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THE DISTRIBUTION OF MOTONEURONES SUPPLYING CHICK HIND LIMB MUSCLES

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SUMMARY

1. The motor nuclei supplying many of the hind limb muscles were localized in late chick embryos (stage 36–37; 10–11 days) by utilizing the technique of retrograde transport of horseradish peroxidase.

2. Each nucleus was found to be localized in a characteristic position in both the rostro-caudal and transverse plane of the spinal cord with only slight individual variation.

3. Each motor nucleus consisted of an elongate, coherent cluster of labelled cells, with few cells occurring outside the cluster. Thus, there did not appear to be extensive overlap of nuclei nor extensive intermingling of motoneurones projecting to different muscles.

4. The position of a motor nucleus in the transverse plane was not correlated with whether its muscle was used as an extensor or flexor; nor were adjacent nuclei necessarily co-activated during normal unrestrained walking movements as deduced from e.m.g. recordings. The position of a motor nucleus also was not correlated in a topographical manner with the adult position in the limb of the muscle to which it projected.

5. Further, while no correlation was found between the rostrocaudal position of a motor nucleus and the embryonic muscle mass from which its muscle was derived, such a relationship existed for the medio-lateral position; all muscles arising from the dorsal muscle mass, regardless of their function or adult position, were innervated by laterally situated motoneurones, all muscles arising from the ventral muscle mass by medially situated motoneurones.

6. It is concluded that motoneurone position is most closely correlated with ontogenetic events presumably involved in the matching of the motoneurone with the correct muscle in the periphery. It can also be inferred that the central connexions onto motoneurones, responsible for their proper activation, cannot be achieved by a simple mechanism based largely on the position of the motoneurone soma.

INTRODUCTION

From the early studies of Sherrington (1892) it has been known that the motoneurones innervating individual cat hind limb muscles are restricted to certain segments of the lumbosacral spinal cord. Romanes (1951, 1964) extended these observations to show that the motoneurones for each muscle were grouped into an

elongate nucleus which was situated in a characteristic position in both the rostro-caudal and the transverse plane. A similar organization has been reported for the human (Sharrard, 1955) and frog (Cruce, 1974) spinal cord.

It has recently come to be appreciated that the spinal cord possesses considerable automatism (but see Brown, 1911); isolated from both descending and afferent input, it is capable of generating a pattern of muscle activation similar to that seen in normal locomotion (Grillner, 1975; Grillner & Zangger, 1975; Shik & Orlovsky, 1976; Szekely, Czéh & Voros, 1969). This implies that much of the neural circuitry underlying normal locomotion is intrinsic to the lumbosacral spinal cord. Thus it becomes more important to fully understand the organization of the lumbosacral cord, including that of the motor nuclei.

The observed arrangement of the motoneurones has sometimes been suggested to reflect their functional relationships. Motoneurones to muscles serving a similar function (flexion or extension) or moving the same joint would be grouped in the cord (Kuypers, 1964; Székely *et al.* 1969; Székely & Czéh, 1967; Bloomfield, 1974). This would greatly simplify the developmental problem of achieving proper central connectivity, since motoneurones with similar function and therefore connectivity would be spatially grouped. Motoneurone arrangement has also been suggested to reflect topology, the anatomical proximity of motoneurones being related to the proximity of their muscles in the limb (Romanes, 1951, 1964). Finally the observed organization has been related not to adult topology but to ontogenetic events (Hughes, 1965; Cruce, 1974). In other words, muscles with a similar embryonic origin would be innervated by motoneurones with a similar embryonic origin in space and/or time.

A previous electrophysiological study (Landmesser & Morris, 1975) showed that the rostro-caudal position of chicken motoneurones was generally similar to the position of mammalian motoneurones innervating homologous muscles (Romanes, 1964). Since birds represent another peak in vertebrate evolution, and since they may use some muscles in a different manner from their homologous counterparts in cats, a more comprehensive study of motoneurone organization seemed justified. The present study was therefore undertaken to determine the arrangement of the motor nuclei in late chick embryos by the retrograde transport of horseradish peroxidase and to relate it to (1) muscle function as deduced from e.m.g. recording, (2) adult anatomical position of muscles and (3) their embryonic origin. This study will also serve as a basis to assess the early development of motoneurone projection patterns as described in the following paper.

METHODS

General procedure

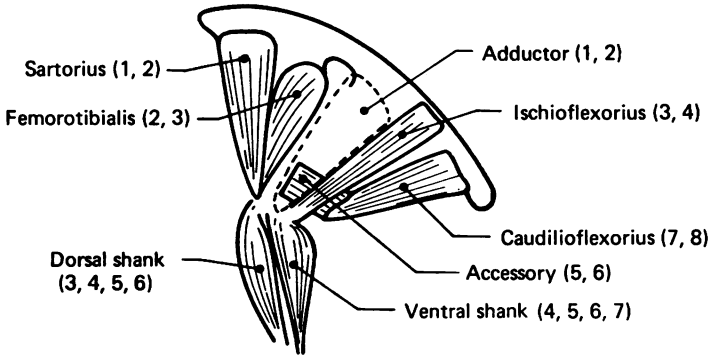
White Leghorn chick embryos were incubated in a forced draft incubator until stage 36–37 (10–11 days) as defined by Hamburger & Hamilton (1951). They were quickly decapitated, eviscerated and placed in a chamber containing oxygenated Tyrode solution at room temperature (20–22 °C); they were further dissected by removing all skin and a ventral laminectomy was performed from high thoracic through lumbosacral levels, to allow adequate oxygenation of the spinal cord. The spinal cord segments rostral to the last but one thoracic segment were removed to aid subsequent localization along the rostro-caudal axis.

All superficial muscles could be visualized by this procedure. However, in order to have access to some deeper muscles (iliofibularis, caudilioflexorius and accessory), overlying muscles had to be

removed; the ischioflexorius in the case of the accessory and caudilioflexorius and the ischioflexorius, caudilioflexorius and accessory in the case of the iliofibularis (see Text-fig. 1).

Injection of horseradish peroxidase

Horseradish peroxidase (HRP) (Sigma Type VI) was dissolved in Tyrode solution to make a 5% wt./vol. solution. This was pressure-injected through glass pipettes whose tip diameter varied between 50 and 100 μm depending on the muscle to be injected. HRP was injected at various positions into the muscle body until the whole muscle was observed to contain it. Generally the fascia and sheaths surrounding the muscle prevented diffusion of HRP to adjacent muscles. In a few cases this was checked by injecting one muscle in the presence of, or following surgical removal of, its neighbouring muscle. The number of motoneurones and their position in the spinal cord was similar in both cases, indicating that HRP was confined to the injected muscle. In some birds, one muscle was injected in one leg, and another in the contralateral leg. This was useful when comparing muscles whose motoneurones arose from overlapping segments of the spinal cord, and made possible comparison of the medio-lateral position of motoneurones projecting to each muscle in single histological sections.



Text-fig. 1. Schematic diagram of the major chick hind limb muscles as viewed from medial side. Adductor (dashed line) has been removed to show underlying accessory. Lumbosacral spinal segments innervating each muscle indicated in parentheses. Not shown: the iliofibularis which occupies a position similar to ischioflexorius but lateral to the caudilio-accessory complex and the iliotibialis, a thin sheet-like muscle covering the lateral surface of the thigh.

Following injection of HRP, the temperature of the Tyrode solution in the chamber was raised to 33–35 °C and maintained there for 5–6 hr, when the cords were fixed in 2% glutaraldehyde containing phosphate buffer, pH 7·2. This time was adequate to allow retrograde transport from the most distal muscles studied, and corresponds to a minimum rate of retrograde transport of HRP of 5 mm/hr. Of the fifty-seven embryos injected, the staining following subsequent histological preparation was considered adequate for motoneurone localization in thirty-five embryos; sixteen of these had one muscle in each limb injected. In most of the others the staining reaction was unsuccessful or of insufficient intensity. In three cases leakage of the HRP to adjacent muscles occurred.

Most of the major muscles of the limb were labelled in this study. However, some small inaccessible muscles were not studied; these included the iliofemoralis-internus, iliofemoralis-externus, iliotrochanterici, obturator, ischiofemoralis and coccygeofemoralis muscles. The muscles throughout are named according to Romer (1927).

The localization of motoneurones at embryonic stage 36–37 was taken to represent the mature condition, since this is after the massive period of cell death when the motoneurone population appears to stabilize (Hamburger, 1975). Only small changes in the number of motoneurones or of axons in the ventral root occur from this time until several weeks after hatching (Hamburger, 1975; Chu-Wang & Oppenheim, 1977). Furthermore, chicks are precocious at hatching and even exhibit co-ordinated motor activity prior to this (Bekoff, 1976).

Estimate of proportion of total lateral motor column sampled by HRP injections

In any study that employs a retrograde labelling technique, such as the HRP method used here, it is necessary to assess the extent to which the results give a complete picture of the actual projection pattern. Therefore the total number of labelled lateral motor column motoneurones was determined by summation of the mean counts for each of the individual muscles. This value of 8373 cells is similar to Hamburger's (1975) count of 10,300 motoneurones in the lumbosacral lateral motor column at a comparable stage. Furthermore, at stage 36 there is a linear relationship between the number of motoneurones projecting to a muscle and the wet wt. of the muscle (L. Landmesser, unpublished observations). Using this relationship, and determining the total wet wt. of the small muscles that were not injected in the present study, a corrected value of 9378 cells was obtained.

Histological processing

All cords were fixed in 2 % glutaraldehyde containing phosphate buffer, pH 7·2, for approximately 12 hr at 4 °C. They were rinsed in many changes of Tris buffer (pH 7·2) for 3 days, and were subsequently incubated in 0·1 % diaminobenzidine (DAB) in Tris (pH 7·2) for 3 hr at 4 °C. Hydrogen peroxide was added to the DAB to make its final concentration ~ 0·03 % and the tissue was incubated at room temperature for an additional 3–5 hr. After several rinses with Tris, the tissue was placed in 70 % ethanol overnight. The next day it was dehydrated and paraffin-embedded according to standard procedures.

Sections (10 μm) were cut transversely to the long axis of the cord from the last but one thoracic segment through the lumbosacral enlargement (segments 21–30; Hamburger, 1958). They were deparaffinized, stained in 3 % cresyl violet and mounted in Permount. In each section every labelled profile consisting of a nucleus containing soma in the ipsilateral ventral horn was counted. Histograms were constructed by plotting the number of labelled cells per 30 μm as % of the total number of labelled cells comprising that motor nucleus. The positions of the lumbosacral segments were determined by the adjacent dorsal root ganglia. For example, lumbosacral spinal segment 1 began midway between the end of the last thoracic and the beginning of the first lumbosacral ganglion and ended midway between the 1st and 2nd lumbosacral ganglia. For each muscle the histograms for three to five animals were pooled by bringing them into register beginning at the first section of the first lumbosacral ganglion. Since the total length of the lumbosacral motor column varied by only a small amount this usually brought the whole column into register and no further normalization procedure was used. Since there were small deviations in the extent of individual dorsal root ganglia the demarcation of the segments represented an average value obtained for any one muscle for all the animals. The data from the individual histograms were then combined and expressed as the mean % of total labelled cells per 30 μm $\pm$ S.E. A more accurate representation of the absolute number of cells was obtained by making a correction for double counting (Abercrombie, 1946). Mean values for each muscle are given in the appropriate Text-figs. in parentheses.

In order to localize the positions of motoneurones in the transverse plane of the cord, camera lucida drawings were made with a Zeiss compound microscope with drawing tube attachment at a total magnification of 400 × for a representative section in each segment contributing to each muscle. Every motoneurone profile in the ventral horn was drawn, unlabelled cells being outlined and labelled cells filled in. The distribution from section to section is quite consistent for any muscle, and wherever any shift in localization of the motor nucleus was noted a camera-lucida drawing was made and the exact position noted.

An additional procedure was used to assess the reproducibility and the extent of overlap of different nuclei. A representative outline of the spinal cord was drawn for each lumbosacral segment and the distribution of motoneurones in each determined as follows: for each muscle contributing to that segment the position of every labelled motoneurone in every alternate or every third section (lined up by using the central canal and white matter as boundaries) was plotted. This was done for three animals in each case, and the number of projected neurones usually exceeded several hundred. Five per cent of the total, representing the most scattered somas, were discarded and a line was then drawn which enclosed 95 % of the labelled cells. The boundaries of each nucleus were thus drawn on each representative segment where they occurred. The

final result, depicted in Text-fig. 6, therefore gives a good idea of the position of each nucleus and its relationship and overlap with adjacent nuclei.

E.m.g. recordings from selected muscle pairs

E.m.g.s were recorded from selected pairs of muscles during flexion and crossed extension reflexes and during spontaneous walking in 1–3 week old hatched chicks. Bipolar Teflon-coated silver wire electrodes (170 μm o.d.) were implanted in pairs of thigh muscles under light ether anaesthesia. The electrode wires which emerged from the thigh were anchored to the back (where a ground electrode was placed) and head with sutures, and then run up over a ring support with flexible cables that allowed the bird to move about an area of 4 sq. ft. The e.m.g. responses were recorded with Grass P15 a.c. amplifiers and displayed on a Tektronix 5030A oscilloscope where they were photographed on moving film.

Usually, sequences of spontaneous movement lasting several minutes were recorded continuously as the bird walked about, stepped over the experimenter's finger, etc. In other cases, series of individual reflex responses were recorded. Flexion of the limb in which the electrodes were implanted was induced by light pinching of the ipsilateral toes or by tactile stimulation to the dorsum of the foot. Weak stimuli usually resulted in knee flexion alone, stronger stimuli in hip flexion as well. Strong stimuli often elicited a series of alternating stepping movements of both hind limbs, following the initial ipsilateral flexion. Crossed extension responses were caused by eliciting flexion of the contralateral limb. After termination of the recording session, the bird was sacrificed and the electrode placement confirmed.

The femorotibialis (quadriceps femoris) muscle has been defined by Bekoff (1976) as a knee extensor. The extensor function of this muscle was confirmed in the present experiments by flexion and crossed extension reflexes. The sartorius muscle, defined in the present experiments as a hip flexor on the basis of similar criteria, was found to alternate with the femorotibialis during spontaneous movement. Each of the following muscles was then paired first with the sartorius, then with the femorotibialis, during a typical recording sequence that included both spontaneous walking and flexion and crossed extension reflexes: caudilioflexorius, iliofibularis, ischioflexorius and p. iliotibialis.

The spontaneous walking sequences were analysed from the continuously filmed records. All e.m.g. bursts having a duration of at least 25 msec and an interburst spike frequency of greater than 100 Hz were included. The phase relationship between two muscles was determined by finding the latency of onset of a burst in muscle 1 (measured with respect to the onset of the preceding burst in muscle 2) and dividing this by the period between the two successive bursts in muscle 2. Phase values were grouped into 10 bins of 0·1 phase unit and histograms constructed. Muscles were defined as co-active if phase values clustered around 0·0 or 1·0; they were defined as alternating if phase values clustered around 0·5. Activity tended to occur in definite sequences with sometimes relatively long intervening silent periods. For phase analysis, only the bursts within each sequence were compared. A sequence was said to terminate when a subsequent silent period exceeded 3 times the longest period within a burst sequence.

In addition, duration-latency scatter diagrams (Bekoff, 1976) were constructed for all pairs of bursts that met the criteria defined earlier. In this treatment, the duration of the burst in one muscle is plotted against the latency of the burst in the second muscle (measured from the onset of the burst in the first muscle). Points which fall along the 45° diagonal (see Text-fig. 9) indicate that the second muscle is activated just coincident with termination of the burst in the first muscle. Points to the right of the diagonal indicate that the second muscle is activated with some delay. Muscles with this type of relationship are defined as being alternating. Points to the left of the diagonal show that the burst in the second muscle begins before termination of the burst in the first muscle, and therefore the muscles are activated with some overlap in time. In all plots, a second line (dashed) has been drawn; points to the left indicate that the second muscle is activated halfway through the burst in the first muscle or earlier. Muscle pairs which had points clustered to the left of the dashed line were defined as co-active, even though the bursts in the two muscles were not entirely co-incident. (See Bekoff, 1976, for further description of this type of analysis.)

For each muscle pair, observations were made on three separate animals. Muscle pairs were then classed as being either alternatingly or co-actively activated. They were further classed as extensor or flexor, based on their activation during the reflex responses. It should be stressed that

these observations are of a preliminary nature and were made so that the position of a motoneurone pool could be related to the general function of its muscle. No attempt has been made to relate the sequence of muscle activation to the bird step cycle, which would require video taping of movements.

RESULTS

Rostro-caudal position of motor nuclei

As described in detail in the Methods section, most of the thigh muscles (see Text-fig. 1) were individually injected with HRP, and the motoneurones projecting to each were localized by observing the distribution in the lateral motor column of labelled cell bodies, virtually all of which contained granular reaction product (Pl. 1). A histogram of the number of labelled motoneurones per 30 μm increment of lumbosacral cord was constructed for each muscle (as shown in the inset at the top right of Text-fig. 2 for the sartorius).

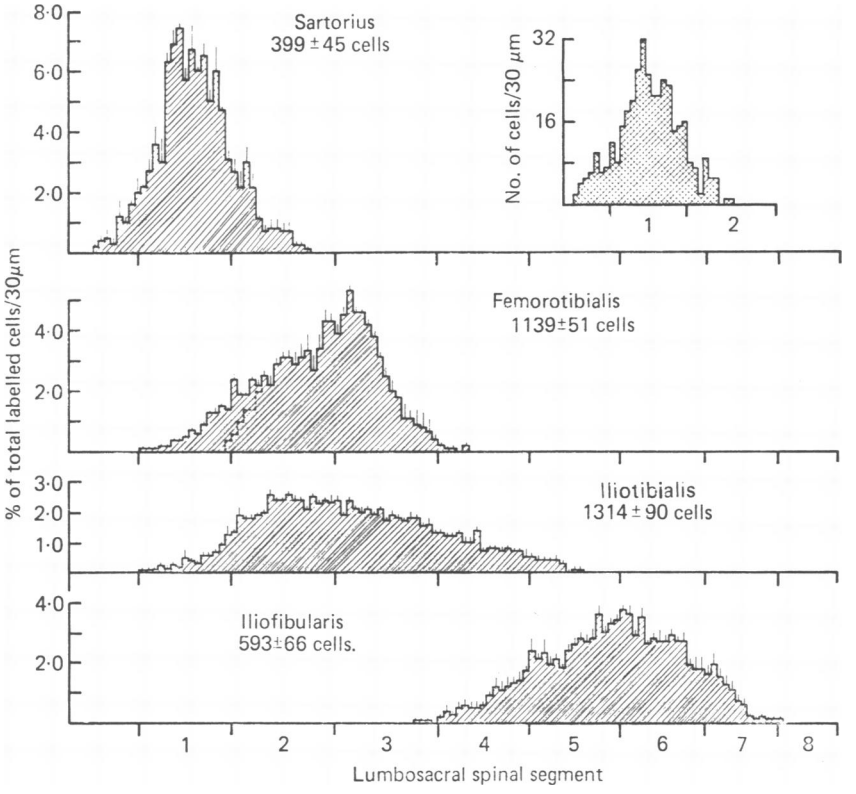
Generally however, since the number of labelled cells varied somewhat among animals, and since the intention of this study was to determine the spatial distribution of motoneurones contributing to a nucleus rather than their absolute number, these data were replotted in a histogram showing the per centage of the total motor nucleus in each 30 μm increment of cord. Then a number of histograms (three to five) could be combined for each muscle and the variation in position of the motor nucleus assessed. Such histograms indicating mean per cent $\pm$ S.E. are shown in Text-fig. 2 for those thigh muscles derived from the embryonic dorsal muscle mass, and in Text-fig. 3 for those originating from the ventral muscle mass.

It can be seen that the rostro-caudal position of the nucleus for each muscle varies only slightly from animal to animal; each occupies a characteristic position that differs from the other nuclei. Some muscles, e.g. the sartorius and ischioflexorius, have a narrow distribution centred around a single segment, while others, e.g. the iliofibularis and iliotibialis, have a broader distribution stretching over 3–4 segments. These findings are in essential agreement with the distributions determined electrophysiologically by spinal nerve stimulation (Landmesser & Morris, 1975), but give a more detailed resolution. (Minor discrepancies are discussed fully by Morris, 1978). Thus while it had been shown that the first lumbosacral spinal nerve activates both the sartorius and adductor muscles, it can be seen in Text-figs. 2 and 3 that the motor nuclei to these two muscles begin at different points: that for the sartorius begins at the end of the last thoracic segment and occupies all of LS 1 while the adductor nucleus only begins halfway through LS 1.

It is clear from these data that muscles in close proximity are not always innervated by motoneurones with similar distributions in the cord. Thus while the adjacent sartorius and femorotibialis muscles have motor nuclei which are located in similar segments, the similarly adjacent ischioflexorius and caudilioflexorius receive from widely divergent sources.

Further there is no consistent correlation between the rostro-caudal position of motoneurones innervating a muscle and the embryonic muscle mass (Romer, 1927) from which the muscle is derived. A greater percentage of motoneurones in the rostral four segments ($\sim 60\%$) do innervate muscles derived from dorsal muscle mass. The converse is true for the caudal four segments where approximately 68% of the motoneurones innervate muscles derived from the ventral muscle mass.

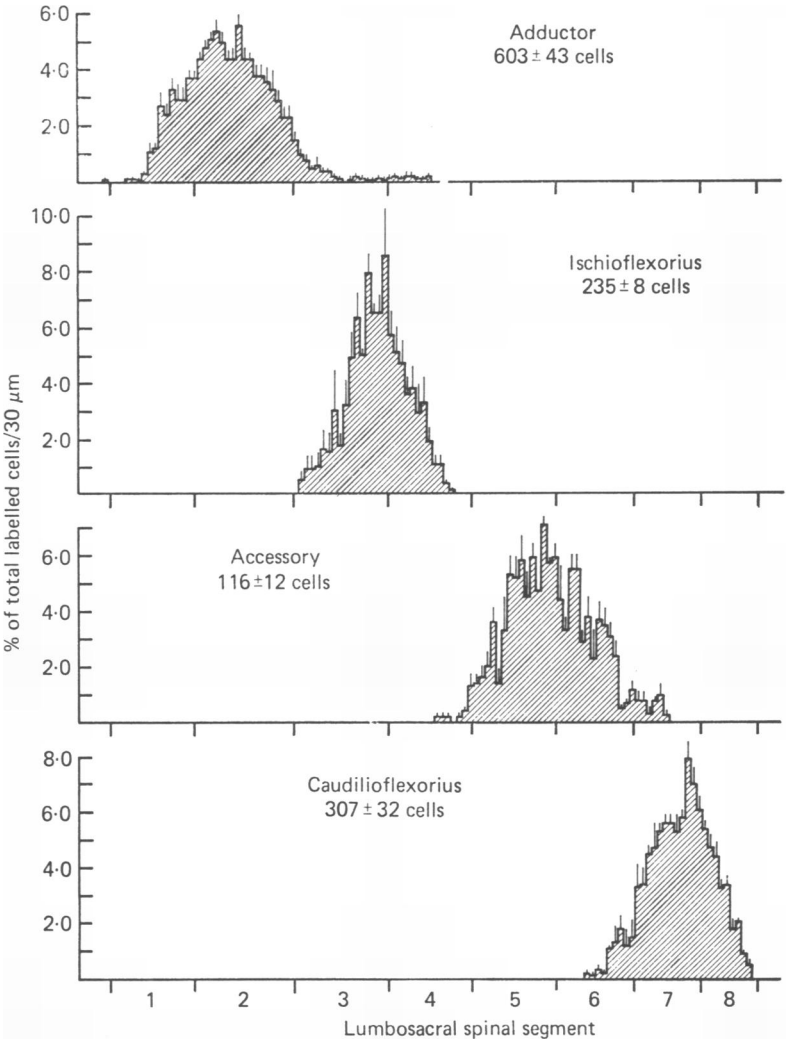
However, motoneurones innervating muscles of dorsal muscle mass origin are distributed along most of the lumbosacral enlargement (LS 1–7), as are those innervating muscles of ventral muscle mass origin (LS 1–8). Similarly, there is no consistent correlation with adult topology. Thus the adductor and ischioflexorius on the posterior 'flexor' surface of the leg have motor nuclei situated nearly as rostrally as do the sartorius and femorobialis on the anterior or 'extensor' surface.



Text-fig. 2. Rostro-caudal position of motor nuclei to thigh muscles derived from dorsal muscle mass. Histograms pooled from three to five embryos showing the mean % of each pool occurring in successive 30 μm increments along the lumbosacral cord; mean number of labelled cells per nucleus ± S.E. also shown. Inset at top right shows raw data for one sartorius muscle expressed as number of cells/30 μm. Femorotibialis histogram (continuous line) also includes cells labelled by injection of ambiens muscle. Dashed line indicates femorotibialis distribution in two muscles where ambiens had been removed prior to HRP injection. Iliotibialis data are for combined anterior and posterior heads which were not separately injected. Error bars drawn in only one direction represent S.E.

Further, if one includes the shank muscles (Text-fig. 4), the classical correlation between the rostro-caudal position of motoneurones and the proximo-distal position of muscles (Romanes, 1964) was not apparent. Motoneurones innervating shank muscles are primarily situated at intermediate levels (LS 4–6), with 83% occurring in LS segments 4, 5 and 6; only 10% occur in LS 3 and 7% in LS 7 and 8. Looked at in another way, the percentage of motoneurones innervating thigh muscles was

75% in LS 3, dropped to 37% over LS 4, 5 and 6 but then rose to 49% in LS 7 and 92% in LS 8.

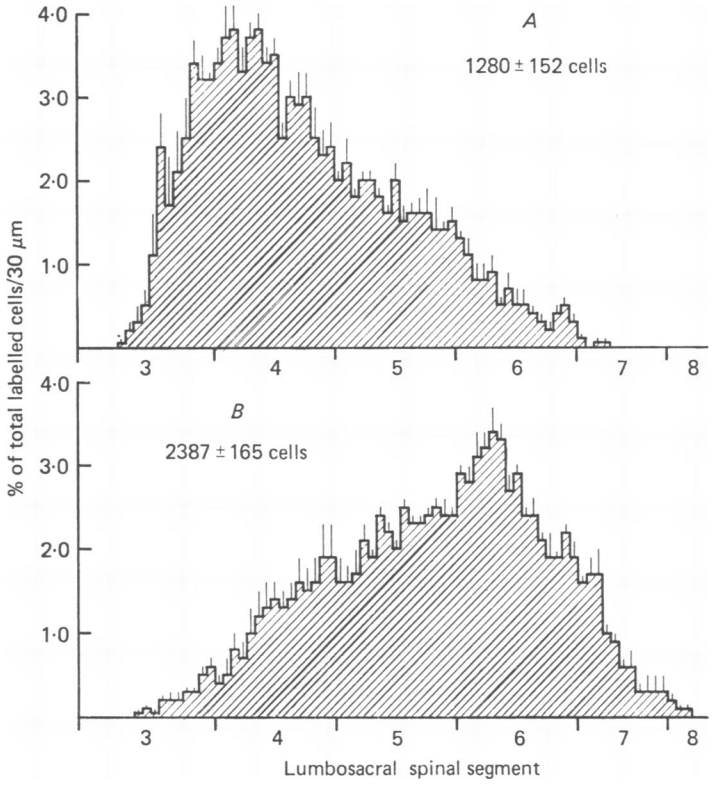


Text-fig. 3. Rostro-caudal location of motor nuclei to thigh muscles derived from ventral muscle mass. Histograms are pooled from three to five embryos and show the mean % of each nucleus occurring in successive 30 μm increments along the lumbosacral cord. Mean number of labelled cells per pool ± S.E. also shown. Error bars drawn in one direction only represent S.E.

Localization of motor nuclei in the transverse plane

Camera lucida drawings of a representative section (Pl. 1) showing all labelled and unlabelled motoneurones in the lateral motor column were constructed for each muscle at the level of each contributing spinal cord segment (see Text-fig. 5). In addition, the boundaries of each motor nucleus (enclosing 95% of the neurones labelled in three separate cases) were determined more quantitatively for each muscle as described in the Methods. These are shown in Text-fig. 6 where the longitudinal

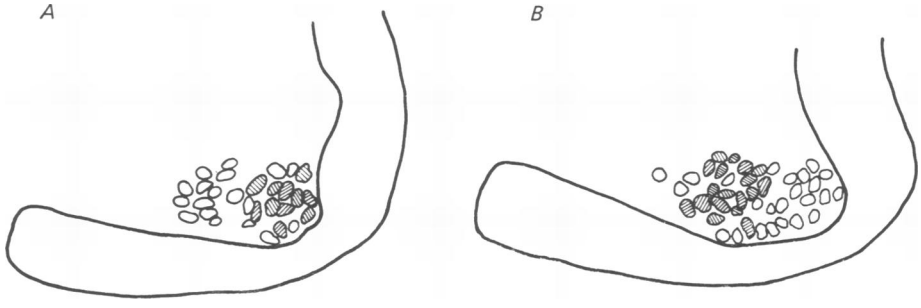
extent of the nuclei is also indicated. There it can be seen that the motoneurones innervating each muscle are clustered in a characteristic position (medio-lateral and dorso-ventral), each nucleus consisting of an elongated cluster or column of cells. Some are relatively broad; for example the adductor nucleus has an approximate medio-lateral width of 80 μm and a length of 780 μm. Others, such as the iliofibularis nucleus, with a width of 45 μm and a length of 1770 μm, are more narrow and elongated.



Text-fig. 4. Rostro-caudal distribution of motoneurones projecting to shank muscles derived from either dorsal (*A*) or ventral (*B*) muscle mass. Histograms are pooled from three embryos and show the mean % of each nucleus occurring in successive 30 μm increments along the lumbosacral spinal cord. Mean number of labelled cells per pool ± S.E. also shown. Errors bars drawn in one direction only represent S.E.

The characteristic locations allow one anatomically to distinguish between motor nuclei that occur at the same segmental level. In LS 1 for example, the sartorius nucleus is located ventro-laterally and the adductor nucleus medially with only slight overlap. In fact the extent of overlap as depicted in Text-fig. 6*A* is probably an over-estimation for the following reasons. First, data from several animals have been pooled and the exact positions of nuclei vary slightly from animal to animal. Secondly, the boundaries of the actual nucleus are not smooth, but somewhat jagged, and vary slightly from section to section (i.e. the sartorius-adductor boundary may be more medial in one section than the next). Finally, for reasons of simplicity, a single representative section has been drawn for each segment, sometimes enhancing

apparent overlap. For example, the femorotibialis nucleus ends as the dorsal shank nucleus begins, so that the bulk of the motoneurones do not overlap. Yet it is true that the few femorotibialis motoneurones occurring at the ends of the nucleus are scattered among dorsal shank ones as indicated.



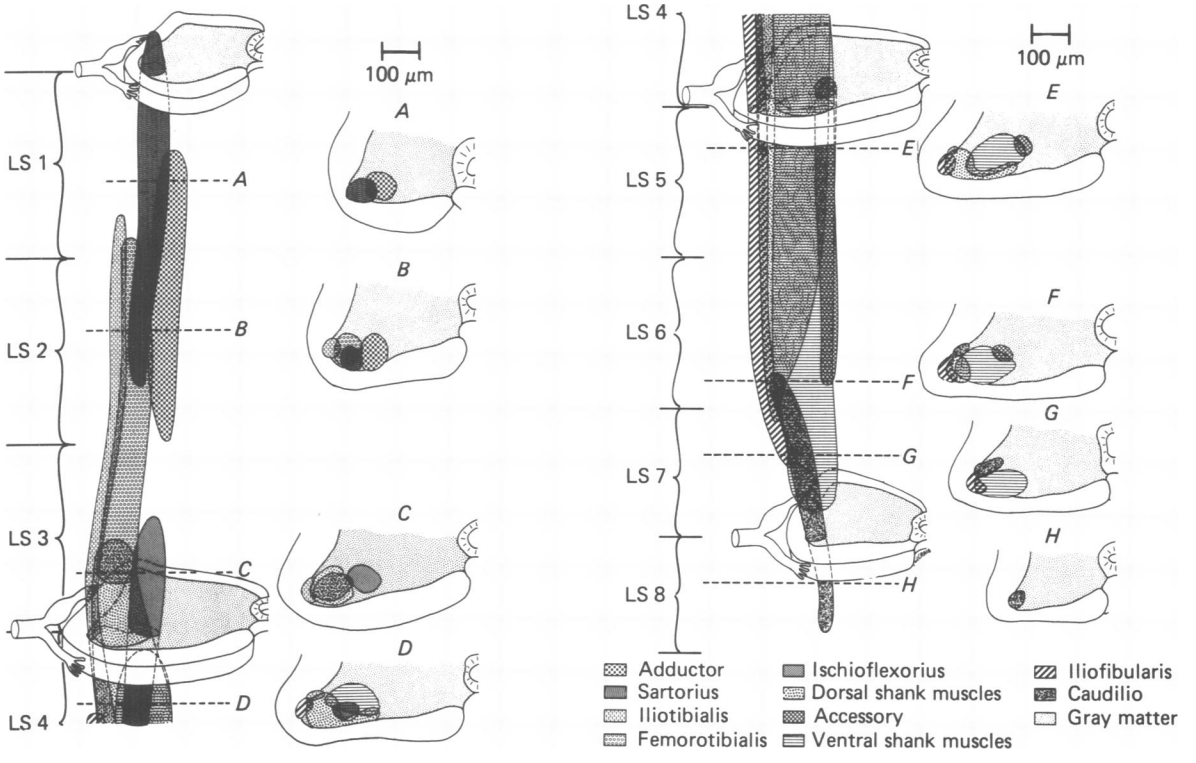
Text-fig. 5. Camera lucida drawings of single sections of left ventral horn showing HRP labelled cells (dashed lines). *A*, sartorius nucleus at level of LS 1; *B*, adductor nucleus at mid LS 2. White matter bounding ventral horn laterally and ventrally shown for orientation.

Romanes in his classical studies (1951, 1964) defined anatomical columns in the cat spinal cord. These columns, which appeared to represent groups of individual motoneurone nuclei, were clusters of cells separated from other clusters by cell-free areas. In the chick there is a tendency for some anatomical clustering to occur, and sometimes such clusters appeared to represent single pools (see the adductor in Pl. 1). However, in other cases nucleus boundaries were not coincident with cell-free areas, and the muscles to which cells along such boundaries projected could only be determined by retrograde labelling techniques, such as the one used. This clearly places limits on the predictability from anatomy as to which muscle any given motoneurone projects.

Some previous studies (Romanes, 1964) have suggested that motoneurones to different muscles might be extensively intermingled. This does not appear to be the case for most of the muscles studied here, each of which had the cells projecting to it situated in a generally coherent cluster, as can be appreciated from Pl. 1. Such coherent clustering was especially evident at the segmental levels representing the peaks of the motoneurone distribution. Thus if one considers the iliofibularis from the middle of LS 4 to the middle of LS 7, only thirty out of 747 labelled cells, or 4 %, were separated from the nucleus by one or more unlabelled cells. At the ends of the motor nucleus, where only a few cells per section were labelled, the clustering was less tight and these cells were often separated by a number of unlabelled cells. Similar results were found for the sartorius, adductor and ischioflexorius muscles with 12, 12 and 15 % respectively of the labelled motoneurones falling outside the coherent motor nucleus.

Shank musculature was not investigated in as much detail as thigh. However, in order to compare the distribution of motoneurones innervating these segments of the limb, all of the anterior shank muscles (arising from the dorsal muscle mass) were injected in one group of birds, and all of the posterior shank muscles (arising

from the ventral muscle mass) in another group, the resulting distributions being shown in Text-fig. 6. As has been observed for mammalian shank musculature (Romanes, 1951, 1964) the anterior muscles are innervated by cells with a general ventro-lateral position, and the posterior muscles by cells located in the dorso-medial position. A small number of dorsal shank motoneurones (13 %) in LS 4 and 5



Text-fig. 6. Location of motor nuclei in the transverse plane. Transverse sections representative of the level designated are shown on the right to indicate the position of motor nuclei for various hind limb muscles; boundaries thus drawn enclose 95 % of the motoneurones comprising each nucleus (see text). For orientation a longitudinal view of the nucleus, based on the histograms of Text-figs. 2–4, is also presented. For clarity the longitudinal extent of nuclei are shown only for regions where each 30 μm increment of cord contains at least 1 % of the total nucleus. This procedure mainly cuts off the ends of each nucleus where only few scattered labelled cells occur (compare Text-figs. 2 and 6). It should also be noted that this Figure indicates only the boundaries of nuclei and gives no indication of the density of labelled cells within pools, for which it is necessary to consult Text-figs. 2–4. Further, while the boundaries in the transverse sections are precise representations of the data for the level indicated, the degree of taper of nuclei shown in the longitudinal view is only approximate.

were situated quite medially, within the boundaries of the medially extending arm of the pool shown in Text-fig. 6. It is not known to which muscles these cells projected.

In summary, the medio-lateral position of motoneurones, unlike the rostro-caudal distribution, was found to be correlated with the derivation of muscles from the embryonic muscle masses. Thus the motoneurones which supply all of the muscles derived from the dorsal muscle mass are situated laterally, while those supplying

the muscles derived from the ventral muscle mass are located medially. This relationship holds irrespective of the segmental level from which the innervation arises.

The classical relationship relating the dorso-ventral position of motoneurones to the proximo-distal position of the muscles they innervate (Romanes, 1951, 1964; Sharrard, 1955) was not generally apparent for the chick. Some thigh muscles are innervated by ventrally located motoneurones (e.g. sartorius) but others (e.g. ischioflexorius, caudilioflexoris, iliofibularis) are situated as dorsally as shank innervating motoneurones from the same segments. (Text-fig. 6, Pl. 1) Of course, since the intrinsic foot motoneurones were not studies, it is possible that they occupy an extreme dorsal position as shown in earlier mammalian studies (Romanes, 1951, 1964; Sharrard, 1955).

Relationship between motoneurone position and muscle function

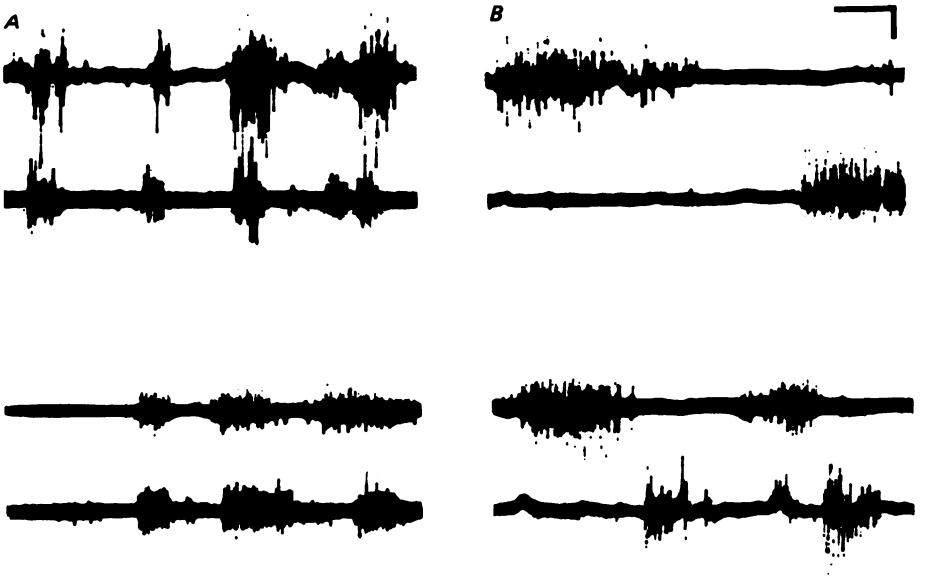
In order to determine whether the position of a motor nucleus was correlated with the function of its muscle, some preliminary observations were made on the function of various thigh muscles during flexion and crossed extension reflexes, and during spontaneous movement. E.m.g.s were recorded from pairs of thigh muscles of 1–3 week-old hatched chicks during flexion and crossed extension reflexes (see Methods for details). For example, in one bird with electrodes implanted in the sartorius and femorotibialis muscles, thirty-two flexion responses elicited by lightly pinching the ipsilateral foot invariably elicited a burst of e.m.g. activity in the sartorius muscle, while any ongoing activity in the femorotibialis was silenced. On the other hand, crossed extension of the leg, caused by eliciting flexion of the contralateral leg always increased the level of activity in the femorotibialis. Similar observations were made on two additional chicks and the femorotibialis was therefore classed as an extensor, the sartorius as a flexor, muscle.

On the basis of similar recordings the p. iliotibialis and caudilioflexorius were classed as extensors, and the iliofibularis as a flexor. The ischioflexorius was activated both during flexion and extension movements, the activity during flexion consisting of a large amplitude and high frequency burst followed by a more prolonged discharge. As has been described by Bekoff (1976), it appears that this muscle is used as both a flexor and extensor. Due to its rather complex activity, it was not analysed further in the present series of experiments.

In addition, the phase relationships between pairs of muscles were investigated during spontaneous unrestrained walking movements. Examples of e.m.g. traces from such spontaneous walking sequences are shown in Text-fig. 7 where it can be seen that the flexors, iliofibularis and sartorius, are co-activated (*A*, top pair) as are the extensors, caudilioflexorius and femorotibialis (*A*, bottom pair). When a flexor and an extensor were paired as in *B*, they were found to alternate, as can be seen in both the top (sartorius and caudilioflexorius) and bottom (iliofibularis and iliotibialis) pairs. The duration of bursts in each of the muscles studied varied considerably, probably because the birds used a wide range of movements during such unrestrained walking (i.e. running, slow walking, stepping over obstacles in contrast to pacing at a constant rate on a treadmill).

Nevertheless, when a more qualitative determination was made of the phase at which one muscle was activated with respect to the preceding, and subsequent

bursts in a second muscle, the results were clear. The histogram in Text-fig. 8*A* shows that the femorotibilialis tended to be activated mid-way between pairs of bursts in the sartorius (i.e. the phase values clustered about 0·5). Clearly these two muscles tended to be activated in an alternating fashion rather than being co-activated.

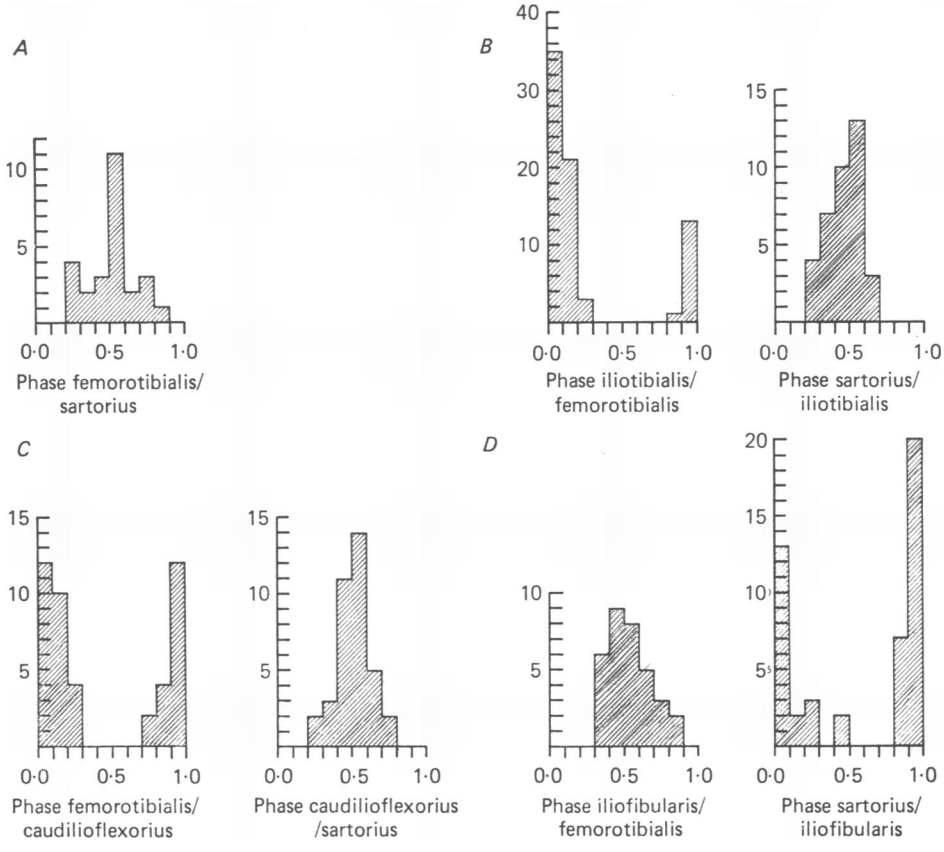


Text-fig. 7. E.m.g. records from muscle pairs during spontaneous unrestrained movement. The top pair of records in *A* shows two flexors which are co-activated: iliofibularis, top trace; sartorius, bottom trace. The lower pair of records shows co-activation of two extensors: caudilioflexorius, top trace; femorotibialis, bottom trace. The muscles in the top pair of *B* (caudilioflexorius, top trace; sartorius, bottom trace) are activated in an alternating fashion, as are the muscles of the bottom pair (iliofibularis, top trace; iliotibialis, bottom trace). Calibration bar is 100 msec and 200 μV.

When the iliotibialis was paired with the femorotibialis (*B*), the phase values clustered around 0·0 or 1·0 indicating co-activation, whereas the sartorius and iliotibialis were activated in an alternating fashion. Similar phase relationships (*C*) with the sartorius and femorotibialis were found for the caudilioflexorius, another extensor. In contrast, the iliofibularis (*D*) was co-activated with the sartorius and was activated out of phase with the femorotibialis, consistent with its assumed flexor action. These results taken together suggest that the sartorius and iliofibularis are flexors and are co-activated during various movements. These two muscles in turn tend to alternate with the extensors femorotibialis, caudilioflexorius and posterior head of the iliotibialis.

Since the phase values in the histograms in Text-fig. 8 are determined from the onset of a burst in one muscle with respect to the onset of a burst in a second one, they do not show in themselves whether the bursts overlap to any extent. Therefore for some muscle pairs, duration latency plots were also constructed (see Methods for detailed description). From these it can be seen that when the following presumed flexors and extensors were paired they tended to be activated in an alternating

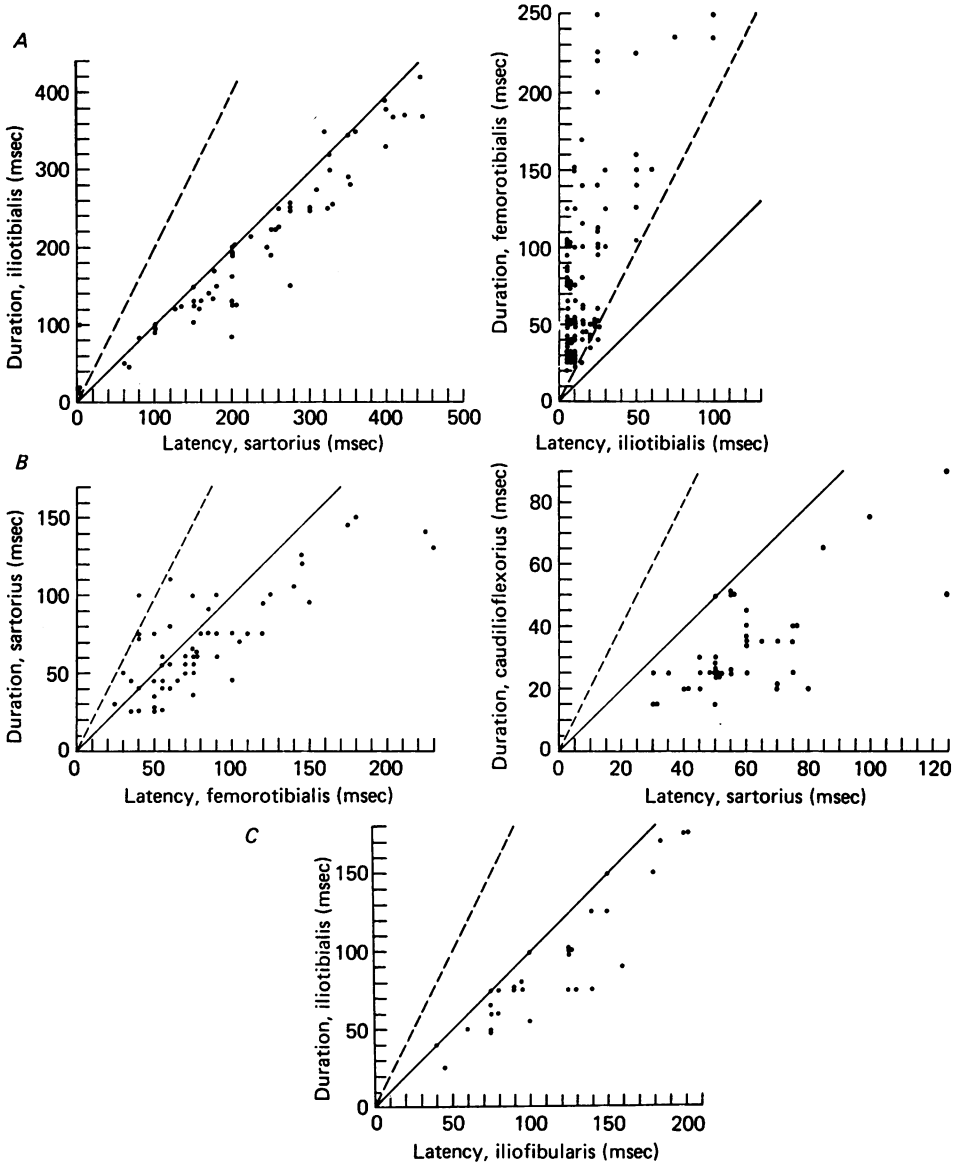
manner, with little or no overlap: sartorius and iliotibialis (Text-fig. 9*A*, left), femorotibialis and sartorius (Text-fig. 9*B*, left), sartorius and caudilioflexorius (Text-fig. 9*B*, right), and iliofibularis and iliotibialis (Text-fig. 9*C*). As would be expected the two presumed synergists, femorotibialis and iliotibialis, both extensors, were co-activated (Text-fig. 9*A*, right), their bursts overlapping considerably in time.



Text-fig. 8. Phase relationships between various muscle pairs. The phase histograms, based on the recording of e.m.g. bursts from pairs of muscles during spontaneous walking movements, show the phase at which one muscle is activated with respect to a second (see text for further explanation). Data for each histogram is pooled from two to three birds aged 1–3 weeks.

DISCUSSION

If one compares the position of a motor nucleus as seen in Text-fig. 6, and the function of its muscle as deduced from e.m.g. experiments, no consistent relationship emerges. The motoneurones innervating the thigh muscles studied physiologically are all located relatively laterally in the classical 'morphological' extensor area (Romanes, 1964). However, two nuclei, the sartorius and iliofibularis, which are situated at the extreme lateral position are actually activated as flexors. Furthermore, adjacent nuclei are not necessarily co-activated. For example, the iliofibularis



Text-fig. 9. Duration–latency scatter diagrams for selected thigh muscles. In each the duration of an e.m.g. burst from one muscle is plotted against the latency of the succeeding burst in the second muscle (see text for further explanation). All data were taken from 1- to 3-week-old hatched chicks during spontaneous walking and during flexion and crossed extension reflexes. Data for each plot is pooled from two to three birds.

and iliotibialis nuclei are elongated, narrow and adjacent to one another in segment 5, both bordering the white matter. Yet the muscle of the former is activated as a flexor, the muscle of the latter as an extensor. Similar observations can be made in the cat for the semi-membranous muscle, whose motor nucleus is situated in a medial 'flexor' position (Romanes, 1951). Yet while the posterior head of this muscle serves

as a flexor (Peters & Rick, 1976), and the motoneurones innervating it are activated accordingly (Engberg & Lundberg, 1969), the anterior head and its motoneurones are activated as extensors (but see Rasmussen, Chan & Goslow, 1978).

The results of this study have shown however that for the bird there exists a somatotopic map which relates the position of lumbosacral motoneurone somas to their axonal termination sites in the limb. These sites are not related to the anatomical position of the mature muscle to which the motoneurone projects. For example, both the iliofibularis and the ischioflexorius occupy a similar position along the AP axis of the limb, yet the former is innervated by the most lateral cells, the latter by the most medial. Rather, these sites appear to relate to a certain part of the premuscle mass from which the muscle arises during early embryogenesis. Thus medial motoneurones project to muscles derived from the ventral muscle mass, and lateral motoneurones to muscles derived from the dorsal muscle mass, similar to the situation observed in the mammal (Romanes, 1964) and amphibian (Lamb, 1976). Furthermore, in the chick, the present HRP studies have shown that within each muscle mass there is an additional topological relationship relating the rostrocaudal position of a motoneurone to the antero-posterior position of its termination site in the limb (see also Text-fig. 1 in following paper, Landmesser, 1978). Some somatotopic mapping within a muscle has also been observed in cats (Swett, Eldred & Buchwald, 1970; Burke, Strick, Kanda, Kim & Walmsey, 1977) although it is not yet clear how extensive this is in cats or birds and therefore at what level of detail the somatotopic relationship exists.

This somatotopic map appears to have been conserved during evolution of different vertebrate lines. The striking similarities in the detailed motor nuclei topography of cat and bird spinal cord can be illustrated with a few examples. The three mammalian hamstring muscles, semi-membranosus, semi-tendinosus, and biceps femoris, arise embryologically from the ventral muscle mass posterior to the adductor region (Lance Jones, 1977 and personal communication). The semi-membranosus derived from the more anterior and medial portion of this area would therefore correspond to the chick ischioflexorius (Romer, 1927, 1970) while the more posterior arising biceps has been defined as the mammalian homologue of the caudilioflexorius. It is therefore interesting to note that the motor nuclei for the homologous semimembranosus (Romanes, 1951) and ischioflexorius are both situated very medial and anterior to the segments which contribute to the biceps and caudilioflexorius. In both species the nuclei for the latter homologues are situated in the same relative position, and even curve in a similar fashion laterally and ventrally as they extend caudad.

Similarly in both animals the adductor nucleus lies medial to and begins slightly rostral to the femorotibialis (or vastus in the cat) nucleus. Finally, the gluteal complex (C. Lance Jones, personal communication) and iliofibularis are derived from similar regions of the dorsal muscle mass and both are innervated by relatively caudally situated motoneurones. Further, both nuclei are located at an extreme lateral position adjacent to the white matter. Although it is not yet possible to make as detailed a comparison, it is probable that similar topographical similarities also exist for the amphibian cord (Cruce, 1974).

What is perhaps most interesting, with respect to the functional organization of

the vertebrate spinal cord, is that this tight topographical relationship has been conserved in vertebrate lines as different as birds and higher mammals, in spite of changes in the function of homologous muscles. For example, the gluteal complex of the cat (Rasmussen *et al.* 1978) and the caudilioflexorius of the chick are both hip extensors. Yet the motoneurones supplying the gluteals are not located in the same relative position as the caudilioflexorius motor nucleus but like the motor nucleus of the iliofibularis. The latter muscle is a probable embryonic homologue of the gluteal complex, but has the opposite function, being a knee flexor. Similarly the bird sartorius and cat rectus are homologous muscles whose motor nuclei occupy similar positions (Romanes, 1951), yet the sartorius is a hip flexor, the rectus a knee extensor (Rasmussen *et al.* 1978). Thus it is possible to conclude that, while the topographical relationship between motor nuclei and specific muscles is rigidly conserved during vertebrate evolution, the function of such muscles, and the activation pattern of their motoneurones (and therefore implicty their central connectivity), can and does change.

What emerges from these considerations is that motoneurone function and topography are not related except perhaps coincidentally, as has been earlier suggested by Romanes (1964). Rather the topography of motoneurone pools is related consistently only to the embryonic site of their axonal termination in the early limb bud. A similar relationship, although in much less detail, has been described for the amphibian (Cruce, 1974; Lamb, 1976). This suggests that some aspect of the motoneurone, tightly related to its position, may be instrumental in allowing the proper matching up of motoneurones and muscles.

Since it can be demonstrated that the functional activation of motoneurones, and therefore their aggregate central connectivity, does not follow the same relationship, some other mechanism not simply related to soma position must be responsible for ensuring proper central connectivity. Of course the central activation of motoneurones is presumably a complex process involving interactions between cutaneous and propriocetive limb afferents, various descending inputs, and the central pattern generator (Edgerton, Grillner, Sjostrom & Zangger, 1976; Deliagina, Feldman, Gelfand & Orlovsky, 1975; Forrsberg, Grillner & Rossignol, 1975; Grillner, 1975, 1976; Perret & Cabelquen, 1976).

A consideration of the distribution of classical excitatory 1A connexions (Eccles & Lundberg, 1958; Perret & Cabelquen, 1976) suggests that cat thigh muscles can be divided into two groups which do not follow the classical functional division into extensors and flexors. It is interesting to note however that these two groups of muscles correspond to different anatomical regions of the limb. Further, the strength of 1A connexions between muscles (Eccles & Lundberg, 1958) appears in a general way to be related to the proximity of the muscles in the limb, but not however to the proximity of their motor nuclei in the cord (Romanes, 1951).

Thus it seems necessary to conclude that 1A connexions and presumably other inputs onto the motoneurone pool as well, including those of the central pattern generator (Deliagina *et al.* 1975; Edgerton *et al.* 1976) are specified by some mechanism not related in a simple fashion to the position of the motoneurone soma. Most mechanisms that have been proposed to specify neuronal connectivity are based on position (i.e. position of a neurone along a gradient of positional information; or cell

birthday, which in the ventral horn is also related to position). Thus it is difficult to imagine what this mechanism might be, although it appears in some way to be related to muscle topography and function.

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REFERENCES

ABERCROMBIE, M. (1946). Estimation of nuclear population from microtome sections. *Anat. Rec.* **94**, 239–247.

BEKOFF, A. (1976). Ontogeny of leg motor output in the chick embryo: a neural analysis. *Brain Res.* **106**, 271–291.

BLOOMFIELD, S. (1974). Addressing the spinal cord. *Brain Res.* **78**, 35–43.

BROWN, T. G. (1911). The intrinsic factors in the act of progression in the mammal. *Proc. R. Soc.* B **84**, 308–319.

BURKE, R. S., STRICK, P. L., KANDA, I. K., KIM, C. C. & WALMSLEY, B. (1977). Anatomy of medial gastrocnemius and soleus motor nuclei in cat spinal cord. *J. Neurophysiol.* **40**, 667–680.

CRUCE, W. L. R. (1974). The anatomical organization of hindlimb motoneurons in the spinal cord of the frog, *R. catesbiana*. *J. comp. Neurol.* **153**, 59–76.

CHU-WANG, I. W. & OPPENHEIM, R. (1977). Cell death of motoneurons in the chick embryo spinal cord. A quantitative and qualitative analysis of degeneration in the ventral root, including evidence for axon outgrowth and limb innervation prior to cell death. *J. comp. Neurol.* **77**, 59–86.

DELIAGINA, T. G., FELDMAN, A. G., GELFAND, J. M. & ORLOVSKY, G. N. (1975). On the role of central program and afferent inflow in the control of scratching movements in the cat. *Brain Res.* **100**, 297–313.

ECCLES, R. M. & LUNDBERG, A. (1958). Integrative pattern of 1a synaptic actions on motoneurones of hip and knee muscles. *J. Physiol.* **144**, 271–298.

EDGERTON, V. R., GRILLNER, S., SJOSTROM, A. & ZANGGER, P. (1976). Central generation of locomotion in vertebrates. *Adv. behav. Biol.* **18**, 439–464.

ENGBERG, I. & LUNDBERG, A. (1969). An electromyographic analysis of muscular activity in the hindlimb of the cat during unrestrained locomotion. *Acta physiol. scand.* **75**, 614–630.

FORSSBERG, H., GRILLNER, S. & ROSSIGNOL, S. (1975). Phase dependent reflex reversal during walking in chronic spinal cats. *Brain Res.* **85**, 103–107.

GRILLNER, S. (1975). Locomotion in vertebrates – central mechanisms and reflex interactions. *Physiol. Rev.* **55**, 247–304.

GILLNER, S. (1976). Some aspects on the descending control of the spinal circuits generating locomotor movements. *Adv. behav. Biol.* **18**, 351–375.

GRILLNER, S. & ZANGGER, P. (1975). How detailed is the central pattern generator for locomotion? *Brain Res.* **88**, 367–371.

HAMBURGER, V. (1958). Regression versus peripheral control of differentiation in motor hypoplasia. *Am. J. Anat.* **102**, 365–410.

HAMBURGER, V. (1975). Cell death in the development of the lateral motor column of the chick embryo. *J. comp. Neurol.* **160**, 535–546.

HAMBURGER, V. & HAMILTON, Y. (1951). A series of normal stages in the development of the chick embryo. *J. Morph.* **88**, 49–92.

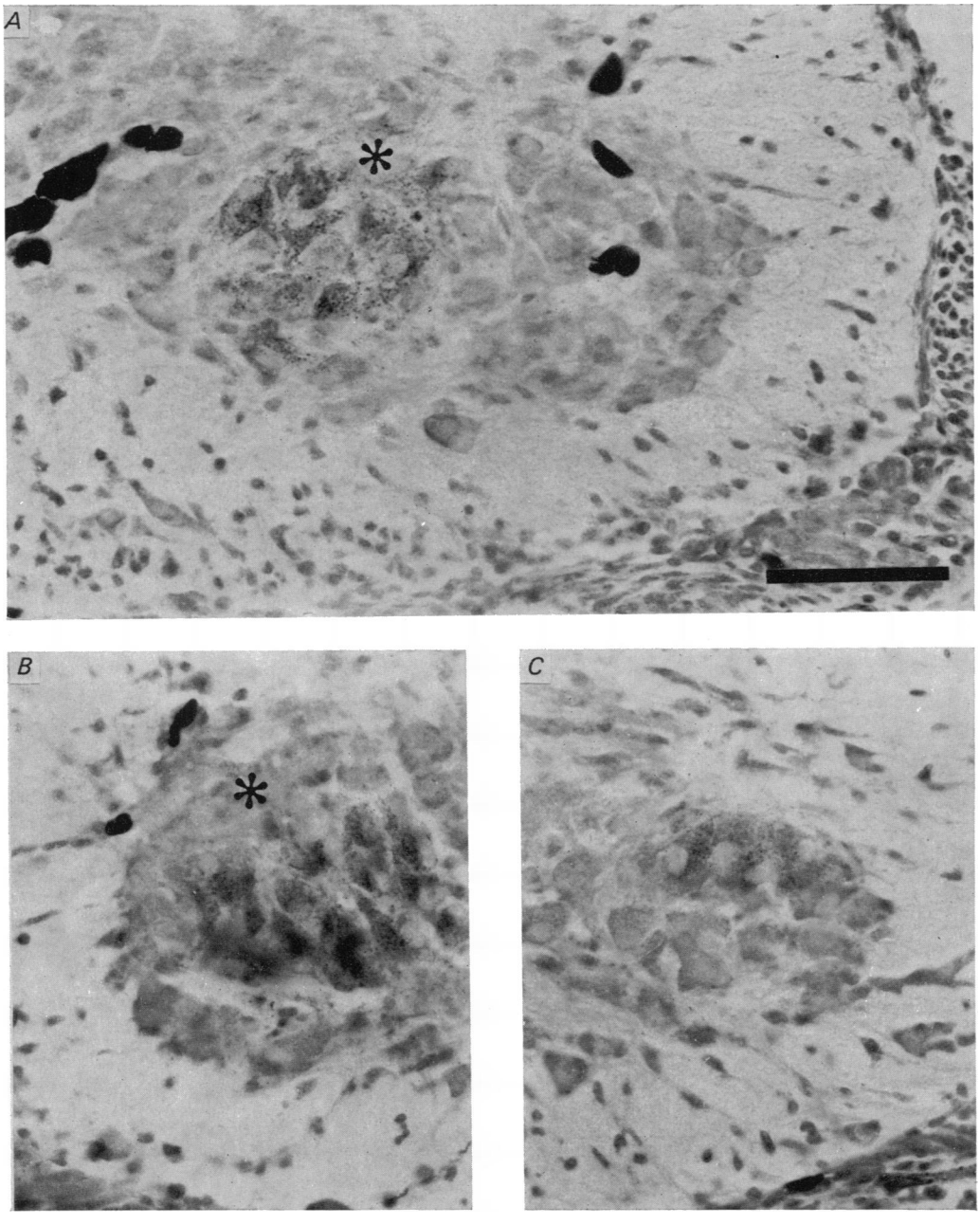
HUGHES, A. (1965). A quantitative study of the development of nerves in the hindlimb of *Eleutherodactylus martinicensis*. *J. Embryol. exp. Morph.* **13**, 9–34.

KUYPERS, H. G. J. M. (1964). The descending pathways to the spinal cord, their anatomy and function. *Prog. Brain Res.* **11**, 178–202.

LAMB, A. (1976). The projection patterns of the ventral horn to the hind limb during development. *Devl Biol.* **54**, 82–99.

LANCE JONES, C. (1977). The morphogenesis of the mammalian hindlimb with special reference to rodent thigh musculature. Doctoral dissertation, University of Massachusetts, Amherst.

LANDMESSER, L. & MORRIS, D. (1975). The development of functional innervation in the hind limb of the chick embryo. *J. Physiol.* **249**, 301–326.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LANDMESSER, L. (1978). The development of motor projection patterns in the chick hind limb. *J. Physiol.* **284**, 391–414.

MORRIS, D. G. (1978). The development of functional motor innervation in supernumerary hindlimbs of the chick embryo. *J. Neurophysiol.* (In the Press.)

PERRET, C. & CABELGUEN, J.-M. (1976). Central and reflex participation in the timing of locomotor activations of a bifunctional muscle, the semi-tendinosus, in the cat. *Brain Res.* **106**, 390–395.

PETERS, S. E. & RICK, C. (1976). The actions of three hamstring muscles of the cat: a mechanical analysis. *J. Morph.* **152**, 315–328.

RASMUSSEN, S., CHAN, A. K. & GOSLOW, G. E. (1978). The cat step cycle: electromyographic pattern for hindlimb muscles during posture and unrestrained locomotion. *J. Morph.* (In the Press.)

ROMANES, C. (1951). The motor cell columns of the lumbosacral spinal cord of the cat. *J. comp. Neurol.* **94**, 313–364.

ROMANES, G. (1964). The motor pools of the spinal cord. *Prog. Brain Res.* **11**, 93–119.

ROMER, A. (1927). The development of the thigh musculature of the chick. *J. Morph.* **43**, 347–385.

ROMER, A. (1970). In *The Vertebrate Body*, ed. Saunders, W. B.

SHARRARD, W. J. W. (1955). The distribution of the permanent paralysis in the lower limb in poliomyelitis. *J. Bone Jt Surg.* **37B**, 540–558.

SHERRINGTON, C. S. (1892). Notes on the arrangement of some motor fibres in the lumbosacral plexus. *J. Physiol.* **13**, 621–772.

SHIK, M. L. & ORLOVSKY, G. N. (1976). Neurophysiology of locomotor automatism. *Physiol. Rev.* **56**, 465–501.

SWETT, J., ELDRED, E. & BUCHWALD, J. S. (1970). Somatotopic cord-to-muscle relations in efferent innervation of cat gastrocnemius. *Am. J. Physiol.* **219**, 762–766.

SZÉKELY, G. & CZÉH, G. (1967). Localization of motoneurones in the limb moving spinal cord segments of Ambystoma. *Acta physiol. hung.* **32**, 3–18.

SZÉKELY, G., CZÉH, G. & VOROS, G. (1969). The activity pattern of limb muscles in freely moving normal and deafferented newts. *Expl. Brain Res.* **9**, 53–62.

EXPLANATION OF PLATE

Transverse views of stage 36 motor nuclei. *A*, adductor nucleus at beginning of LS 2 consists of a cluster of medial cells (below asterisk) containing granular HRP reaction product. Lateral cells are unstained. White matter borders lateral motor column at bottom and right of picture. Large dark bodies scattered in grey matter are red blood cells. The motor nuclei shown in *B* and *C* are taken from opposite sides of the cord from the same section at the middle of LS 7. *B*, injection of the right ventral shank musculature labelled a central cluster of cells, leaving a band of unstained cells bordering the white matter dorsally (asterisk). It can be seen in *C* that this region on the opposite side of the cord was labelled by injection of the left caudilioflexorius muscle. Calibration bar for all photographs = 50 μm.