamimoff, 1968, 1973; Mallart and Martin, 1968). However, changes in $[Ca^{++}]_o$ seem to have little or no affect on facilitation in crayfish or crab neuromuscular junctions (Linder, 1973; Zucker, 1974) and we found no clear-cut effects when $[Ca^{++}]_o$ or $[Mn^{++}]_o$ were varied to give 2- to 12-fold increases in PSP amplitude at squid synapses. Hence, our results do not appear to support the residual calcium theory.

Some facilitation could also be due to a secondary release of calcium from intracellular stores such as mitochondria and subsurface cisternae which are known to have calcium binding sites (Landis et al., 1973; Llinas and Nicholson, 1975; Baker et al., 1971). A similar mechanism may cause release of calcium from sarcoplasmic reticulum in muscle cells (Ford and Podolsky, 1970; Endo et al., 1970) and from photoreceptor outer segments (Hendricks et al., 1974). Such a release might be dependent on extracellular calcium and could produce the second component of facilitation (the hump) by a delayed release of Ca^{++}. This secondary release of Ca^{++} might be small at $t = 0$ and then accelerate to a maximum after which Ca^{++} could be removed by an uptake mechanism to yield the slow decay (T_2) of facilitation.

Other mechanisms that might account for facilitation include progressive changes in the voltage of successive action potentials and (or) their associated ionic currents. In the following paper (Charlton and Bittner, 1978), we examine the possibility that changes in presynaptic action potentials can explain most of the observed facilitation.

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