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The Development of Sensory Projection Patterns in Embryonic Chick Hindlimb under Experimental Conditions

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In the chick, sensory neurons grow to their segmentally appropriate target sites in the hindlimb from the outset during normal development. To elucidate the underlying mechanisms, we performed various manipulations of the neural tube, including the neural crest, or of the hindlimb, before axonal outgrowth and assessed the resulting sensory projections using retrograde and anterograde HRP labeling and electrophysiological techniques. Previous experiments had shown that motoneurons are specified to project to their appropriate target muscles prior to axon outgrowth and that they respond to cues in the limb in order to grow to those targets (C. Lance-Jones and L. Landmesser, 1980, *J. Physiol. (London)* **302,** 559-602; C. Lance-Jones and L. Landmesser, 1981, *Proc. R. Soc. London, B* **214,** 19-52). When several segments of neural tube and neural crest were deleted, sensory neurons in the remaining segments still projected along their correct pathways, as did motoneurons. In situations in which motoneurons grew to their correct targets from altered positions with respect to the limb (e.g., small neural tube reversals), sensory neurons also tended to project along the segmentally appropriate pathways both to skin and to muscle. In situations in which motoneurons were displaced greater distances from their normal point of entry into the limb and made wrong connections (e.g., large neural tube reversals, anterior-posterior limb reversals), sensory neurons also projected incorrectly. The patterns of sensory projections to muscles were, in each situation, generally similar to the motoneuron projections. These results are consistent with the possibility that sensory neurons, like motoneurons, are specified with respect to their peripheral connectivity. Alternatively, the results suggest that motoneurons may play a role in the process of pathway selection by sensory neurons.

INTRODUCTION

Motoneurons projecting to specific target muscles in the chick hindlimb are localized in characteristic longitudinal columns within the spinal cord and project along discrete pathways in the limb to innervate those muscles. The pattern of axonal outgrowth is correct and precise from the earliest stages (Landmesser, 1978b; Lance-Jones and Landmesser, 1981a; Hollyday, 1983a; Tosney and Landmesser, 1985a). Further, when operations are performed to shift, by a few segments along the anterior-posterior axis, the positions from which motoneurons enter the limb, motoneurons are able to alter their courses within the limb to project to their embryologically appropriate target muscles (Lance-Jones and Landmesser, 1980b, 1981b). This result shows that motoneurons are specified with respect to their peripheral projections prior to axonal outgrowth and limb bud formation. However, following larger displacements, motoneurons are sometimes unable to correct for changes in their positions and instead project to inappropriate muscles (Lance-Jones and Landmesser, 1981b). The conclusion reached from these and a number of other experimental studies has been that motoneurons can actively respond to cues in the limb in order to project to their correct targets (Lance-Jones and Landmesser, 1980b, 1981b; Ferguson, 1983; Laing, 1984; Tosney and Landmesser, 1984; Stirling and Summerbell, 1985). However, the distance over which such cues can be sensed or axons can respond appears to be limited (Lance-Jones and Landmesser, 1981b).

We wanted to study how sensory axons are guided to their targets in the limb. Sensory neurons projecting to a particular target are widely distributed throughout individual dorsal root ganglia (DRGs) (Honig, 1982). Thus, their position within a ganglion cannot be used to infer their identity. However, afferents projecting along a given muscle or cutaneous nerve are restricted in distribution to a characteristic set of contiguous DRGs (Honig, 1982). By making use of this observation, we had previously shown that sensory neurons grow to the segmentally correct targets from the outset and that neither cell death nor retraction of axons is necessary for the development of the appropriate connectivity patterns (Honig, 1982; see also Scott, 1982).

Such selective innervation could result if afferents, like motoneurons, were specified to innervate particular targets prior to outgrowth and actively responded to cues in the limb to reach those targets. However, it seemed possible that the rules governing the formation

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of sensory projections might differ from those for motoneuron projections because many properties of neural crest-derived neurons are affected by interactions with the environment (e.g., see Le Douarin, 1982). We therefore examined sensory projections following a variety of experimental manipulations and asked whether sensory neurons could project to their correct targets when we removed part of the neuronal supply to the limb and when we altered their anterior-posterior positions with respect to the limb.

The results we obtained, while consistent with the possibility that sensory neurons are specified and actively respond to guidance cues in the limb, also suggested an alternative hypothesis. The axons of sensory neurons may interact with motoneuron axons, which themselves are responsive to limb-associated guidance cues. This latter possibility is directly tested in the following paper (Landmesser and Honig, 1986). Here we describe the results of the earlier manipulations and the extent to which they allow us to distinguish among various mechanisms. Preliminary reports of some of these results have been presented elsewhere (Honig, 1979).

MATERIALS AND METHODS

Surgical Procedures and Their General Consequences

White Leghorn chick embryos were incubated until stage (st) 15–20 (Hamburger and Hamilton, 1951) when one of the operations described below was performed. Many but not all of these embryos were used to examine motoneuron projections in other studies (Lance-Jones and Landmesser, 1980a,b, 1981b; Landmesser and O'Donovan, 1984).

(1) Small neural tube deletions. A portion of the neural tube was deleted at st 15–16, as described by Lance-Jones and Landmesser (1980a). The deleted region generally extended from the last thoracic segment (T7) through the first two or three lumbosacral (LS) segments. This resulted in a gap in the spinal cord but the morphology of the remaining cord segments was normal. Because the operation included the neural crest, DRGs did not form in the operated region although, in some embryos, a small DRG was present slightly rostral to the resumption of the lateral motor column. The absence of DRGs is consistent with the results of Hammond and Yntema (1947), Nawar (1956), Castro (1963), and Scott (1984) and confirms that DRGs arise from neural crest that is situated at the corresponding axial level of the embryo.

As a result of the operation, some muscles were left completely uninnervated (see Results). In these cases, neuronal labeling contralaterally after injection of another muscle (whose source of innervation arose from segments situated more caudally than the deletion) ensured that the reaction procedure had been carried out successfully.

(2) Anterior-posterior neural tube reversals. A portion of the neural tube was reversed 180° along the anterior-posterior (A-P) axis in st 15–16 embryos as described by Lance-Jones and Landmesser (1980b, 1981b). Spinal cord development proceeded normally after this operation, as described by Lance-Jones and Landmesser (1980b). The lateral motor column of each segment acquired the shape and size characteristic of its origin. We were able to determine the extent of the reversed cord since, upon dissection, the borders of the graft were usually visible as small indentations on the ventral surface of the cord. In addition, some disorganization at boundary regions could be seen histologically. Nevertheless, motoneurons within such reversed grafts exhibit normal patterns of activation following stimulation of descending pathways at more rostral levels (Vogel, 1985).

Reversal of the neural tube at st 15–16 also reversed the neural crest. As previously noted, ablation experiments show that DRGs are formed by crest cells situated at the corresponding axial level of the embryo. Further, transplanted crest cells migrate along pathways that are determined by the level of the neuroaxis to which they are transplanted (Weston, 1970; Le Douarin, 1982). Therefore, in the embryos with neural tube reversals it is likely that, even after migration, DRG cells originating from the posterior part of the transposed crest (e.g., LS3) would be situated at a more anterior level (e.g., LS1). Thus we could infer the sequence of the original positions of sensory neurons by comparison with the identities of the reversed cord segments. However, we could not be certain about the original segmental identities of all the cells in individual ganglia for two major reasons. First, segmentation of the crest might not have proceeded normally since this process might be a consequence of the segmental nature of the somites (Weston, 1963) rather than an intrinsic property of the neural crest (Thiery *et al.*, 1984). Due to postoperative changes in the alignment of neural tube segments, the crest cells that normally coalesce in a single ganglion might come to be situated in both the posterior part of one DRG and the anterior part of the adjacent DRG. Second, a "presumptive ganglion" might have been split by the operation such that only part of it was transposed. The remaining postreversal crest cells (e.g., posterior DRG3) might then form either a separate ganglion or a ganglion which also contained cells from the new posterior border of the reversal region (e.g., the original DRG1). These possibilities complicate the interpretation of some results as will be seen later.

It should also be noted that DRGs in the reversal region were sometimes drastically reduced in size. It is

possible that all the reversed ganglia had fewer than the normal complement of neurons, due to either damage to the crest cells themselves or to disruption of the environment through which the crest migrates. However, this was not assessed quantitatively. A reduction in the size of DRGs was also found following neural tube transplants by Wenger (1951), but not by Weston and Butler (1966).

(3) A-P limb reversals. The limb bud was reversed 180° along the anterior–posterior axis in st 17–20 embryos as described by Landmesser and O'Donovan (1984). This was done by transplanting the right limb bud of one embryo to the left side of a second embryo, whose left limb had previously been removed. In this way, the dorsal–ventral axis of the limb with respect to the body was not altered.

(4) Limb shifts. At st 17–18, prior to axonal outgrowth into the limb, the limb bud was cut free from the surrounding tissue, slid forward a distance of several somites, and pressed against a slit made into the body wall (Lance-Jones and Landmesser, 1981b). In all embryos chosen for study the limb developed normally in a position one to four segments more anterior than normal.

Analysis of Connectivity Patterns

Embryos were incubated until st 27–38, when they were removed from the egg and dissected to expose the spinal cord and limbs as described in Landmesser (1978a). In most embryos, we first assessed the projections of motoneurons by stimulating the ventral surface of the spinal cord at different segmental levels and scoring which muscles were activated by observing contraction of individual muscles or by recording extracellularly from the muscles as described previously (Landmesser, 1978b; Lance-Jones and Landmesser, 1980a). This was useful in determining the extent of each operation, in identifying segments, and in providing a comparison for the results on sensory projections. Sensory neuron projections were then assessed using one or more of the procedures described below.

(1) Individual muscles were injected with horseradish peroxidase (HRP) and after allowing time for retrograde transport to occur, embryos were fixed, processed for HRP, and sectioned. Labeled neurons were counted as described in Landmesser (1978a,b) and Honig (1982).

(2) Nerve patterns within the limb were reconstructed from histological sections as described in Lance-Jones and Landmesser (1980a, 1981a).

(3) Individual DRGs were injected with HRP. After histological processing and sectioning, orthogradely labeled sensory axons were traced into the limb as described in Honig (1982).

(4) To obtain a measure of the relative number of axons from each spinal nerve projecting along each of the two major cutaneous nerves, the lateral and medial femoral cutaneous nerves, compound action potentials were recorded successively from individual spinal nerves in response to sequential stimulation of each of those cutaneous nerves, as in Honig (1982). The area under each compound action potential response was measured and for each spinal nerve, the response from each cutaneous nerve was expressed as a percentage of the sum of the responses from the two cutaneous nerves. In some embryos, this figure was estimated using the percentage contribution to each cutaneous nerve obtained from spinal nerve stimulation (Honig, 1982) and the relative cross-sectional areas of the two cutaneous nerves (measured from camera lucida tracings from histological sections through the limb).

RESULTS

The chick hindlimb is normally innervated by eight lumbosacral spinal cord segments (LS1–8) and the corresponding DRGs. As shown in Fig. 1, the spinal nerves from LS4–8 and the posterior part of LS3 join together in the ischiadic plexus and subsequently innervate posterior thigh, shank, and foot. Spinal nerves from LS1–3 and occasionally the last thoracic segment (T7) join together in the crural plexus and project primarily to the anterior thigh. For these studies, we have focused on the anterior spinal nerves and on the peripheral nerves that arise from them. In particular, we have examined projections to three anterior thigh muscles, the sartorius, the adductor and the femorotibialis, and along two nerves that project to skin, the lateral femoral and medial femoral cutaneous nerves. As detailed previously (Landmesser, 1978a; Honig, 1982), for each of these targets or nerves there is a distinct pattern of segmental projections. The sartorius and adductor both receive innervation from motoneurons in LS1 and LS2 with the sartorius being innervated mainly by motoneurons in LS1 and the adductor mainly by motoneurons in LS2. The femorotibialis is innervated by motoneurons in LS2 and LS3 (Landmesser, 1978a). The sensory innervation of each of these muscles arises from the corresponding DRGs; the percentage contributions to a given muscle from each segment as determined by retrograde HRP labeling are fairly similar for motoneurons and sensory neurons (Honig, 1982; here see Fig. 2 inset and control data in Table 2). Both of the cutaneous nerves that arise from the crural plexus contain axons from LS1, LS2, and LS3. However, as with muscle innervation, the segmental contributions to these nerves are also distinguishable. The lateral femoral cutaneous nerve receives its major contribution from LS1, whereas the medial femoral cutaneous nerve receives larger contributions

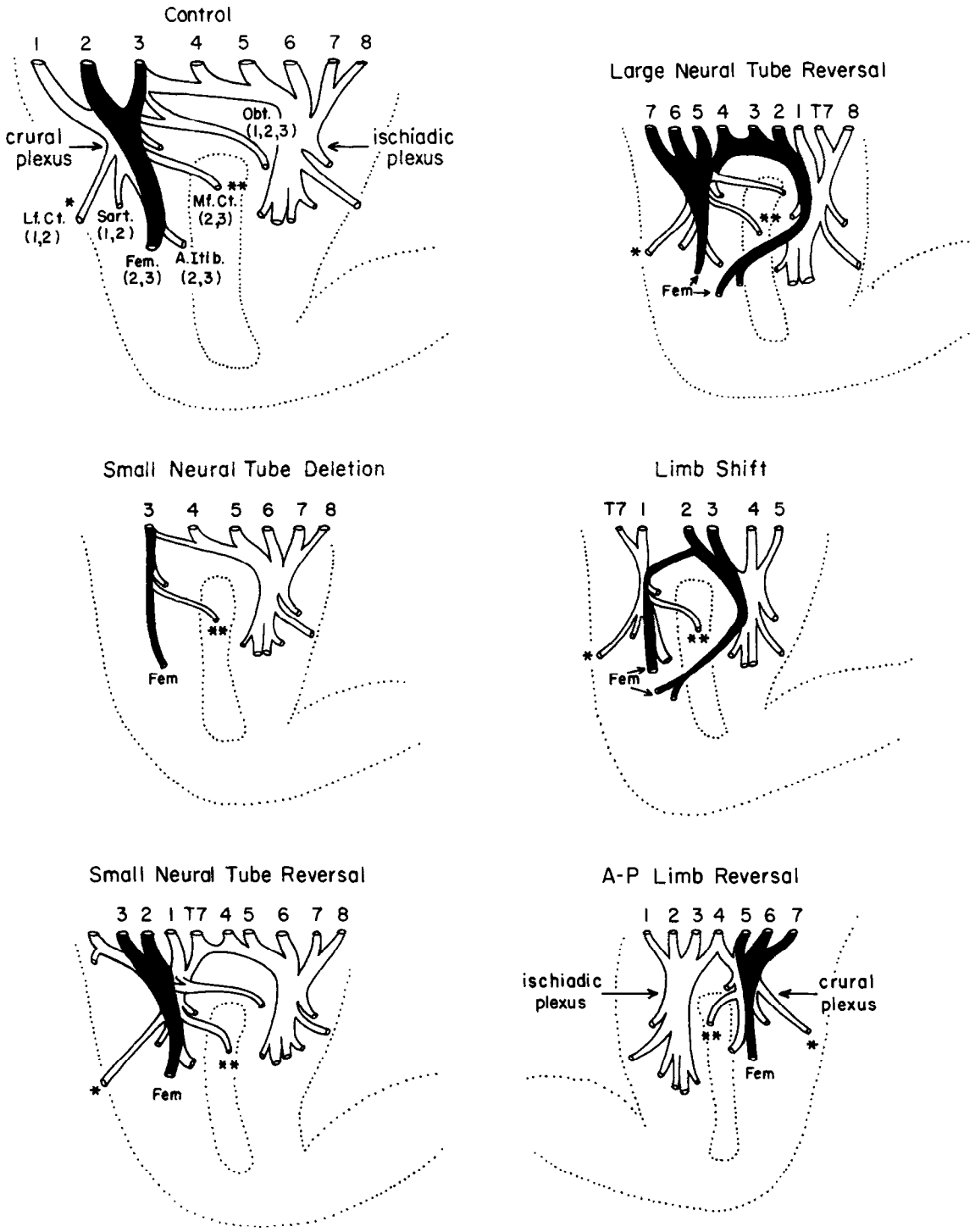

FIG. 1. Schematic representation of the gross anatomical pattern and specific nerve pathways in normal and experimental chick hindlimbs. The diagram of the control embryo shows the spinal nerve contributions to the two plexuses and the segments that normally provide the major source of axons to each peripheral nerve. To illustrate the basic pattern of motoneuron projections in the control and following each type of operation, the segments containing motoneurons that innervate the femorotibialis (Fem.) and the pathways those motoneurons take in the limb are shown darkened. Following small neural tube deletions, small neural tube reversals, and limb shifts, appropriate motoneurons innervate the femorotibialis. Following limb reversals, the femorotibialis is innervated by inappropriate motoneurons. Following large neural tube reversals, projections from both appropriate and inappropriate motoneurons are found. The thigh and femur are outlined. The obturator nerve (Obt.) innervates the adductor and obturator muscles. The single and double asterisks indicate the lateral femoral cutaneous nerve (Lf. Ct.) and the medial femoral cutaneous nerve (Mf. Ct.), respectively. Additional abbreviations: Sart., sartorius; A. Itib., anterior iliotibialis.

from LS2 and LS3 (Honig, 1982; here see Table 3 for control values).

Small Neural Tube Deletions

To determine if interactions among neurons arising from different segments are involved in the establishment of appropriate sensory projections, we deleted several lumbosacral neural tube segments (including the neural crest) prior to axonal outgrowth. We knew that motoneurons in the remaining segments still projected along the appropriate pathways to their correct targets (Lance-Jones and Landmesser, 1980a; but see Hollyday, 1983b). As shown schematically by the example in Fig. 1, removal of LS1 and LS2 leaves the sartorius, a muscle normally innervated by those segments, uninnervated. The femorotibialis, normally innervated by LS2 and LS3, receives innervation only from the remaining LS3. Thus, motoneurons from remaining segments do not innervate inappropriate muscles.

We examined the projections of the remaining afferents by injecting HRP into selected muscles, whose normal source of motor and sensory innervation had been completely or partially removed. Table 1 shows that, in most cases, neither motoneurons nor afferents in the remaining inappropriate segments projected to the experimental muscle. As a result, some muscles were left completely uninnervated whereas other muscles, deprived of only part of their normal innervation, were innervated by afferents and motoneurons situated in only the appropriate remaining segments (see, for example, Fig. 2). Minor projections from a given segment (e.g., LS3 for the sartorius, LS3 and LS4 for the adductor) were not greater than normal when the muscle's main source of innervation had been deleted. These results suggest that appropriate pathway selection has occurred and that there has not been extensive sprouting in the periphery. Neurons in remaining segments did not appear to compensate for the partial or the complete removal of the normal source of a muscle's innervation. In one embryo (D374) the results appeared to be somewhat different in that the femorotibialis received a larger projection from LS4 than is typical for normal embryos (Table 2; Landmesser, 1978a; Honig, 1982), although occasionally projections of this magnitude have been seen (Lance-Jones, 1986).

Most embryos listed in Table 1 were examined at st 35–36, toward the end of the period of sensory neuron cell death (Hamburger *et al.*, 1981). Therefore, it is possible that some sensory neurons had initially projected inappropriately and subsequently died. However, even in the three embryos examined at st 28–30, shortly after the onset of cell death, muscles were innervated only by appropriate segments. Thus, in the experimental embryos, as in normal embryos (Honig, 1982), afferents appeared to grow along only the appropriate pathways and ramify only within appropriate regions of the undivided muscle mass.

TABLE 1
PROJECTIONS TO MUSCLES FOLLOWING PARTIAL NEURAL TUBE DELETIONS

	Number of labeled neurons (DRG/MN) in each segment				
	LS1	LS2	LS3	LS4	LS5–8
Sartorius					
Control	216/276	156/100	12/2	0/0	0/0
D341	—/—	—/—	—/—	0/0	0/0
D356*	—/—	—/—	0/0	0/0	0/0
D337	—/—	—/—	5/—	0/0	0/0
D342	—/—	—/—	5/(0)	0/0	0/0
D320	—/—	—/—	28/—	0/0	0/0
D382L	276/(0)	259/616	52/27	0/0	0/0
D382R	255/(0)	116/402	14/3	0/0	0/0
Femorotibialis					
Control	33/115	394/600	485/626	0/0	0/0
D338	—/—	94/(0)	37/70	0/0	0/0
D354*	—/—	88/(21)	72/93	0/0	0/0
D339	—/—	24/(11)	116/405	0/0	0/0
D377R	—/—	95/(211)	145/119	0/0	0/0
D337L	—/—	71/(25)	221/317	0/0	0/0
D374L	—/—	—/—	63/(132)	112/79	0/0
D374R	—/—	—/—	20/(99)	5/81	0/0
Adductor					
Control	29/121	373/589	129/94	36/35	0/0
D364*	—/—	—/—	—/—	—/0	0/0
D348	—/—	—/—	0/—	0/0	0/0
D349	—/—	—/—	18/61	8/6	0/0

Note. HRP was injected into selected limb muscles in embryos in which a few segments of neural tube (and neural crest) had been deleted at st 15–16. Labeled neurons were counted in the remaining segments. Values indicated by an asterisk are from st 28–30 embryos. All other embryos were st 35–36. For each segment, the number of labeled neurons in the DRG is shown first followed by the number of labeled motoneurons (MN). A dash indicates a completely deleted segment. Parentheses enclose values for cord segments that were substantially depleted. Control values are from st 35–36 embryos (Honig, 1982); the minor contributions of T7 to the control muscles are not indicated.

Two additional lines of evidence support the view that sensory neurons grew along the appropriate muscle nerves at early stages and suggest that sensory neurons also chose the appropriate cutaneous pathways. First, we reconstructed the nerve patterns in six experimental embryos at st 26½–31. In these embryos, the cutaneous and muscle nerves that normally contain axons originating primarily from the deleted segments (e.g., the lateral femoral cutaneous nerve, the sartorius muscle nerve, and the obturator) were either markedly reduced in diameter or absent (see also Fig. 8 in Lance-Jones

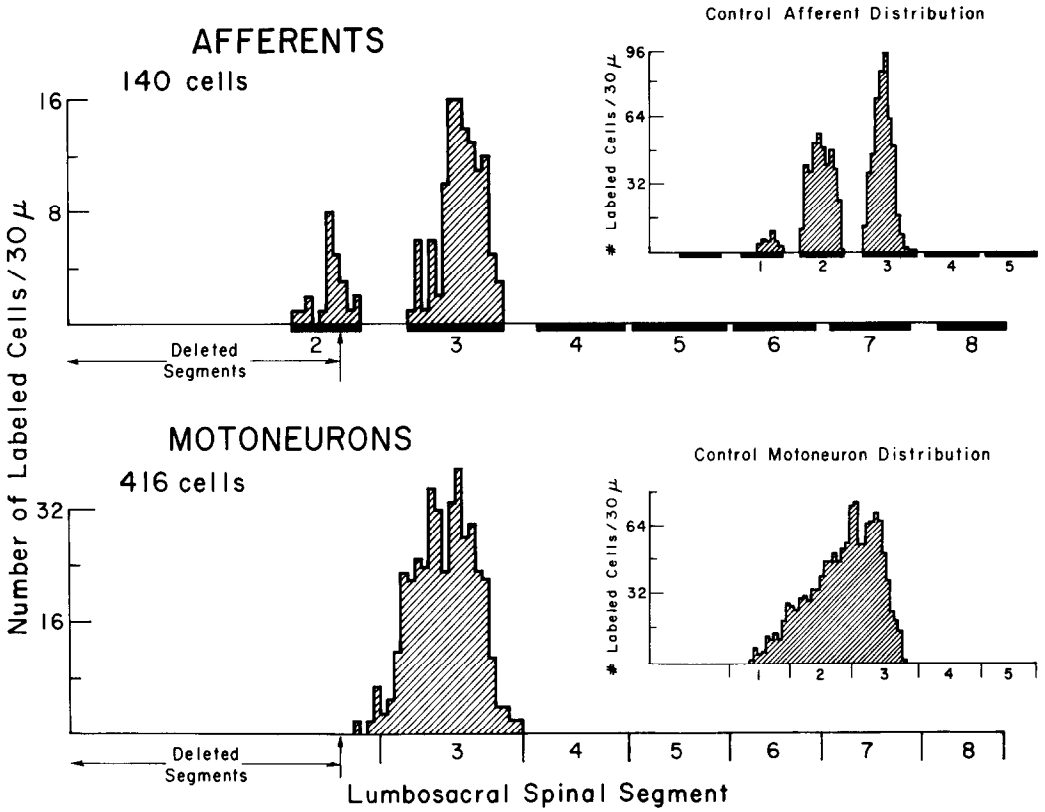

FIG. 2. Anterior-posterior positions of labeled afferents and motoneurons following small neural tube deletions. HRP was injected into the femorotibialis muscle of a st 36 embryo from which LS1-2 had been deleted at st 15-16. DRG2 formed at the end of the deleted region anterior to the return of the lateral motor column. Insets show distributions of femorotibialis afferents and motoneurons in a normal st 36 embryo. Inappropriate caudal segments did not project to the experimental femorotibialis. Labeled neurons were found only in appropriate segments LS3 and the small part of LS2 which remained. Note that the actual number of labeled cells in the experimental embryo (D339) was less than in the control (see Table 1).

and Landmesser, 1980a). Second, we used orthograde HRP labeling to examine the projections of remaining DRGs. While we did not quantify these results, they also suggested that neurons in the remaining DRGs projected normally. For example, when LS1 or LS1 and LS2 were removed (five limbs at st 29-31), DRG3 still sent more axons down the medial femoral cutaneous nerve than down the lateral femoral cutaneous nerve, just as it does in normal embryos. Thus, from the earliest stages of outgrowth, sensory cells in remaining segments appear to grow along only the appropriate peripheral pathways. The results of the deletion experiments, therefore, suggest that afferents demonstrate regional differences by the time of their initial outgrowth into the limb. To further test this possibility, we assessed sensory projections following anterior-posterior reversals of several segments of neural tube and neural crest.

Small Neural Tube Reversals

After reversal of the first few lumbosacral segments about the anterior-posterior axis, motoneuron axons enter the appropriate (crural) plexus but from the wrong positions. The displaced motoneurons alter their pathways in the plexus and major nerve trunks and project to the originally appropriate muscles (Lance-Jones and Landmesser, 1980b). For instance, as shown in Fig. 1, although LS3 motoneurons are now situated anteriorly, they still project to the appropriate femorotibialis muscle. Motoneurons in cord segments rostral and caudal to the reversal region innervate the same muscles as in normal embryos.

Effect of small neural tube reversals on sensory projections to muscles. Sensory projections to muscles were analyzed using HRP retrograde transport. As shown in Table 2, in three embryos in which the sartorius and femorotibialis muscles were injected on opposite sides, the majority of neurons projecting to the femorotibialis were situated in more anterior segments than were the neurons to the sartorius. This is the reverse of the normal situation. In four additional embryos in which the projections to a single muscle were assessed, the majority of sensory neurons projecting to the sartorius were situated more posteriorly than normally, and the ma-

TABLE 2
PROJECTIONS TO MUSCLES FOLLOWING PARTIAL NEURAL TUBE REVERSALS

	% labeled neurons (DRG/MN) in each segment							Number (DRG/MN) of labeled neurons
	Control values							
	T6	T7	LS1	LS2	LS3	LS4	LS5-8	Mean
Sartorius ($n = 5$)	0	3/5	54/69	40/26	3/1	0/0	0/0	462/538
Femorotibialis ($n = 4$)	0	0.08/0.0	8/11	36/42	56/47	0.2/0.1	0/0	902/1066
Adductor ($n = 6$)	0	0.2/0.03	9/29	61/63	24/8	5/1	0/0	318/718
	Experimental values							
	←	LS3	LS2	LS1	T	LS4	LS5-8	Total
CR 141 Sart.	13/1	10/4	68/46	9/49	0/1		0/0	77/402
Femoro.	18/25	34/62	32/12	16/2	0/0		0/0	324/785
CR 142 Sart.		5/8	23/29	43/40	29/23	0/0	0/0	107/911
Femoro.		30/23	43/48	14/28	14/2	0/0	0/0	365/463
CR 117 Sart.		0/0	1/0	48/93	51/7	0/0	0/0	125/115
Femoro.		0/6	56/90	1/3	—/2	43/0	0/0	191/108
CR 116 Sart.	0/0	—/0	0/2	41/60	59/38	0/0	0/0	351/457
CR 202 Femoro.	0/13	0/56	18/28	77/4		5/0	0/0	22/170
CR 45 Femoro.	—/2		40/47	42/10	1/1	18/40[a]	0/0	205/933
CR 94 Femoro.			88/72	6/10	6/0	—/18[a]	0/0	17/208
CR 116 Add.	0.3/0	9/4	22/54	21/31	31/4	7/6	*	387/193

Note. HRP was injected into selected muscles in embryos in which the first three lumbosacral neural tube segments had been reversed about the anterior-posterior axis at st 15-16. The number of labeled dorsal root ganglion (DRG) neurons and motoneurons (MN) in each segment is expressed as a percentage of the total number of labeled neurons of each type. Percentages greater than one have been rounded off. Experimental embryos were st 35-36 except CR 94 which was st 30. Control values are from st 35-37 embryos (see Honig, 1982). Values from ganglia contributing >30% to a given muscle are shown in boldface. Reversed segments are shown in their new positions, which are roughly comparable to those indicated above for controls, but with their original identities indicated. Reversals in different embryos varied in extent; sometimes thoracic cord and/or LS4 was included in the reversal. Shaded boxes indicate regions at the borders of the reversals. Occasionally two small ganglia were present at border regions; their percentage contributions have been summed. Sometimes a ganglion did not form at a given segmental level; this is indicated by a dash. A segment left blank was not present in the position indicated because the extent of the particular reversal was different from that listed above. In CR 45 and CR 94 T7-LS2 were reversed; the values marked by superscript [a] for LS4 actually represent the LS3 contribution (no labeled cells were found in LS4). The asterisk indicates there were labeled neurons in LS5-8 in this embryo as follows: LS5, DRG 0%, MN 1%; LS6, DRG 5%, MN 0%; LS7, DRG 5%, MN 0%; LS8, DRG 0%, MN 0%. n = number of embryos.

jority of those projecting to the femorotibialis were situated more anteriorly than normally. Thus, like motoneurons, afferents tended to project to muscles in accord with their origins rather than in accord with their new positions. However, some exceptions were found. In one case the adductor was innervated by neurons in many segments. In addition, the pattern of afferent projections usually was not a mirror image of the normal pattern as was generally true for motoneuron projections (Lance-Jones and Landmesser, 1980b).

Effects of small neural tube reversals on cutaneous projections. We assessed cutaneous projections in a series of st. 35-38 embryos by determining electrophysiologically the relative number of axons each DRG in the reversal region sent down the lateral femoral cutaneous and medial femoral cutaneous nerves (Table 3). In most animals many afferents now situated in an anterior position projected in accord with their origin. For instance, normally T7 does not contribute any axons to the medial femoral cutaneous nerve (13/14 st 36-38 control embryos) and the occasional contribution is very small (Honig, 1982; Scott, 1984). In 12 out of 14 experimental cases the ganglion (originally DRG3) now situated in the position of T7 made a significant contribution to the medial femoral cutaneous nerve. This contribution was, in some cases, as large as the projection made by DRG3 in normal animals. Thus the displaced DRG3 contributed to the medial femoral cutaneous nerve in a manner consistent with its origin as DRG3.

The ganglion in the position of DRG1 also projected in a manner more consistent with its origin (as DRG2) than with its new position. Normally the contribution

TABLE 3
SEGMENTAL CONTRIBUTIONS TO CUTANEOUS NERVES FOLLOWING SMALL NEURAL TUBE REVERSALS

Expt no.	% contribution of the medial femoral cutaneous nerve to spinal nerve compound action potentials			
	Control values			
	T7	LS1	LS2	LS3
	<2	18 ± 10	54 ± 21	82 ± 14
	Experimental values			
	← a. LS3	LS2	LS1	T7 p. LS3 →
224L	85	12	50	50
230R	80	60	47	100
240L	59	64	16	100
228R	36	49	53	69
228L	15	65	64	81
233R	0	11	64	54
	← LS3	LS2	LS1	T7 →
232L	100	79	9	84
231R	91	78	38	80
236L	90	28	1	23
232R	52	74	26	87
231R	48	100	16	52
236R	44	48	20	91
237L	12	68	63	77
237R	0	16	74	100
Mean	50.9	53.7	38.6	74.9

Note. Compound action potentials were recorded from spinal nerves in embryos in which T7-LS3 had been reversed about the anterior-posterior axis at st 15-16. The lateral and medial femoral cutaneous nerves were stimulated successively. The areas under the compound action potentials produced in each spinal nerve were measured. For each spinal nerve, the contribution to the medial femoral cutaneous nerve is expressed as a percentage of the arithmetical sum of the action potentials elicited by both cutaneous nerves. This provides a measure for each spinal nerve of the number of axons that project along the medial femoral cutaneous nerve relative to the lateral femoral cutaneous nerve. Control values (mean ± SD) are from st. 36-38 embryos; experimental values are from st 35-38 embryos. Crosshatched boxes indicate the borders of the reversals. Original identities of the reversed segments are indicated; their positions are comparable to those shown above for control embryos. Reversals varied slightly in extent. In the first group of experimental embryos, only the anterior part of LS3 (a. LS3) was included in the operation; the posterior part (p. LS3) remained in its normal position. In the second group of embryos, reversals included all of LS3.

of DRG1 to the medial femoral cutaneous nerve is a fairly small percentage (18 ± 10%, mean ± SD) of its total contribution to both cutaneous nerves. In the experimental animals the ganglion in this position projected much more heavily to the medial femoral cutaneous nerve (53 ± 27%), in a manner appropriate for its origin as DRG2 (54 ± 21%).

In contrast, the ganglion in the position of DRG3 usually contributed many more axons to the medial femoral cutaneous nerve than expected based on its origin. These axons may have projected along cutaneous pathways in accord with their new position. Thus, as with sensory projections to muscles, the pattern of projections was usually not the mirror image of the normal pattern.

Despite this exception, it is clear that many cutaneous sensory neurons do not project along peripheral nerves in accord with their new position. Thus, like muscle afferents, some cutaneous afferents can project to their originally appropriate targets when their positions are altered with respect to the limb.

Sensory Projections to Muscles following Large A-P Neural Tube Reversals, A-P Limb Reversals, and Limb Shifts

When motoneurons are displaced to a greater degree than following the small neural tube reversals such that their axons enter the limb through the wrong plexus, they either grow along inappropriate pathways to innervate inappropriate muscles or they take aberrant paths within the limb to reach the correct muscles (Lance-Jones and Landmesser, 1981b). In the experiments described below, we asked whether sensory neurons exhibited similar behavior.

Large A-P neural tube reversals. Following reversals of six to eight segments of lumbosacral neural tube (and neural crest), posterior segments (e.g., LS4-7) come to be situated anteriorly. As shown schematically in Fig. 1, motoneurons from these originally posterior segments project to inappropriate muscles in the anterior thigh, in this case the femorotibialis. HRP injections into selected anterior thigh muscles labeled afferents as well as motoneurons in these originally posterior segments (Table 4). Thus, sensory neurons in these cases also grow to incorrect muscles.

Some muscles also receive innervation from the correct motoneurons which project to that muscle along an aberrant path as shown schematically in Fig. 1. Here the femorotibialis is innervated by appropriate LS2 and LS3 motoneurons which first grow through the ischiadic plexus and then course anteriorly. In embryos with large neural tube reversals, the centrally located adductor muscle more frequently receives a projection from the appropriate motoneurons following an aberrant path than do the anteriorly situated sartorius or femorotibialis (Lance-Jones and Landmesser, 1981b). The results in Table 4 show that neurons in LS1 and LS2, even though situated in a posterior position, still innervated the adductor. Although we did not determine the segmental contributions to the plexuses, the extreme posterior positions of the labeled afferents make it probable that these sensory neurons had projected along aberrant paths.

TABLE 4
PROJECTIONS TO MUSCLES FOLLOWING LARGE NEURAL TUBE REVERSALS

	% of labeled neurons (DRG/MN) in each segment										Total number (DRG/MN) of labeled neurons
	T6 ▼										
Adductor											
CR252		LS8	LS7	LS6	LS5	LS4	LS3	LS2	LS1	T7	
		0/0	0/0	0/8	27/45	9/41	9/6	0/0	0/0	55/0	11/234
CR254			LS7	LS6	LS5	LS4	LS3	LS2	LS1	LS8	
			0/0.4	0/6	0/19	63/53	13/10	0/11	25/2	0/0	8/256
CR207			LS6	LS5	LS4	LS3	LS2	LS1	LS7	LS8	
			0/0	0/2	5/23	24/45	6/8	9/19	58/3	0/0	106/554
CR246		LS7	LS6	LS5	LS4	LS3	LS2	LS1	LS8		
		0/0	5/1	24/42	43/26	24/19	5/12	0/0	0/0		21/294
Femorotibialis											
CR256	LS6	LS7	LS6	LS5	LS4	LS3	LS2	LS1	T7	T6	
	0/0.3	9/8	8/11	44/40	41/39	0/0	0/0	0/0	0/0	0/0	181/788
CR266		LS7	LS6	LS5	LS4	LS3	LS2	LS1	T7	LS8	
		0/0	7/3	23/35	45/60	17/10	0/0	0/0	0/0	0/0	73/432
CR263	T6	LS7	LS6	LS5	LS4	LS3	LS2	LS1	T7	LS8	
	23/0	1/8	61/37	8/25	6/29	0/0	0/0	0/0	0/0	0/0	83/813
Sartorius											
CR266		LS7	LS6	LS5	LS4	LS3	LS2	LS1	T7	LS8	
		0/3	22/4	18/9	34/14	26/70	0/0	0/0	0/0	0/0	123/231
CR263	T6	LS7	LS6	LS5	LS4	LS3	LS2	LS1	T7	LS8	
	88/0.4	4/14	4/32	3/35	0/18	0/0.3	0/0	0/0	0/0	0/0	162/711

Note. HRP was injected into selected muscles in embryos in which six to eight lumbosacral neural tube segments had been reversed about the anterior-posterior axis at st 15-16. The number of labeled dorsal root ganglion (DRG) neurons and motoneurons (MN) in each segment is expressed as a percentage of the total number of labeled neurons of each type. Percentages greater than one have been rounded off. Experimental embryos were st 35-36. Control values are given in Table 2. Crosshatched boxes indicate borders of the reversals. Original identities of the reversed segments are indicated. The column on the far left represents the position of segment T6. In CR 263, one labeled DRG neuron was found in T5 following injection of the sartorius.

A-P limb reversals. Following A-P reversals of the hindlimb, most motoneurons enter a foreign region of the limb and grow to incorrect muscle targets (Landmesser and O'Donovan, 1984). For example, as shown in Fig. 1, the femorotibialis muscle now situated in a posterior position is inappropriately innervated by motoneurons from LS5, LS6, and LS7. Following retrograde transport of HRP from selected muscles, motoneurons in inappropriate segments were labeled; afferents in the corresponding DRGs were also labeled (Table 5). Thus, in this situation too, sensory neurons projected to incorrect muscles. In addition, the segmental pattern of projections for sensory neurons was strikingly similar to that of the motoneuron projection.

Limb shifts. In embryos subjected to limb shifts, spinal nerves enter the limb in a more posterior position than normal such that spinal nerves LS2 and LS3 (and sometimes LS1), which normally enter the limb through the crural plexus, join with more posterior spinal nerves (usually LS4 and LS5) to form an ischiadic plexus (Figs. 1 and 3). Anterior thigh muscles are innervated by appropriate motoneurons which project in either or both of two ways, depending on the extent of the shift (Lance-Jones and Landmesser, 1981b).

First, some lumbosacral motoneurons still enter the limb through the crural plexus and project to their correct muscles. The nerve patterns in the experimental limbs suggest that sensory neurons too may have projected appropriately (Table 6). In embryos in which the crural plexus contained axons from part or all of LS1 but lacked its normal complement of axons from LS2 and LS3, the femorotibialis nerve, which normally contains axons mostly from LS2 and LS3, was seen very rarely (3 out of 14 cases). In contrast, the sartorius nerve, normally formed mostly by LS1 and LS2, was found more frequently (9 out of 14 cases). Thus, the presence or absence of muscle nerves seemed to reflect the segmental origin of the neurons that normally contribute to those nerves.

Second, some motoneurons project through the ischiadic plexus and then along an aberrant path to innervate the appropriate muscle in the anterior thigh.

TABLE 5
PROJECTIONS TO MUSCLES FOLLOWING LIMB REVERSALS

	% labeled neurons (DRG/MN) in each segment							Total number (DRG/MN) of labeled neurons
	LS1	LS2	LS3	LS4	LS5	LS6	LS7	
	Ischiadic			Crural				
Sartorius (LS1-LS2)	0/0	0/0	0/0	52/54	38/46	10/0	0/0	21/123
	Ischiadic			Crural				
P. Iliotibialis (LS4-LS5)	11/11	47/42	43/48	0/0	0/0	0/0		47/191
	Ischiadic				Crural			
Iliofibularis (LS4-LS7)	17/2	74/89	9/9	0/0	ND			144/100

Note. HRP was injected into selected muscles in embryos in which the limb bud had been reversed about the anterior-posterior axis at st 17-20. The number of labeled dorsal root ganglion (DRG) neurons and motoneurons (MN) in each segment is expressed as a percentage of the total number of labeled neurons of each type. The segments that provide each muscle's major source of innervation in normal embryos are indicated in parentheses. Experimental embryos were st 36. Segmental positions and identities were not altered by this operation. However, anterior spinal nerves now formed an ischiadic plexus and grew into posterior thigh regions while posterior spinal nerves formed a crural plexus and innervated anterior thigh regions. ND, not determined.

To determine whether sensory neurons grew along these aberrant paths, we examined three embryos with limb shifts following injection of HRP into anterior thigh muscles (Table 7). In each of these, labeled sensory neurons as well as labeled motoneurons were found in segments that projected into the limb through the ischiadic plexus, indicating that these neurons must have grown along aberrant pathways to the injected muscles.

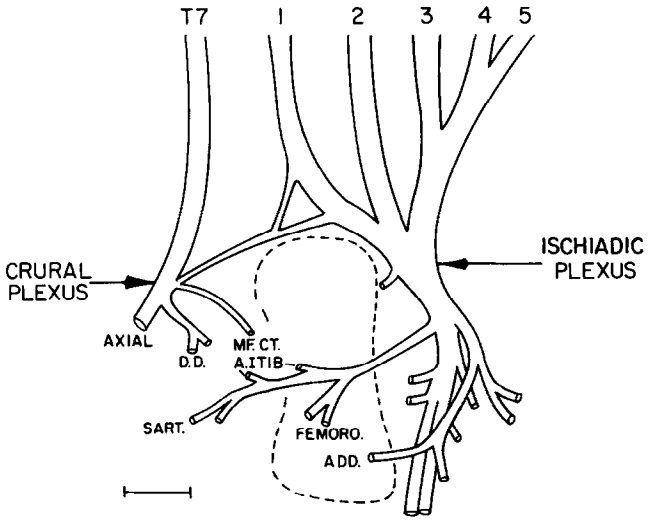

FIG. 3. The nerve pattern at st 30 in a limb that had been shifted several segments anteriorly at st 17½. Reconstructions were made from camera lucida drawings of cross sections through the limb. Spinal nerves T7 and LS1 formed a crural plexus which gave rise to only two nerves that entered the limb, the medial femoral cutaneous nerve (MF. CT.) and a branch to the deep dorsal muscles (D.D.). The other nerves which normally arise from the crural plexus were absent. The remainder of LS1 and LS2-5 converged in the ischiadic plexus. Several aberrant nerve trunks arose from the ischiadic plexus and coursed anteriorly to innervate the sartorius (SART.), the femorotibialis (FEMORO.), the anterior iliotibialis (A. ITIB.), and the adductor (ADD.) muscles. Calibration bar = 150 μm.

Comparison of sensory neuron and motoneuron contributions to muscles. Tables 4, 5, and 7 also show that the afferents projecting to a given muscle were for the most part located in the same segments as the motoneurons projecting to that same muscle. In some embryos, the actual percentage contributions were very similar for motoneurons and sensory neurons although in other embryos they were somewhat different. When all the data in these tables were considered, the percentage contribution of sensory neurons from a given segment projecting to a particular muscle was found to be correlated with the motoneuron contribution (correlation coefficient = 0.502; $P < 0.005$). A significant correlation was also found for the small neural tube reversals shown in Table 2 (correlation coefficient = 0.603; $P < 0.005$).

The Formation of Cutaneous Nerves following Large A-P Neural Tube Reversals, A-P Limb Reversals, and Limb Shifts

Following large neural tube reversals and limb reversals, posterior lumbosacral segments formed the crural plexus. It is clear from the overall nerve patterns that the lateral and medial femoral cutaneous nerves were then formed by segmentally inappropriate afferents. For instance as shown in Fig. 1, sensory neurons in inappropriate segments LS4, LS5, LS6, and/or LS7

TABLE 6
FORMATION OF CRURALLY DERIVED NERVES FOLLOWING LIMB SHIFTS

Expt No. (Stage)	Peripheral nerves present					
	Sartorius	Lateral fem. cutaneous	Ant. iliotibialis	Femorotibialis	Deep dorsals	Medial fem. cutaneous
141 (33½)	+	+	–	+	+	+
275 (36)	+	+	–	–	?	+
140 (33)	–	–	–	–	+	+
121 (32)	+	+	–	+	+	+
55 (34)	+	+	+	–	+	+
184 (34)	+	+	–	–	+	+
214 (36)	+	–	–	–	+	+
94 (34½)	+	–	–	+	+	+
225 (35)	+	+	?	–	–	+
239 (36)	–	–	–	–	+	+
69 (30)	–	–	–	–	+	+
12 (33)	+	+	–	–	?	–
270 (36)	–	–	–	–	+	+
131 (32)*	–	–	–	–	–	+
Total	9/14	7/14	1/13	3/14	10/12	13/14

Note. Cross sections of limbs were examined for the presence (+) or absence (–) of specific crurally derived nerves in embryos in which the limb had been shifted anteriorly at st 17-18. This table lists only those embryos with limbs shifted such that part or all of LS1, T7, and sometimes also T6 formed the crural plexus; LS2 and LS3 now entered the limb through the ischiadic plexus. Only those nerves which arose from the crural plexus are indicated. The obturator is not listed because its formation seems to be dependent on the presence of specific pelvic girdle elements (Tosney and Landmesser, 1984). The femorotibialis also includes the ambiens muscle. (?) Indicates uncertainty due to poor section quality. *A second aberrant medial femoral cutaneous nerve arising from the ischiadic plexus was also present in this embryo.

must have contributed axons to the lateral and medial femoral cutaneous nerves in both these situations. In some embryos, only one of the two cutaneous nerves was present but which of the two nerves formed did not seem to depend in any simple way on the segments contributing to the crural plexus. Motoneurons did not project down the cutaneous nerves in this or any other experimental situation examined (Lance-Jones and Landmesser, 1981b).

Similarly, following limb shifts, it is probable that crurally-derived cutaneous nerves frequently contained inappropriate afferents. For example, the medial femoral cutaneous nerve was formed in 13/14 embryos in which the crural plexus was composed of axons from part or all of LS1, T7, and sometimes T6 as well (Fig. 3 and Table 6). Since this nerve normally receives only a small contribution from LS1, it is unlikely that the experimental medial femoral cutaneous nerves were composed

TABLE 7
PROJECTIONS TO MUSCLES FOLLOWING LIMB SHIFTS

	% labeled neurons (DRG/MN) in each segment						Total number (DRG/MN) of labeled neurons
	T7	LS1	LS2	LS3	LS4	LS5	
	Crural		Ischiadic				
Femorotibialis (LS 121)	0/0	0/10	94/30	6/54	0/6		18/160
	Crural		Ischiadic				
Femorotibialis (LS 141)	0.2/0	17/3	39/36	44/61	0.5/0	0/0	563/488
	Crural		Ischiadic				
Adductor (LS 140)	6/0	53/5	18/95	24/0	0/0		34/124

Note. HRP was injected into selected muscles in embryos in which the limb bud had been shifted anteriorly at st 17-18. Some anterior spinal nerves now entered the posterior part of the limb and converged in the ischiadic plexus. The number of labeled dorsal root ganglion (DRG) neurons and motoneurons (MN) in each segment is expressed as a percentage of the total number of labeled neurons of each type. Experimental embryos were st 32-33½. Control values are in Table 2.

solely of appropriate axons from LS1; they probably contained a sizable number of inappropriate axons from T7 and/or LS1. In addition, the lateral femoral cutaneous nerve formed less frequently (7/14 embryos) than the medial femoral cutaneous nerve (13/14 embryos) even though the lateral femoral cutaneous nerve normally receives a larger contribution than does the medial femoral cutaneous nerve from DRG T7 and DRG1. The results were similar in six additional embryos in which, in addition to T7 and LS1, part of LS2 formed the crural plexus. The medial femoral cutaneous nerve was present in all six, the lateral femoral cutaneous nerve in only three. Thus, the formation of cutaneous nerves seemed not to depend on the specific outgrowth of the appropriate afferents. Instead, as suggested by the results in Table 6, the formation of each of the two cutaneous nerves appeared to be associated with the presence of the muscle nerve that diverges from the same general region of the main nerve trunk as the cutaneous nerve. For example, in 10 out of 12 cases in which the medial femoral cutaneous nerve was present, the nerve to the deep dorsal muscles was also present. Both normally arise from the posterior part of the crural trunk. More strikingly, the lateral femoral cutaneous nerve, which arises from the anterior part of the crural nerve trunk, never formed in the absence of the sartorius muscle nerve, which also arises from this anterior region, even though the nerve to the sartorius muscle was present only 64% of the time.

In five additional embryos with limb shifts, including four in which the crural plexus probably contained axons from thoracic segments only, crurally derived axons grew toward but did not penetrate far into the limb; neither muscle nor cutaneous nerves were formed. In contrast, as just discussed, thoracic sensory neurons grew into the limb and along cutaneous pathways following slightly less extensive shifts, when lumbosacral neurons were present and when muscle nerves formed. Thus, in both situations, whether or not cutaneous nerves form might be associated with the presence or absence of muscle nerves or of motoneurons.

We also wanted to determine whether sensory neurons could take aberrant paths from the ischiadic plexus to grow along cutaneous pathways normally occupied by crurally derived axons. Such aberrant cutaneous nerves arising from the ischiadic plexus and projecting to the anterior thigh were generally not found following limb shifts even when aberrant muscle nerves were present (14 out of 17 embryos). However, in each of three embryos in which few or no crurally derived axons grew directly into the anterior thigh, an aberrant nerve that grew distally on the medial surface of the thigh between the femorotibialis and adductor muscles, along the normal pathway of the medial femoral cutaneous nerve, was found. In two embryos, the sensory neurons projecting along this aberrant path appeared to have been initially accompanied by motoneuron axons, since the nerve trunk that diverged from the ischiadic plexus also sent a branch to anterior thigh muscles. However, in the third embryo, the aberrantly projecting nerve branch followed only the medial femoral cutaneous nerve pathway, suggesting that it contained only sensory neuron axons.

DISCUSSION

Passive Spatiotemporal Mechanisms and Competition

In the chick hindlimb sensory neurons grow specifically along precise pathways from the outset to project to their segmentally correct targets (Honig, 1982; Scott, 1982). The results presented here allow us to exclude several possible spatiotemporal mechanisms by which this may be achieved. Jacobson (1978) suggested that axons that enter the limb first occupy the most proximal pathways while those axons that grow out later occupy successively more distal and still available pathways. This possibility seemed unlikely based on descriptions of the actual projections in normal limbs (Honig, 1982) and can now be excluded based on the results of small neural tube deletions. Sensory neurons in the remaining segments grew only to their normal targets and not to more proximal targets whose source of innervation had been removed. The projections of remaining limb neurons did not expand to innervate the entire target area, as is found for retinal ganglion cells (see reviews by Gaze, 1978; Willshaw and Malsburg, 1979). Generally similar results have been reported for the chick by Castro (1963) and, following partial neural crest deletions, by Eide *et al.* (1982) and by Scott (1984). In contrast, Frank and Westerfield (1982) have observed expansion of sensory neurons into denervated areas in the frog.

Since muscle afferents did not sprout to innervate adjacent uninnervated muscles, competition for target sites cannot normally restrict afferents to appropriate muscle regions. Similarly, cutaneous afferents do not appear to be confined to their normal pathways by competition with other cutaneous afferents. However, competition among axons may have some effect in determining the relative distribution of sensory axons within the pathways they normally occupy. Scott (1984) has found that while the projections of DRGs immediately adjacent to the deleted region were generally normal following small neural crest deletions, the axonal projections of DRGs several segments away from the deleted region appeared to shift toward but not into inappropriate "denervated" pathways. Whether motoneuron pathways (which were undisturbed in Scott's experiments though not in ours) and sensory cell death play any role in this shift is un-

clear. Nevertheless, the presence of neighboring sensory neurons does not seem to be a necessary component of how sensory neurons select the appropriate pathways.

We can also rule out a mechanism proposed by Horder (1978). He suggested that limb-innervating neurons maintain constant topographical relationships with each other as they course through the spinal nerves and plexus and that they subsequently are channeled into peripheral nerves depending on their position in space and time. If this were true, then following small neural tube reversals, neurons should project in accord with their new positions. Instead we found that many sensory neurons grew along pathways that were the correct ones based on their original position. To do so they had to grow in different directions than normally and some axons had to cross the paths of other axons as also occurs during normal development (Honig, 1982).

In other experimental situations, some sensory axons showed even more dramatic changes in their trajectories. Following large neural tube reversals and limb shifts, when axons were displaced far from their normal point of entry into the limb and entered it through the wrong (ischiadic) plexus, some axons grew along novel pathways to reach skin and muscle targets in the anterior thigh. Such changes in pathways cannot be explained by a simple spatiotemporal mechanism.

However, we also found following some of these large displacements (for example, in large neural tube reversals and limb reversals), that many sensory axons that had entered the limb through the wrong plexus, projected along inappropriate pathways, perhaps simply being nonselectively or passively channeled into those nerves (see also Lance-Jones and Landmesser, 1981b). Thus, we do not mean to imply that channeling of sensory axons based on their position in major nerve trunks never occurs. We also do not intend to completely exclude the effects that the temporal sequence of outgrowth or that motoneuron axons may have on the development of sensory projections. These issues will be addressed later. However, the projections we find cannot arise simply by sensory axons maintaining constant topographical relationships with each other.

Regional Differences among DRGs

Our results indicate that DRGs demonstrate regional differences prior to axonal outgrowth. For example, following small neural tube reversals, sensory neurons generally projected along pathways in accord with their segmental origins. DRG3 neurons tended to form different projections than DRG1 neurons even when they were situated in the position that DRG1 normally occupies.

These projections could have been the result of random initial outgrowth followed by selective cell death, rather than a consequence of appropriate pathway selection. Since we did not examine the embryos with small neural tube reversals until near the end of the period of sensory cell death, it is possible that their sensory projections had initially been inappropriate. However, many sensory neurons that grew to inappropriate targets in other situations survived the cell death period, showing that contact with the wrong target does not necessarily lead to cell death. It therefore seems likely that, as they grow into the limb, sensory neurons behave in a manner characteristic for their segmental origin.

A striking observation was that sensory neurons and motoneurons usually responded in a similar manner to each experimental manipulation. Following small neural tube deletions, the remaining motoneurons and sensory neurons projected along only the appropriate pathways. Following small neural tube reversals, motoneurons altered their paths in the plexus region to grow to their correct targets; many sensory neurons also projected appropriately. In large neural tube reversals and limb reversals, some motoneurons and sensory neurons entered the limb through the wrong plexus and projected to the wrong targets. Other motoneurons projecting into the limb through the wrong plexus (following limb shifts and large neural tube reversals) were able to grow along novel pathways to reach their correct targets; some sensory neurons also grew along aberrant paths to muscle and to skin. Following each type of operation, the afferents projecting to a given muscle were generally located in the same segments as the motoneurons projecting to that muscle (Tables 1, 2, 4, 5, and 7). In some embryos, most notably those with limb reversals (Table 5), the actual percentage contributions were very similar for motoneurons and sensory neurons. In addition, overall the contribution of sensory neurons from a given segment was correlated with the motoneuron contribution from that segment.

This observation is compatible with two types of mechanisms. First, the regional differences demonstrated by DRGs may be intrinsic to the neurons themselves. Sensory neurons may be specified with respect to their peripheral targets prior to axonal outgrowth and actively respond to cues in the limb in a manner similar to the way motoneurons respond. After small displacements sensory neurons may be able to alter their pathways to reach the correct targets; after large displacements they may be unable to sense or respond to the necessary cues and may be passively channeled along the wrong pathways.

A second possibility is that sensory neuron outgrowth is somehow directed by motoneuron outgrowth and that the regional differences shown by DRGs consequently reflect the specific identities of the motoneurons situated at the same segmental levels. Data presented in the fol-

lowing paper (Landmesser and Honig, 1986) provides support for this possibility by showing that the absence of motoneurons has profound effects on sensory neuron outgrowth. Therefore, in the paragraphs below, we describe how motoneurons may influence the pattern of sensory neuron projections and discuss whether the experiments presented here allow us to distinguish between these two types of mechanisms.

Based on the temporal and spatial pattern of normal development, we have previously proposed that some sensory neuron axons may become associated with motoneuron axons in the spinal nerves and be guided by them to muscles (Honig, 1980; 1982). The segmental contributions of motoneurons and sensory neurons to muscles would then be similar not only in normal embryos but also in embryos with various experimental manipulations. Differences in segmental contributions would be possible with this mechanism if, for example, in embryos with small neural tube deletions, some sensory neurons instead become associated with motoneurons from nearby segments which they first encounter in the plexus.

Interactions with motoneuron axons could also explain the results on cutaneous projections. Following deletions of anterior segments, sensory neurons may project along the appropriate cutaneous pathways because, in the absence of motoneurons, they do not have access to nerve pathways arising from the anterior part of the crural plexus. Similarly, following small neural tube reversals, DRG neurons of posterior origin now situated anteriorly, may be able to grow into the posterior part of the plexus (and hence along their appropriate posterior cutaneous pathways) because of the presence of motoneuron axons which show similar pathway alterations.

Can we distinguish between these mechanisms based on the kind of data presented here? Since the segmental distributions of sensory neurons and motoneurons projecting to each muscle normally are very similar, the two possibilities lead to basically the same predictions for all of the experimental manipulations. For instance, according to either mechanism, only muscle nerves that normally originate from LS1 should form in the crural region when limbs were shifted such that the crural plexus contained axons from LS1 but none from LS2 and LS3. In general this was the case, and the number of retrogradely labeled sensory neurons in segments that projected via crural paths was within the normal range for each muscle (Table 7). However, we would have to examine more retrogradely labeled embryos to ascertain whether only the segmentally appropriate sensory neurons had projected along these muscle nerves. Thus, we cannot distinguish whether in this situation sensory neurons had grown specifically to the appropriate muscles or whether they had simply grown along pathways established by the segmentally appropriate motoneurons. Similarly, in embryos with small neural tube deletions, we could ask whether sensory neurons from remaining appropriate segments projected to muscles that completely lacked motoneuron innervation. In three out of five such cases, there in fact appeared to be a small sensory projection. However, in view of the limitations of retrograde HRP labeling and variability between embryos in the anterior-posterior extent of their motoneuron and sensory neuron pools, these data do not unequivocally show that sensory neurons projected to muscles that completely lacked motoneuron innervation or that a sensory projection was absent when a small one should have been present. Thus it is exceedingly difficult to distinguish between these mechanisms based on this type of analysis.

Another approach would be to ask if aberrant nerves form that contain sensory neuron axons but not motoneuron axons. In fact, in three embryos with limb shifts, aberrant cutaneous nerves were present, but in at least two of these cases motoneurons may have directed sensory neuron axons away from the ischiadic nerve trunk and toward the anterior thigh where separate muscle and cutaneous branches formed. While sensory neuron axons may have responded independently of motoneurons to cues emanating from the uninnervated anterior thigh, we would need to know whether aberrant sensory nerves can form when motoneurons are absent to be certain of this.

Some characteristics of the nerve patterns in embryos with limb shifts clearly cannot be explained by sensory neurons being specified. Many sensory neurons that entered the limb through the crural plexus did not grow along the segmentally appropriate cutaneous nerve pathways. That is, afferents in thoracic DRGs sent considerable numbers of axons to the lateral femoral cutaneous nerve along which only a few of these neurons normally project and to the medial femoral cutaneous nerve along which they seldom normally project (Honig, 1982; Scott, 1984). In addition, projections along the medial femoral cutaneous nerve were observed more frequently even though this pathway is more inappropriate for these neurons than is the lateral femoral cutaneous nerve. Thus, factors other than the origin of sensory neurons seem to govern the formation of and projections along cutaneous nerves in this situation. Further, this pattern of projections could not have been produced by the indiscriminant channeling of sensory neurons along all available pathways. Rather, one possible explanation for the formation of cutaneous nerves in this situation is suggested by the frequent cooccurrence of particular muscle and cutaneous nerves that arise from the same part of the plexus region. Motoneurons may affect the choice of cutaneous pathways by guiding the growth of

all sensory neurons through the plexus region. The precise positions of sensory neurons in the plexus and nerve trunks may in turn determine along which cutaneous pathways they grow. For example, motoneurons projecting to the deep dorsal muscles may guide sensory neurons toward the posterior part of the plexus region. Once in that posterior region, sensory neurons may be more likely to project along the medial femoral cutaneous nerve, which also arises from the posterior part of the plexus, than along the lateral femoral cutaneous nerve, which arises from the anterior part of the plexus. Other environmental features such as skeletal elements (Tosney and Landmesser, 1984) and blood vessels (Landmesser and Honig, 1986) may also affect the positioning of nerves and pathway selection.

Thus several aspects of the data presented here suggest, but do not prove, that motoneurons may influence the pattern of sensory projections. Further evidence in support of this idea comes from recent findings of Scott (1985). She found that when the neural crest was reversed along the anterior–posterior axis such that many motoneurons were left in their original position, the displaced sensory neurons tended to grow along cutaneous nerves more in accord with their new positions than with their origins. Thus the choice of one cutaneous nerve pathway over another seems to be influenced by the pattern of motoneuron projections.

It is important to note that there were some differences between the results on sensory neurons and motoneurons which are not compatible with either mechanism. First, the segmental contributions of sensory neurons and motoneurons to muscles were not always similar. One reason for this might be that following most of the operations some DRGs clearly were depleted. Missing or small projections might therefore merely reflect that depletion and the relative contributions of other DRGs could then be large even though this might not actually involve a greater number of neurons forming the projections than in normal embryos. That depletion in fact could be a problem is illustrated by one embryo (CR 263) in which it was striking that the DRG that contributed the most to the sartorius, was the only DRG in the region not drastically depleted by the operation. Further, the similarity in segmental contributions of afferents and motoneurons was the greatest in embryos with limb reversals, an operation which did not affect DRG formation.

Second, in small neural tube reversals, the pattern of projections was usually not a mirror image of the normal pattern. As just described, depletion of some DRGs might partly explain this. Uncertainties regarding the exact segmental composition of the DRGs (see Materials and Methods) might be an additional complicating factor. For example, in some embryos, the ganglion in the position of DRG3 might have contributed heavily to the medial femoral cutaneous nerve because it actually contained some DRG 3 neurons (not displaced by the operation) whose normal pathway is the medial femoral cutaneous nerve. In addition, some neurons in this ganglion probably were of thoracic origin which, not normally having any targets in the limb, might have been channeled along the medial femoral cutaneous nerve in accord with the posterior position they would occupy as they grew in the plexus and nerve trunks. Similarly, in some cases (233R, 237L, 237R), the ganglion in the position of T7 might have contained cells (either from DRG3 or the anterior part of DRG4) that normally entered the ischiadic plexus and now responded as they would following a "large" neural tube reversal and grew along the lateral femoral cutaneous nerve in accord with their anterior position.

Possible Constraints on Sensory Neuron Outgrowth

Despite these qualifications of the data, it seems likely that some afferents might have actually responded differently than did motoneurons to the manipulations and that sensory neurons were more readily channeled down incorrect pathways than were motoneurons. This may indicate that there are considerable constraints on sensory neuron outgrowth. For example, after small neural tube reversals, LS1 afferents enter the limb in the posterior part of the crural plexus. Some of them may be unable to cross the other axons to reach the anterior part of the plexus and project to the appropriate sartorius muscle. As a result they would continue to course in the posterior part of the nerve trunk and subsequently be channeled into a muscle nerve (i.e., the femorotibialis) when femorotibialis motoneurons which eventually attain a posterior position exit from the nerve trunk. Other LS1 afferents may similarly be confined to the posterior part of the plexus and thereby be able to project only to the medial femoral cutaneous nerve and not to the appropriate lateral femoral cutaneous nerve.

Constraints on the ability of sensory neurons to alter their pathways may be due in part to the later stages at which many sensory neurons grow into the limb as compared to motoneurons (Honig, 1982; Landmesser and Honig, 1986) and may simply reflect the presence of so many more additional axons. We do not know if older sensory neurons, which grow out at relatively early stages, tend to project to their correct targets more frequently than do the younger sensory neurons, which grow out later. Sensory neurons may also be less able than motoneurons to alter their pathways because their growth cones are smaller than those of motoneurons (Tosney and Landmesser, 1985b; Landmesser and Honig, 1986) and they exhibit lower levels of the neural cell

adhesion molecule, NCAM (Tosney *et al.*, 1986; Landmesser and Honig, 1986).

An additional interesting finding was that following the more extensive limb shifts, thoracic sensory neurons (and motoneurons) did not seem to grow into the limb to a significant extent. Occasionally, following motoneuron removals, we also found that sensory neuron axons did not enter the limb (Landmesser and Honig, 1986). One possible interpretation of these results is that motoneurons may normally facilitate the growth of sensory neurons through limb tissue (see also Landmesser and Honig, 1986).

Specification of Sensory Neurons

The results presented here suggest that an orderly pattern of sensory projections may come about as a result of interactions with motoneuron axons, rather than as a consequence of specificity. Based on experiments showing that neurons made to innervate foreign muscles in the frog limb subsequently form central projections that match their peripheral ones, Frank and Westerfield (1982) have also suggested that sensory neurons may not be specified with respect to their peripheral connectivity. However, neither our experiments nor theirs provide conclusive proof against specificity. In fact, most of our data could just as well be explained by a mechanism in which sensory neurons are specified and actively respond to cues in the limb. The remainder of our data could also be explained with this mechanism if sensory neuron outgrowth is subject to the constraints just discussed and/or if sensory neurons are less rigidly specified than are motoneurons. The cues sensory neurons respond to may in large part be the same cues that motoneurons use. Although we do not know what those cues are for either motoneurons or sensory neurons, it seems unlikely that they could be provided solely by muscle (Lewis *et al.*, 1981; Lance-Jones and Landmesser, 1981b; Tosney and Landmesser, 1984; Landmesser, 1984; Lance-Jones, 1986), by skin (Kitson and Roberts, 1983), or by nerve growth factor (Letourneau, 1982; Lumsden and Davies, 1983). It is also possible that motoneuron axons provide a major source of cues for sensory neurons, although other cues would be required for the formation of cutaneous pathways.

A mechanism in which motoneurons direct sensory neuron outgrowth does not rule out the possibility that sensory neurons are specified. For example, sensory neurons may vary with respect to their interactions with specific motoneurons. Therefore, these two possibilities are not necessarily mutually exclusive. The following paper (Landmesser and Honig, 1986) provides additional insight into this problem and discusses the possible nature of the interactions between sensory neurons and motoneurons.

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