

OPTIMAL TETANIC CONDITIONING OF HETERONYMOUS MONOSYNAPTIC REFLEXES

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Post-tetanic potentiation of the monosynaptic reflex response was first described by Lloyd (1949) who recorded from spinal ventral roots and demonstrated the phenomenon by testing after long bursts of tetanic stimulation of the afferent pathway. Eccles & Rall (1951*a*), testing at brief time intervals after short bursts of tetanic stimulation, obtained increased reflex responses which were due to 'early potentiation'. Rall (1955) and Araki, Eccles & Ito (1960) subsequently made use of this technique to raise reflex discharges in monosynaptic pathways to sizes which were convenient for their experiments.

Job (1953) and Beswick & Evanson (1954) showed that during the 'late' potentiation (Lloyd, 1959), which follows prolonged tetanic stimulation, single volleys in the afferent fibres of one muscle could cause the discharge of motoneurons of synergistic muscles: potentiated monosynaptic reflex responses recorded from ventral roots did not, however, permit a distinction to be made between homonymous and heteronymous motoneurons. Beswick & Conroy (1961) recording from muscle nerves, found that repeated short bursts of tetanic stimulation of muscle afferents produced an increasing heteronymous response to single stimuli. With 'optimal conditioning' (Beswick & Conroy, 1964*a*), the heteronymous responses reached a maximum which could then be maintained for periods up to 5 hr.

In the experiments described here, the circumstances under which such repetitive tetanic conditioning produces a maximum effect are defined and some characteristics of such conditioning described.

METHODS

The experiments were performed on decerebrate cats, using the techniques described by Beswick & Evanson (1955*a*). The animals' temperatures were maintained between 37 and 39° C. Two synchronized stimulators each capable of delivering square pulses of 0.1 msec duration, the voltage of which could be varied between 0 and 22 V, were used. Each stimulator could deliver single pulses or trains of pulses at frequencies from 1 to 2000 c/s. The duration of the trains of pulses could be fixed at any value up to 5 sec. A master oscillator governed the repetition rate of the stimulators and triggered the oscilloscope.

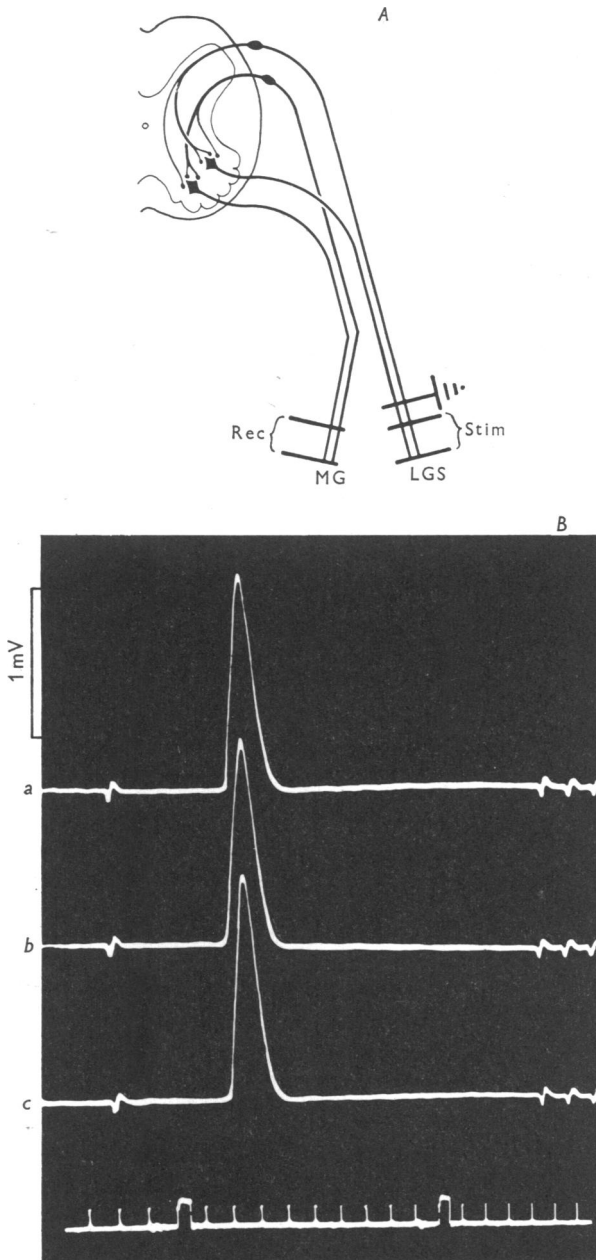


Fig. 1. *A.* Peripheral stimulating and recording arrangements. See text. *B.* Heteronymous monosynaptic reflex responses as recorded from MG during an experiment in which LGS was stimulated. *a*, *b* and *c* represent responses to testing stimuli at 5 sec intervals between successive conditioning trains of volleys. Time 10/1msec.

Stimuli were applied to a nerve supplying one of the extensor muscles of the ankle: monophasic records were made from the nerve to a synergist divided peripherally and the presence or absence of a discharge observed. The nerves most frequently used were those to the medial head of gastrocnemius (MG) and to the lateral head of gastrocnemius and soleus (LGS) although combinations of other pairs of nerves supplying the ankle extensors were also employed (nerve to deep posterior muscles of leg, DPM and nerve to plantaris, Plan). A typical arrangement of recording and stimulating electrodes is shown in Fig. 1A.

After records of the effect of single testing shocks had been made, the nerve was subjected to conditioning by repeated bursts of tetanus delivered from the second stimulator. Usually the timing of the stimuli was adjusted so that a testing stimulus fell at the commencement, and the first few stimuli of the next conditioning train at the end of an oscilloscope traverse (see Fig. 1B). Stimulus strengths were adjusted to give maximal reflex responses, necessitating voltages between three and four times afferent threshold prior to tetanization. Eccles & Rall (1951*b*) showed that the afferent volley in the dorsal root declined over 100–150 msec during repetitive stimulation at 3–500 c/s even when the stimulus strength was adjusted to 3.0 times afferent threshold for group I fibres. This they attributed to successive stimuli falling within the relatively refractory period of the nerve fibres (Gasser, 1935; Gasser & Grundfest, 1936) or to interruption of conduction in the dorsal root ganglion (Brücke, Early & Forbes, 1941). A similar explanation is probably applicable to these experiments where large conditioning and testing stimuli were required when the highest frequencies of stimulation were needed to evoke maximal responses. Testing stimuli of similar strengths were needed to evoke maximum reflex responses, confirming the observations of Gasser & Grundfest (1936) and Jefferson & Benson (1953) that the thresholds of group I fibres were increased in the post-tetanic phase.

There was no significant spread of the stimulus from the activated to other nerves, as was shown by the absence of peripherally conducted action potentials in the nerve used for recording. Indeed, before and after each experiment, the shock strength was raised and an increase of 16- to 32-fold was necessary before any such spread occurred. It was noteworthy that increasing the conditioning or testing stimulus strengths by an amount just insufficient to cause spread of the stimulus did not change the characteristics of the reflex discharge.

Initially, the repetition rate of 12/min, used by Beswick & Evanson (1955*a*), was chosen, to avoid the post-activation depression which follows a single volley in the same pathway, as the minimum time interval at which an undepressed testing response occurs is about 3 sec (Jefferson & Schlapp, 1953). Hence, the interval between the last stimulus of the tetanus and the testing stimulus, which is referred to as the 'Tetanus-test' or 'T-t interval', should be at least 3 sec and, in fact, a T-t interval of 4 sec was used initially.

For some minutes following the application of the first tetanus, every sweep of the oscilloscope was photographed (Fig. 2A). Thereafter, records were commonly made of every third or sixth sweep until such time as tetanization was discontinued. On some occasions every response was photographed and the average of six (Fig. 2B), or twelve (Fig. 7), successive responses plotted. When a series of controls was required, photographs were taken every 5 sec.

RESULTS

Effects of trains of different frequencies

Records were made of the effects of changing the frequency of the conditioning trains of tetanus when their duration and repetition rate were kept constant, at 1 sec and 12/min, respectively. A frequency was chosen and the conditioning was applied until interpolated single testing stimuli elicited heteronymous reflex discharges of constant magnitude. The

frequency of the tetanus was then changed and the conditioning maintained at this new frequency until the size of the reflex response was again steady. The procedure was repeated, in random order, several times with different frequencies.

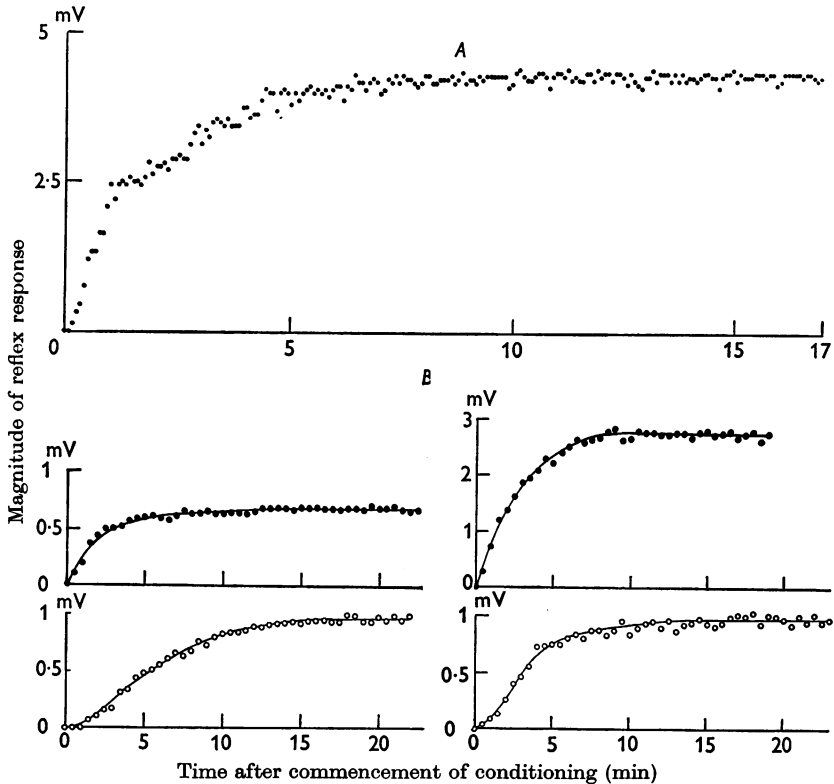


Fig. 2. Increase in magnitude of reflex responses on the synergistic nerve during optimal conditioning. Preparations had not received any previous conditioning. Stim MG, rec LGS—●; stim LGS, rec MG—○. A, all responses plotted; B, average of every six successive responses plotted. Results from four animals.

Figure 3 illustrates graphically the results obtained from two of the experiments. It may be seen that the steady spike potential increased in size with the frequency of the conditioning tetanus as it was raised above 90 c/s. The largest responses were observed when the tetanus frequency was about 850 c/s, but above this the spike potential fell off slowly as the frequency was raised. At 2000 c/s, the highest frequency tested, the spike height was about 70% of the maximum. In eleven experiments where comparisons were made, maximal responses were consistently attained when frequencies of 800–900 c/s were employed.

Effects of trains of different durations and the rate of their repetition

Tetanus durations up to 1 sec, or repetition rates less than 12/min could be examined without altering the T-t interval from 4 sec, but increasing either duration or repetition rate above these values involved reduction of the T-t interval. It was important, therefore, to determine the minimum T-t interval at which the testing response remained at its steady value when the conditioning was maintained at 850 c/s for 1 sec duration repeated twelve or less times per minute. No alteration in the magnitude of the testing response was found when the T-t interval lay between 4 and 2 sec: reduction of the T-t interval to 1.5 sec was, however, usually accompanied by a reduced response. If this were to be avoided, the minimum period occupied by one complete cycle of stimulation could not be less than 3 sec which limited the maximum repetition rate to 20/min.

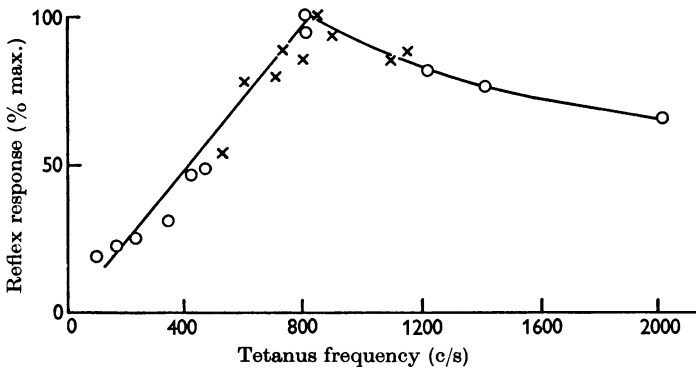


Fig. 3. Relation of reflex response to frequency of conditioning trains of volleys. Results from experiments on two animals are illustrated. Stim LGS, rec MG. See text for further details.

Trains of volleys of 850 c/s and 1 sec duration were applied at a chosen repetition rate until steady responses to single stimuli were obtained. The repetition rate was then changed and conditioning maintained until the height of the reflex response was again steady. The whole procedure was repeated several times at different repetition rates. Figure 4 illustrates the results of one experiment, typical of nine such experiments, from which it may be seen that the largest responses were obtained with repetition rates of 9–15/min with a peak at about 12/min.

Records were made of the effects of changing the bursts of tetanus to durations which ranged between 50 msec and 3 sec when their frequency and repetition rate were kept constant at 850 c/s and 12/min, respectively. As the duration of the bursts was increased so the T-t interval became less; the maximum duration, therefore, which could be examined at a

repetition rate of 12/min was 3 sec, since this gave the minimum T-t interval of 2 sec. An effective duration within these limits was chosen and conditioning applied until a steady heteronymous reflex response to the single stimulus was obtained. The duration of the tetanus was then changed and conditioning maintained until the height of the reflex response was again steady. The procedure was repeated several times with the duration altered in random order and Fig. 5 illustrates graphically the results obtained from two of nine such experiments. It may be seen that the steady spike potential increased in size as the duration of the conditioning tetanus was lengthened to about 1 sec; further increase in the duration decreased the magnitude of the spike potential.

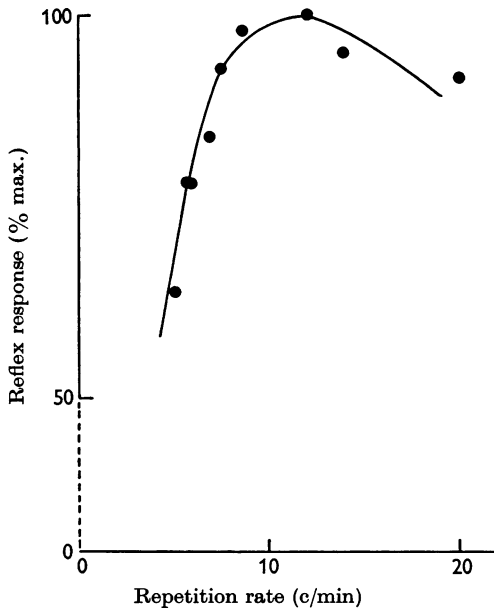


Fig. 4. Relation of reflex response to rate at which conditioning trains were repeated. Stim MG, rec LGS. See text for further details.

Taken together with the results observed on altering the duration and frequency of the conditioning tetanus, these findings suggest that there is a definite range of tetanus frequencies, durations, repetition rates and T-t intervals within which maximal heteronymous monosynaptic reflex responses may be obtained. Conditioning by interrupted tetanus of 800–900 c/s of 0.1–1.1 sec duration which when repeated about 12 times per minute produces a maximal response at a T-t interval of 2–4 sec, has therefore been termed 'optimal conditioning'.

Responses during optimal conditioning

Onset and growth. A series of experiments on 37 different preparations which had not received any conditioning whatsoever was performed.

The rise of the reflex response is shown graphically in Fig. 2 where the magnitude of the monosynaptic reflex action potential on the ordinate has been plotted against time from the beginning of the first conditioning train on the abscissa. In Fig. 2*A* every response was plotted, but in 2*B* 0.5 min average responses were plotted. It was found that the first measurable heteronymous response occurred when from one to 40 bursts of tetanus had been applied. Once a response was evoked, it first rose rapidly and then more slowly as it approached its maximum size which was usually attained between 10 and 20 min from the onset of conditioning, irrespective of the ultimate size of the maximum response or the time of its first appearance.

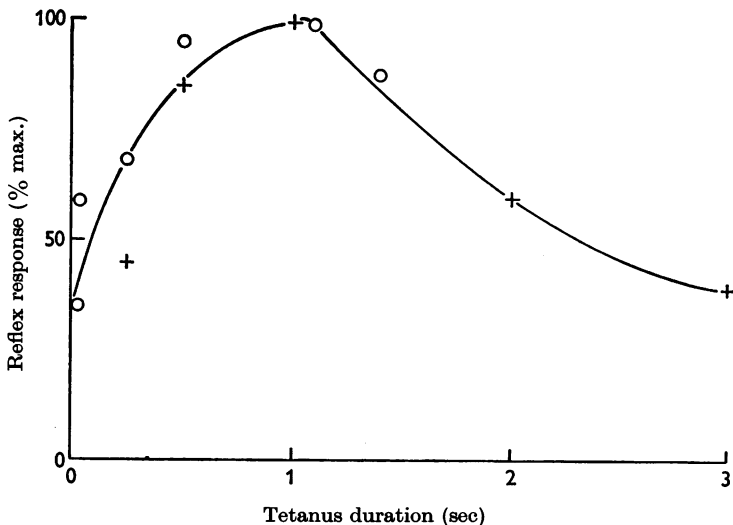


Fig. 5. Relation of reflex response to duration of conditioning trains. Results from similar experiments on two animals are shown. Stim LGS, rec MG. See text for further details.

The heteronymous reflex responses obtained by Beswick & Evanson (1955*a*) were small, measuring only a few microvolts. They rose to a maximum and fell away over a time course similar to that of Lloyd's ventral root responses (Lloyd, 1949). The responses obtained by optimal conditioning (Fig. 2) were at least ten times larger than Beswick & Evanson's (1955*a*). They varied only slightly in magnitude and were repeatable when the conditioning had been temporarily discontinued.

There were considerable differences in the magnitudes of the responses evoked using different pairs of nerves and when the same pairs were examined in different preparations. Also, it was noteworthy that responses appeared later and grew more slowly when certain pairs of nerves were used, that is, the responses on LGS rose more rapidly on conditioning MG than did the MG response when LGS was conditioned (Fig. 2*B*). These findings presumably reflect the anatomical arrangement and excitatory power of the collateral connexions between the afferent fibres of one muscle and its synergists and are complementary to those of Lloyd & McIntyre (1955). The largest heteronymous responses were not consistently observed in any particular muscle nerve, although it was generally found that the interaction between anatomically closely related synergists was greater than between those which were more separated (e.g. LGS and MG and Plan and DPM than between LGS and DPM).

Maintenance. When the reflex response had reached a maximum, it remained at this level for long periods of time provided that the nature of the conditioning was unaltered. This maximum has been maintained regularly for 2 hr but on one occasion for 5 hr when over three million conditioning volleys had been applied. In a small number of instances, however, the response fell off gradually after about an hour's conditioning, but usually regained its maximum if tetanization was discontinued for about half an hour and then recommenced (Beswick & Conroy, 1964*b*).

Responses after discontinuance of optimal conditioning

Residual potentiation; (i) Overt. On cessation of optimal conditioning, the height of the testing response at first diminished rapidly, but the initial sharp fall was followed by a gradual decline towards zero. When optimal conditioning had been maintained for 1 hr and then discontinued (Fig. 6*A*), the testing response often did not return to zero within an hour. On the other hand (Fig. 6*B*), on testing after only 1 min optimal conditioning, the response returned to zero within 1 min. Although a direct relation has not been demonstrated between the number of conditioning bursts and the persistence of the testing response after conditioning ceased, in general it may be said that the more prolonged the conditioning, the greater the persistence of the testing response. Whenever conditioning had been discontinued for a period and then reapplied, the responses to single testing stimuli returned to their previous maximum size, provided that a sufficient number of conditioning tetani were delivered, and that the stimulating and recording arrangements and the state of the preparation were unaltered.

(ii) *Concealed.* When a testing stimulus no longer evoked a reflex response, after discontinuance of conditioning, residual potentiation of the pathway

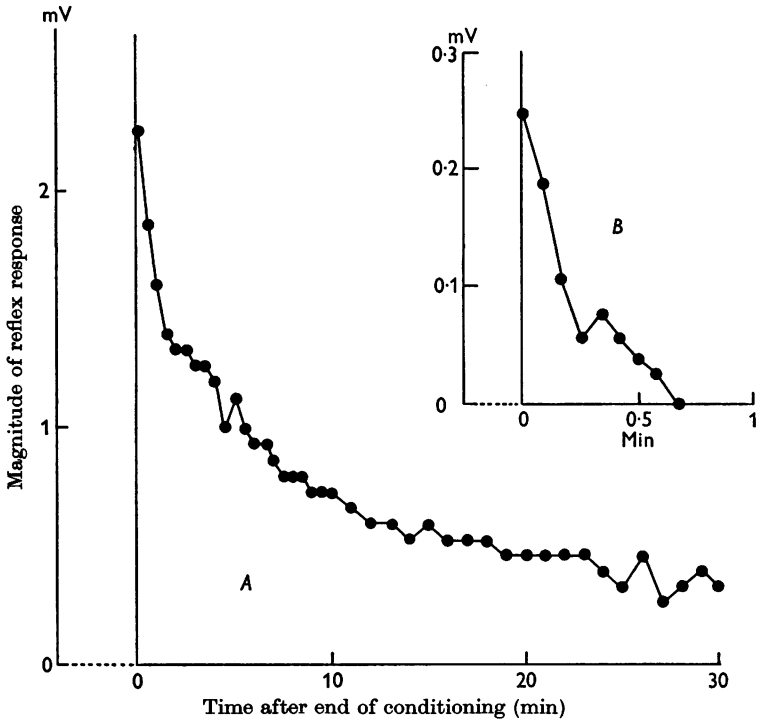


Fig. 6. Duration of overt residual potentiation after cessation of optimal conditioning. *A*, 720 conditioning trains; *B*, 12 conditioning trains, both indicated by dotted lines; zero time 4 sec after last train. Stim MG, rec LGS.

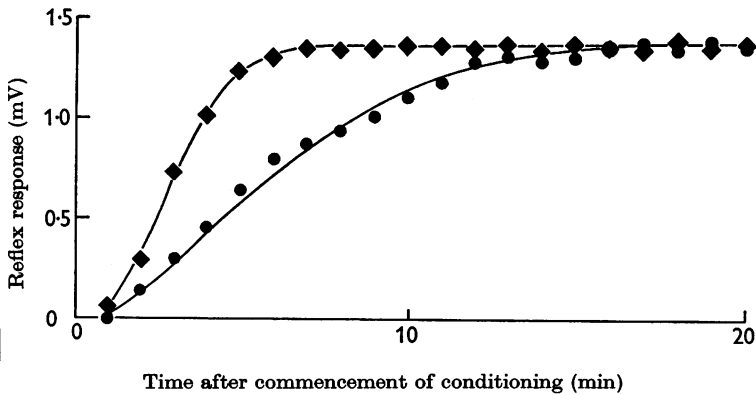


Fig. 7. Concealed residual potentiation. Increase in size of reflex response in absence of prior conditioning (●). Conditioning discontinued at 20 min; 30 min after testing response reached zero conditioning recommenced—reflex responses plotted (■). Responses from each minute averaged: stim MG, rec LGS.

could still be demonstrated by recommencing conditioning, for the heteronymous response appeared earlier, rose more quickly and reached maximum in a shorter time than when the first conditioning was given (Fig. 7). This 'concealed' residual potentiation was demonstrable in some instances for a period of up to $1\frac{1}{2}$ hr after testing responses had disappeared.

DISCUSSION

Reflex responses from a typical experiment are shown in Fig. 1*B* from which it may be seen that the latency of the action potentials was less than 5 msec and the duration of the potential not more than 2 msec; these times accord with the responses being monosynaptic (Renshaw, 1940; Lloyd, 1943). It is unlikely that the responses could be dorsal-root reflexes (DRRs) (Matthews, 1934; Toennies, 1938) since the DRR latency on muscle nerves usually exceeds 5 msec even during post-tetanic potentiation (PTP) and their duration often reaches 20 msec (Eccles, Kozak & Magni, 1961). Preparations were maintained at temperatures at which DRRs are usually small (Brooks & Koizumi, 1956).

In the experiments reported here, the duration of the tetanus exceeded 0.15 sec in all instances and even though stimulus strengths were maintained at 3–4 times afferent threshold it is likely that the later afferent volleys in each train would be reduced by 20% or more (Eccles & Rall, 1951*b*) and also that stimulation frequencies above 500 c/s would cause further reductions. However, the total potentiation, as judged by the magnitude of the testing response, increased with frequency (Fig. 3) and if it be assumed that the degree of potentiation is related to the number of impulses reaching the synaptic knobs in unit time, the results suggest that, in spite of some reduction in the size of the afferent volley, the total number of impulses reaching excitatory terminals with each burst continued to increase until a frequency of 800–900 c/s was reached. It should be noted that the responses which are plotted in Fig. 3 were recorded only when a steady state had been reached after each change of frequency had been made, suggesting that the potentiating process is cumulative and is a function of the total number of impulses reaching the synaptic knobs during each cycle of conditioning and testing. The fact that the maximum effect of this form of conditioning occurs with a frequency between 800–900 c/s agrees with the observation of Lloyd (1959) that the maximum of both early and late potentiation is reached with a stimulation frequency of 820 c/s. It follows that it is not possible to assess whether the potentiation seen in these experiments is early or late or a combination of both.

At stimulation frequencies above 900 c/s the degree of potentiation fell

off to a value of about 65 % maximum at 2000 c/s, the maximum frequency tested. Gasser & Grundfest (1936) showed that although mammalian class 1 fibres may be activated for short periods at frequencies of about 2000 c/s, a proportion of the impulses so generated is not conducted along the fibres. At frequencies above 1000 c/s maximal compound spike potentials could not be maintained in mammalian peripheral nerves since stimuli fell within the refractory periods of previous ones and alternation of response occurred where individual fibres generated propagated responses to every second or third stimulus (Gasser & Grundfest, 1936). However, it is likely that such afferent impulses as arrived in the cord would traverse the fine non-myelinated terminal fibres and reach the synaptic knobs (Eccles & Rall, 1951*b*). Since potentiation fell away less rapidly as the frequency neared 2000 c/s it seems probable that the afferent fibres were approaching an alternating rhythm of response which allowed a steady stream of impulses to enter the cord.

The results of the experiments in which the duration of the conditioning tetanus and the rate of its repetition were examined suggest that the time course over which the impulses are applied also plays a considerable part in determining the amount of potentiation (Figs. 4 and 5). As the duration of the conditioning tetanus or the repetition rate was increased, the number of impulses reaching the synaptic knobs would increase. However, with durations above 1.1 sec and repetition rates over 10 min the reflex response became less as the tetanus duration or repetition rate was raised in spite of increased synaptic bombardment. In both instances the length of the T-t interval never fell below 2 sec. This diminution in reflex discharge could only be explained by there being a depression of conduction within the reflex arc. It is unlikely that early or late depression (Lloyd, 1959; Brooks, Downman & Eccles, 1950) were responsible since the former lasts only 1 sec and is related to the P wave of the after-hyperpolarization (Gasser, 1935) and the latter yields to late potentiation by brief tetani (Beswick & Evanson, 1955*b*; Lloyd, 1959). It is more likely that the depressive process represents 'post-tetanic block' which follows prolonged tetanic stimulation (Lloyd, 1959).

It is noteworthy that post-tetanic potentiation may persist for periods of as long as half-an-hour after the cessation of optimal conditioning (Fig. 6), and even after this time its incomplete disappearance may be demonstrated by recommencement of conditioning (Fig. 7), during a further period of 1½ hr. It may be seen, therefore, that the accumulation of post-tetanic effects requires that results which have been obtained by sporadically repeated tetanization be interpreted with care.

The reflex responses evoked by optimal conditioning are very large and suggest that the collateral fibres of the stimulated nerve have their

excitatory powers greatly increased. It is possible, therefore, that observations of the magnitude of such reflex responses evoked in the nerves of synergists might give an indication of the strength of the collateral connexions to that motoneurone pool. It is also possible that hitherto undetected anatomical connexions might be revealed by this method of conditioning.

SUMMARY

1. Monosynaptic reflex responses in heteronymous motoneurone pools have been examined by means of a peripheral stimulating and recording technique.

2. Pathways were conditioned by trains of volleys repeated at regular intervals.

3. An investigation of the effects of changing the frequency and duration of the conditioning tetanus and its repetition rate has been made. Within certain narrow limits of these parameters maximal monosynaptic reflex responses to single testing stimuli following the tetanus by not less than 2 sec were obtained. This form of repetitive conditioning has been termed 'optimal conditioning'.

4. In the absence of previous conditioning the responses reached a maximum between 10 and 20 min after the onset of optimal conditioning. These reflex responses were large and remained at a constant size for periods of up to 5 hr.

5. The responses to single stimuli persisted for over half-an-hour after prolonged conditioning had been discontinued. When a reflex response could no longer be elicited by a single stimulus, the presence of what has been termed 'concealed residual potentiation' was demonstrable for periods of up to 1.5 hr.

6. The findings are discussed and suggestions made concerning the application of optimal conditioning.

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