

Long-lasting Depression and the Depletion Hypothesis at Crayfish Neuromuscular Junctions

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Summary. 1. Synaptic depression was studied at the neuromuscular junctions of the crayfish giant motoneurone onto the abdominal fast flexor muscles. The kinetics of depression were compared quantitatively to predictions of the depletion hypothesis of synaptic depression.

2. Synaptic transmission was depressed about 71% following a single motoneurone impulse. Transmission recovered to normal along an exponential time course with an average time constant of 285 s (Fig. 1).

3. This recovery curve was used to predict the depression that repeated responses would be expected to suffer. Even after correction for a superimposed facilitation (Fig. 2) and non-linear postsynaptic summation, the response to repeated stimulation at frequencies at or above once per minute were less depressed than expected (Figs. 3, 4). Increasing the frequency above this rate did not enhance depression, contrary to predictions. The discrepancy was not due to a mobilization of transmitter into a releasable store (Fig. 5).

4. The depression caused by each stimulus in a train was much less than that following a single impulse. This contradicts the depletion hypothesis and suggests that depression is accompanied by a large change in the fraction of the releasable transmitter store liberated by an impulse, with very little accompanying depletion.

5. When transmitter release is reduced in high magnesium solution, there is no change in the depression observed to repeated stimulation, contrary to the depletion hypothesis (Fig. 6).

6. This discrepancy could be due to an effect of magnesium on mobilization. However, no difference was found in the slow recovery rates following an impulse in different magnesium concentrations (Fig. 7).

7. It is concluded that the depletion hypothesis is untenable as a basis for depression at this neuromuscular junction.

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Introduction

In 1953, in an appendix to a paper by Liley and North, Wilfrid Rall proposed a model for synaptic depression which he used to estimate the amount of transmitter in nerve terminals and the effectiveness of nerve spikes in releasing it. This model, called the depletion model of synaptic depression, has come to form the foundation of later research and the cornerstone of our thinking about the processes underlying synaptic depression. All subsequent studies of depression have attempted to account for the phenomenon in terms of this model, or have modified the model in order to account for discrepancies between predictions and observations.

Basically, the depletion hypothesis proposes that depression occurs because each nerve impulse releases an appreciable fraction of a finite store of transmitter substance available for release, and that this store is incompletely replenished or refilled at the time of a subsequent impulse. Thus each impulse is seen as releasing a constant fraction of a shrinking store, until a steady-state is reached in which the rate of replenishment of the store between impulses is equal to the rate of release of transmitter by impulses. Transmitter release is depressed because the store is depleted, relative to its size following a long period of rest. This model is appealing because it is intuitive, simple, and seems like a natural account of what must be happening.

In 1965, Kennedy and Takeda described a very pronounced and long-lasting synaptic depression at a neuromuscular junction in crayfish. After only a few impulses at 0.5 to 5 Hz, transmission was reduced to a small fraction of its initial level, as measured by the amplitude of the postsynaptic excitatory junctional potential (e.j.p.). Full recovery from this depression required more than ten minutes. They tentatively ascribed this depression to a severe depletion of the releasable transmitter store.

The depression in this preparation seems not to be of postsynaptic origin. In fact, no modifications in membrane resistance can be observed during the depression (Bruner and Kennedy, 1970). Also, although the desensitization to ionophoretic injections of L-glutamate can be observed, at least in sites of non-giant motoneurone terminals, the full recovery is much faster (10–20 s) than that of synaptic depression (Thieffry and Bruner, in prep.).

Bruner and Kennedy (1970) found that reduction of the size of the e.j.p. in high magnesium solutions failed to have an effect on the depression observed to stimuli which generated single impulses once per minute. The locus of the magnesium effect on transmission was presumably presynaptic, reducing the amount of transmitter released by each impulse (Wernig, 1972). Thus magnesium would be expected to relieve the depletion of transmitter stores caused by transmitter release, and reduce depression in proportion to the reduction in transmission (Thies, 1965). The failure to observe this effect was taken as evidence against the depletion hypothesis for depression at this neuromuscular junction.

Unfortunately, this study suffered from several shortcomings which tend to weaken the conclusion. First, the responses were not corrected for the non-linear relation between transmitter release and e.j.p. amplitude (Martin, 1955). This tends to selectively underestimate the magnitude of the undepressed re-

sponses in normal magnesium, and thus to underestimate the depression of transmission referenced to those responses. The data were interpreted as showing an equivalent depression of normal responses and reduced responses obtained in high magnesium. However, this data may actually represent a reduced depression of small responses in high magnesium, compared to an underestimated larger depression in normal solution.

A second problem with this earlier work is that only low frequencies of stimulation were used in comparing the depression at different levels of junctional transmission. The effect of magnesium on reducing the amount of transmitter released could be counteracted by an effect of reducing the rate of replenishing the releasable store from the reserve store. If magnesium were to have both these effects, and depression were measured only by responses to a few stimuli at low frequency, then the depression observed in different magnesium solutions might appear similar. Thus the results are still compatible with a modified depletion hypothesis.

In order to clarify and test this possibility, we have decided to re-examine long-lasting depression at this crayfish neuromuscular junction to see whether or not its behavior can be explained by some form of a depletion model.

Methods

The preparation used was the neuromuscular junction between the giant motoneurone and the abdominal fast flexor muscles of the crayfish *Procambarus clarkii*. The abdomen was pinned ventral side up on a Sylgard disc in a chamber containing crayfish saline (195 mM NaCl, 5.4 mM KCl, 13.5 mM CaCl_2 , 2.6 mM MgCl_2 , 10 mM tris maleate buffer, pH 7.3). The temperature in most experiments was held to $15 \pm 1^\circ \text{C}$. The fast flexor muscles and their innervation were exposed in segments 2, 3 and 4. The preparation was illuminated with both transmitted and reflected light.

The fast flexor muscles are twitch muscles. In order to study them electrophysiologically for prolonged periods, their contraction must be blocked. We tried using solutions of high osmotic pressure (394 mM sucrose added) (Takeuchi and Onodera, 1973), a high magnesium to calcium ratio (52 mM Mg^{2+} , 2.5 mM Ca^{2+}) or containing 5 mM L-glutamic acid γ -methyl ester, a glutamate receptor blocker (Lowagie and Gerschenfeld, 1974). We also tried detubulating the muscle fibres with glycerol or ethylene glycol (Sevcik and Narahashi, 1972; Papir, 1973). None of these procedures blocked the contraction in the body of the flexor muscles, even when a single muscle head and its innervation were dissected free and placed alone in a chamber with circulating fluid. We presume that these procedures failed because of a diffusion barrier between the bathing medium and the extracellular space in the muscle body (cf. Zucker, 1974a, b). We finally settled on a procedure of presoaking the abdomen with muscles exposed in a H^+ - and Ca^{2+} -buffered saline containing 1 mM EGTA (ethyleneglycol-bis-(β -aminoethyl ether), N, N'-tetra-acetic acid), 52 mM MgCl_2 and 0.5 mM CaCl_2 (free calcium $3 \mu\text{M}$ at pH 7.3). After 15 min in this solution, the abdomen was rinsed in the experimental solution, which was continually perfused through the chamber throughout the experiment (bath volume of 65 ml replaced every 30 min).

This procedure effectively removed calcium from the extracellular spaces of muscle fibres deep in the muscle. In order to control the magnitude of transmitter released by spikes in the giant motoneurone, experiments were performed in various concentrations of magnesium (usually 5 times or 20 times the normal level, i.e., 13 mM or 52 mM), with the calcium concentration left at the normal level of 13.5 mM. This method of controlling synaptic function was found more reliable than varying the calcium concentration. In order to reduce synaptic transmission to a low level, calcium had to be reduced to such an extent that neuronal repetitive firing ensued (cf. Zucker, 1974b, c), which had profound effects on the synaptic depression we were studying.

The increase in osmotic pressure in high magnesium solutions was compensated by an appropriate reduction in the NaCl concentration.

Synaptic potentials were recorded with glass microelectrodes filled with 3 M KCl, having an average resistance of 15 Mohms measured in crayfish saline. Each muscle fibre of the abdominal fast flexors is innervated by an inhibitor and a non-giant motoneurone, as well as the giant motoneurone. Only the motor giant and the inhibitor innervate all parts of the muscle. The first and the second anterior oblique muscles of the fast flexor group share no common non-giant motoneurons (Selverston and Remler, 1972). By recording from one of these muscles, and stimulating the nerve to the other with a suction electrode, unitary e.j.p. responses due to activity of the motor giant alone were obtained (Bruner and Kennedy, 1970).

Calculations of predictions of the depletion hypothesis, and various corrections for facilitation and postsynaptic nonlinear summation, were performed automatically on a Hewlett-Packard 9830 minicomputer. Each type of computation was checked at least once by hand.

Results

The Depletion Hypothesis

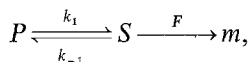
In the classical depletion model (Liley and North, 1953), depression of synaptic transmission is due simply to a reduction in the amount of transmitter available for release, as a consequence of part of the store having already been released by previous nervous activity. Each nerve impulse releases a constant fraction (F) of the store available for release. If the store contains S quanta of transmitter, then the number of quanta released (m) is a direct measure of the size of the store,

$$m = FS. \quad (1)$$

Suppose the first impulse in a train releases m_1 quanta from an initial store size S_1 . Then, if no replenishment of the store occurs, the second impulse will release fraction F of a depleted store $S_2 = S_1 - m_1 = S_1 (1 - F)$; or $m_2 = FS_1(1 - F) = m_1 (1 - F)$. The third impulse will release fraction F of a further depleted store, $S_3 = S_2 - m_2 = S_1 (1 - F) - FS_1 (1 - F) = S_1 (1 - F)^2$; or $m_3 = m_1 (1 - F)^2$. In general, the k th impulse will release $m_k = m_1 (1 - F)^{k-1}$ quanta. Thus transmission will exhibit a depression in which successive responses decline geometrically (the discrete form an exponential) to zero.

If some time passes between successive stimuli, a part of the store released by previous impulses will be replenished from some larger depot store, often called a 'precursor store', of size P . If enough time elapses, the releasable store will be fully recharged from the depot store. It is simplest to imagine that an equilibrium becomes established between the depot and releasable stores, and that first-order kinetics govern the refilling of S from P . Then the rate of replenishing the store will be proportional to the amount of depletion of S that has occurred, while the time constant of refilling will not vary. It is assumed that the depot store is very large and constant, not influenced by short trains of neuronal electrical impulses.

All the factors which influence the size of S can be summarized by the schema



where k_1 and k_{-1} are the forward and backward rate constants governing the $P-S$ equilibrium. At rest, $S_r = k_1 P / k_{-1}$, where S_r is the resting level of S .

If the nerve has been stimulated one or more times, then the store size will recover from its initial value S_i to the level S_r according to the equation

$$S = S_r + (S_i - S_r) e^{-k_{-1}t}, \quad (2)$$

where the initial store S_i is measured at time $t=0$. Thus the releasable store should recover exponentially with time constant $1/k_{-1}$ after any number of stimuli.

Suppose the nerve is stimulated repetitively at frequency f . Then transmitter is being released from S at the rate fFS . The dynamics of S are governed by its mass action relation to P and by its depletion due to release. This can be represented by the equation

$$\frac{dS}{dt} = k_1 P - k_{-1} S - fFS, \quad (3)$$

whose solution is

$$S = S_s - (S_r - S_s) e^{-gt}, \quad (4)$$

where

$$S_s = \frac{k_1 P}{fF + k_{-1}} \quad \text{and} \quad g = fF + k_{-1}. \quad (5)$$

Thus the releasable store size S will decline exponentially with time constant $1/g$ to a non-zero steady-state level S_s . Both S_s and g depend on the effectiveness of nerve impulses in releasing a fraction of the store, on the frequency of stimulation, and on the reverse rate constant for the $P-S$ equilibrium.

A key attribute of this depletion model, and a major reason for its popularity, is that the behavior of synaptic transmission during repeated stimulation can be entirely predicted from measurements of the recovery of transmission following a single stimulus. Since the fraction of the store released by an impulse does not change in depletion-mediated depression, transmitter release is a direct measure of S , $m = FS$. Transmitter release (m) can be estimated from the amplitude of the postsynaptic response, corrected, if necessary, for the nonlinear relation between transmitter release and e.j.p. amplitude (Martin, 1955). Since e.j.p.s in this muscle are of fairly long duration (10 ms half-width) and have a reasonably rounded peak, the usual Martin correction is applicable (Jack et al., 1975). We assumed a value of 0 mV for the e.j.p. reversal potential (Taraskevich, 1971).

Recovery from Depression

The first prediction of the depletion hypothesis is that immediately following one stimulus, transmission (measured by the response to a second stimulus)

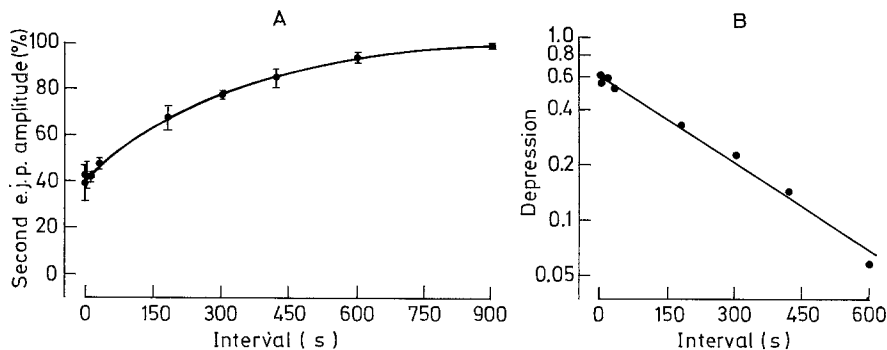


Fig. 1A and B. Recovery from depression following a single e.j.p. The giant motoneurone was stimulated twice at the interval shown on the abscissa. **A** The second response after Martin-correction is plotted on the ordinate as a percentage of the first response. The average amplitude of the first e.j.p. was 9.5 mV. The first two points were at intervals of 1 s and 10 s. Paired stimuli were separated by 15 min rests. Each point is the average of several measurements ($N \geq 5$). Bars are standard errors. The line is the best-fit exponential by regression analysis. **B** Semi-logarithmic plot of depression shown by second responses (ordinate) *vs.* interval between stimuli (abscissa). Magnesium concentration, 20 times normal. Data from 53 fibres in 35 animals

should be depressed, and as the interval between the two responses is increased, the second response should grow exponentially to its resting level. This prediction is tested in Figure 1, where amplitudes of the second e.j.p. relative to the first are plotted *vs.* the interval between the two responses. Apparently, full recovery requires 15 min, so only a few measurements could be obtained from any one preparation. In order to obtain several measurements at a number of different intervals, it was necessary to pool data from several experiments.

The results fit the exponential prediction quite satisfactorily and k_{-1} is estimated as $1/285 \text{ s}^{-1}$. F can be estimated by extrapolating the best fit exponential back to $t=0$, yielding a value of $F=0.60$. F can also be estimated simply from the 'zero-time depression', or the depression measured at such short intervals that virtually no replenishment of stores will have occurred. Then $m_2/m_1 = 1 - F$ or $F = 1 - m_2/m_1$. But $1 - m_2/m_1$ is the usual measure of depression, D evinced by response m_2 . By this definition, D is the fractional reduction in m_2 compared to m_1 , and D is a direct measure of F . The zero-time depression for the fibres shown in Figure 1 was measured at 0.71 ± 0.15 (S.E.), using paired stimuli separated by a few milliseconds.

Depression to Repeated Stimulation

From this data, it is possible to predict the successive responses that should be obtained by stimulating the motoneurone at any frequency. The responses ought to fall on an exponentially declining curve with rate constant g , predicted from Equation 5, and with steady-state responses which are the following fraction of the resting response:

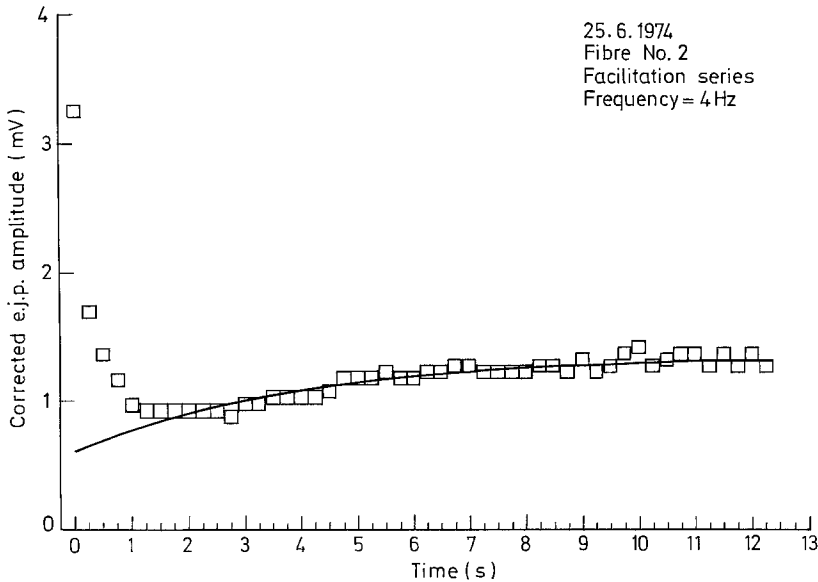


Fig. 2. E.j.p.s (squares) evoked by motor giant impulses at 4 Hz. From the sixth response onward, e.j.p.s were averaged in successive groups of three (to reduce scatter); and a single exponential (solid line) was fitted to these points using regression techniques. From the equations of Mallart and Martin (1967), the increment of facilitation induced by each impulse was estimated as $0.075 e^{-0.25t}$. Magnesium concentration, 20 times normal

$$\frac{S_s}{S_r} = \frac{k_{-1}}{g}. \quad (6)$$

Before comparing predictions to observations, account has to be taken of the fact that at the moderate frequencies at which we wished to study depression, these neuromuscular junctions display a super-imposed synaptic facilitation (Bruner and Kennedy, 1970). This facilitation may be observed in the responses to stimuli delivered at 4 Hz, shown in Figure 2. It can be seen that there is an early rapid depression that appears to reach steady-state after only a few stimuli. This is, in fact, a general property of the long-lasting depression shown by this synapse, as will become evident later (see Figs. 3 and 5). Following the phase of depression, the responses slowly grow to a new increased level, reflecting an accumulating facilitation of transmission.

In order to take account of this facilitation, we need to measure its properties. Unfortunately, it is not possible to study facilitation in isolation, because we were not able to block depression by the usual ionic manipulations (see below). However, it appears from Figure 2 that after the first 5 responses, there is no further depression, and all subsequent changes are due entirely to facilitation. We have decided to estimate the parameters of facilitation by fitting it to the model of Mallart and Martin (1967). In this description of facilitation, it is assumed that each impulse adds a constant increment to a facilitation factor which multiplies unfacilitated transmission, and that these increments

decay with a double exponential time course. Although this model describes facilitation at frog neuromuscular junctions fairly accurately (Magleby, 1973), it is less valid for crayfish neuromuscular junctions (Linder, 1974; Zucker, 1974b). However, at frequencies below 5 Hz, it is an excellent approximation, and the rapid component of the decay of facilitation can be ignored (Zucker, 1974b). Thus, we felt justified in applying the simple model of Mallart and Martin, using a linear addition rule for the accumulation of tetanic facilitation and assuming a simple exponential time course for the decay of facilitation, and its growth in a tetanus. Using the procedure of Mallart and Martin (1967), we estimated the parameters of the facilitation caused by each impulse from the growth of facilitation during repeated stimulation. The facilitation parameters measured in these fast flexor muscles were somewhat different from those reported for the tonic claw opener (Zucker, 1974b). In the fast flexors, each impulse contributed a facilitation of about 0.2, lasting about 0.5 s. Facilitation is much more pronounced in the claw opener.

Each experiment on a new fibre began with an estimate of the parameters of facilitation, as in Figure 2. Then the fraction of release for the magnesium concentration used was estimated from the zero-time depression, measured from the responses to two stimuli separated by 20 ms. The second response was corrected for the expected effects of facilitation and recovery from depletion before computing F . Using this measurement, and the estimate of k_{-1} from the pooled fibre data (Fig. 1), the parameters g and S_s/S_r were computed for a particular frequency of stimulation f , and the effects of depression on successive responses were calculated from Eqs. 1 and 4. The pooled-data estimate of k_{-1} had to be used because it was too difficult to collect enough data from one fibre to obtain an accurate estimate of k_{-1} . This parameter did not appear to vary more than two-fold between fibres. The predicted successive responses were next corrected for the effects of facilitation, using the facilitation parameters derived for the given fibre and assuming that the effects of facilitation and depression are independent and multiplicative (Thies, 1965). These predictions were further corrected for a nonlinear relation between transmitter release and postsynaptic potential. These fully-corrected predictions of the depletion hypothesis were then compared to the observed responses at stimulus frequency f .

Figure 3 shows the results of a typical experiment. At very low frequencies of stimulation (1/200 Hz), there is excellent agreement between the observed depression and the predictions of the depletion hypothesis. At moderate frequencies (1/50 Hz), there is a clear discrepancy between the results and the predictions. At higher frequencies (1/5 Hz and 1 Hz), the depletion hypothesis fails completely to predict either the time-course or the final magnitude of the depression. It is also evident that the predicted effect of facilitation at the frequencies used is negligible. At 1 Hz, when facilitation becomes significant, the predicted depletion is so severe as to reduce all responses to a negligible level.

When the different series of responses are superimposed (Fig. 4), it may be seen that depression fails to increase for frequencies above 1/50 Hz, contrary to predictions of the depletion hypothesis. Thus the facilitation that is expected at 1 Hz may be observed because it is not masked by depression, since the

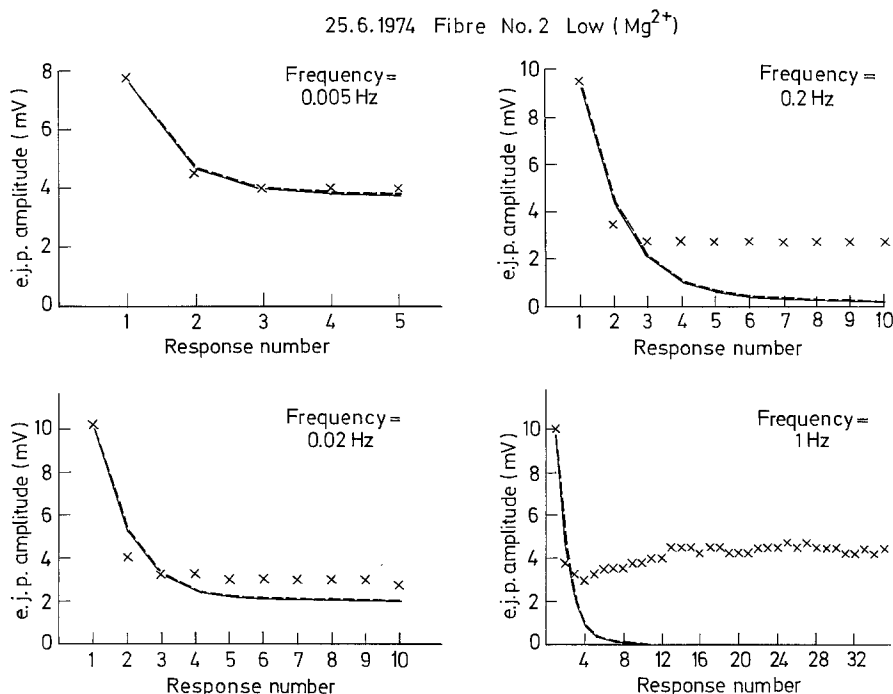


Fig. 3. Successive e.j.p.s to giant motoneurone stimuli at four different frequencies. Crosses represent observed e.j.p. amplitudes; lines plot the predicted responses. The solid line includes corrections for facilitation; the dashed line does not. Predictions ignoring facilitation are larger because the estimate of F is smaller when facilitation is neglected. The different series of stimuli were separated by 15 min rests. Magnesium concentration, 5 times normal

large predicted depression fails to materialize. Results similar to those illustrated were obtained in five complete experiments, and several partial experiments in which fewer frequencies were tested.

A possible explanation of these results is that there exists an impulse- or release-activated extra mobilization of transmitter into the releasable store during a tetanus. That is, the kinetics of replenishing the releasable store may be more rapid during a tetanus than suggested from the recovery of depression following one impulse. In that case, the recovery from depression immediately following a short tetanus, such as those of Figures 3 and 4, would be more rapid than the recovery illustrated in Figure 1. In 11 experiments, we tested this idea by measuring the responses to single stimuli delivered at various intervals after a sequence of five stimuli at 0.2 Hz. In 8 of these experiments, we also observed the recovery following a single stimulus. In no case was a significant difference observed between the recovery rate following one stimulus and that following repeated stimulation. The pooled results of 5 experiments with identical paradigms are shown in Figure 5.

Another significant deviation from the depletion hypothesis is revealed by this experiment. According to the depletion hypothesis the fraction of the releasable store released by an impulse is a constant and can be estimated from

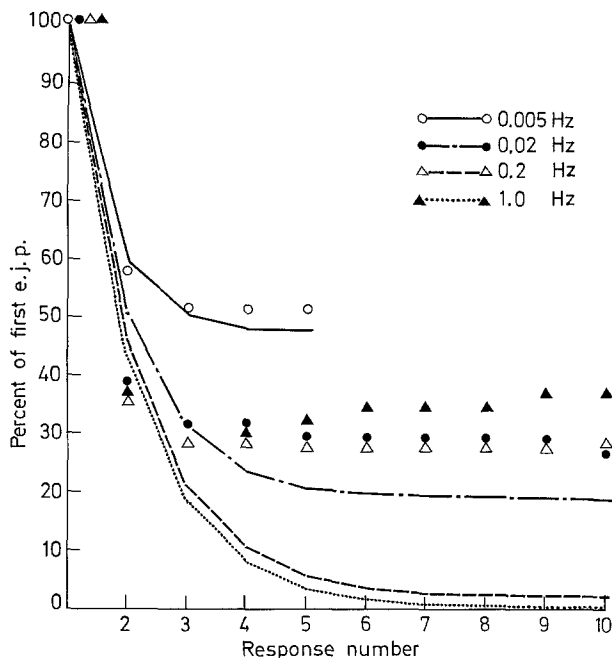


Fig. 4. Observed and predicted responses from Figure 3 are replotted with responses expressed as a percentage of the initial e.j.p. amplitude

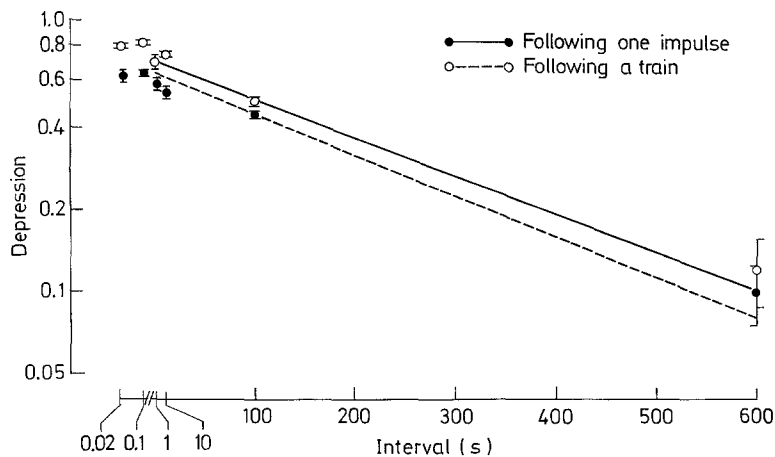


Fig. 5. Comparison of recovery from depression induced by a single impulse and a train (5 impulses at 0.2 Hz). The ordinate measures depression with respect to the initial undepressed response. The interval between the last tetanic response (or the single conditioning response) and the test response is represented on the abscissa. The first points are at 20 and 100 ms followed by responses at 1, 10 and 600 s, 15 min rest between sequences; 20 times normal magnesium. Regression lines fitted to the responses at 1, 100 and 600 s. The slopes of the regression lines correspond to time constants of 289 (●) and 310 (▲) s, and they are not significantly different ($p > 8$). Data from 5 fibres

the zero-time depression using paired stimuli. Thus, the extra depression of a test response immediately following an already depressed tetanic response should be the same as the depression experienced by a test response immediately following a single impulse. Using data from some of the above experiments, we found that the average zero-time depression following a single impulse, corrected for the effects of facilitation and recovery, was 0.71 ± 0.02 (S.E.). The added zero-time depression of a test response immediately following a tetanically depressed response was only 0.33 ± 0.12 (S.E.) These values are significantly different (t -test, $P < 0.05$).

Depression and Level of Synaptic Transmission

Our final test of the depletion hypothesis was to reduce transmission with elevated concentrations of magnesium and observe the effects on depression. If magnesium acts by reducing the fraction of the store released by an impulse, then a certain percentage reduction in the undepressed e.j.p. will represent the same reduction in F .

Our procedure was to estimate the zero-time depression in low magnesium solution and predict and observe the responses to repeated stimulation as before. Then we changed to a high magnesium solution, estimated what the reduced value of F should now be from the reduction in undepressed transmission, and again predicted and observed the responses to a similar train of stimuli.

Figure 6 illustrates a typical result. Raising the magnesium concentration from 13 mM to 52 mM reduced single e.j.p.'s from 19.7 mV to 5.5 mV. Depression is predicted to develop more gradually and to a lesser extent in the high magnesium solution. In fact, reducing the level of synaptic transmission had virtually no effect on depression. This result was confirmed in five fibres for various frequencies of stimulation.

It could be argued that raising the magnesium concentration not only reduces the fraction of release, but also retards the kinetics of replenishing the releasable store, for example, by reducing k_1 or P . This would tend to reduce the size of the expected steady-state response, which would tend to cancel the effect of a reduction in F (see Eq. 5). Any such change in the kinetics of refilling S would be reflected as a change in the rate of recovery of the store following an impulse (Eqs. 2 and 3).

We looked for evidence of such a retarding effect of magnesium on the kinetics of mobilization by comparing the time courses of recovery from depression in different concentrations of magnesium. In all these experiments, we found that the slow portion of the recovery curve following one impulse by 1 s or more was unchanged. On a depletion model, one would expect zero-time depression to drop by 70% in high magnesium, but a much smaller decrease, about 20% on the average, was observed in the extrapolated zero-time depression (Fig. 7).

There was one major difference, however, in the recovery curves at different levels of transmission. In high magnesium, we observed a brief period of facilitation after a single stimulus (cf. Fig. 2). In low magnesium the facilitation was

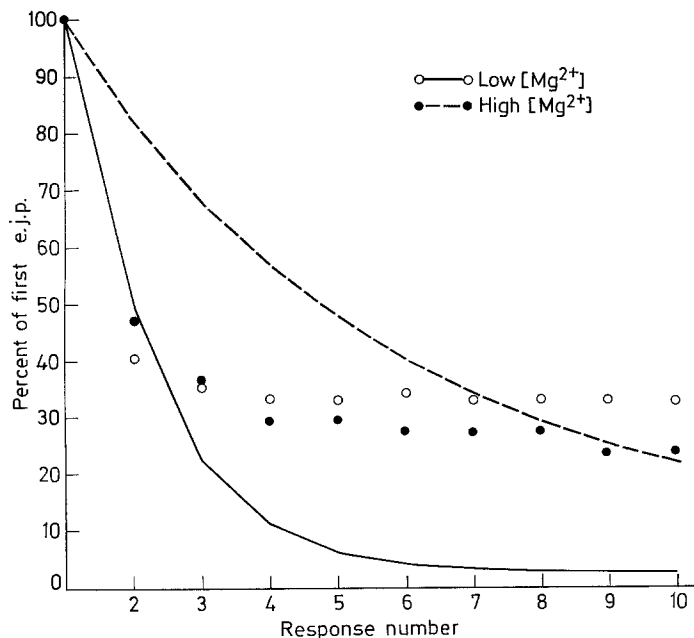


Fig. 6. Effect of varying the magnesium concentration on the predicted (lines) and observed (points) depression of 10 responses at a frequency of 0.2 Hz. Responses are expressed as a percentage of the amplitude of the first e.j.p. which was 19.7 mV in low magnesium solution and 5.5 mV in high magnesium solution. 15 min between sequences

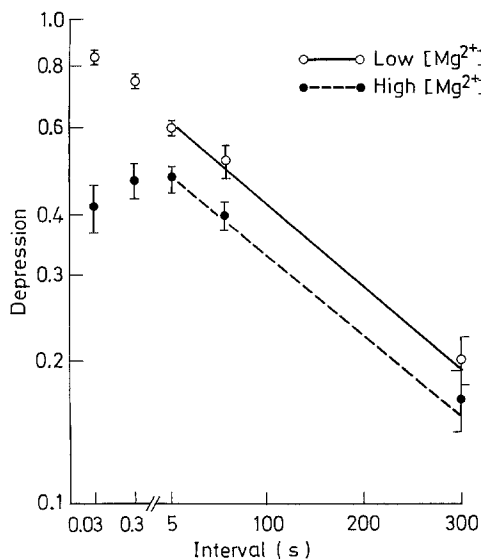


Fig. 7. Semilogarithmic plot of points showing depression (ordinate) following a single impulse, measured by a test response occurring at various intervals (abscissa) after the response to the first impulse. The average first e.j.p. was 9.0 mV in low magnesium and 33.2 mV in high magnesium, after Martin correction. The first three points are at 30 ms, 300 ms, and 5 s. Straight lines fitted to measurements at the last three time points by regression techniques. The slopes are not significantly different ($p > 0.8$). 15 min between sequences. Data from 8 fibres. Bars are standard errors

masked by an additional short-lasting depression, with a time constant of less than 500 ms. This may well be a depletion-type of depression since it depends on magnesium concentration, augmenting substantially in low magnesium solution (Czternasty and Bruner, 1975). It does not concern us here, however, where we concentrate only the longlastic depression, whose recovery time constant is 4 to 5 min.

Discussion

Liley and North (1953) showed that at rat diaphragm neuromuscular junctions both the tetanic accumulation of depression and the subsequent recovery were exponential. They did not, however, determine whether the rate constant for the build-up of tetanic depression, and the steady-state reached, were in agreement with predictions from the recovery following one impulse.

The first attempt to compare quantitatively the observed tetanic depression to that predicted from the recovery curve was made by Takeuchi (1958). He found that at frog sartorius neuromuscular junctions, the steady-state tetanic depression was larger than expected. He attributed this discrepancy to the effects of facilitation, for which he made no correction.

Elmqvist and Quastel (1965) found that the brief tetanic run-down of transmission at human intercostal neuromuscular junctions was also exponential. They found a qualitative agreement between results and theory, in that the steady-state level of transmission declined as frequency increased. They also reported a negative correlation between the first and second tetanic response amplitudes, which is consistent with a depletion hypothesis. However, they did not measure the depression following a single impulse, nor attempt to predict tetanic depression from any independent parametric estimates.

Elmqvist and Quastel (1965) estimated S by plotting successive responses in the rapid tetanic run-down against total transmitter released, and extrapolating the initial linear portion of the curve to the abscissa for a zero response. Then, by assuming a depletion model, they could estimate F . They found that manipulating the level of transmitter release by changing calcium and magnesium concentrations changed only F , not S .

Thies (1965) also observed that when the quantum content of synaptic transmission at guinea-pig neuromuscular junctions was changed by altering the calcium or magnesium concentration, depression was changed in the manner predicted by the depletion hypothesis. As quantum content was reduced to near zero, so was depression, and the two were linearly related. Similar but less extensive demonstrations of such a relationship have been reported at rat diaphragm and frog sartorius neuromuscular junctions, as well as central nervous synapses in leech, *Aplysia* and squid (Lundberg and Quilisch, 1953; Takeuchi, 1958; Otsuka et al., 1962; Hubbard, 1963; Nicholls and Purves, 1972; Schlapfer et al., 1974; Kusano and Landau, 1975).

Recently, however, there have been some careful tests of the depletion hypothesis which have detected some difficulties in the application of the classical model to neuromuscular depression. Christensen and Martin (1970) used a

binomial statistical analysis to measure n and p of the statistical process presumed to underlie transmitter release at rat diaphragm neuromuscular junctions. They found that the statistical estimates of p were smaller than those of F obtained either by the method of Elmqvist and Quastel (1965) or from the zero-time depression. However, in recent discussions of the physical meaning of the binomial parameters n and p (Zucker, 1973, 1977), it was pointed out that p is a compound probability, influenced both by the probability that the release site is activated by an impulse (which is related to F) and by the probability that a release site is occupied by a quantum of transmitter (which is related to S). It is expected that p will be less than F , even if depression is due entirely to depletion. Christensen and Martin (1970) also reported a non-linear relationship between depression and excess amount of transmitter release. However, in most of their experiments the total transmitter released was varied by adding conditioning shocks, which may have had effects on the size of the releasable store due to a possible mobilization component of posttetanic potentiation (Magleby and Zengel, 1975). In any case, it cannot be determined from their data whether reduction of transmission to near zero would have reduced depression proportionately, as predicted by the depletion hypothesis.

Betz (1970) has found more serious difficulties with the depression hypothesis at frog neuromuscular junctions. He also found a slightly non-linear relationship between level of release and depression. More significantly, he found that the zero-time depression following an already depressed response was less than that following a single undepressed response. In the classical depletion model, zero-time depression is a measure of F , which is a constant. Thus, this result contradicts a basic assumption of the hypothesis, and all estimates of F and S based on the simple depletion model will be in error.

Rather than discard the depletion model, Betz (1970) proposed a modification in which there is a change in both the size of the releasable store and in the fraction of that store released by an impulse. He suggested that both F and S were reduced by the same percentage in depression, i.e. $\Delta F/F = \Delta S/S$. Using this assumption, he showed that if r is the ratio of the second (m_2) to the first (m_1) of two responses used to measure zero-time depression following a single impulse, and if r' is the ratio (m'_2/m'_1) used to measure zero-time depression following conditioning stimuli that reduce the first response to a level of depression D where $D = 1 - m'_1/m_1$, then the following relation should hold:

$$\frac{r'}{r} = \frac{1 - F\sqrt{1-D}}{1-F}. \quad (7)$$

In this model, F is estimated from

$$F = 1 - \sqrt{\frac{m_2}{m_1}}. \quad (8)$$

Betz (1970) confirmed this prediction for the effects of various conditioning trains, causing various degrees of depression D , on the zero-time depression.

Christensen and Martin (1970) used a similar model to describe their data for tetanic depression, which also deviate from a simple depletion hypothesis.

This model of Betz is, of course, no longer purely a depletion model. Rather, it states that only half the depression is due to depletion, the other half being caused by some other process, perhaps coupled to depletion, but one which affects the releasing potency of a nerve impulse.

We also found that the zero-time depression following depressed responses was less than that following one impulse. We decided to see whether Betz' model, which seemed to account for his results, would also predict our own. From Equations 7 and 8, it is easy to show that if the undepressed zero-time depression is 0.71 (i.e., if $r=0.29$), then the zero-time depression following responses showing a 67% depression should be reduced to 0.61 ($r'=0.39$). In fact, we found that the zero-time depression following such depressed responses was reduced to 0.33.

Not yet willing to abandon the Betz formulation, we supposed that there still occurred a reduction in F that was proportional to—and somehow tied to—the reduction in S due to depletion. However, we let the proportionality constant be unknown, so $\Delta F/F = C\Delta S/S$. Using a derivation analogous to that of Betz, it follows that F and C can be estimated from

$$F = \frac{(1-r')}{\sqrt{r(1-D) + (1-r')}} \quad \text{and} \quad C = \frac{r}{(1-F)^2}. \quad (9)$$

From our measurements of r , r' and D , we compute $F=0.775$ and $C=14.0$. In other words, there is 14 times as much change in F as there is a depletion of S .

It is certainly true that such a partial depletion model would predict less depression to repetitive stimulation than the classical depletion model. However, even this model predicts more steady-state depression as the frequency is raised, which was not observed for frequencies above 0.02 Hz (Figs. 3 and 4). This partial depletion hypothesis also predicts that as transmission is reduced toward zero, both zero-time and tetanic depression should vanish. Furthermore, a parallel compartment model, in which there are two releasable stores only one of which is subject to rapid depletion (Birks and MacIntosh, 1961; Richards, 1972; Friesen, 1975), would also be subject to relief from depression in high magnesium. All these predictions were contradicted by our results (Fig. 6). For this reason, we do not believe the Betz hypothesis, or our generalization of it, or a parallel compartment model provide a useful explanation for depression at the crayfish neuromuscular junctions of the giant motoneurone.

Finally, we have shown that the deviations of our results from a depletion hypothesis are not likely to be due to an altered mobilization of transmitter into the releasable store, either following repeated stimulation (Fig. 5) or induced by magnesium ions (Fig. 7). In recent experiments on the squid giant stellate synapse, Kusano and Landau (1975) found good agreement between the effects of calcium concentration on transmission and depression, and predictions of the depletion hypothesis. However, they observed a deviation of the steady-state level of synaptic depression from that predicted on a classical or Betz-type depletion hypothesis. They proposed that a rapid phase of mobilization, triggered

only by repetitive stimulation, was operative. Similar suggestions have been made by other authors to account for this sort of failure of the classical depletion model to describe correctly an observed tetanic depression (Elmqvist and Quastel, 1965; Hubbard et al., 1971; Richards, 1972; Lass et al., 1973). Our results show that if such a rapid mobilization exists here, it must be ended by 20 ms following each impulse.

The failure of elevated magnesium to diminish long-lasting depression, either its accumulation during repetitive stimulation or its magnitude and time course following one impulse, is very difficult to reconcile with any depletion hypothesis which attempts to relate the amount of depression to the degree of depletion of transmitter previously released. This independence of depression and level of transmission sets this synapse apart from all the others studied in the context of a depletion model, and forces us to conclude that the depletion hypothesis does not provide a useful basis of explanation of our results.

There are a number of alternative possibilities that may account for depression. We have not been able to perform a quantal analysis of depression in this preparation, as we cannot eliminate postsynaptic desensitization as a factor. Other non-specific postsynaptic factors have been eliminated (Bruner and Kennedy, 1970). Presynaptically, there may be a change in the shape of the nerve impulse after activity, or a failure of propagation into terminal branches. We were unable to record nerve terminal potentials from motor terminals. Finally, there may be a change in some other step in the depolarization-secretion process. For example, the calcium influx accompanying nerve impulses may decline. Without additional results, further speculation seems fruitless.

The synaptic depression at the crayfish giant motoneurone neuromuscular junction may not be so unique in its unaccountability in terms of a depletion model. Other synapses exhibiting long-lasting depression with relevance to behavioural habituation have been described (Kandel et al., 1970; Zucker, 1972b) whose kinetics resemble those of the present neuromuscular junction. Experiments on these central nervous synapses indicate that their depression is presynaptic in origin (Zucker, 1972b; Castellucci and Kandel, 1974a). Preliminary work suggests that these synapses also show a depression independent of the amount of transmitter released (Zucker, 1972a; Castellucci and Kandel, 1974b). Thus it appears that at these synapses, whose plasticity is responsible for the most rudimentary form of learning, a depletion model is also unsatisfactory.

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