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Segmental Specificity of Chick Sympathetic Preganglionic Projections Is Influenced by Preganglionic Neurons from Neighboring Spinal Cord Segments

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Sympathetic preganglionic neurons of the chick are located between the brachial and lumbosacral enlargements of the spinal cord. Their axons exit the spinal cord via their adjacent ventral roots and project rostrally or caudally along the sympathetic trunk to innervate sympathetic ganglia. The projections of sympathetic preganglionic neurons are segmentally specific. Neurons from the 16th cervical (C16) and the first thoracic (T1) spinal cord segments project predominantly in the rostral direction, whereas those from the fifth thoracic (T5) to the first lumbar (L1) spinal segments project predominantly in the caudal direction. Neurons from intervening spinal cord segments (T2–T4) project in rostral and caudal directions. In the present study, neural tube manipulations show that the direction of preganglionic projections is altered by both the elimination and addition of preganglionic neurons projecting into the sympathetic trunk from neighboring segments. The present study also compares the projections of preganglionic neurons from transplants of multiple neural tube segments with those from transplants of single neural tube segments reported in a previous study (Yip, 1987). In the previous study when single thoracic neural tube segments were transplanted to the cervical level, preganglionic neurons did not maintain their original projection patterns. The present study found that, when contiguous neighboring segments were transplanted to the cervical level, preganglionic neurons maintained projection patterns characteristic of their original segmental levels. These results indicate that the direction of preganglionic projections can be influenced by neurons from neighboring segments, suggesting that the formation of segmentally specific preganglionic projections during embryogenesis may involve the interactions of preganglionic neurons with those from neighboring spinal cord segments.

Key words: axon guidance; competition; transplantation; autonomic neurons; chick embryo; neuronal interactions

Projections of sympathetic preganglionic neurons in the sympathetic trunk of birds and mammals are segmentally specific (Langley 1892, 1904; Lichtman et al., 1980; Rubin and Purves, 1980; Yip, 1990; Forehand et al., 1994; Yip et al., 1998). In the chick, for example, preganglionic neurons arising from the first thoracic spinal cord segment (T1) project predominantly in the rostral direction, whereas those arising from the last thoracic spinal cord segment (T7) project predominantly in the caudal direction. The mechanisms underlying the establishment of these segmentally specific projection patterns are not fully understood. Our recent study shows that the projections of these neurons are not determined intrinsically by the segmental origins of their cell bodies in the spinal cord (Yip et al., 1998). A previous study has shown that the segmentally specific projections of preganglionic neurons do not require their target neurons—sympathetic ganglion cells (Yip, 1987). Because neither the intrinsic properties of preganglionic neurons nor their target cells appear to be responsible for the segmentally specific projections, it is likely that some factor or factors along the projection pathway in the sympathetic trunk may play a role in the development of preganglionic projection patterns.

In addition to sympathetic ganglion cells, the sympathetic trunk also contains axons of preganglionic neurons and Schwann cells. Schwann cells, however, do not seem to be required for the formation of segmentally specific preganglionic projections because preganglionic projections are not affected by neural crest removal (Yip, 1987). Because each ganglion is innervated by preganglionic neurons arising from several spinal cord segments (Langley, 1904; Njå and Purves, 1977; Yip, 1986; Forehand, 1994), axons of preganglionic neurons from multiple spinal cord segments share the same pathway to arrive at their target ganglia. In the development of the nervous system it has been shown that axonal projections can be influenced by other axons. For example, axons may be guided by pioneer fibers (Bentley and Keshishian, 1982). Axon guidance, moreover, may be mediated by fasciculation with existing fibers (Raper et al., 1983) as well as by repulsive interactions with other fibers (Kapfhammer and Raper, 1987). Are the segmentally specific preganglionic projections influenced by axons from neighboring spinal segments?

A previous study has shown that, when single segments of spinal cord containing preganglionic neurons are transplanted to a cervical level that contains no preganglionic neurons, the transplanted preganglionic neurons do not maintain their original projection patterns (Yip, 1990). The altered projection patterns of preganglionic neurons that have been transplanted to locations that have no preganglionic neighbors suggest that preganglionic projections may be influenced by interactions with neighboring preganglionic neurons. In the present study we test this possibility by adding or eliminating preganglionic neurons in neighboring segments of normal thoracic spinal cord. Additionally, contiguous neighboring segments containing preganglionic neurons are

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transplanted to the cervical level. Results from these studies suggest that the segmental specificity of sympathetic preganglionic projections is influenced by preganglionic neurons from neighboring spinal cord segments.

MATERIALS AND METHODS

White leghorn eggs (Keystone Mills, Ephrata, PA) were used in the present study. All embryos were incubated at 37°C and 70% humidity in a forced-draft incubator and staged according to Hamburger and Hamilton (1951). Embryo manipulations were performed at stage 14. For embryo manipulation, a window was opened in the shell of the egg. Segmental levels were determined by counting somites (Levi-Montalcini, 1950). Carbon particles were used to mark the rostrocaudal boundaries of the neural tube segments of interest. Neural tube segments then were removed from embryos with a tungsten needle and transplanted to different spinal levels. After surgical manipulation the windows were sealed with cellophane tape, and the embryos were returned to the incubator until death at stage 30/31. For each type of surgical manipulation performed, an additional set of sham-operated embryos was used. In these control embryos the neural tube segments were removed and reinserted into the same embryo. Projection patterns in all sham-operated embryos were determined to be the same as in normal embryos.

To determine segmental levels of the spinal cord at the time of death, we took the T1 segmental level to be the spinal level just caudal to the brachial plexus. The boundaries of each segment were taken to be halfway between the midpoints of adjacent dorsal root ganglia. Only those embryos that showed manipulations at the appropriate levels were used for data analysis.

Specificity of sympathetic preganglionic projections from each spinal cord level

The sympathetic chain ganglia, which are arranged segmentally from cervical throughout the sacral levels, lie along each side of the vertebral column. Ganglion cells are innervated by preganglionic neurons that are located between the brachial and lumbosacral enlargements of the spinal cord. Preganglionic neurons exit the spinal cord via their adjacent ventral roots and enter into the sympathetic trunk where they project rostrally or caudally to innervate the chain of ganglia (Yip, 1990). To visualize the preganglionic cell column, we sectioned stage 30 embryos at 20 μm in the horizontal plane with a cryostat and immunostained with E/C8, a monoclonal antibody against neurofilaments (a generous gift of Dr. G. Ciment, Oregon Health Sciences University, Portland, OR). Detailed immunostaining procedures have been described in a previous publication (Yip et al., 1995). The mature pattern of preganglionic projections from each spinal cord segment was examined in stage 30/31 embryos by anterograde labeling with horseradish peroxidase (HRP) or DiI. The details of axonal tracing techniques are described below.

Removal of neighboring neural tube segments

The T2–T4 neural tube segments were removed unilaterally or bilaterally and replaced with a similar length of cervical neural tube (C11–C13) that does not give rise to sympathetic preganglionic neurons (see Fig. 2*A*). This eliminated the caudal neighbors of the T1 segment and the rostral neighbors of the T5 segment. The contralateral side served as a control. Only those embryos that at the time of death clearly showed boundaries of surgery at the T2 and T4 levels were used. To ensure that this surgical manipulation eliminated preganglionic neurons from the T2–T4 spinal levels, we sectioned one set of operated embryos at 20 μm in the transverse or horizontal plane with a cryostat and immunostained with E/C8 as described in a previous publication (Yip et al., 1995). In another set of embryos, preganglionic projections from the T1 or T5 spinal cord segments were evaluated by using anterograde labeling with HRP or DiI on both the control and experimental sides of the embryo. Finally, because the T3 ganglion normally receives innervation from preganglionic neurons at the T2–T4 spinal levels, retrograde labeling with DiI was used to evaluate changes in preganglionic axonal projections to the T3 ganglia in another set of embryos. To quantify the number of T1 and T5 preganglionic neurons projecting to the T3 ganglia, we used retrograde labeling with fluorescent dextran amine dyes. Details of HRP, DiI, and dextran amine labeling are described below.

Addition of neighboring neural tube segments

To increase preganglionic projections from neighboring segments rostral to the T1 segment, we removed the cervical neural tube from the C13–C16 spinal levels of a host embryo bilaterally and replaced it with a thoracic neural tube from the approximate T1–T4 spinal levels of a similarly staged donor embryo (see Fig. 5*A*). Both experimental and donor embryos were returned to the incubator until death. Donor embryos were examined to assure that the transplanted neural tube segments were from the thoracic level. Only those host embryos that received thoracic spinal cord were analyzed. Anterograde labeling with HRP and DiI was used to determine the projection pattern of preganglionic neurons from the T1 spinal cord segment of the host embryos.

Transplantation of multiple neural tube segments to a novel environment

The cervical level of the spinal cord normally does not contain preganglionic neurons. When several contiguous thoracic spinal cord segments were transplanted into the cervical level, the transplanted neurons were situated in a novel environment. For these transplantations the C9–C12 neural tube segments were removed bilaterally from host embryos and replaced with the T1–T4 neural tube segments from similarly staged donor embryos. Anterograde labeling with HRP was used to assess preganglionic axonal projections from the transplanted T1 and T4 segments.

Axonal tracing with HRP and DiI

For neuronal labeling the embryos were eviscerated in Tyrode solution, and a dorsal laminectomy was performed to expose the spinal cord. For all anterograde studies the sections were cut in the sagittal plane; for retrograde studies the sections were cut in the horizontal plane.

HRP labeling. For anterograde labeling of preganglionic axons with HRP, ~0.2 μl of 30% HRP/1% lysolecithin solution was pressure-injected with a micropipette (20 μm tip diameter) into the appropriate spinal cord segment(s). For retrograde labeling of preganglionic neurons, a similar volume of HRP was injected into the T3 ganglia. Injected embryos were maintained in oxygenated Tyrode solution at 31°C for 5–7 hr to allow for transport of HRP (Landmesser, 1978); thereafter, they were fixed for 1 hr with a phosphate-buffered fixative consisting of a mixture of 1% paraformaldehyde, 2.5% glutaraldehyde, and 4% sucrose; they were equilibrated in 30% phosphate-buffered sucrose; and they were sectioned serially with a cryostat at 30 μm. All sections were mounted on Superfrost Plus slides (Fisher Scientific, Pittsburgh, PA) and reacted for the presence of HRP, using diaminobenzidine as the chromogen (Adams, 1981).

DiI labeling. Embryos were placed in a fixative consisting of 4% paraformaldehyde in 0.1 M phosphate buffer. For anterograde labeling of preganglionic axons, DiI crystals (Molecular Probes, Eugene, OR) mixed in silicone grease (Lubriseal, Thomas Scientific, Swedesboro, NJ) (Mirnics and Koerber, 1995) were embedded in the central canal of the appropriate spinal cord segment(s) (Yip et al., 1998). All neural tube segments that were not embedded with DiI were removed to eliminate diffusion of the dye. For retrograde labeling of preganglionic neurons, DiI (0.25% in 100% alcohol; Honig and Hume, 1986) was pressure-injected into the T3 ganglia. All DiI-treated embryos were incubated at 37°C for 3–4 d, embedded in 7% agar, and sectioned at 150 μm with a vibratome.

Fluorescent dextran amine labeling for cell counts

Because individual preganglionic neurons in the spinal cord are difficult to distinguish with retrograde DiI labeling, retrograde labeling with fluorescent dextran amines was used to evaluate changes in the percentages of preganglionic neurons at the T1 or T5 spinal cord segment that project to the T3 ganglia. Preganglionic neurons projecting to the T3 ganglia were retrogradely labeled by injecting ~0.2 μl of 25% rhodamine-conjugated dextran amine in 1% Triton X-100 into the T3 ganglia. The preparation was incubated for 5–6 hr at 31°C in oxygenated Tyrode solution to allow for retrograde transport of the dye. To delineate the boundaries of the T1 and T5 spinal cord segments, we then cut the ventral roots on either side of the T1 and T5 segments. The preparation was reincubated for an additional ½ hr to allow the cut nerves to seal. Then ~0.2 μl of 25% FITC-conjugated dextran amine in 1% Triton X-100 was injected into the T1 and the T5 ganglia; this effectively labeled all preganglionic neurons in those segments. The preparation was reincubated again for an additional 5–6 hr. Embryos then were fixed in 4% paraformaldehyde in 0.1 M phosphate buffer overnight at 4°C, equilibrated in 30% phosphate-buffered sucrose, and serially sectioned with a cryostat at 20 μm in the transverse plane. The percentage of T1 and T5

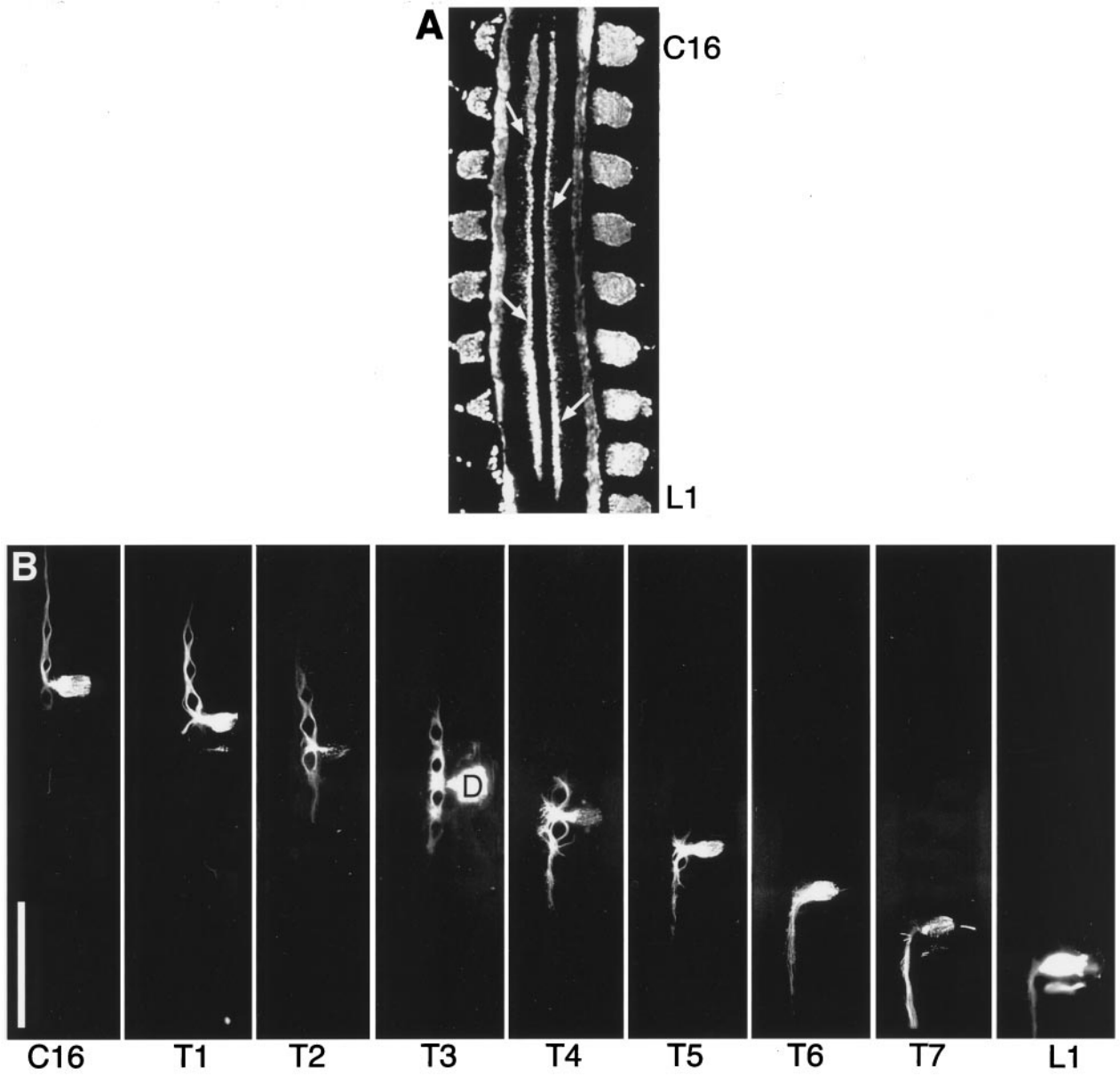

Figure 1. *A*, Location of the preganglionic cell column in the spinal cord. The micrograph shows a horizontal section from a stage 30 embryo that has been immunostained with monoclonal antibody E/C8. Preganglionic neurons (*arrows*) cluster around the central canal of the spinal cord and extend from the C16 to the L1 spinal cord segments. *B*, Specificity of sympathetic preganglionic projections from each spinal cord segment. Micrographs show sagittal sections through the sympathetic trunk of stage 30 embryos. Anterograde labeling with DiI shows that, within the sympathetic trunk, preganglionic neurons from rostral segments (*C16* and *T1*) project predominantly in the rostral direction, whereas preganglionic neurons from caudal segments (*T5–L1*) project predominantly in the caudal direction (rostral is *up*; dorsal is to the *right*). Intervening spinal cord segments (*T2–T4*) show rostral as well as caudal projections. Note that DiI injected into the spinal cord anterogradely labels sympathetic preganglionic axons and also retrogradely labels dorsal root ganglia. In this plane of section, only labeled sympathetic preganglionic axons and dorsal root ganglia (*D*) are visible. The spinal cord, where DiI was injected, is medial to this plane of section and is not visible. A similar plane of section showing retrogradely labeled dorsal ganglia is found also in Figures 4 and 5. Scale bar, 1 mm.

preganglionic neurons projecting to the T3 ganglia was calculated by dividing the number of rhodamine-labeled cells in the T1 and T5 segments by the sum of fluorescein-labeled cells and rhodamine-labeled cells in those segments.

RESULTS

Preganglionic projections from each spinal cord segment are specific

The preganglionic cell column of the chick (column of Terni) was identified in the thoracic spinal cord by Terni (1924) and also by Levi-Montalcini (1950), using silver staining. In the present study, immunostaining with monoclonal antibody E/C8 was used to visualize the column of Terni in stage 30 embryos ($n = 5$). The column of Terni extends from the C16–L1 spinal levels (Fig. 1*A*). The width of the column appears uniform throughout the thoracic levels but tapers off toward the rostral (C16) and caudal (L1) ends. The projections of preganglionic neurons from individual spinal cord segments were anterogradely labeled with either DiI or HRP in stage 30 embryos. Only one spinal cord segment was labeled in each embryo. As shown in Figure 1*B*, the projection pattern of these neurons is segmentally specific. Projections from

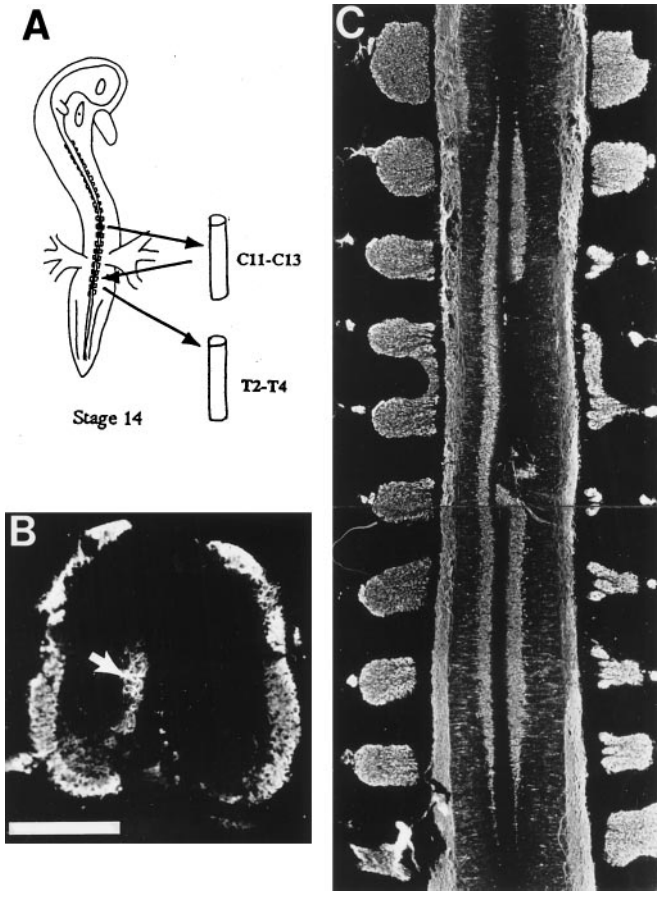

Figure 2. Removal of neighboring neural tube segments. *A*, The *T2–T4* neural tube segments were removed unilaterally from a stage 14 embryo and replaced with the *C11–C13* neural tube segments. The contralateral side served as a control. *B*, Transverse section from an operated stage 30 embryo. Immunostaining with E/C8 shows the absence of preganglionic neurons on the operated (*right*) side of the embryo. The *arrow* shows preganglionic neurons on the control (*left*) side of the embryo. *C*, Horizontal section from an operated stage 30 embryo. Immunostaining with E/C8 shows a discontinuous preganglionic cell column on the operated (*right*) side of the embryo. Scale bars: *B*, 250 μm; *C*, 500 μm.

rostral spinal cord segments (C16 and T1) are predominantly rostral in the sympathetic trunk, whereas projections from caudal spinal cord segments (T5–L1) are predominantly caudal. Intervening spinal cord segments (T2–T4) show rostral as well as caudal projections.

Specificity of preganglionic projections is altered with removal of neighboring neural tube segments

To test whether preganglionic neurons from neighboring spinal cord segments can affect the segmental specificity of preganglionic projections, we examined the T1 and T5 preganglionic projections in embryos that had the T2–T4 spinal cord segments on one side replaced with the C11–C13 spinal cord segments. Because cervical spinal cord does not contain preganglionic neurons, this effectively eliminates preganglionic neurons in the T2–T4 spinal levels (Fig. 2*A*). In one set of operated embryos ($n = 6$) immunostaining was used to show that preganglionic neurons were indeed absent between the T2 and T4 spinal levels on the experimental side (Fig. 2*B,C*).

The pattern of preganglionic projections was determined by using retrograde labeling with DiI and dextran amines and anterograde labeling with DiI. Retrograde labeling with DiI injected into the T3 ganglia revealed that the T3 ganglion on the control side is supplied mostly by preganglionic axons from the T2–T4 spinal cord segments, with very little contribution from the T1 and T5 spinal cord segments. The T3 ganglion on the operated side, in contrast, is supplied almost exclusively by preganglionic neurons from the T1 and T5 spinal cord segments ($n = 24$) (Fig. 3). Thus, in the absence of preganglionic neurons from the T2 to T4 spinal cord segments, more T1 preganglionic neurons now project caudally and more T5 preganglionic neurons project rostrally to the T3 ganglion. Differences in the number of T1 or T5 preganglionic neurons projecting to the T3 ganglion on the experimental and the control sides of operated embryos were quantified further by using retrograde fluorescent dextran amine labeling ($n = 8$; data not shown). Results show that 19 ± 3.1% (mean ± SD) of the total number of T1 preganglionic neurons on the experimental side sent axons to the T3 ganglion, whereas only 3 ± 1.2% of the T1 preganglionic neurons on the control side sent axons to the T3 ganglion. Additionally, an average of 36 ± 4.2% of the T5 preganglionic neurons on the experimental side sent their axons to the T3 ganglion, whereas only 3 ± 1.5% of the T5 preganglionic neurons on the control side sent their axons to the T3 ganglion.

Anterograde labeling studies also were done subsequent to the removal of the T2–T4 spinal segments. In the absence of their rostral neighbors, T5 preganglionic neurons on the experimental side projected 3½ to six segments rostrally, compared with only ½ to 1½ segments rostrally on the control side. Caudal projections were similar on both control and experimental sides (Fig. 4*B,C*). Although less striking, results from anterograde labeling of T1 preganglionic neurons also showed a change in projection patterns (Fig. 4*A*). In 8 of 11 cases, T1 preganglionic neurons responded to the absence of caudal neighbors and projected 1½ to two segments caudally, compared with their normal caudal projections of not more than one segment (data not shown). Rostral projections from the T1 segment of experimental embryos were similar to those of normal embryos. Together, these results show that the segmental specificity of preganglionic projections can be altered with the removal of neighboring spinal cord segments.

Specificity of preganglionic projections is altered with the addition of preganglionic neurons in neighboring neural tube segments

In normal embryos, preganglionic neurons that are located at the rostral boundary of the preganglionic cell column (C16–T1) have few or no rostral neighbors and tend to project predominantly in the rostral direction. To explore the possibility that the directional projections of preganglionic neurons at the rostral boundary of the cell column are influenced by the fact that they confront fewer axons from neighboring segments, we extended the normal preganglionic cell column to the cervical level by replacing cervical spinal cord segments in host embryos with thoracic neural tube from donor embryos (Fig. 5*A*). In the operated embryos, axons from T1 preganglionic neurons encountered more axons from the transplanted segments. Preganglionic projections from both sides of the native T1 spinal cord segment were evaluated to determine the effects of additional neighbor axons. In all cases ($n = 20$), preganglionic neurons from the native T1 spinal cord segment did not retain their predominantly rostral projections but projected instead in both directions (Fig. 5*B*). On average, they projected two to three segments caudally instead of the not more

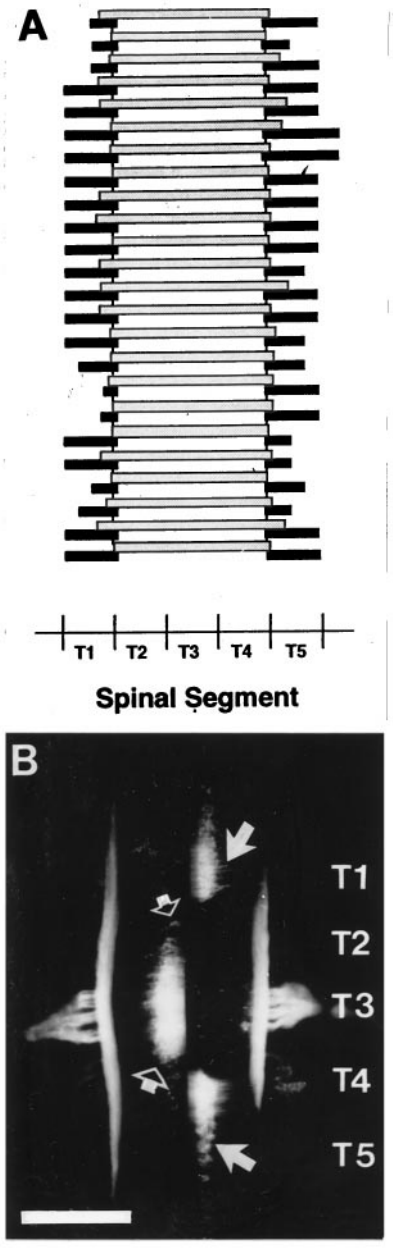

Figure 3. T2–T4 neural tube removal changes the distribution of preganglionic neurons projecting to the T3 ganglia. *A*, Distribution of preganglionic neurons projecting to the T3 ganglia of operated stage 30/31 embryos as determined by retrograde labeling with DiI ($n = 24$). Each set of *bars* represents one embryo. *Shaded bars* represent the control side, and *filled bars* represent the experimental side of the embryo. On the control side the majority of preganglionic neurons projecting to the T3 ganglia is from the T2–T4 spinal segments, with few preganglionic neurons projecting from the T1 or T5 segments; those T1 and T5 neurons that did project to T3 ganglia were found close to the T2 and T4 borders, respectively. On the experimental side, T2–T4 neural tube removal resulted in the absence of preganglionic neurons from those segments; only preganglionic neurons from the T1 and T5 spinal cord segments projected to the T3 ganglia, and these neurons were found throughout the entire T1 and T5 segments. *B*, Micrograph of a horizontal section from one of the experimental embryos. Note the extensive labeling at the T1 and T5 spinal levels on the experimental (*right*) side of the embryo (*filled arrows*). Labeling on the control (*left*) side of the embryo was confined, for the most part, to the T2–T4 spinal levels (*open arrows*). Scale bar, 500 μm.

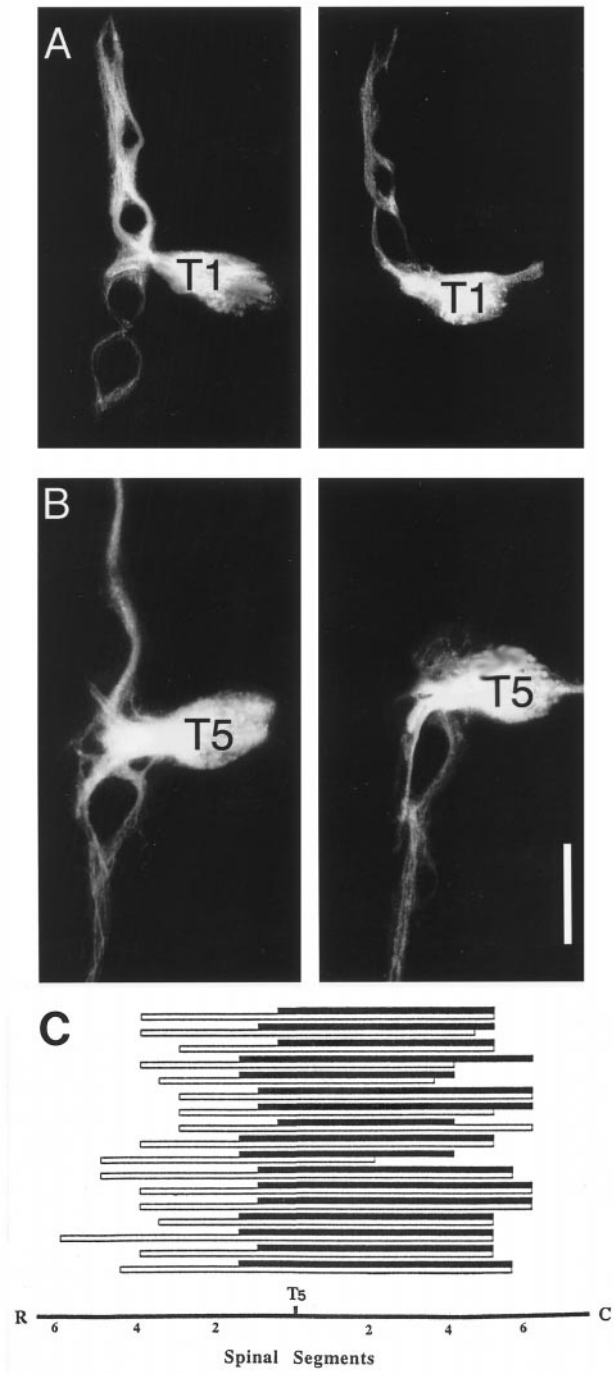

Figure 4. Specificity of preganglionic projections is altered with removal of the neighboring neural tube segments. *A*, Anterograde DiI labeling of T1 preganglionic neurons in a stage 30 neural-tube-removed embryo shows more caudal projections on the experimental side (*left*) than on the control side (*right*). *B*, Anterograde DiI labeling of T5 preganglionic neurons in a stage 30 neural-tube-removed embryo shows both rostral and caudal projections on the experimental side (*left*), compared with mostly caudal projections on the control side (*right*). *C*, T5 preganglionic projections in neural-tube-removed stage 30/31 embryos as determined by anterograde labeling with DiI ($n = 17$). Each set of *bars* represents one embryo. The *filled bars* show projections on the control side, and the *open bars* show projections on the experimental side. Taking T5 as the origin, rostral projections (*R*) are to the *left*, and caudal projections (*C*) are to the *right*. T5 projections on the control side were predominantly caudal, extending four to six segments caudally and not more than 1.5 segments rostrally. Projections on the experimental side, however, were bidirectional, extending three to six segments rostrally and four to six segments caudally. Scale bar, 500 μm.

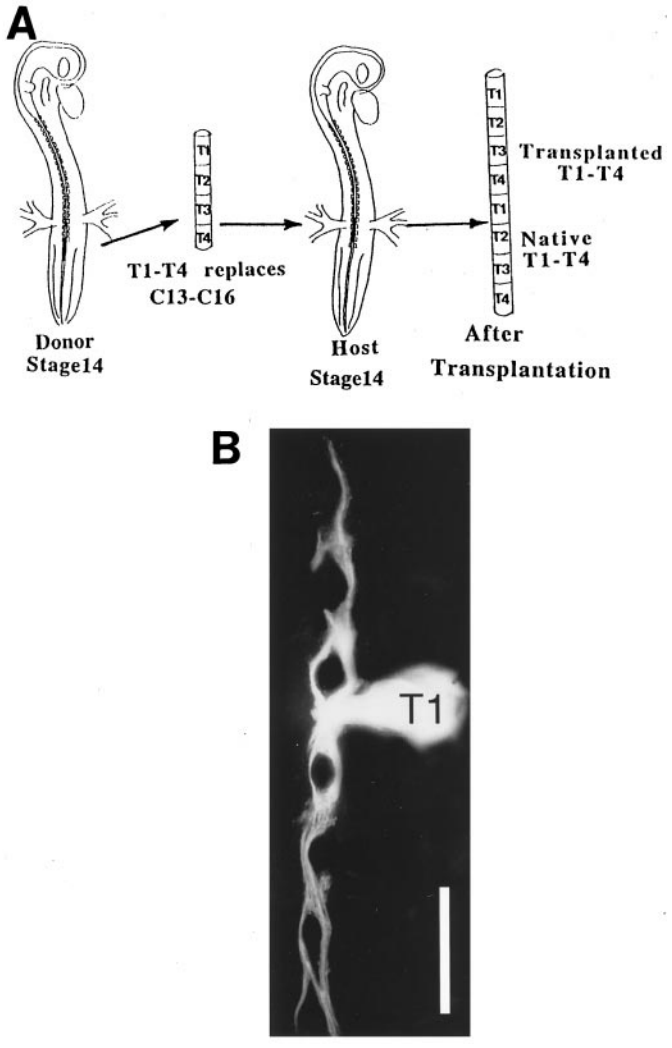

Figure 5. The addition of preganglionic neurons in neighboring neural tube segments alters the projections of preganglionic neurons. *A*, The T1–T4 neural tube segments were removed from a stage 14 donor embryo and transplanted to the C13–C16 spinal level of a similarly staged host. This operation results in transplanted T1–T4 spinal segments immediately rostral to the native T1–T4 spinal segments of the host embryo. *B*, Anterograde DiI labeling of native T1 preganglionic neurons in a stage 30 experimental embryo shows projections in both rostral and caudal directions. Note that T1 preganglionic projections in the normal embryo (see Fig. 1*B*) are predominantly rostral. Scale bar, 500 μm.

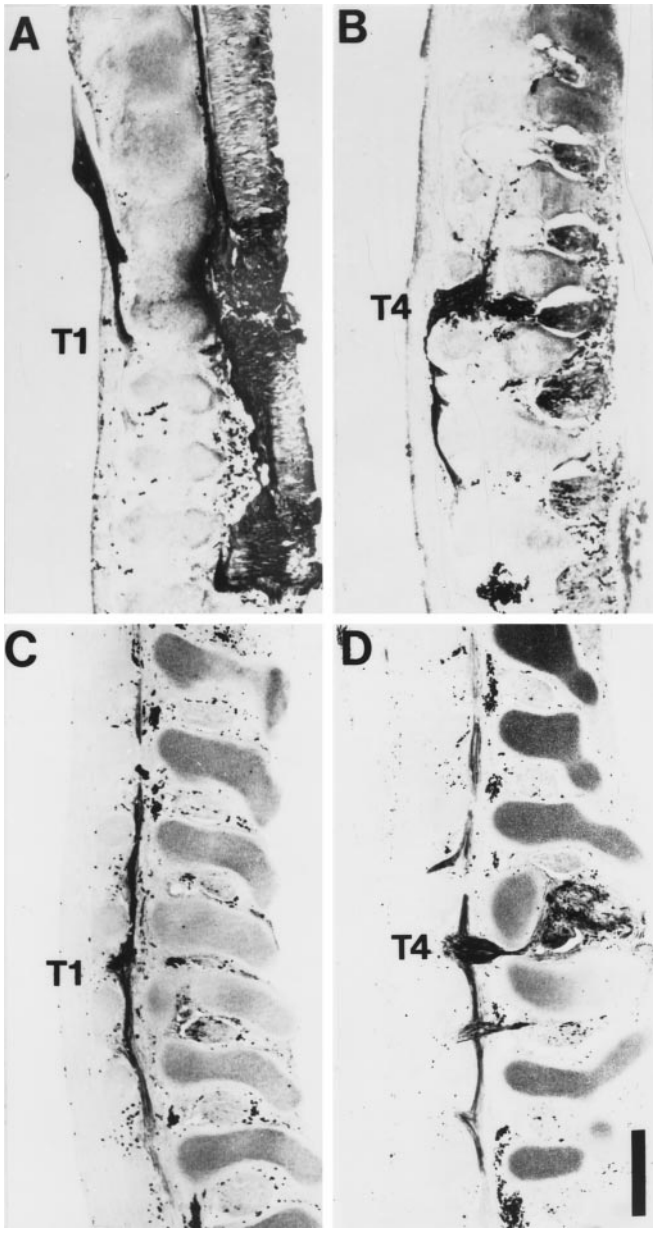

Figure 6. Transplantation of neural tube segments to a novel environment. Anterograde labeling with HRP was used to determine projection patterns. When contiguous T1–T4 neural tube segments were transplanted to the cervical level (C9–C12), the transplanted T1 preganglionic neurons projected predominantly in the rostral direction (*A*), and T4 preganglionic neurons projected predominantly in the caudal direction (*B*). These results differ from those of a previous study showing that, when single T1 neural tube segments were transplanted to the C9 level without their neighboring segments, the transplanted T1 preganglionic neurons projected more or less equally in both rostral and caudal directions (*C*). Similarly, when single T4 neural tube segments were transplanted to the C9 level, T4 preganglionic neurons also projected more or less equally in both directions (*D*). *C* and *D* are reprinted from Yip (1990). Scale bar, 400 μm.

than one segment found in normal embryos (Yip, 1990). However, rostral projections from native T1 preganglionic neurons appeared normal. The increased caudal projections from T1 preganglionic neurons in these experimental embryos show that the specificity of preganglionic projections also can be altered with the addition of preganglionic neurons in neighboring spinal cord segments.

Specificity of preganglionic projections is retained with the transplantation of multiple neural tube segments to a novel environment

A previous study showed that, when a single neural tube segment (T1 or T4) was transplanted to the cervical level, the transplanted preganglionic neurons projected more or less equally in both directions (Fig. 6*C,D*) instead of in their normal predominantly rostral or caudal direction (Yip, 1990). The change in directional projection of these neurons may be attributable to the lack of interaction with neurons from neighboring spinal cord segments. To investigate this possibility, we transplanted contiguous T1–T4 spinal cord segments to the C9–C12 level. Results showed that, when the T1–T4 spinal cord segments were transplanted as a whole ($n = 10$), the transplanted T1 neurons projected predom-

inantly in the rostral direction (Fig. 6*A*), and the transplanted T4 neurons projected predominantly in the caudal direction (Fig. 6*B*). This result further shows that the specificity of preganglionic projections can be influenced by neurons from neighboring spinal cord segments.

DISCUSSION

The establishment of the segmentally specific projection patterns of preganglionic neurons is likely to involve many factors. It has been shown that these patterns are not determined by the segmental origins of preganglionic neurons (Yip et al., 1998). It also has been shown that specific projections of preganglionic neurons do not require target sympathetic ganglia (Yip, 1987). In the present study the elimination and addition of preganglionic axons from the sympathetic trunk, as well as the transplantation of neighboring segments to a novel environment, all show that preganglionic axons from neighboring segments can influence projection patterns. Changes in the preganglionic projection patterns reported here were observed in stage 30/31 embryos, which is before the period of preganglionic cell death (stages 34–36; Oppenheim et al., 1982). These results, therefore, suggest that changes in preganglionic projection patterns are likely the result of the redistribution in the number of rostrally or caudally projecting neurons rather than the selective survival of neurons that have projected into the denervated territories.

That the pattern of axonal projections can be influenced by neurons from a neighboring segment also has been observed in the leech (Gao and Macagno, 1987a,b). During development, several identified neurons (HA, AP, and AE neurons) have processes that initially overlap with their homologs in adjacent ganglia. Normally, the processes from the homologs eventually retract. However, if these neurons are ablated, their homologs in adjacent ganglia retain their extraneous processes and take over the territories of the ablated neurons. These results suggest that the pattern of axonal projections in the leech may result from the competitive interactions among homologous neurons. Indeed, a more recent study in another identified leech neuron (P_o neuron) suggests that the extent and direction of its axonal growth depend on the inhibitory interactions between the segmental homologs (Gan and Macagno, 1995). In the avian sensory system the competitive interactions among axons from neighboring dorsal root ganglia (DRG) also have been implicated in the development of sensory projection and innervation patterns in the chick hind limb (Scott, 1984). When selected DRG were deleted through neural crest removal, the distribution of axonal projections from neighboring intact DRG were shifted toward the deleted pathways, and the dermatomes of the intact DRG were enlarged. The establishment of specific neuronal patterns in these systems, therefore, appears to involve the competitive interactions of neurons with each other. However, this is not the case in the somatic motor system. After partial deletion of the spinal cord, the projection pattern of the somatic motor neurons in the remaining segments was unaltered, and muscles for which the innervation source was removed by spinal cord deletion remained uninnervated (Lance-Jones and Landmesser, 1980).

The reason that somatic motor and autonomic neurons differ in their response to partial deletion of the spinal cord is not clear but may be attributed to functional differences between the somatic motor and the autonomic systems. In the somatic motor system the neurons must be able to innervate their respective target muscles selectively for the control of fine motor movements. Such degree of specificity may not be necessary for autonomic neurons. Sympathetic neurons innervate smooth muscles of blood vessels and skin that are distributed throughout the body. Because preganglionic neurons in different spinal cord segments will elicit autonomic responses such as vasoconstriction and piloerection at different rostrocaudal levels, neurons of the same class must span multiple segments, if not the entire preganglionic cell column. Neuronal interactions among preganglionic neurons from neighboring segments during outgrowth would ensure that all target cells become innervated.

The present study does not address how preganglionic neurons from neighboring segments might interact to produce segmentally specific projections. However, in a preliminary study that used HRP to label a small number of neurons, preganglionic axons have been observed to make hairpin turns near the point at which axons enter the sympathetic trunk (Yip et al., 1996), suggesting that axons can make rostrocaudal choices depending on what they encounter along their pathway. Axons from neighboring segments may influence these choices directly through axon–axon interactions or indirectly through substrate modification or competition for space or trophic factors. Anterograde labeling with lipophilic dyes has shown that during normal development preganglionic axons from neighboring spinal cord segments do appose one another during outgrowth in the sympathetic trunk (Yip et al., 1996); thus the effects of axon–axon interactions cannot be ruled out. It is also possible that preganglionic axons compete for trophic factors along their pathway. A previous study showed that the cells in the local environment of the preganglionic pathway are derived from the somite. Moreover, in the absence of the somitic mesoderm, many preganglionic axons fail to project to their target region (Yip, 1996). This finding suggests that the projection of preganglionic neurons depends on some factors in the somitic mesoderm. Limited supplies of such factors could result in competition among preganglionic neurons. Results from the present study are consistent with the hypothesis that preganglionic neurons compete for some factor(s) along their pathway. For example, when the T2–T4 spinal cord segments were removed, decreased competition in the pathway caudal to T1 and rostral to T5 might explain the caudal projection of some T1 neurons and the rostral projection of T5 neurons. Conversely, when additional axons were introduced in the pathway rostral to the T1 segment, increased competition in the rostral pathway may have increased the caudal projection of T1 neurons. Finally, when single segments—regardless of segmental origin—were transplanted to the cervical level where preganglionic neurons would encounter no competition, no specific preference of rostral caudal projections was observed (Yip, 1990). In contrast, when multiple segments were transplanted to the cervical level, increased competition among axons from the transplanted neighboring segments could explain why normal projection patterns were maintained.

Thus the normal segmentally specific projections seen in the sympathetic system may be explained by a competition hypothesis. In the normal embryo, sympathetic preganglionic neurons are restricted mainly to the thoracic spinal cord, neurons from rostral segments will tend to project rostrally for lack of competition from cervical levels, and neurons from caudal segments will project caudally for lack of competition from lumbosacral levels. Neurons from intervening spinal cord segments will compete with their neighbors from both rostral and caudal levels, resulting in rostral and caudal projections from these segments.

Finally, our current view on the development of segmentally specific sympathetic preganglionic projections in the chick can be

summarized as follows. Sympathetic preganglionic axons, along with somatic motor axons, exit the spinal cord in the ventral roots. Outside the spinal cord the preganglionic axons are guided to the sympathetic trunk area. Rostral or caudal preganglionic projections in the sympathetic trunk, however, are independent of target cues (Yip, 1987) and are not determined intrinsically by the segmental origin of the neurons in the spinal cord (Yip, 1990; Yip et al., 1998). Instead, they appear to be influenced by the competition of preganglionic axons with each other for factors in the somitic mesoderm.

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