J. Physiol. (1960), **154**, *pp.* 68–88
With 7 text-figures
Printed in Great Britain

MONOSYNAPTIC EXCITATORY ACTION ON MOTONEURONES REGENERATED TO ANTAGONISTIC MUSCLES

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(*Received* 27 *April* 1960)

There have been many attempts to discover if the transformation of reflex function by tendon transposition or by nerve cross-union effects any change in the simplest reflex pathways. A comprehensive and critical review of the literature led Sperry (1945) to conclude that there was little, if any, acceptable evidence that such a 'plastic' change occurred in mammals, even when very young, a conclusion supported by more recent investigations (Sperry, 1947; McIntyre & Robinson, 1958).

In contrast, there are many accounts of remarkable plastic changes in reflex phenomena following various operative procedures, including nerve cross-union, on teleosts and larval amphibians. These experimental investigations have generally been concerned with complex reactions such as movements of whole limbs (Sperry, 1950; Sperry & Deupree, 1956) or of the jaw (Arora & Sperry, 1957), and not with the contractions of isolated muscles. Under such conditions unrecognized feed-back mechanisms are likely to distort the responses and greatly to complicate their interpretation. Moreover, such movements merely signal the net result of the contractions of the antagonistic muscles controlling movement at each joint; hence it is hazardous to use such observations in establishing that there have been changes in the synaptic connexions on to individual nerve cells. Nevertheless, these investigations form a striking contrast to comparable mammalian experiments and suggest that the central nervous systems of teleosts and larval amphibians have well developed plastic properties. The experiments on mammals have given no indication of such plastic changes in the central nervous system (Sperry, 1941, 1942, 1945, 1947; McIntyre & Robinson, 1958); and it has been suggested by Sperry that embryonic mammals may have a comparable plasticity, which is already lost about the time of birth.

Hitherto there has been no investigation of the possibility of plastic modification of monosynaptic pathways from primary afferent fibres to motoneurones, which are now particularly well suited to quantitative

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evaluation. A fairly precise measure of the monosynaptic activation of a motoneurone is provided by the size of the excitatory post-synaptic potential (EPSP) as recorded by an intracellular micro-electrode. In this way it has been possible to define the pattern of monosynaptic activation for over a thousand motoneurones supplying the hind limb and forelimb muscles of the cat (Eccles, Eccles & Lundberg, 1957*b*; R. M. Eccles & Lundberg, 1958). With rare exceptions, it has been found that the monosynaptic activation from the afferent fibres (Group Ia) of any particular muscle operating at the knee or ankle joints is restricted to the motoneurones concerned in the movements subserved by that muscle, i.e. to the motoneurones of that muscle and of synergic muscles. A systematic investigation of the monosynaptic EPSPs thus provides the most delicate possible test for any change in central pathways that might result from an inversion of motoneurone function consequent on nerve cross-union. For example, if the function of a motoneurone normally innervating a pre-tibial muscle is changed by the regeneration of its axon to an ankle extensor muscle, intracellular recording from this motoneurone would be able to detect very small developments of monosynaptic innervation from the afferent fibres of other ankle extensor muscles.

METHODS

Factors in the design of the experiment

Several factors have to be taken into account in choosing muscle nerves for the cross-union operation (cf. Fig. 1A): (1) the two nerves N_1 and N_2 must be so placed that the cross-union operation is anatomically possible and they must supply muscles (M_1 and M_2) that are not synergists and preferably are antagonists. (2) M_1 and M_2 must each have one or more synergic muscles MS_1 and MS_2. The nerves NS_1 and NS_2 of MS_1 and MS_2 are not molested in the initial operative cross-union, and hence their afferent fibres provide the control afferent pathways that are employed in testing the motoneurones with changed functions. (3) As a rule afferent volleys in NS_1 or NS_2 will normally evoke considerable monosynaptic EPSPs in the respective synergic group of motoneurones, MN_1 or MN_2, which is being subjected to the cross-union procedure, i.e. these volleys will produce heteronymous EPSPs (cf. Eccles *et al.* 1957*b*). It is very important, however, that monosynaptic EPSPs should very rarely be produced normally by NS_1 volleys on the motoneurones of group MN_2 or by NS_2 volleys in MN_1, because, in the interpretation of the experimental results, such aberrant EPSPs (Eccles *et al.* 1957*b*, p. 41) provide the criterion for the establishment of new connexions as a consequence of the cross-union between N_1 and N_2. The sensitivity of this criterion would be reduced if such aberrant responses were commonly observed in the control experiments in which there was no operative cross-union. (4) Finally our experiments have indicated that it is very important for the motoneurones with changed function (say MN_1) to be in close proximity to the motoneurones (MNS_2) belonging to the antagonist test nerve (NS_2 in this case).

It is possible to design several experiments on the cat's hind limb that conform reasonably closely with these specifications. In eleven kittens, from 1 to 25 days old, the nerve to the peroneal muscles, peroneus longus, brevis and tertius (Per), was cross-united to the nerve to the medial gastrocnemius (MG), as illustrated in Figs. 1B, 2B. The deep peroneal (DP) nerve to the remaining pre-tibial muscles, and the nerves to the remaining post-tibial

extensor muscles—lateral gastrocnemius-soleus (LG), plantaris (Pl) and flexor digitorum longus (FDL)—serve as the respective control afferent pathways. Cross-unions were also performed in six kittens between the Per and LG nerves. More recently another cross-union has been attempted, DP to MG, but some months must elapse before the final experimental tests.

None of these experiments provides ideal conditions. For example, in the LG-to-Per cross-union the motoneuronal nuclei belonging to the two testing nerves, MG and DP, are

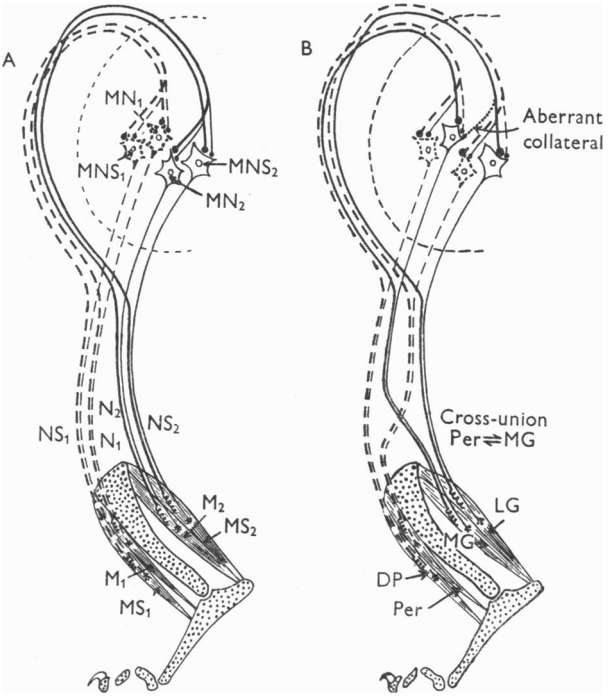

Fig. 1A. Diagram showing the monosynaptic pathways for two pretibial muscles and two post-tibial muscles, labelled in accordance with the text description. Only one Group Ia afferent fibre and one motor fibre is drawn for each muscle, the afferent fibres being distinguished by their thicker lines. The afferent and efferent pathways for muscles functioning as flexors are shown by broken lines. It will be seen that the afferent fibres have monosynaptic action on the motoneurone of their own muscle and, to a less extent, on that of the synergic muscle, as shown by the thinner collateral branch. B. Diagram showing changed peripheral connexions when regeneration has followed the cross-union. The change in function is shown by the change in broken line designation. In the spinal cord the dotted line shows the aberrant connexions that developed from the Ia afferent fibres of post-tibial extensors to the motoneurones of the peroneal muscles when nerves to medial gastrocnemius and to the peroneal muscles were cross-united.

each some distance from the respective nuclei under test, the MG nucleus being caudal to the Per motor nucleus; while the DP nucleus is a considerable distance dorsolateral to the LG nucleus and somewhat more rostral (cf. Romanes, 1951). The Per-to-MG cross-union provides one test situation of close proximity; the Per and LG nuclei are juxtaposed at the same segmental level (L7), the Per nucleus being just lateral to that for LG. The other

test situation, MG-to-DP, is most unfavourable, as the respective nuclei are at different segmental levels, and there is also considerable transverse separation. As a consequence our experimental testing has been concentrated on Per motoneurones that have regenerated to innervate the MG muscle, as in Fig. 1B. This situation has the disadvantage that the Per muscles make a negligible contribution to ankle flexion (peroneus brevis being in some ankle positions even a very weak extensor), which may presumably be correlated with the normal occurrence of a rather high proportion of aberrant monosynaptic excitatory connexions from some post-tibial extensors to Per motoneurones (cf. particularly from MG; Table 2). However, most Per motoneurones have synaptic connexions that indicate not only synergism with the DP motoneurones (monosynaptic EPSPs from DP nerve), but also antagonism to the post-tibial extensors (Ia IPSPs from one or other of these). It was surprising to find that the frequency of Ia IPSPs from post-tibial extensor muscles was as high (22/49) for Per motoneurones as it was for the DP motoneurones supplying the much more effective antagonists (25/56). An earlier investigation (Eccles *et al.* 1957*b*, Table 4) showed that peroneus longus motoneurones received much more monosynaptic excitation from the DP nerve than did the other peroneus motoneurones. In the present investigation it was not possible to discriminate between the three peroneal muscles, because the nerve to all three was cross-united to the MG muscle.

Experimental procedures

The nerve cross-union was performed in an initial aseptic operation. In order to prevent the nerves growing back into their own muscles, the two nerves to be crossed were severed as far distally as possible, and the central ends were freed as far proximally as was necessary to allow suturing in the crossed position without undue tension (cf. Fig. 2B). A single strand of nylon thread was employed to tie the cut ends together and the sheaths of both stumps were torn open by fine forceps so that nerve regeneration would not be impeded. In the final experimental tests 21 to 38 weeks after the cross-union, it was invariably found that the muscles were appropriately innervated and that there were no aberrant connexions to other mucles. For example, in the main series of Per-to-MG cross-union, the Per nerve innervated only MG and the MG nerve only the Per muscles. At the end of the intracellular investigation the various nerves, MG, LG and peroneal, were dissected from the sciatic and stimulated. The responses were recorded from the peripheral nerves that had been employed for stimulation during the experiment. Again it was found that there was no evidence of even one stray nerve fibre. For example, with the cross-union described above, the nerve innervating MG muscle had all its fibres in the peroneal nerve, while the nerve innervating the Per muscles had all its fibres in the MG nerve.

The experimental technique in the final experiment resembled that already described for a survey of the monosynaptic excitatory fields of normal motoneurones (Eccles *et al.* 1957*b*). The cross-united nerves were severed approximately at the site of the suture and the central ends were dissected free sufficiently far to enable the cathode of the stimulating electrodes to be applied well proximal thereto. Since the micro-electrodes were usually filled with 3 M-KCl, IPSPs were sometimes inverted to depolarizing potentials on account of the diffusion of Cl^- out of the electrode. Such inverted IPSPs were readily distinguished from EPSPs by their conversion back to hyperpolarizing potentials when a steady depolarizing current was passed through the micro-electrode. At the end of each intracellular investigation of a motoneurone the micro-electrode was withdrawn to a position just extracellular to the particular neurone and the potentials produced by the various nerve volleys were again recorded. The potential changes actually occurring across the cell membrane are given by the difference between the intracellular and the corresponding extracellular record. As is shown in the illustrative figures, each record is made by the superposition of many faint traces, much of the background noise being thus rejected. Post-tetanic potentiation was also employed in order to lift the post-synaptic potentials above the noise level. The conditioning tetanus was standardized at 450/sec for 15 sec.

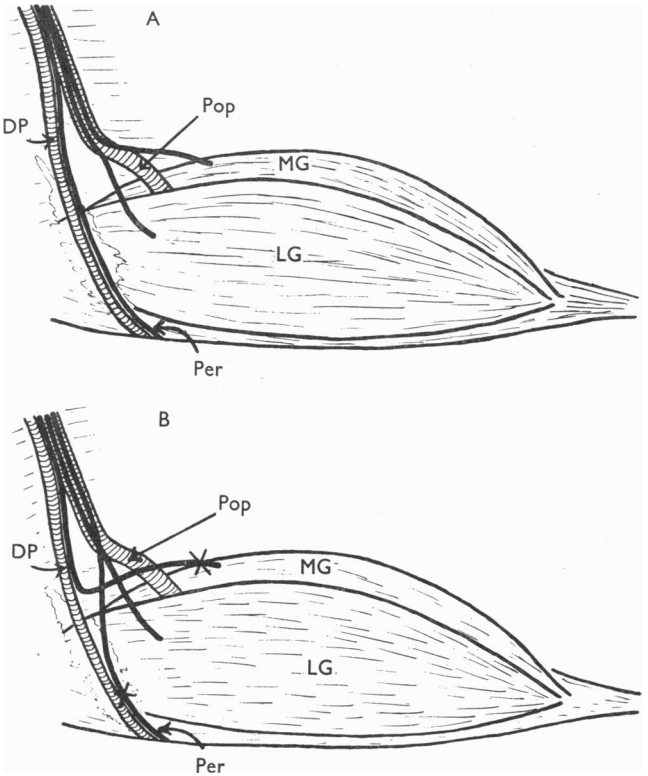

Fig. 2. Drawing of the field of operation when the nerve to the peroneal muscles is cross-united to the medial gastrocnemius nerve, A being before and B after the cross-union, the crosses showing the positions of the sutures. MG, LG mark the positions of the medial gastrocnemius and lateral gastrocnemius muscles and Per labels the nerve to the peroneal muscles; DP is the deep peroneal nerve supplying tibialis anterior, extensor digitorum longus and extensor digitorum brevis muscles, and Pop is the popliteal nerve.

RESULTS

The ventral roots were left intact so that each motoneurone penetrated by the micro-electrode could be identified by antidromic invasion from its motor axon (cf. Eccles *et al.* 1957*b*). For example, in Fig. 3A the stimulus to the Per nerve (re-innervating the MG muscle) was just at threshold for the motor axon of the cell. In about half the superimposed traces this was excited, giving the large truncated spike-potential, which is seen in full at the much lower amplification of the traces in 3E. In the remaining half of the traces in Fig. 3A the monosynaptic EPSP alone was generated. The time course of the potential change across the cell membrane was obtained by projecting and tracing so as to superimpose on the intracellular record the extracellular record (the third line of A) taken at the same stimulus

strength and immediately after withdrawal of the electrode from the cell. The difference between the two traces gives the time course of the membrane potential change, and is shown below each set of recordings of Fig. 3 A–D and F–I.

In this case of reinnervation of MG muscle by the Per nerve it is immediately evident that depolarizing potentials with the brief latency characteristic of monosynaptic EPSPs were set up by LG, Pl and FDL nerves in

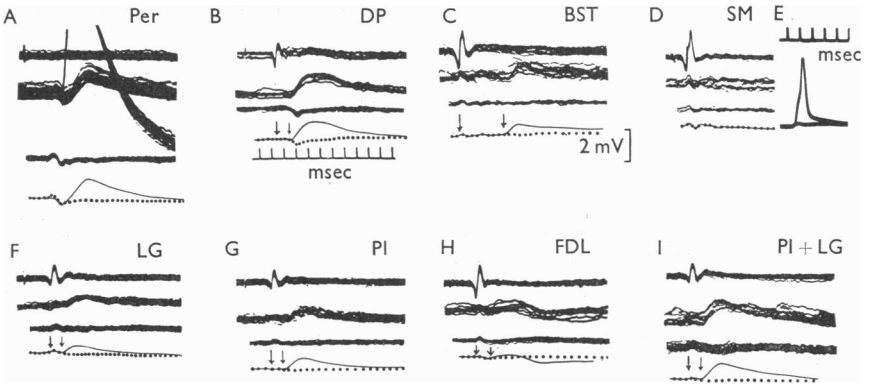

Fig. 3. Intracellular responses of a Per motoneurone reinnervating MG muscle. The three records of each ensemble A–D and F–I are formed by the superposition of about twenty faint traces and show, from above downwards: the potential produced by an afferent volley at the dorsal root entry at mid L 7 segmental level, negativity being upwards; the intracellular potential of a Per motoneurone having a membrane potential of −75 mV and spike potential of 109 mV, positivity being upwards; the potential produced by the same volley, but with a just extracellular position of the micro-electrode. Below the three records there is a superimposed tracing of the intracellular (continuous line) and extracellular (dotted line) records showing the time course of the potential change across the neuronal membrane. With A the stimulus to the Per nerve was just at threshold for the axon of the motoneurone, producing an antidromic spike potential in about half the intracellular traces (middle series), which is shown at much lower amplification in E. B–D and F–I were produced by a maximum Group I afferent volley in nerves indicated by the symbols; DP, deep peroneal; BST, posterior biceps-semitendinosus; SM, semimembranosus + anterior biceps; LG, lateral gastrocnemius + soleus; Pl, plantaris; FDL, flexor digitorum longus. With Pl + LG the two volleys were synchronized. Same time scale for all records and same potential scale for all intracellular and extracellular records except E. The two arrows on the superimposed traces give the central synaptic delay.

F, G and H respectively, in addition to the EPSPs set up by the Per and DP volleys in A and B respectively. On the other hand, in C and D, BST and SM volleys failed to set up monosynaptic EPSPs. The EPSP produced by the LG plus Pl volleys (I) was nearly as large as that generated homonymously in A or by the afferents from the normal synergic muscles in B.

Enhancement of the EPSPs by post-tetanic potentiation was regularly employed in order to increase the size of the EPSPs, and was particularly

valuable in attempting to lift very small EPSPs above the noise level so that they could be identified and their latency and subsequent time course determined. In Fig. 4 the records in the rows marked Con and PTP are respectively the control and the most potentiated. In every case stimuli maximal for Group I were applied to the afferent nerve. Besides the Ia EPSPs, polysynaptic EPSPs are shown in C, G and D, H to be potentiated,

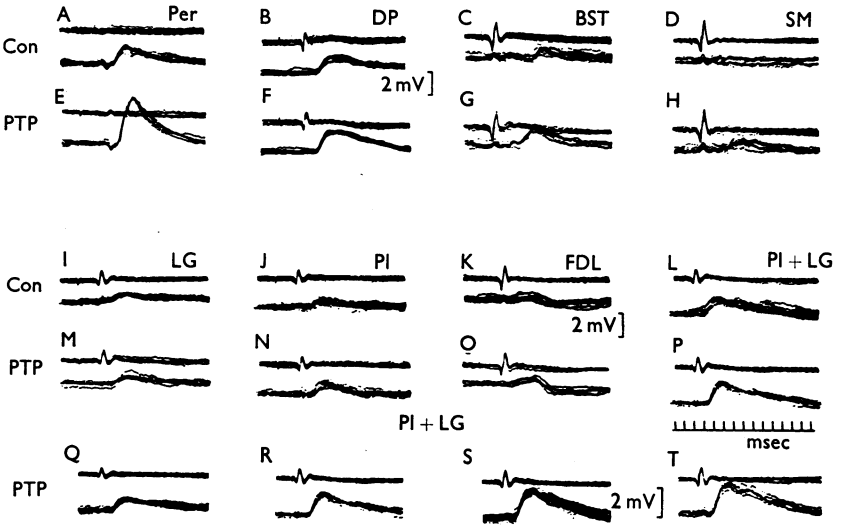

Fig. 4. Each pair of records of A–P corresponds to the upper pair of each ensemble in Fig. 3, and is from the same Per motoneurone, with the same afferent nerve identification. In addition they are arranged in rows labelled Con and PTP, which are respectively the control EPSPs and those at the height of the post-tetanic potentiation following stimulation at 450/sec for 15 sec. Q–T show responses to PI + LG volleys at the height of post-tetanic potentiation, being evoked by stimuli of increasing strength from Q to T, Q being so weak that it was submaximal for Group Ia and below the Ib threshold. Same time scale for all intracellular records; Q–T being at higher amplification than A–P, as shown by the respective potential scales.

while in K, O the polysynaptic IPSP was also potentiated. This Per motoneurone thus received a considerable monosynaptic excitation from the nerves to the post-tibial extensors (I–P), an aberrant effect of a size never observed in normal Per motoneurones. The series with graded stimulus strengths Q–T shows that this monosynaptic EPSP was produced by the lowest threshold Group I afferent fibres (cf. also Fig. 5A–C, G–K), and hence is attributable to the Group Ia fibres (cf. Eccles, Eccles & Lundberg, 1957*a*, *b*).

The hyperpolarizing IPSPs in Fig. 4K and O reveal that the IPSPs of this motoneurone had not been reversed by the diffusion of Cl^- ions out of the KCl electrode (cf. Coombs, Eccles & Fatt, 1955). All membrane

depolarizations in Figs. 3 and 4 would thus be EPSPs. However, in many experiments Cl^- diffusion had converted IPSPs to depolarizing potentials, and it was necessary to depolarize the motoneurone by the application of

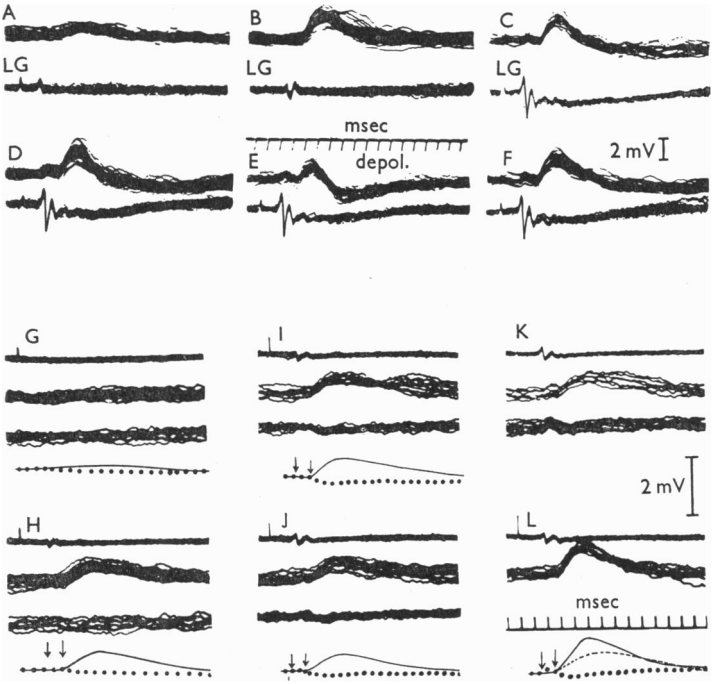

Fig. 5. A–G. Upper tracings are intracellular records from another Per motoneurone reinnervating MG muscle (membrane potential −55 mV), responses being evoked by LG volleys, the lower tracings being of the incoming afferent volley at the mid L7 level. The LG stimulus was just above the Ia threshold in A, and submaximal and maximal for Group I in B and C respectively. D–F were evoked by a maximum Group I volley, the neuronal membrane being depolarized in E by passage of a steady current through the micro-electrode. Note in E the large hyperpolarization of the IPSP which cuts into the rising phase of the monosynaptic EPSP, an effect which is much less obvious in the control records D and F.

G–L are intracellular records from another Per motoneurone reinnervating MG muscle (membrane potential −60 mV, spike potential 79 mV), the responses evoked by LG volleys, which in G–K are set up by progressively increasing strengths of stimulation as in A–C, J being maximal for Group Ia and K for group I. L shows effect of post-tetanic potentiation of EPSP set up by a maximal Group I volley. Superimposed traces of intracellular and extracellular records reveal the time course of the membrane potential; the two arrows give approximately the central synaptic delay.

a current through the micro-electrode in order to convert IPSPs to hyperpolarizing potentials (Fig. 5 D–E) and so to discriminate them from EPSPs (cf. Eccles, Eccles & Lundberg, 1957*c*). The IPSPs invariably had a central latency in excess of 1·3 msec (Eccles *et al.* 1957*c*), whereas the

latency of normally occurring EPSPs was not longer than 1·0 msec (cf. Figs. 3B, 4B, 7F).

EPSPs generated in Per motoneurones by volleys in the nerves of the post-tibial extensor muscles have often had central latencies in the range 1·0–1·3 msec. The small size of most of these EPSPs would result in a rather late estimate being made of their origin in time, and hence account, at least in part, for such high figures for monosynaptic latency. But even apart from this factor the latencies appear to be abnormally long. All transitions exist between undoubted monosynaptic EPSPs and EPSPs with latencies in excess of 1·5 msec (Fig. 7H, I), which are certainly due to Ib disynaptic action. In practice all EPSPs with a latency in excess of 1·3 msec have been regarded as not being monosynaptically evoked. The second criterion has been the threshold discrimination between Ia and Ib afferent fibres. Even when there has been no appreciable difference of Ia and Ib conduction velocity, afferent volleys evoked by stimuli up to 1·2 times threshold only produce monosynaptic Ia EPSPs in motoneurones (Eccles *et al.* 1957*c*). As is shown in Figs. 4Q–S, 5A–C, G–K, the lowest threshold afferent fibres in the nerves to the post-tibial extensor muscles were responsible for the brief-latency EPSPs in Per motoneurones reinnervating MG muscle. It has always been found that the lowest threshold (Ia) afferent fibres were responsible for the monosynaptic EPSPs in the Per motoneurones with changed function, which is precisely the situation for the EPSPs of normal motoneurones (Eccles *et al.* 1957*a*).

Altogether, in the eleven experiments, satisfactory intracellular investigations were made on 102 Per motoneurones which had reinnervated MG muscle. In addition 59 normal Per motoneurones in normal cats were investigated in order to give a contro series. The entries for Per motoneurones in the second rows of Tables 1 and 2 are derived from these two series. The monosynaptic EPSPs of the other species of motoneurones involved in the cross-union (MG) or synergic with those cross-united (DP and LG) are given also in the other rows of the tables. The entries in each column relate to the monosynaptic EPSPs produced by maximum Group I volleys in the various muscle nerves indicated by the letters at the top. Each series of entries for the action of each species of nerve on each species of motoneurone gives, first, the number in which monosynaptic EPSPs were observed out of the total number of motoneurones so investigated, and secondly the mean EPSP calculated by dividing the aggregate EPSPs by the total number of those motoneurones. For example, the aggregate EPSP for the action of LG volleys on Per motoneurones was 20·3 mV, which gives a mean of 0·20 mV for the 102 motoneurones so investigated.

Comparison of the Per motoneurones in Tables 1 and 2 reveals that the

control nerves to the post-tibial extensor muscles evoked monosynaptic EPSPs far more frequently in the Per motoneurones with changed function (see and compare framed sections of each Table), the increase being

TABLE 1. Assembled results for the eleven experiments with cross-union of Per with MG. Maximum monosynaptic EPSPs in motoneurones (arranged in rows) by volleys in eight different muscle nerves (arranged in columns). The upper entry gives the number of cells responding by recognizable monosynaptic EPSPs over the total so investigated. The lower entry gives the mean EPSP for the total cells so investigated. Cells with very low membrane potentials (less than −40 mV) were not included in this Table

	Nerve volleys							
Motoneurones	DP	Per	MG	LG	PL	FDL	BST	SM
DP	107/107	48/107	0/107	2/107	3/107	8/102	0/107	0/94
(mV)	2·96	0·22	0	0·01	0·01	0·03	0	0
Per	92/102	85/100	3/101	41/102	17/101	23/95	2/101	0/91
(mV)	1·50	1·17	0·03	0·20	0·05	0·08	0·01	0
MG	1/31	2/31	31/32	31/33	2/31	0/28	0/31	0/26
(mV)	0·02	0·07	2·1	2·05	0·01	0	0	0
LG	0/31	1/31	25/31	31/31	18/31	0/29	1/31	1/22
(mV)	0	0·01	0·59	3·6	0·84	0	0·03	0·01

In both tables the abbreviations for nerves and motoneurones are as follows: DP, deep peroneal; Per, peroneus longus, brevis and tertius; MG, medial gastrocnemius; LG, lateral gastrocnemius + soleus; Pl, plantaris; FDL, flexor digitorum longus + flexor hallucis longus; BST, posterior biceps plus semitendinosus; SM, semimembranosus + anterior biceps.

TABLE 2. Assembled results from twelve normal cats, tabulated identically with Table 1. Note the contrast between the corresponding entries which are framed in the two Tables

	Nerve volleys							
Motoneurones	DP	PER	MG	LG	PL	FDL	BST	SM
DP	69/69	35/70	0/51	0/51	0/52	1/51	0/42	0/17
(mV)	3·90	0·90	0	0	0	0·01	0	0
PER	40/56	59/59	8/53	3/52	1/54	6/54	0/42	0/29
(mV)	0·94	3·66	0·08	0·015	0·005	0·04	0	0
MG	0/27	1/24	42/42	43/43	16/40	5/33	0/27	0/16
(mV)	0	0·01	4·85	2·04	0·20	0·06	0	0
LG	0/25	1/20	34/34	34/34	33/34	4/32	0/24	0/14
(mV)	0	0·05	2·27	4·7	1·1	0·02	0	0

particularly large with the LG nerve (from 6 to 40 %), but also considerable with Pl and FDL motoneurones, as is seen in Figs. 3 and 4. This is associated with an increase in the mean size of the monosynaptic EPSPs produced

by LG volleys in Per motoneurones by more then tenfold, from the normal value of about 0·015 mV to 0·20 mV for the Per motoneurones with changed function, and there were also large increases in EPSPs evoked by the Pl and FDL afferent volleys. Usually the LG volleys were the most effective, as is illustrated in Figs. 6 and 7. Comparison of Tables 1 and 2 shows further that there was virtually no alteration in the monosynaptic action of volleys in DP, BST and SM nerves on the Per motoneurones with changed function (cf. Figs. 3, 6, 7; Tables 1 and 2).

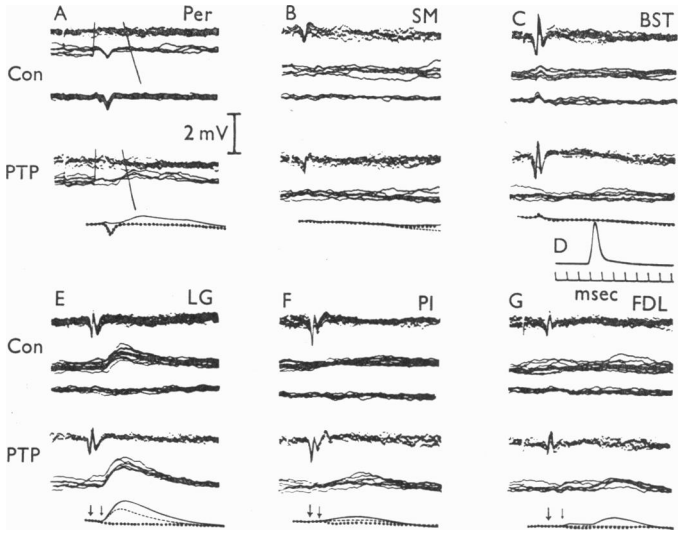

Fig. 6. A–C and E–G show both intracellular and extracellular records from a Per motoneurone that reinnervated MG muscle (membrane potential −76 mV, spike potential 76 mV), arranged to show intracellular records before (row labelled Con) and after post-tetanic potentiation (row labelled PTP). Bottom trace of Con in each ensemble is the corresponding extracellular record; below PTP are the superimposed tracings of the two intracellular (broken line, Con; continuous, PTP) and the extracellular (dotted line) records, the arrows giving the approximate central synaptic delays. The afferent volleys are identified as in Fig. 3. D shows the intracellular antidromic spike evoked by a Per volley, and recorded at much lower amplification. Same time scale for all records, and all but D at same amplification. The afferent volley records are at mid L7 level, negativity being upwards.

Finally, both the MG and Per nerves had diminished actions on the Per motoneurones in Table 1 as compared with the control actions in Table 2. This diminution is also evident for the actions of these two nerves on the other motoneurones of Tables 1 and 2. Undoubtedly the principal factor concerned in this effect is the chromatolytic degeneration of a considerable proportion of the Group I afferent fibres of Per and MG nerves. Chromatolysis, both of spinal ganglion cells and of motoneurones, is particularly severe after peripheral nerve section in young animals, and

it has been reported that it causes the death of a very large proportion of these nerve cells (Nonidez, 1944; Romanes, 1946). Correspondingly, the surface lead from the dorsal root at cord entry shows only a very small spike for afferent volleys in Per and MG nerves (Figs. 3A; 4A; 6A; 7A, B). In a few experiments monophasic recording from dorsal roots was employed in estimating the proportion of surviving Group I afferent fibres in the cross-united nerves, the values being in the range of 20–40 %. The depressions of monosynaptic excitatory actions in Table 1 as compared with

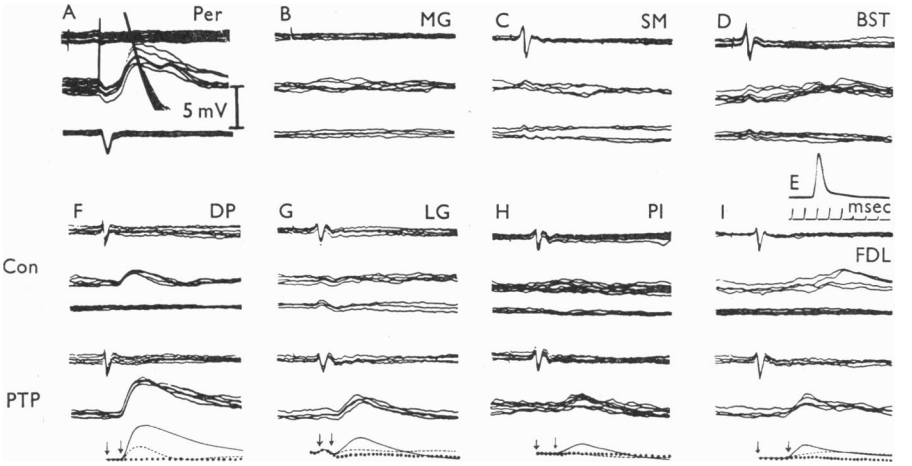

Fig. 7. Series as in Fig. 6, but for another Per motoneurone reinnervating MG muscle (membrane potential −74 mV, spike potential 82 mV). Post-tetanic potentiation is only shown for F–I. The afferent volley records are from the mid L7 level, negativity being downwards.

Table 2 accord well with this proportion of survival. Thus the homonymous action of the Per afferent volley is depressed to 0·31 (1·17:3·66) and the heteronymous action on DP motoneurones to 0·24 (0·22:0·90), while for the MG volley the corresponding homonymous and heteronymous ratios are 0·43 (2·1:4·85) and 0·26 (0·59:2·27). Presumably the reduction of the aberrant MG action on Per motoneurones to about one third (0·03:0·08) may be accounted for in the same way.

The post-tetanic potentiation of EPSPs following a long high-frequency tetanus (Eccles, Krnjević & Miledi, 1959; Curtis & Eccles, 1960) has been employed regularly in defining more accurately the time course of small EPSPs, especially in the measurement of central latency. It has been of particular importance when there was uncertainty about the presence of an EPSP. Post-tetanic potentiation would then either increase the EPSP so that its existence was undoubted (Fig. 6, F, G; Fig. 7, G), or alternatively would show that the EPSP had such a long latency that it was not monosynaptic; e.g. it was shown in this way to be 1·5 and 2·5 msec

in Fig. 7H and I respectively. Sometimes the relative potentiation has been abnormally high (3·8 in Fig. 7G) but often the EPSPs produced by the post-tibial extensor nerves in Per motoneurones were not potentiated more than were EPSPs from the DP nerve to Per motoneurones, as may be seen by comparing in Fig. 4, B with F, I with M, J with N and L with P. With the Per motoneurones of Table 1 the mean potentiation ratio was 2·5–2·6 for monosynaptic EPSPs produced by nerves of the post-tibial extensors, and about 2·15 for the DP nerve. Rarely, post-tetanic potentiation revealed a monosynaptic EPSP that was otherwise undetectable, there being for example three instances of this in the 41 EPSPs reported for LG afferent volleys on Per motoneurones in Table 1. As a result of the relatively high degree of post-tetanic potentiation, the mean monosynaptic EPSP for the combined action of the nerves to the post-tibial extensors was almost 1·0 mV for the 102 Per motoneurones which had been changed to function as ankle extensor motoneurones.

The responses of MG cells in Table 1 show little, if any, increase in aberrant connexions from the acquired synergists, the muscles innervated by DP nerve, and are thus in striking contrast with the responses of Per cells. A similar absence of aberrancy from nerves to the acquired synergists was observed when LG and Per nerves were cross-united in six kittens. In a total of 21 LG and 5 Per motoneurones there were no aberrant monosynaptic connexions. It was noteworthy that not only had the cells retained their original monosynaptic activation by Group Ia afferent fibres, but also that the Ia disynaptic inhibitory actions on them from the original antagonists (the acquired synergists) were at least as frequent as in the control investigations.

The negative results of such cross-union operations account for the very low proportion of aberrancy (4/50) reported in a preliminary account of this present investigation (Eccles, 1959). Changed function in these 50 motoneurones resulted from the LG–Per cross-union in 26, and from the MG–Per cross in 24. Of these latter only 10 were Per motoneurones; they displayed much the same proportion of aberrancy as the much larger series of Table 1, 4/10 for LG and 1/10 for Pl.

Hitherto attention has been restricted to the evidence that change in motoneurone function by operative cross-union results in a change of the receptive field from which the motoneurone draws its monosynaptic activation. But operative cross-union also entails a change of the muscle from which an afferent fibre receives its activation. For example, as a consequence of the nerve cross-union of Table 1, the Per afferent fibres were excited by receptors in MG muscle and the MG afferent fibres by receptors in the Per muscles (cf. McIntyre & Robinson, 1958). Comparison of Tables 1 and 2 shows that, as a result of this changed peripheral con-

nexion, there was no development of central connexions, of these afferent fibres to the motoneurones of their acquired synergists, from the Per and MG nerves to LG and DP motoneurones respectively. There was merely a diminution in the sizes of the EPSPs normally produced by both afferents, which, as we have seen, is sufficiently explained by the chromatolytic death rate.

DISCUSSION

In general, the experimental results as presented in Tables 1 and 2 show that after birth the mammalian spinal cord has only a small degree of plasticity, in so far as this is exhibited by changes of monosynaptic reflex pathways following cross-union of nerves to muscles. For example, in two experiments there was no trace of any aberrant connexions to the 23 Per motoneurones that had reinnervated MG muscle. In three other experiments there were aberrant connexions to more than half of the Per motoneurones with changed function. Yet the mean values for the aberrant EPSPs produced in Per motoneurones from the various nerves to the post-tibial extensors (LG, 0·5 mV; Pl, 0·3 mV; FDL, 0·4 mV) show that the formation of new synaptic connexions usually occurred only to a slight extent. There was no correlation between the age of the kitten at operation (range 1–25 days) and the frequency and magnitude of the aberrant EPSPs.

The present experimental evidence of a small degree of plastic change in the synaptic connexions of the mammalian spinal cord is not in conflict with the negative results of earlier investigations. For example, the small changes here reported in the Ia receptive fields of motoneurones would not be detectable in investigations on reflex discharges in ventral roots (McIntyre & Robinson, 1958) or in reflex responses of limbs (Sperry, 1941, 1942, 1947).

The aberrant EPSPs have tended to differ from normal EPSPs in two respects, longer central latency and larger post-tetanic potentiation. The longer latency might be expected to occur on account of increased conduction time in the newly developed presynaptic fibres. Presumably also the newly developed synaptic terminals may be relatively ineffective liberators of the chemical transmitter, and hence show more potentiation as a consequence of the transmitter mobilization ensuing after a conditioning tetanus (cf. Curtis & Eccles, 1960). The mean PTP ratio for the aberrant EPSPs, 2·6, was much higher than the normal ratio of 1·5:2·0. It may be of significance that there was also an abnormally high level of potentiation for the EPSPs evoked by DP and P volleys on Per motoneurones, which had mean ratios of 2·1:2·5 respectively; for it will later be suggested that in part such EPSPs were also produced by newly developed synaptic connexions.

It seems certain from Tables 1 and 2 that, when Per motoneurones are transformed to have an 'ankle-extensor' function, there is an increase in the aberrant monosynaptic connexions from the post-tibial extensor muscles to them. Before considering these changes as arising on account of the transformed motoneurone function and being specifically related thereto, it should be considered whether there is an entirely non-specific explanation. Since the cross-union operation resulted in the chromatolytic death of about two thirds of the spinal ganglion cells, their degenerating central terminals may have stimulated sprouting of adjacent Ia afferent fibres which then established the aberrant synaptic connexions on the Per motoneurones. This effect of degenerating nerve fibres was first demonstrated in partial muscle denervation (Hoffman, 1950, 1951; Edds, 1950, 1953), and it has also been found in the partially denervated sympathetic ganglion (Murray & Thompson, 1957) and in the spinal cord when spinal cord section caused degeneration of the descending tracts (McCouch, Austin, Liu & Liu, 1958).

If this non-specific central sprouting caused the increased aberrancy of monosynaptic connexion to Per motoneurones seen in Table 1, it is possible that proximity of the motor nuclei provides a sufficient explanation of the preponderance of the LG aberrant connexions. Each motoneuronal nucleus has a wealth of fine branches of Group Ia primary afferent fibres, particularly from the muscle it innervates; hence motoneuronal nuclei in close proximity are optimally placed for the operation of any influence that degenerating Ia afferent fibres in one may have in causing the sprouting of Ia fibres in the other. Thus LG motoneurones are as a rule closer to Per motoneurones than are the Pl and FDL motoneurones; and degeneration of central terminals has not been associated with the development of aberrant connexions when the respective nuclei were more remote, e.g. from DP nerve to MG motoneurones in Table 1.

It seems unlikely for several reasons that the observed increase in aberrancy can be fully explained by this postulated process of non-specific sprouting. First, the Ia fibres most exposed to the postulated influence would be those actually in the Per nucleus, i.e. the surviving Per fibres and those DP fibres which evoke the heteronymous EPSP (cf. Table 2 and Eccles *et al.* 1957*b*). The mean EPSP of the latter was increased (from 0·94 to 1·50 mV), while with the former there was also possibly a small increase, because chromatolytic destruction reduced the mean Per EPSP on DP motoneurones to 24 %, and on Per motoneurones to 31 %. Thus under optimal conditions non-specific sprouting appears to have increased only to a small extent (by about one third) the EPSPs produced by DP and Per afferents on Per motoneurones, which suggests that it could make but a negligible contribution to the much larger relative increases (over tenfold)

in the EPSPs evoked in Per motoneurones by nerves to the post-tibial extensors (Table 1). Secondly, non-specific sprouting would be expected to increase the aberrant connexions from BST and SM Ia afferent fibres to Per motoneurones, for in the L7 segment their motor nuclei are at least as close to the Per motor nuclei as the FDL nucleus is, yet the observed aberrancy rates in Table 1 are negligible, 2:101 and 0:91 respectively. Thirdly, non-specific sprouting would be expected to increase the aberrancy of monosynaptic connexions not only to Per motoneurones, but also to MG motoneurones—e.g. from the contiguous BST motoneurones—and this has not been observed (Table 1).

There are two further experimental investigations of the postulated effect of degenerating primary afferent fibres in causing sprouting of adjacent fibres. It would be expected that the central degeneration caused by partial severing of the dorsal roots central to the spinal ganglia would cause a large development of aberrant monosynaptic connexions to motoneurones. This possibility is at present being fully investigated. In a preliminary investigation the dorsal root operation was performed on two adult cats and 25 weeks later there were no aberrant monosynaptic connexions on the 96 motoneurones from which intracellular records were made. In the alternative control experiment the Per and MG nerves have been severed, as in the cross-union operation, but have been rejoined to their own peripheral stumps. Under such conditions there will be the same chromatolytic destruction of spinal ganglion cells as in the cross-union operation, but no change in motoneurone function. Comparison of the aberrancies under such conditions with those of Table 1 should establish the degree to which non-specific sprouting is responsible for the aberrancies shown for Per motoneurones in Table 1. Operations of this type have been performed on young kittens, but some months must elapse before the final experimental tests, which will be separately reported.

Following a comprehensive investigation of the receptive fields for the monosynaptic activation of many different species of motoneurones (Eccles *et al.* 1957*b*), the following question was raised, but not answered. What developmental processes are responsible for establishing the highly selective connexions which have been shown to exist? For example, the nuclei of BST motoneurones on the one hand and of the MG and LG motoneurones on the other are in close apposition, yet their respective Ia receptive fields are usually quite independent. Still more remarkable are the connexions made between widely separated but functionally related nuclei, the best instance being the reciprocal gracilis-semitendinosus linkage (Eccles *et al.* 1957*b*; R. M. Eccles & Lundberg, 1958), which occurs despite the location of the respective nuclei at L5 and L7 segmental levels. It does not seem possible to postulate that these highly specific

intersegmental connexions are established by the ingrowth of fibres from the primitive spinal ganglia along the easiest channels between tissue planes. On such an explanation aberrant connexions would be expected to be far more frequent than is actually observed.

One possible explanation of the development of selective connexions is that some specific chemical or physico-chemical property of the Group Ia fibres provides a 'selective contact guidance' during growth; and furthermore, limits the possibility of their functional connexion, so that connexions are made only to those motoneurones that have a complementary specificity (Sperry, 1951*a*; Weiss, 1950). An influence of a muscle on the motoneurones innervating it was postulated by Wiersma (1931) and by Weiss (1936, 1947, 1952). This postulate of a 'modulating' influence of muscle on motoneurones, 'myotypic specification', has been developed by Sperry (1951*a*, *b*; 1958) and Weiss (1952) into the concept that the motoneurone could be conditioned in this way to attract and retain one type of synaptic contact rather than another, there being some kind of chemical affinity of 'specification' between a nerve cell and the synaptic knobs in contact with it. This concept certainly provides a plausible explanation for the development of reciprocal monosynaptic connexions between muscles operating synergically at only one joint; but it encounters difficulty in explaining the central connexions of double-joint muscles, which may receive monosynaptic connexions from the muscles restricted to either of the two joints, and also activate motoneurones which innervate muscles operating either joint (Eccles *et al.* 1957*b*; R. M. Eccles & Lundberg, 1958). The complex monosynaptic linkages between knee and hip flexors and extensors (R. M. Eccles & Lundberg, 1958) provide still greater difficulties for an explanation by specification. Yet, on the other hand, specification as a controlling factor is suggested by the finding that no motoneurone receives both excitation and inhibition from the Group Ia fibres of any particular muscle (R. M. Eccles & Lundberg 1958). The postulated myotypic specification must be distinguished from the chemical transmitter mechanism at synaptic junctions; the former is responsible for the actual existence of the synapses, the latter for their functional operation. Yet it seems very probable that there is a relationship, otherwise the specification could cause the development of synaptic contacts that were functionless because the chemical transmitter was ineffective.

At present the only alternative to the explanation by specification is that initially all varieties of functional connexion are made by the ingrowing Ia fibres, and that this virtual randomness of connexion is gradually shaped to the specific adult patterns by a process of 'resculpturing', by means of which all inappropriate connexions regress, while there is a complementary

enhancement of those synaptic connexions 'corresponding' to proper function (Sperry, 1951*a*).

When considering the experimental results in relation to the alternative hypotheses of 'specification' or 'resculpturing', it seems clear that the former hypothesis alone is supported by the monosynaptic excitatory connexions. The resculpturing hypothesis would predict that there would be a loss of monosynaptic connexions from the control synergic muscles which functioned as antagonists after the cross-union. For example, there should be decreases in the EPSPs produced by DP nerve on the transformed Per motoneurones, and by LG nerve on transformed MG motoneurones, whereas comparison of Tables 1 and 2 shows that there was either a slight increase or no change. On the other hand, the large increase (over tenfold) of the aberrant LG connexions to Per motoneurones can be accounted for by the hypothesis of myotypic specification. It has to be postulated that, as a consequence of the innervation of MG muscle, the Per motoneurones develop a changed surface specificity that attracts LG synapses. However, until the further control experiments evaluate the contribution of non-specific sprouting to the increased aberrancy, the present experiments do not provide convincing evidence for this hypothesis of linked modulation and specification.

The effect of the changed function of Per motoneurones on the incidence of disynaptic Ia inhibition upon them also indicates the existence of some specific factor. In the normal control experiments the Ia IPSP from the antagonist muscles (the post-tibial extensors) was as frequently observed with Per (22:49) as with DP (25:56) motoneurones. With Per motoneurones reinnervating MG muscle there was a large reduction to 9:106 in the incidence of Ia IPSP from the post-tibial extensors, which have been transformed from antagonists to the acquired synergists of the Per motoneurones. In contrast, the Ia IPSPs on the adjacent DP motoneurones were just as frequent (46:107) as normally. There appears to be some incompatibility between Ia inhibitory and excitatory action by any particular nerve on the same motoneurone (cf. R. M. Eccles & Lundberg, 1958). Hence the observed shedding of Ia inhibitory connexions to the transformed Per motoneurones may be a necessary preliminary before Ia excitatory connexions can be established from the acquired synergists. Evidently such suggestions imply that a specific 'modulating' influence from the MG muscle operates on the Per motoneurones reinnervating it, which would be a further example of the process of myotypic specification postulated by Weiss and by Sperry. The apparent shedding of inhibitory synapses is in accord with the resculpturing hypothesis; but these observations must be regarded with caution, unless substantiated by further investigations.

However, even if myotypic specification is responsible for the observed

changes in Table 1, the magnitude of this effect is far less than would be expected for a process that is postulated to account for the embryological development of specific connexions. Possibly specification is much more effective at the very early stage of development. As postulated by Weiss (1947) and Sperry (1951*a*, *b*, 1958), one can envisage the growing nerve fibre as chemically sensing the surfaces along which it grows, and being specifically attracted by the chemical properties of some surfaces and not by others. There are now many lines of evidence which suggest that the development of synaptic connexions can be explained only by some such postulate of surface specificity. For example, Group Ia and Ib primary afferent fibres of muscle appear never to make functional synaptic connexions on the same nerve cell, whether this be a motoneurone, an interneurone of the intermediate nucleus, a cell of Clarke's column, or a cell of origin of the ventral spinocerebellar tract (Oscarsson, 1957; Lundberg & Winsbury, 1960; Eccles, Eccles & Lundberg, 1960). Apparently these two types of fibre differ in respect of the surfaces which attract them. In the regeneration of peripheral nerve fibres, not only do sensory and motor fibres display discrimination in the choice of pathway (Weiss & Edds, 1945; Gutmann, 1945), but there are also discriminations between the various sensory modalities, e.g. between taste buds, lateral-line organs and cutaneous endings (Sperry, 1951*a*). It remains to be determined whether the developments of aberrant monosynaptic connexions here reported are further instances of the influence of specific chemical affinities, i.e. of the postulated specification process, and, in addition, of changes which muscles can produce in motoneuronal specification, the postulated myotypic specification.

SUMMARY

1. In eleven kittens 1–25 days old, nerves to antagonistic leg muscles were cross-united, and some months later intracellular recording of the monosynaptic excitatory post-synaptic potentials was employed to test if any change had been produced in the synaptic connexions to motoneurones with inverted function. The motoneurones and afferent fibres of synergic muscles were employed as controls in the experimental tests.

2. After crossing of the nerve to the peroneal muscles (peroneus longus, brevis and tertius) to medial gastrocnemius muscle, 40 % of the peroneus motoneurones (41:102) were monosynaptically activated from their acquired synergist, lateral gastrocnemius, whereas normally this aberrant connexion was rare (3:52). There was also a large increase in monosynaptic activation from other post-tibial extensors.

3. Correspondingly, there appeared to be a considerable decrease in the inhibitory action exerted by Group Ia afferent volleys from the post-tibial extensor muscles to peroneus motoneurones.

4. No equivalent change occurred in the monosynaptic excitatory action on the medial gastrocnemius motoneurones reinnervating the peroneus muscles.

5. There was a loss of about two thirds of the Group I afferent fibres of the cross-united nerves, presumably on account of chromatolytic death of the spinal ganglion cells.

6. The degeneration of the fine central terminals of these afferent fibres probably caused sprouting of adjacent fibres, so providing a partial explanation of the increased aberrancy of post-tibial extensors to peroneus motoneurones; however this effect appeared to account for only a small fraction of the observed aberrancy.

7. The alternative explanation attributes the aberrancy to a change in peroneus motoneurones consequent on their reinnervation of medial gastrocnemius muscle, whereby they attract synaptic endings from the Ia afferent fibres from their acquired synergists (the process of myotypic specification of Weiss and Sperry). It is hoped that further experimental investigations will allow a relative evaluation of these alternatives.

8. Brief consideration was given to the relation of these findings to the factors responsible for embryonic development of specific connexions in the central nervous system.

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