J. Physiol. (1975), **245**, *pp.* 13–32
With 6 text-figures
Printed in Great Britain

DEPRESSION AND RECOVERY OF TRANSMISSION AT THE SQUID GIANT SYNAPSE

BY K. KUSANO AND E. M. LANDAU*

From the Marine Biological Laboratory, Woods Hole, Massachusetts, and the Department of Biology, Illinois Institute of Technology, Illinois, U.S.A.

(*Received 23 November 1973*)

SUMMARY

1. The process of synaptic depression and recovery were studied in the squid (*Loligo pealii*) giant synapse with intracellular recording and stimulating electrodes in the presence of tetrodotoxin (10^{-7} M).

2. When the synapse was stimulated at 50 Hz, depression occurred rapidly. Recovery after the tetanus was a first-order process with an average time constant of 4·9 sec. The rate of recovery was independent of the amplitude of the post-synaptic potential (p.s.p.) or the degree of depression.

3. For the first five to seven p.s.p.s in the train there was a linear relationship between depression and the total amount of transmitter previously released. This may indicate that depression in this preparation was caused by the depletion of the presynaptic store of transmitter (S).

4. Assuming that this interpretation was correct, we could show that recovery from depression during the tetanus (i.e. 'mobilization') proceeded about 10 times faster than after the end of the tetanus.

5. When the amplitude of the p.s.p. was varied by changing the bathing calcium concentration, [Ca], the degree of depression was correlated to the amplitude of the p.s.p.

6. When the amplitude of the p.s.p. was increased by increasing presynaptic depolarization, synaptic depression was found to increase as well. However, synaptic depression increased less than the amplitude of the p.s.p., the relationship between these two measures being non-linear.

7. This finding is interpreted to indicate that the transmitter stores, S, are closely related to the *area* of the presynaptic membrane which is sufficiently depolarized to release transmitter.

* Permanent address: Department of Physiology and Pharmacology, Tel-Aviv University Sackler School of Medicine, Tel-Aviv, Israel.

INTRODUCTION

A phenomenon encountered at many synapses is that of synaptic depression. When the synapse is stimulated repetitively, particularly under conditions of high quantal release, subsequent post-synaptic potentials (p.s.p.s) become smaller, recovering slowly after the end of the stimulation. This has been found at various neuromuscular preparations (Eccles, Katz & Kuffler, 1941; Lundberg & Quilisch, 1953; Liley & North, 1953; Thies, 1965; Elmqvist & Quastel, 1965*b*; Bruner & Kennedy, 1970) and also at synapses on to guinea-pig olfactory cortex cells (Richards, 1972), cat lateral geniculate neurones (Bishop, Burke & Hayhow, 1959), cat dorsal spino-cerebellar tract cells (Eccles, Oscarsson & Willis, 1961), cat spinal motor neurones (Curtis & Eccles 1960) sympathetic ganglion cells in cat (McCandless, Zablocka-Esplin & Esplin, 1971), guinea-pig (Bennett & McLachlan, 1972*a*, *b*) and rabbit (Eccles, 1955). Depression is also encountered at synapses on to crayfish tactile interneurones (Zucker, 1972), lobster cardiac ganglion cells (Hagiwara & Bullock, 1957), leech segmental ganglion motoneurones (Nicholls & Purves, 1972), *Aplysia* central neurones (Wachtel & Kandel, 1967; Castellucci, Pinsker, Kupferman & Kandel, 1970; Gardner & Kandel, 1972) and at synapses on to giant neurones in cockroach (Callec, Guillet, Pichon & Boistel, 1971), *Aplysia* (Bruner & Tauc, 1966), hatchet fish (Auerbach & Bennett, 1969; Highstein & Bennett, 1973) and squid (Bullock & Hagiwara, 1957; Bryant, 1958; Takeuchi & Takeuchi, 1962; Katz & Miledi, 1967; Horn & Wright, 1970). A number of studies have been devoted to examining the ionic dependence of depression (Thies, 1965; Elmqvist & Quastel, 1965*b*; Hubbard, Jones & Landau, 1971) and the effects on it of temperature variations (Hubbard *et al.* 1971), of drugs (Hubbard & Wilson, 1970; 1973) and of conditioning presynaptic polarization (Hubbard & Willis, 1962, 1968).

We now present a study of this phenomenon at the squid giant synapse, where the relationship between presynaptic depolarization and depression could be examined. In particular, we have been interested in the rate of mobilization of transmitter during repetitive stimulation (tetanus) and have found evidence for the assumption that such mobilization proceeds much faster than mobilization after the end of the tetanus. We have also been interested in the relationship between synaptic depression and the amplitude of the post-synaptic potential (p.s.p.), the latter being varied by changing the level of presynaptic depolarization. We have found that although both depression and the PSP increase with presynaptic depolarization, the former increases less than the latter, the relationship between the two being distinctly non-linear. This finding is interpreted in terms of the existing depletion models (Elmqvist & Quastel, 1965*b*; Betz, 1970;

Ginsborg, 1970) where each presynaptic depolarizing pulse is assumed to release a fraction (F) of a presynaptic store of transmitter (S) and the depression is then caused by the depletion of S. Our findings lead us to formulate an hypothesis as to the physical meaning of S and F. A preliminary note of our results has been published (Landau & Kusano, 1972).

METHODS

The common squid (*Loligo pealii*), available at the Marine Biological Laboratory in Woods Hole, was used for the experiments. The technique of dissection of the stellate ganglia was essentially similar to that of Bullock (1948). The method of mounting the preparation and the experimental chamber have been described in a previous paper (Kusano, Livengood & Werman, 1967). The recording and the stimulation were performed with intracellular micro-electrodes, filled with 1 M-K citrate. One recording electrode was inserted into the post-synaptic giant axon and two electrodes, one for recording and the other for the application of current, were inserted into the presynaptic giant nerve within the terminal area and were separated by no more than 300 μm. The synapse was stimulated by single pulses or short tetanic trains delivered from a Grass S-48 stimulator. The pulse duration was either 0·5 or 5 msec. The biological signals were amplified and recorded using conventional electrophysiological equipment. Bathing solutions were as follows.

(1) Natural sea water (NSW). At the beginning of the experiments the preparation was always perfused with filtered natural sea water. The pH was 7·5.

(2) Artificial sea water (ASW). This was of the following composition: NaCl, 423 mM; KCl, 9 mM; $CaCl_2$, 10 mM; $MgCl_2$, 23 mM; $MgSO_4$, 25 mM; $NaHCO_3$, 2 mM; Tris buffer, 10 mM. The pH was 7·5–7·8. In a few experiments the calcium concentration was reduced to 5 and 2·5 mM. Tetrodotoxin was added in all experiments (up to 10^{-7} M) and the temperature of the medium was kept at 16–18° C.

All post-synaptic potential (p.s.p.) amplitudes were corrected for non-linear summation (Martin, 1955), assuming a transmitter reversed potential of +20 mV (Miledi, 1969).

RESULTS

Some observations on synaptic facilitation at the squid giant synapse

To induce synaptic depression, short tetanic trains were produced by presynaptic stimulation. In a preliminary experiment we found that the depression produced by repetitive stimulation was maximal at stimulation rates between 25 and 75 Hz and we therefore chose to do most of our tetanizations at a stimulation rate of 50 Hz. At lower stimulation rates there was evidence for recovery from depression between pulses (see Fig. 2 and the next section). At higher rates (up to 200 Hz) the depression was less, probably because of the effect of synaptic facilitation.

Facilitation is the short-term increase in transmitter release that is found upon stimulation of a synapse with closely spaced stimuli, and is superimposed on the processes of depression and recovery from depression (Mallart & Martin, 1968). This phenomenon has been found at the squid giant synapse by a number of workers (Takeuchi & Takeuchi, 1962; Miledi

& Slater, 1966; Bloedel, Gage, Llinas & Quastel, 1966; Katz & Miledi, 1967). We studied the phenomenon in three preparations equilibrated for 15–30 min in low calcium solutions (5–2·5 mM), where depression but not facilitation should be reduced or absent (see Fig. 4 and also Hubbard *et al.* 1971). In two preparations we studied short tetani (four p.s.p.s) and in the third we observed longer tetani (16 p.s.p.s). The rate of stimulation was 50 Hz. The facilitation of the *i*th p.s.p., FT_i, was defined as

$$FT_i = \frac{V_i - V_1}{V_1},$$

where V_1 and V_i were the amplitude of the first and the *i*th p.s.p. of the tetanus, respectively. This was the total facilitation that had been contributed by the preceding p.s.p.s. The total facilitation of the fourth p.s.p. in these experiments was on the average $0{\cdot}14 \pm 0{\cdot}02$ (S.D.). In the experiment where we examined long tetani, the facilitation of the 16th p.s.p. was 0·26. If each impulse is assumed to contribute a residual facilitation, f_i, whose magnitude and time course are identical for each impulse in the train, and these residual facilitating effects are assumed to sum linearly to form the total facilitation, FT_i, then it is possible to compute the residual facilitation function from the total facilitation according to the method of Magleby (1973). Using this method on the long tetani in low calcium we found that the time course of decay of residual facilitation was approximately exponential with a half-decay time of 20 msec. There was no evidence for a late phase of facilitation, residual facilitation becoming less than 0·02 after about 100 msec. In these experiments we also elicited test pulses at 100 msec or 1 sec after the end of the tetanus, demonstrating that synaptic depression was very small or absent.

It could be concluded that facilitation at this synapse, although small, was not negligible. Therefore, to study depression unaffected by facilitation we usually elicited a test pulse at 100 msec after the end of the tetanus, when facilitation was almost over. However, we now had to study the time course of recovery after the tetanus in order to estimate how much recovery may have occurred by the time of the test pulse.

The time course of recovery from synaptic depression

When a short train (tetanus) of depolarizing current pulses (Fig. 1, bottom traces) was applied to a presynaptic terminal in normal [Ca] at a rate of 50 Hz, a corresponding train of presynaptic depolarizing potentials was produced (Fig. 1, upper traces). These in turn resulted in a series of p.s.p.s (Fig. 1, middle traces). It was evident that when their amplitude was large (Fig. 1*D–F*, high level of transmitter release), consecutive p.s.p.s diminished rapidly, without there being a corresponding

change in the amplitude of the presynaptic pulses. The recovery from this depression was studied by applying test pulses at various intervals after the tetanus. After each tetanus only one test pulse was applied (Fig. 1) and therefore the whole sequence had to be repeated many times in order to construct the time course of recovery as shown (Fig. 2*A*, *B*). In this figure both the depression within the conditioning train and the recovery were drawn for two levels of transmitter release at the same synapse. The

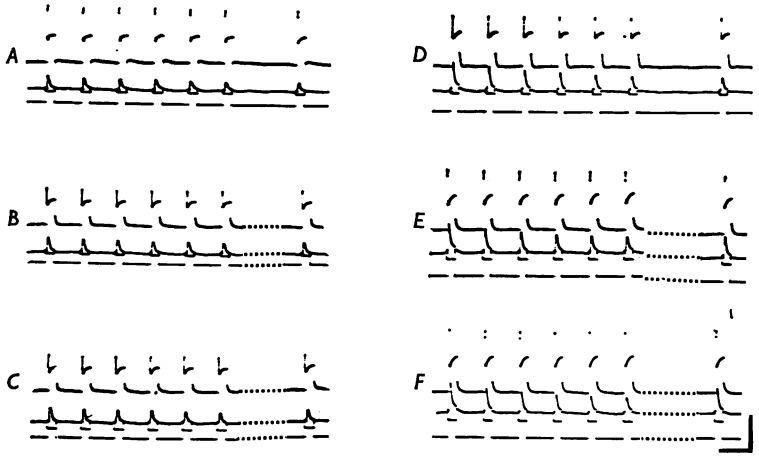

Fig. 1. Synaptic depression and recovery. The consecutive p.s.p.s in a tetanic train at 50 Hz are shown together with a test pulse. The upper line in each drawing represents the presynaptic depolarizing pulse, the middle line represents the p.s.p. and the bottom line shows the current pulse applied to the presynaptic electrodes. The presynaptic depolarizations in the left row were small and in the right row were large. The test intervals for both the rows from above downward were 50 msec, 0·9 sec and 4 sec. The vertical bar represents 34 mV or 4×10^{-6} A and the horizontal bar represents 20 msec.

control p.s.p. was 31·7 mV in Fig. 2*A* and 13·7 mV in Fig. 2*B*. The experiment was repeated in four other preparations.

In all the experiments the recovery appeared to proceed with an exponential time course. However, in two out of five experiments we had the impression that the p.s.p.s recovered at first to only 98 % or 99 % of the control level and that the last few per cent of recovery occurred with a much longer time constant. We fitted the data by eye to the following expression:

$$V_t = V_1 - (V_1 - V_D) \exp -t/T, \quad (1)$$

where V_1, V_D and V_t were the amplitudes of the control, 'zero time' test and test p.s.p.s respectively, and t and T were the time and time constant of recovery. The value of T in Fig. 2*A* (high level of release) was 3·2 sec

and in Fig. 2*B* (low level of release) was 6 sec. On the other hand, in a second similar experiment, *T* was shorter at the low level of release than at the high level (4·6 *vs.* 5·6 sec). In a third experiment the time constant of recovery was equal for both release levels (5·5 sec). In the remaining two experiments the time course of recovery was examined at only one level of release. In our experiments, therefore, there was no clear relationship between the rate of recovery from depression and the level of transmitter release, or the degree of synaptic depression. This finding was

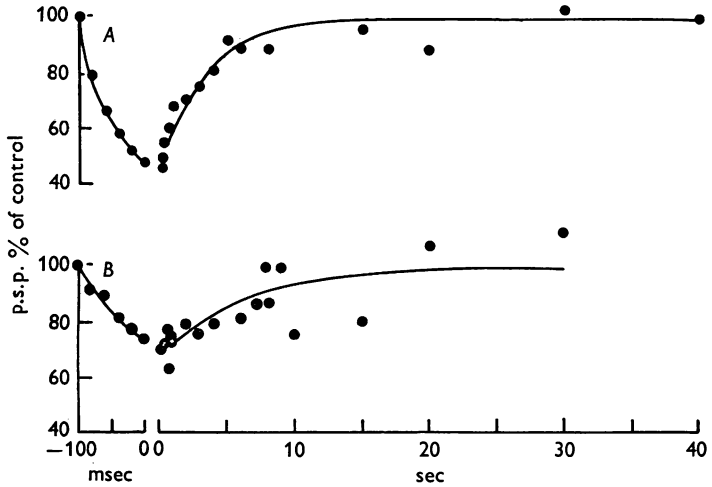

Fig. 2. The time course of recovery from synaptic depression. In the control p.s.p. was 31·7 mV and in *B*, 13·7 mV. Both experiments were performed at the same synapse. The first six points in each curve represent the average p.s.p. values in a short tetanic train at 50 Hz. The other points, drawn on a different time scale, represent the test p.s.p.s various times after the end of the tetanus. The p.s.p. amplitudes are expressed relative to the control p.s.p. The solid lines in the recovery period were drawn according to eqn. (1). In *A*, *T* was 3·2 sec, and in *B*, 6 sec.

consistent with those of Thies (1965) and Betz (1970) in two different nerve–muscle preparations. Altogether, we performed five recovery experiments with a total of eight time courses. The *t* values ranged between 1·5 and 6·7 sec with a mean and standard deviation of $4{\cdot}9 \pm 2{\cdot}4$ sec. These values were again very similar to those found by Thies (1965), Betz (1970) and Christensen & Martin (1970) in neuromuscular preparations.

Finally we examined in detail the recovery of p.s.p. amplitudes within the first 100 msec after the end of the tetanus. This was done by producing a conditioning train of p.s.p.s at 50 Hz, followed by a test p.s.p. occurring after 100 msec. The number of stimuli in the conditioning tetanus was increased stepwise from 1 to 8 (one experiment) or from 1 to 15 (two

experiments). The test p.s.p. after a given train (test interval: 100 msec) could then be compared to the last p.s.p. of the next train (test interval: 20 msec). We found in three experiments that the p.s.p.s coming after 100 msec were consistently a few per cent (average: 3·8 %, range, 2–7 %) larger than those coming after 20 msec. This increase could fully be explained by the slow process of recovery that has just been described. Even at the longest trains, we never found the p.s.p.s at 20 msec to be larger than the corresponding p.s.p.s at 100 msec. In only one time-course experiment did we study test p.s.p.s at shorter intervals of 6–11 msec after the tetanus (50 Hz, three p.s.p.s). The p.s.p.s at 6–11 msec were indeed slightly larger than those at 88–160 msec, the average difference being 6 % for the large p.s.p.s (31 mV) and 16 % for the smaller ones (27 mV). The difference between the early and late test p.s.p.s was significant at the 0·01 level (*t* test) only for the smaller p.s.p.s.

Our failure to detect facilitation at test intervals of 20 msec may indicate either that facilitation was decreased in normal Ca concentration (cf. Mallart & Martin, 1968; Rahamimoff, 1968), or that there was an early phase of rapid recovery from depression which cancelled the effects of facilitation. We cannot, at present, distinguish between these two possibilities.

The relationship between synaptic depression and the amount of transmitter released previously

One explanation of synaptic depression is the 'depletion hypothesis', according to which depression is produced by the depletion of an immediately available store of quanta (*S*) (Liley & North, 1953; Thies, 1965; Elmqvist & Quastel, 1965*b*). Elmqvist & Quastel predicted that if the fraction (*F*) of the store released by each impulse was constant and mobilization into the store was negligible, the depression (*D*) defined as

$$D = 1 - V_t/V_1 \qquad (2)$$

should be linearly related to the amount of transmitter released previously. Here V_1 was the amplitude of the control p.s.p. and V_t was that of the test p.s.p. They found that in the human neuromuscular junction, this prediction was true for the first four to seven end-plate potentials (e.p.p.s) but later e.p.p.s deviated from the predicted linear relationship presumably due to mobilization of quanta into the store. Betz (1970) and Christensen & Martin (1970) made a strong case for a non-linear model of depression where both '*F*' and '*S*' become reduced during repetitive stimulation. According to this model, depression should be defined as

$$D' = 1 - \sqrt{V_t/V_1} \qquad (3)$$

for the same prediction to hold.

Both these predictions are based on the assumption that depression reflects the depletion of the initial presynaptic store of quanta. The depletion can be defined as $1-S_i/S_1$, where S_1 and S_i are the store existing before the first and ith stimuli, respectively. This is the fraction of the initial store that has been released before the ith stimulus and should therefore be proportional to the total amount of transmitter released by the first $i-1$ stimuli, provided no mobilization had occurred. The relationship between depression and depletion is derived as follows. First, the quantum content of the p.s.p., m, is equal by definition to $F \times S$. If we assume that $V_i/V_1 = m_i/m_1$, then the depression according to the linear model and equation (2) is

$$1-\frac{FS_i}{SF_1} = 1-\frac{S_i}{S_1}.$$

According to the non-linear model, $F_i/F_1 = S_i/S_1$ (Betz, 1970), and therefore the depression according to eqn. (3) is again

$$1-\sqrt{\frac{F_iS_i}{F_1S_1}} = 1-\frac{S_i}{S_1}.$$

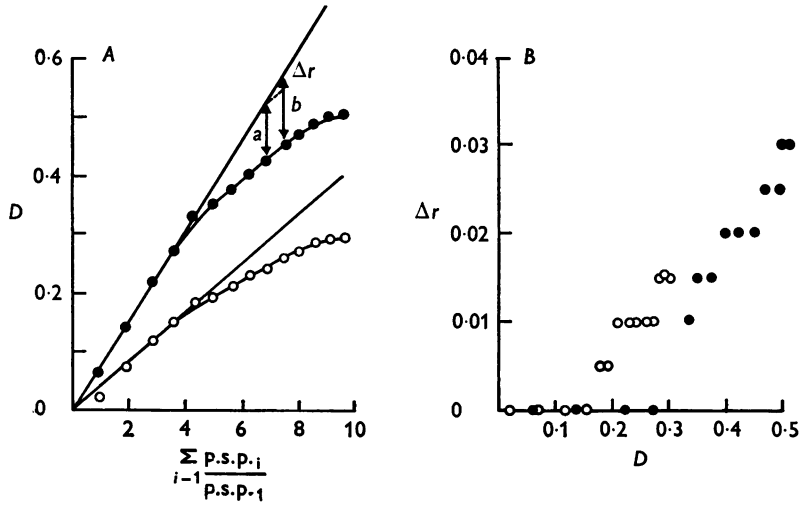

Fig. 3. The relationship between synaptic depression, previously released transmitter and recovery within the period of the tetanus. *A* shows the relationship between the depression (ordinate) (filled circles – according to eqn. (2); open circles – according to eqn. (3)) and the sum of all previous p.s.p.s normalized to the control p.s.p. value (abscissa). Lines were drawn by eye. The vertical lines show how the increment of recovery, Δr, was derived from these data. For explanation, see text. *B* shows the recovery increments, Δr_i (ordinate), plotted against the depression; D_i (abscissa) defined according to eqn. (2) (filled symbols) or (3) (open symbols).

It was clearly of interest to examine these predictions at the squid giant synapse. An example of such an experiment is shown in Fig. 3*A*. Here the D (filled circles) and D' (open circles) values for each p.s.p.s of a tetanic train (at 50 Hz) were plotted against the sum of all previous p.s.p.s. It can be seen that the predicted linearity was indeed found for the first six

p.s.p.s, no matter what definition of depression we have chosen. Thus, we could not say from our data that one definition was preferable to the other. Moreover, the fact that predictions from the 'depletion hypothesis' could be substantiated in the squid giant synapse did not prove that this was the mechanism of synaptic depression in the squid. However, these findings did point to a great similarity between the depression in the squid and at various neuromuscular preparations. It also induced us to interpret our data with special reference to these two depression models.

The present analysis did not consider the possible occurrence of facilitation together with the depression. Facilitation is believed to increase F, the fraction each pulse releases from the store (Mallart & Martin, 1968), and would thus lead to an underestimation of the depletion $(1-S_i/S_1)$. One could correct for the effect of facilitation by dividing each p.s.p. by the appropriate facilitation factor $(1+FT_i)$ derived from the experiment mentioned in the first section. The effect of this correction would be to increase the initial slope of the curves of Fig. 3*A* (by about 40 %) and to elevate the final level of depression (by about 20 %). However, the general shape of the curves would remain unaltered. We did not introduce this correction into the results because facilitation in normal [Ca] could be less than that found in low [Ca].

Recovery of transmitter stores during the tetanus

If either of the two depression models, linear or non-linear, were applicable, then the deviation from the straight-line relationship should indicate the amount of accumulated recovery or 'mobilization' (r). The *rate* of recovery would then be given by the increment in recovery (Δr), that had occurred between a given p.s.p. and the next, divided by the appropriate time interval.

The method of derivation can be explained as follows: the store, S_i, existing before the ith stimulus can be described as:

$$S_i = S_1 - \sum_{i-1} m_i + k_i, \tag{4}$$

where S_1 and S_i are the initial store and the store preceding the ith stimulus respectively, m_i is the quantal content of the ith p.s.p. and k_i the total number of quanta that have been mobilized into the store before to the ith stimulus. Another equation describing S_i is

$$S_i = m_i/F \tag{5}$$

assuming a constant release fraction, F, according to the linear model. Combining eqns. (4) and (5), dividing by m_1 and rearranging, we obtain

$$1-\frac{m_i}{m_1} = F\frac{\sum_{i-1} m_i}{m_1} - \frac{k_i}{S_1}. \tag{6}$$

However, if the amplitude of the quantum is assumed to be constant, then $m_i/m_1 = V_i/V_1$, and the left-hand side of eqn. (6) equals the observed depression ($D_{i,\,obs}$) according to eqn. (2). The second term in equation (6),

$$F\sum_{i-1} m_i/m_1,$$

represents the expected depression, $D_{i,\,exp}$, i.e. the depression that would have been caused by the first i–1 stimuli had there been no mobilization. This is so because this term can be written as

$$\sum_{i-1} m_i/(m_1/F) \quad \text{which equals} \quad \sum_{i-1} m_i/S_1,$$

indicating what fraction of the initial store was released by the first i-1 stimuli.

Finally, k_i/S_1 is r_i, the fraction of the initial store which has been replenished before the i-th stimulus. Therefore eqn. (6) may be rewritten as

$$r_i = D_{i,\,exp} - D_{i,\,obs}. \tag{7}$$

The same equation holds for the non-linear model except that the observed depression must be computed according to eqn. (3), and F in eqn. (6) is equal to F_1. The fraction of the initial store recovered between the $(i-1)$th and the ith stimuli is

$$\Delta r_{i-1,\,i} = r_i - r_{i-1}. \tag{8}$$

The method of graphic determination of r_i and $\Delta r_{i-1,\,i}$ according to eqns. (7) and (8) is shown in Fig. 3*A*. The straight thin line represents the expected depression, $D_{i,\,exp.}$. This is the extension of the initial straight part of the curve which follows the expected relationship between D (or D') and $\sum_i m_i/m_1$ with no added mobilization. The curve represents the observed depression, $D_{i,\,obs}$ and the vertical straight lines, a and b represent the differences between the expected and observed depression for two consecutive p.s.p.s.

Thus, a and b are consecutive estimates of r_i and the difference between them is $\Delta r_{i-1,\,i}$ – the increment in recovery that has occurred between these two consecutive p.s.p.s. The value of $\Delta r_{i-1,\,i}$, divided by the observed D_i, yields the percentage of the store released before the ith stimulus which is recovered between the $(i-1)$th and ith stimuli. When the values of $\Delta r_{i-1,\,i}$ were plotted (Fig. 3*B*) against the corresponding depression values (D_i), the rate of recovery appeared to show a delayed onset. Until three to five p.s.p.s were elicited, no recovery was evident, but after that its rate increased rapidly until a fairly large percentage of the depression was recovered between the 14th and 15th p.s.p.s (6 % for the linear and 5 % for the non-linear model).

The function shown in Fig. 3*B* depends on how the theoretical straight line describing $D_{i,\,exp}$ is drawn in Fig. 3*A*, and the apparent delay in the onset of recovery could conceivably be due to some underestimation of the straight line slope (F) in Fig. 3*A*. This slope has to be increased by less than 10 % to produce a straight line (first order) relationship between recovery and depression in Fig. 3*B*. Such an increase in slope would result in a small increase in the values of $\Delta r_{i-1,\,i}$ and make for

an even higher rate of recovery during the tetanus. On the other hand, the synaptic depression observed during the tetanus would be only slightly smaller than expected, provided only a small number of stimuli is given.

The recovery during the tetanus had a remarkable feature. Its rate was very much higher than the rate of recovery after the end of the tetanus. As shown in Fig. 3*B*, 5–6 % of the depression was recovered between the 14th and the 15th impulse, and the percentage of recovery was largely independent of which model of depression (linear or non-linear) was chosen. A similar percentage of recovery was found in three more experiments, the average value for the linear model being $7{\cdot}9 \pm 3{\cdot}3$ % S.D. (range in four experiments: 5–12·5 %). The slow process of recovery described in the previous section was not sufficient to account for these percentages of recovery. One would predict that only 0·4 % of the depression could be recovered in the first 20 msec after the end of the tetanus, if recovery were a first-order process with a time constant of 5 sec. Clearly the rate of recovery during the tetanus was at least 10 times faster than that.

This conclusion remains valid even if some facilitation is present during the tetanus. When the values of Δr_1 were computed after correcting the p.s.p.s for possible facilitation, the percentage of recovery between the 14th and 15th pulse was somewhat larger, but did not exceed the original estimates by more than 20 %. It would thus appear that during the tetanus a much faster process of recovery was active than that acting after the end of the tetanus. In two experiments we were able to compare the delay in the onset of and the rate of recovery at two different levels of transmitter release at the same synapse. It turned out that both these parameters were independent of the amplitude of the p.s.p.s, and in this respect the fast process of recovery during the tetanus resembled the slower process occurring within the post-tetanic period.

The effect of Ca on the release fraction and synaptic depression

In this experiment we modified the p.s.p. amplitudes by varying the bathing Ca concentration. We measured the synaptic depression produced by a short conditioning train (usually 3–4 pulses, except in one experiment where 16 pulses were given). We computed the depression (D) according to eqn. (2) which is derived from the linear model of Elmqvist & Quastel (1965*b*). We also computed F, the fraction of the store released by the first impulse, according to the model of Betz (1970), employing the following expression (cf. eqn. (6)):

$$F = \frac{1-\sqrt{(V_t/V_1)}}{\frac{\sum_i V_1 - k_1}{V_1}} = \frac{D'}{\frac{\sum_i V_1 - k_1}{V_1}}, \tag{9}$$

where V_t was the test p.s.p. at 100 msec after the tetanus, V_1 was the control p.s.p. and $\sum_i V_i$ was the sum of all p.s.p.s in the conditioning train. k_1 was the total amount of transmitter (in mV) recovered during the conditioning train and in the period preceding the test pulse, and for short trains was set to zero. This was based on the assumptions that the fast process of recovery, attending tetanic stimulation, did not start until a few p.s.p.s had occurred (Fig. 3*B*) and that the slow post-tetanic recovery could have decreased the depression by a few per cent only (Fig. 2).

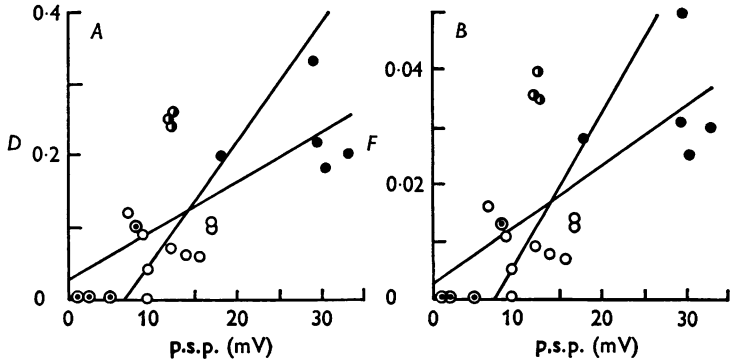

Fig. 4. The relationship between depression (*D*, Fig. 4*A*) or release fraction (*F*, Fig. 4*B*) on the ordinate and the amplitude of the p.s.p. (V_1) of eqn. (2), on the abscissa). Depression was calculated from eqn. (2) and *F* from eqn. (9). Half-filled circles represent p.s.p.s where [Ca] was being reduced from 10 to 5 mM. Filled circles: [Ca] returned to 10 mM. Dotted circles: [Ca] reduced to 5 mM. Open circles: [Ca] returned to 10 mM. The two lines drawn in *A* or *B* represent the regression of *D* or *F* on the p.s.p. and the regression of the p.s.p. on *D* or *F*. The correlation coefficient (r = 0·64) is the geometrical mean of the two regression slopes. Ordinates: *D* or *F*; abscissa: amplitudes in mV.

The results of one of our experiments were drawn in Fig. 4. Here we changed the [Ca] from 10 to 5 mM, changed it back to 10 mM, then again to 5 mM and again to 10 mM, each change-over lasting some 20–30 min. The presynaptic depolarizing pulses were maintained in the plateau region of the input–output transfer characteristic (Katz & Miledi, 1967). The scatter of the data in this type of experiment was very large (Fig. 4*A*, *B*), probably due to the long time intervals between samplings required by the slow changes in [Ca] in this preparation (Miledi & Slater, 1966; Katz & Miledi, 1970). We therefore computed the correlation coefficients between the depression (*D*) and the p.s.p. amplitude (r = 0·64) and between the release fraction, *F*, and the p.s.p. (r = 0·64). These correlations were significant at the 0·01 level (n = 21). In two other experiments the r values for *D* were 0·96 (n = 17, a highly significant correlation) and 0·01 (not

significant, but the number of experimental points was small, $n = 9$). We took these results to indicate that there probably was a correlation between the p.s.p. amplitude and D or F, although more experiments were needed to establish this point firmly. As the changes in the p.s.p. amplitude were produced by changes in [Ca], it could be concluded that Ca^{2+} affected D, the synaptic depression, and F, the release fraction. However, the scatter of the data was too large to permit us to establish whether here, as in various neuromuscular preparations, one of these relationships was strictly linear (Thies, 1965; Elmqvist & Quastel, 1965*b*; Hubbard *et al.* 1971).

Presynaptic depolarization and synaptic depression

The effects of varying the amplitude of the presynaptic depolarizing pulses were studied in eight experiments. The results of one such experiment are shown in Fig. 5. In the inset we show sample traces demonstrating the presynaptic current pulses (bottom traces), the resulting presynaptic depolarizations (upper traces) and the p.s.p.s (middle traces). In each run, a conditioning train of four pulses and a test pulse 100 msec

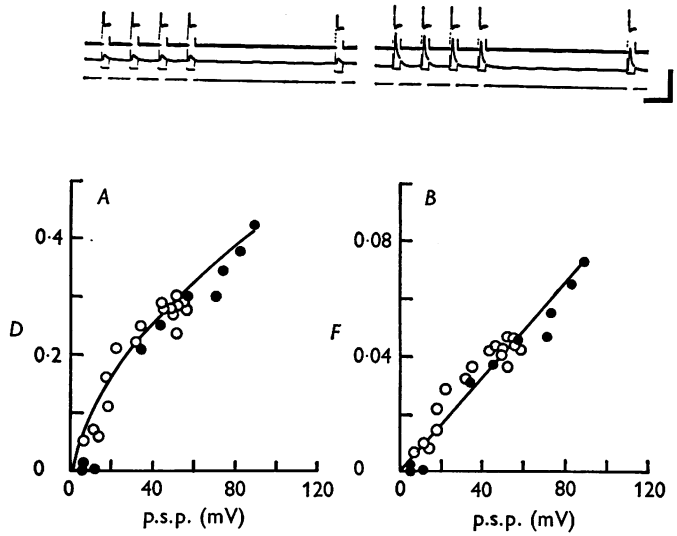

Fig. 5. The relationship between synaptic depression and the amplitude of the p.s.p. Inset: sample records. The convention of the recording is the same as in Fig. 1. The test interval here was 100 msec. The vertical calibration is 34 mV or 4×10^{-6} A and the horizontal calibration is 20 msec. *A*, the relationship between D (according to eqn. (2), the ordinate) and the amplitude of the p.s.p. (V_1 of eqn. (2), in mV, the abscissa). Open circles: stimulating pulses of 0·5 msec; filled circles: stimulating pulses of 0·5 msec. *B*, same data as in *A*; Here F (from eqn. (9), the ordinate) is plotted against the amplitude of the p.s.p. (in mV, the abscissa).

later were recorded. As the p.s.p. amplitude increased so did the synaptic depression. The graph in Fig. 5*A* shows the results of the whole experiment, where both long (5 msec, open circles) and short (0·5 msec, filled circles) current pulses were employed. In this experiment we were interested primarily in the relationship between *D* and the amplitude of the p.s.p. The relationship between the presynaptic potential and the p.s.p. amplitude was similar to that found by others (Katz & Miledi, 1967; Kusano *et al.* 1967). We expected the relationship between *D* and the p.s.p. amplitude to be linear similar to the situation at the neuromuscular junction, when the amplitude of the e.p.p. is varied by changing [Ca] (Thies, 1965; Elmqvist & Quastel, 1965*a*; Hubbard *et al.* 1971). However, as can be seen in Fig. 5*A*, this was not the case – the relationship between *D* and p.s.p. being distinctly non-linear. This was found in all eight experiments devoted to this problem. In Fig. 5*B* an attempt was made to

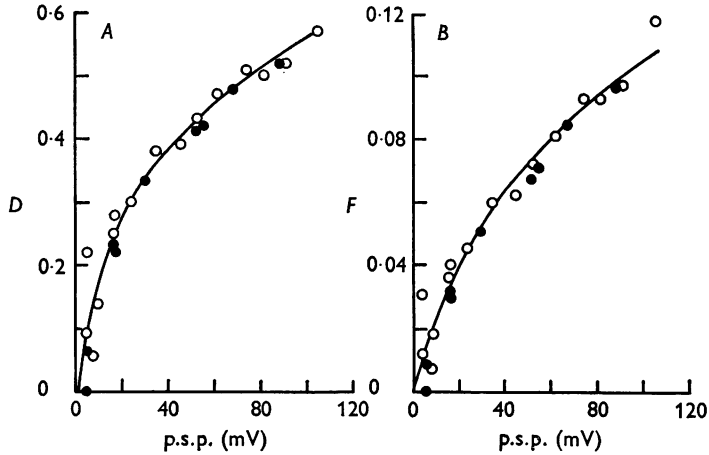

Fig. 6. Another experiment like that of Fig. 5. The same co-ordinates as in Fig. 5. Open and filled circles denote the results from two different series of measurements at the same synapse.

explain this non-linearity by applying the model of Betz (1970). According to this model, the release fraction (*F*) and hence the depression (*D*) become smaller for consecutive pulses in a tetanic train. Hence we would expect *D'* (eqn. (3)) or *F* (eqn. (9)) to be linearly related to the amplitude of the p.s.p. This correction, as applied in Fig. 5*B*, indeed produced a linear relationship, and this was also obtained in three more experiments.

However, in four further experiments (e.g. Fig. 6*A*, *B*) the relationship between *D* and the p.s.p. amplitude was markedly non-linear (Fig. 6*A*) and the non-linearity did not disappear when the Betz correction was

applied (Fig. 6*B*). It is interesting to interpret this non-linearity in terms of S and F. If it is assumed that the quantal size, q, remains constant throughout the tetanic train, then

$$\text{p.s.p.} = q.m, \tag{10}$$

where m is the quantal content. If it is further assumed that $m = F.S$, then we obtain

$$F = \frac{1}{q.S}.\text{p.s.p.} \tag{11}$$

and a decrease in the slope of the relationship between F and p.s.p. indicates that an increase in S may have occurred. The fact that presynaptic depolarization and calcium were not exactly equivalent could also be demonstrated by another type of experiment. Here a prepulse of 34 mV in 10 mM calcium produced a p.s.p. of 25 mV. The depression of the sixth p.s.p. in a train (rate of stimulation: 50 Hz) was 0·3 (according to eqn. (2)). Now the bathing calcium was reduced to 2·5 mM and the prepulse increased to 60 mV to produce a p.s.p. of 20 mV (80 % of control). However, the depression was now only 0·05 (17 % of control). This experiment could be interpreted to indicate that when [Ca] was reduced, F became smaller, and when the prepulse was increased it affected mainly S.

DISCUSSION

The depression in the squid giant synapse is probably due to a reduced quantal release. Two other mechanisms, namely desensitization of post-synaptic receptors (cf. Wachtel & Kandel, 1967; Gardner & Kandel, 1972) or a diminution of the released quantum (cf. Auerbach & Bennett, 1969; Highstein & Bennett, 1973) seem to be less probable. The first appears unlikely because desensitization is a slow process (Katz & Thesleff, 1957) whereas the duration of transmitter action in our experiments was probably very brief (the rise time of the p.s.p. was about 1 msec; e.g. Fig. 1). Moreover, it has been shown repeatedly that presynaptic hyperpolarization can alleviate synaptic depression in the squid (Hagiwara & Tasaki, 1958; Takeuchi & Takeuchi, 1962; Miledi & Slater, 1966) and this would tend to rule out a post-synaptic mechanism of depression. The second possibility is also unlikely, because the reduction of quantal size by depletion usually requires a large number of stimuli (Elmqvist & Quastel, 1965*a*), whereas in our experiments depletion could be observed after a single p.s.p. However, both these mechanisms cannot be ruled out completely, until miniature p.s.p.s can be studied in a tetanized giant-squid synapse.

It should also be pointed out that the present findings do not constitute

evidence that the probable reduction of quantal release by repetitive stimulation is caused by the depletion of presynaptic transmitter stores. However, this seems to be the simplest model to explain the effects of previous release, calcium and presynaptic depolarization on depression. Within the framework of this model one can conclude that recovery from depletion ('mobilization') is about ten times faster during the train (at 50 Hz) than after its termination (Figs. 2, 3). This is also evident from observing the rate of mobilization of quanta in the rat neuromuscular preparation. At room temperature the maximum rate of mobilization is 2000 quanta/sec (Hubbard *et al.* 1971). The time constant of recovery from a depression of 0·4 is 4 sec (Christensen & Martin, 1970). One can compute from this an initial rate of recovery of about 100 quanta/sec, assuming a store, S, of 1000 quanta (Christensen & Martin, 1970; Hubbard *et al.* 1971).

Although these estimates are very approximate, they also indicate that during stimulation a second, more rapid, process of mobilization becomes prominent and serves to adapt the rate of mobilization to the rate of stimulation (Elmqvist & Quastel, 1965*b*; Hubbard *et al.* 1971; Bennett & MacLachlan, 1972*b*; Richards, 1972). One could visualize the operation of the fast recovery as follows. Suppose that immediately after the p.s.p. a process of fast recovery starts to function for a short time (20–50 msec). If no additional p.s.p.s occur, the fast process is switched off and the recovery continues with a slow time course, lasting many seconds. If the fast process were proportional to the depletion of transmitter (as Fig. 3*B* tends to show) this would constitute a simple mechanism of feed-back, enabling the synapse to match release and mobilization. Moreover, the existence of such a process could explain why mobilization becomes the limiting factor for transmission when the rate of stimulation is increased above a certain level (Curtis & Eccles, 1960; Hubbard *et al.* 1971; Richards, 1972). As long as the stimulus interval is greater than the duration of the fast process, an increase in the rate of stimulation will cause a proportional increase in transmitter release. When the stimulus intervals fall within the span of the fast process, an increase in frequency will cause a reduction of mobilization and no increase in release will occur. Our inability to find evidence for facilitation at short test intervals (20 msec) when depression was present, may be interpreted as indicating the existence of a fast, short-lasting, recovery process which cancels the effects of facilitation. However, a decrease of facilitation in high bathing calcium could be an alternative explanation (Rahaminoff, 1968; Mallart & Martin, 1968). In this case, a fast recovery may still have occurred but may have been over within 20 msec after the end of the tetanus.

We do not know where the mobilized quanta are derived from. They may come from another store, termed 'mobilization store', by Elmqvist &

Quastel (1965*b*). This store may be a dynamic rather than a static concept, and may include all the vesicles that are being recycled after discharging their contents (Heuser & Reese, 1973). In order to study the rate of recovery of the 'mobilization store', a large number of stimuli is required (Elmqvist & Quastel, 1965*b*; Bennett & McLachlan, 1972*a*, *b*; Lass, Halevy, Landau & Gitter, 1973). Our present analysis was confined to short tetani and did not study the behaviour of the synapse over prolonged periods of stimulation or when presented with recurrent tetanic trains (Horn & Wright, 1970; McCandless *et al.* 1971). It is thus likely that the 'mobilization store' was not greatly altered and its rate of recovery did not affect the present results.

Finally, the non-linear relationship between D, the depression, F, the release fraction and the amplitude of the p.s.p. (Figs. 5, 6) merits discussion. One possible interpretation is that as the presynaptic depolarization increases so do both F, the release fraction, and S, the store of available quanta (see Results). An increase of F is easy to explain in terms of the 'calcium hypothesis' (Katz & Miledi, 1968). It has been shown by previous workers that changes in external [Ca] modify F mainly (Elmqvist & Quastel, 1965*a*; Hubbard *et al.* 1971; see also Fig. 4). Presynaptic depolarization, which facilitates the entry of Ca^{2+} ions into the terminal (Katz & Miledi, 1968), will presumably also increase F, the fraction of the store to be released. The increase in S is more difficult to explain. It is possible that depolarization mobilizes quanta in some manner. However, a simpler interpretation is also possible. If we assume that S represents the vesicles that are at or near the presynaptic membrane (Hubbard & Kwambunbumpen, 1968), perhaps at membrane release sites (Zucker, 1973), then it is possible to relate S to the *area* of the presynaptic membrane which is activated by depolarization (Kuno, Turkanis & Weakly, 1971). If we note that the synaptic input–output characteristic has a virtual threshold (Katz & Miledi, 1967; Kusano *et al.* 1967) and that a focal depolarizing pulse becomes markedly attenuated along the terminal (K. Kusano, unpublished), then it is easy to see that for relatively small pulses only a part of the terminal will participate in the release process. When the pulse is made bigger, the area of the terminal which attains threshold increases and so does S (Fig. 6). It would therefore be interesting to test whether procedures that modify the space constant of the nerve terminal also affect synaptic depression in a manner predicted by our hypothesis.

This work has been supported in part by U.S.P.H.S. Research Grant (NS-09618) and UPC grant (SG-28-72).

REFERENCES

AUERBACH, A. A. & BENNETT, M. V. L. (1969). Chemically mediated transmission at a giant fibre synapse in the central nervous system of a vertebrate. *J. gen. Physiol.* **53**, 183–210.

BENNETT, M. R. & MCLACHLAN, E. M. (1972*a*). An electrophysiological analysis of the storage of acetylcholine in preganglionic nerve terminals. *J. Physiol.* **221**, 657–668.

BENNETT, M. R. & MCLACHLAN, E. M. (1972*b*). An electrophysiological analysis of the synthesis of acetylcholine in preganglionic nerve terminals. *J. Physiol.* **221**, 669–682.

BETZ, W. J. (1970). Depression of transmitter release at the neuromuscular junction of the frog. *J. Physiol.* **206**, 629–644.

BISHOP, P. O., BURKE, W. & HAYHOW, W. R. (1959). Repetitive stimulation of optic nerve and lateral geniculate synapses. *Expl Neurol.* **1**, 534–555.

BLOEDEL, J., GAGE, P. W., LLINAS, R. & QUASTEL, D. M. J. (1966). Transmitter release at the squid giant synapse in the presence of tetrodotoxin. *Nature, Lond.* **212**, 49–50.

BRUNER, J. & KENNEDY, D. (1970). Habituation: occurrence at a neuromuscular junction. *Science N.Y.* **169**, 92–94.

BRUNER, J. & TAUC, L. (1966). Habituation at the synaptic level in Aplysia. *Nature, Lond.* **210**, 37–39.

BRYANT, S. H. (1958). Transmission in squid giant synapses: the importance of oxygen supply and the effects of drugs. *J. gen. Physiol.* **41**, 473–484.

BULLOCK, T. H. (1948). Properties of a single synapse in the stellate ganglion of squid. *J. Neurophysiol.* **11**, 343–364.

BULLOCK, T. H. & HAGIWARA, S. (1957). Intracellular recording from the giant synapse of the squid. *J. gen. Physiol.* **40**, 565–577.

CALLEC, J. J., GUILLET, J. C., PICHON, Y. & BOISTEL, J. (1971). Further studies on synaptic transmission in insects. II. Relations between sensory information and its synaptic integration at the level of a single giant axon in the cockroach. *J. exp. Biol.* **55**, 123–149.

CASTELUCCI, V., PINSKER, H., KUPFERMAN, I. & KANDEL, E. R. (1970). Neuronal mechanisms of habituation and dishabituation of the gill-withdrawal reflex in Aplysia. *Science, N.Y.* **167**, 1745–1748.

CHRISTENSEN, B. N. & MARTIN, A. R. (1970). Estimates of probability of transmitter release at the mammalian neuromuscular junction. *J. Physiol.* **210**, 933–945.

CURTIS, D. R. & ECCLES, J. C. (1960). Synaptic action during and after repetitive stimulation. *J. Physiol.* **150**, 374–398.

ECCLES, R. M. (1955). Intracellular potentials recorded from a mammalian sympathetic ganglion. *J. Physiol.* **130**, 572–584.

ECCLES, J. C., KATZ, B. & KUFFLER, S. W. (1941). Nature of the 'end plate potential' in curarized muscle. *J. Neurophysiol.* **4**, 362–387.

ECCLES, J. C., OSCARSSON, O. & WILLIS, W. D. (1961). Synaptic action of group I and II afferent fibres of muscle on the cells of the dorsal spinocerebellar tract. *J. Physiol.* **158**, 517–543.

ELQVIST, D. & QUASTEL, D. M. J. (1965*a*). Presynaptic action of hemicholinium at the neuromuscular junction. *J. Physiol.* **177**, 463–496.

ELMQVIST, D. & QUASTEL, D. M. J. (1965*b*). A quantitative study of end plate potentials in isolated human muscle. *J. Physiol.* **178**, 505–529.

GARDNER, D. & KANDEL, E. R. (1972). Diphasic post-synaptic potential: a chemical synapse capable of mediating conjoint excitation and inhibition. *Science, N.Y.* **176**, 675–678.

GINSBORG, B. L. (1970). The vesicle hypothesis for the release of acetyl-choline. In *Excitatory Synaptic Mechanisms*, ed. ANDERSON, P. & JANSEN, J. K. S., pp. 77–82. Oslo: Universitetsforlaget.

HAGIWARA, S. & BULLOCK, T. H. (1957). Intracellular potentials in pacemaker and integrative neurons of the lobster cardiac ganglion. *J. cell. comp. Physiol.* **50**, 25–47.

HAGIWARA, S. & TASAKI, I. (1958). A study on the mechanism of impulse transmission across the giant synapse of the squid. *J. Physiol.* **143**, 114–137.

HEUSER, J. E. & REESE, T. S. (1973). Evidence for recycling of synaptic vesicle membrane during transmitter release at the frog neuromuscular junction. *J. cell Biol.* **57**, 315–344.

HIGHSTEIN, S. M. & BENNETT, M. V. L. (1973). Fatigue at the Mauthner fiber–giant fibre synapse of the Hatchet fish. *Fedn Proc.* **32**, 443.

HORN, G. & WRIGHT, M. J. (1970). Characteristics of transmission failure in the squid stellate ganglion: a study of a simple habituating system. *J. exp. Biol.* **52**, 217–231.

HUBBARD, J. I., JONES, S. F. & LANDAU, E. M. (1971). The effect of temperature change upon transmitter release, facilitation and post-tetanic potentiation. *J. Physiol.* **216**, 591–609.

HUBBARD, J. I. & KWANBUNBUMPEN, S. (1968). Evidence for the vesicle hypothesis. *J. Physiol.* **194**, 407–420.

HUBBARD, J. I. & WILLIS, W. D. (1962). Hyperpolarization of mammalian motor nerve terminals. *J. Physiol.* **163**, 115–137.

HUBBARD, J. I. & WILLIS, W. D. (1968). The effects of depolarization of motor nerve terminals upon the release of transmitter by nerve impulses. *J. Physiol.* **194**, 381–405.

HUBBARD, J. I. & WILSON, D. F. (1970). Reduction of the quantum content of end-plate potentials by atropine. *Experientia* **26**, 1234–1235.

HUBBARD, J. I. & WILSON, D. F. (1973). Neuromuscular transmission in a mammalian preparation in the absence of blocking drugs and the effect of D-tubocurarine. *J. Physiol.* **228**, 307–325.

KATZ, B. & MILEDI, R. (1967). A study of synaptic transmission in the absence of nerve impulses. *J. Physiol.* **189**, 407–436.

KATZ, B. & MILEDI, R. (1968). The role of calcium in neuromuscular facilitation. *J. Physiol.* **195**, 481–492.

KATZ, B. & MILEDI, R. (1970). Further study of the role of calcium in synaptic transmission. *J. Physiol.* **207**, 789–801.

KATZ, B. & THESLEFF, S. (1957). A study of the 'desensitization' produced by acetylcholine at the motor end-plate. *J. Physiol.* **138**, 63–80.

KUNO, M., TURKANIS, S. A. & WEAKLY, J. N. (1971). Correlation between nerve terminal size and transmitter release at the neuromuscular junction of the frog. *J. Physiol.* **213**, 545–556.

KUSANO, K., LIVENGOOD, D. R. & WERMAN, R. (1967). Correlation of transmitter release with membrane properties of the presynaptic fibre of the squid giant synapse. *J. gen. Physiol.* **50**, 2579–2601.

LANDAU, E. M. & KUSANO, K. (1972). Depression and facilitation in the squid giant synapse. *Biol. Bull. mar. biol. Lab., Woods Hole* **143**, 467.

LASS, Y., HALEVI, Y., LANDAU, E. M. & GITTER, S. (1973). A new model for transmitter mobilization in the frog neuromuscular junction. *Pflügers Arch. ges. Physiol.* **343**, 157–163.

LILEY, A. W. & NORTH, K. A. K. (1953). An electrical investigation of effects of repetitive stimulation on mammalian neuromuscular junction. *J. Neurophysiol.* **16**, 509–527.

LUNDBERG, A. & QUILISCH, H. (1953). Presynaptic potentiation and depression of neuromuscular transmission in frog and rat. *Acta. physiol. scand.* **30**, suppl. III, 111–120.

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

MALLART, A. & MARTIN, A. R. (1968). The relation between quantum content and facilitation at the neuromuscular junction of the frog. *J. Physiol.* **196**, 593–604.

MARTIN, A. R. (1955). A further study of the statistical composition of end-plate potential. *J. Physiol.* **130**, 114–122.

MCCANDLESS, D. L., ZABLOCKA-ESPLIN, BARBARA & ESPLIN, D. W. (1971). Rates of transmitter turnover in the cat superior cervical ganglion estimated by electrophysiological techniques. *J. Neurophysiol.* **34**, 817–830.

MILEDI, R. (1969). Transmitter action in the giant synapse of the squid. *Nature, Lond.* **223**, 1284–1286.

MILEDI, R. & SLATER, C. R. (1966). The action of calcium on neuronal synapses in the squid. *J. Physiol.* **184**, 473–498.

NICHOLLS, J. G. & PURVES, D. (1972). A comparison of chemical and electrical synaptic transmission between single sensory cells and a motoneuron in the central nervous system of the leech. *J. Physiol.* **225**, 637–656.

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

RICHARDS, C. D. (1972). Potentiation and depression of synaptic transmission in the olfactory cortex of the guinea-pig. *J. Physiol.* **222**, 209–231.

TAKEUCHI, A. & TAKEUCHI, N. (1962). Electrical changes in the pre- and postsynaptic axons of the giant synapse of Loligo. *J. gen. Physiol.* **45**, 1181–1193.

THIES, R. E. (1965). Neuromuscular depression and the apparent depletion of transmitter in mammalian muscle. *J. Neurophysiol.* **28**, 427–442.

WACHTEL, H. & KANDEL, E. R. (1967). A direct synaptic connection mediating both excitation and inhibition. *Science, N.Y.* **158**, 1206–1208.

ZUCKER, R. S. (1972). Crayfish escape behavior and central synapses. II. Physiological mechanisms underlying behavioral habituation. *J. Neurophysiol.* **35**, 621–637.

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