DEVELOPMENTAL BIOLOGY 186, 27–35 (1997)
ARTICLE NO. DB978583

Muscle Sensory Innervation Patterns in Embryonic Chick Hindlimbs Following Dorsal Root Ganglion Reversal

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Previous studies suggest that sensory innervation of muscles is patterned by motor innervation. Muscle afferent projections mirror motor projections after various experimental manipulations and muscle afferents fail to project to muscle in the absence of motoneurons. It is not known, however, whether muscle afferents are specified with respect to the corresponding motoneurons or target muscles. To test this possibility we rotated three to four segments of neural crest in St. 15–17 chick embryos, leaving motoneurons intact, to reverse the rostrocaudal order of dorsal root ganglia (DRGs) T7/LS1–LS3. This caused sensory neurons derived from one segmental level to grow into the limb with motor axons from a different level. The resulting innervation patterns were assessed at St. 28–37 by injecting DiI and DiA into the sartorius and femorotibialis muscles or into the spinal cord and DRG. DiI labeling of crest prior to rotation showed that DRGs in the operated region were derived primarily from rotated cells. Muscle afferents from rotated DRGs grew to muscles in accord with their new rostrocaudal position, together with "inappropriate" motor axons from the same segmental level. The segmental distribution of sensory neurons innervating each muscle was more widespread in embryos operated at older than at younger stages. In contrast, sensory axons projected to the appropriate muscles in accord with their embryonic origin when segments of the whole neural tube, including motoneurons, were rotated, as reported previously. Thus, sensory neurons do not appear to be selectively matched with motoneurons or target muscles at stages when the corresponding motoneurons have clear identities.

INTRODUCTION

During embryogenesis both sensory and motor innervation patterns are established precisely and correctly from the outset, and neither cell death nor retraction of axons plays a role in the development of appropriate connectivity (Landmesser, 1978; Honig, 1982; Scott, 1982). It is well established that motoneurons destined for a particular muscle acquire specific identities (i.e., become specified) at early embryonic stages, which allows them to actively seek out the appropriate pathways and targets (Lance-Jones and Landmesser, 1980, 1981; Matise and Lance-Jones, 1996), even when experimentally displaced from their usual position. Much less is known about the mechanisms that ensure the establishment of appropriate sensory innervation.

Sensory neurons, like motoneurons, tend to project along their usual pathways to reach their appropriate targets following a variety of experimental manipulations (Honig *et al.*, 1986; Scott, 1992). One interpretation of these findings is that sensory neurons, like motoneurons, are inherently specified with respect to their peripheral connectivity. An alternative explanation, however, is that sensory neurons are guided or directed to the periphery by neighboring motoneurons (Honig *et al.*, 1986; Landmesser and Honig, 1986). This latter interpretation is supported by the observation that muscle afferent outgrowth and patterning appear to be absolutely dependent on motoneurons. Sensory axons grow out together with, but slightly later than, motor axons (Tosney and Landmesser, 1985b; Landmesser and Honig, 1986) and afferents are unable to project to muscles when motoneurons are removed prior to axon outgrowth (Taylor, 1944; Landmesser and Honig, 1986; Swanson and Lewis, 1986; Scott, 1988; Tosney and Hageman, 1989). Moreover, when the rostrocaudal order of dorsal root ganglia (DRGs) is reversed leaving motoneurons in place, *skin* sensory neurons tend to establish innervation patterns in accord with their new position (Scott, 1986), as if they are guided by cues from nearby intact motoneurons rather than their own inherent specificity. Muscle afferent projections were not examined in these embryos. Thus, it is not known whether muscle

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sensory neurons are specified or selectively matched with respect to the corresponding motoneurons or target muscle.

In the present study, we reversed the rostrocaudal order of DRGs by rotating the neural crest (Scott, 1986) and focused on the development of muscle sensory innervation patterns. In order to confirm the origin of DRGs from rotated neural crest, we injected neural crest in a selected segment with the carbocyanine dye DiI prior to rotation in some embryos. The resulting muscle sensory innervation patterns, mapped with retrograde and orthograde transport of DiI and DiA, showed that muscle afferents projected in accord with their new position and did not selectively associate with their embryologically appropriate motoneurons or target muscles. Some of these results have been reported previously in abstract form (Wang and Scott, 1996).

MATERIALS AND METHODS

General

Fertile White Leghorn chick eggs from a local supplier were incubated in a humidified, forced-draft incubator at 38°C. Embryos were staged according to Hamburger and Hamilton (1951) at the time of surgery and at sacrifice.

Surgical Manipulations

Several types of surgical manipulations were performed on embryos between St. 15 and 17 [Embryonic Day 2.5 (E2.5)]. A window was cut in the shell over the embryo, the embryo was lightly stained with 0.5% neutral red in PBS, and the vitelline membrane was opened. The desired portion of neural tissue was excised with sharpened tungsten needles and rotated and replaced or discarded, as described below. The embryo was moistened with several drops of Ringer's solution of the following composition (m*M*): NaCl, 150; KCl, 3; $NaHCO_3$, 17; $MgCl_2$, 1; $CaCl_2$, 3; dextrose, 12; Hepes, 10; pH 7.4. Eggs were sealed with paraffin and a coverslip and returned to the incubator until the desired stage.

Neural crest rotations. The goal of our experiments was to reverse the rostrocaudal order of DRGs from the last thoracic (T7) through third lumbosacral (LS3) segments, leaving the corresponding motoneurons intact. Thus, we needed to rotate neural crest opposite somites 26–29. However, the neural crest is too fragile to be surgically manipulated alone, so the neural crest together with approximately the dorsal half of the neural tube was excised, rotated 180° about the rostrocaudal axis, and reimplanted, as illustrated in Fig. 1. To aid in orientation, the rostral end of the piece was marked with carbon particles prior to excision. In younger embryos the caudal end of the excised region had to be estimated, because somite segmentation had not yet progressed to that level. Of the 167 embryos that underwent this type of neural crest rotation, 58 survived to the desired stage, developed DRGs throughout the operated region, and were normal in gross appearance. Fifty-two of these were successfully injected with dyes at St. 35–37 or St.28–30, as described below.

Because we rotated only the dorsal neural tube, it was sometimes difficult to assess the exact extent of the operated region when we dissected the embryos for dye injection. If we misjudged the number of somites at the time of surgery, our rotations could be displaced one segment rostrally or caudally from the intended location. Moreover, the rotated piece could be shifted somewhat relative to the somites when it was replaced. To determine whether this amount of variability would affect the results obtained, in 4 embryos we deliberately rotated the "wrong" region of neural crest, that opposite somites 28–31, to reverse the rostrocaudal order of DRGs LS2–LS5. In addition, in 6 embryos we rotated only three segments of neural crest to reverse DRGs LS1–LS3.

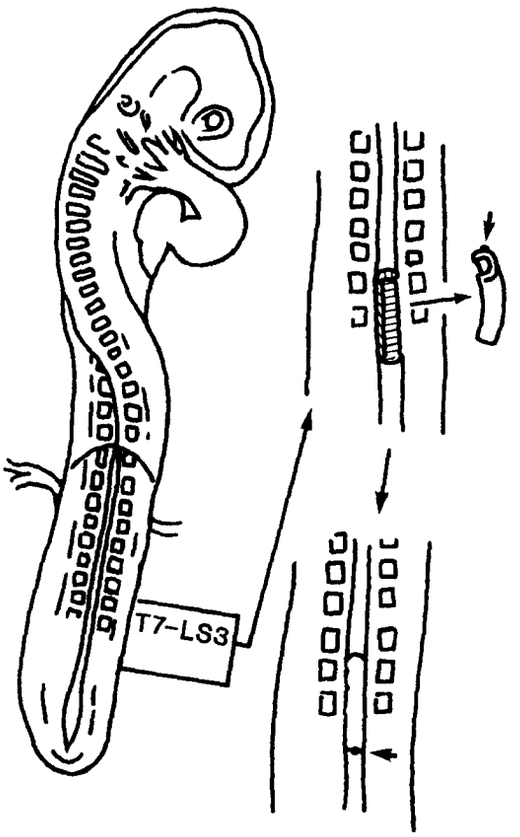


FIG. 1. Schematic representation of surgical procedures. Diagram of a St. 15 (E2.5) embryo (left) shows the segmental level of dorsal neural tube that was rotated. Neural crest together with dorsal neural tube was removed opposite somites 26–29, keeping the ventral neural tube intact (top right), rotated 180° about the rostrocaudal axis, and replaced (bottom right). The black dot (short arrow) represents carbon particles that we placed on the rostral end of the dorsal neural tube prior to excision to aid in orientation.

Neural crest injections. In order to be certain that our experimental manipulations did not alter the migratory pattern of neural crest, in one group of embryos we injected DiI (see below) into the crest opposite somite 26 (corresponding to segment T7) prior to excision. Embryos were allowed to develop until St. 28–30, when they were fixed and sectioned (see below) and the location of labeled DRG cells was identified.

Neural tube rotations. As an additional control, the entire neural tube and neural crest opposite somites 26–29 or 27–29 was excised, rotated, and reimplanted in St. 14–16 embryos. Notochords were also rotated for ease of manipulation, but did not appear to affect the resulting innervation patterns (see also Lance-Jones and Landmesser, 1980, 1981; Scott, 1986; Matise and Lance-Jones, 1996).

Retrograde and Orthograde Labeling with DiI and DiA

Operated embryos were incubated until St. 28–37, when they were removed from the eggs, staged, and placed in a chamber containing oxygenated Ringer's at room temperature (20–22°C). Embryos were quickly decapitated and eviscerated and a ventral laminectomy was performed from the high thoracic through the lumbosacral levels to allow adequate oxygenation of the spinal cord. Embryos were dissected further to expose both the sartorius and the femorotibialis muscles, the spinal cord, and DRGs or cutaneous nerves for dye injection.

Retrograde labeling. The sartorius and the femorotibialis muscles, which receive both motor and sensory innervation from segments LS1–LS2 and LS2–LS3, respectively (Landmesser, 1978; Honig, 1982), were injected with DiI [1,1'-dioctadecyl-3,3,3',3-tetramethylindocarbocyanine perchlorate (Molecular Probes, Inc., Eugene, OR); 2.5 mg/ml dimethylformamide (DMF)] or DiA [4-(4-dihexadecylaminostyryl)-*N*-methylpyridinium iodide; 3 mg/ml DMF] at St. 35–37 to identify the source of their sensory and motor innervation. Injected embryos were maintained in oxygenated L15 medium (GIBCO BRL, Grand Island, NY) at 28°C for 6 hr, when they were fixed in 4% paraformaldehyde in phosphate-buffered saline (PBS), pH 7.4. The embryos were stored in the same fixative at 37°C for another 2–3 weeks to allow thorough retrograde labeling. DiI always labeled neurons more robustly than did DiA. However, in all embryos, DiI was injected into a sartorius muscle on one side and a femorotibialis muscle on the opposite side, allowing us to assess the pattern of sensory and motor innervation of these muscles, even when DiA labeling was weak.

In 3 operated embryos the cutaneous femoralis lateralis (CFL) was injected with DiI to label skin sensory neurons. After injection, all procedures were the same as those for muscle injections.

Orthograde labeling. To trace sensory and motor projections in younger embryos, DiI and DiA were injected into the spinal cord and DRGs, at segment LS1 or LS3 in St. 28–30 embryos. If the motor pools were injected with DiI, the DRGs were injected with DiA and vice versa. After injection, all procedures were the same as those for retrograde labeling.

Histology

All injected embryos were first observed as whole mounts with fluorescence optics to assess the overall pattern of transported dyes. Subsequently a block containing spinal cord and DRGs from T6 through the lumbosacral enlargement was cut out and embedded in gelatin–albumin. Blocks were hardened overnight with 1% glutaraldehyde and serially sectioned at 80 μm with a vibratome. Most embryos with neural crest or muscle injections were cut transversely, although a few such embryos were cut longitudinally. Embryos injected for orthograde labeling were cut sagittally. Sections were mounted in 90% glycerol/10% PBS containing 0.1% *p*-phenylenediamine (Johnson and Araujo, 1981) to retard fading.

Labeling was viewed with a Zeiss Axioskop microscope or a Bio-Rad MRC-600 confocal scanning laser microscope. Because the sections were thick, it was not possible to count individual labeled DRG cells. Instead, we scored every section throughout the operated region for the presence or absence of DiI- and DiA-labeled sensory and motor neurons. The sizes of DRGs varied from segment to segment in both control and operated embryos. Thus, to generate the summary diagrams shown in Figs. 3 and 5, we normalized DRG size by displaying the distribution of labeled DRG neurons along an axis of equal length for all DRGs.

RESULTS

Muscle Sensory Projection Patterns in Normal Embryos

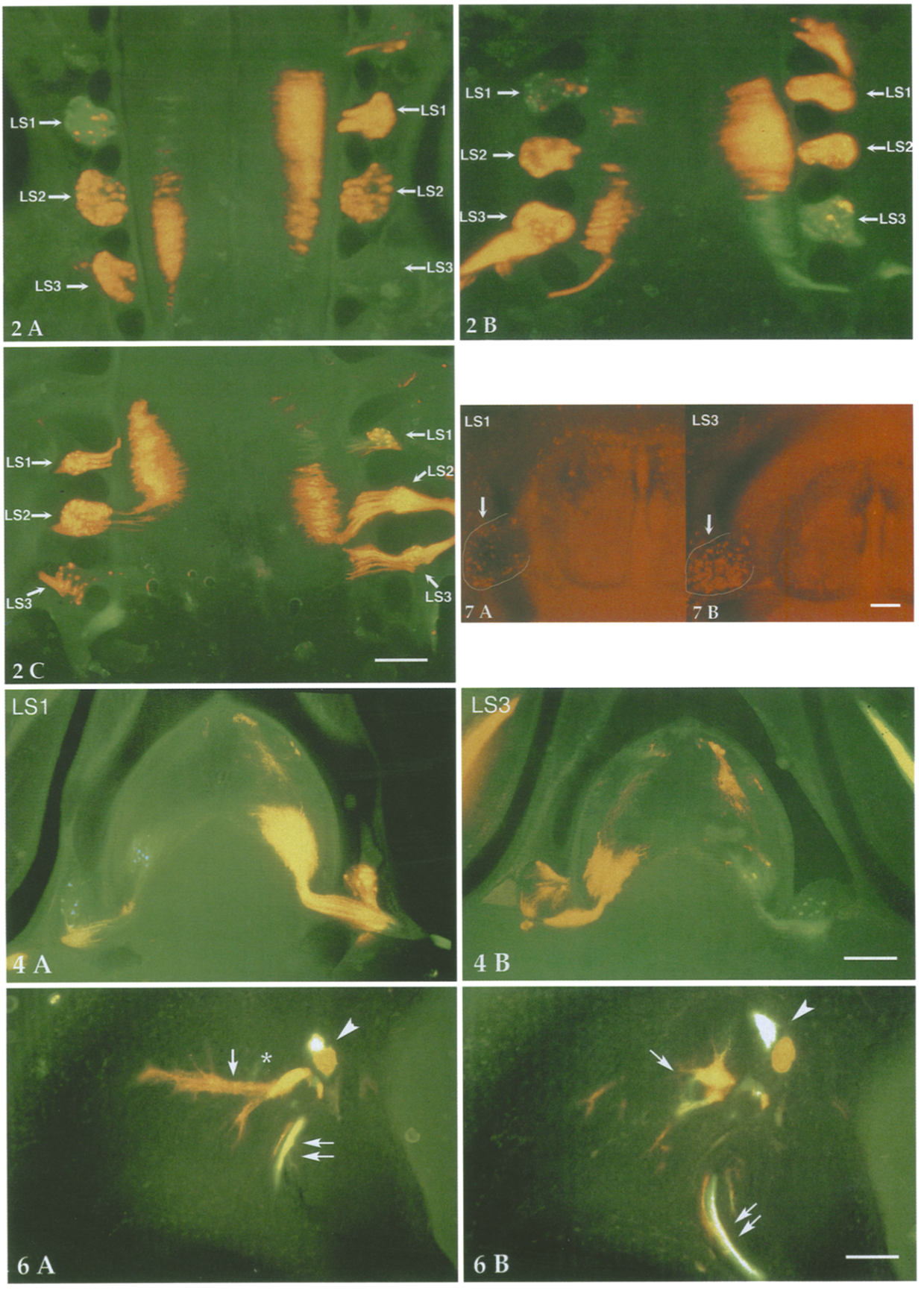
In normal embryos, muscles receive both sensory and motor innervation from the same segmental levels (Honig, 1982; Scott, 1992), presumably because motor axons direct the outgrowth of sensory axons to the appropriate muscle (Honig *et al.*, 1986; Landmesser and Honig, 1986). The goal of the present experiments was to determine whether there is any matching between sensory neurons and the corresponding motoneurons or muscles. As a first step, we simply injected DiI and DiA into the sartorius and femorotibialis muscles in one St. 30 and five St. 36 unoperated control embryos to verify our ability to distinguish between the innervation patterns of these muscles. As previously reported, and shown here in Figs. 2A and 3, the sartorius muscle is innervated predominantly by sensory neurons in DRGs LS1 and LS2, whereas the femorotibialis muscle is innervated predominantly by sensory neurons in DRGs LS2 and LS3; occasionally a few neurons in DRG T7 or LS3 project to the sartorius, and a few neurons in DRG LS1 or LS4 project to the femorotibialis. Motoneurons were labeled in the same segments, but were located slightly rostral to the sensory neurons (see also Swett *et al.*, 1970; Honig, 1982).

Muscle Sensory Projection Patterns Following Reversal of DRGs T7-LS3

To test whether outgrowing muscle afferents selectively associate with the appropriate motoneurons or muscles, we reversed the rostrocaudal order of DRGs T7 through LS3, leaving motoneurons intact. This caused sensory neurons derived from one segmental level to grow into the limb together with motoneurons derived from a different segmental level and displaced sensory neurons from their usual target muscles. If muscle afferents selectively associated with their normal complement of motoneurons or projected to muscles in accord with their embryonic origin, we would expect the femorotibialis muscle to be innervated by neurons in DRGs located rostral, rather than caudal, to those that innervated the sartorius muscle.

Instead, sensory neurons projected to muscle in accord with their new position in all operated embryos examined at St. 35–36, almost as if no rotation had been made, as shown in the examples in Figs. 2B, 4A, and 4B. The sartorius muscle was always innervated by sensory neurons located predominantly at segmental levels LS1 and LS2, whereas the femorotibialis muscle was innervated by sensory neurons located predominantly at segments LS2 and LS3, despite the novel embryonic origin of these DRGs. Thus, the majority of muscle afferents in rostrocaudally reversed DRGs appeared to ignore their usual motoneurons and muscles and grow into the limb in accord with their new position. The results of all such operated embryos examined at St. 35–36 are summarized in Fig. 5.

Although the overall pattern of sensory innervation of


LS1
LS2
LS3
2 A
2 B
2 C
7 A
7 B
4 A
4 B
6 A
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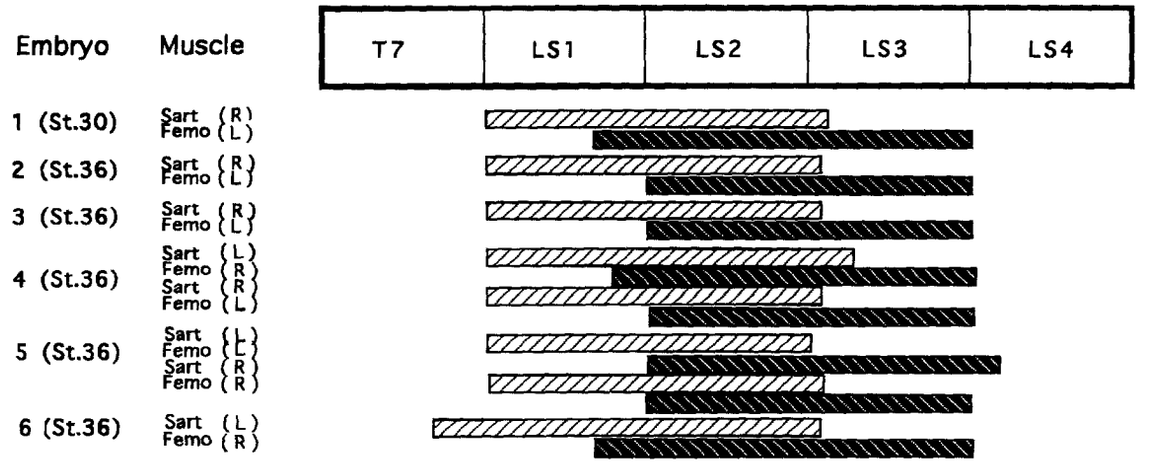

FIG. 3. Bar graph summarizing sensory projections to the sartorius and femorotibialis muscles in one St. 30 and five St. 36 unoperated control embryos. The sartorius muscle is innervated predominantly by sensory neurons in DRGs LS1 and LS2; whereas the femorotibialis muscle is innervated predominantly by sensory neurons in DRGs LS2 and LS3 R, right limb; L, left limb.

muscle was generally normal in operated embryos, the fine details of the pattern appeared to depend on the age at which the surgery was performed. In the embryos that were slightly older at the time of surgery (i.e., those with the most somites—embryos 217, 236, 240, 147 in Fig. 5), the segmental distribution of sensory neurons was more widespread along the rostrocaudal axis compared to those operated at younger stages. Possible reasons for this are discussed later.

Rotated muscle afferents appeared to project in accord with their new position from the outset, as innervation patterns mapped with orthograde labeling (see Materials and Methods) at St. 28–30, prior to the bulk of sensory cell death, also appeared normal. Following neural crest rotation, motor and sensory axons from a given segmental level entered the limb and traveled to the periphery together despite being derived embryologically from different segmental levels (Figs. 6A and 6B). We saw no evidence of sensory neurons taking aberrant pathways to associate with their usual complement of motoneurons or muscles. Thus, sen-

FIG. 2. Longitudinal sections through the lumbosacral spinal cord and DRGs of three St. 36 embryos. In all three panels, DiI (orange) injections into the sartorius muscle and DiA (green) injections into the femorotibialis muscle are shown on the right, and DiA injections into the sartorius and DiI injections into the femorotibialis muscle are shown on the left. Injections labeled both motor and sensory neurons. (A) A control unoperated embryo. Note that the sartorius muscle is innervated by motor and sensory neurons from segments LS1 and LS2, whereas the femorotibialis muscle is innervated by segments LS2 and LS3. (B) An embryo in which the dorsal neural tube was rotated from segments T7–LS3. Sensory neurons projected to muscles in accord with their new position, as if no rotation had been done. (C) An embryo in which the whole neural tube was reversed from segments T7–LS3. Both motor and sensory neurons projected to muscles in accord with their embryonic origin. Note that, in contrast to control embryos and embryos with dorsal neural tube rotations, both motor and sensory projections to the sartorius arose from segments caudal, rather than rostral, to those of the femorotibialis. Scale bar is 100 μm.

FIG. 4. Cross sections through the lumbosacral spinal cord and DRGs of a St. 36 embryo in which the dorsal neural tube was reversed from segments T7–LS3. The right side of each panel shows the outcome of injecting DiI (orange) into the sartorius muscle and DiA (green) into the femorotibialis muscle; the left side of each panel shows the outcome of injecting DiA into the sartorius and DiI into the femorotibialis muscle. (A) Both sensory and motor neurons at segment LS1 were labeled by dyes injected into the sartorius muscle, but not by femorotibialis injections. (B) In contrast, at segment LS3, both sensory and motoneurons were labeled only from the femorotibialis. This pattern is identical to that of control embryos. The ventral edge of the spinal cords was distorted somewhat during embedding. Scale bar is 130 μm.

FIG. 6. Sagittal sections of a St. 30 embryo in which the dorsal neural tube was rotated to reverse the rostrocaudal order of DRGs T7–LS3. DiI (orange) was injected into the motor pool at the segment LS1 and DiA (green) was injected into the DRG at the same segmental level. (A) Motor and sensory axons entered the proximal limb together in the spinal nerve (arrowhead) and traveled together to the sartorius (arrow) and adductor (double arrow) muscles. Note the sensory axons branching into the muscle from the main sartorius nerve (asterisk). (B) A deeper section shows motor and sensory axons ramifying in the sartorius muscle and the continuing trajectory of sensory and motor axons in the adductor nerve. Scale bar is 275 μm.

FIG. 7. Cross sections through the lumbosacral spinal cord and DRGs of a St. 30 embryo in which DiI was injected into neural crest at segment T7 before the dorsal neural tube was removed and rotated. (A) There are only a few DiI-labeled neurons in DRG LS1. (B) By contrast, there are many labeled neurons in DRG LS3. Scale bar is 400 μm.

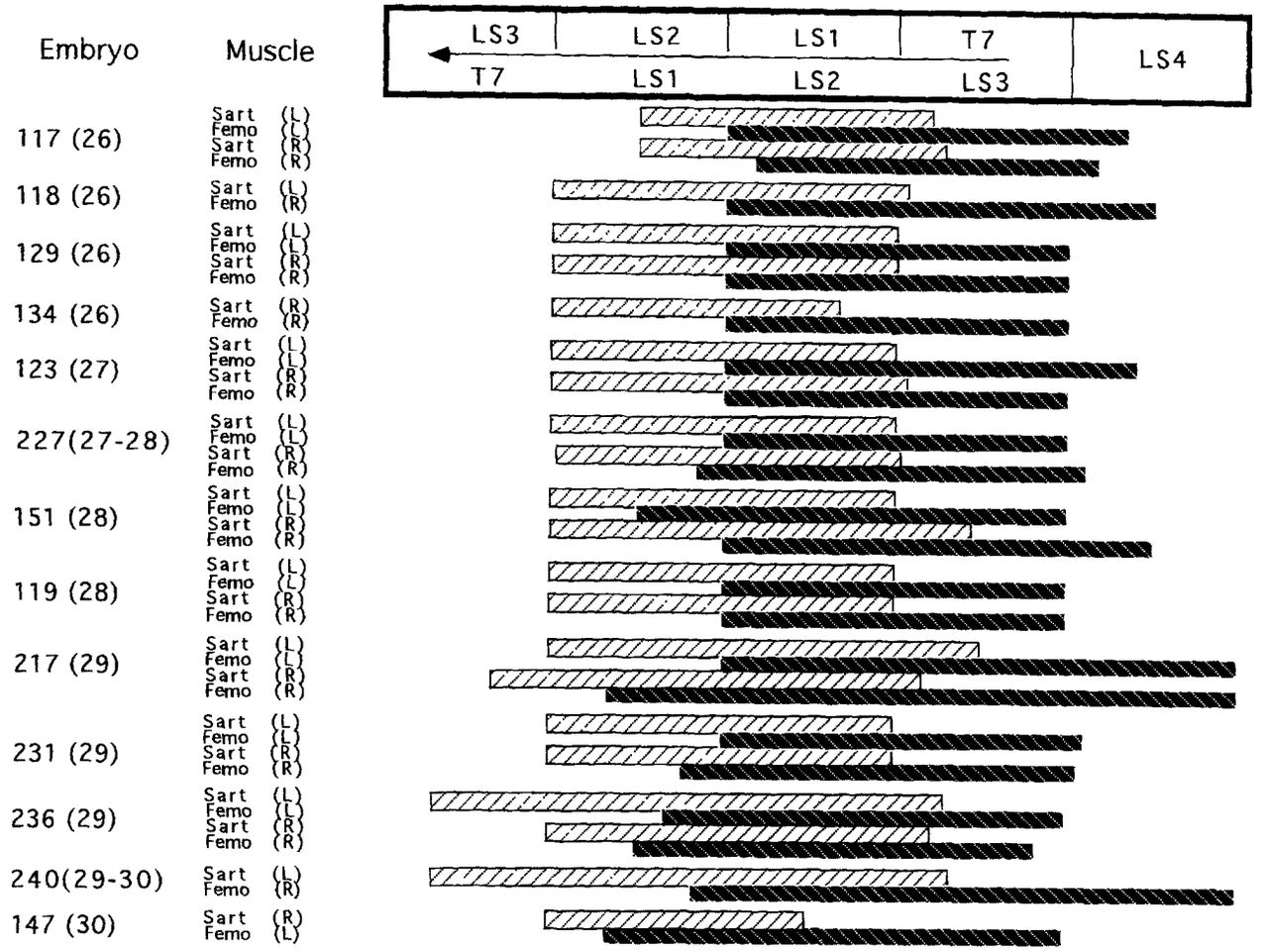

FIG. 5. Bar graph summarizing sensory projections to the sartorius and femorotibialis muscles following dorsal neural tube rotations that reversed the order DRGs T7–LS3 but left motoneurons intact. In embryos operated at the youngest stages, the sensory projection pattern was almost identical to controls (see Fig. 3). In embryos that were slightly older at the time of surgery, the segmental distribution of sensory neurons was somewhat more widespread along the rostrocaudal axis, although the sartorius was still innervated predominantly by DRGs LS1 and LS2 and the femorotibialis by DRGs LS2 and LS3. The numbers in parentheses are the number of somites at the time of surgery. L, left limb; R, right limb.

sory axons from rotated DRGs take pathways through the limb and project to muscles that are appropriate for their segmental position rather than segmental origin.

Skin sensory innervation patterns were also mapped in three embryos with neural crest rotations by injecting DiI into the CFL nerve. As in normal embryos (Honig, 1982; Scott, 1982), CFL injections labeled neurons in DRGs LS1 and LS2, but not in LS3. Thus, cutaneous neurons also tended to project in accord with their new position following neural crest rotation, as previously reported (Scott, 1986).

Effects of Other Manipulations on Muscle Afferent Projections

These findings differ markedly from earlier studies in which several segments of the entire neural tube were rotated. Following neural tube rotation, both motor (Lance-Jones and Landmesser, 1980, 1981) and muscle sensory (Honig *et al.*, 1986) neurons change their trajectories through the limb and project to their embryologically appropriate muscle. The major difference between the present and earlier studies is that we rotated only the neural crest and dorsal neural tube, leaving motoneurons intact. To be certain that these differences arose as a consequence of rotating neural crest independent of motoneurons, we did a series of control experiments to rule out other possibilities.

One possible explanation for the relatively normal pattern of muscle afferent projections is that the DRGs in our operated embryos were not actually derived from rotated neural crest, but instead arose from neurons that had migrated prior to surgery, from the remaining ventral neural tube, or from rotated crest cells that migrated rostrally or caudally back

to their original position. To test these possibilities we removed the neural crest and dorsal neural tube in six embryos but did not replace it. No DRGs were observed throughout the operated region at either St. 30 (E7, $n = 3$) or St. 36 (E10, $n = 3$), although small sympathetic ganglia were occasionally present (see also Yntema and Hammond, 1947). We can, therefore, exclude the possibility that DRGs in the operated region contained significant numbers of neural crest cells that had migrated prior to rotation. These embryos also confirmed that we accurately assessed somite number and position at the time of surgery in most embryos. In five of the six embryos in which dorsal neural tube was removed and not replaced, the operated region clearly extended from T7 to LS3; in the last, from LS1 to LS4.

To test the possibility that rotated crest cells migrated back to their original segmental level, we injected DiI into neural crest at segment T7, before the piece was excised and reversed, and examined the distribution of labeled cells at St. 28–30 in eight embryos. DiI-labeled cells were abundant in DRG LS3 (Fig. 7B), although a few labeled cells were also present in DRGs T7 and LS1 (Fig. 7A). Thus, following neural crest rotation most crest cells migrated ventrolaterally to form DRGs at the same segmental level, as in normal embryos, and did not wander rostrally or caudally back to their original position.

Because we were unable to distinguish the exact extent of rotated tissue in some embryos with dorsal neural tube rotations, we were concerned that we may have misjudged the site of operation in some embryos. To test whether such errors might have influenced our data, we deliberately rotated neural crest from segments LS2–LS5. Muscle afferents innervated the sartorius and femorotibialis in accord with their new position in all four embryos assessed at St. 35–36 (not shown), indicating that slight variability in the location of the rotation would not affect our results.

Lance-Jones and Landmesser (1981) found that motoneuron axons failed to form patterned connections with the appropriate muscles when displaced too far from their normal position in the embryo. Perhaps muscle sensory neurons projected in accord with their new position because rotating four segments of neural crest displaced them too far from their usual cues. To test this possibility we reversed three segments of neural crest, from LS1–LS3, in six embryos for comparison. Muscle sensory innervation in these embryos was indistinguishable from that of control embryos or embryos with larger neural crest rotations. As usual the sartorius was innervated by more rostral DRGs than the femorotibialis (not shown). Thus, muscle afferents appeared to project in accord with their new position following neural crest rotation regardless of the location or extent of neural crest that was rotated.

Muscle Sensory Projection Patterns Following Neural Tube Rotation

In contrast, muscle afferents projected to their embryologically appropriate muscles when three to four segments of the *entire* neural tube were rotated at St. 16 (27–28 somites), in agreement with earlier studies (Honig *et al.*, 1986). In these embryos both motor and sensory axons altered their pathways through the limb such that axons from segments LS1–LS2 projected to the femorotibialis muscle, while axons from segments LS2–LS3 projected to the sartorius muscle, as shown in the example in Fig. 2C.

The segmental distribution of both motor and sensory projections became more widespread along the rostrocaudal axis as neural tube was rotated in progressively younger embryos, but at all stages motor and muscle sensory innervation patterns were similar. Following neural tube rotation at St. 15 (24–25 somites), both sartorius and femorotibialis muscles were innervated by neurons throughout segments T7–LS3, but the majority of sartorius innervation was from more caudal segments than the majority of femorotibialis innervation. Thus, at St. 15 most motor and muscle sensory neurons projected to muscle in accord with their embryonic origin. In contrast, following neural tube rotation at St. 14 (22–23 somites) the majority of sartorius innervation was from more rostral segments than the majority of femorotibialis innervation. Thus, in embryos operated at the youngest stages, both motor and muscle sensory neurons projected in accord with their new position, although their segmental distributions were more widespread than usual. Our findings are similar to those reported recently for motoneurons by Matise and Lance-Jones (1996).

DISCUSSION

We have shown that when the rostrocaudal order of three to four DRGs is reversed, leaving motoneurons intact, sensory neurons project to muscles in accord with their new position rather than in accord with their embryonic origin, as if no rotation had been made. These results are similar to those obtained previously (Scott, 1986) for *skin* sensory neurons. They differ markedly, however, from studies involving rotation of several segments of the entire neural tube, in which both sensory neurons (Honig *et al.*, 1986) and motoneurons (Lance-Jones and Landmesser, 1980, 1981; Matise and Lance-Jones, 1996) changed their trajectory through the limb to project to the embryologically appropriate muscles. We have ruled out a number of trivial explanations for the difference from earlier studies, and it thus appears that the normal outgrowth of muscle afferents observed here arose as a consequence of rotating neural crest independent of motoneurons.

Origin of Rotated DRGs

A meaningful interpretation of our results depends on DRGs in the operated region being derived from rotated neural crest. We are confident that this is so. No DRGs developed in the operated region when neural crest was removed and not replaced. Moreover, DiI injection into neural crest at T7 prior to rotation led to large numbers of labeled cells in DRG LS3, as predicted if neural crest cells migrated normally following rotation. Some dye-labeled

cells were also observed in DRGs T7 and LS1 in these embryos. The presence of labeled cells in more rostral DRGs was unexpected and requires some explanation. One possibility is that our dye injections labeled some cells deeper in the neural tube, below the neural crest, which were not excised and rotated and which subsequently migrated into the DRG. Such late migration of neural tube cells into DRGs has been recently observed in the chick (Sharma *et al.*, 1995). Alternatively, some rotated neural crest cells may have migrated rostrally back to their embryologically correct position. Such wide-ranging migration has not been reported for DRG precursors (Teillet *et al.*, 1987), but is well documented for sympathetic precursors (Yntema and Hammond, 1947). Regardless of the source of these labeled cells, they are few in number, and the vast majority of labeled cells appeared to migrate along normal pathways. Thus, DRGs in the operated region are derived predominantly from rotated neural crest.

Effects of Embryonic Age on Innervation Patterns

Although most sensory neurons in rotated DRGs innervated muscle in accord with their new position, innervation patterns appeared to be influenced by the age at which the surgery was performed. The segmental distribution of sensory neurons was somewhat more widespread in embryos operated at older stages (St. 17) compared to those operated at younger stages (St. 15). One possible explanation for this phenomenon is that sensory neurons may become specified with respect to motoneurons or their target muscles after they coalesce into DRGs and that the enviornmental signal disappears around St. 17, the age of our oldest embryos. Thus, axons from rotated crest cells that migrate later may not become specified for their new position and would, therefore, grow out in accord with their original position or randomly. Such temporally restricted expression of signaling molecules during development is well documented (Dodd *et al.*, 1988; Placzek *et al.*, 1990).

A second possibility is that sensory precursors may become specified at the time that they leave the neural crest and begin to migrate. The reported timing of neural crest migration in the lumbosacral region (Weston and Butler, 1966; Bronner-Fraser, 1986) suggests that at the time of surgery some crest cells may have begun to migrate in our oldest embryos. When the dorsal neural tube was reversed, cells that have begun to migrate but which are still associated with the dorsal neural tube would be displaced to the new position. If neural crest cells are specified at this stage, they may project in accord with their embryonic origin, to innervate their original target, leading to a more widespread sensory innervation pattern in embryos operated at the oldest stages.

A third possibility is that the cells that migrate from the neural tube to the DRG at later stages (Sharma *et al.*, 1995) acquire their identity at about St. 17. If these late-migrating neurons were rotated with the dorsal neural tube, they might project according to their embryonic origin in older embryos but according to their new position prior to their specification in younger embryos, which would broaden muscle sensory innervation patterns in older embryos. At present we cannot distinguish among these various possibilities.

Muscle sensory innervation patterns following whole neural tube rotations were also influenced by the age at which the surgery was performed. In embryos operated at St. 15 most motor and muscle sensory neurons projected to muscle in accord with their embryonic origin, whereas in embryos operated at St. 14 most neurons projected in accord with their new position. Similar results have been reported recently for motoneurons and have been taken as evidence that motoneurons acquire their identity just before St. 15 (Matise and Lance-Jones, 1996). In our studies, sensory patterns always corresponded to the motor patterns. Sensory neurons may simply follow the motoneurons to their target muscles, as previously suggested (Honig *et al.*, 1986; Landmesser and Honig, 1986); alternatively, sensory neurons may respond to the same cues as the motoneurons at early embryonic stages (see below).

Specification of Sensory Neurons?

Our findings that sensory neurons project to muscles in accord with their new position following neural crest rotation indicate that sensory precursors in the neural crest were not rigidly specified with respect to their connectivity at the time of surgery. Do sensory neurons in DRGs ever become specified and make active pathway or target choices as do motoneurons? The present experiments cannot answer this question. Our findings could result from two very different scenarios. First, muscle sensory neurons in DRGs may have no specific identity prior to outgrowth, but simply associate nonselectively with nearby motor axons, which direct them to a muscle. The target muscle would then determine the sensory neuron's identity, such as its central connections (Wenner and Frank, 1995). During normal development sensory axons appear to grow on the axons of adjacent motoneurons in segmental nerves (Tosney and Landmesser, 1985a). However, they have the opportunity to interact with axons from both appropriate and inappropriate motoneurons in the plexus region, where axons from different segmental levels come together, intermix, and sort out to project to different peripheral targets. Clearly, sensory projections are influenced by interactions with other axons within the plexus region, as experimental perturbation of fasciculation among outgrowing axons in the plexus disrupts the usual sensory projection pattern (Honig and Rutishauser, 1996). Whether normal patterning involves *selective* recognition and fasciculation remains unknown.

Alternatively, sensory neurons may acquire their identity sometime after their precursors leave the neural crest but before their axons project to the periphery. If muscle sensory neurons do become specified prior to axon outgrowth, their identity must be influenced by (or overridden by) the adjacent ventral neural tube or motoneurons, since the sensory neurons always project to the same muscles as the neighboring motoneurons, regardless of whether these are segmentally appropriate, and fail to project to muscle in the

absence of motoneurons. Preliminary results from our laboratory suggest that sensory neurons do indeed become specified after coalescing into DRGs, allowing them to behave independently from nearby motoneurons. Some progress has been made in elucidating the genes and molecules involved in motoneuron specification and pathfinding (Tsuchida *et al.*, 1994; Daston and Koester, 1996; Ebens *et al.*, 1996). An understanding of whether similar mechanisms are involved in patterning of sensory projections awaits future research.

ACKNOWLEDGMENTS

We thank David Adams for expert technical assistance and Dr. Thomas N. Parks and Sharon M. Cahoon for helpful comments on the manuscript. This work was supported by NS16067 to S.A.S.

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Received for publication January 21, 1997
Accepted April 3, 1997