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The Localization of Motoneurons Supplying the Hindlimb Muscles of the Mouse

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THE LOCALIZATION OF MOTONEURONS SUPPLYING THE HINDLIMB MUSCLES OF THE MOUSE

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The method of retrograde axonal transport of horseradish peroxidase (HRP) has been used to localize the motoneurons that innervate the mouse hindlimb musculature. Motoneurons were labelled following either intramuscular injection of an HRP solution or application of HRP to the cut end of a muscle nerve. When intramuscular injection was used the nerves to adjacent muscles were cut and deflected from the injection site to prevent motoneurons projecting to these muscles being labelled with HRP. For some muscles this procedure was inadequate since the nerves to adjacent muscles were too short to enable adequate deflexion. The motoneurons projecting to these muscles were labelled by the method of cut nerve exposure.

The motoneurons that project to a single muscle or a group of muscles were organized as longitudinal columns. The positions of such motonuclei within the lateral motor column were similar in different animals for any given muscle or muscle group. Motoneurons innervating the anterior and medial femoral muscles were located in spinal segments L1 and L2. Motoneurons innervating the remaining hindlimb muscles were found in segments L3–L5. Topographic relationships between muscle motonuclei were in general found to be similar to those described for the cat. The principal differences to be noted between the two species were that the adductors motonucleus did not overlap with the hamstrings motonucleus in the mouse. Also the motonuclei supplying the deep flexors of the crural musculature and intrinsic musculature of the foot were located more ventrally relative to the posterior crural motonucleus in the mouse as compared to the cat.

Consideration of muscle homologies between vertebrate classes enabled comparisons of the localization of motonuclei between the mouse and the other species studied. It was found that topographical relations between motonuclei were similar in all the species so far studied.

There was no absolute correlation between the rostrocaudal position of a motonucleus and the position in the hindlimb of the muscle that it innervated. In general, motonuclei innervating muscles derived from the dorsal muscle mass were located lateral to motonuclei innervating muscles derived from the ventral muscle mass. Furthermore, within each muscle mass there is a relationship between rostrocaudal position of a motonucleus and the anteroposterior position of the muscle it supplies. Thus there is a relation between position of a motonucleus within the spinal cord and the derivation from the embryonic muscle mass of the muscle that it supplies.

1. Introduction

The problem of motoneuronal localization has been studied since Sherrington (1892) showed that motoneurons innervating a given muscle are confined to one or two segments of the spinal cord. Early studies (reviewed by Strauss (1946) and Romanes (1951)) showed that the moto-

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neurons supplying the appendicular musculature were confined to the lateral motor columns in the lumbar and cervical enlargements but these studies failed to clarify the relationships between individual muscle motonuclei. Romanes (1951) in his study of the cat used the chromatolysis that follows nerve section as a marker and showed that each motonucleus supplying a muscle or group of muscles in the hindlimb is organized as a longitudinal column. These columns have a clearly defined and reproducible position in the lateral motor columns. Sharrard (1955) in examination of *post mortem* poliomyelitis cases extended these observations to the human spinal cord. Essentially similar results have been obtained in three non-mammalian species: the frog (Cruce 1974); *Xenopus* (Lamb 1976); and chick (Landmesser 1978*a*). The information on motoneuronal localization in the rodent spinal cord (Romanes 1946; Kaizawa & Takahashi 1970; Brushart & Mesulam 1980) is incomplete. Such information was required as part of a larger study on motoneuronal development in normal and neurological mutant mice and this paper reports the results of such a study in this species.

The majority of earlier investigations into this problem have used chromatolysis following either nerve crush or section, or limb amputations accidental or experimental. Romanes (1964) has observed that chromatolysis is unreliable in adult rodent spinal motoneurons. This fact and the demonstration that retrograde transport of horseradish peroxidase (HRP) from muscle to motoneurons (Kristensson & Olsson 1971) might be a quantitatively reliable technique to examine central locations of motoneurons (Burke *et al.* 1977) led us to use HRP as a cell marker in our experiments.

A preliminary report of some of this work has been published (Biscoe & McHanwell 1979).

2. Methods

2.1. *Animals used in this study*

The experiments were performed on 88 mice of the C129 ReJ strain, 2–3 months of age and of either sex. They were bred and maintained in the animal house at Bristol and given food and water *ad libitum*.

2.2. *Horseradish peroxidase experiments*

2.2.1. *Application of horseradish peroxidase*

The animals were anaesthetized with sodium pentobarbital (60 μg g^{-1}). A cannula was inserted into the peritoneum to maintain anaesthesia. The mice were then subjected to one of two experimental procedures. In the majority of cases preliminary experiments (see §3.1) showed that spread of HRP to adjacent muscles following intramuscular injection could be controlled by section of nerves to these muscles. In these cases the muscle was injected with a solution of HRP (100 mg ml^{-1}, Sigma type VI) dissolved in sterile 0.15 M NaCl solution. A syringe assembly consisting of a glass pipette (tip diameter 50–70 μm) attached to a side filling 10 μl syringe was used to inject the muscles. The volume injected varied from 7 to 20 μl according to the muscle size and an attempt was made to infiltrate the entire muscle or muscle group as judged by discoloration of both surfaces. Multiple injections were necessary to achieve this and care was taken to confine the injection to the muscle(s) being investigated.

It was not possible to sever all the branches of the gluteal and inner pelvic muscle nerves without damaging the remainder of the sciatic nerve. To ensure that HRP was not taken up at

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these sites nor at the cut ends of other nerves a series of control experiments was performed in which the nerve to the muscle injected was also severed before injection.

Where the results of these experiments (see §3.1) showed that uptake at the cut ends of nerves to adjacent muscles could occur, an alternative method was used. The cut end of the muscle nerve was placed in a small polyethylene tube and solid HRP (1–2 mg) was applied to the cut end. After 20 min the tube and HRP were removed and the end of the nerve was washed with sterile saline (Oldfield & McLachlan 1980). Control experiments in which the nerve was sectioned proximal to the application site and before application resulted in no labelled motoneurons being found in the spinal cord.

Following either procedure the wound was washed with sterile saline and closed in layers. The animals were then allowed to recover. In the majority of experiments HRP was applied bilaterally, though in three experiments unilateral applications were made.

2.2.2. *Fixation*

After a survival period of either 24 or 48 h the animals were reanaesthetized with sodium pentobarbitae. The chest wall was opened and the right auricle was cut to permit drainage. A 20 G stub adaptor attached to a syringe was inserted into the aorta via the left ventricle and perfusion was done by hand. The animals were perfused with 20 ml of 0.15 M NaCl solution (*ca.* 37 °C) followed by 60 ml of 4% (by volume) glutaraldehyde solution (*ca.* 4 °C); both solutions were made up in 0.1 M phosphate buffer, pH 7.4.

The spinal cords were removed immediately after perfusion. A laminectomy was performed from L6–T9 and the spinal segments were identified and marked, before removal of the tissue, in the following way. The dorsal roots were dissected back to their point of origin into the dorsal horn, and opposite the most caudal rootlet of each dorsal root the spinal cord was pierced through to the ventral horn and as near normal to the surface of the cord as possible. The markers were made on the right side of the cord but close to the midline. The dorsal roots were numbered from the next rostral vertebral body to the dorsal root ganglion from which they originated. The spinal cord segments L6–T13 were then removed as a single block.

The tissue was postfixed in 4% (by volume) glutaraldehyde solution for 3 h, transferred to 0.05 M sucrose solution for 1 h and then to 0.99 M sucrose solution overnight; all solutions were made up in 0.1 M phosphate buffer, pH 7.4, and kept at 4 °C.

2.2.3. *Demonstration of horseradish peroxidase*

Serial horizontal frozen sections of 50 μm were cut on an M.S.E. freezing microtome with a Pelcool stage. The sections were transferred to 0.1 M phosphate buffer, pH 7.4, mounted in serial order on gelatin-coated slides and incubated for demonstration of horseradish peroxidase. In the majority of experiments the method used for demonstrating the retrogradely transported HRP was that of Hanker *et al.* (1977). The sections were washed for 10 min in 0.1 M Tris–HCl (Analar, B.D.H.) buffer, pH 7.6. The concentration of combined *p*-phenylenediamine (1 part by mass) catechol (2 parts by mass) reagent was 12 mg in 100 ml of 0.1 M Tris–HCl, pH 7.6 (both reagents were obtained from Sigma). The slides were washed, dehydrated and mounted without staining in Permount. The use of DPX was found to result in rapid fading of the reaction product. In three experiments the method of Mesulam (1978), which employs 3-3′,5-5′-tetramethylbenzidine as the chromogen, was used to demonstrate the retrogradely transported

HRP. Four control spinal cords from non-injected animals were incubated for HRP by the method of Hanker *et al.* (1977); in two animals the sciatic nerve was cut unilaterally 24 h before the animals were killed, while in the other two animals the sciatic nerve was cut 48 h before killing.

2.2.4. *Analysis of the location of motoneurons*

All the experiments were analysed in the following manner. Sections containing labelled structures were drawn by means of a camera lucida with a $\times 16$ eyepiece attached to a Reichert Biopan microscope with a $\times 4$ objective; the height of the drawing surface was adjusted to produce an overall magnification of $\times 63$. The section outlines and white–grey matter boundaries were drawn and the positions of the segmental markers were indicated. The positions of labelled structures were marked on these diagrams with use of a $\times 10$ objective with a $\times 6.3$ eyepiece in the camera lucida. The segmental markers were followed through the sections and their positions on the diagrams were corrected if the segment markers had not been made normal to the cord surface.

The sections were next examined at high power ($\times 40$) on a Zeiss Photomicroscope II by means of both bright field and phase contrast illumination. The following criteria were used to identify HRP-labelled structures as motoneuron soma: the presence of a granular brown reaction product confined to the cytoplasm with a nucleus visible as an area relatively clear of reaction product and the presence within the nucleus of a nucleolus surrounded by reaction product (see figures 2 and 3). It was found that the nucleolus was often visible with bright field illumination but could be more easily visualized with phase contrast illumination. The nucleolus had to be surrounded by reaction product to exclude the possibility that it originated from an unlabelled profile above or below the labelled structure in question. Figures 2 and 3 illustrate these criteria. Only structures satisfying these criteria were retained on the diagrams, from which total cell counts were then made. All the labelled cells were then plotted onto a single representative horizontal section so as to indicate the segmental extent of the motonucleus and the cellular density at any given region within it.

For some experiments reconstructions were made in the transverse plane to localize the position of the motonucleus more accurately in the dorsoventral axis; at least two such reconstructions were made for each muscle or muscle group injected. All the sections were drawn and aligned with respect to the segment markers, and section outlines and reconstructions were made at three or more levels of the motonucleus.

2.3. *Reconstruction of cell columns*

2.3.1. *Preparation of material for light microscopy*

Twelve mice were employed in this study. The animals were perfused, spinal segments were marked and tissue was processed as described in §2.2.2. Five spinal cords were cut horizontally, four were cut sagittally and three were cut coronally at 50 μm on a M.S.E. freezing microtome with a Pelcool freezing stage. All spinal cords were cut serially and mounted from 0.1 M phosphate buffer, pH 7.4, onto gelatin-coated slides in serial order. The sections were stained overnight in gallocyanin at room temperature (Einarson 1932).

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2.3.2. *Method of reconstruction*

The cell columns were reconstructed according to a method modified from Elliott (1942). Camera lucida drawings were made of the sections, and the positions of motoneurons with a clearly defined nucleolus were marked. The relatively small size of mouse motoneurons meant that it was only necessary to superimpose two 50 μm sections to be able to define the nuclear groupings, rather than up to 3 mm of sections as has been employed in some other studies; indeed nuclear groupings could frequently be defined by examining single 50 μm sections.

3. Results

3.1. *Control experiments*

Control experiments were performed to examine the possibility of transfer of HRP to motoneurons via routes other than retrograde axonal transport from the muscle injected and to ensure that mouse spinal motoneurons or interneurons did not contain endogenous peroxidase activity.

There was no evidence for endogenous peroxidase activity in spinal motoneurons, interneurons or non-neuronal structures other than erythrocytes in spinal cords taken from animals either where the muscle nerves had been left intact before death or where they had been cut 24 or 48 h previously. Unilateral injection of HRP into hindlimb muscles resulted in labelled motoneurons being found on the ipsilateral side only. Thus there was no evidence for any contralateral motoneuronal projections in the mouse nor was there any evidence for vascular transfer of HRP to motoneurons. Further evidence against such a vascular route came from experiments in which the hindlimb was entirely denervated before injection (see below), when no HRP-labelled motoneurons could be found in the ventral horn.

In ten preliminary experiments the nerves to adjacent muscles were left intact before the injection of HRP. Injections were made into two muscle groups, posterior crural musculature and hamstring muscles, and the animals were killed 24 or 48 h later. In the posterior crural muscles failure to section adjacent muscle nerves resulted in labelling in two columns additional to, and ventral and lateral to, the motoneurons labelled when the adjacent muscle nerves had been sectioned. These results were later interpreted, on the basis of the detailed results of the motoneuronal localization experiments, as being due to uptake from the hamstring and anterolateral crural muscles (see figure 29). In the hamstring muscles, leaving adjacent muscle nerves intact resulted in labelled motoneurons being observed in a more rostral position and this was interpreted as being due to uptake from the adductor muscles. Such spurious labelling was not controlled by careful injection of the muscle or by a reduction of either the volume or concentration of the HRP solution injected except to amounts where the labelling of the target motonucleus was also reduced. It was clear from these experiments and the results of other workers (Burke *et al.* 1977; Richmond *et al.* 1978) that only the section of nerves to adjacent muscles could control the spread of HRP from the injection site.

However, HRP does not require the presence of an intact neuromuscular junction for its uptake since the cut end of a nerve can be an effective site. This fact, together with the difficulty of cutting all the branches of the nerves to the gluteal and inner pelvic musculature without endangering the sciatic nerve, necessitated a further series of control experiments. In these experiments, in addition to the nerves to the adjacent muscles, the nerve to the target muscle

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group was cut before injection of HRP. The following muscle groups were injected: quadriceps femoris, adductors, hamstring, posterior crural, anterolateral crural and intrinsic muscles of the foot. At least two experiments at both 24 and 48 h survival times were performed for each muscle group examined. In all these experiments care was taken to deflect the cut ends of the nerves from the site of injection. There were no labelled motoneurons in any of the target motonuclei, as defined tentatively on the basis of preliminary experiments, for each of the muscle groups injected. Also there were no labelled motoneurons in the position of the gluteal motonucleus. However, the injection of quadriceps femoris and adductor muscles invariably resulted in labelled motoneurons being found in a position more rostral (L1–T13) and medial to the main motonucleus (see figure 1). Labelled motoneurons were also found in this position in some of the experiments in which the hamstring muscles were injected. These motoneurons were presumed to supply the psoas group of muscles (see Sharrard 1955). Their position was sufficiently distinct to prevent inclusion within other motonuclei and they were excluded from further analysis. In the experiments where the hamstring or adductor muscle groups were injected labelled motoneurons were also found in a ventral and caudal position. These motoneurons were presumed to supply the inner pelvic musculature and this was later confirmed by direct injection of the muscles concerned. While the motoneurons supplying this muscle were located close to the hamstring motonucleus their position was again sufficiently distinct to prevent their inclusion with that motonucleus. Thus the uptake of horseradish peroxidase from cut nerve ends was not a complicating factor where the nerve stump could be deflected from the injection site.

It is not always possible to deflect the cut nerve stump away from the injection site where the branch from a nerve supplying a particular muscle is very short. This is frequently so in the mouse where the terminal branches of the major nerve trunks branch to supply individual muscles. In some experiments where an attempt was made to fractionate a motonucleus supplying a muscle group by the injection of individual muscles that compose that group it was clear that uptake must have occurred at the cut ends of nerves. This was investigated in a final series of control experiments in which the nerves to the posterior crural musculature were cut in the popliteal fossa close to the muscles that they supply and the medial gastrocnemius muscle was injected. The nerves were cut either at the time of injection or 24 or 48 h earlier and the animals were killed 24 or 48 h after injection; at least two experiments were performed in each case. The remaining muscles in the hindlimb were denervated at the time of injection. The results showed that in no case was it possible to prevent uptake of horseradish peroxidase. The labelling was confined to the posterior crural and foot motonuclei; the posterior tibial nerve was also sectioned in the popliteal fossa. As the time interval between nerve section and death was extended motoneuron labelling became diffuse rather than granular but occasional neurons showing granular labelling could always be found. Motoneurons showing both types of labelling were distributed throughout the motonuclei labelled. If the medial gastrocnemius nerve was left intact then a discrete column of motoneurons showing dense, granular labelling was found in a reproducible position within the main motonucleus. This column of neurons could be distinguished at 48 h survival times from another group of motoneurons surrounding them showing diffuse labelling though, as would be expected, some granular labelled motoneurons were found outside the main column of motoneurons showing this type of labelling.

Such an approach is not ideal, however, because these experiments showed that granular labelling was not invariably associated with uptake of enzyme at an intact endplate. Con-

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sequently in all subsequent experiments where the cut ends of nerves to adjacent muscles could not be deflected some distance from the injection site then a control experiment was also performed in which the nerve to the target muscle was additionally cut. Only if the results of such control experiments showed no evidence of labelled motoneurons in the spinal cord was the method of intramuscular injection used to label motoneurons supplying that particular muscle or muscle group. If the results of such control experiments were not satisfactory then the motoneurons were labelled by the application of HRP to the cut end of the muscle nerve.

3.2. *The appearance of labelled motoneurons*

The motoneurons supplying any given muscle were arranged as a longitudinal column with a defined and reproducible position in the lateral ventral horn. Figure 1 is a low-power photomicrograph of one horizontal section through the ventral horn taken from an experiment in which the adductors and quadriceps femoris muscle groups had been injected on the right and left sides respectively and shows the appearance of these columns; also visible are a group of motoneurons in a rostral and medial position which are presumed to have supplied the psoas group of muscles (see §3.1).

The criteria that were applied in determining whether labelled profiles should be counted as motoneurons have been discussed in §2.2.4. Figures 2 and 3 show three labelled profiles in the inner pelvic muscle motonucleus made visible by means of phase contrast (figure 2) or bright field (figure 3) illumination. The horseradish peroxidase reaction product is granular and appears confined to the cytoplasm. The large and small profiles on the left of the figure both contain nucleoli within the nucleus. The nucleoli appear as dense, refractile bodies clearly seen only by means of phase contrast illumination and this emphasizes the importance of examining all labelled profiles by such illumination. The profile on the right does not have a nucleolus and is interpreted as being a fragment of a larger neuron, the nucleolus of which appears in an adjacent section; such a fragment would not be included in a count of the total number of motoneurons. The question of whether there is a bimodal distribution of motoneuronal size will be dealt with in the subsequent paper (McHanwell & Biscoe 1981).

The density of reaction product within motoneurons varied both between experiments and within an individual motonucleus. In general labelling was heavier and more consistent after a 24 h survival period than after a 48 h survival period. It was also observed that the labelling within motoneurons labelled after exposure of the cut ends of their nerves was lighter and more variable than the labelling obtained after intramuscular injection. Many motoneurons in the former case exhibited granular labelling but other motoneurons could be observed to be labelled diffusely. This finding is in agreement with those obtained by other workers (Richmond *et al.* 1978).

3.3. *The anatomy of motoneuronal nuclei*

Figures 4–27 show the density and segmental location of motoneurons supplying given muscles together with reconstructions in the transverse plane localizing the motonuclei in the dorsoventral and mediolateral axes. Figure 28 is a summary figure constructed on the same general plan as that prepared by Romanes (1951) for the cat to facilitate the comparison between his work and that of the present study. Figures 29 (*a–e*) show photomicrographs of Nissl-stained preparations of the spinal cord in transverse section, beside which are drawings outlining the motoneuronal columns described in §3.5 and indicating the projections to the

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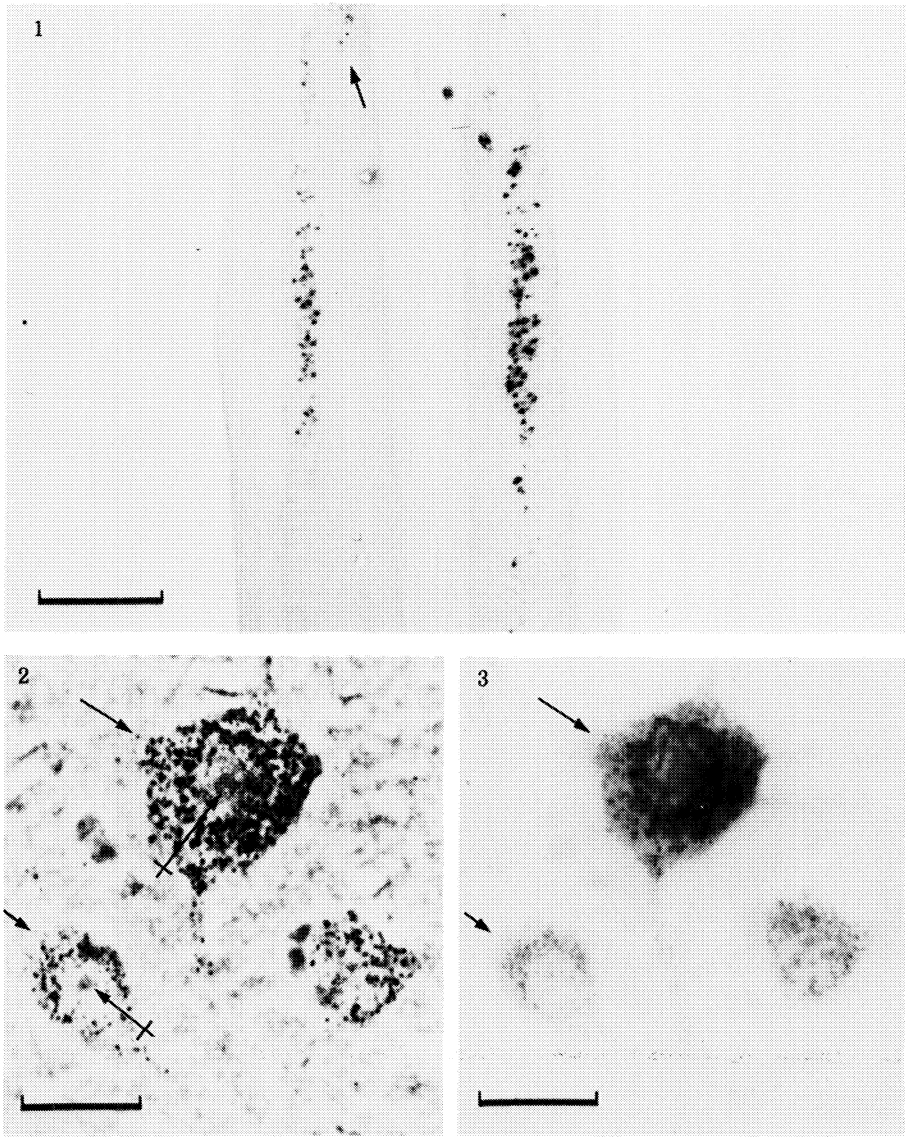

FIGURE 1. A low-power bright field photomicrograph of a 50 μm horizontal section through the ventral horn of lumbar segments L1 and L2. Horseradish peroxidase containing motoneurons in the quadriceps femoris (left) and adductors (right) motonuclei can be seen. Motoneurons in a more rostral and medial position are also visible (arrow). These are presumed to be psoas motoneurons. Calibration bar, 500 μm.

FIGURE 2. A light micrograph of a 50 μm horizontal sections showing three horseradish peroxidase labelled profiles in the inner pelvic motonucleus viewed with phase contrast illumination. The small profile on the left (shorter arrow) has a clearly defined nucleolus (crossed arrow) and a soma area of less than 275 μm^2 and is presumed to be a gamma motoneuron. The large profile (longer arrow) also has a clearly defined nucleolus (crossed arrow) but a soma area of more than 275 μm^2 and is presumed to be an alpha motoneuron. The small profile on the right does not have a clearly defined nucleolus and is presumed to be a fragment of a larger motoneuron. Only the first two profiles would be counted as motoneurons. Calibration bar, 20 μm.

FIGURE 3. A light micrograph of the same neurons as in figure 2 but viewed with bright field illumination. The nucleoli are less easily distinguished with this method of illumination.

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muscles that they supply defined on the basis of the HRP experiments. These figures illustrate the relations between the motoneuronal nuclei and emphasize that, with hindsight, it is possible to examine Nissl-stained sections of the mouse spinal cord and attribute to particular groups of ventral horn motoneurons projections to particular muscle groups.

The results of motonucleus localizations from the 24 and 48 h survival time experiments were similar and will be treated together in this section. Only one set of transverse reconstructions is shown for any motonucleus since it was found that the localization in the dorsoventral and mediolateral axes of any given motonucleus was remarkably constant between experiments. The rostrocaudal position of the motonuclei did vary between experiments. The majority of mice used in this study had six lumbar vertebrae. When the results from experiments involving these animals were treated as a group the maximum variation in segmental localization for a given motonucleus was half a segment; these variations will be discussed separately for each motonucleus. From the results of experiments where bilateral injections were made it was observed that both motonuclei on either side of the spinal cord would show a similar degree of rostrocaudal displacement, that is, their rostrocaudal relationships were preserved. Romanes (1951) reported much greater variations in the cat in the segmental position of motonuclei but similarly observed parallel shifts in rostrocaudal position of different motonuclei.

Some of the mice used in this study have five lumbar vertebrae (see also McClaren & Michie 1954). In these animals the position of the motonuclei was consistently shifted by up to a full segment more rostral. Since only five such animals were discovered the positions of only four motonuclei in this group of animals were studied: quadriceps femoris, adductors, hamstring and inner pelvic muscles. The results of these experiments were excluded from the main body of results and are not illustrated; one example will suffice. The position of the adductor motonucleus was observed to be from upper L1 to mid-L2 in one such experiment. This can be contrasted to its more usual position from lower L1 to upper L3. There was no evidence for a reduction in the total number of motoneurons.

The anatomy of each motonucleus will now be described in turn.

3.3.1. *Anterior femoral muscles*

3.3.1.1. *Quadriceps femoris*. This group of muscles was injected in nine experiments and figure 4 shows the localization of the motonucleus supplying these muscles. The majority of neurons are located in L2 segment, with some cells in the upper half of L3 and some isolated cells in the lower third of L1. The other experiments showed that the nucleus started in the upper third to base of L1 and that it terminated in the upper third to half of L3. The transverse reconstructions show that the rostral region of the nucleus is located in a ventrolateral position and that as the nucleus extends caudally the nucleus expands both dorsally and laterally so that at mid-L2 the nucleus accounts for over half the motoneurons in the lateral ventral horn. There is a suggestion, from the reconstructions, that two separate columns compose the quadriceps motonucleus though this is not entirely clear except in the caudal most transverse reconstructions, where the main nucleus has split into a ventrolateral and dorsomedial portion.

The sartorius muscle is only present in mice, as a vestige, up to the 13th day of embryonic life (Lance Jones 1979) and thus its motonucleus location was not determined in these experiments.

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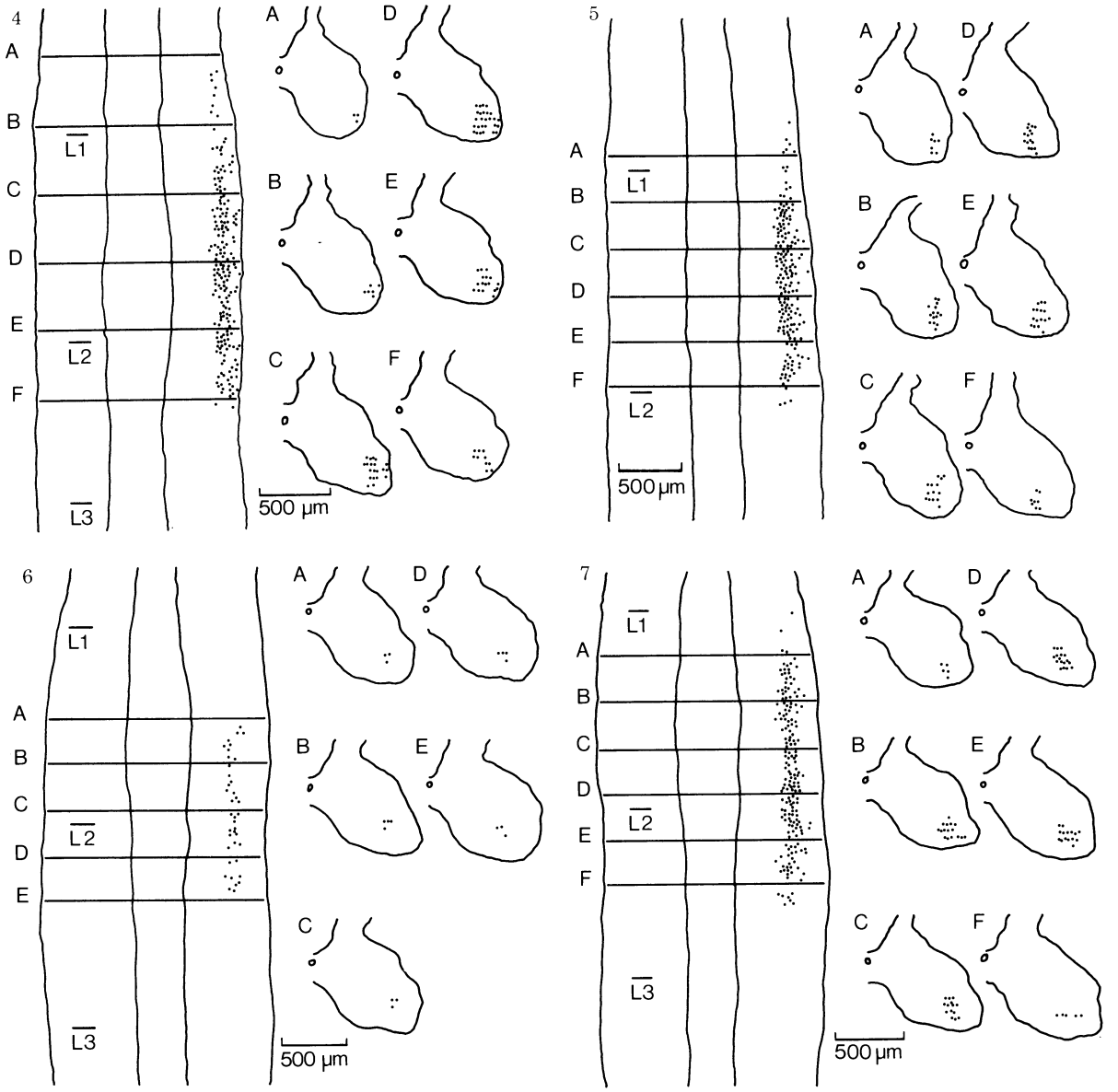

FIGURE 4. A reconstruction of the quadriceps femoris motonucleus from one experiment; the reconstruction was prepared as described in §2.2.4. This and all the subsequent reconstructions of motonuclei are all presented in a similar way. The diagram on the left is a horizontal section of the spinal cord on which are indicated (by dots) the positions of motoneuronal soma plotted from camera lucida drawings. The short horizontal lines show the lower border of the segment indicated determined as described in §2.2.4. The longer horizontal lines indicate the rostrocaudal levels at which transverse reconstructions were made. These transverse reconstructions are shown on the right side of the figure; the letters indicate the level at which each reconstruction was made. The motoneuron soma indicated on these reconstructions were those occurring 100 μm rostral and 100 μm caudal to the level of reconstruction. In all these diagrams the grey–white matter boundaries only are shown.

FIGURE 5. A reconstruction of the adductors and gracilis motonucleus.

FIGURE 6. A reconstruction of the gracilis motonucleus.

FIGURE 7. A reconstruction of the adductors motonucleus.

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3.3.2. *Medial femoral muscles*

3.3.2.1. *Adductors and gracilis*. This muscle group was injected in 15 experiments. Figure 5 shows the localization of the motonucleus supplying the adductors and gracilis muscles. The motoneurons supplying this group of muscles were located predominantly in L2 with some in L1; in the experiment illustrated some motoneurons were found in L3. The other experiments showed that the motonucleus could start in the upper half of L1, terminating at the base of L2 or in the upper half of L3. The transverse reconstructions show this motonucleus to occupy a medial position throughout its length. As the nucleus extends caudally it occupies a more ventral and medial position in the lateral column. The lateral border of this nucleus is contiguous with the medial border of the quadriceps motonucleus, though the results of experiments in which these two muscles were injected on opposite sides show that the motonuclei do not overlap at any given rostrocaudal level.

The adductors of the thigh and the gracilis muscles were injected separately in two experiments. The localization of motoneurons supplying the gracilis muscle is shown in figure 6. The gracilis motonucleus in this experiment was located in lower L2 and upper L3 and in the dorsal region of the main motonucleus. A comparison of figures 6 and 7 shows that there is a considerable overlap of the gracilis and adductors motonuclei except for the caudal region of the gracilis motonucleus, which is situated dorsally with respect to the adductors motonucleus.

3.3.2.2. *Pectineus*. In the mouse the pectineus muscle is supplied by a branch of the femoral nerve; very rarely is it innervated by a branch of the obturator nerve (Lance Jones 1979; McHanwell 1980). This muscle was injected in four experiments. Figure 8 shows the localization of the motonucleus supplying this muscle. The motoneurons supplying pectineus were located in the lower half of L1 and the upper half of L2. The other experiments showed that this motonucleus could start anywhere within the lower half of L1 and terminate anywhere within the lower half of L2. It was situated in a lateral and ventral position throughout its length.

3.3.3. *Gluteal muscles*

This muscle group was injected in eight experiments. Figure 9 shows the localization of motoneurons supplying the gluteal muscles. This motonucleus is situated caudally with respect to the previously described motonuclei. The motoneurons supplying this group of muscles are located in L3 and L4. The other experiments showed that the nucleus could start in the upper quarter of L3 and could terminate in the lower quarter of L4, never extending beyond the base of that segment. The upper region of this motonucleus is located in a ventrolateral position but throughout the remainder of L3 and upper L4 extends more medially and dorsally. The most caudal region of this motonucleus again occupies a ventrolateral position.

3.3.4. *Quadratus femoris and inner pelvic muscles*

This group of muscles was injected in four experiments while the inner pelvic musculature was injected in a further two experiments. Figure 10 shows the localization of motoneurons from an experiment in which both the quadratus femoris and inner pelvic muscles were injected. Two motonuclei were labelled, a rostral motonucleus located in L3 and a caudal motonucleus located in L4 and L5. In the two experiments in which the inner pelvic musculature was injected after sectioning the nerve to quadratus femoris labelling was confined to the caudal group

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of motoneurons. This confirmed that this group of motoneurons supplied the inner pelvic musculature while the rostral group of motoneurons supplied the quadratus femoris muscle.

The quadratus femoris motonucleus is located ventromedially in L3 and it overlaps, at its dorsal border, with the semimembranosus motonucleus. In one experiment a few motoneurons were found in L2, in two experiments some motoneurons were found in L4, but in the majority of experiments the inner pelvic motonucleus was located in L5. The rostral part of this motonucleus is located in a ventromedial position but caudally occupies a more ventrolateral position.

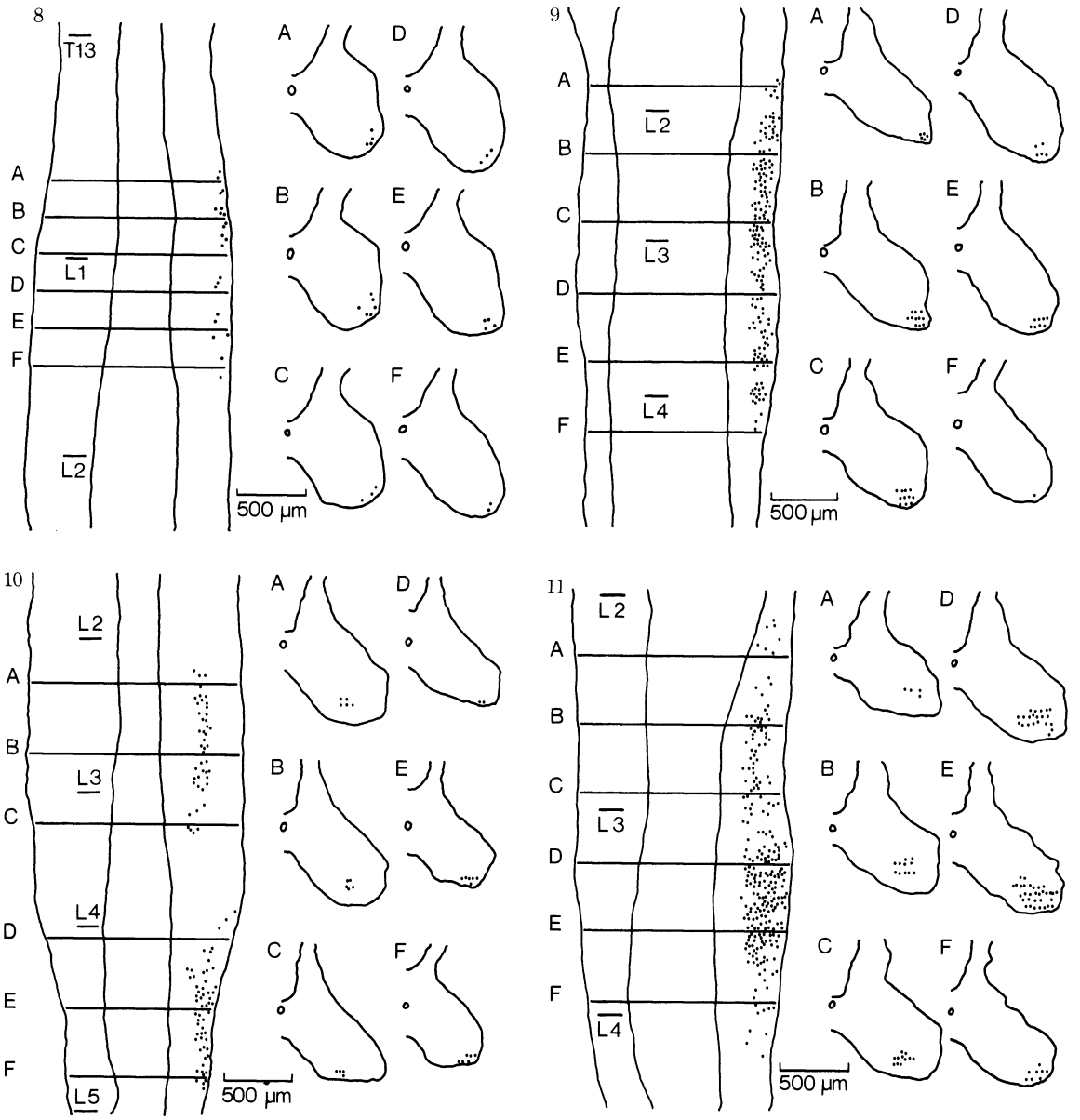

FIGURE 8. A reconstruction of the pectineus motonucleus.

FIGURE 9. A reconstruction of the gluteal muscle motonucleus.

FIGURE 10. A reconstruction of the quadratus femoris and inner pelvic muscle motonuclei.

FIGURE 11. A reconstruction of the hamstring muscles motonucleus.

An explanation of the method of preparation and presentation of these figures is given in the legend to figure 4.

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3.3.5. *Hamstring muscles*

This muscle group was injected in nine experiments. The muscles that compose this group are the biceps femoris, including its anterior head, the principal and accessory heads of semitendinosus, caudofemoralis and semimembranosus, and they form the largest, by bulk, muscle group in the mouse. Figure 11 shows the localization of the motonucleus supplying this group of muscles.

This motonucleus is located within L3 and L4 segments. The other experiments showed that this nucleus started in the lower quarter of L2 or upper quarter of L3 and never extended beyond the base of L4. The rostral half of the nucleus is located dorsomedially but the caudal half expands laterally and ventrally to occupy much of the ventral half of the ventral horn and it terminates in a ventrolateral position. Thus there appears to be some overlap between this motonucleus and the gluteal motonucleus in L4. Since these two muscles are anatomically contiguous within the hindlimb it should be emphasized that control experiments in which the hamstring muscle nerves were sectioned before injection showed no labelled motoneurons in this region. Consequently it can be concluded that the motonuclei do in fact overlap.

3.3.5.1. *Semimembranosus and caudofemoralis*. This overlap is due to the caudofemoralis motonucleus. The caudofemoralis and semimembranosus muscles were injected as a group in two experiments. The nerve to caudofemoralis was too short a branch from the semimembranosus nerve to enable these muscles to be injected separately. Figure 12 shows the localization of motoneurons supplying these two muscles. Labelled motoneurons were found in two motonuclei, one located dorsomedially in lower L2 and L3 and the other situated laterally in L4 in a similar location to the gluteal motonucleus. By analogy with the results of Romanes (1951) it was assumed that the rostral nucleus in L3 supplied the semimembranosus while the caudal motonucleus supplied caudofemoralis. Without injection of these muscles separately this identification must remain tentative but it is of interest to note that the caudofemoralis motonucleus overlaps the gluteal motonucleus in the cat.

3.3.5.2. *Biceps femoris*. The anterior head of biceps receives a separate innervation from the remaining hamstring muscles via a branch of the inferior gluteal nerve. This small muscle was injected in three experiments and figure 13 shows the localization of the motoneurons that supply it. The motonucleus in this experiment was situated in the lower half of L3 and in L4. A similar location was observed in the other experiments. This small motonucleus is located in an extreme ventral and lateral position within the ventral horn.

The remaining heads of biceps femoris are supplied by branches from the main hamstring nerve. It was necessary to use the application of HRP to the cut ends of these branches to localize the motoneurons that supply these muscles (see §2.2.3). Figure 14 shows the result from one such experiment. It can be seen that the motoneurons that supply biceps femoris and located in the caudal half of the main hamstring motonucleus and predominantly, though not exclusively, in the lateral half of that nucleus.

3.3.5.3. *Semitendinosus*. The semitendinosus muscle can be divided into two heads in the mouse. The long head of semitendinosus receives a separate innervation via a branch of the sciatic while the short head is innervated from branches of the main hamstring nerve. The motoneurons that supply the long head of semitendinosus could be labelled satisfactorily by intramuscular injection. The motoneurons that supply the short head of semitendinosus were labelled by application of HRP to the muscle nerve. Figure 15 shows the localization of the motonucleus that supplies the long head of semitendinosus while figure 16 shows the localization

of the motonucleus that supplies the short head. In both cases the motonuclei are situated in the caudal half of the main hamstring motonucleus and in a medial position. There is a degree of overlap between the semitendinosus and biceps femoris motonuclei at their mediolateral border, as shown from experiments in which the two motonuclei were labelled on opposite sides of the spinal cord in one animal.

3.3.6. *Posterior crural muscles*

This group of muscles was injected in six experiments. The posterior crural group of muscles denotes all those muscles in the shank supplied by the tibial division of the sciatic nerve.

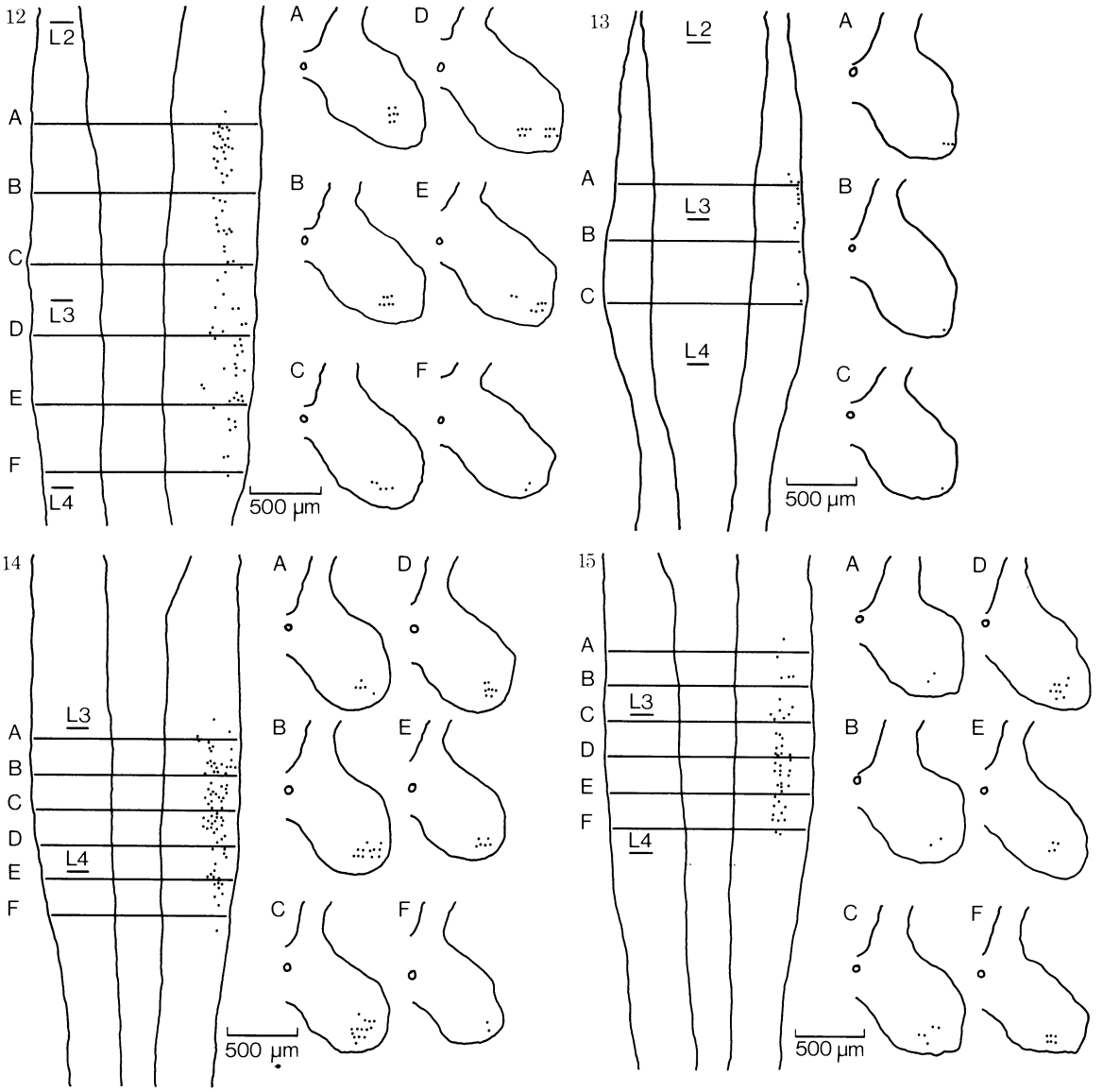

FIGURE 12. A reconstruction of the semimembranosus and caudofemoralis motonuclei.

FIGURE 13. A reconstruction of the motonucleus innervating the anterior head of biceps femoris.

FIGURE 14. A reconstruction of the biceps femoris motonucleus.

FIGURE 15. A reconstruction of the motonucleus innervating the principal head of semitendinosus.

An explanation of the method of preparation and presentation of these figures is given in the legend to figure 4.

Figure 17 shows the localization of the motonucleus supplying this group of muscles. The majority of motoneurons supplying this muscle group are located in L3 and L4 with some motoneurons in the lower part of L2 and upper part of L5 segments. The other experiments on the localization of this motonucleus showed that it started in the lower quarter of L2 or upper quarter of L3 and that it terminated at the base of L4 or upper half of L5. The rostral part of this motonucleus is located in the dorsomedial region of the lateral column and it expands dorsally and ventrally as it extends through L3. In L4 the dorsal region of the nucleus terminates while the ventral region extends into L5 and terminates in a ventromedial position.

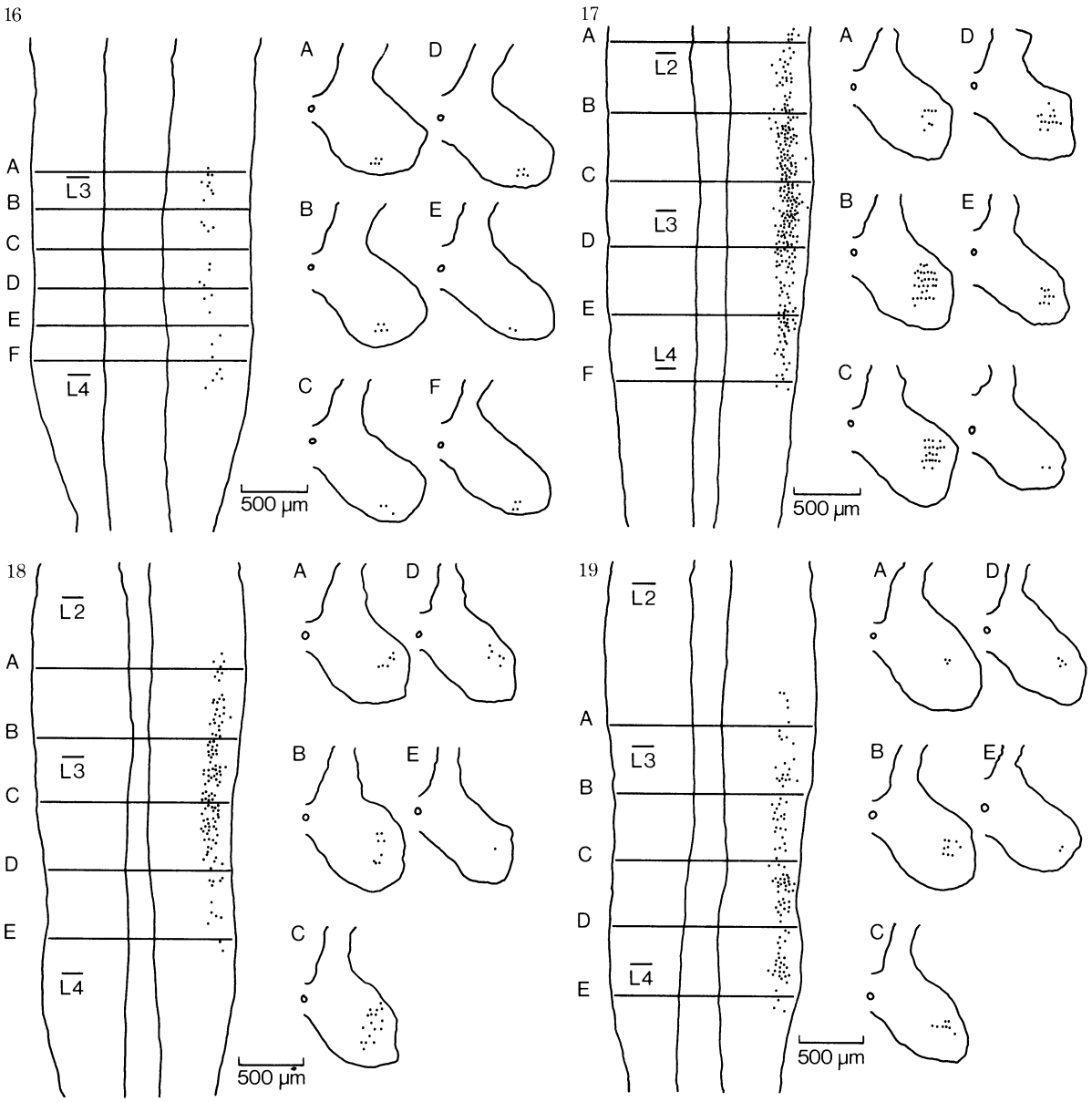

FIGURE 16. A reconstruction of the motonucleus innervating the accessory head of semitendinosus.

FIGURE 17. A reconstruction of the posterior crural muscle motonucleus.

FIGURE 18. A reconstruction of the motonucleus innervating the deep posterior crural muscles.

FIGURE 19. A reconstruction of the motonucleus innervating the triceps surae muscles.

An explanation of the method of preparation and presentation of these figures is given in the legend to figure 4.

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The muscles that compose this group can be further subdivided into a superficial and a deep group. The superficial group comprises the lateral and medial heads of gastrocnemius, soleus and plantaris. The deep group comprises flexor digitorum longus, flexor hallucis longus, tibialis posterior and popliteus. The distributions of the motoneurons that supply these two groups were examined separately.

The deep posterior muscles of the shank were injected in two experiments and figure 18 illustrates the localization of motoneurons supplying these muscles. In both experiments the

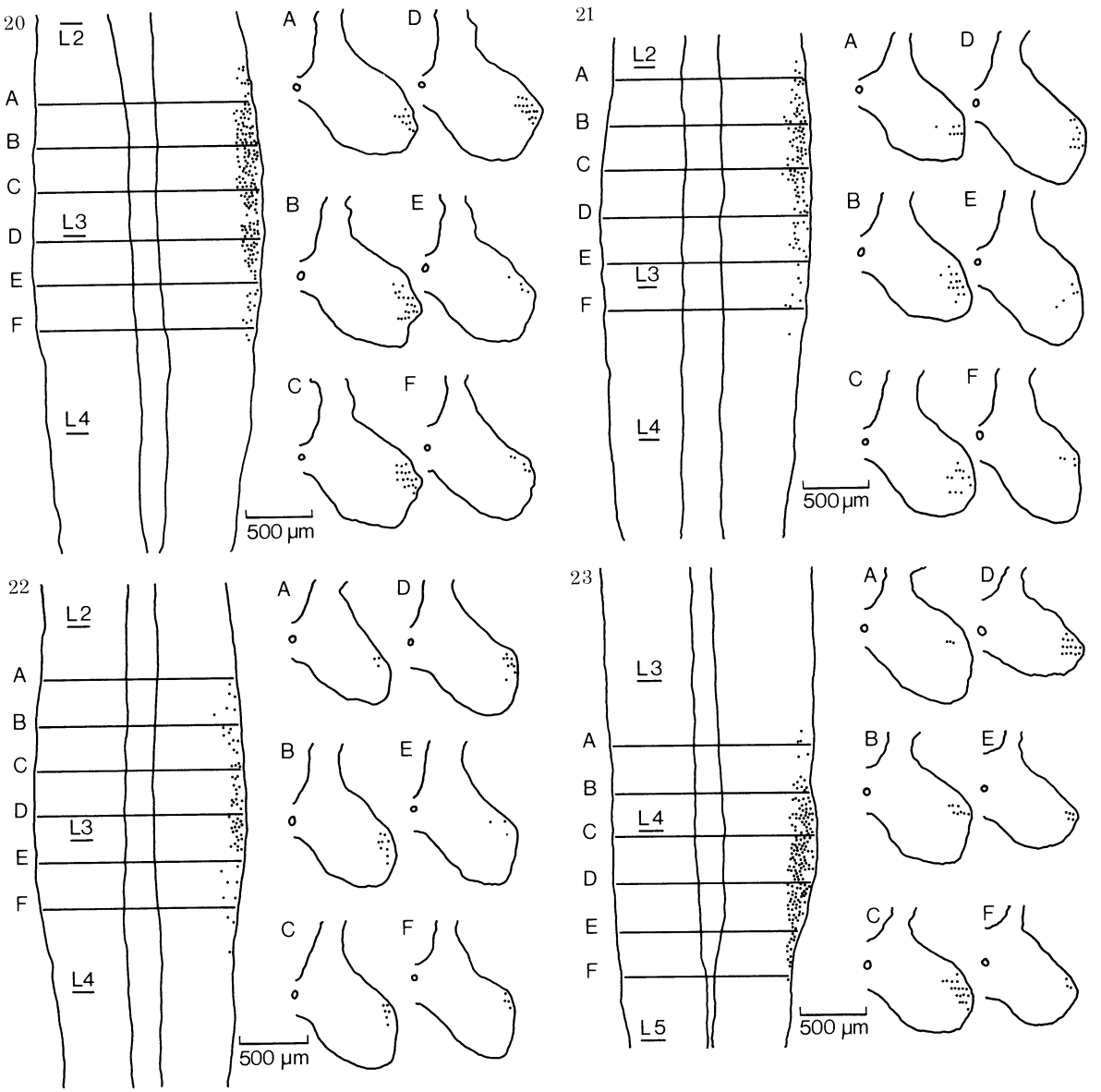

FIGURE 20. A reconstruction of the anterior and lateral crural muscle motonucleus.

FIGURE 21. A reconstruction of the motonucleus supplying muscles innervated by the deep peroneal nerve.

FIGURE 22. A reconstruction of the motonucleus supplying muscles innervated by the superficial peroneal nerve.

FIGURE 23. A reconstruction of the motonucleus innervating the intrinsic muscles of the foot.

An explanation of the method of preparation and presentation of these figures is given in the legend to figure 4.

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motonucleus was found to start in the upper third of L3 and terminate in the lower third of L4. The motoneurons supplying these muscles were located dorsally in the main posterior crural motonucleus, with a small separate column located ventrally. The superficial group were also injected in two experiments and figure 19 illustrates the localization of the motoneurons supplying these muscles. In both experiments the motonucleus was observed to start in the lower half of L3 and terminate in the upper half of L5. The motoneurons supplying these muscles were located ventrally in the main posterior crural motonucleus.

3.3.7. *Anterior and lateral crural muscles*

This muscle group was injected in seven experiments. The anterior and lateral crural group of muscles denotes those muscles in the shank supplied by the peroneal division of the sciatic nerve. Figure 20 shows the localization of the motonucleus supplying this group of muscles. The motonucleus is contained entirely within L3 and L4, with the majority of motoneurons located in L3. In the other experiments the motonucleus was observed to start from the lower border of L2 to the upper half of L3 while it terminated in the lower quarter of L4. The rostral portion of this motonucleus expands ventrally in the lateral column but the caudal region of this motonucleus is located at the extreme dorsal margin of the lateral column. At all rostrocaudal levels the anterior and lateral crural motonucleus is situated laterally to posterior crural motonucleus.

The peroneal nerve can be further divided into a deep and superficial division. The deep peroneal nerve in the mouse supplies tibialis anterior, extensor digitorum longus, extensor hallucis longus and extensor digitorum brevis, while the superficial peroneal supplies peroneus longus, brevis and tertius.

The locations of motoneurons that supply these two groups of muscles were examined by the application of HRP to the cut end of the deep and superficial peroneal nerves in three experiments. Figures 21 and 22 illustrate the results of one such pair of experiments for the deep and superficial peroneal nerves respectively. The motoneurons whose axons travel in the deep peroneal nerve are located predominantly within L3 and throughout the anterolateral crural motonucleus at these levels. The motoneurons whose axons travel in the superficial peroneal nerve are located somewhat more caudally and dorsolaterally but there is extensive overlap between the motonuclei supplying the two groups of muscles.

3.3.8. *Intrinsic musculature of the foot*

This muscle group was injected in seven experiments. Figure 23 illustrates the localization of the motonucleus supplying this group of muscles. The motonucleus extends over two segments, L4 and L5, starting in the upper half of L4 and terminating near the lower border of L5. In the other experiments this motonucleus was observed to start in the upper half of L4 and terminate within the lower half of L5. The rostral part of this motonucleus occupies a dorsal and medial position in the lateral column but as the motonucleus extends caudally it progressively occupies a more lateral and ventral position.

3.3.8.1. *Dorsal musculature*. The musculature of the foot can be further subdivided into dorsal and plantar muscles. There is one muscle on the dorsal surface of the foot, the extensor digitorum brevis, that receives its innervation from a branch of the deep peroneal nerve. The localization of the motoneurons that supply this muscle was examined separately in three experiments and figure 24 illustrates the result of one such experiment. The motoneurons are localized in the lower half of L3 and in an extreme dorsolateral position in the lateral ventral

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horn. The other experiments showed that the motoneurons could be situated anywhere within L3 but were always in a dorsolateral position.

3.3.8.2. *Plantar musculature*. The plantar musculature of the foot is innervated by three nerve branches from the sciatic; lateral and medial plantar nerves and the sural nerve via a lateral branch to the lateral plantar (McHanwell 1980). The localization of motoneurons whose axons are contained in each of these three nerves was examined in nine experiments. Figures 25 and 26 show the localization of motoneurons whose axons are contained in the lateral and medial

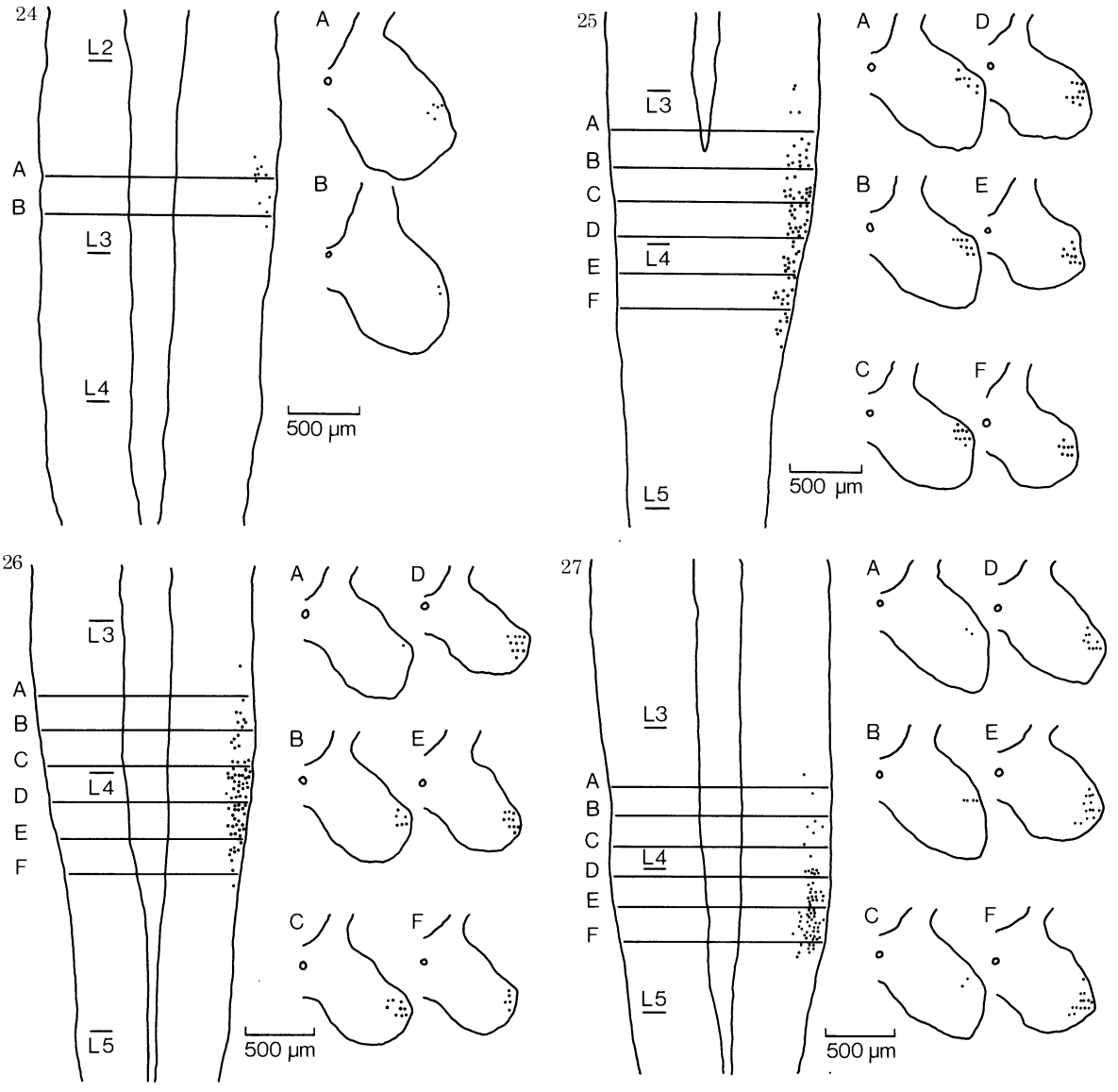

FIGURE 24. A reconstruction of the motonucleus innervating extensor digitorum brevis muscle.

FIGURE 25. A reconstruction of the motonucleus supplying the intrinsic musculature of the foot innervated by the lateral plantar nerve.

FIGURE 26. A reconstruction of the motonucleus supplying the intrinsic musculature of the foot innervated by the medial plantar nerve.

FIGURE 27. A reconstruction of the motonucleus supplying the intrinsic musculature of the foot innervated by the sural nerve.

An explanation of the method of preparation and presentation of these figures is given in the legend to figure 4.

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plantar nerves respectively; the small anastomotic branch from the lateral to the medial plantar nerve, which is given off near the tendons of the triceps surae, was included with the medial plantar nerve. It can be seen from figures 25 and 26 that these motoneurons are located in the rostral two-thirds of the main foot motonucleus and that there is no discernible localization of motoneurons within the motonucleus. The localization of motoneurons whose axons are contained in the sural nerve is shown in figure 27. The majority of these motoneurons are localized in the caudal third of the main foot motonucleus but again there is no discernible localization of motoneurons within a specified region of the motonucleus.

TABLE 1. NUMBERS OF MOTONEURONS IN MOTONUCLEI

survival time/h	24		48	
motonucleus	mean	range	mean	range
quadriceps femoris	196(2)	180–213	172(4)	140–209
adductors/gracilis	199(4)	185–213	192(8)	155–225
adductors			150(2)	130–171
gracilis			35(3)	27–39
pectineus	—		20(4)	13–27
quadratus femoris	—		42(4)	39–44
inner pelvic	—		46(6)	28–64
gluteal	198(2)	190–206	146(6)	109–184
hamstrings	242(2)	222–262	252(7)	225–283
semimembranosus	—		58(2)	55–61
caudofemoralis	—		28(2)	27–28
anterior head biceps	—		13(3)	11–16
biceps femoris	71(3)	64–79	—	
semitendinosus	40(3)	23–52	—	
accessory semitendinosus	—		33(2)	32–33
posterior crural	297(2)	195–299	251(4)	208–308
deep posterior muscles	—		151(2)	131–172
triceps surae	—		154(2)	116–193
anterior and lateral crural	184(2)	177–191	188(5)	185–195
superficial peroneal	68(3)	65–71	—	
deep peroneal	106(4)	81–117	—	
intrinsic foot musculature	210(2)	201–219	190(5)	167–219
extensor digitorum brevis	13(3)	11–15	—	
lateral plantar nerve	94(3)	72–122	—	
medial plantar nerve	76(3)	58–87	—	
crural nerve	55(3)	35–75	—	

3.4. *The number of motoneurons in motonuclei*

Table 1 shows the numbers of motoneurons within motonuclei, compiled from the results of the experiments described above. The mean appears to be greater after a 24 h survival period than after a 48 h survival period, except in the anterolateral crural muscle motonucleus. A χ^2 test, however, showed that there was no difference between the two sets of results ($P = 0.34$). An inspection of these results in table 1 shows that only in the gluteal motonucleus did the number of motoneurons obtained after a 24 h survival exceed the maximum number obtained after a 48 h survival. This suggests that the labelling obtained at 24 h was simply more consistent and did not reveal greater numbers of motoneurons *per se*. This suggestion is supported by the qualitative observation that labelling within motoneurons after a 24 h survival period

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was generally denser than that obtained after a 48 h survival period. Thus in the subsequent experiments on the location of individual muscle motonuclei either a 24 h or a 48 h survival time was used.

Localization of individual muscles or smaller muscle groups within the seven major groups showed that the sum of the number of motoneurons obtained in each case equalled that obtained after labelling the entire muscle group. This observation suggests that the labelling of motoneurons by retrograde transport of HRP allowed detection of a substantial fraction of motoneurons projecting to a given region. This result further suggests that labelling of motoneurons via the cut ends of their axons was also a quantitatively reliable technique despite the reduction in intensity of the label.

A second observation that this may be so comes from the use of the TMB method of Mesulam (1978), which has been reported as being far more sensitive in the detection of HRP within neurons than any other histochemical procedure currently available (Mesulam & Rosene 1979). The use of this procedure did not reveal greater numbers of neurons in either the quadriceps femoris (173, range 153–193) or adductor motonuclei (205, range 189–222, cf. table 1). This leads to the conclusion that the method of Hanker *et al.* (1977) used in the majority of experiments was capable of detecting all the motoneurons containing horseradish peroxidase that could be revealed by any method at present available.

The total number of motoneurons in the lateral ventral horn labelled with horseradish peroxidase was 1650; this figure includes pectineus, quadratus femoris and the inner pelvic motonuclei. This value is 78 % of the total number of motoneurons (2152) counted in the lateral ventral horn in Nissl-stained preparations (McHanwell 1980). Clearly the HRP technique labelled a substantial fraction, though not all, of the motoneurons in the lateral ventral horn. The possible reasons for this discrepancy will be discussed later.

3.5. *Motoneuronal columns in the ventral horn*

Motoneuron groupings may be observed in Nissl-stained preparations of the spinal cord. Their arrangement was investigated and related to the motoneuron nuclei defined in the previous section. Romanes (1946) has described these groupings in the mouse but he did not indicate their segmental extent. In this study eight separate longitudinal motoneuronal columns could be identified in the ventral horn. As noted by Romanes these groupings were sometimes indistinct and not every column was present in all transverse sections. The columns and their extend are shown in figure 28. They are numbered in the order of their cephalocaudal appearance according to the nomenclature devised by Romanes (1946); in addition their segmental location is described.

Column 1 appears at the middle of the L1 segment and is located at the extreme ventrolateral margin of the ventral horn. It retains this position throughout the lumbosacral enlargement, expanding dorsally and medially in L3 and the upper half of L4 but becoming smaller and more localized near its termination at the lower border of L4.

Column 2 begins caudal to column 1 at the lower border of L1 and is located dorsally and medially with respect to it. It expands in segments L3 and L4 and fuses with column 3 in L3 and column 6 in L4 and terminates just rostral to the lower border of L4.

Column 3 begins just caudal to column 2 and is situated lateral to it. The boundary between these two columns can be difficult to discern and at the lower border of L2 column 3 is found more lateral and fuses with the dorsolateral margin of column 2.

Column 4 appears in the upper half of L3 as a large group of cells located at the dorsolateral

border of the ventral horn and dorsal to columns 2 and 3. As the column extends caudally through L3 it is found more dorsally and it terminates in the upper half of L4 as a small group of cells at the extreme dorsolateral margin of the ventral horn.

Column 5 begins just caudal to column 4 in the upper half of L3 and is located dorsally to column 2 and medially to column 4. At the base of L3 it appears to be partially divisible into a dorsal and ventral portion but this division is not recognizable in all reconstructions. In the lower half of L4 the column decreases in size and now in a more ventral position terminates just caudal to columns 2 and 6 and just ventral to column 1.

Column 6 begins at the base of L3, lateral to column 2, and appears partially to fuse with it in L4. The division between columns 2 and 6 is not apparent at the level of mid-L4 and column 6 terminates with column 2 just cranial to the base of L4.

Column 7 arises just caudal to the termination of column 4 and at the level of mid-L4. The column extends caudally into L5, is expanded laterally and ventrally and terminates in the lower half of that segment.

Column 8 appears just caudal and medial to the termination of column 1 and terminates laterally at the base of L5, caudal to the termination of column 7.

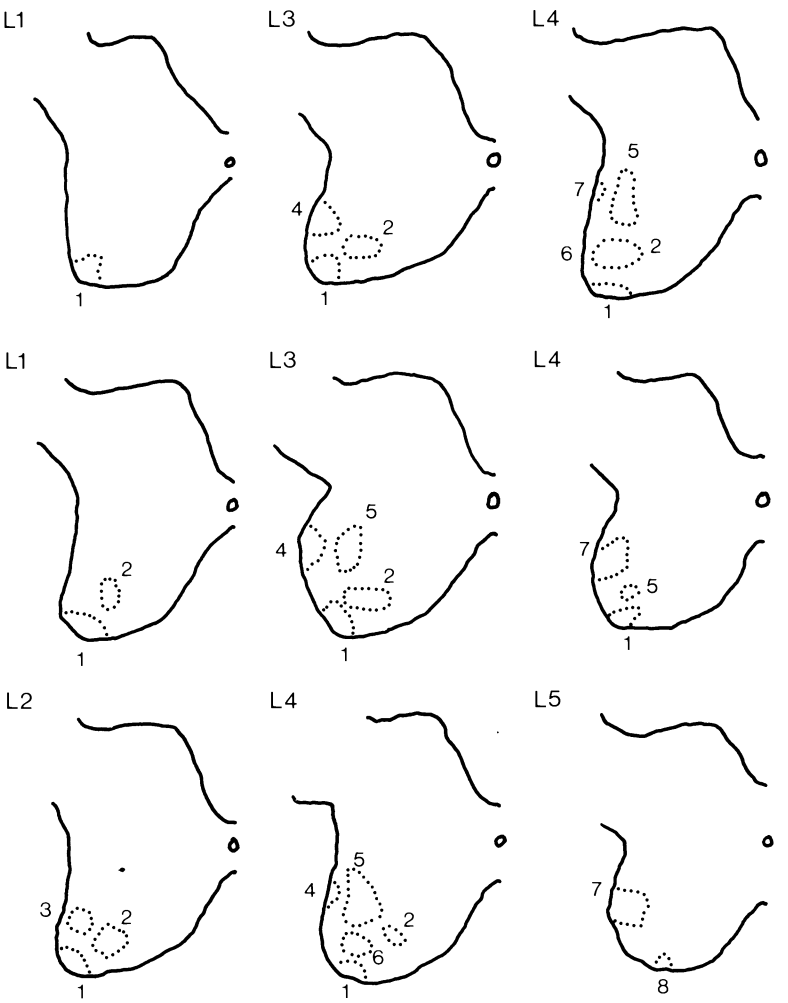

FIGURE 28. The motoneuron columns in the lateral motor column of the lumbosacral ventral horn. The columns are numbered in the rostrocaudal order of their appearance. Calibration bar, 500 μm.

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3.6. *A summary of the organization of motoneuronal nuclei*

Figure 29 summarizes the preceding results on the anatomy of motoneuron nuclei in the mouse lumbosacral lateral ventral horn. It has been prepared according to the same general plan as that of Romanes (1951) to facilitate the comparison between his results and those of the present study. Since the lengths of individual segments and the segmental position of motonuclei varied between animals it was necessary when superimposing the results from different

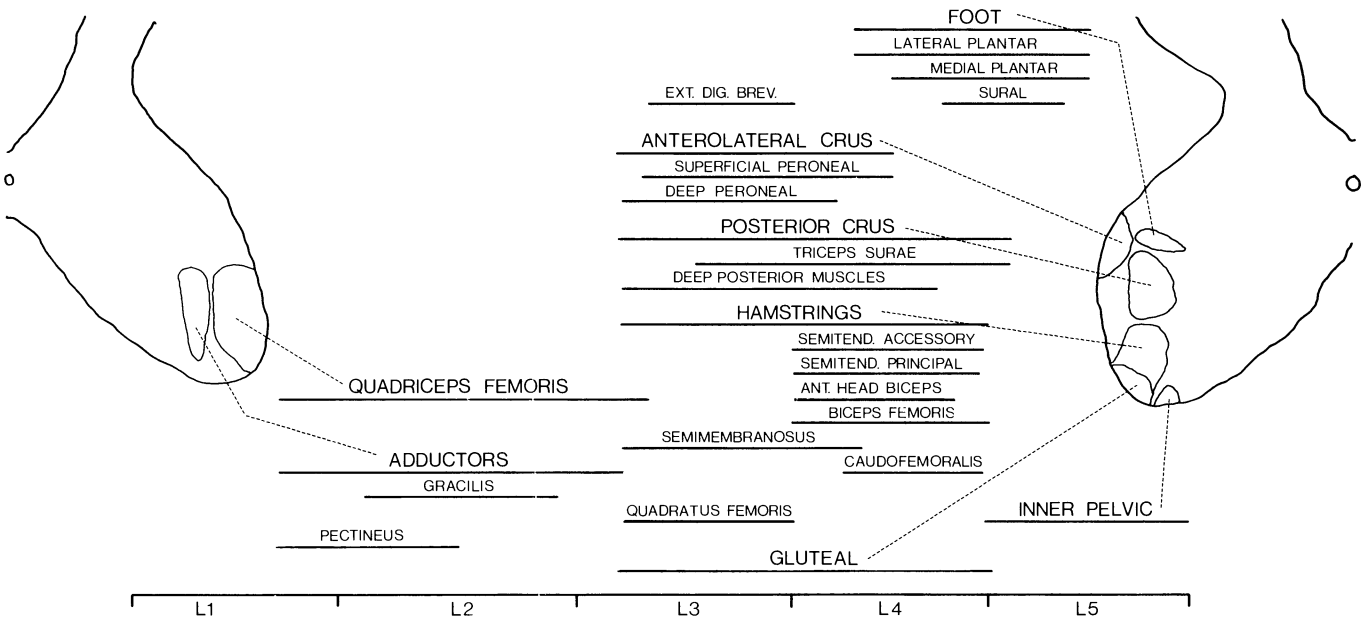

FIGURE 29. A diagram showing the segmental location and position in the transverse axis of the motonuclei studied. The horizontal bar indicates the segmental extent of the motonucleus. The position of motonuclei in the dorsoventral and mediolateral axis are illustrated on the idealized transverse sections of the spinal cord on the right and left. The left transverse section is at the level of mid-L2 while the right transverse section is at the level of the base of L4.

experiments to use a fixed reference point. The results of bilateral injections showed that the adductors motonucleus never overlapped the motonuclei innervating the sciatic nerve. Thus the base of the adductors motonucleus or start of the sciatic motonuclei served as the reference point for the alignment of the results from different experiments. The length of a given motonucleus was consistent between experiments and it was felt that such an alignment would preserve the relationships between motonuclei which would have been distorted if absolute segmental locations had been used. The segmental locations shown on figure 29 represent an approximate midpoint for any motonucleus.

Two general statements can be made about the relationships between motonuclei. First,

DESCRIPTION OF PLATE 2

FIGURE 30. Left: light micrographs of 50 μm transverse sections of the spinal cord from the lumbar segments indicated; sections stained with gallocyanin. Right: camera lucida drawings of the same sections in which the motoneuronal columns in the lateral ventral horn are outlined; the muscle groups to which these columns project are also indicated. Calibration bar, 500 μm.

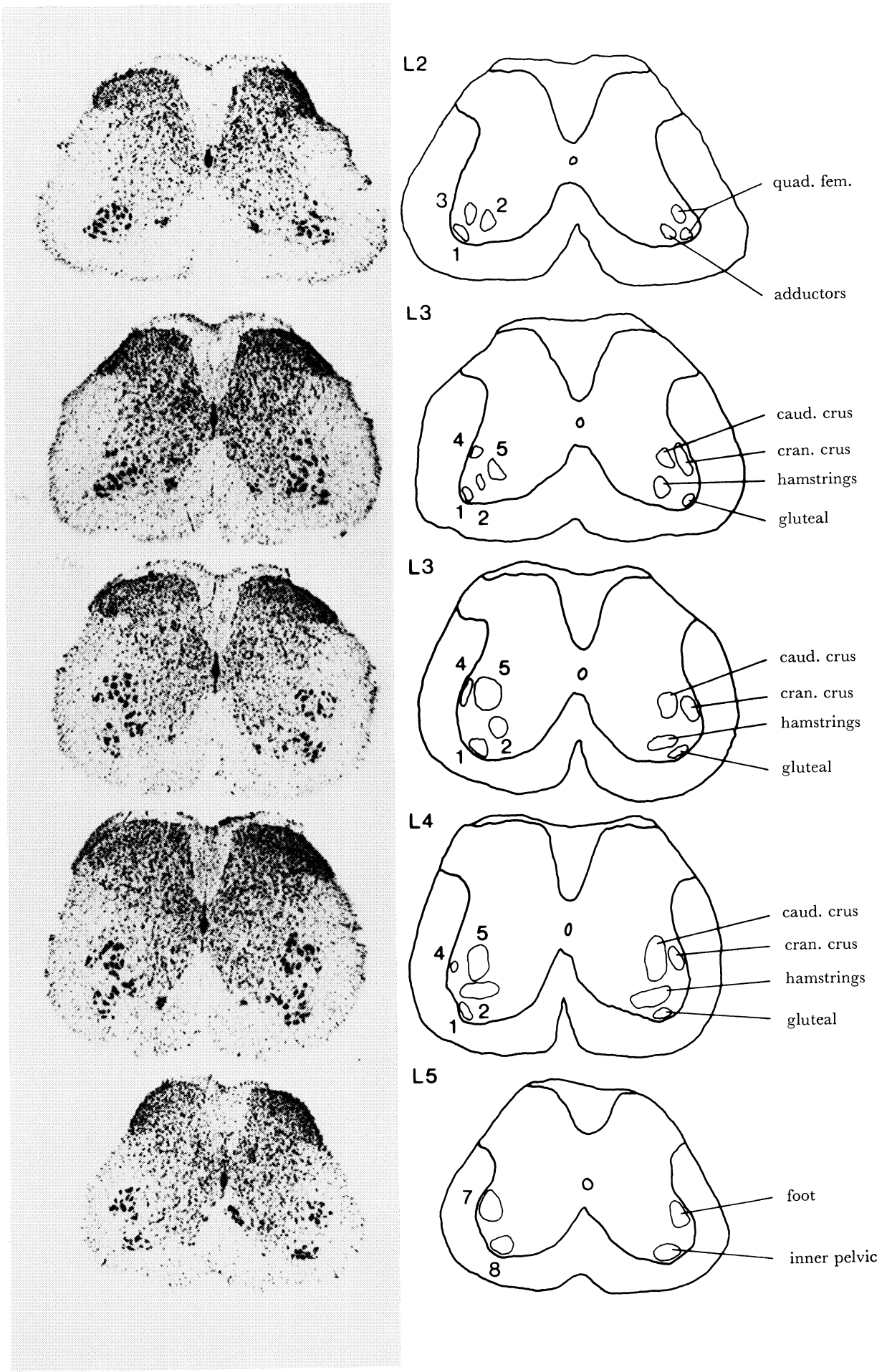

FIGURE 30. For description see opposite.

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motoneurons that supply the distal musculature within the hindlimb tend to be located more dorsally and caudally in the ventral horn. It must be stressed, however, that there is no absolute correlation between the rostrocaudal position of a motoneuron and the proximodistal position of the muscle it supplies. Secondly, there is a relationship between the embryonic muscle mass from which a muscle is derived and the location within the ventral horn of the motoneuron that supplies it. Thus quadriceps femoris, gluteal muscles and craniolateral crus muscles are all derived from the dorsal muscle mass (Lance Jones 1979) and their motoneurons are situated laterally in the ventral horn. The adductors, semimembranosus, semitendinosus and posterior crural muscles are derived from the ventral muscle mass (Lance Jones 1979) and their motoneurons are located medially in the ventral horn.

Figure 30 shows the relations between the motonuclei defined on the basis of horseradish peroxidase experiments and the motor cell groupings observed in Nissl-stained preparations. In segments L1 and L2 three motoneuron columns could be defined and it can be seen that the motoneurons that they contain supply two major muscle groups; columns 1 and 3 supply the quadriceps femoris muscle group while column 2 supplies the adductors motonucleus. Though these three columns are closely associated throughout L1 and L2 the horseradish peroxidase experiments have shown that the motonuclei that they supply do not overlap at any given level. In L3 two further columns could be defined; column 4, which supplies the anterior and lateral crural musculature, and column 5, which supplies the posterior crural musculature. Column 5 appears to be partially divisible into a dorsal and ventral portion throughout its length and this corresponds to the division of the posterior crural motonucleus into divisions supplying the deep posterior muscles of the leg and the triceps surae respectively. Also in L3 column 3 fuses with the dorsolateral margin of column 2. The dorsal part of this composite column supplies the semimembranosus while its ventral portion supplies quadratus femoris. Column 1, which supplies part of quadriceps femoris rostrally, supplies the gluteal musculature caudally in this segment until it terminates in L4. In L4 two further columns could be recognized: column 6, which partially fuses with column 2 at its medial edge, these columns together supplying the biceps femoris and semitendinosus muscles, and column 7, which supplies the intrinsic musculature of the foot. Finally, in L5 column 8 could be recognized and this column supplies the inner pelvic musculature.

From these results it can be seen that a given motoneuronal column does not supply a single muscle group throughout its length; neither are the motonuclei supplying the major muscle groups necessarily confined to one column. Thus the motoneuronal groupings that may be defined by the reconstruction method do not correspond absolutely to the topographic localization of the lateral motor column defined on the basis of the retrograde mapping experiments.

4. Discussion

The localization of motoneurons that supply hindlimb musculature has been studied in a number of species. Before comparison of the results described here in the mouse with those obtained in these other species some methodological considerations in the use of HRP as a retrograde label for identifying the projection patterns of motoneurons will be discussed.

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4.1. *Methodological considerations*

4.1.1. *Application of horseradish peroxidase*

Motoneurons can be labelled with HRP after either intramuscular injection (Kristensson & Olsson 1971; Burke *et al.* 1977) or application of the enzyme to the cut end of the muscle nerve (Kristensson & Olsson 1976; Richmond *et al.* 1978). Each method has its own limitations. Following intramuscular injection HRP can readily diffuse across perimysial barriers (Richmond *et al.* 1978) and this has been shown to complicate the interpretation of the results of such experiments (Burke *et al.* 1977; Richmond *et al.* 1978). Burke *et al.* (1977) cut the nerves to adjacent muscles so as to limit the possible effects of spread of HRP. However, Richmond *et al.* (1978) suggested that this procedure may not always prevent spurious labelling because of the possibility of uptake at the cut ends of nerves. Application of HRP directly to the cut end of a nerve circumvents these difficulties, but Richmond *et al.* (1978) found that motoneurons labelled in this way tend to show fainter and more variable amounts of HRP reaction product than motoneurons labelled following intramuscular injection.

The results presented in this paper confirm that nerve section cannot always control spurious labelling of motoneurons projecting to other than the muscle injected. This was found to be so where nerve branches were too short to be adequately deflected from the injection site. The labelling observed was shown to be due to the uptake of HRP at the cut ends of these nerves. The section of nerves 24 or 48 h before the injection of HRP did not prevent such uptake. These results are in contrast to those of Kristensson & Olsson (1976), who showed marked reductions in the number of spinal ganglion neurons labelled with HRP if the application of enzyme to the sciatic nerve was delayed until 30 min after nerve crush. Halperin & LaVail (1975) have shown that injured neurons in the isthmo-optic nucleus accumulate more HRP after injury to their axons despite an initial decrease in the amount of uptake. These workers also showed, however, that at longer survival times neurons whose axons had been damaged contained less label. In the present experiments a qualitative examination suggested that any reduction in the amount of HRP in motoneurons whose axons had been cut was insufficient to distinguish them from motoneurons with intact axons.

Other studies have suggested that uptake of HRP from injured axons may result in diffuse rather than granular labelling (Adams & Warr 1976; Lamb 1977). In the results described here such diffuse labelling was present in many though not all motoneurons whose axons had been injured, this pattern of labelling being particularly obvious at 48 h survival times when the nerves had been sectioned before injection. Even in these latter experiments, however, some neurons with granular reaction product could always be seen. Furthermore, the deliberate application of HRP to cut nerve endings frequently resulted in motoneurons containing granular reaction product, though such labelling was often fainter than that in motoneurons labelled after intramuscular injection. Thus in the present experiments it could not be concluded that the presence of a granular rather than diffuse reaction product within motoneurons was the result of uptake of HRP at intact endplates. A similar result has been observed by Richmond *et al.* (1978).

Taken together these results show that there are no certain criteria that allow recognition of uptake of HRP from injured rather than intact axons. Also, that it is unlikely that uptake of HRP from adjacent muscles can, in every case, be prevented by nerve section. These limitations

should be borne in mind when using the method of intramuscular injection as a means of labelling motoneurons and emphasize the need to ensure that uptake at other than the target site has not occurred.

4.1.2. *Number of labelled motoneurons*

Any method of marking motoneurons may fail to label all of them in a particular motonucleus. If these failures are distributed throughout the nucleus then the extent of that particular nucleus would be correctly estimated, though the number of cells that it contained would be underestimated. On the other hand if the nucleus itself is topographically organized then a failure to label all the subgroups would also incorrectly identify the extent of the whole motonucleus. Where the cells are marked by chromatolytic changes following nerve section, equivocal changes are scattered throughout the nucleus and so its extent is likely to be correctly shown (Romanes 1951). However, the high degree of somatotopic order within the lateral motor column, which may extend even to single muscle motonuclei (Strick *et al.* 1976; Burke *et al.* 1977), means that failure to label motoneurons resulting from incomplete infiltration of a muscle may result in an underestimate of the extent of that motonucleus as well as the number of cells marked. This may represent a further limitation in the use of intramuscular injection to label motoneurons with HRP. Application of HRP to the cut end of the nerve might be expected to circumvent this difficulty because it may equalize the exposure of all axons to HRP.

One method of validating a retrograde transport method is to compare the numerical results obtained with an independent estimate of the number of neurons in that structure. Burke *et al.* (1977) showed that the number of alpha motoneurons labelled with HRP in the cat medial gastrocnemius and soleus motoneuclei was 90% of the number of alpha motor axons in the deafferented muscle nerves (Boyd & Davy 1968). Landmesser (1978*a*) showed the number of motoneurons labelled with HRP in the chick lumbosacral lateral ventral horn to be similar to the number counted in Nissl-stained preparations. These results suggest that HRP may be a quantitatively reliable technique to label motoneurons and thus will also indicate the extent of motonuclei reliably.

The total number of motoneurons in the lumbar cord labelled with HRP injected into hindlimb muscles in the experiments reported here was 1650. This is 78% of the total number of motoneurons (2152) counted in Nissl-stained spinal cord sections (McHanwell 1980). The discrepancy may be accounted for by a failure to label all gamma motoneurons (Burke *et al.* 1977; McHanwell & Biscoe 1981). Indirect evidence for completeness of labelling in these experiments is that a reduction in survival time did not increase the maximum number of motoneurons detected, suggesting that a plateau had been reached in retrograde labelling at the survival times employed. Mesulam & Rosene (1979) have suggested that the method of Mesulam (1978) is the most sensitive currently available for the demonstration of HRP. The use of this method did not result in the detection of larger numbers of motoneurons, which suggests that the method of Hanker *et al.* (1977) was reliable enough to allow detection of all the neurons that could be made visible by any method presently available. The results taken together suggest that the localization of motonuclei was not affected by a failure to label substantial numbers of motoneurons.

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4.2. *Somatotopic organization of the mouse lumbosacral lateral ventral horn; a comparison with other species*

The conservation of nerve muscle connections within different vertebrate species has been recognized for many years (Strauss 1946). By comparing the organization of motoneuron columns in the lateral ventral horn, Romanes (1964) suggested that the somatotopic organization of motoneurons might also be similar between vertebrate species. To compare motoneuronal organization between species it is necessary to establish their muscle homologies. Within a single class of vertebrates such as Mammalia, gross innervation is a considerable aid to establishing homologies (Strauss 1946), but such criteria are of less help in comparisons between vertebrate classes. Other criteria that have been used include skeletal origins and insertions of muscles and their topography. Lance Jones (1979) has studied the patterns of cleavage of muscles in the ontogeny of the mouse thigh. On the basis of a comparison of these results with similar studies made in the chick and *Lacerta* (Romer 1927, 1942), Lance Jones (1979) has proposed an ontogenetic basis for muscle homologies between these three species. Correlations between motoneuron position and the derivation of the muscle that it supplies have already been observed in other species (Cruce 1974; Lamb 1976; Landmesser 1978*a*). In view of these correlations the comparisons of motoneuron topography that follow will be discussed in the light of muscle homologies proposed from ontogenetic evidence by Lance Jones (1979).

4.2.1. *Mammalia*

Romanes (1946) used the motor cell changes that followed amputation of limb segments, muscle removal and sciatic nerve section to provide some limited data on the topographic organization of the mouse lumbosacral spinal cord. His results showed clearly that the motoneurons supplying the foot were located in a caudal and dorsal column. Similarly Tsang (1939) reported increased numbers of motoneurons in this column in a polydactylous strain of mouse. Both these results are in agreement with those reported here. Romanes (1946) showed that following removal of the anterolateral or posterior crural muscles consistent chromatolytic changes were found only in a lateral column of motoneurons. The position of this column appears to correspond with the position of the column in the present study defined as innervating the anterolateral crural muscles. However, the results described here showed that motoneurons innervating the posterior crural muscles were located medial to this column. Romanes (1946) only detected a few motoneurons showing chromatolytic changes in this column following knee amputation and found no altered neurons in this column following removal of the gastrocnemius and soleus muscles. Amputation of the leg at the level of the hip by Romanes (1946) resulted in altered motoneurons being detected in several columns in the ventral horn. When the changes that would have been expected on the basis of removal of the foot and shank musculature have been discounted these results do not correspond well with the present study. There were no altered motoneurons observed in the ventral half of the lateral motor column, which would have been expected from the results described here, though Romanes (1946) did observe some altered motoneurons in a medial location that may correspond to part of the hamstring motonucleus defined on the basis of HRP labelling. The nature of these operations performed does not allow further definition of the topography. Thus results of Romanes (1946) are, in some respects, similar to those described here. The significance of the differences cannot be further commented on due to the incomplete nature of Romanes's (1946) results.

Three further studies on the location of motoneurons supplying rodent hindlimb musculature

should be mentioned. Kiesel (1938) showed the motoneurons that supply the anterolateral crural muscles to be located dorsolaterally in the guinea pig ventral horn. The motoneurons supplying the posterior crural muscles were located medially to this group. This is similar to the results described here for the mouse. Kiesel showed the motoneurons innervating the hamstring musculature to be in a position in the guinea-pig comparable to that reported here in the mouse. Kaizawa & Takahashi (1970) and Brushart & Mesulam (1980) have studied the localization of motoneurons that supply the posterior and anterolateral crural muscle groups in the rat. The results of both these studies were similar to those of Kiesel (1938) and to those described here in locating the anterolateral crural motoneurons laterally to the posterior crural motoneurons and in the dorsal half of the lateral motor column.

The most complete study of lateral ventral horn organization in any species is that by Romanes (1951) in the cat. Comparisons between the results for the mouse and those for the cat show that the topography of the ventral horn is strikingly similar in the two species. The motoneurons innervating muscles supplied by the lumbar plexus are located in the rostral ventral horn in both species. The motoneurons innervating the quadriceps femoris group (rectus and the vasti) are located in two columns in the lateral ventral horn (though the two columns are not always distinct in the mouse) while the motoneurons innervating the adductors of the thigh are located medially to these columns in both species. The other muscle of this group is sartorius. This muscle is only present as a vestigial structure in the mouse, disappearing before birth, and there is no information as to the location of motoneurons that may transiently supply it (Lance Jones 1979).

The motoneurons supplying muscles innervated by the sciatic plexus are also similarly located in both species. The motoneurons that innervate the gluteal musculature are located laterally and ventrally in the lateral motor column. Dorsal to this group are motoneurons innervating the hamstring musculature while dorsal to the hamstring motonucleus are the motoneurons that innervate the posterior crural muscles. Lateral to both these groups are the motoneurons that innervate the anterolateral crural musculature. The most caudal and dorsal motonucleus is that which innervates the intrinsic musculature of the foot. The relationships between the motonuclei that supply the seven major muscle groups of the hindlimb are similar in the mouse and cat. The motoneurons supplying the muscles innervated by the sciatic plexus are located caudally with respect to lumbar plexus motoneurons.

Some differences in motoneuron topography between the two species can be observed. In the cat the motonucleus innervating adductor magnus overlap extensively the motonucleus innervating semimembranosus but in the results reported here for the mouse no such overlap could be observed. An explanation for this discrepancy may lie in the known variability of innervation of adductor magnus in mammals (Lance Jones 1979). In the guinea-pig (Cooper & Schiller 1975) and in man (Last 1978) adductor magnus is partially innervated by a branch of the sciatic nerve but this is not so in the mouse (Lance Jones 1979; McHanwell 1980). A sciatic innervation of this muscle might result in some of the motoneurons that supply it being located caudally. A second difference is that in the mouse the quadriceps femoris motonucleus extends medially at its caudal end to fuse with the semimembranosus motonucleus, whereas in the cat it remains lateral in position throughout its length to fuse with anterolateral crural motonucleus. In the cat the motonuclei that supply the deep and superficial divisions of the posterior crural muscles are clearly separable into two distinct columns, whereas in the mouse the columns are fused at their dorsoventral boundary. The motoneurons innervating the deep posterior muscles

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of the leg are predominantly dorsal in position in the mouse but they are extensively intermingled with the motoneurons that innervate triceps surae. In addition the motoneurons supplying the deep posterior musculature do not extend laterally to the edge of the ventral horn in the mouse as they do in the cat. A final difference is in the location of the motonucleus that innervates the musculature of the foot. In the cat this motonucleus is located dorsally to all other motonuclei whereas in the mouse only the caudal two-thirds of this motonucleus is so located. The rostral part of the nucleus is located more ventrally and lateral to the dorsal half of the posterior crural motonucleus. Despite these minor differences in the detailed relationships between some motonuclei, the general pattern of motoneuronal organization is similar in the mouse and the cat.

The only other detailed study of motoneuronal organization in the mammal for this region of the spinal cord is by Sharrard (1955) in the human. Romanes (1964) has compared his earlier results in the cat with those obtained by Sharrard (1955) for the human spinal cord. He concluded that the somatotopic organization of motoneurons was similar in these two species despite some minor differences in the relative positions of some motonuclei. These results also show that the hamstring and adductors motonuclei overlap in the human spinal cord. This correlates with the innervation of adductor magnus by a branch of the sciatic nerve in man (Last 1978).

A surprising finding in the organization of the mouse lateral motor column was that as many as 30% of motor axons innervating the intrinsic musculature of the foot were present in the sural nerve. There have been no other reports of such an extensive somatic efferent component of the sural in any mammalian species though Betz *et al.* (1979) report an innervation of the rat 4th lumbrical muscle by an anastomotic branch of the sural nerve that joins the lateral plantar nerve. Section of plantar nerves, in the mouse, results in no motoneurons innervating the foot being labelled with HRP following intramuscular injection. Thus it would seem that this anastomotic branch of the sural nerve, which is also present in the mouse, innervates the foot musculature.

4.2.2. *Aves*

The organization of motoneurons that supply the hindlimb musculature of the chick has been studied by Landmesser (1978*a*). From a consideration of the ontogenetic homologues between mammalian and avian hindlimb muscles (Lance Jones 1979) this author detailed the similarities between the cat and chick motonucleus organization. She concluded that the somatotopic maps were similar in the two species and clearly these similarities can now be extended to the mouse. Thus the motoneurons supplying the chick homologues of the mammalian adductors, quadriceps femoris, gluteal musculature and hamstring muscles are all located in similar relative positions in the spinal cord. Nevertheless some differences in motoneuron organization between mammals and chick can be noted. The motoneurons that innervate the shank musculature of the chick are organized in a manner similar to that in the mouse and other mammals in that the motoneurons that innervate dorsal muscle mass derived muscles are located laterally to the motoneurons that innervate ventral muscle mass derived muscles. In the chick, however, these motonuclei extend to the ventral border of the ventral horn whereas in mammals they are confined to the dorsal half of the ventral horn except in their most caudal segments, but even at that level they do not reach the ventral margin of the ventral horn. Also the rostral dorsal shank motonucleus extends ventromedially beneath the ventral shank motonucleus in the chick

whereas in the mammal it always remains lateral in position. A second difference between mammalian and chick motonucleus topography may be noted in the position of the motonucleus that innervates the mammalian homologue of biceps and ventral semitendinosus caudilioflexorius. In the chick this motonucleus is located dorsally with respect to the motonuclei that innervate the shank musculature, in contrast to its ventral position relative to these motonuclei in the mammal.

4.2.3. *Anuran Amphibia*

Comparisons between the species so far described and the anuran vertebrates is complicated by two factors. First there is no available information on the detailed cleavage patterns of the major muscle masses for this vertebrate class. Consequently muscle homologies cannot be made on the basis of similarities in embryonic development. The muscle homologies that have been proposed on the basis of similarities of innervation and embryonic derivation have yielded conflicting results (Cruce 1974; Romer 1970). The second factor is that the columnar organization of the spinal cord is more rudimentary in these species in comparison with higher vertebrates. Cruce (1974) has examined the localization of motoneurons innervating hindlimb muscles in the frog. Similar studies have been made in *Xenopus* by Lamb (1976). Again, a number of similarities can be recognized between these species and the others previously discussed. Thus the motoneurons supplying the homologues of quadriceps femoris, which in the frog and *Xenopus* are cruralis and gluteus magnus, are located rostrally and laterally in the ventral horn. The homologues of the mammalian adductor muscles, gracilis semimembranosus and semitendinosus in the frog are adductor magnus, semitendinosus, gracilis and semimembranosus respectively. In both vertebrate classes the motoneurons supplying these muscles are situated medially in the ventral horn, though in the anuran species this group of motoneurons does not extend so far caudally as in the mammal and the chick. The major difference between the frog (Cruce 1974) and other species is in the position of the motoneurons supplying the flexors of the toe which are situated caudally and medially in comparison to a more rostral and lateral location in other species. It is of interest to note that Lamb (1976) could not observe this difference in *Xenopus*; thus there may be a genuine species difference between the frog and other vertebrates.

4.3. Conclusions

From the preceding discussion it can be seen that the topographical organization of motoneurons innervating hindlimb muscles is similar in all the vertebrate species so far described. There is no evidence that there are consistent relationships between motoneurons that supply muscles of similar function (Cruce 1974; Landmesser 1978*a*). Neither is there a consistent relationship between motoneuron position in the spinal cord and position of muscles in the adult hindlimb (Landmesser 1978*a*). Thus, for example, in the mouse motoneurons that supply the inner pelvic musculature are located quite caudally with respect to muscles of the gluteal complex. The most consistent relationship that emerges, however, is that between the embryonic muscle mass from which a muscle derives and the position of its motoneuron soma in the spinal cord. Romanes (1964) observed that motoneurons that supply morphologic extensors lie in the lateral part of the lateral motor column while motoneurons that supply morphologic flexors lie in the medial part of the lateral motor column. In the mouse it has

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already been observed that motoneurons innervating dorsal muscle mass derived muscles lie laterally with respect to motoneurons that supply ventral muscle mass motoneurons. Similar relationships have been observed in the other species studied (Cruce 1974; Lamb 1976; Landmesser 1978*a*). Landmesser (1978*a*) has observed that this relationship holds between different classes of vertebrate even when the muscle homologues have assumed different functions as is so with the chick sartorius and cat rectus muscles, which are homologous muscles occupying similar positions in the spinal cord yet have different functions.

However, even this relationship would appear to have its exceptions. The biceps femoris and caudofemoralis muscles are derived from the ventral mass in the mouse (Lance Jones 1979) yet lie laterally in the ventral horn. It is interesting to note that the chick homologue of biceps, caudilioflexorius, is similarly located laterally in the ventral horn. The significance of this deviation of these muscles from the general pattern is not clear. It can be noted, however, that in the mouse both caudofemoralis and biceps femoris arise from a similar region of the ventral muscle mass close to the dorsal muscle mass (Lance Jones 1979). The cleavage patterns of the intrinsic musculature of the foot have not been described and so it is not possible to determine whether these muscles also conform to the general pattern.

The relationships between motoneuron positions and the sites of their terminations in the embryonic hindlimb suggest that it is motoneuron position that is a prime determinant of connectivity patterns observed. It further suggests that these mechanisms may operate similarly in different vertebrate species. The mechanisms by which motoneuron axons reach their correct positions in the hindlimb have been reviewed by Landmesser (1980). A simple timed outgrowth hypothesis in which rostral axons grow out first to contact proximal musculature seems unlikely in view of the fact, noted above, that there is no absolute correlation between rostrocaudal position of a motoneuron and the proximodistal location of the muscle it supplies. Migrations of muscles, their insertions and rotation of the hindlimb (Lance Jones 1979) may partially obscure such relations in adult, which may have occurred in the foetal animal. It will be necessary to look closer at such relations in the development of the foetal hindlimb. Lance Jones & Landmesser (1980*a*, *b*) have provided evidence that passive mechanisms alone are not able to explain the connectivity patterns between motoneurons and muscles in the chick. Adult motoneuron topography can only provide clues to the mechanisms that give rise to them. To examine their development it is necessary to map motoneuronal projections during development and to examine the effects of various surgical interventions upon motoneuronal development. It will be interesting to compare such processes in the mouse with those described in the chick (Landmesser 1978*b*; Lance Jones & Landmesser 1980*a*, *b*).

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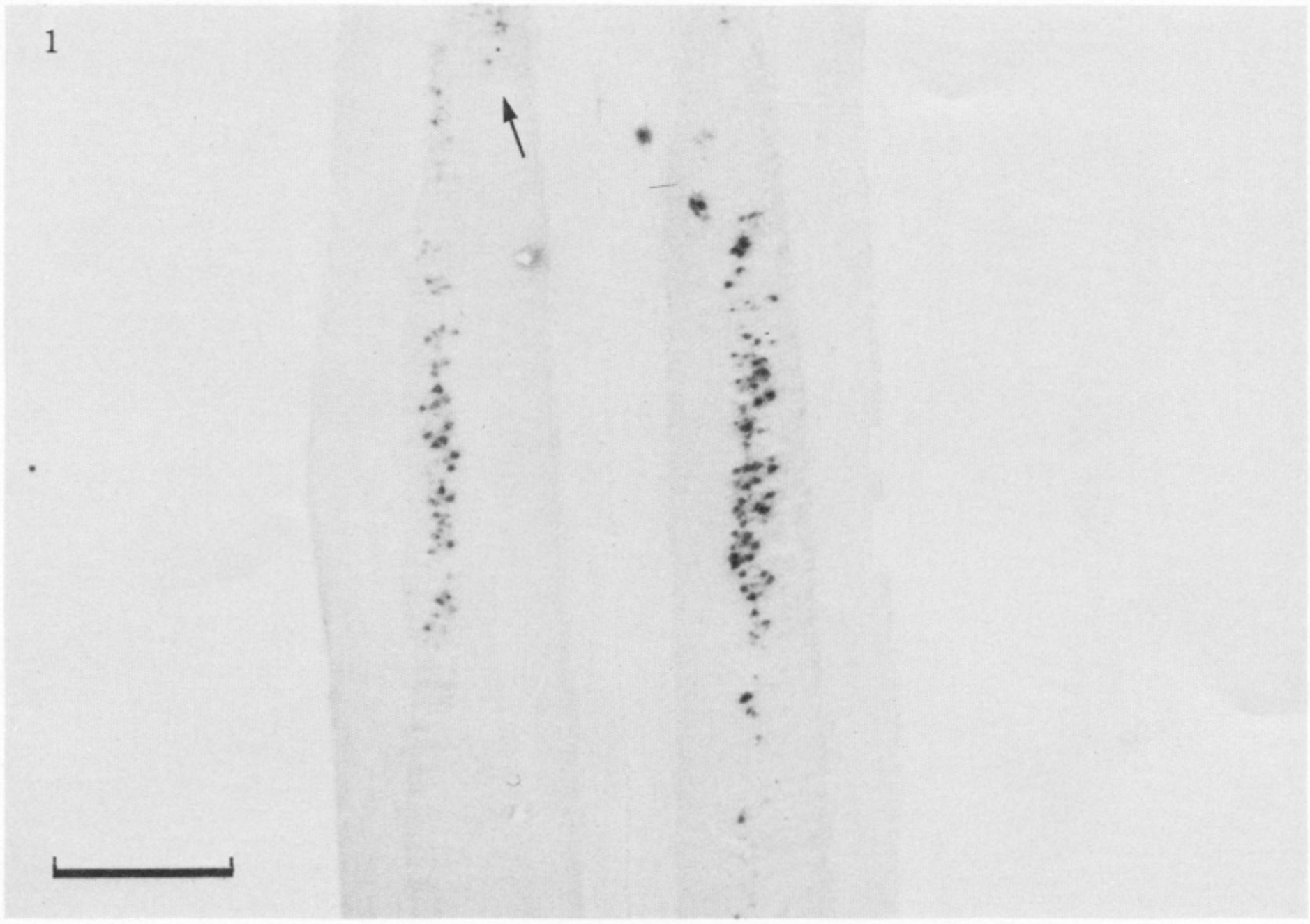
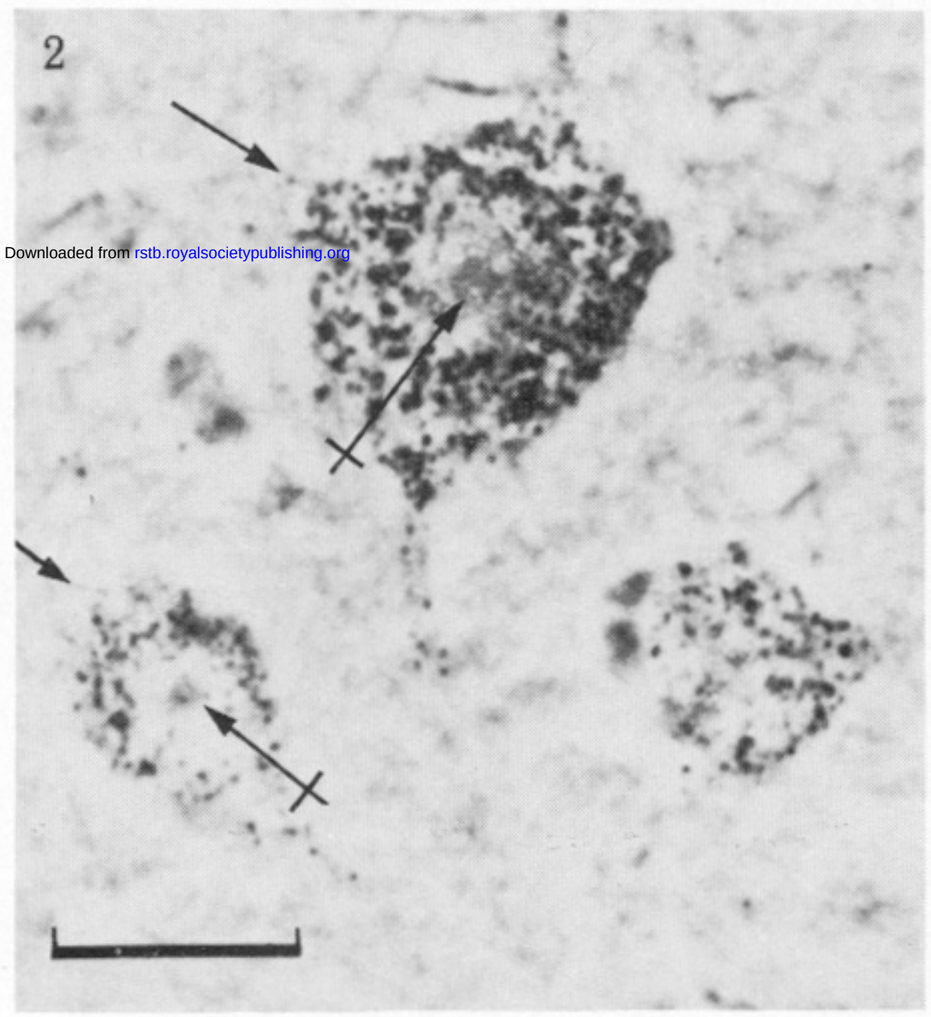




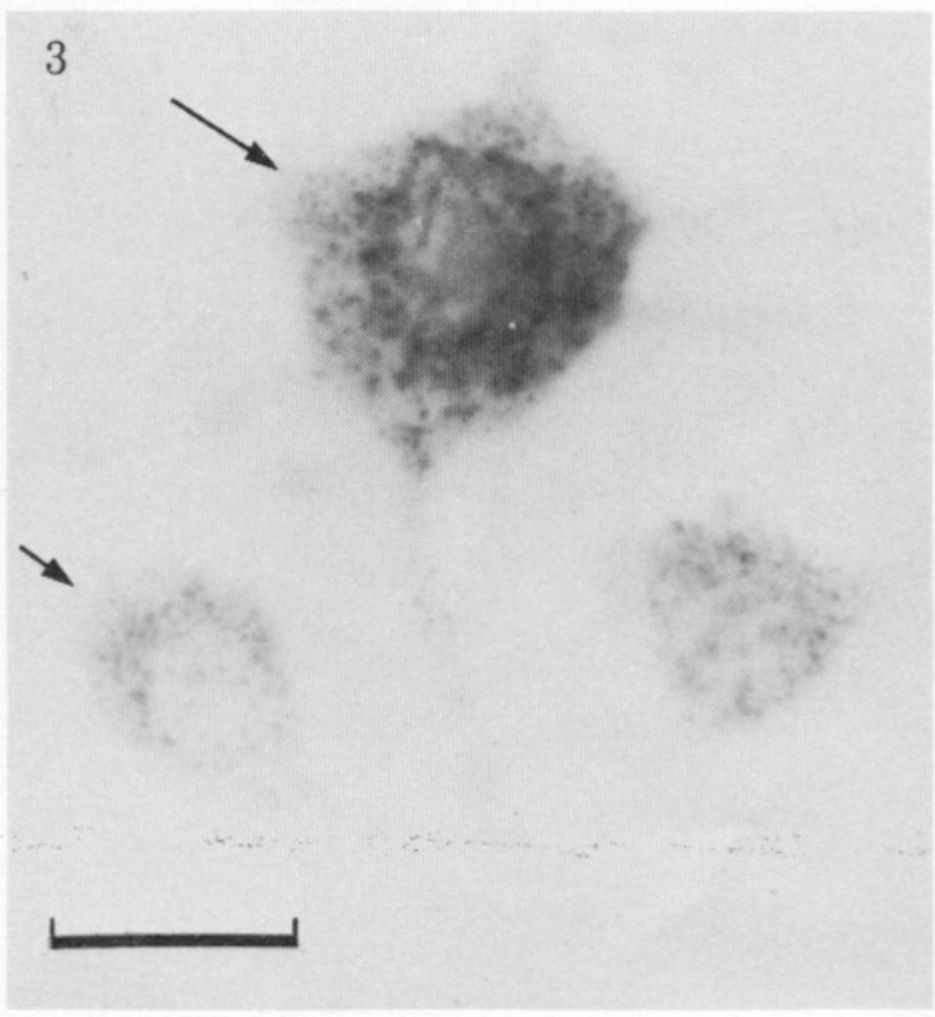
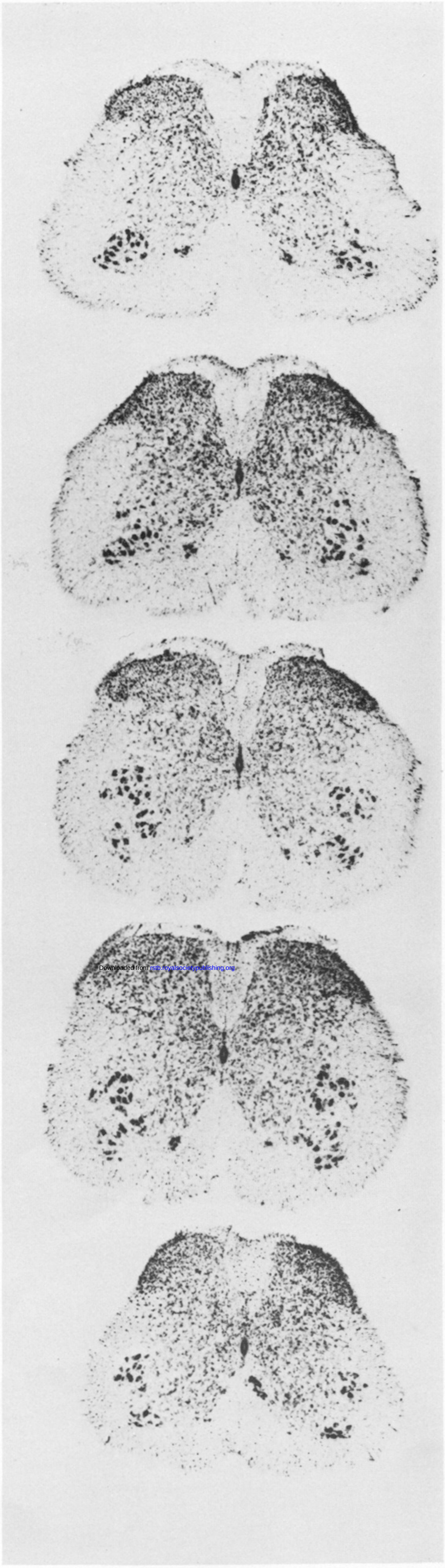


FIGURE 1. A low-power bright field photomicrograph of a 50 μm horizontal section through the ventral horn of lumbar segments L1 and L2. Horseradish peroxidase containing motoneurons in the quadriceps femoris (left) and adductors (right) motonuclei can be seen. Motoneurons in a more rostral and medial position are also visible (arrow). These are presumed to be psoas motoneurons. Calibration bar, 500 μm.

FIGURE 2. A light micrograph of a 50 μm horizontal sections showing three horseradish peroxidase labelled profiles in the inner pelvic motonucleus viewed with phase contrast illumination. The small profile on the left (shorter arrow) has a clearly defined nucleolus (crossed arrow) and a soma area of less than 275 μm^2 and is presumed to be a gamma motoneuron. The large profile (longer arrow) also has a clearly defined nucleolus (crossed arrow) but a soma area of more than 275 μm^2 and is presumed to be an alpha motoneuron. The small profile on the right does not have a clearly defined nucleolus and is presumed to be a fragment of a larger motoneuron. Only the first two profiles would be counted as motoneurons. Calibration bar, 20 μm.

FIGURE 3. A light micrograph of the same neurons as in figure 2 but viewed with bright field illumination. The nucleoli are less easily distinguished with this method of illumination.



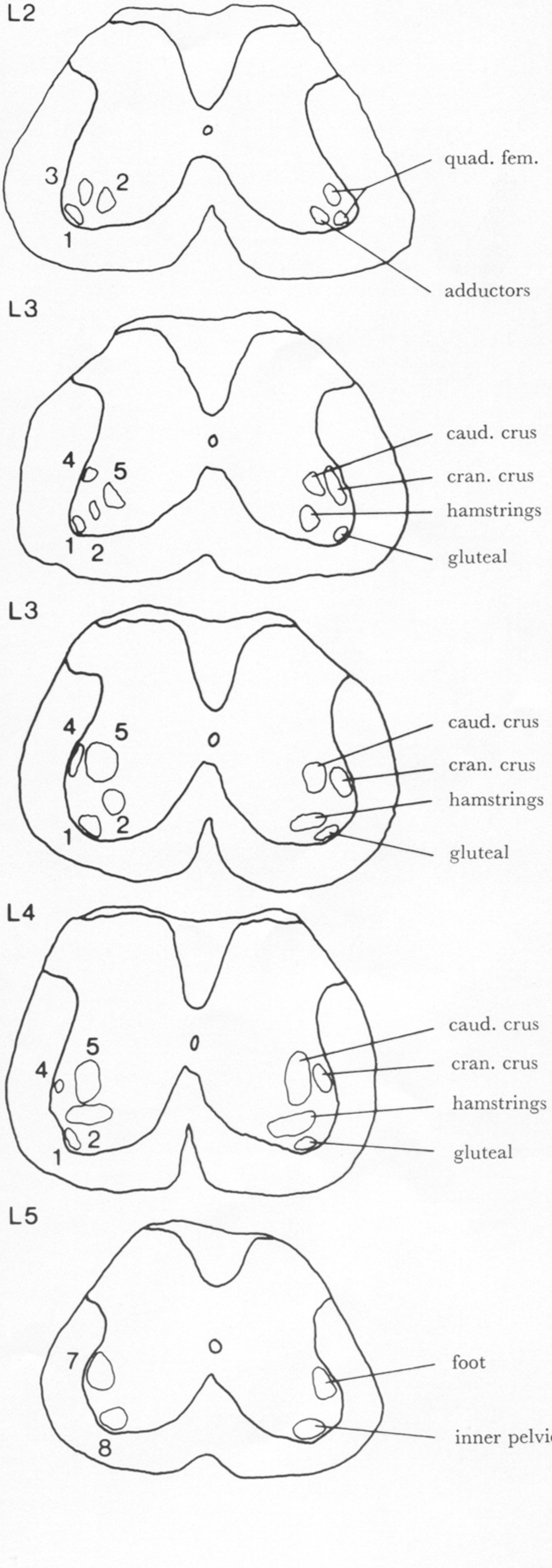


FIGURE 30. For description see opposite.