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SYNAPTIC ACTION DURING AND AFTER REPETITIVE STIMULATION

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Under natural conditions synapses are activated by trains of impulses that may be of relatively high frequency. For example the annulo-spiral endings of muscle spindles often discharge impulses at frequencies as high as 200/sec (Matthews, 1933; Hunt & Kuffler, 1951; Eldred, Granit & Merton, 1953; Granit, 1955); and in conditions of extreme stress with activation by the gamma efferents the discharge frequency may be much higher (Matthews, 1933; Eldred *et al.* 1953) so that synapses may be activated at frequencies up to 500/sec. Presumably it is functionally desirable that such high frequencies should result in a greater rate of output of transmitter substance, the higher frequency of receptor organ discharge resulting in a greater synaptic excitation of motoneurones (cf. Granit, 1955). Repetitive synaptic activation of motoneurones has been studied by the monosynaptic reflex discharges generated thereby (Hagbarth & Naess, 1951; Eccles & Rall, 1951*a*; Jefferson & Schlapp, 1953; Alvord & Fuortes, 1953; Fuortes & Hubel, 1956; Evanson, 1956; Lloyd & Wilson, 1957; Lloyd, 1957*a*, *b*) and by the synaptic potentials recorded either extracellularly or after electrotonic spread to the ventral root (Eccles, 1946; Eccles & Rall, 1951*a*). In the present investigation repetitive synaptic activation has been studied in detail by intracellular recording from motoneurones. An investigation of the response to a second volley over a wide range of intervals leads on to a study of a wide range of frequencies of activation.

There have been many investigations of the changes in synaptic efficacy after single and repetitive stimulation (cf. Hughes, 1958). For example, there have been comprehensive studies by Bernhard (1947), Lloyd (1949, 1952), Ström (1951), Eccles (1946), Brooks, Downman & Eccles (1950), Brock, Eccles & Rall (1951), Eccles & Rall (1951*b*), Beswick & Evanson (1955*a*, *b*) and Granit (1956) of changes signalled by the sizes either of monosynaptic reflexes or of the extracellular synaptic potentials in the spinal cord. By employing intracellular recording it has been possible to

achieve a more analytical investigation of this post-activation potentiation or depression. Intracellular recording of post-activation potentiation of monosynaptic activity has already been reported for standard conditioning tetani (Eccles, 1953; Eccles, Krnjević & Miledi, 1959). However, this present investigation has concerned the post-activation changes induced by a wide range of frequencies and durations of conditioning synaptic stimulation, from single stimuli to high frequencies of 30 sec duration.

Furthermore, an attempt has been made to relate the changes in synaptic activity during repetitive stimulation to the changes observed post-tetanically. In the light of this information the effect of repetitive stimulation on the mobilization of transmitter substance will be discussed. Since the magnitude of post-synaptic potentials, particularly their rate of rise, is a measure of the action exerted by the synaptic transmitter (Eccles, 1957; Curtis & Eccles, 1959), the present experiments give evidence directly relating to the amount of synaptic transmitter released under the varying circumstances of the investigations.

METHODS

All experiments were performed upon motoneurones located within the lumbosacral segments of cats lightly anaesthetized with pentobarbital sodium. The spinal cord was divided in the lower thoracic region. The general methods were those in standard use in this laboratory (cf. Brock, Coombs & Eccles, 1952; Eccles, Fatt, Landgren & Winsbury, 1954; Coombs, Eccles & Fatt, 1955*a*). The intracellular responses were recorded by means of glass micro-electrodes filled with 0·6 M-K_2SO_4 solution. Various muscle nerves were mounted on stimulating electrodes in a warmed paraffin pool. The stimuli were invariably above maximal for Group Ia afferent fibres, and were several times maximal during the long conditioning tetani. Except in special cases, excitatory post-synaptic potentials have been evoked by afferent volleys in the nerve supplying the muscle that is innervated by the motoneurone under investigation. The ventral roots of the L6, L7 and S1 segments were cut. The frequencies of the stimuli used were checked against a standard oscillator and were accurate to $\pm 5\%$.

RESULTS

Synaptic activation by a second volley

As is illustrated by the intracellular records of Fig. 1*A*, the monosynaptic EPSP's (excitatory post-synaptic potentials) set up by two afferent volleys in the same muscle nerve do not sum until the volley interval is less than about 20 msec. At shorter intervals the size of the second EPSP may be determined by subtracting the initial control response from the summed response. This procedure is justified because an analysis of the summed EPSP's produced by two different volleys converging on the same motoneurone has revealed that the second EPSP suffers a negligible depression on account of the superposition (Eccles, Eccles & Lundberg, unpublished

observations). In Fig. 1*B* the EPSP produced by a second volley in the same afferent fibres was a little larger than the control for volley intervals up to 25 msec. Usually the second EPSP was depressed at all brief intervals (Fig. 2*A*, open circles; cf. Eccles, 1946), this depression passing off with volley intervals in excess of 1 sec. There is a corresponding depression of a testing monosynaptic reflex (Bernhard, 1947; Brooks *et al.* 1950; Brock *et al.* 1951). However, depression of the EPSP was usually less with volley intervals briefer than 50 msec (Fig. 2*A*), there being thus a relative potentiation corresponding to the phase of considerable potentiation displayed with other motoneurones (Figs. 1*B*, 2*B*). At volley intervals from 50 msec to 1 sec depression was regularly observed.

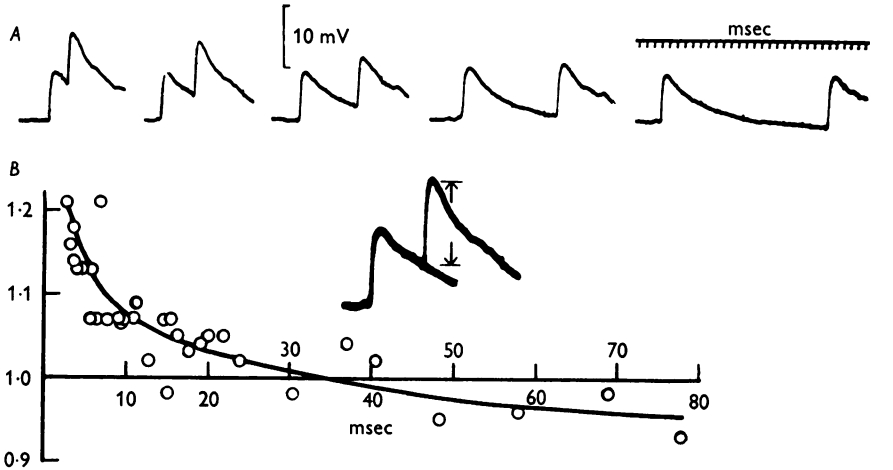

Fig. 1. *A*. Monosynaptic EPSP's set up in a gastrocnemius motoneurone (resting potential, −74 mV) by two maximal Group Ia volleys in the gastrocnemius nerve. The second stimulus was several times maximal strength for Group Ia in order to be maximal early in the refractory period; hence the polysynaptic waves on declining phase of its EPSP. Note calibration scale for d.c. amplifier and time in milliseconds. *B*. Plotting of relative size of EPSP's evoked by second volley against volley interval for series partly illustrated in *A*. Measurement of size of second EPSP is shown in inset (higher amplification than *A*).

When synaptic transmission was blocked by curare, a comparable phase of potentiation (usually 15–30 %) for a second synaptic potential was found in the cat stellate ganglion (Eccles, 1943), and in the turtle superior cervical ganglion (Laporte & Lorente de Nó, 1950). In the absence of curarization block a second volley caused an increased discharge from the cat stellate ganglion for volley intervals as long as 2 sec (Larrabee & Bronk, 1947), while at shorter intervals of activation (up to 0·3 sec) it was shown that a potentiated synaptic action was superimposed on the depression of ganglion cells resulting from their discharge to the conditioning preganglionic volley (Job & Lundberg, 1953).

Mammalian neuromuscular transmission provides an even closer parallel to the findings of Figs. 1, 2. At intervals up to several seconds the second volley sets up a diminished (range 60–90 % of control) end-plate potential (Eccles, Katz & Kuffler, 1941; Liley & North, 1953; Lundberg & Quilisch, 1953); but with volley intervals briefer than 100 msec there is

often a relative potentiation above this depression (Liley & North, 1953), which may even be large enough to give an absolute potentiation (Lundberg & Quilisch, 1953; Hubbard, 1959), just as in Figs. 1*B*, 2*B*.

Thus the response evoked by the testing nerve impulse reveals that activation of both the monosynaptic and mammalian neuromuscular synapses is followed by two opposed processes: a briefer phase of enhanced action is superimposed on a more prolonged depression; and the relative

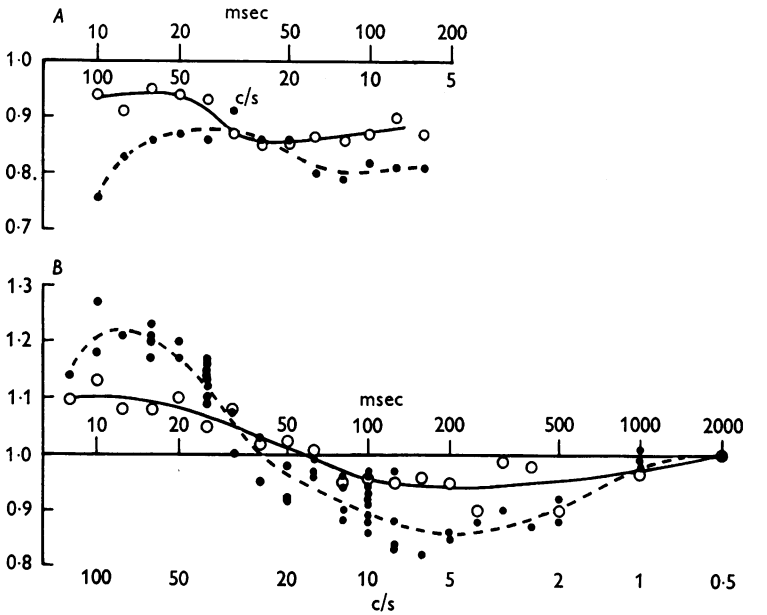

Fig. 2. *A* and *B* are respectively biceps-semitendinosus and gastrocnemius motoneurones with resting potentials of −65 mV and −78 mV. ○, size of second EPSP against volley interval, as in Fig. 1*B*, except that the abscissa scale is logarithmic (note upper scales in milliseconds). ●, relative sizes of EPSP's during the steady state obtaining after the first few responses at various frequencies of stimulation (cf. Fig. 3), as shown by the lower logarithmic scales in cycles per second, the two logarithmic scales being in fact identical. Note that in both *A* and *B* there is a considerable degree of correspondence between the curves for the second EPSP and the repetitive series of EPSP's.

intensities of these two processes vary considerably in different preparations, so that one or the other may be dominant at brief intervals. Potentiation is much more prominent after a single activation of the amphibian neuromuscular junction, particularly when it is curarized (Schaefer & Haass, 1939; Feng, 1941; Eccles *et al.* 1941; del Castillo & Katz, 1954*a*, *b*), but probably it does not differ qualitatively from the potentiation at mammalian synapses.

Repetitive synaptic stimulation

Repetitive stimulation evokes standard responses in those motoneurones in which the monosynaptic EPSP's produced by maximum Group Ia volleys are virtually uncontaminated by superimposed polysynaptic EPSP's or IPSP's. When the temporal summation of EPSP's does not evoke the discharge of impulses, a steady state is attained after the first few EPSP's, even over a wide range of frequencies (Fig. 3; cf. Eccles & Rall, 1951*a*); and this steady state is maintained for hundreds of responses.

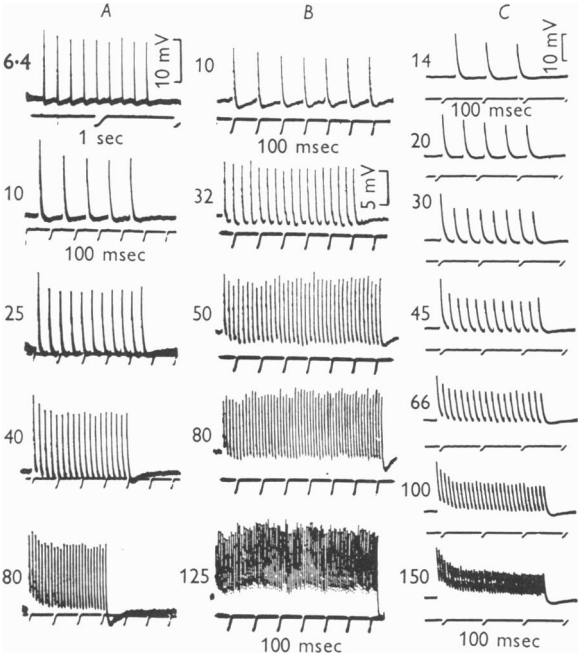

Fig. 3. *A*, *B*, *C* show repetitive monosynaptic EPSP's (intracellular) evoked in biceps-semitendinosus (resting potential, −65 mV), gastrocnemius (resting potential, −78 mV) and deep peroneal (resting potential, −70 mV) motoneurones respectively; frequencies of stimulation are marked on each record. Time scales are in 100 msec, except for scale in seconds on top record of *A*; d.c. amplification throughout. Note potential scales for *A*, *B* and *C*.

When the frequency is below 50/sec, there is virtually no overlap of the successive EPSP's, and it is usually observed that there is a progressive decline in size over the first few responses until the uniformly depressed size is attained (Fig. 3*A*, *C*). It will be seen in Fig. 3 that following each EPSP there is a prolonged after-hyperpolarization which sums during the repetitive stimulation (Brock *et al.* 1952; Coombs, Eccles & Fatt, 1955*b*). With higher frequencies the second and subsequent EPSP's are, in addi-

tion, superimposed on the residuum of preceding EPSP's. As a consequence these EPSP's may attain a higher summit than the first, even though individually depressed in size (Fig. 3*A* at 80/sec). In Fig. 3 the plateaux of both the onsets and the summits of the individual EPSP's have reached a steady value within 200 msec. In different preparations the plateau may be reached, as in Fig. 3*A*, *C*, after an initial phase of rise and decline; or there may be a continued rise to the plateau (Fig. 3*B* at the higher frequencies). In part this initial phase is attributable to summation of the earliest EPSP's, but it must also arise on account of the initial period of adjustment of the EPSP to the steady-state size characteristic of that frequency, and there is also the background of summed after-hyperpolarizations. Two procedures have been adopted in order to measure accurately the sizes of EPSP's during the steady-state response to repetitive stimulation.

The first method is applicable only during the steady state and underestimates the size with those frequencies giving appreciable summation of successive EPSP's, i.e. above 50/sec. If the sweep speed is sufficiently high, sweeps can be repeated at frequencies up to 100/sec, so giving superimposed traces of EPSP's. By a suitable delayer device the frequency can be set at as low a level as one pleases. In this way the superimposed traces of EPSP's were obtained over a wide range of frequencies (cf. Fig. 4*A*). With frequencies of 1/sec or faster the camera shutter was opened after the first few responses in order that all the superimposed traces would occur during the steady state. With low frequencies there were five superimposed traces, but the number was much larger for the higher frequencies. When the sizes of the superimposed EPSP's of the whole series partly illustrated in Fig. 4*A* were plotted against frequency or volley interval (scaled logarithmically), the points on the extreme right reveal that there was no appreciable change in size of EPSP's until the frequency was in excess of 0·4/sec (Fig. 4*B*). There was a progressive depression as the frequency was raised to 5–10/sec. With further increase in frequency, the EPSP increased to a maximum at about 50/sec, being then almost as large as at the lowest frequencies. The decline of the individual EPSP's at still higher frequencies would be in part attributable to superposition on the preceding EPSP's (cf. Fig. 3*B*, at 125/sec). A curve similar to Fig. 4*A* was obtained if the steepest rising slope of each EPSP was measured instead of its height.

This investigation of the sizes of EPSP's during the steady state over a wide range of frequencies has been applied to fourteen motoneurones. The curves so obtained corresponded to Fig. 4*B* in general features; but, as in Fig. 5*A*, the size at the optimal high frequency response was sometimes much below the control level at the lowest frequencies, though with three

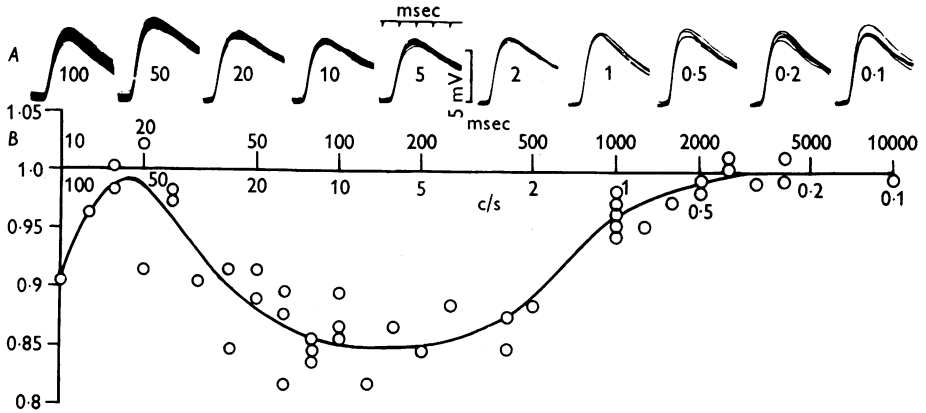

Fig. 4. *A*. Intracellularly recorded EPSP's from a biceps-semitendinosus motoneurone with a resting potential of −62 mV; the superimposed traces were obtained during the steady state of repetitive responses at the indicated frequencies per second. *B*. The EPSP's partly illustrated in *A* are expressed as fractions of the mean size obtaining at 0·4 c/s or slower and plotted against the respective stimulus frequencies on a logarithmic abscissal scale as in Fig. 2. Above the frequency scale the corresponding stimulus intervals are shown in milliseconds.

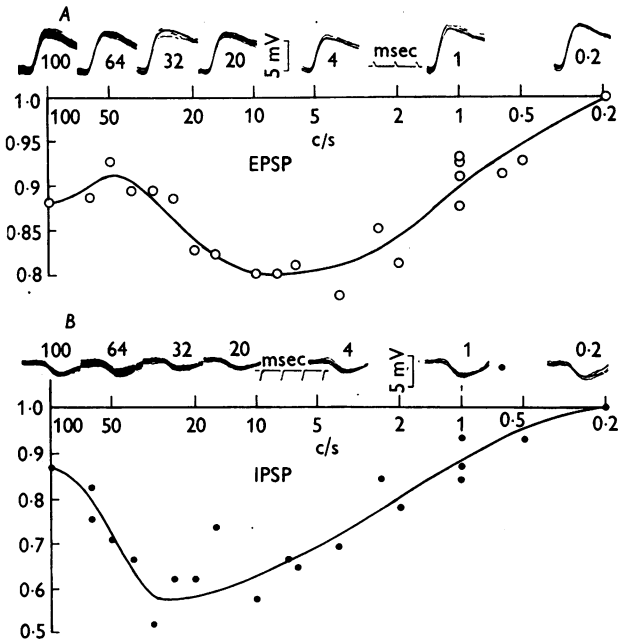

Fig. 5. Plotting as in Fig. 4 of repetitive EPSP's (*A*) and IPSP's (*B*), intracellularly recorded from a biceps-semitendinosus motoneurone with resting potential of −72 mV. Above each series are shown representative records formed by superimposed traces, as in Fig. 4*A*, and at the frequencies indicated in cycles per second. The EPSP's and IPSP's are evoked by maximum Group Ia volleys in biceps-semitendinosus and quadriceps nerves respectively.

motoneurones it was larger. Invariably there were depressed EPSP's at the intermediate frequencies, as in Figs. 4, 5*A*, the range being from 70 to 85 % of the control at frequencies in the range of 4–20/sec. The range of the maximum at higher frequencies was from 77 to 128 %, the optimal frequency lying between 30 and 100/sec. Repetitive EPSP's from the experiment with one of the largest potentiations are illustrated in Fig. 3*B* and plotted in Fig. 2*B* (filled circles). There is depression at 10/sec, 32/sec is transitional and at 50/sec and 80/sec there is a large potentiation of the EPSP's after the initial period of adjustment.

When EPSP's have been investigated both with double volleys and during the steady state to repetitive stimulation, there was a good correlation between these two responses. For example, a second volley evoked a potentiated EPSP at intervals less than 50 msec in Fig. 2*B* (open circles), and correspondingly the EPSP's evoked at frequencies of 40–100/sec were potentiated well above the control level (Figs. 2*B*, 3*B*). On the other hand, when the potentiation of the second of two EPSP's at brief intervals was inadequate to overcome the depression (Fig. 2*A*, open circles), depression also dominated the repetitive response at high frequencies (Fig. 3*A*, Fig. 2*A* filled circles).

A similar investigation has been performed for the IPSP's set up in two motoneurones by Group Ia afferent impulses (direct inhibition). There was in both a decline with rising frequency to a minimum at 10–30/sec and an increase at still higher frequencies (cf. Fig. 5*B*). Since there is an interneurone on the inhibitory pathway, the frequency–response curve may be significantly modified by this interpolated synaptic relay, temporal facilitation at this synapse contributing at least in part to the increased IPSP at higher frequencies (cf. R. M. Eccles & Lundberg, 1958). Nevertheless, the general similarity of the curves does suggest that inhibitory synapses are affected by the frequency of activation in much the same way as excitatory synapses. The large depression at slow frequencies in Fig. 5*B* contrasts with the finding that under such conditions there is little or no depression of reflex inhibition (Beswick & Evanson, 1957; Wilson, 1958).

In the second method the size of the EPSP during the steady state of the repetitive response (cf. Fig. 3) was determined by expanding the end of the repetitive response at a fast sweep speed (Fig. 6*A*). It can be assumed that during the steady state the decline of the penultimate EPSP of the tetanus follows the same time course as the last; hence by subtraction of this assumed curve the size of the last EPSP can be determined. This method can be applied at high frequencies (Fig. 6*A*), and reveals that the apparent size of the added EPSP declines with increasing frequency, particularly beyond 220/sec. The relationship is best expressed by plotting this size against the stimulus interval, as in Fig. 6*B* (filled circles). There is

approximately a direct proportionality between size of EPSP and stimulus interval for frequencies in excess of about 300/sec and up to 660/sec, which was the highest level applied in our tests. A similar result has been reported for synaptic potentials electrotonically conducted to the ventral root (Eccles & Rall, 1951*a*). The relationship of direct proportionality indicates that the rate of output of transmitter reaches a maximum with

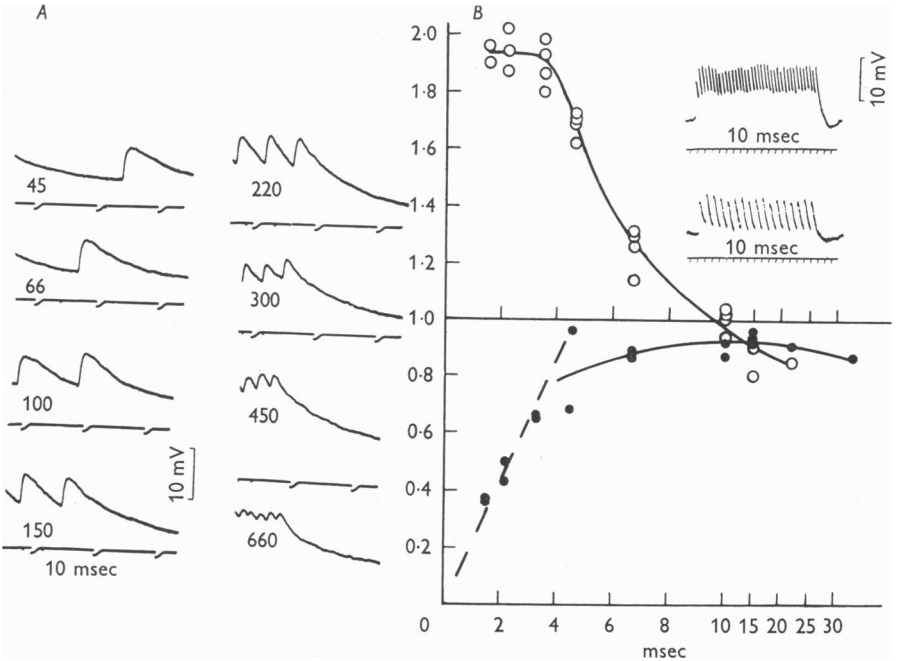

Fig. 6. *A*. Repetitive EPSP's of a gastrocnemius motoneurone recorded intracellularly, resting potential, −68 mV. The records show the end of a repetitive response at the indicated stimulus frequency, which was applied for 170 msec, so that a steady state of repetitive response was attained (cf. Fig. 3); d.c. amplification at the indicated potential scale; time in 10 msec. *B*. ●, sizes of the terminal responses of the repetitive series partly illustrated in *A*, measured relative to the mean value of EPSP's after rest periods of several seconds; the straight line through zero origin shows that there is approximately a direct proportionality at high frequencies. ○, plot of plateau height against stimulus interval for the repetitive EPSP series of the same motoneurone. Inset shows specimen records at 150 and 300/sec.

frequencies in excess of 300/sec. The attainment of a maximum rate of output independent of frequency is also indicated by the curve obtained by plotting the heights of the steady-state plateau against the stimulus intervals (Fig. 6*B*, open circles). There is a rapid increase in plateau height as the frequency rises from 100 to 300/sec, but virtually no change from 300 to 660/sec.

Correlation with the changes in monosynaptic reflexes during repetitive stimulation is only feasible at relatively low frequencies, because at frequencies above 10/sec each successive reflex response is depressed by the after-hyperpolarization that follows a motoneuronal discharge (Brock *et al.* 1952; Coombs *et al.* 1955*a*). At slower frequencies there is good agreement between the two types of investigation. For example, Jefferson & Schlapp (1953) and Evanson (1956) found that reflex depression occurred with frequencies above 0·3/sec, and progressively deepened as the frequency was increased. By employing very sensitive techniques Lloyd & Wilson (1957) and Lloyd (1957*b*) have been able to detect slight depressions with frequencies as low as 0·1/sec. Again, they found increasing depression as the frequency was increased up to 10/sec. After the onset of any frequency of repetitive stimulation a steady state of reflex depression was rapidly attained, just as has been observed for the EPSP's (cf. Fig. 3). At still higher frequencies there was a further deeper depression of reflex discharge, which on experimental evidence was attributed to after-hyperpolarization of the discharging motoneurones. However, at still higher frequencies of activation (usually 60–150/sec, but even as low as 20/sec), there was a phase of relatively less depression, the higher the frequency the less the depression (Alvord & Fuortes, 1953; Lloyd, 1957*a*, *b*). Doubtless much of this effect is due to temporal summation of EPSP's, as suggested by these investigators, but part would also arise on account of the potentiation of EPSP's which contributes to the increased rate of transmitter output for frequencies in excess of 30/sec (Figs. 2*B*, 3*B*, 4, 5*A*). This latter mechanism can be revealed only by intracellular recording.

Repetitive responses of the mammalian neuromuscular junction have been dominated much more by depression (Hutter, 1952; Lundberg & Quilisch, 1953; Liley & North, 1953). However, an effective potentiation was observed when the end-plate potential was depressed by low Ca or high Mg (Lilley, 1956*b*). Potentiation has been a dominating feature with repetitive activation of the amphibian neuromuscular junction (Feng, 1941), particularly during treatment by high magnesium and low calcium (del Castillo & Katz, 1954*b*). Under these conditions there was such a prolonged period of progressive potentiation that a ceiling was still not attained after 50 responses. Since the miniature end-plate potentials showed no increase in size, it was concluded that the potentiation was due to progressive increase in the number of quanta of transmitter emitted by the successive impulses. In contrast, during repetitive activation of normal and curarized amphibian junctions the initial phase of potentiation rapidly gave place to depression of the end-plate potentials, this effect again being due to a corresponding change in the number of quanta emitted by an impulse (del Castillo & Katz, 1954*b*).

Synaptic potentiation and depression after a conditioning tetanus

Recently (Eccles *et al.* 1959) there has been a description and illustration of post-tetanic potentiation of monosynaptic EPSP's after standard conditioning tetani of 400/sec for 10 sec. The present investigation explores the effect of variations in the frequency and duration of the conditioning tetanus. When the post-tetanic potentiation caused the EPSP to generate a spike potential, the steepest part of the rising phase was employed as a measure of the intensity of synaptic action. Probably this is more satisfactory than the height of the EPSP, but this latter measurement has been more convenient, so it has been plotted for those series uncomplicated by spikes. Since there was usually a considerable prolongation of the rising phase of the potentiated EPSP (cf. Eccles *et al.* 1959), larger relative values were obtained for potentiations measured by heights than by slopes.

With increase in the duration of the conditioning tetanus there was at first an increase in both the height and the duration of post-tetanic potentiation; but a ceiling was reached for the height of the potentiation (at 640/sec for 10 sec in Fig. 7*B*), and conditioning tetani of longer duration (*C*, *D*) merely slowed the rise and decline of the potentiation. The potentiations of the EPSP thus provide an exact parallel to the potentiations of the monosynaptic reflex discharges (Lloyd, 1949, Fig. 9). Similar observations have been made on the post-tetanic potentiation both of synaptic

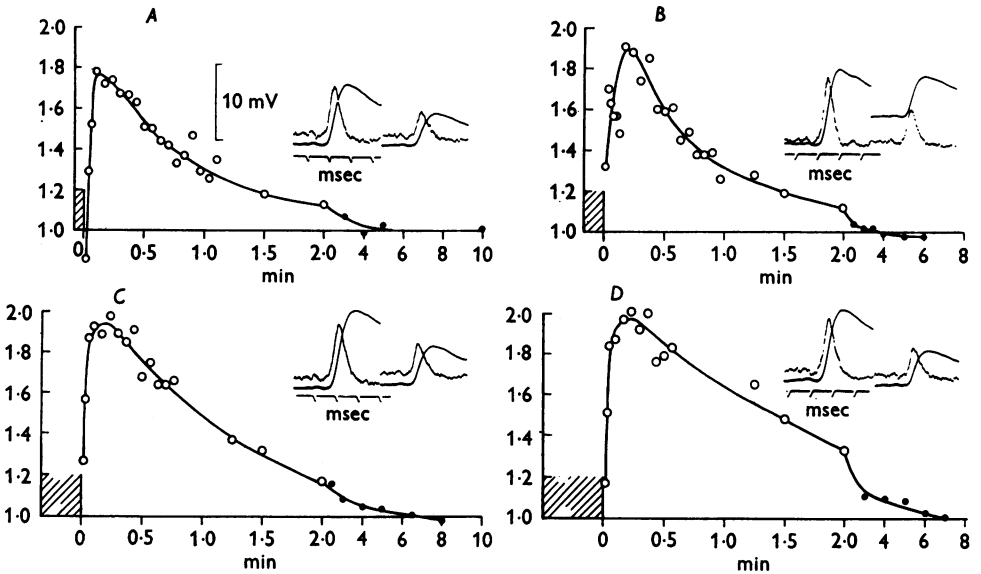

Fig. 7. Post-tetanic potentiation curves of monosynaptic EPSP's of a biceps-semitendinosus motoneurone, resting potential −62 mV. The conditioning tetani were uniformly at 640/sec, the durations being for *A* 5, *B* 10, *C* 20 and *D* 30 sec. Inset records show the EPSP at maximum potentiation and at the initial control level together with the electrically differentiated record. Sizes of EPSP's are calculated relative to the initial mean level which was at least 10 min after a previous tetanic conditioning; durations of the tetani are shown by the initial hatched blocks. Time scale was greatly compressed after 2 min and the symbols were altered to filled circles. All points except those on the rapid rising phase are the means of three consecutive testing responses.

transmission through a sympathetic ganglion (Larrabee & Bronk, 1947) and of mammalian end-plate potentials (Liley & North, 1953). The maximum potentiation of the EPSP was usually in the range 1·5–2·0 times the control (Figs. 7, 8, 10; Eccles *et al.* 1959). Much larger potentiations are usually displayed by reflexes (Lloyd, 1949), because the changes in synaptic efficacy are sampled by the response of a population of motoneurones and not directly, as when the size of the EPSP is employed as the criterion.

When the number of stimuli in the conditioning tetanus was held constant, but the frequency was varied, the maximum potentiation was lower when the frequency was below a critical level of about 300/sec (Fig. 8*B*; cf. Lloyd, 1949). After the lower conditioning frequencies the potentiation followed a slower time course with a later summit and slower decline (Fig. 8*C*, *D*). In these respects also the post-tetanic potentiation of the EPSP parallels the potentiation of monosynaptic reflexes (cf. Lloyd, 1949, Figs. 11, 12). However, potentiation of EPSP's was not usually observed for conditioning frequencies much below 100/sec even when continued for several seconds, though Lloyd reported slight post-tetanic potentiation of reflex discharges after conditioning tetani of 50–75/sec.

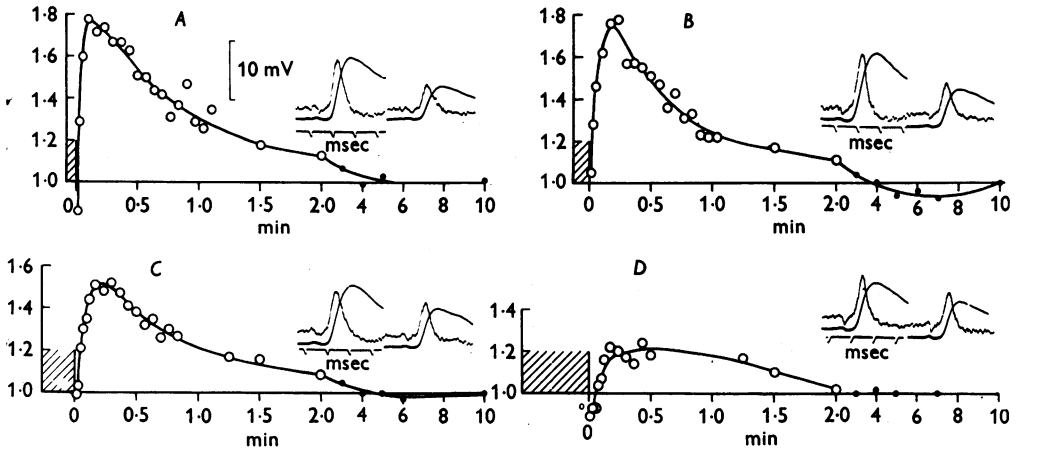

Fig. 8. Series as with Fig. 7 and for same motoneurone, but the conditioning tetanus was in every case 3200 volleys, *A* at 640/sec for 5 sec, *B* 400/sec for 8 sec, *C* 200/sec for 16 sec and *D* 100/sec for 32 sec.

With the mammalian neuromuscular junction there was no potentiation after repetitive activation at 50/sec or lower and 350/sec was adequate for maximum potentiation (Liley & North, 1953).

When post-tetanic potentiation was sampled by a brief repetitive stimulation, there was a rapid decline in the relative potentiation of the successive responses at any one test interval. In one example, when the first EPSP was potentiated by about 60 % relative to the control, the relative potentiations declined progressively for the subsequent responses, being only 20 % with the last (cf. Eccles, 1957, Fig. 7*A*, *B*). An even more rapid decline occurred when a repetitive stimulus tested the post-tetanic potentiation at the curarized mammalian neuromuscular junction (Liley & North, 1953). A very rapid decline during a repetitive test was also found by Ström (1951) during post-tetanic potentiation of a monosynaptic reflex.

These results may be taken to indicate that large reserves of available transmitter are not mobilized during post-tetanic potentiation.

Late depression. It was reported and illustrated without comment (Eccles *et al.* 1959, Figs. 3, 6) that some minutes after the conditioning tetanus, the post-tetanic potentiation occasionally passed over to a depression that persisted for many minutes. A comparable depression of the EPSP occurred in Fig. 8*B* from 4 to 7 min post-tetanically, and it had passed off by 10 min. Since the sizes of the control EPSP's had not varied by more than 2 % for a period of 30 min, including the 10 min period of Fig. 8*B*, it can be presumed that there was a genuine depression of as much as 7 % in synaptic efficacy and not some transient defect in the intracellular recording. Such transient defects frequently arise when movement causes the micro-electrode to be no longer sealed effectively in the motoneuronal membrane. As would be expected, all excitatory synaptic potentials are then similarly diminished, and there is a simultaneous fall in the membrane potential. Such defects of intracellular recording can be detected and allowed for if a synergic excitatory path is employed as a control test throughout the whole of the post-tetanic period.

In each sweep of the inset records of Fig. 9*A* two EPSP's were set up in a gastrocnemius motoneurone, the first by a volley from the lateral gastrocnemius-soleus (LGS) nerve, the second by a medial gastrocnemius (MG) volley. Both before and after the conditioning tetanization of the MG nerve (400/sec for 10 sec at the arrow) the monosynaptic EPSP's were evoked every 2 sec and three traces were superimposed on each record. As would be expected, the large increase in the MG EPSP at 4 sec post-tetanically contrasted with the absence of any appreciable change in the LGS EPSP, which remained virtually constant throughout the whole post-tetanic test period. Each of the points plotted for the post-tetanic period in Fig. 9*A* was obtained by expressing the ratio of the MG EPSP to the LGS EPSP at that time relative to the ratio obtaining before the conditioning tetanus. Since the effects of any transient changes in the intracellular recording were thus eliminated, the prolonged post-tetanic depression of EPSP in Fig. 9*A* (cf. the inset records at 154 and 220 sec) must be due to a diminution of synaptic efficacy. Usually the post-tetanic depression of the EPSP was much less (cf. Fig. 9*B*) and sometimes it was not detectable. The series of Fig. 7*A–D* suggests that post-tetanic depression may be submerged beneath the prolongation of the post-tetanic potentiation that occurs after a long conditioning tetanus. Possibly the degree of submergence beneath the potentiation accounts for the variations in the prominence of the depression between different series both on the same and on different motoneurones.

IPSP potentiation. The IPSP (inhibitory post-synaptic potential) pro-

duced by 1a impulses is also often potentiated after a conditioning tetanus (R. M. Eccles & Lundberg, 1958), corresponding to the post-tetanic potentiation of direct inhibition (Lloyd, 1949; Wilson, 1958), but the potentiation was usually much less than for EPSP's of the same motoneurone (Fig. 10). The differences in amount and time course of the potentiation need not indicate that inhibitory synapses differ from excitatory, but may instead be attributable to the interneurone that is interpolated in the inhibitory pathway (cf. R. M. Eccles & Lundberg, 1958).

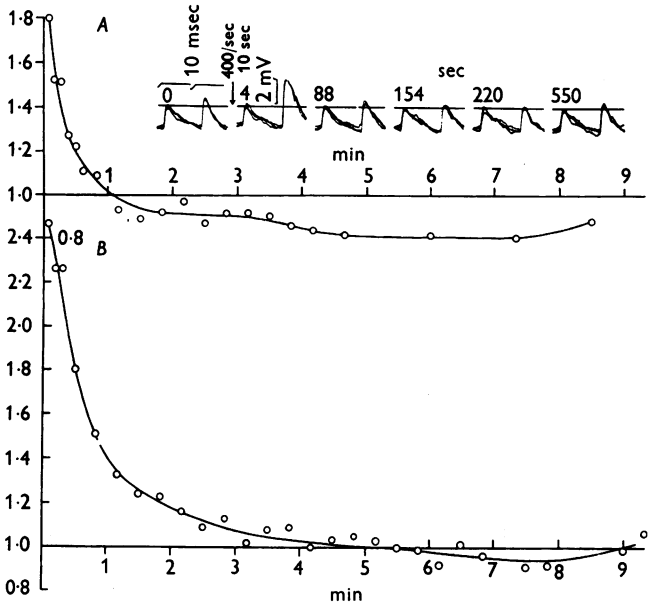

Fig. 9. *A*. Post-tetanic potentiation of monosynaptic EPSP's generated in a gastrocnemius motoneurone (resting potential, −65 mV) by lateral gastrocnemius and medial gastrocnemius volleys, as shown in the inset records. The conditioning tetanus (400/sec for 10 sec) was applied to the medial gastrocnemius nerve as shown and the subsequent records are at the indicated intervals in seconds after the end of the tetanus. Note post-tetanic depression of the testing EPSP relative to the control at 154 and 220 sec. The size of the MG EPSP is plotted relative to the LG EPSP, as described in the text. *B*. Similar plot for another gastrocnemius motoneurone.

Early potentiation. Brief conditioning tetani at a sufficiently high frequency are followed by a very early and evanescent potentiation, which was originally revealed by increases both of monosynaptic reflexes and of extracellular synaptic potentials (Eccles & Rall, 1951*b*; Lloyd, 1952; Beswick & Evanson, 1955*b*; Wilson, 1958). When studying the changes in synaptic efficacy within 2 sec after brief repetitive stimulations, only one testing stimulus could be employed after each conditioning tetanus, and

the tetani had to be spaced at least 10 sec apart so as to minimize cumulative effects during a prolonged experimental series. As shown in the inset records of Fig. 11*A–C*, the testing intracellular responses were photographed at high speed by expanding the part of the trace on which they occurred. It was thus possible to measure the steepest part of the rising slopes of the EPSP's, and so to determine the potentiation of the EPSP before the slope was increased by the superimposed spike potential. In some experiments the maximum slope was measured as the summit height of electrically differentiated records (cf. the inset records of Figs. 7, 8, 10).

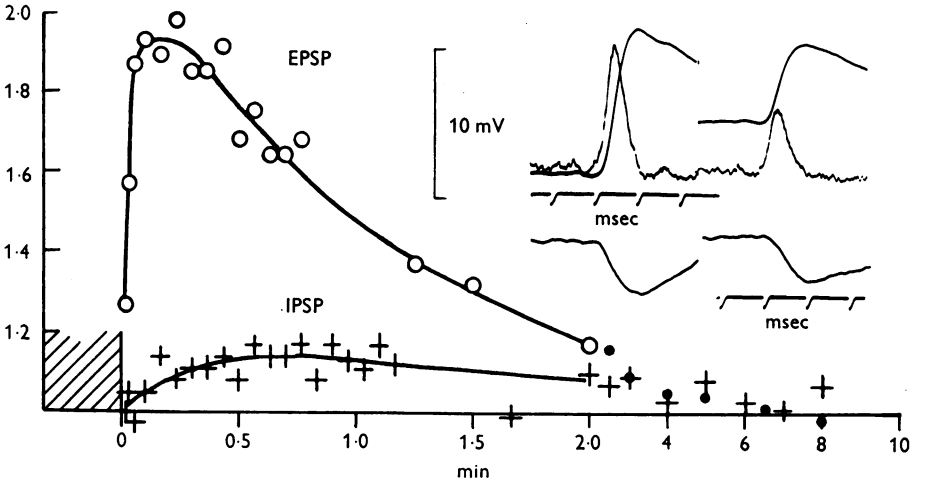

Fig. 10. ○, Post-tetanic potentiation of EPSP for the motoneurone of Fig. 7; +, potentiation of an IPSP generated in the same motoneurone by a quadriceps Group Ia volley. The conditioning tetanus in both cases was 640/sec for 20 sec, the insets showing the EPSP and IPSP at maximum potentiation, and the control. As in Fig. 7, the EPSP has been differentiated.

There were 40 conditioning stimuli for each of the series of Fig. 11*A–C*, but the frequencies were 640/sec, 400/sec and 100/sec for *A*, *B* and *C* respectively. The conditioning tetanus at 400/sec was almost as effective as that at 640/sec, but there was no potentiation whatever after 40 impulses at 100/sec, as also may be seen in the inset records. A conditioning tetanus at 200/sec was not employed in the series of Fig. 11*A–C*, but in other experiments it always gave a small potentiation, whereas 100/sec was always followed by a depression. For example, the curves of Fig. 11*D* give the time courses of the conditioning produced by 40 impulses over a wide range of frequencies in another experiment. Post-tetanic depression was regularly observed after slow conditioning frequencies. Thus the curves of Fig. 11*E* show that there was actually an increasing depression after 57/sec for 0·2 sec whereas after higher frequencies for the same duration

there was considerable potentiation at 200/sec and virtually no change at 100/sec. In this series the size of the last EPSP of the conditioning series was plotted (the initial points on the curves), it being assumed that this would be approximately the size of a test response at the stimulus interval after the end of the conditioning tetanus. There was thus an increasing depression for some time after the slow conditioning tetanus; but after 100/sec there was a potentiation above the level obtaining during the conditioning tetanus.

These potentiations of the EPSP after brief conditioning tetani correspond closely with the observations on reflex potentiation under comparable conditions (Eccles & Rall, 1951*b*; Lloyd, 1952; Beswick & Evanson, 1955*b*). For example, after a high-frequency tetanus of 40–100 volleys

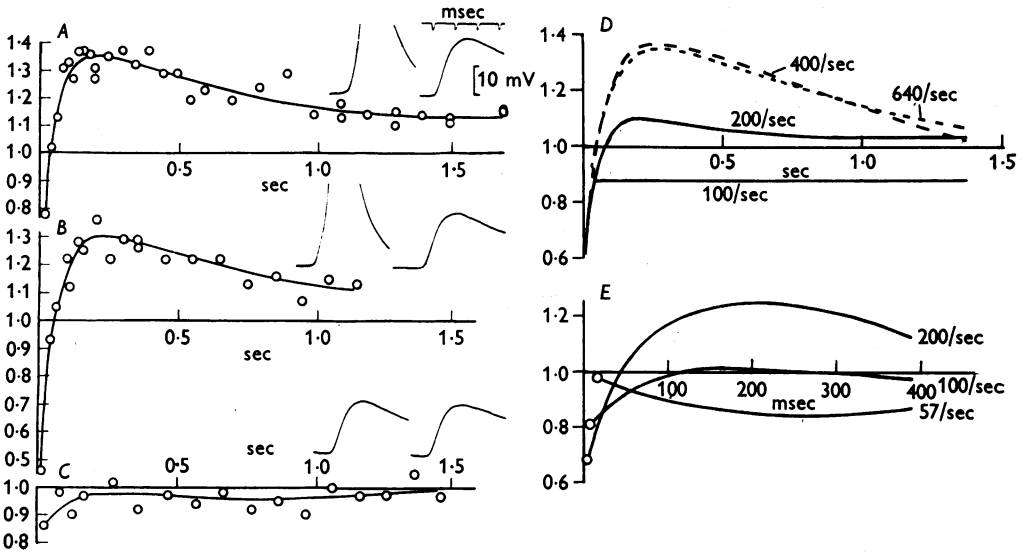

Fig. 11. Early post-tetanic potentiation and depression following brief conditioning tetani. In *A–C* the points plot the potentiation of the steepest part of the rising slope of the EPSP's as a fraction of the mean control; intracellular records from the biceps-semitendinosus motoneurone (resting potential, −60 mV) are shown as insets, the first being the response at about 0·2 sec post-tetanically, the second the control; note spike origin in *A* and *B*. Conditioning tetani of 40 impulses, the respective frequencies being 640/sec, 400/sec and 100/sec for *A*, *B* and *C*. *D* gives curves plotted on the same co-ordinates for a series such as *A–C* in another biceps-semitendinosus motoneurone (resting potential, −67 mV). The conditioning tetani are again 40 impulses. Virtually the same potentiation is produced by conditioning at 640 and 400/sec, 200/sec is intermediate, and 100/sec purely depressant. *E* gives curves on same co-ordinates for the post-tetanic effect of brief tetani at different frequencies for the same duration (0·2 sec). The initial points mark the size of the last EPSP of the conditioning tetanus. Note potentiation after 200/sec, depression after 57/sec with an intermediate response after 100/sec.

the potentiation of monosynaptic reflexes began at about 50 msec, reached a maximum at 200 msec, and thereafter rapidly declined for 1–2 sec.

It is of particular significance that the frequency of the conditioning tetanus plays such a decisive part in setting the level of post-tetanic potentiation (cf. Beswick & Evanson, 1955*b*). Since the total duration of the conditioning tetanus was only 0·4 sec in Fig. 11*C*, and since the potentiations in *A* and *B* had declined very little by 0·4 sec after the tetanus, it is evident that the increased temporal spread of the conditioning volleys was not responsible for the absence of potentiation in Fig. 11*C*. It is the frequency rather than the number of impulses that is of particular significance in producing post-tetanic potentiation. However, to some extent number of impulses can compensate for low frequency; for example in Fig. 8*D*, 3200 impulses at 100/sec gave a potentiation of 1·25 which ran a very prolonged time course, whereas after 40 impulses at 100/sec the potentiation was inadequate to compensate for the depression (Fig. 11*C*, *E*). Similarly Lloyd (1949) reported that post-tetanic potentiation of monosynaptic reflexes always occurred after prolonged repetitive stimulation at a frequency as low as 50/sec.

DISCUSSION

The present investigation has been concerned solely with the potentials generated in motoneurones by afferent impulses successively activating the same synapses, and has been almost entirely restricted to monosynaptic excitatory action. According to present concepts, the various potentiated and depressed states of synaptic action could occur in four ways: (i) block (or relief of block) of impulse transmission at sites of low safety factor in the presynaptic pathways, particularly at branching points (cf. Krnjević & Miledi, 1958); (ii) increase in size of the presynaptic impulse during the after-hyperpolarization that follows repetitive activity (Lloyd, 1949, 1952; Eccles & Rall, 1951*b*; Liley & North, 1953; Wall & Johnson, 1958; Eccles & Krnjević, 1959*a*, *b*); (iii) change in the availability of transmitter substance in the presynaptic terminals, more or less being liberated by a given size of presynaptic impulse (Brown & Feldberg, 1936; Perry, 1953; Emmelin & Macintosh, 1956; Liley & North, 1953; Liley, 1956*a*, *b*; Eccles, 1957; Hubbard, 1959); (iv) depression of the effectiveness with which the transmitter causes post-synaptic depolarization, an effect which has been called receptor desensitization (Thesleff, 1955; Katz & Thesleff, 1957; Axelsson & Thesleff, 1958). It should be noted in this context that during post-tetanic potentiation there is no appreciable increase in the sensitivity of the post-synaptic membrane to the transmitter substance (Larrabee & Bronk, 1947; Hutter, 1952). Likewise, during post-tetanic depression, testing of the motoneurones by a converging excitatory path

showed that there was no change in the excitability (Beswick & Evanson, 1957).

In many respects the end-plate potentials generated at the mammalian neuromuscular junction during and after repetitive stimulation (Lundberg & Quilisch, 1953; Liley & North, 1953; Liley, 1956*a*, *b*) exhibit phases of potentiation and depression resembling those here described for the potentials generated at central synapses. In addition, after repetitive stimulation the frequency of miniature end-plate potentials parallels the potentiation of the neurally evoked end-plate potentials (Liley, 1956*a*, *b*; Hubbard, 1959). The frequency of miniature end-plate potentials can be regarded as a measure of the availability of transmitter; hence this parallelism provides strong support for factor (iii) above. In the first instance it will therefore be expedient to attempt to explain the experimental observations during and after brief tetani by postulated changes in the availability of transmitter (cf. Liley & North, 1953). This procedure is also chosen because factors (i), (ii) and (iv) probably achieve significance only after prolonged stimulation.

In the simplest experiment it has been shown that, after a single conditioning impulse, a brief phase (up to 200 msec) of potentiation is superimposed on a depression of more than 1 sec duration (Figs. 1, 2). An important correlation is that at the mammalian neuromuscular junction the frequency of miniature end-plate potentials is increased for as long as 200 msec after a single impulse (Liley, 1956*a*; Hubbard, 1959). Both the potentiation and depression are cumulative during repetitive stimulation so that at frequencies above 10/sec a steady state of synaptic efficacy is established in about 0·2 sec. At low frequencies (1–10/sec) depression is dominant, but with the higher frequencies there is increased summation of the briefer potentiating phases (Figs. 2, 4, 5) and at frequencies of 40–100/sec the potentiation may even dominate the depression (Figs. 2*B*, 3*B*). With still higher frequencies the individual synaptic actions are depressed, the approximate inverse relationship to frequency indicating that the actual rate of liberation of transmitter reaches a maximum at frequencies in excess of 250/sec (Fig. 6*B*).

It seems possible to correlate these changes in synaptic efficacy during stimulation with the changes observed within 1–2 sec after these brief tetani (Fig. 11). On termination of repetitive synaptic stimulation at slow frequencies (100/sec or less), there is a subsequent prolonged period (seconds) of depression of the testing EPSP. This depression is greatest after frequencies that are optimal for the depression during repetitive stimulation, i.e. for frequencies of 5–20/sec (cf. Figs. 3, 4, 5). Evidently this is the same depression which was observed after single conditioning EPSP's (Figs. 1, 2) and which occurs during slow frequencies of stimulation.

It is postulated that EPSP's are depressed under all these conditions because there is a preponderant effect of depletion of the available transmitter. At higher frequencies mobilization of the available transmitter appears to become progressively more dominant, so that at about 100/sec there is an approximate balance between potentiation and depression for a second or so after the brief conditioning tetani (cf. Fig. 11*C*, *D*, *E*). After still higher frequencies potentiation is dominant, there being virtually the same high level of potentiation after brief tetani in the frequency range of 300 to 800/sec (Figs. 11*A*, *B*, *D*). This range corresponds approximately to the range for optimum rate of liberation of transmitter during repetitive stimulation.

On termination of repetitive synaptic stimulation at 250/sec or higher, there is a sudden cessation of the liberation of transmitter, which had been running at the maximum attainable rate; albeit with an output per impulse much below the level for a single impulse under resting conditions. It would therefore be expected that, after cessation of this maximum drain of transmitter from the synaptic knob, there would be an accumulation of available transmitter therein above the resting level; as a consequence a testing impulse would cause an increased liberation of transmitter, so giving the potentiated EPSP seen in Fig. 11. The time course of this early post-tetanic potentiation indicates that available transmitter goes on accumulating for as long as 200 msec after repetitive synaptic stimulation has ceased and then slowly declines over several seconds. During lower frequencies of synaptic stimulation (150–200/sec) there is a lower rate of liberation of transmitter (Fig. 6*B*), and correspondingly there is a lower potentiation after cessation (Fig. 11*D*).

Thus in general the EPSP's generated during and after brief repetitive stimulation can be satisfactorily accounted for by the postulate that each presynaptic impulse exerts two opposing actions on the transmitter mechanism, a depletion of available transmitter and a mobilization which may be more or less than compensatory. At any frequency of presynaptic activation a balance is soon reached between these two effects, so giving the steady state (Fig. 3). It seems furthermore necessary to postulate that with impulses in quick succession (40/sec or more), there is some serial facilitatory action causing a more effective mobilization of available transmitter and a consequent potentiated output of transmitter due to the mobilization process dominating the depletion.

In preliminary observations Liley (1956*b*) showed that after as few as 100 impulses (200/sec for 0·5 sec) there was a considerable increase in frequency of miniature end-plate potentials at the mammalian neuromuscular junction, the relative increase being larger than the potentiation of the end-plate potential. Systematic investigations with brief conditioning tetani of various durations and frequencies have shown that the

post-tetanic increase in frequency of miniature potentials was always more than the potentiation of the end-plate potential; hence it can be concluded that the post-tetanic increase in available transmitter is sufficient to explain both the size and duration of the potentiation (Hubbard, 1959).

It has been observed that after cessation of a prolonged (10 sec or longer) high-frequency tetanization of synapses, there was a phase of depressed synaptic transmission for as long as 1 sec, maximum post-tetanic potentiation occurring as late as 30 sec after the tetanus (Fig. 7*D*; Lloyd, 1949, 1952; Eccles & Rall, 1951*b*; Ström, 1951; Eccles *et al.* 1959). This delayed attainment of maximum potentiation also occurred after prolonged tetanization of sympathetic ganglia (Larrabee & Bronk, 1947) and of the curarized mammalian neuromuscular junction (Liley & North, 1953; Liley, 1956*a*; Hubbard, 1959). In the latter situation the frequency of miniature end-plate potentials was usually maximal immediately after the tetanus, indicating that available transmitter was then at a maximum, yet there was not a corresponding release by the testing impulse. Probably this discrepancy is attributable to failure of the presynaptic impulse to invade all the synaptic terminals, an explanation (cf. factor (i), p. 390) which also seems likely for the delayed summit of post-tetanic potentiation of central synaptic transmission.

Two explanations (factors (ii) and (iii) above) are available for the prolonged potentiation that follows a long high-frequency tetanus (cf. Figs. 4, 5). It has now been established that after a tetanus there is a large and prolonged after-hyperpolarization of the presynaptic fibres, with, as a consequence, an increase in the presynaptic spike potential (Lloyd, 1949; Eccles & Rall, 1951*b*; Wall & Johnson, 1958; Eccles & Krnjević, 1959*a*, *b*). Such an increased presynaptic spike can be expected to have an increased synaptic excitatory action (cf. Lloyd, 1949; Eccles & Rall, 1951*b*), and it is now known that under such conditions there is an increased emission of quanta of transmitter (del Castillo & Katz, 1954*c*; Liley, 1956*b*). A particularly large increase in synaptic action was observed in the stellate ganglion of *Loligo* when the presynaptic spike was increased as a consequence of an applied anelectrotonus (Hagiwara & Tasaki, 1958). Thus there is an impressive array of evidence indicating that prolonged post-tetanic potentiation arises on account of the increased size of the presynaptic impulse (factor (ii) above).

Yet there is no less strong evidence in support of an explanation by the increased availability of transmitter (factor (iii) above). If it be assumed that the frequency of miniature end-plate potentials is a measure of availability of transmitter, there is a close correlation between the magnitude and time course of the prolonged post-tetanic potentiation and the increase in available transmitter, the only significant discrepancy being the delayed

rise of the former (Liley, 1956*a*, *b*; Brooks, 1956; Hubbard, 1959). Further evidence of a negative character also supports an explanation by factor (iii). In many cases (Liley & North, 1953; Eccles & Rall, 1951*b*) potentiation of the presynaptic spike potential runs a briefer time course than the synaptic transmission. Sometimes, however, the agreement may be very close (Lloyd, 1949; Wall & Johnson, 1958).

In conclusion, it may be accepted that post-tetanic potentiation after a prolonged conditioning tetanus is attributable both to potentiation of the presynaptic impulse and to increased availability of transmitter. It has been suggested above that the latter explanation accounts satisfactorily for the early post-tetanic potentiation after a brief conditioning tetanus (about 20–200 stimuli at high frequency); and presumably, as the conditioning tetanus is prolonged, after-hyperpolarization with a consequent potentiation of the presynaptic spike becomes an increasingly important factor. Possibly the depression and eventual abolition of the early potentiation that occurs when the conditioning tetanus is increased beyond a few hundred impulses (Eccles & Rall, 1951*b*; Lloyd, 1952) are due to the presynaptic after-hyperpolarization causing an initial post-tetanic phase of partial presynaptic block.

Brief reference should be made to the small and inconstant phase of late depression that follows the potentiation after a long conditioning tetanus (cf. Fig. 9; Eccles *et al.* 1959). Much larger post-tetanic depressions of this type have been found with synaptic transmission through the lateral geniculate ganglion (Evarts & Hughes, 1957; Hughes, 1958; P. O. Bishop & W. Burke, unpublished observations). As a preliminary suggestion it seems likely that the receptor desensitization found at the neuromuscular junction (Thesleff, 1955; Katz & Thesleff, 1957; Axelsson & Thesleff, 1958) provides the most likely explanation (factor (iv) above). The prolonged synaptic stimulation can be envisaged as causing a blockage of many receptor sites on the post-synaptic membrane, just as occurs during prolonged treatment of the neuromuscular junction with a low concentration of acetylcholine. Such a depression would not be manifest until after the decline of the potentiation due to increase in available transmitter.

The principal interest in the present investigation arises in relation to the mechanism ensuring effective synaptic action at high frequency of activation. Previous investigations have given rise to the suggestion that repetitive synaptic activation involves two opposing processes, depletion of the available transmitter and a compensatory mobilization of transmitter (Feng, 1941; Larrabee & Bronk, 1947; Hutter, 1952; Lundberg & Quilisch, 1953; Liley & North, 1953; Eccles, 1953, 1957; del Castillo & Katz, 1954*b*; Beswick & Evanson, 1955*b*; Liley, 1956*b*). Del Castillo & Katz (1954*a*) made the important observation that a presynaptic impulse

induced potentiation even when no transmitter liberation occurred; thus depletion of available transmitter is not a prerequisite for a reaction giving an excess. Since the increase in available transmitter often overcompensates for any depletion that arises during repetitive stimulation (Figs. 2*B*, 3*B*; del Castillo & Katz, 1954*b*), depletion is also contra-indicated as a cause of the mobilization. Apparently the presynaptic impulse initiates the process of transmitter mobilization, and there is a large facilitation of this process when presynaptic impulses follow at high frequency. In this way high frequency of synaptic activation calls forth a mobilization of transmitter that ensures a greater synaptic effectiveness for rates of synaptic activation up to 250/sec (cf. Fig. 6).

If, as seems likely, the quantal liberation of transmitter is due to the bursting of synaptic vesicles into the synaptic cleft (del Castillo & Katz, 1956; Katz, 1958), the available transmitter at any one instant would be the transmitter in vesicles in immediate juxtaposition to the presynaptic membrane, for only such vesicles could be ejected by an impulse. There may be very few vesicles in such a strategic position; consequently depletion may be evident even after only one impulse (cf. Figs. 1, 2). Besides causing the ejection of some vesicles the presynaptic impulse can be envisaged as causing other vesicles to move into the strategic zone. This movement would be greatly potentiated by impulses in quick succession, but no such interaction occurs with longer intervals (30 msec or longer) between successive presynaptic impulses; hence depletion then dominates mobilization (cf. Figs. 4, 5). Electron-microscopic investigations may eventually provide a crucial test for these speculations on the structural correlations of the depletion and mobilization phenomena of repetitively activated synapses.

SUMMARY

1. Repetitive synaptic stimulation has been studied in detail by intracellular recording of the excitatory post-synaptic potentials (EPSP's) of motoneurones activated monosynaptically, it being assumed that these synaptic potentials give a measure of the size of the transmitter action.

2. A single synaptic activation is followed by two opposed processes; a brief phase of enhanced action (200 msec) is superimposed and usually submerged by a depression lasting for several seconds.

3. During repetitive stimulation (10–600/sec) a steady state is attained in about 200 msec. There is usually depression below the initial size, but with some EPSP's there may be potentiation.

4. There is increasing depression of the EPSP's as the frequency of activation is raised above 0·3/sec, and a minimum of 70–85 % occurs in the range 4–20/sec. At higher frequencies there is always a relative potentiation, a maximum of 77–128 % being attained at 30–100/sec. There is a

good correlation between responses to two volleys on the one hand and the steady state during repetitive stimulation on the other.

5. At higher frequencies of stimulation there is a progressive decline of the individual EPSP's, and above 250/sec the size of the EPSP is directly proportional to the stimulus interval, indicating the attainment of a maximum rate of transmitter output.

6. The changes in synaptic efficacy following repetitive stimulations of a wide range of frequencies and durations have been measured by the sizes of the EPSP's evoked by a testing volley at various times thereafter.

7. Brief tetani of 200/sec or higher are followed by an early potentiation, just as with monosynaptic reflexes.

8. After long conditioning tetani there was also a close parallel between potentiation of EPSP's and reflexes, the potentiation being low with frequencies below 300/sec. A later post-tetanic depression of EPSP's is also described.

9. There is a general discussion of the relationship of these observations to the mode of operation of synaptic knobs under various frequencies of activation. Post-tetanic potentiation is discussed in relation to the alternative explanations, transmitter mobilization in synaptic knobs, and after-hyperpolarization giving larger presynaptic spikes.

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