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THE ACTIVATION PATTERNS OF EMBRYONIC CHICK MOTONEURONES PROJECTING TO INAPPROPRIATE MUSCLES

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SUMMARY

1. Chick lumbosacral motoneurones were caused to innervate foreign muscles by surgically rotating or shifting the limb bud about the anterior–posterior axis in stage 17–18 embryos.

2. The activation pattern of such wrongly projecting motoneurones was assessed at stages 35–38 by recording electromyographic activity from muscles in an isolated spinal cord/hind limb preparation. Muscle activity was classed as flexor- or extensor-like according to the characteristics of the patterned sequence of bursts elicited by a single shock to the thoracic cord.

3. Wrongly projecting motoneurones did not have their activation pattern altered to one appropriate for the muscle innervated; therefore in some cases a particular muscle was activated with a pattern similar to its original one, and in other cases in an opposite manner. Mixed flexor–extensor-like activation of a single muscle was, however, rare.

4. The identity of motoneurones projecting to a muscle was determined by their cord location following retrograde labelling with horseradish peroxidase. This allowed us to conclude that motoneurones could develop their normal pattern of activation even when projecting to foreign muscles.

5. It is concluded that the cord circuits (presumably composed of local interneurones responsible for the activation of motoneurones in the isolated cord preparation are not altered by retrograde influences from the muscle.

6. Wrongly projecting motoneurones, which were maintained throughout the normal cell death period, were activated during spontaneous embryonic movements, and in many cases were found to have a behaviourally inappropriate activation pattern. These observations are discussed in relation to proposed mechanisms by which developmental errors in connectivity are corrected.

INTRODUCTION

To explain his observations of homologous response by supernumerary amphibian limbs, Weiss (1937) proposed that the peripheral target could determine the central

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connexions and therefore the activation patterns of motoneurones. Subsequently other explanations of this phenomenon have been favoured, among them selective reinnervation of muscles by their original neurones (Grimm, 1971; Kleinbeckel, 1979) as well as the observation that electromyographic (e.m.g.) recordings actually reveal differences in the behaviour of homologous muscles not apparent at the behavioural level (Szekely & Czeh, 1971).

Eccles and his co-workers (1960, 1962) reported some minor alterations in the central connexions of kitten motoneurones which had been cross-united to foreign muscles. However, a subsequent study (Mendell & Scott, 1975) failed to detect measurable changes in IA input onto such cross-united motoneurones. Nonetheless, such surgical alterations were performed long after the basic circuits of the spinal cord have formed, and therefore these studies do not address whether the periphery can influence the laying down of initial connexions.

In the frog Frank & Westerfield (1982) have described changes in the central projections of dorsal root ganglion cells surgically caused to innervate the limb in young tadpoles (see also Hollyday & Mendell, 1975). However, no one has tested the central connexions of vertebrate motoneurones which have been caused to innervate foreign muscles from early in development. In the chick it is possible surgically to produce inappropriate innervation from the time of initial axon outgrowth, and to determine the identity of such motoneurones with reasonable accuracy from their cord position (Lance-Jones & Landmesser, 1981; Hollyday, 1981).

In addition to serving as a measure of the degree of plasticity in the embryonic chick cord, an analysis of the activation pattern of wrongly projecting motoneurones should also contribute to our understanding of how errors in synaptic connectivity are removed. Initial errors in connectivity in *Xenopus* are removed during the normal motoneurone cell death period (Lamb, 1976, 1979). However, in the chick, following a variety of manipulations, wrongly projecting motoneurones are maintained throughout the normal cell death period (Lance-Jones & Landmesser, 1981; Hollyday, 1981; Summerbell & Stirling, 1981). It is possible that, following such embryonic manipulations, motoneurones are no longer activated to threshold, and motoneurone cell death is thereby prevented (Pittman & Oppenheim, 1979). Alternatively, changes in the central connexions of such wrongly projecting motoneurones might result in appropriate activation of the muscle. Such peripheral errors would then be without behavioural consequence, and might go undetected by an error-removal mechanism that in some way sensed appropriate muscle behaviour.

To determine the consequence of an altered peripheral target on the central connectivity of spinal motoneurones, and to relate this to error-removal, we assessed the behaviour of chick motoneurones innervating foreign muscles following early limb manipulations, using e.m.g. recordings from the isolated spinal cord/limb preparation described in the preceding paper (Landmesser & O'Donovan, 1984).

METHODS

Embryonic surgery

Embryonic surgery was performed on 166 embryos (stage 17–20: Hamburger & Hamilton, 1951) in a sterile glove box. A small hole was made in the shell, the shell membrane and amnion opened and one of several limb manipulations was performed. In the earlier experiments, the limb bud of

an embryo was transected at the level of the somites and shifted approximately three to five somites forwards (see Fig. 1). This caused the first three lumbosacral nerves, which normally form the crural plexus, to enter the posterior part of the limb where they formed the sciatic plexus (see Lance-Jones & Landmesser, 1981, for further details).

However, since variable numbers of the spinal nerves which normally form the sciatic plexus (i.e. nerves 4–8), also contributed to the plexus in this case, and since we wanted to optimize the

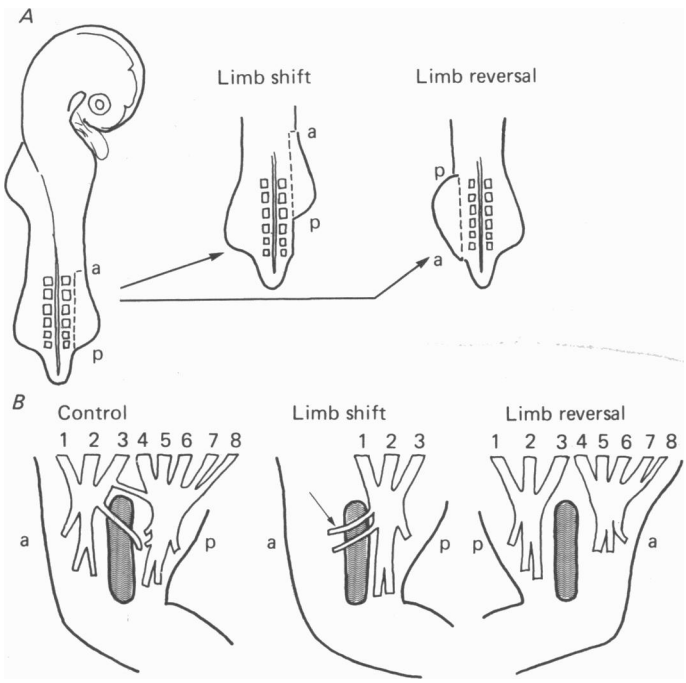

Fig. 1. Embryonic surgery and resultant hind limb nerve patterns. A schematic view of the control nerve pattern formed by the eight lumbosacral spinal cord segments innervating the chick hind limb is shown in *B* at the left. Following a shift of the limb bud anteriorly (a), the first three spinal nerves now enter the posterior (p) part of the limb, and while some motoneurones project to their original muscles by taking novel pathways (arrow in middle section of *B*), most project to foreign muscles. Following an a–p reversal of the limb bud, most spinal nerves project into the wrong part of the limb and synapse with foreign muscles (*B*, right).

conditions for muscles to be innervated by foreign motoneurones, in most of the later experiments we reversed the limb bud about the anterior–posterior (a–p) axis. This was accomplished by transplanting the right limb bud of one embryo to the left side of a second embryo, whose left limb bud had been previously removed. In this case the dorsal–ventral (d–v) axis was conserved. In many cases this resulted in the crural spinal nerves (1–3) forming the sciatic plexus, while the sciatic spinal nerves (4–8) entered the anterior part of the limb and contributed to the crural plexus. However, due to slight variations in positioning of the limb, the nerve component contributing to each plexus was somewhat variable.

Following the operation, the hole in the egg was sealed with a coverslip and the egg returned to the incubator until stages 35–38. Of the operated embryos fifty-one survived to these stages and had essentially normal limbs, and these were studied further.

E.m.g. recording

Embryos were decapitated, eviscerated and a ventral laminectomy was performed, after placing the embroys in a bath of oxygenated Tyrode solution at room temperature (18–22 °C). The limbs were skinned and the muscles freed of connective tissue. The sciatic and crural plexuses were usually dissected so that the spinal nerve contribution to each could be visualized. After several hours at a bath temperature of *ca.* 30 °C, the thoracic or cervical cord was stimulated with single shocks at 10 min intervals, each shock setting off a sequence of hind limb kicks. The e.m.g. activity associated with these kicks was recorded with suction electrodes, and displayed on an oscilloscope and pen recorder. It was also taped for further analysis. For further details of the isolated cord preparation and the recording procedure see Landmesser & O'Donovan (1984).

We obtained satisfactory e.m.g. recordings from forty-nine muscles in thirty-seven different embryos. The activation pattern of the muscle was classified as flexor-like or extensor-like primarily according to the length of the period of inhibition following the synchronous discharge that begins a burst (for additional details see Landmesser & O'Donovan, 1984). Usually e.m.g. recordings were made simultaneously from a muscle in the contralateral limb. This served as a control for comparison with the e.m.g. pattern of the experimental muscle.

For more quantitative comparison of e.m.g. patterns the following procedure, described in Landmesser & O'Donovan (1984), was used. Briefly, the time following the synchronous discharge that began a burst was divided into 40 ms intervals, and a series of bursts from a given muscle was aligned at the synchronous discharge. Histograms were then constructed which showed the proportion of cases in which a muscle was active during each interval (see for example Fig. 3). In assessing muscle activation patterns we excluded the first one or two bursts of a series; these tend to occur synchronously in most muscles and do not exhibit the proper phasing shown in subsequent bursts (see for example Fig. 2*B*). The e.m.g. recording sessions often lasted for 5 h or more, and the activity patterns from a given muscle were quite similar throughout, the isolated cord preparation remaining viable for long periods.

Anatomical analysis of motoneurone pools

In order to determine the location of motoneurones innervating a particular muscle, and thereby infer their original identity (Landmesser, 1978), we labelled them retrogradely with horseradish peroxidase (HRP). After we had recorded from a series of muscles in the experimental limb we would choose one to be injected with 10% HRP. After five additional hours at 32 °C the preparation was removed and the cord and limbs fixed in 2% glutaraldehyde. The cords were processed, reacted for HRP and sectioned according to previously published procedures (Landmesser, 1978).

The HRP staining procedure worked satisfactorily in twelve embryos. In these the labelled motoneurones on the experimental and control sides were counted in serial sections at a total magnification of × 630. Histograms showing the number of labelled cells for each 30 μm increment of cord, allowed localization of the pool along the a–p axis (see for example Fig. 6). To localize a pool in the transverse plane, camera lucida drawings were made for a series of sections at a given level. The sections were aligned by the central canal and the outer boundaries of the white matter, and a dot was made at the position of each stained cell. Every section of the experimental pool and every other section of the control pool was thus analysed (see for example Fig. 6).

Total motoneurone counts

To determine the effect of our experimental manipulation on motoneurone cell death, in six embryos we compared the total number of motoneurones per section in the lateral motor column on the experimental and control sides, for those segments that contributed innervation to the experimental limb. Since the experimental limb was often smaller than the control limb, we carried out this analysis only for embryos where the experimental limb was completely normal in form and more or less equivalent in size to the control limb.

Counts were made at × 630, and all large cell profiles which contained a nucleus and were located in the lateral motor column were counted. Nuclear diameters were measured on experimental and control sides and were found not to differ ($P \leqslant 0{\cdot}001$). Since comparisons were only made within a single embryo, no corrections for double counting were made.

Histology of limbs

In many cases (in all cases where motoneurone pools were determined by retrograde HRP labelling) the limbs were sectioned transversely to confirm the identity of muscles, especially those that had been injected with HRP. In some of the later experiments the limbs were frozen, sectioned on a freezing microtome and reacted for myosin ATPase to identify fast and slow myotubes. The results of this procedure will be reported in a subsequent paper.

RESULTS

E.m.g. activation patterns of muscles innervated by foreign motoneurones

While we did not quantify the *in ovo* behaviour of experimental limbs, it was clear that they showed movement during the periods of spontaneous activity that chick embryos exhibit (Hamburger & Balaban, 1963; Oppenheim, 1973; Bekoff, 1976; Landmesser & O'Donovan, 1984). The experimental embryos were usually checked daily and in all cases where the embryos were active the experimental limb was observed through the window in the shell to move vigorously. Also when the embryos were killed the experimental limb was observed to move with approximately the same frequency as the control limb.

To quantify better the behaviour of individual muscles, e.m.g. recordings were made in isolated cord/hind limb preparations from various muscles in the experimental limb. Muscles were classified as flexor- or extensor-like primarily according to whether they exhibited a prolonged silent period following the synchronous discharge that normally begins an e.m.g. burst in the isolated cord preparation. (While this must be confirmed with intracellular recordings, we are assuming that the silent period represents inhibition.) Extensors show only a very brief period of inhibition; for example the burst in the two muscles shown in Fig. 2*A* begins almost immediately after the synchronous discharge (indicated by arrows). Flexors on the other hand (for example the control sartorius muscle in Fig. 2*B*), show a period of inhibition lasting approximately 200–500 ms, before they exhibit burst activity. (For additional details of this scheme of classification see Landmesser & O'Donovan, 1984.)

We also always compared the e.m.g. activation pattern of an experimental muscle with the patterns from a control muscle in the contralateral limb. In the isolated cord preparation homonymous stage 35–36 muscles in both limbs are activated in tight synchrony (Cooper, 1983). Therefore it was useful to compare the behaviour of an experimental muscle with the behaviour of its homologous muscle, or with another flexor or extensor muscle in the opposite limb.

The e.m.g. records shown in Fig. 2 indicate our general findings. In the majority of cases (38/49) muscles showed the same class of behaviour as they normally would have. For example extensors exhibited extensor behaviour and flexors flexor behaviour. The experimental caudilioflexorius (Fig. 2*A*), an extensor, is activated very similarly to the contralateral posterior iliotibialis, another extensor. Specifically neither muscle exhibits prolonged inhibition following the synchronous discharge (arrow). However, in a minority of cases (7/49) muscles exhibited an e.m.g. pattern very different from their normal behaviour. It is obvious that the experimental sartorius in Fig. 2*B* is activated out of phase with the control sartorius and fails to show the long period of inhibition following the synchronous discharge. In this case

the synchronous discharge at the onset of the first two bursts is indicated by arrows as it is not clearly visible on this time base.

The iliofibularis muscle is difficult to classify because during walking behaviour in hatched chicks it exhibits both flexor and extensor bursts (Jacobson & Hollyday, 1982). We have also observed flexor and extensor activity from this muscle during

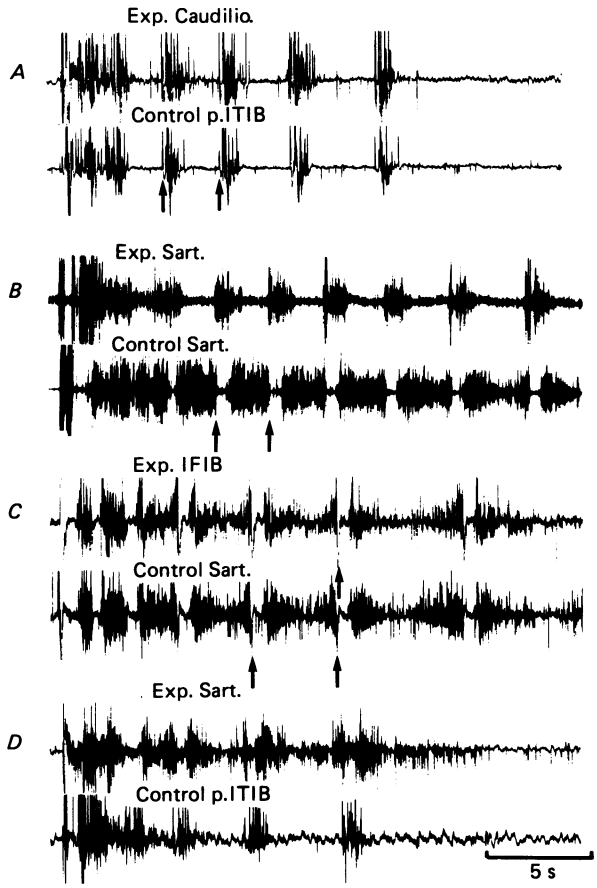

Fig. 2. E.m.g. records from pairs of muscles in experimental embryos. In most cases an experimental (Exp.) muscle innervated by foreign motoneurones is paired with a control muscle from the unoperated limb. All sequences of bursts were elicited by a single stimulus to the thoracic cord, at the onset of the record. Time of occurrence of some of the synchronous discharges (described in more detail in the text) is indicated by arrows. Amplitude of e.m.g. responses was approximately 1–3 mV. Caudilio., caudilioflexorius; IFIB, iliofibularis; p. ITIB, posterior iliotibialis; Sart., sartorius.

in ovo recordings from stage 35–36 embryos (Landmesser & O'Donovan, 1984). However, in the isolated cord preparation the iliofibularis muscle demonstrates a highly characteristic activation pattern that differs from the *in ovo* behaviour; usually there is only a pronounced synchronous discharge at the start of each cycle, with little or no subsequent activity. We recorded from ten iliofibularis muscles and in all cases found that the activation pattern was different from its normal behaviour

in the isolated cord. We observed extensor activity in two cases and flexor activity in six cases. An example of the latter behaviour is shown in Fig. 3*C* where it is paired with the contralateral sartorius. It should be noted that the behaviour of this iliofibularis closely parallels the activity of the sartorius muscle in all respects, even in the amplitude modulation that occurs during the burst. Since the sartorius is the only muscle in the thigh that shows this activation pattern (Landmesser & O'Donovan, 1984), one might reasonably infer that in this case the iliofibularis had been innervated by sartorius motoneurones which maintained their original activation pattern. The sciatic nerve which innervates the iliofibularis was formed in this case by the first three lumbosacral segments, which contain the sartorius motoneurone pool.

Finally, while the e.m.g. activation pattern of some experimental muscles was somewhat more ragged than that of the controls, we only rarely (3/49 cases) observed obvious mixed flexor and extensor behaviour in a muscle. An example is shown in Fig. 2*D*, where the experimental sartorius muscle exhibits prolonged bursting similar to normal but without the silent period characteristic of flexors at the initiation of the burst. This period, which is usually silent in flexors (compare with Fig. 2*B* and *C*), is filled in with low-level activity.

Although the basic activation pattern of a given muscle could be characterized from inspection of records such as that shown in Fig. 2, to quantify the results better we constructed histograms showing the probability of various muscles being active at different times following the synchronous discharge. (For additional details see Methods and Landmesser & O'Donovan, 1984.)

Confirming what is apparent from the e.m.g. records shown in Fig. 2*A*, the histograms in Fig. 3*A* show that in this case the experimental caudilioflexorius behaves very similarly to the control posterior iliotibialis; both muscles begin their activity at the occurrence of the synchronous discharge, show only a brief period of partial inhibition, and have similar burst durations. In contrast, the activation pattern of the sartorius muscle of this same embryo was opposite to the normal sartorius pattern, and was in fact quite similar to the example from another embryo shown in Fig. 2*B*. As the histograms of Fig. 3*B* show, its activation differed markedly from normal sartorius behaviour, there being no build-up of activity preceding the synchronous discharge, no period of prolonged inhibition, and no activity at later times in the cycle when the control sartorius was active. Thus foreign-innervated muscles could show behaviour similar to, or quite different from, their normal behaviour.

Fig. 3*C* and *D* are taken from another embryo and illustrate several points. In this case both the experimental iliofibularis and experimental sartorius were activated in a similar fashion and may in fact have been innervated by the same motoneurone pool. In both cases, however, the activation pattern differed from normal; for the iliofibularis, markedly so, for the sartorius only subtly so. Nonetheless the briefer burst duration as well as the shorter period of inhibition allowed the experimental sartorius pattern to be distinguished from the normal sartorius response, even though both of these patterns would be classified as flexor-like.

Another point, illustrated by the control sartorius records in Fig. 3*B* and *D*, is that the activation patterns of muscles in the contralateral limb were not affected by our

experimental manipulations. These control sartorius histograms were strikingly similar to ones obtained from unoperated embryos of the same stage (Landmesser & O'Donovan, 1984). Similar results were found for a number of other contralateral muscles. We also observed that when muscles in the experimental limb happened to become innervated by their own motoneurone pools their activation patterns did not differ from normal.

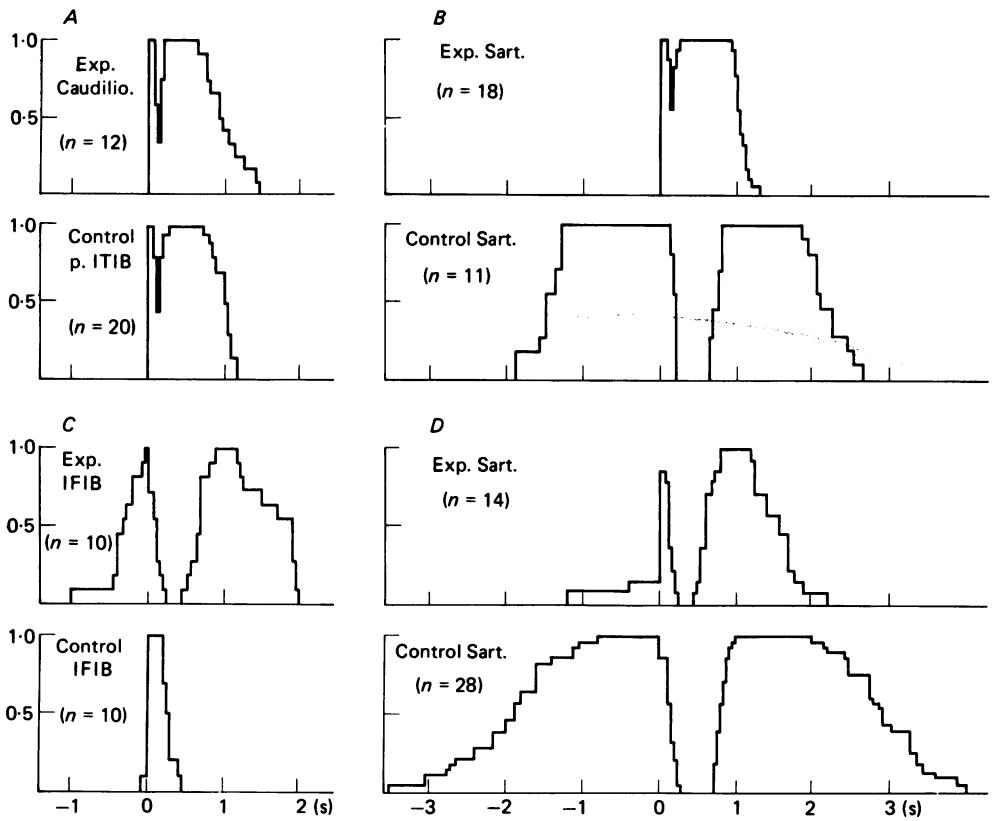

Fig. 3. Histograms showing the temporal relationships of the bursts in experimental (Exp.) and control muscles. The ordinate indicates the probability of a given muscle being active at various times preceding or following the synchronous discharge (which is time 0). The number of bursts contributing to each histogram (n) is given in parentheses. Abbreviations as in Fig. 2. (See text for further explanation.)

Finally a given muscle could not only exhibit extensor- or flexor-like activation patterns in different embryos, but could be activated with distinctive patterns within this classification. For example, the iliofibularis muscles of Fig. 3*C* and Fig. 4 both exhibit flexor patterns. The latter has prolonged bursts characteristic of the sartorius but a somewhat shorter silent period characteristic of the anterior iliotibialis. This muscle was later found to be innervated by both species of motoneurones (see Fig. 6*A*). From its activation pattern we would conclude that the iliofibularis muscle shown in Fig. 3*C* was innervated by non-sartorius flexor motoneurones, probably

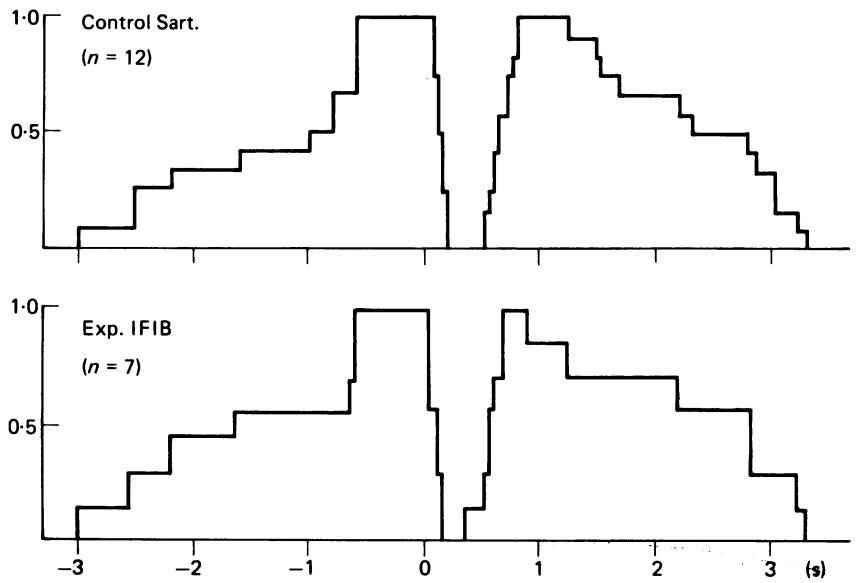

Fig. 4. Activity histograms of an experimental iliofibularis muscle (Exp. IFIB) paired with a control sartorius (Control Sart.) muscle. The experimental muscle was later shown to be innervated by sartorius motoneurones. Other details as in Fig. 3.

TABLE 1. E.m.g. activation patterns of inappropriately innervated muscles*

Muscle	Flexor-like	Extensor-like	Mixed
Adductor (extensor)	0	3	0
Accessory (extensor)	0	5	0
Ischioflexorius (extensor)	0	2	0
Caudilioflexorius (extensor)	1	6	0
Sartorius (flexor)	7	5	1
Femorotibialis (medial) (extensor)	0	3	0
Iliofibularis (flexor–extensor)	6	2	2
Posterior iliotibialis (extensor)	1	5	0

* Muscles in the first group are derived from the embryonic ventral muscle mass, those in the second from the dorsal muscle mass.

belonging to the anterior iliotibialis pool. However, since we did not label the pool in this case its identity could not be determined with certainty.

Table 1 summarizes our results for the forty-nine muscles from which we obtained satisfactory recordings. It also divides muscles into those derived from either the dorsal or ventral embryonic muscle mass (Romer, 1927). It is noticeable that almost all of the muscles which showed the opposite class of behaviour from normal were derived from the dorsal muscle mass. Only one out of the seventeen ventrally derived muscles was so activated. One simple explanation for this may be that all of the ventral muscles studied were extensors, and it has been shown that following a variety of manipulations (Lance-Jones & Landmesser, 1981; Hollyday, 1981; Ferguson, 1981) motoneurones that would normally innervate ventral muscles will preferentially innervate other ventral muscles.

In contrast, the dorsally derived sartorius, a flexor, showed extensor-like behaviour in 5/13 cases. It is important to point out that even in the seven cases in which flexor behaviour was observed, in only one case was it clearly sartorius-like. The other six muscles exhibited flexor behaviour that differed from normal sartorius behaviour in one or more respects: usually the inhibitory period was shorter than normal, and often the bursts were quite short as well. Similarly, although the iliofibularis normally exhibits a short flexor burst in the hatched chick (Jacobson & Hollyday, 1982), in all six cases the experimental flexor behaviour differed from this and was often indistinguishable from either sartorius or anterior iliotibialis behaviour.

Finally, muscles that clearly exhibited both extensor and flexor behaviour were rare. Even considering only the dorsally derived muscles we observed such behaviour in only three out of thirty-two muscles. Extensor motoneurones quantitatively predominate over flexors in the chick cord. This can be roughly computed from the number of motoneurones in each pool (Landmesser, 1978) and the physiological classification of that pool (Jacobson & Hollyday, 1982). However, in segments 1 to 3, which formed the sciatic plexus in many of our experimental embryos, flexor and extensor motoneurones occur in relatively equal numbers. Had motoneurones randomly innervated muscles, one might have expected the adjacent iliofibularis and posterior iliotibialis muscles to be innervated by a combination of flexor and extensor motoneurones in most cases. Yet this was rarely found (2/14 cases).

Furthermore, motoneurones did not have a strong tendency to innervate muscles of appropriate type. For example the sartorius, a flexor, was innervated by extensor motoneurones in five cases and by flexor motoneurones in seven cases. However, extensor and flexor motoneurones did have a tendency not to innervate the same muscle, as reflected by the sartorius receiving such mixed input in only one out of thirteen cases. In fact in only two muscles showing mixed behaviour did we record extensor and flexor activation patterns at a single recording site. In the other, an iliofibularis muscle, whose responses are shown in Fig. 5, we observed extensor behaviour when we recorded from the lateral surface of the muscle, whereas the medial surface was activated in an entirely different flexor-like manner. This part of the muscle did not behave exactly like the contralateral sartorius, but had a shorter inhibitory period like the anterior iliotibialis (see Landmesser & O'Donovan, 1984). This was the only case in which different parts of a muscle had different activation patterns. While we did not sample the entire surface of an experimental muscle, usually in the course of a recording session we would record from several different sites, and the activation patterns from the different sites were never found to vary significantly.

In summary, the main finding is that when motoneurones innervated a foreign muscle, their activation pattern was not altered to one appropriate for that muscle. If one restricts the analysis to muscles derived from the dorsal muscle mass, which include both flexors and extensors, only six out of thirty-two muscles exhibited an opposite class of behaviour (i.e. flexor rather than extensor) from normal. However, fully twenty-two out of thirty-two exhibited an activation pattern that could be distinguished from normal.

Anatomical characterization of motoneurone pools projecting to inappropriate muscles

We characterized motoneurone pools projecting to foreign muscles for several reasons. First, while the physiological results showed that the activation pattern of motoneurones was not altered to match the muscle they had innervated, we could not assess, without knowing their identity, whether the motoneurones had developed the activation pattern they normally would have had. Secondly, it was possible that

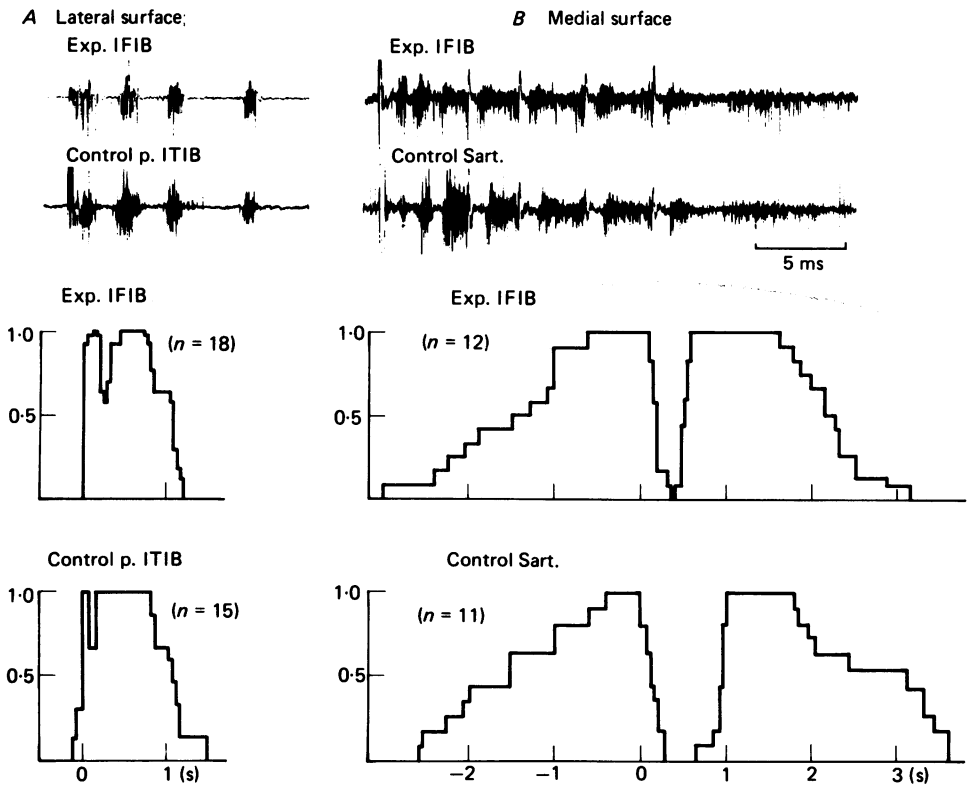

Fig. 5. E.m.g. activation patterns of the two surfaces of an experimental iliofibularis muscle. The lateral surface was in this case activated with an opposite (extensor) pattern from the medial surface (flexor). Details as in Fig. 3.

the reason we had failed to observe mixed flexor–extensor behaviour within a muscle was that when both flexors and extensors projected to a muscle, the activation of one or the other tended to be suppressed. We therefore determined the anatomical location within the cord of the motoneurones innervating twelve foreign muscles whose behaviour we had previously characterized. Table 2 summarizes the results.

Since the motoneurone pools projecting to the chick thigh muscles had been previously characterized (Landmesser, 1978; Hollyday, 1980) we could identify motoneurones by their position in the cord. However, it should be pointed out that the unambiguous assignment of a labelled motoneurone to a single pool was not always possible, especially in cases where a labelled cell was located along the border

of a motoneurone pool, or in a region where two pools normally show overlap (see Landmesser, 1978). When a muscle clearly received innervation from a motoneurone pool the name of that pool is shown in the right-hand column of Table 2. Motoneurone pools that possibly also contributed to the innervation are shown in square brackets. The physiological behaviour of the muscle, and of the pools innervating it, is also noted.

TABLE 2. Activation patterns of experimental muscles and pools innervating them

Embryo no.	Experimental muscle (original behaviour)	Activation pattern of experimental muscle	Pool(s) contributing to innervation of experimental muscle
226	IFIB (synchronous discharge)	Extensor	Add. (ext.)
275	p. ITIB (extensor)	Extensor	Femoro. (ext.) [Add. (ext.)]
283	p. ITIB (extensor)	Extensor	Add. (ext.)
296	p. ITIB (extensor)	Extensor	Femoro. (ext.)
321	Sart. (flexor)	Extensor	p. ITIB (ext.)
293	Sart. (flexor)	Extensor	Caudilio. (ext.) Shank (flex. + ext.)
300	Sart. (flexor)	Extensor	Caudilio. (ext.) Shank (ext. + flex.)
308	Sart. (flexor)	Flexor	Int. Femoro. (flex.) Shank (flex. + ext.)
335	IFIB (synchronous discharge)	Extensor (lateral surface) and flexor (medial surface)	a. ITIB (flex.) [Sart. (flex.)] Femoro. (ext.) } 10 % p. ITIB (ext.) }
238	IFIB (synchronous discharge)	Flexor	Sart. (flex.) a. ITIB (flex.) Femoro. (ext.) 10 %
327	p. ITIB (extensor)	Extensor	Femoro. (ext.) Sart. (flex.) a. ITIB (flex.) } 43 %
338	IFIB (synchronous discharge)	Flexor	Sart. (flex.) [a. ITIB (flex.)] Femoro, (ext.) 27 %

In all cases (12/12) the identity of the motoneurones was consistent with the behaviour recorded from the muscle. Thus, no muscle which exhibited flexor behaviour was unambiguously innervated by predominantly extensor motoneurones. We did, however, find some cases (4) where although the majority of labelled neurones was consistent with the behaviour of a muscle, a smaller fraction belonged to a pool that normally exhibits contrasting behaviour.

An example of this is shown in Fig. 6*A*. Approximately 90 % of the motoneurones innervating this iliofibularis were found in lumbosacral (LS) segments 1 and 2, and from the camera lucida reconstructions were located in the sartorius and/or anterior iliotibialis pools. This muscle exhibited flexor behaviour characteristic of these pools. However, 10 % of the cells, those located in LS3, appeared to be in the medial

femorotibialis and/or posterior iliotibialis pools, which are normally both extensors. It is possible that this small fraction of the pool simply did not contribute significantly to the e.m.g. we recorded, or that there was some partitioning of inputs within a muscle. We consider it less likely that these femorotibialis/posterior iliotibialis motoneurones had had their activity suppressed or altered, be we cannot exclude the possibility at this time.

Another example is shown in Fig. 6*B*. In this embryo the experimental iliofibularis was activated in a manner indistinguishable from the sartorius (see responses in Fig. 2*C*). The labelled cells were predominantly located in LS1 and LS2, and at this level were clearly within the sartorius pool, which is consistent with the muscles' physiological behaviour. However 50/186 labelled cells (those located in LS3) could have been femorotibialis or posterior iliotibialis motoneurones. In either case had these extensor motoneurones contributed extensively to our e.m.g. recording we would have expected a shorter inhibitory period than we observed.

Finally, while the experimental iliofibularis shown in Fig. 5 was innervated by both flexor and extensor motoneurones, these were partitioned to different sides of the muscle (see Fig. 7*A*). The majority of the cells (90%) were located in LS2 and, as seen from the camera lucida reconstruction, most were more lateral than sartorius motoneurones are at this level (Landmesser, 1978). They were clearly in the anterior iliotibialis motoneurone pool, which is consistent with the shorter inhibitory period shown by this side of the muscle when compared with the control sartorius (see Fig. 5*B*). The labelled cells in LS3 were found in the femorotibialis motoneurone pool, and their activity histogram (shown in Fig. 5*A*) is quite like that of the normal lateral femorotibialis (see Landmesser & O'Donovan, 1984).

We have already presented evidence that the activation pattern of a motoneurone pool is not altered to match the muscle it innervates. However, to determine whether it develops its normal pattern of activation in these circumstances it was necessary to find cases in which a single foreign pool innervated the experimental muscle. In one previously illustrated example (Fig. 3*A* and *B*) the activity patterns for the experimental sartorius and control posterior iliotibialis were shown to be very similar. The experimental pool was localized in LS segments 4 and 5 in the position of the control posterior iliotibialis pool (Fig. 7*B*). Camera lucida reconstructions were made for the experimental sartorius and the contralateral posterior iliotibialis from the beginning of LS4 to mid-LS5. It can be seen that with the exception of one cell the labelled cells projecting to the sartorius are located in the position of the posterior iliotibialis pool.

From these observations, as well as the numerous examples of 'clean' sartorius or anterior iliotibialis behaviour patterns recorded from foreign muscles, it seems reasonable to conclude that a motoneurone pool can develop its characteristic e.m.g. activation pattern even when it innervates a foreign muscle.

Total number of motoneurones in lumbosacral segments projecting to foreign limb regions

From the results presented so far, it is possible to conclude that motoneurones do not inevitably die when they synapse with a foreign muscle, whether this belongs to their own or to the opposite functional class. To determine whether there might be more subtle effects on the extent of cell death, and especially to determine whether

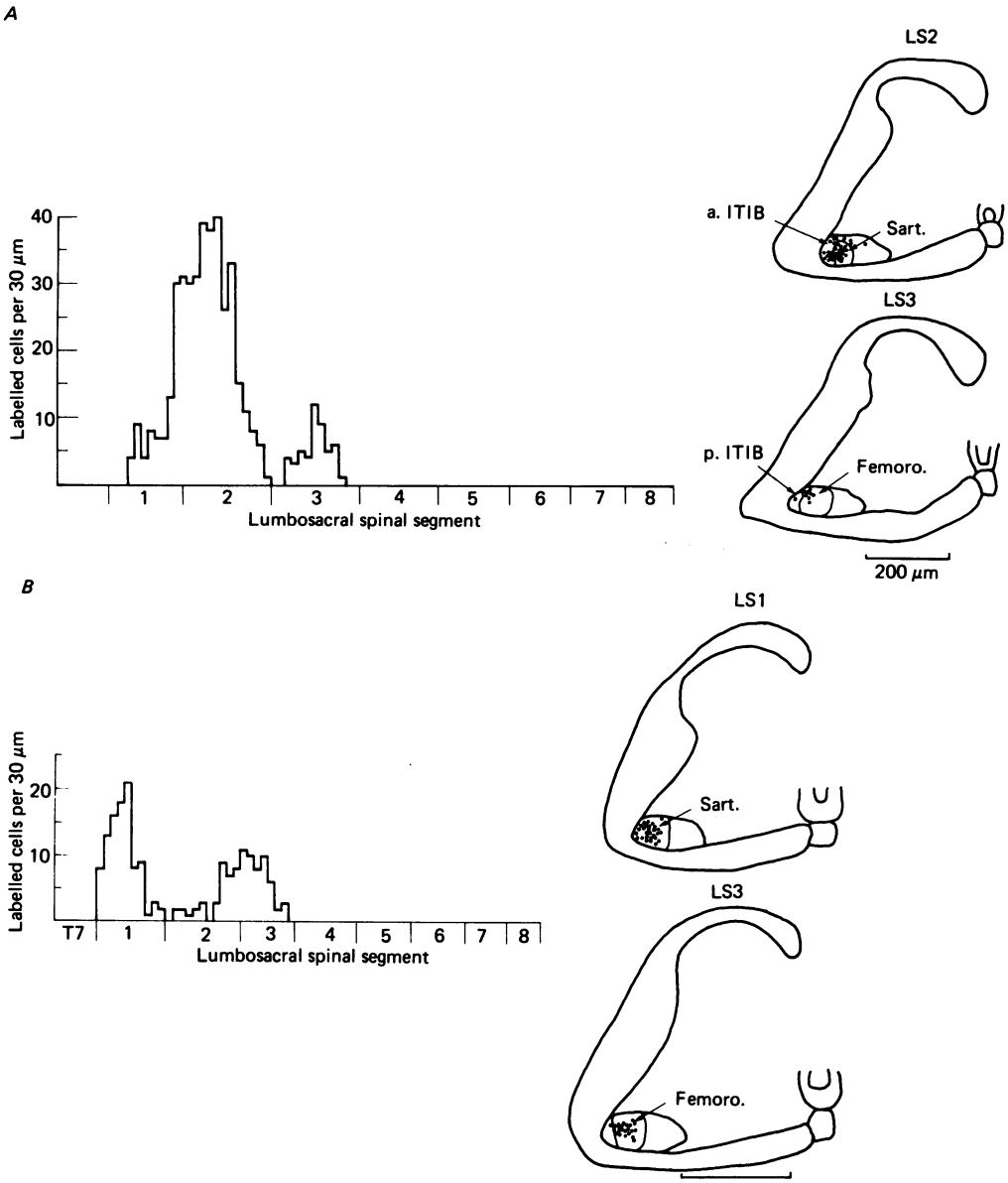

Fig. 6. Anatomical localization of motoneurones projecting to two experimental iliofibularis muscles. The histograms at the left show the total numbers of labelled cells per each 30 μm along the a–p axis of the lumbosacral cord following retrograde labelling with HRP. The total number of cells contributing to the histograms was 440 in *A* and 186 in *B*. The location of labelled motoneurones in the transverse plane for selected segments is shown at the right. Each dot represents one labelled motoneurone. The entire lateral motor column is enclosed by a heavy line. This has been subdivided into separate motoneurone pools and these are labelled with arrows. The calibration bar in both *A* and *B* represents 200 μm. The normal iliofibularis pool extends from LS4 to LS7. Therefore all labelled cells in both experimental muscles are foreign. a. ITIB and p. ITIB, anterior and posterior iliotibialis; Femoro., femorotibialis; Sart., sartorius.

our experimental procedures might have prevented cell death, we counted the total number of motoneurones in segments projecting to foreign limb regions in six stage 36–41 experimental embryos.

As shown in Table 3 for LS343 the total number of lumbosacral motoneurones did not differ between the experimental and control side. Similar results were found for

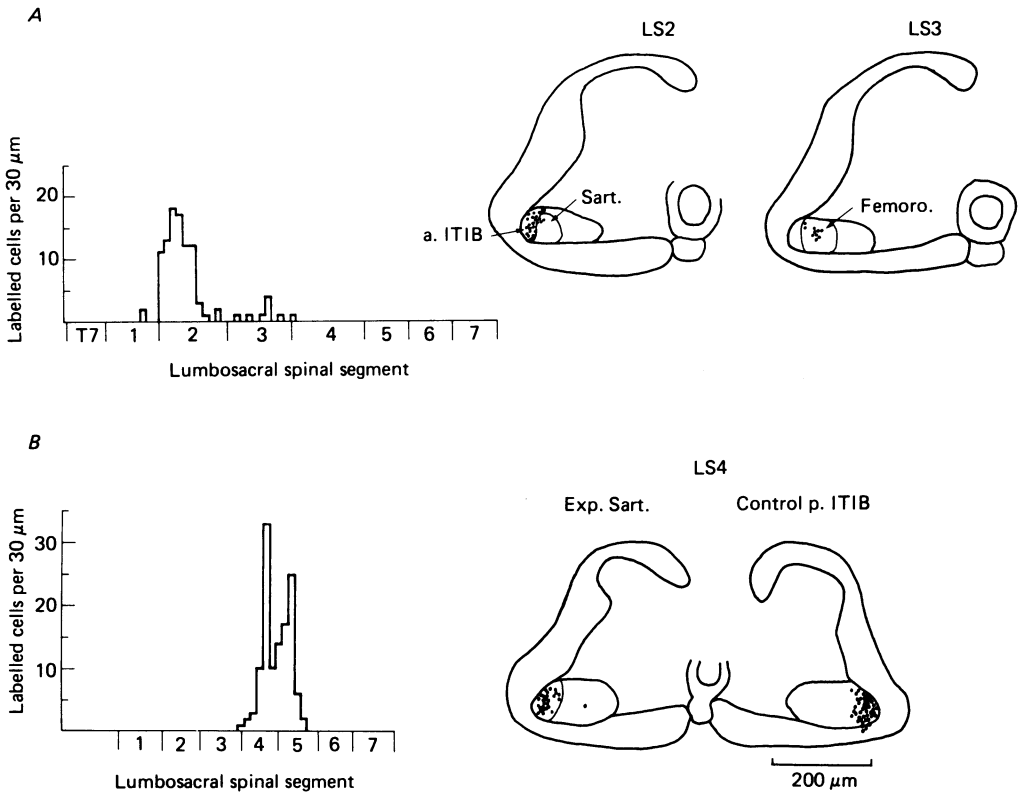

Fig. 7. Location of motoneurones projecting to experimental iliofibularis (*A*) and experimental sartorius (*B*) muscles. Location of HRP-labelled cells along the a–p axis is shown in the histograms at the left. The total number of cells contributing to the histograms was 100 in *A* and 121 in *B*. Their location in the transverse plane is shown at right. The e.m.g. pattern of the iliofibularis muscle (*A*) can be seen in Fig. 5, that of the sartorius muscle (*B*) in Fig. 3*B*. All labelled cells in *A* and *B* were foreign, as the normal iliofibularis pool is located in LS4 to LS7, the sartorius pool in LS1 to LS2. In *B*, the location of cells projecting to the control posterior iliotibialis muscle located in the contralateral unoperated limb is also shown. The calibration bar applies to both *A* and *B*. Abbreviations as in Fig. 6. (See text for further details.)

three additional embryos at stages 35, 36 and 38 (not shown in Table). These control counts were similar to those reported at comparable stages by Hamburger (1975) and by Oppenheim, Chu-Wang & Maderdrut (1978). In one embryo (LS335) the total number of motoneurones on the experimental side was somewhat less than the control, and in one other embryo (LS349) appreciably higher in some segments.

We do not yet understand the reason for this variation. However, it is clear that

motoneurone cell death was not as a rule prevented on the experimental side, and in some cases occurred to exactly the same extent as on the control side. On the other hand, there was no tendency for motoneurone cell death to be enhanced by motoneurones innervating foreign muscles, at least up to stage 41. This was well after the normal cell death period (stages 30–36).

TABLE 3. Total number of motoneurones per section (mean ± S.D.)

Embryo (stage)	Lumbosacral spinal segment 1	2	3	4	5
LS335 (stage 38)					
Exp.	26·2 ± 8·2	55·4 ± 11·9	44·7 ± 12·4	—	—
Control	38·1 ± 8·2	64·2 ± 11·8	63·7 ± 9·1	—	—
LS343 (stage 40)					
Exp.	39·4 ± 4·0	46·8 ± 3·9	48·8 ± 3·7	32·8 ± 4·3	—
Control	39·2 ± 5·0	50·6 ± 6·1	45·8 ± 3·3	46·4 ± 6·1	—
LS349 (stage 41)					
Exp.	46·6 ± 6·9	56·4 ± 7·8	72 ± 9·9	68·4 ± 5·0	47·0 ± 6·6
Control	16·2 ± 2·8	34·6 ± 4·5	53·8 ± 5·9	51·6 ± 4·0	48·4 ± 3·9

The number of motoneurones innervating individual muscles within the experimental limb was usually less than normal (Landmesser, 1978). This may have resulted because in many cases the number of spinal segments contributing to the limb was less than normal. In addition, the segments innervating a given region often contained fewer motoneurones than the segments which would normally innervate that region. For example, LS1–3 which contains approximately 2500 motoneurones often formed the sciatic plexus which would normally be formed by approximately 6000 motoneurones. We did not, however, observe any consistent trend, such as significantly lower counts for motoneurone pools projecting to muscles of opposite function.

DISCUSSION

These results indicate that wrongly projecting chick lumbosacral motoneurones are activated during embryonic movements, and further that their activation pattern is not altered to correspond to the foreign muscle innervated. We can therefore exclude several explanations for the retention of inappropriate peripheral synapses in this system.

The first explanation we can reject is that we have prevented normal motoneuronal cell death by blocking neuromuscular activity. Oppenheim and his co-workers have shown that paralysis of chick embryos with neuromuscular blocking agents can delay (Pittman & Oppenheim, 1979) and possibly even prevent (Oppenheim, 1982) motoneurone death. Such cell death has been proposed to be at least one of the mechanisms by which peripheral errors in connectivity are corrected (Lamb, 1976; Pettigrew, Lindeman & Bennett, 1979).

However, we have found that wrongly projecting motoneurones are activated

during spontaneous embryonic activity. Furthermore in the isolated cord preparation, motoneurones were activated in bursts of approximately normal duration and intensity. Minor changes cannot be excluded, but alterations in activity levels comparable to those which prevent motoneurone cell death (Pittman & Oppenheim, 1979) were not found. In addition, total motoneurone counts from limb-reversed embryos in which virtually all motoneurones were projecting wrongly did not consistently differ from normal. While we cannot rule out some selective cell death within individual motoneurone pools, death of most wrongly projecting neurones was not prevented.

The possibility that the central connexions of wrongly projecting motoneurones had been altered to result in behaviourally appropriate activation patterns, and thereby the retention of such peripheral projection 'errors', can also be excluded. In many cases muscles were innervated by foreign motoneurones which produced inappropriate activation patterns. Such motoneurones were also retained throughout the normal cell death period (stages 30–36: Hamburger, 1975; Oppenheim *et al.* 1978). Since we did not assess individual muscles later than stage 38, it is possible that such errors might ultimately be corrected. Total motoneurone counts indicate that most wrongly projecting motoneurones survive as late as stage 41. Nonetheless, it is possible that when these spinal circuits begin to be used for purposeful behaviour such as hatching or locomotion, such errors might be corrected by some mechanism involving, or independent of, motoneurone cell death.

What is clear is that formation of an incorrect peripheral synapse by itself does not result in the death of a motoneurone, a result supported by a variety of other studies (Lance-Jones & Landmesser, 1981; Hollyday, 1981; Summerbell & Stirling, 1981). Since we have now ruled out several other reasons for the retention of such incorrect synapses, it seems reasonable to conclude that a simple recognition process between motoneurones and muscle does not play a dominant role in correcting errors should they occur. More complex mechanisms involving interactions between motoneurones would seem to be required.

The second main conclusion to be drawn from the results is that the basic spinal circuitry responsible for activating motoneurones as flexors or extensors is not influenced by the muscle innervated, as was proposed by Weiss (1937). Motoneurones were shown to develop an activation pattern consistent with their original identity even when projecting from the outset to foreign and in many cases antagonistic muscles.

Without intracellular recordings, we cannot exclude some alterations in the central connexions of wrongly projecting motoneurones. However, the method we have used provides a good measure of how motoneurones are actually activated and therefore a reasonable estimate of certain interneuronal inputs to them. It should be stressed that the inputs we are assessing in the isolated cord preparation are local circuits of interneurones (Cooper, 1983; Landmesser & O'Donovan, 1984). Such circuits are considered to be of prime importance in generating the basic pattern of motoneurone activation during locomotion (Grillner & Zangger, 1979). It is, however, possible that other inputs such as afferent or descending ones may be altered. Tsukahara & Fujito (1976) have in fact reported changes in cortical inputs to cat rubrospinal interneurones following muscle nerve cross-union. Morris (1976) also observed that wrongly

innervated muscles in chick supernumerary limbs were activated appropriately during cutaneous reflexes. It will therefore be important to test other inputs to such motoneurones, as these might be involved in any subsequent compensation the chick is able to make for such behaviourally inappropriate lumbosacral cord circuits (see also Cohen, 1978).

Finally, the rareness with which we observed mixed flexor and extensor inputs projecting to the same muscle deserves comment. As detailed in Results, it seems unlikely that this could occur simply by the chance parcelling out of axons into different muscle nerves during development. It is possible that axons make some selective pathway choices based on appropriate function, as proposed by Hollyday (1981), although other studies show that this does not invariably occur (Lance-Jones & Landmesser, 1981). Alternatively flexor and extensor motoneurones may have a tendency not to innervate the same muscle or muscle region, due to some form of interaction between motoneurones.

We did observe one case where foreign flexor and extensor motoneurones distributed to different parts of a muscle (the iliofibularis muscle: Fig. 5). One explanation for the partitioning of motoneurones exhibiting different activation patterns to different regions of a muscle could be the distribution of fast and slow muscle fibres within these regions. Myosin ATPase staining characteristics (McLennan, 1983; M. Vogel, L. Landmesser & M. J. O'Donovan, unpublished observations) have shown that the anterior-medial part of the stage 36 iliofibularis contains mostly slow fibres, the posterior-lateral surface mostly fast fibres. If there were some selectivity in the establishment of synapses between, for example, slow motoneurones and slow muscle fibres, then a motoneurone pool containing predominantly slow motoneurones might be restricted to the slow portion of the muscle.

It is also possible that this partitioning of inputs within a muscle could be achieved by some interaction between motoneurones. For example motoneurone terminals that are activated in a temporally similar way may tend to co-distribute. A similar mechanism has been suggested for the competitive rearrangement of synaptic terminals in other systems (Purves & Lichtman, 1980).

Another possibility which we do not favour, but at this time cannot exclude, is that when flexor and extensor motoneurones project to the same muscle region one input, perhaps the minority one, is silenced or altered to conform to the majority input. This possibility is consistent with our failure in some cases to detect physiologically inputs which were later found by anatomical techniques to be projecting to the muscle.

We cannot completely exclude a more trivial explanation for these observations: leakage of HRP into adjacent muscles. Yet this is not likely to be an adequate explanation. First, in all cases where muscles were injected with HRP, limbs were sectioned and in only a few cases was leakage apparent. These were not included in the analysis. Further, in several hundred HRP-injected muscles used in previous studies by this laboratory, HRP leakage to adjacent muscles was only rarely observed (Landmesser, 1978; Lance-Jones & Landmesser, 1981). When it did occur, it was easily detected because the motoneurones stained by leakage to adjacent muscles were always less intensely labelled.

It should be noted that discrepancies between physiological and anatomical

characterization of motoneurone pools were not common (2/12 cases) and we do not wish to over-emphasize their importance. However, since they may indicate interesting developmental phenomena, the reason for their occurrence should be resolved; whether this is due to inputs being functionally altered or silenced, or to partitioning of inputs within a single muscle. These possibilities are currently being tested in muscles innervated by foreign motoneurones following large cord reversals. Anatomical evidence (Lance-Jones & Landmesser, 1981) indicates that these muscles are innervated by widely distributed motoneurones belonging to both flexor and extensor pools, and should therefore provide an optimum preparation for testing these ideas.

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