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DELAYED EFFECTS OF PERIPHERAL SEVERANCE OF AFFERENT NERVE FIBRES ON THE EFFICACY OF THEIR CENTRAL SYNAPSES

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It is now generally assumed that, with increased use, synaptic linkages between nerve cells develop a greater functional efficiency (Cajal, 1911; Hebb, 1949; Konorski, 1948, 1950; Toennies, 1949; Jung, 1953; Eccles 1953). Unfortunately this postulate has not yet been subjected to sufficient experimental testing. With the simplest reflex actions it is difficult to arrange for conditions in which there is excessive synaptic use and in which the necessary controls are available. It has been easier to investigate the effects of disuse on synaptic function, but it has not yet been possible to establish the effects of disuse uncomplicated by other factors. Thus total disuse can be secured by severing afferent fibres just distal to their cell stations in the dorsal root ganglia, but this operative procedure causes in addition a chromatolytic change in the dorsal root ganglion cells, with an accompanying shrinkage and slower conduction velocity of their afferent fibres (Eccles & McIntyre, 1953). Nevertheless, it was considered that disuse was at least partly responsible for the three changes observed in monosynaptic reflexes several weeks post-operatively: (1) the relatively small size, (2) the abnormally prolonged time course of post-tetanic potentiation and (3) the very prolonged potentiation (residual potentiation) that often persisted for many hours after a post-tetanic potentiation. It was concluded that disuse had reduced synaptic efficacy and also rendered synapses more susceptible to the adjuvant effects of activity.

With the present series of experiments disuse of synapses has been effected by severing the nerves to muscles, a procedure which also introduces the complications of chromatolysis of the dorsal root ganglion cells and shrinkage of the afferent fibres, as well as chromatolysis of the motoneurones whose

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axons are involved. However, it has the advantage of allowing a comparison to be made between the intracellularly recorded potentials evoked in the same motoneurone by normal afferent pathways and those changed by operation.

In one type of experiment the motoneurone itself was normal (i.e. not chromatolysed). For example, the nerve to medial gastrocnemius was severed, with the consequence that some of the monosynaptic excitatory synapses on a lateral gastrocnemius motoneurone belonged to afferent nerve fibres severed at this preliminary operation. Some weeks later the animal was set up for intracellular recording and the effectiveness of these synapses was demonstrated by the size and time course of the excitatory post-synaptic potential (EPSP) evoked in a lateral gastrocnemius motoneurone by a maximal Group Ia afferent volley from medial gastrocnemius nerve. The EPSP evoked in this same motoneurone by a maximal Group Ia afferent volley from lateral gastrocnemius nerve served as a control.

In another type of experiment, the motoneurone was chromatolysed. For example, EPSP's evoked by the two respective volleys were recorded in a medial gastrocnemius motoneurone. These EPSP's were often distorted by the partial responses that are superimposed on the EPSP's of chromatolysed motoneurones (Eccles, Libet & Young, 1958), since the medial gastrocnemius motor axons had been severed together with the afferent fibres. Nevertheless, the responses to monosynaptic stimulation via normal and severed afferent pathways exhibited the same differences as those found in the experiments using normal motoneurones.

METHODS

In the initial operations one branch of each synergic nerve pair was completely severed in the left hind limb of a cat with full aseptic precautions. One such pair was the medial gastrocnemius (MG) and lateral gastrocnemius-soleus (LGS), the former being severed. The nerve to flexor digitorum longus (FDL) was also severed, the nerve to flexor hallucis longus (FHL) being left intact. The other two nerve pairs were provided by the nerves to semitendinosus and posterior biceps, the long branches (LST and LBP) in each case being severed, the short branches (SST and SPB) serving as controls.

In seven animals the standard intracellular recording (Eccles, Eccles & Lundberg, 1957) was performed after 13–25 days, in five after 33–39 days, and in four experiments after only 6–10 days. In all but the first two experiments the ventral roots were severed in order to eliminate complications produced in the intracellularly recorded potentials by antidromic impulses in motor axons, i.e. the spike potentials of the motoneurone under investigation and of neighbouring motoneurones, and also the inhibitory synaptic action through the Renshaw cell system.

Special precautions were taken to ensure that the severed afferent pathways were stimulated as little as possible before the actual experimental tests. During the insertion of the micro-electrode, sufficient guidance was provided by the field potentials generated by volleys in the normally used afferent lines and in the appropriate ventral root. When insertion into a motoneurone was signalled by the resting potential and by the responses evoked orthodromically and antidromically, the effects of afferent volleys in the severed lines were cautiously tested at the frequency of 0·5 c/s, which was regularly employed in all testing, both before and after the conditioning tetanus. After a sufficient number of initial control observations, the standard conditioning tetanus of 400 c/s

for 10 sec was applied and the testing continued thereafter, usually for several minutes. Since it was important to have maximal Group I a volleys throughout, the strength of the testing stimulus was at least twice the maximal for Group I a. Still stronger stimuli (about four times maximal) were employed during the conditioning tetanus. The Grass camera was usually arranged to expose each frame for 5 sec and then rapidly to turn on to the next frame. There were thus two or three superimposed sweeps on each frame, as may be seen in Figs. 4–6. It proved convenient and accurate to measure the maximum slopes of EPSP's by aligning the records on graph paper. The significance of differences between mean values of maximal potentiation was estimated on the null hypothesis by means of the *t* test.

RESULTS

It was essential in the first place to choose a post-operative period that was sufficiently long, and yet not so long as to introduce complications on account of re-innervation of receptor organs. Such conditions obtained for our initial series of thirty-three motoneurones in seven experiments, where the experimental testing occurred 13–25 days after the initial operative lesion. This series consisted of twenty-one motoneurones which, judging by their chromatolytic type of response (Eccles *et al.* 1958), presumably supplied the denervated muscle, and twelve motoneurones with normal responses. Since the responses of this latter group are not complicated by changes due to motoneuronal chromatolysis, they will be considered first.

Monosynaptic activation of normal motoneurones through normal and operatively severed paths

In the first two experiments of this series the ventral roots were intact. As a consequence, when a motoneurone was impaled by the micro-electrode, the motor nerve containing its axon could be identified with certainty by antidromic invasion (Eccles *et al.* 1957). For example, the antidromic spike potential in Fig. 1 *A* and *B* reveals that the motor axon was in the short nerve to posterior biceps (SPB), which had not been severed in the preliminary operation. Fortunately the threshold stimulus for the motor axon in the SPB nerve was 1·4 times maximal for the Group I a afferent fibres, so that with Fig. 1 *C*, *D* the stimulus to the SPB nerve was well above maximum for Group I a afferent fibres, as shown by the records of the dorsal root spike, and yet below threshold for the motor axon.

It is seen in Fig. 1 *C* that a much larger monosynaptic EPSP (3·5 mV) was evoked by an SPB volley than by maximal volleys from the long branch of posterior biceps nerve (*E*, 1·0 mV) and from the anterior biceps nerve (*H*, 1·1 mV). In the LPB records the brief initial diphasic wave was due to extracellular fields generated by antidromic invasion of adjacent motoneurones, since the same wave was recorded when the micro-electrode was just withdrawn from the cell (Fig. 1 *G*). Unfortunately this superimposed field potential makes it impossible to compare accurately the onsets and rising phases of the

EPSP's generated respectively by normal synapses (Fig. 1 *C*, *H*) and by those belonging to peripherally severed axons (Fig. 1 *E*). However, Fig. 1 *D*, *F* and *I* shows typically that these last synapses differ from normal in that a conditioning tetanus caused a greater potentiation of the monosynaptic EPSP, the ratios for maximum potentiation being respectively 2·9 (*F*) as against 1·4 (*D*) and 1·6 (*I*) for normal synapses.

In all later experiments field effects from antidromic invasion were eliminated by severing the ventral roots at the time of the final investigation. It was no longer possible to identify motoneurones by antidromic invasion, but

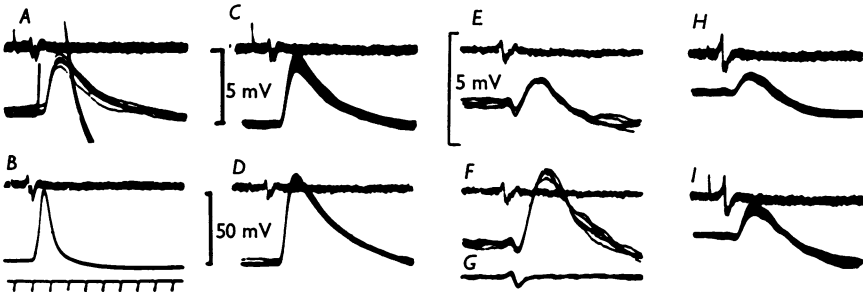

Fig. 1. In all except *G* (which is just extracellular) lower traces are intracellular records from a short posterior biceps (SPB) motoneurone, as is indicated by the antidromic spike potential seen truncated in some traces of *A* and at low amplification in *B*. The upper traces are recorded by an electrode making contact with the L 7 dorsal root as it enters the spinal cord, negativity being signalled downwards. *A–D* are evoked by volleys in the SPB nerve, *E–G* by volleys in the LPB nerve which had been severed 25 days previously, and *H*, *I* by volleys in the anterior biceps nerve. *D*, *F* and *I* are taken at the height of the post-tetanic potentiation following a conditioning tetanus of 400 c/s for 10 sec, *C*, *E*, and *H* being the control records. Same time scale for all records. *E*, *F*, and *G* are at higher amplification, as shown by the 5 mV scale. The 50 mV scale is for *B* only.

as a rule the chromatolysed motoneurones with axons in the severed nerves were readily identified by their characteristic responses.

In Fig. 2 monosynaptic EPSP's have been evoked in an FHL motoneurone by maximal Group Ia volleys homonymously in FHL nerve (*A*) and heteronymously in the nerve to the synergist, FDL *B*. Typically the EPSP set up through the severed afferent line (FDL) has a longer latent period and a later summit. It is also much smaller and has a slower rising phase. Probably the longer latent period is sufficiently explained by the slower conduction velocity of the afferent fibres, as may be seen when the afferent volley is simultaneously recorded from the dorsal root entry zone (cf. Figs. 1, 4, 5). There is no evidence that nerve section is associated with a lengthening of synaptic delay.

The smaller size and less steep slope of the EPSP evoked from the severed nerve might conceivably be due to the fact that the comparison is between a heteronymous and a homonymous EPSP, the former being smaller in all but

very exceptional circumstances (Eccles *et al.* 1957). Two procedures have been employed in an attempt to provide a quantitative test of this possible explanation. One procedure is to investigate post-tetanic potentiation of the normal and severed afferent lines respectively. The other procedure is described in the Discussion.

As is shown in Fig. 2, the maximum post-tetanic potentiation of the EPSP evoked by the severed line was relatively much greater than that evoked by

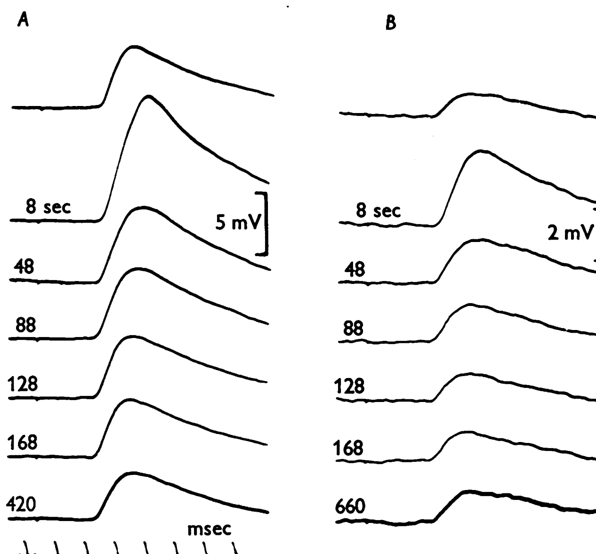

Fig. 2. Intracellular EPSP's evoked in a FHL motoneurone by maximum Group Ia volleys in FHL nerve (*A*), and in FDL nerve (*B*) which had been severed 15 days previously. Top records in *A* and *B* were taken before the conditioning tetanus 400 c/s for 10 sec, and the subsequent records at the indicated intervals after the tetanus. Same time scale throughout, but different voltage scales for the two series as indicated.

the normal line, as was also seen in Fig. 1, the respective ratios being 2·8 and 1·95 for the heights of the EPSP's and 2·5 and 1·4 for their maximum rates of rise. In both the maximum was observed 8 sec after the end of the tetanus. When the whole series was plotted both for heights and slopes (Fig. 3*A*, *B*), the post-tetanic potentiation exhibited a slower decline for the severed line (open circles); as may also be seen in the sample EPSP's of Fig. 2. When the heights of the EPSP's were being measured, the respective times for decline to half the maximum potentiation were 48 sec as against 37 sec for the normal line (Fig. 3*A*), whereas for the maximum slopes of the EPSP's the respective half-times were 44 sec and 30 sec. Finally, there appeared to be a small residual potentiation of the height of the former EPSP even after 11 min (cf. Fig. 2), whereas the normal EPSP was depressed below the initial size from 2 till 14 min after the conditioning tetanus (cf. Fig. 2).

It cannot be assumed that the relative magnitudes of excitatory synaptic actions on a nerve cell are directly proportional to the heights of the respective EPSP's. When two EPSP's are simultaneously produced by two sets of synapses, the recorded EPSP is a few per cent less than the sum of the two EPSP's individually recorded, and it has been shown that this small degree of occlusion is predictable from the postulate that excitatory synapses act as virtual short-circuits of the post-synaptic membrane (Eccles, Eccles & Lundberg, unpublished observations). This error arising from occlusion becomes negligible if maximal slopes of the EPSP's are used for assessment of their size. However, another error is then introduced, because this measurement is very sensitive to variations in the amount of temporal dispersion obtaining for the actions of the individual synapses. For example, after the severe conditioning

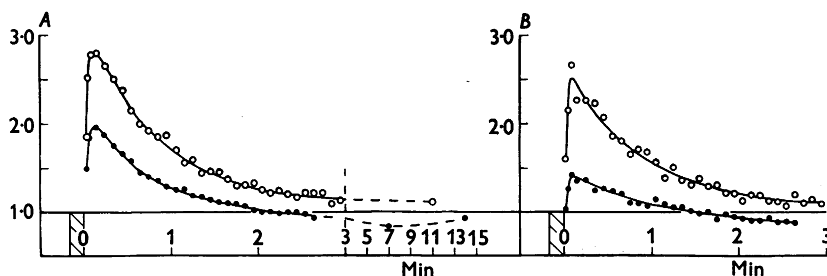

Fig. 3. Plots of the time courses of post-tetanic potentiations of EPSP's produced by a conditioning tetanus of 10 sec at 400 c/s, as indicated by the hatched columns. Specimen records are shown in Fig. 2. In Fig. 3*A* the heights of the EPSP's are plotted relative to the control height, open circles being for the operatively severed pathway (FDL) and filled circles for the control pathway (FHL). Fig. 3*B* as for Fig. 3*A*, but for maximum slopes of the rising phases of the EPSP's. Note that in Fig. 3*A* the time scale is greatly shortened after 3 min.

tetanus, usually 400 c/s for 10 sec in our experiments, there may be a considerable increase in the temporal dispersion of the testing afferent volley, and the degree of this dispersion may be larger for the operatively severed afferent pathway. Nevertheless, except for the first two experiments, where the rising phase of the EPSP was complicated by antidromic spike potentials (cf. Fig. 1), the maximum slope of the EPSP has been adopted as the best criterion of the magnitude of the synaptic excitatory action. In particular, this slope has been the most convenient measurement with chromatolysed cells, because it is made early in the course of the EPSP's before they are distorted by superposition of the partial responses that so frequently are produced by these cells (Eccles *et al.* 1958).

Comparison of Fig. 3*A* and *B* typically shows that the potentiation is significantly larger for the heights of the EPSP's than for the maximum slopes of the EPSP's. This discrepancy occurs because the rising phases of the potentiated EPSP's are considerably lengthened, for example, in Fig. 2*A* from 1·17 to 1·64 msec. As the potentiation declines, the rising phase

correspondingly shortens until it attains the initial duration. There is a comparable lengthening during the post-tetanic potentiation of the EPSP's set up from the severed afferent path (cf. Fig. 2*B*).

Occasionally monosynaptic EPSP's have been produced in a semitendinosus or posterior biceps motoneurone by four converging afferent paths, two of which had been operatively severed (LST and LPB), while two (SST and SPB) were normal. As is shown in Fig. 4, the maximum potentiation of the EPSP's generated by both the severed afferent pathways (3·5 for *D–C* and 3·2 for *H–G*) was much greater than for the two normal pathways (1·8 for *B–A* and 1·4 for *F–E*).

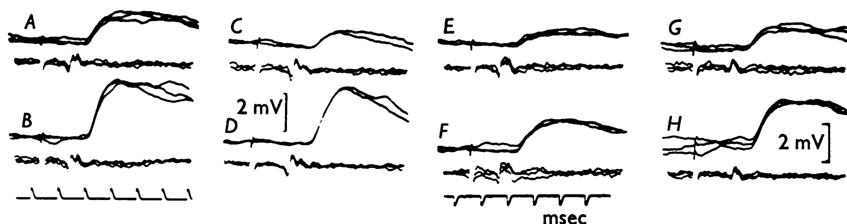

Fig. 4. Intracellular records as in Fig. 1 from a motoneurone (probably ST) in which four afferent nerves produced EPSP's: SST, *A* and *B*; LST, *C* and *D*; SPB, *E* and *F*; LPB, *G* and *H*. The first of each of these paired responses was the control and the second was at the maximum potentiation after 10 sec at 400 c/s. The LST and LPB nerves had been severed 13 days previously. Same time and voltage scale throughout.

Table 1 summarizes the results of comparison of the post-tetanic potentiation ratios for the twelve normal motoneurones in which EPSP's were evoked both from normal and operatively severed afferent lines. It will be seen that there has been a highly significant increase in the post-tetanic potentiation of EPSP's evoked from the operatively severed afferent paths, the respective mean control values being 1·69 and 2·81.

Monosynaptic activation of chromatolysed motoneurones through normal and severed afferent paths

It has already been reported that in severely chromatolysed motoneurones the EPSP's are reduced in size and often have superimposed small irregular action potentials that probably arise in highly excitable patches momentarily appearing on the soma and dendrites (Eccles *et al.* 1958). When the motor axons are severed peripherally in the muscle nerves, the motoneurones also show the histological changes characteristic of chromatolysis, but in general to a much less degree or even none at all (Buzzard & Greenfield, 1921; Bucy, 1928); correspondingly, the responses of these motoneurones were less abnormal. However, the EPSP's appeared to be reduced in size and superimposed partial responses were not infrequent (Fig. 5*B*, *C*).

Just as with normal motoneurones, the post-tetanic potentiation of synapses activated from the operatively severed nerves was much greater than with normal synapses, as may be seen by comparing Fig. 5*E*, *F* with *B*, *C*. It will be noted that in Fig. 5*F* the potentiated EPSP generated a spike potential after the very long latency (up to 2·3 msec after arrival of the afferent volley

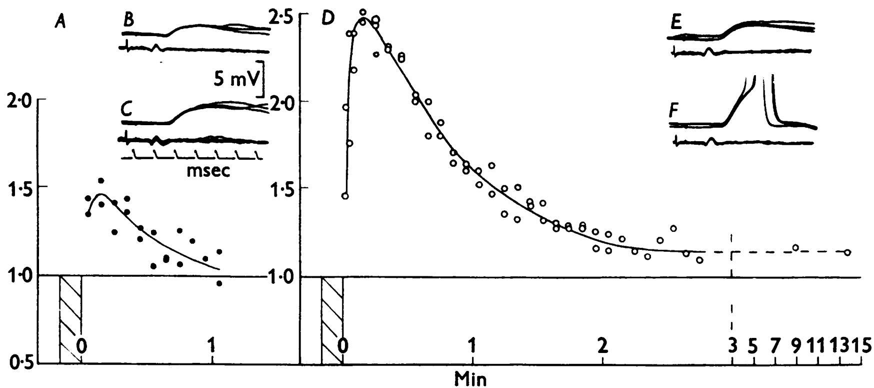

Fig. 5. Recording from an ST motoneurone 15 days after section of the long semitendinosus (LST) nerve—*A* and *D* show the post-tetanic potentiations following 10 sec stimulation at 400 c/s and measured by the slopes of the EPSP's as in Fig. 3*B*. Two successive series are plotted for each curve. Curve *A* is for the control afferent path, short semitendinosus nerve (SST); specimen records *B* and *C* being respectively the control EPSP and the EPSP at maximum potentiation. Curve *D* is for the severed afferent path (LST); specimen records of control and potentiated EPSP's are shown in *E* and *F*. Same potential and time scales for all inset records.

TABLE 1. Post-operative changes in post-tetanic potentiation of EPSP's

			Maximum post-tetanic potentiations			
					(Pooled results)	
Motoneurones	Days post-operative	Number of moto-neurones	Normal afferent (mean S.E.M.)	Severed afferent (mean S.E.M.)	Normal (mean S.E.M.)	Severed (mean S.E.M.)
Normal	13–25	12	1·69 ± 0·072	2·81 ± 0·19	1·65 ± 0·042	2·68 ± 0·058
Chromatolysed	13–25	21	1·62 ± 0·052	2·61 ± 0·14		
Normal	33–39	15	1·74 ± 0·055	2·18 ± 0·10	1·75 ± 0·050	2·35 ± 0·073
Chromatolysed	33–39	18	1·76 ± 0·075	2·47 ± 0·10		
Normal plus chromatolysed	6	23	—	—	1·53 ± 0·040	1·61 ± 0·055
	10	10	—	—	1·72 ± 0·063	2·05 ± 0·085
	13	8	—	—	1·56 ± 0·098	2·54 ± 0·21

at the cord) characteristic of chromatolysed motoneurones. The time course of the post-tetanic potentiation produced by the standard conditioning tetanus (400 c/s for 10 sec) is shown in Fig. 5*A* for the control normal synapses and in Fig. 5*D* for the synapses belonging to the severed afferent nerves. The results of two tetani are shown in each case; there was adequate time for recovery between the two tetani to any one afferent. The curves for the chromatolysed motoneurone closely parallel the curves for normal motoneurones

(cf. Fig. 3*B*). Not only was the maximum potentiation of the EPSP evoked by the severed afferent pathway larger (2·45:1·48), but it declined more slowly, the respective times of decline to half values being 50 sec and 30 sec. Finally, there appeared to be a small residual potentiation.

Table 1 summarizes the measurements made on twenty-one chromatolysed motoneurones 13–25 days after operation. The maximum post-tetanic potentiation of the EPSP's evoked by normal afferent pathways had a mean value of 1·62 (S.E. $\pm$ 0·052), which was not significantly different ($P > 0{\cdot}25$) from the mean value of 1·69 (S.E. $\pm$ 0·072) for the EPSP's evoked by these pathways on normal motoneurones. Correspondingly, the potentiation of EPSP's evoked by severed afferent nerves, with a mean value of 2·61, was not significantly different ($P > 0{\cdot}25$). It is therefore justifiable to pool the two series, as shown at the right of Table 1; this gives mean potentiation ratios of 1·65 and 2·68 for EPSP's evoked by normal and operatively severed afferent lines on the thirty-three motoneurones tested at 13–25 days post-operatively, which is a highly significant difference ($P < 0{\cdot}001$).

Investigations after longer post-operative intervals

When the post-operative interval was as long as 33–39 days, stimulation of several of the severed nerves caused contraction of their corresponding muscles, i.e. there was functional motor regeneration. There may also have been a functional regeneration of the afferent fibres to their receptor organs, but no attempt was made to investigate this possibility.

In most cases post-tetanic potentiation was much larger for the EPSP's evoked by the severed afferents. However, with seven of the thirty-three motoneurones the post-tetanic potentiation was not very different (within 10%), as in Fig. 6*B*, *C* and *E*, *F*, where the time course of the potentiation also was not much changed (Fig. 6*A* and *D*). It is of interest that in different motoneurones (normal and chromatolysed) the same normal and severed afferent pathways exhibited very divergent behaviour. For example, with two other gastrocnemius motoneurones in the same experiment as Fig. 6, the post-tetanic potentiation of the EPSP's was as usual much larger for the severed path (3·0:2·08 and 2·07:1·52), and one other (with ratios 1·95:1·96) resembled that of Fig. 6.

Apart from a possible regression of chromatolytic changes (Bucy, 1928), an attractive explanation of the exceptional observations of similar post-tetanic potentiations is that there has been functional regeneration of the severed afferent pathway, with the result that the synapses have been normally activated for some time. The discrepancies between different motoneurones could be attributed to the recovery of some only of the afferent fibres, certain motoneurones being activated almost exclusively by the recovered fibres. This postulate of functionally recovered afferent pathways

could be tested by investigating at still longer post-operative intervals, when a larger proportion of recovered afferent paths would be expected. It should also be possible to prevent functional regeneration during a relatively prolonged period by taking special precautions or by severing again the regenerating nerves.

Investigations after shorter post-operative intervals

In a few experiments the experimental testing was performed at shorter post-operative intervals in order to gain information on the time course of development of the changes fully exhibited after 13 days. As is shown in Table 1, there was no significant increase ($P > 0{\cdot}1$) in the post-tetanic potentiation at 6 days, but at 10 days post-operatively almost half the maximum effect had been attained ($P = 0{\cdot}005$).

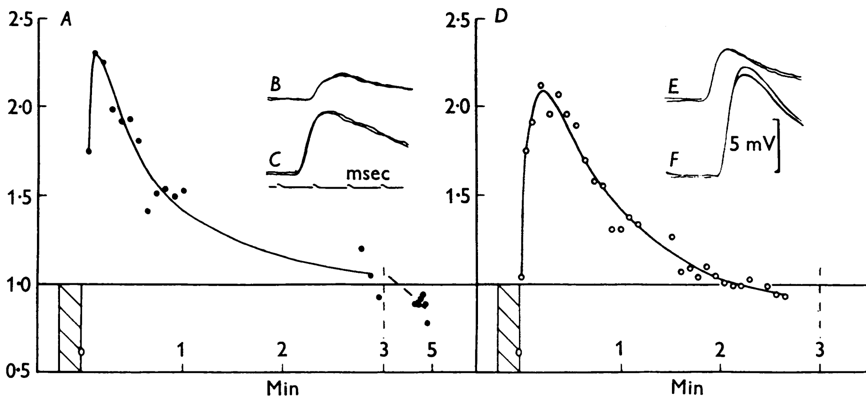

Fig. 6. Intracellular recording from a medial gastrocnemius (MG) motoneurone 35 days after section of the MG nerve. *A* and *D* are curves as in Fig. 5 with corresponding inset records. *A*, *B*, *C* are for the normal afferent path, the nerve to lateral gastrocnemius and soleus (LGS), while *D*, *E*, *F*, are for the severed path, MG. Same time and potential scales for all traces.

Investigations with variations in the conditioning tetani, and in the testing for potentiation

The standard conditioning tetanus of 400 c/s for 10 sec was chosen because it is well within the range that gives maximum potentiation (Lloyd, 1949; Eccles & Rall, 1951). With a longer tetanus, e.g. 30 sec, the maximum potentiation of the EPSP's was unchanged for both normal and severed pathways, but it was reached later and the decline was slower. With briefer conditioning tetani, e.g. 2 sec, the maximum potentiation was lower and the decline faster.

There is evidence in Figs. 2, 3 and 5 that synapses of the severed afferent pathways exhibited a small residual potentiation for several minutes after the conditioning tetanus, that is, at a time when there was often a post-tetanic depression of the control synapses. This residual potentiation of EPSP's would account for the residual potentiation of monosynaptic reflexes observed

after comparable post-operative periods (Eccles & McIntyre, 1953). A further possible correlation is that the severance of the afferent nerves causes a regression of synaptic function over several days (see Discussion) simply because there is a deprivation of the cumulative effects of the residual potentiations following bursts of normal activation from the peripheral receptor organs. In a few motoneurones the conditions of recording have been stable for such a prolonged period that it was possible to test for this postulated summation of residual potentiations. For example, in Fig. 7, seven tetani of 400 c/s for 10 sec were repeated at intervals of about 150 sec. There was no evidence of any residual potentiation in the decline of the last of this series. After 7 min the EPSP had declined to the initial value and remained virtually

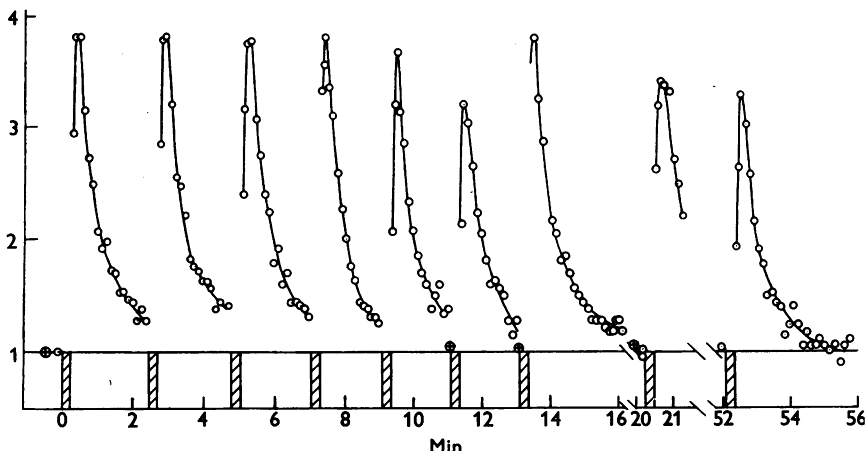

Fig. 7. Plotting of a series of post-tetanic potentiations for the same neurone as in Fig. 3, and for the heights of the EPSP's generated by volleys in the severed afferent pathway, as in Fig. 3*A*. The conditioning tetani, each 10 sec at 400 c/s, are shown by the hatched columns. The occasional circles with inset crosses show the control EPSP of the other afferent path in order to check the stability of the EPSP responses of the motoneurones.

unchanged during a rest period of 30 min. A final conditioning tetanus was followed by a potentiation that did not differ significantly from the initial series.

The absence of a cumulative residual potentiation in Fig. 7 contrasts with the experiments on monosynaptic reflexes (Eccles & McIntyre, 1953). However, it should be pointed out that reflexes should provide a more delicate test of small residual changes in synaptic function than do EPSP's even under very stable conditions of intracellular recording.

DISCUSSION

Sizes of EPSP's evoked from severed afferent nerves

The experiments have been designed so that the monosynaptic EPSP produced by a volley in a severed afferent path can be compared with the monosynaptic EPSP produced in the same motoneurone by a volley in a normal

afferent path. When any synergic nerve pair is investigated in normal animals, there is a considerable variation in the relative sizes of the EPSP's produced on the same species of motoneurones (Eccles *et al.* 1957); but when the ratios are determined for a considerable number of motoneurones, for example, ten or more, the mean ratio becomes highly significant. Unfortunately, normal mean ratios have been determined only for one of the nerve pairs used in the present research, the gastrocnemius-soleus group. Thus calculations based on Table 3 of Eccles *et al.* (1957) and other, unpublished, data, show that a volley in the medial gastrocnemius (MG) nerve evoked an EPSP in LGS motoneurones which was on the average 0·51 times that produced by an LGS afferent volley, whereas in the reciprocal investigation on MG motoneurones the MG afferent volley had an effectiveness 2·3 times that of the LGS afferent volley.

In the present experiments the EPSP's evoked by MG afferent volleys, that is, by the severed afferent path, can likewise be expressed relative to the EPSP's produced by LGS volleys on the same motoneurones. For example, at 13–25 days after dividing the MG nerve, an afferent volley in that nerve produced EPSP's in LGS motoneurones which had a mean value only 0·24 times that evoked by an LGS afferent volley, which is about half of the normal ratio (0·51). Correspondingly, the MG volley was also less effective on MG motoneurones, for it evoked an EPSP with a mean value of 1·2 times that produced by an LGS afferent volley, in contrast to the normal ratio of 2·3. Thus the MG synapses have only about half the effectiveness of normal MG synapses when measured relative to the normal LGS synapses both on normal (LGS) and chromatolysed (MG) motoneurones. The value so calculated for relative synaptic efficacy has been plotted as the dotted line in the middle of Fig. 8*A* and *B*, from 13–25 days. It is also shown by the dotted line at 10 days post-operatively that synaptic function is depressed less (to about 70 %), but after 33–39 days the dotted lines indicate some recovery, at least for the synapses on LGS motoneurones (*A*).

Figure 8 further shows that during the maximum post-tetanic potentiation of synapses belonging to the severed and normal afferent paths, the former synapses recover about half of their lost potency, for example, in the 13–25 days series from a mean value of about 50 % to about 70 %. This is, of course, merely a corollary of the larger post-tetanic potentiations exhibited by the severed afferent pathways.

With the three other synergic pairs the normal ratios have not been measured; hence it was not possible to evaluate the effects of operative severance as in Fig. 8. However, the sizes of the EPSP's relative to the control normal values also suggested that synaptic efficacy was depressed to about half at 13–39 days after nerve severance. From Fig. 8 it seems justifiable to assume that the increase in the post-tetanic potentiation of synapses activated from the severed afferent paths provides an approximate measure of their depressed function.

Post-tetanic potentiation

In the histogram of Fig. 9 the shaded areas show the mean increase in the post-tetanic potentiation up to the thirteenth day after severance of peripheral axons, and the recession at 33–39 days post-operatively (cf. Table 1). It will be noted that the mean post-tetanic potentiation of the control afferent

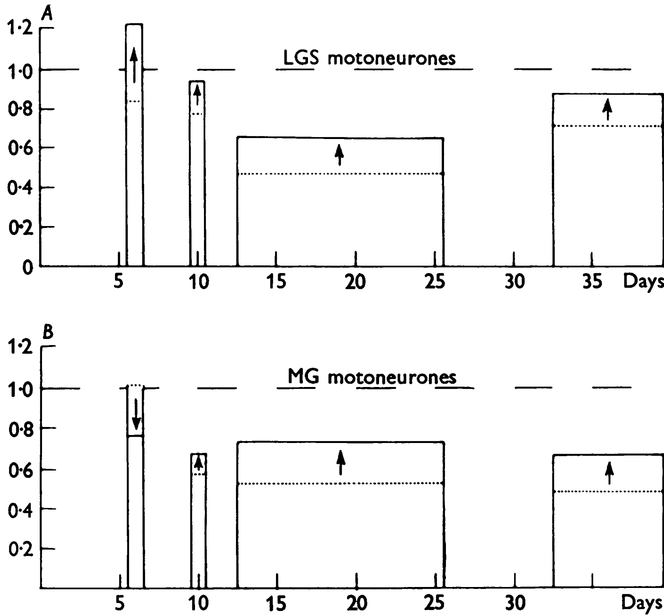

Fig. 8. Delayed reduction in mean synaptic efficacy of afferent volley in nerve from medial gastrocnemius (MG), at various times after peripheral severance. EPSP's evoked in same motoneurones by maximal monosynaptic stimulation of severed (MG) and normal lateral gastrocnemius-soleus (LGS) nerves were first compared. Relative efficacy of severed (MG) nerves was then calculated by further comparison of mean ratios so obtained with corresponding normal ratios estimated in experiments in which both MG and LGS nerves were intact. Arrows point to higher levels of synaptic efficacy at peaks of post-tetanic potentiation, calculated as above (except at 6 days). Separate estimates of synaptic efficacy of MG nerves were made, based on EPSP's recorded in LGS motoneurones (*A*), and in MG motoneurones (*B*). Note small and irregular changes at 6 days, and gradual recovery of synaptic efficacy at 33–39 days.

paths (indicated by the unshaded area) shows no significant trend over the whole period, the minimum value being 1·53 and the maximum 1·75 (Table 1).

The change in the post-tetanic potentiation of synapses in Fig. 9 has approximately the same time course as that for the progressive depression of their function after operative severance (Fig. 8). Using this criterion the depression was fully developed in the eight motoneurones investigated at 13 days post-operatively (Table 1) with a mean potentiation ratio of 2·54

(control ratio, 1·56), but was only about half developed at 10 days (ten motoneurones), and was not significant at 6 days (twenty-three motoneurones).

Activation and efficacy of excitatory synapses

Before discussing the possible general significance of the present results in relation to the possible effect of use on synaptic efficacy, it should be pointed out that the operatively severed afferent paths may have a depressed synaptic action as a consequence of shrinkage of their synaptic knobs brought on by

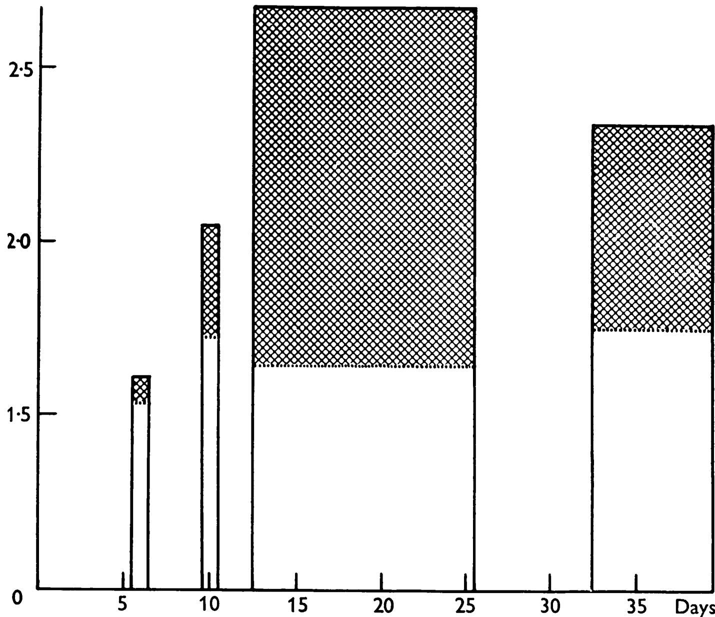

Fig. 9. Histogram showing mean post-tetanic potentiations for the control afferent paths (dotted line) and for the severed afferent paths (continuous line). The shaded areas represent the additional potentiations attributable to the nerve severance. The results of all experiments are pooled into the four post-operative periods shown on the abscissa (cf. Table 1). The results for chromatolysed and normal motoneurones are also pooled for each period, as is shown in the right-hand portion of Table 1.

the peripheral section of their axons. Such peripheral section certainly causes a central shrinkage of both the motor and sensory axons (Greenman, 1913; Gutmann & Sanders, 1943; Weiss, Edds & Cavanaugh, 1945; Sanders & Young, 1946; Sanders & Whitteridge, 1946; Eccles & McIntyre, 1953; Szentagothai & Rajkovits, 1955). In our present experiments this axonal shrinkage was also suggested by the invariable diminution of conduction velocity of both the motor and sensory axons (cf. Eccles & McIntyre, 1953); the mean diminution was about 25% relative to the control member of the synergic pair. This observation is not necessarily in conflict with the

finding of Sanders & Whitteridge (1946) that the conduction velocity was 10–15% faster in the central stumps of severed nerves, for their observations were made at 56–123 days post-operatively, whereas 39 days was the maximum period in the present experiments. It is especially relevant that Szentagothai & Rajkovits (1955) have found that the synaptic knobs in the intermediate zone of the L6 level of the spinal cord were significantly smaller when the limb nerves had been severed some 5 months before in the 6-day-old animal and precautions had been taken to prevent regeneration. However, most of these investigations on nerve fibres and synaptic knobs were made at much longer intervals after the initial nerve section, and the indications are that the changes would be much smaller at the briefer intervals of the present investigation, particularly at 13 days, when the depressed efficacy was already fully established. For example, Gutmann & Sanders (1943) found that the shrinkage was much greater at 100 days than at 50 days, which was their earliest observation, and the small size of the synaptic knobs (Szentagothai & Rajkovits, 1955) is more properly attributed to stunted growth than to a shrinkage.

It is certainly desirable to design an experiment in which disuse occurs uncomplicated by the shrinkage that arises secondarily to the operative severance of the afferent fibres. It has, however, not yet been possible to produce a virtually total silence of the afferent fibres without severing them. Nevertheless, experiments by Szentagothai & Rajkovits (1955) suggest the possibility that disuse may itself cause a regression of synaptic function on account of fibre shrinkage. In a young animal (7–14 days old) the long bones were excised without injuring the nerves to one hind limb, which consequently could no longer be effectively used. Some months later both motor and sensory fibres were found smaller than in the control limb. However, this deficiency may again be the result of stunted growth. Moreover, the present results differ in several respects from the depression of synaptic function that would be expected to occur with a simple shrinkage. There is, first, the great increase in post-tetanic potentiation, and secondly, its slower decline (Figs. 3–5). Finally, there is the prolonged residual potentiation which can be observed even at a time when normal synapses are exhibiting a post-tetanic depression (Figs. 3, 5).

On account of these differences it may provisionally be assumed that the depression of synaptic function is at least partly due to disuse (cf. Eccles & McIntyre, 1953). Besides thus confirming this earlier work in which synaptic function was tested by reflexes, the present experiments make quantitative estimates of synaptic function possible and also reveal the time course of the onset of the depression of function.

If 10–13 days of inactivity causes a depression of synaptic function, it follows that the normal activation of the synapses by receptor organs causes an increment of synaptic function that persists for 10–13 days. It may now be asked, 'Why is this prolonged increase not evident after the periods of

intense activation provided in the present experiments, and particularly in experiments such as that of Fig. 7, where there were many periods of intense activation?' A partial answer may be provided by comparison of the number of impulses involved in the day-to-day activation of synapses and in our experimental stimulations. Under ordinary conditions of activity with the background activation of muscle spindles, a frequency of 20 c/s is probably an underestimate of the mean frequency of discharge of an annulospiral ending (cf. Granit, 1955); nevertheless, there would be more than 10^6 discharges in 14 hr. Compared with that figure the 4000 impulses in one of our conditioning tetani are negligible and in Fig. 7 the total would still be only 36,000 impulses during the total period of one hour. Another factor could be the frequency of activation of the synapses. The high frequencies are extremely effective in producing potentiations lasting for a few minutes, but the low frequencies characteristic of the usual level of receptor organ discharge may be more efficacious in producing potentiations persisting for days.

SUMMARY

1. Nerves to muscle have been severed peripherally by an initial aseptic operation and the resulting changes in synaptic function have been tested by intracellular recording from motoneurones usually 13–25 days later, but also after shorter and longer periods.

2. Since only one nerve of a pair supplying synergic muscles was severed, it was possible to compare the monosynaptic EPSP's evoked in the same motoneurone from operatively severed and normal afferent paths.

3. After 13–25 days synaptic function was depressed by about half. Recovery was sometimes observed at 33–39 days post-operatively, but this could be due to peripheral regeneration of the afferent fibres. There was no significant depression of synaptic function at 6 days, but it was about half developed after 10 days.

4. Post-tetanic potentiation of synapses activated from the severed nerves was much increased, from a mean value of 1·64 to 2·68. For the different post-operative periods this increased potentiation paralleled the depression of synaptic function. The decline of the potentiation was abnormally slow and there was sometimes evidence that a slight residual potentiation persisted for as long as 15 min.

5. All these effects of peripheral nerve section were observed equally well in normal motoneurones and in those motoneurones that were chromatolysed on account of the original operation.

6. The significance of these results is discussed in relation to the possibility that the original nerve section caused chromatolysis of the dorsal root ganglion cells and probably shrinkage as well as disuse of the primary afferent fibres.

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