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Pathfinding by zebrafish motoneurons in the absence of normal pioneer axons

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Summary

Individually identified primary motoneurons of the zebrafish embryo pioneer cell-specific peripheral motor nerves. Later, the growth cones of secondary motoneurons extend along pathways pioneered by primary motor axons. To learn whether primary motor axons are required for pathway navigation by secondary motoneurons, we ablated primary motoneurons and examined subsequent pathfinding by the growth cones of secondary motoneurons. We found that ablation of the primary motoneuron that pioneers the ventral nerve delayed ventral nerve formation, but a normal-appearing nerve eventually formed. Therefore, the secondary motoneurons that extend axons in the ventral nerve were able to pioneer that pathway in the absence of the pathway-specific primary motoneuron. In contrast, in the absence of the primary motoneuron that normally pioneers the dorsal nerve, secondary motoneurons did not pioneer a nerve in the normal location, instead they formed dorsal nerves in an atypical position. This difference in the ability of these two groups of motoneurons to pioneer their normal pathways suggests that the guidance rules followed by their growth cones may be very different. Furthermore, the observation that the atypical dorsal nerves formed in a consistent incorrect location suggests that the growth cones of the secondary motoneurons that extend dorsally make hierarchical pathway choices.

Key words: *Brachydanio rerio*, axonal guidance, identified vertebrate neurons, single cell ablation.

Introduction

The development of appropriate synaptic connections requires that neuronal growth cones navigate successfully to their correct target cells. Many different types of cues have been implicated as sources of information for navigating growth cones, including the axons of early-developing neurons which act as pioneers in establishing the first axonal pathways (Bentley and Keshishian, 1982; Goodman et al., 1982; Kuwada, 1986; Bentley and Caudy, 1983; Raper et al., 1983; Ghosh et al., 1990). Pioneer neurons have been described in the central nervous systems of mammals (McConnell et al., 1989), fish (Kuwada, 1986; Wilson and Easter, 1991), grasshopper (Ho and Goodman, 1982), and leech (Kuwada and Kramer, 1983; Kuwada, 1985) and in the periphery in chicks (Tosney and Landmesser, 1985), fish (Eisen et al., 1986; Myers et al., 1986), and insects (Bate, 1976; Ho and Goodman, 1982; Bentley and Keshishian, 1982; Tix et al., 1989). In experiments in which the role of pioneer neurons in guidance of later-developing neurons has been directly tested, three general categories of results emerge: (1) pioneer neurons appear unnecessary for guidance of later-developing neurons (Keshishian and Bentley, 1983; Tix et al., 1989); (2) pioneer neurons facilitate, but are unnecessary for, pathfinding by later-developing neurons (Schubiger and Palka, 1985; Chitnis and Kuwada, 1991); (3) pioneer neurons appear to be necessary for normal pathfinding by later-developing neurons (Raper et al., 1984; Kuwada, 1986; Klose and Bentley, 1989; Ghosh et al., 1990). Based on these studies, it seems likely that ablation of pioneer neurons may have variable effects on navigation by later-developing neurons depending upon the system studied. Therefore, the guidance function of any particular pioneer neuron must be assessed on an individual basis.

In this study, we examined the role of pioneer motoneurons in the establishment of motor nerves in the trunk of the embryonic zebrafish. Previous studies have characterized a small group of early-developing, individually identifiable primary motoneurons whose growth cones pioneer the peripheral nerves. The growth cones of the primary motoneurons project directly to their cell-specific target muscles along stereotyped pathways (Myers et al., 1986; Eisen et al., 1986). For the work described in this paper, we studied the development of two distinct nerves, the ventral nerve and the dorsal nerve, each of which is pioneered by a single identified primary motoneuron. The ventral

and dorsal nerves extend along the medial surface of the myotome. Previous studies showed that the growth cone of the CaP primary motoneuron pioneers the ventral nerve while the growth cone of the MiP primary motoneuron pioneers the dorsal nerve (Westerfield et al., 1986; Eisen et al., 1986; Myers et al., 1986). The axons of secondary motoneurons are added to these nerves progressively during embryonic development (Myers, 1985). To test whether the primary motoneurons are necessary for proper establishment of the motor nerves, we ablated them and examined subsequent development of the motor nerve. We found that the secondary motoneurons that extend ventrally were able to form normally positioned ventral nerves in the absence of CaP whereas, the secondary motoneurons that extend growth cones dorsally fail to form normally positioned dorsal nerves in the absence of MiP. Instead, most dorsal nerves extended within the myotome, rather than along the medial surface. These results indicate that within this class of motoneurons, different growth cones are probably following different guidance cues.

Materials and methods

Animals

Embryos of the zebrafish, *Brachydanio rerio*, were obtained from our laboratory colony and maintained as described in Myers et al. (1986). Embryos were staged by hours postfertilization at 28.5°C (h). Segments were numbered as described in Hanneman et al. (1988). A hemisegment refers to a single somite and the corresponding half of the spinal cord.

Single-cell ablations

Primary motoneurons were ablated by laser-irradiation as described in Eisen et al. (1989). Ablations were performed either before or shortly after axogenesis, but before the primary motoneuronal growth cones had reached the horizontal septum. Previous studies (Eisen et al., 1989, 1990; Pike and Eisen, 1990) showed that when primary motoneurons are ablated at the stages used in this study, they are not replaced. Some spinal hemisegments contain an additional primary motoneuron in the CaP position, called VaP (Eisen et al., 1990). In hemisegments in which both cells were present, both cells were ablated. Ablations were performed in one to three spinal hemisegments on one side of the embryo only, so that the neighboring hemisegments and the opposite side of the spinal cord served as controls. Since we performed some ablations after axogenesis, we also determined the effect of primary motoneuron cell body ablation on the motor axons. In three cases, CaPs were labeled with DiI or intracellularly by blastomere injection with rhodamine-dextran (Eisen et al., 1986) and allowed to develop an axon that extended to the region of the horizontal septum, which later divides the dorsal and ventral regions of the myotome. These three CaPs were ablated by laser-irradiation of their cell bodies and then viewed with fluorescence optics within one hour. In all three cases, the axon of the ablated CaP had disappeared; flecks of fluorescence were visible in the periphery, but they were not obviously distributed along the axonal pathway. These observations indicate that ablating the cell bodies of CaPs that have already undergone axogenesis effectively eliminates the axon as well as the cell body. Thus, the axon is unavailable as a guidance cue for the growth cones of the secondary motoneurons.

The success of all CaP, or CaP and VaP, ablations described in this paper was verified by Nomarski DIC observation of the cell bodies as described in Eisen et al. (1989). In contrast, the MiP cell body is often more difficult to identify with Nomarski optics. To determine the percentage of MiPs successfully ablated, we ablated putative MiP cell bodies, allowed the embryos to develop for 6 hours, labeled the embryos with the zn-1 monoclonal antibody (mAb) which recognizes the cell bodies and axons of primary motoneurons (Myers et al., 1986), and scored the experimental segments for the presence or absence of MiPs. MiP ablations were successful in approximately 70% (9/13) of experimental segments.

Antibody labeling

Secondary motoneurons were labeled with the zn-5 monoclonal antibody (Trevarrow et al., 1990) as described in Eisen et al. (1989).

Fluorescent labeling

Secondary motoneurons were labeled by extracellular application of the fluorescent lipid-soluble dyes, DiI and DiO using the methods described in Pike and Eisen (1990) or by intracellular iontophoresis of Lucifer yellow or sulforhodamine using the methods described in Eisen et al. (1989).

Anterograde labeling with DiI

DiI was applied to the region of the spinal cord containing the cell bodies of secondary motoneurons that project in either the ventral or dorsal nerves. Embryos were fixed for 5-10 minutes in 4% paraformaldehyde prior to DiI application. This technique labels a subpopulation of the axons in either the ventral or dorsal nerve. The axons of primary motoneurons were also labeled in some cases; they were readily distinguished from the axons of the secondary motoneurons by their larger diameters (Myers, 1985). DiI anterograde labels were only feasible up to about 40 h; at later stages the secondary motoneuron cell bodies were less accessible to the DiI-containing micropipette.

Retrograde labeling with DiI

The cell bodies of secondary motoneurons were labeled by application of DiI to the ventral or dorsal nerve using techniques described in Eisen et al. (1990).

Results

Strategy

We examined the ability of the growth cones of secondary motoneurons to pioneer two distinct regions of their peripheral pathways. First we determined whether the growth cones of secondary motoneurons were able to pioneer the ventral root in the absence of primary motoneurons (see Fig. 1). To test this, we ablated all of the primary motoneurons within a single hemisegment prior to ventral root formation and determined whether the growth cones of secondary motoneurons established a ventral root. Second, we determined whether the growth cones of secondary motoneurons were able to pioneer the ventral and dorsal motor nerves in the absence of the individual primary motoneurons that usually pioneer these pathways. To test this, we ablated either CaP, which

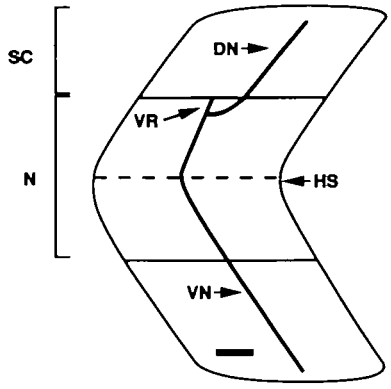


Fig. 1. Drawing of motor nerves. The chevron-shaped outline represents a single somite. The ventral root (VR) exits the spinal cord (SC) and then splits to form the ventral nerve (VN) and the dorsal nerve (DN). N, notochord; HS, horizontal septum. Scale bar, 10 μm.

pioneers the ventral nerve, or MiP, which pioneers the dorsal nerve, and determined whether secondary motoneurons extended growth cones along the appropriate trajectories to form these nerves (Fig. 1).

Axonal pathways in control segments

Development of the ventral root

Each spinal hemisegment in the trunk of the embryonic zebrafish contains a ventral root which is normally pioneered by CaP (Eisen et al., 1986, Myers et al., 1986) at approximately 17 h. To determine when the growth cones of the first secondary motoneurons extended out the ventral root, we labeled the ventral root by retrograde application of DiI and determined when it first contained the axons of secondary motoneurons, as judged by labeling of their cell bodies in the spinal cord. The first secondary motoneuron to extend an axon out the ventral root does so at about 22 h (n=21). In the majority of cases, a single secondary motoneuron was labeled at this stage (18 of 21). Over time, more secondary motoneurons extend growth cones into the periphery, as will be described in the next section.

Development of the ventral nerve

We characterized addition of secondary motor axons to the ventral nerve by counting the number of cell bodies labeled by application of DiI to the ventral nerve. We found that secondary motoneurons, whose cell bodies were positioned close to the CaP cell body, extended their axons in the ventral nerve (25/25), but never in the dorsal nerve (0/25). The earliest stage at which we observed secondary motoneurons with axons in the ventral nerve was 23 h; we found that 1-2 secondary motoneuron cell bodies were labeled (n=8). By 27.5 h, 3-5 secondary motoneuron cell bodies were labeled (n=5); the growth cones of some of these cells extended to the ventral aspect of the notochord and in one case a growth cone had extended to the ventral edge of the myotome. By 30 h, at least 15 secondary motoneuron cell bodies were labeled (n=7); the growth cones of some of these cells extended to the ventral edge of the myotome. While we could not determine whether we labeled all of the motoneurons that contributed to the ventral nerve at these stages, it is apparent that over time an increasing number of secondary motoneurons contribute axons to this nerve.

To determine the spatial relationship between the CaP axon and the axons of the secondary motoneurons that extend ventrally, we labeled individual CaPs and secondary motoneurons by intracellular iontophoresis of Lucifer yellow and rhodamine. We found that the growth cones and axons of some secondary motoneurons were closely apposed to the CaP axon (Fig. 2A), suggesting that they fasciculated with the CaP axon during extension. We also observed secondary motor axons parallel to the CaP axon but distant from it by several μm (Fig. 2B). Our interpretation of this observation is that the space between the axons of these motoneurons is occupied by the axons of other secondary motoneurons.

Development of the dorsal nerve

We estimated the minimum number of secondary motor axons in the dorsal nerve at an early developmental stage by counting the number of cell bodies labeled by application of DiI to the dorsal nerve. We found that individual secondary motoneurons whose cell bodies were positioned close to the MiP cell body often extended axons in the ventral nerve as well as in the dorsal nerve (3/4). In the remaining case the secondary motoneuron extended an axon only in the dorsal nerve. The earliest stage at which we observed labeled cell bodies of secondary motoneurons was 23 h (n=4); in these cases there was only a single secondary motoneuron that had extended an axon in the dorsal nerve. Previous studies showed that by 5 days there are at least 6 secondary motor axons in the dorsal nerve (Myers, 1985). Thus, we conclude that over time an increasing number of secondary motoneurons contribute axons to this nerve. However, there appear to be consistently fewer axons in the dorsal nerve than in the ventral nerve throughout embryonic and larval development.

To determine the spatial relationship between the MiP axon and secondary motoneurons that extend axons dorsally, we labeled MiP and secondary motoneurons with dorsal axons with DiI and DiO (Fig. 2C). As seen in the ventral nerve, secondary motoneurons extended axons dorsally along the pre-existing MiP axon (n=7).

Axonal pathways in experimental segments

Formation of the ventral root following primary motoneuron ablation

To determine whether the growth cones of secondary motoneurons could pioneer the ventral root, we ablated all of the primary motoneurons within a hemisegment before axogenesis. We found that, in 92% (24/26) of these experimental hemisegments, the growth cones of secondary motoneurons were able to pioneer the ventral root. In two experimental hemisegments, secondary motoneurons failed to establish a ventral

Table 1. *Ventral nerve formation following CaP ablation*

	Control (40 h)	40 h	48 h	60 h	72 h
H (%)	0	17	0	0	0
N (%)	0	52	19	3	0
V (%)	0	16	27	17	0
VM (%)	100	15	54	80	100
Total no. of nerves	50	108	59	30	39

Ventral nerves in experimental segments were observed at progressively later developmental stages (h) and were scored as falling into one of the following 4 categories: H, Ventral nerves ended in the region of the horizontal septum; N, ventral nerve ended in the region of the ventral aspect of the notochord; V, ventral nerve ended in the region of the ventral myotome, proximal to the ventral edge of the myotome; VM, Ventral nerves extended to the ventral margin of the myotome; the length of the experimental nerve appeared indistinguishable from control nerves. Control nerves had all reached the VM by 40 h.

root. Instead, cells in the normal position of secondary motoneurons extended axons caudally within the spinal cord.

Formation of the ventral nerve following CaP ablation

We ablated CaP either before or just after axogenesis and observed secondary motoneuron outgrowth at progressive developmental stages. The results from these experiments are summarized in Table 1. Our first observation was made at 40 h, approximately 22 hours following CaP ablation. We found that extension of the growth cones of secondary motoneurons was delayed in most experimental segments (Table 1, Figs 3, 4A). Many of the delayed ventral nerves stopped at the ventral aspect of the notochord, a location where the CaP axon typically makes a prominent varicosity (Eisen et al., 1986). At later developmental stages, we observed fewer perturbations in ventral nerve formation (Table 1). By 48 h, many of the secondary motoneurons had extended axons ventral to the notochord (Fig. 4B). By 60 h, the majority of ventral nerves in experimental hemisegments were indistinguishable from control ventral nerves (Fig. 4C). Thus, the initial perturbation in ventral nerve formation was transient. By 72 h, almost all of the experimental ventral nerves appeared normal (Fig. 4D). In some of the experimental hemisegments with delayed secondary motoneuron outgrowth, processes projected along aberrant pathways (Figs 3,4B,C). The trajectory and location of these aberrant projections were highly variable among experimental hemisegments. Some axons extended medially between the myotome and notochord as well as laterally into the myotome.

Formation of the dorsal nerve following MiP ablation

We ablated MiPs and examined dorsal nerve formation at 52 h, 60 h and 72-96 h (Table 2). We found that at 52 h, approximately 33 hours following MiP ablation, most of the dorsal nerves were shorter than the dorsal nerves

Table 2. *Dorsal nerve formation following MiP ablation*

	52 h	60 h	72–96 h
Short (%)	80	0	0
Aberrant (%)	0	73	75
No Effect (%)	20	27	25
Total no. of nerves	5	15	8

Experimental dorsal nerves were scored as follows: SHORT, not extending to the dorsal limit of the pathway, as compared with controls; ABERRANT, following a characteristic abnormal trajectory through the dorsal myotome instead of extending along the normal medial pathway; or NO EFFECT, there was no obvious difference between experimental and control dorsal nerves.

in control hemisegments (Fig. 5A,B). By 60 h, all of the dorsal nerves were as long as controls and were located in the appropriate dorsal muscle region. However, most of them had an aberrant trajectory that formed at a consistent location within the myotome, instead of along the normal medial pathway (Fig. 5C,D). In control hemisegments, dorsal nerves were not observed in this location. At the latest developmental stages observed, the dorsal nerves were still in an aberrant location in the majority of experimental hemisegments (see Table 2; Fig. 5E,F).

Discussion

The results presented in this paper suggest that in the absence of primary motoneurons, the growth cones of secondary motoneurons are able to form the ventral root. Moreover, two different identified primary motoneurons appear to have distinct roles in guiding the growth cones of secondary motoneurons. The CaP axon appears to facilitate pathfinding by secondary motoneurons, however, this axon appears unnecessary for eventual ventral nerve formation. In contrast, the MiP axon appears to be required for proper pathfinding by the secondary motoneurons. The lesson from this result is that pioneer neurons can assume a variety of roles in development and, therefore, generalizations about the role of one type of pioneer neuron in guidance of later-developing neurons may not hold true for other pioneer neurons, even within the same system.

The ability of the growth cones of secondary motoneurons to pioneer their normal ventral pathway has interesting implications. Following CaP ablation, secondary motoneurons extended growth cones ventrally along their normal pathway, rather than along the axonal pathways of other primary motoneurons or through regions not typically traversed by motor growth cones. These observations suggest that the secondary motoneurons may be committed to extend growth cones along specific pathways or in a specific direction. Further, it seems possible that the growth cones of the secondary motoneurons may recognize the same guidance cues that are used during pathfinding by the primary motoneurons. Previous studies showed that primary motoneurons are committed to extend their

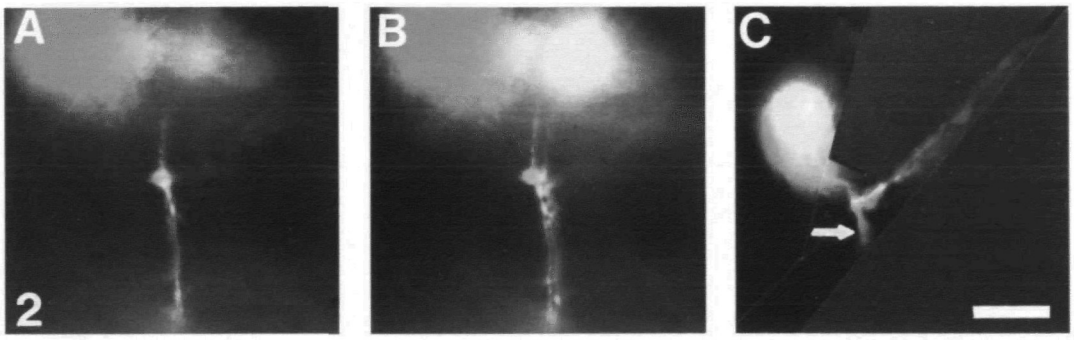
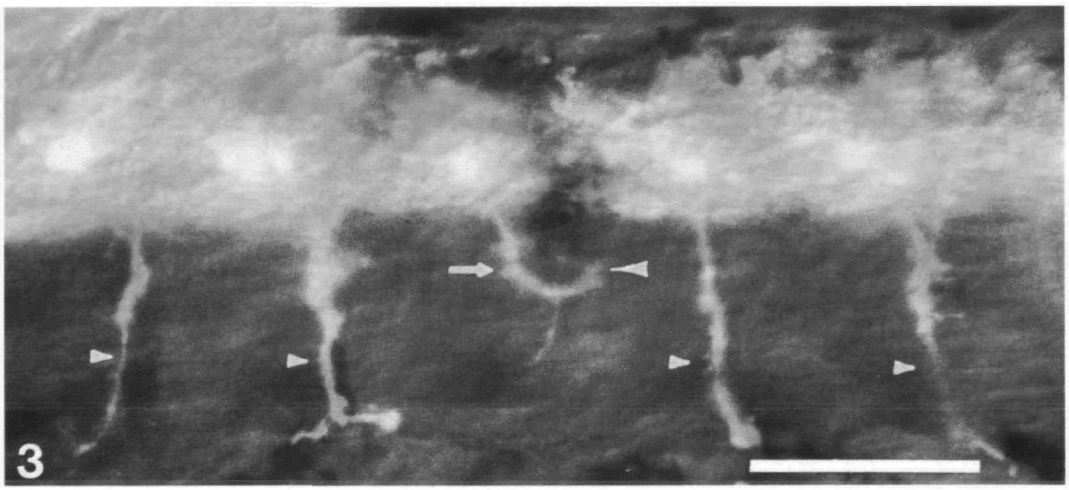
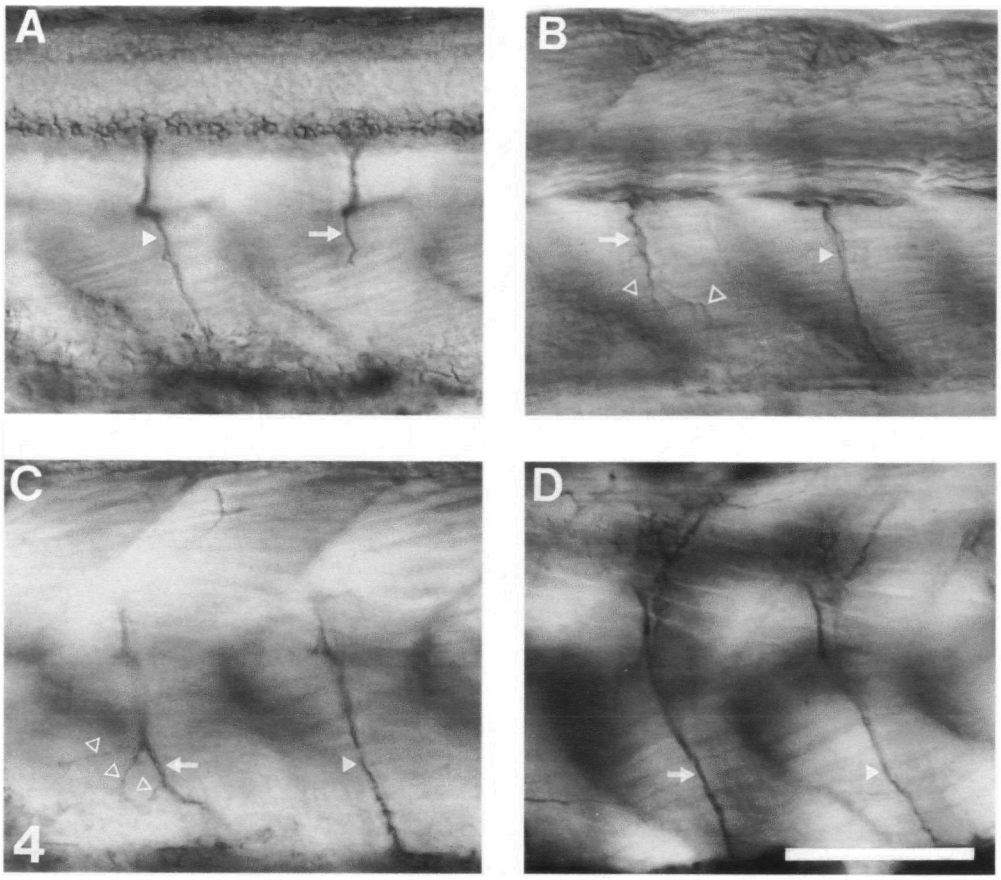


Fig. 2. The axons of secondary motoneurons are associated with the axons of primary motoneurons. Side views of 28 h live embryos showing labeled primary and secondary motoneurons. Each cell was labeled intracellularly with either sulforhodamine (red) or Lucifer yellow (yellow) in A and B or DiI and DiO in C and viewed in many focal planes to ascertain the relationship between the axons of the labeled cells. In these photomicrographs, the cell bodies of all of the motoneurons are out of the plane of focus. (A) The CaP axon (yellow) and secondary motoneuron axon (red) are closely apposed. (B) In this case, the CaP axon (yellow) and secondary motoneuron axon (red) extend along parallel trajectories but do not appear to contact one another. (C) This MiP axon (yellow) and secondary motoneuron axon (red) are closely apposed. The secondary motoneuron has a ventral projection at this stage (arrow). Scale bar, 10 μm.

Fig. 3. Ventral nerve formation is delayed following CaP ablation. Ventral nerves were labeled anterogradely with DiI in control (small arrowheads) and experimental (arrow) hemisegments of lightly fixed 40 h embryos. As shown in this example, at this developmental stage the ventral nerves in control hemisegments extended to the ventral edge of the muscle. The ventral nerve in the experimental hemisegment failed to extend as far as the control nerves; in this case, the ventral nerve also extended a process along an abnormal trajectory (large arrowhead). Scale bar, 50 μm.

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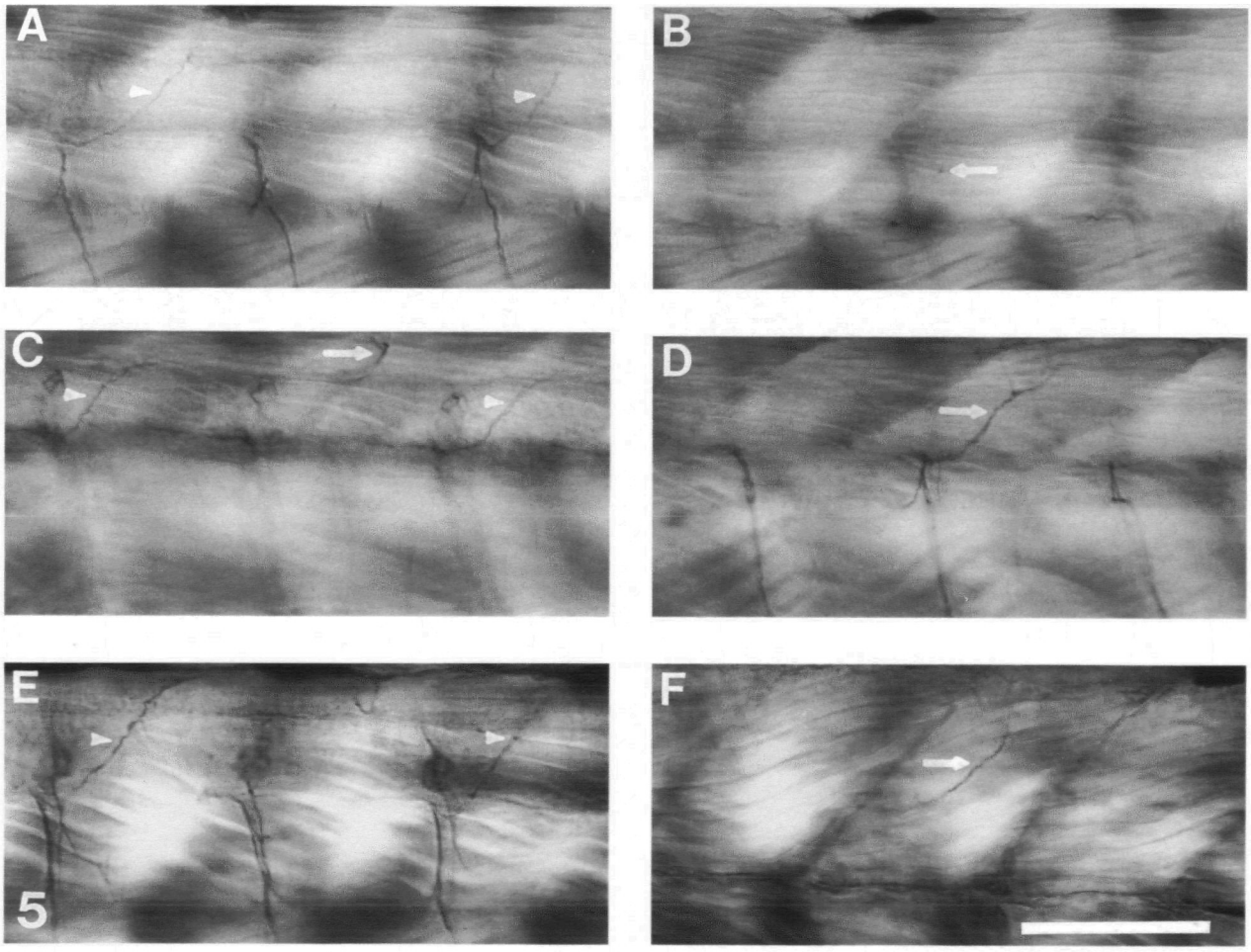
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Fig. 4. Normal nerves eventually form following CaP ablation. Photomicrographs of embryos labeled with the zn-5 mAb and viewed with Nomarski optics. (A) Experimental and control ventral nerves at 40 h. The ventral nerve in the experimental hemisegment (arrow) is delayed compared to the ventral nerve in the neighboring control hemisegment (arrowhead). (B) Experimental and control ventral nerves at 48 h. In this case the experimental ventral nerve (arrow) is delayed compared to the neighboring control (arrowhead) and it has extended processes along abnormal trajectories at its leading edge (open arrowheads). (C) Experimental and control ventral nerves at 60 h. In a few cases, such as the one illustrated here, experimental ventral nerves are still abnormal at this developmental stage. This experimental ventral nerve (arrow) has abnormal branches (open arrowheads) and appears to be slightly delayed compared to the neighboring control nerve (arrowhead). (D) Experimental and control ventral nerves at 72 h. By this stage, all of the experimental ventral nerves (arrow) had extended the full length of the ventral pathway and the majority were indistinguishable from controls (arrowhead). Scale bar, 50 μm.

Fig. 5. Dorsal nerves follow an abnormal trajectory following MiP ablation. Photomicrographs of embryos labeled with the zn-5 mAb and viewed with Nomarski optics. The panels on the left (A,C,E) show medial views that correspond to the lateral views of the same nerves shown in the panels on the right (B,D,F). (A) Side view of 3 hemisegments at 52 h, showing dorsal nerves in their normal location in control hemisegments (arrowheads). The dorsal nerve is not apparent in this plane of focus. (B) This more lateral focal plane shows the experimental dorsal nerve beginning to project laterally through the dorsal muscle (arrow). (C) At 60 h, the dorsal nerve in the experimental hemisegment is not present along the normal medial pathway and is only evident near the dorsal edge of the hemisegment (arrow). Control nerves occupy the normal medial pathway (arrowheads). (D) A more lateral plane of focus reveals that the dorsal nerve in the experimental hemisegment extended lateral to the normal medial pathway (arrow). There is no nerve present along this pathway in the neighboring control hemisegments. (E) At 88 h there is still no dorsal nerve occupying the medial pathway in this experimental hemisegment; control nerves (arrowheads) are normal. (F) A more lateral plane of focus reveals that the dorsal nerve (arrow) projects in the same lateral region seen at 60 h. Again, no nerves are seen in this region in control hemisegments. Scale bar, 50 μm.

growth cones along specific pathways before axogenesis and suggested that cell body position in the spinal cord plays a role in the commitment process (Eisen, 1991). Similarly, the spatial organization of secondary motoneuron cell bodies could play a role in determining their axonal trajectories. The observation that secondary motoneurons near the MiP cell body position, but not near the CaP cell body position, extend their axons in the dorsal nerve is consistent with this hypothesis.

In the absence of the CaP axon, the ventral nerves formed more slowly and the growth cones of the secondary motoneurons formed aberrant branches. These observations suggest that although the CaP axon is unnecessary for proper pathfinding by the secondary motoneuron growth cones, it nevertheless facilitates their outgrowth. This delay in outgrowth is reminiscent of what was observed following ablation of pioneer neurons in the CNS of grasshoppers (Raper et al., 1984) and fish (Kuwada, 1986). In both cases, following ablation of a pioneer neuron, axons of later-developing neurons were stunted. However, it is unclear from those studies whether the stunted axons were arrested or simply delayed in their outgrowth. In either case, the results are similar to ours in that the axons of the later-developing neurons were oriented in the correct direction, suggesting that there are directional cues in addition to the pioneer axon that later-developing growth cones can recognize.

In contrast to the ventrally projecting secondary motoneurons, the secondary motoneurons that extend dorsally were typically unable to pioneer a normal dorsal nerve. This result suggests that unlike CaP, MiP may be required for proper guidance of the growth cones of secondary motoneurons along their appropriate dorsal pathway. It is interesting that the secondary motoneurons formed a nerve that was located in a consistent, though abnormal, position. This observation suggests that these secondary motoneurons may make hierarchical pathway choices. Their preference is to extend dorsally along the MiP axon; in the absence of the MiP axon, they select a specific, atypical pathway. In other systems, this type of hierarchical pathway choice has not been observed (Raper et al., 1984; Bastiani et al., 1985; Klose and Bentley, 1989). In fact, in some cases when pioneers are ablated, follower growth cones appear to select a variety of incorrect pathways, most of which have previously been pioneered by other axons (Chitnis and Kuwada, 1991). However, in the cat CNS, the absence of pioneer neurons results in later-developing neurons bypassing their normal target and extending along an atypical pathway (Ghosh et al., 1990). Thus, in the absence of the subplate, geniculocortical axons fail to make a trajectory change that would take them into the overlying cortical plate, and instead continue to extend through the intermediate zone, a direct continuation of their pathway which is normally taken by other thalamic axons. The important difference between these results and ours is that, in the cat, the axons of later-developing neurons extend along an alternate pathway *away* from their normal target whereas, in the zebrafish, the axons of secondary motoneurons extend along an alternate pathway, *but still reach* the vicinity of their normal target. Thus, our results suggest that the growth cones of these secondary motoneurons may choose among several possible pathways that lead toward their targets, in a hierarchical manner.

In a few cases, dorsal nerves formed in a normal location following MiP ablation. Similar observations have been made following ablation of pioneers in the zebrafish brain; these results have been interpreted to mean that cues provided by the pioneers are not required for proper guidance of follower growth cones (Chitnis and Kuwada, 1991). Although this may also be the case for the secondary motoneurons, we favor

another explanation. Since the percentage of cases in which the secondary motoneurons formed a normal dorsal nerve following MiP ablation was about the same as our failure rate for MiP ablation (see Methods), it seems likely that in those cases in which a normal dorsal nerve formed, we failed to ablate MiP.

The prevailing model for growth cone navigation is that a number of different cues work in a combinatorial manner to guide growth cones along appropriate pathways (Berlot and Goodman, 1984; Schubiger and Palka, 1985; Bastiani et al., 1987; Bixby et al., 1987; Dodd and Jessell, 1988; Harrelson and Goodman, 1988; Tomaselli et al., 1988; Elkins et al., 1990). We have described the variable roles played by the zebrafish primary motoneurons in guiding the growth cones of later-developing secondary motoneurons. Our results suggest that, in addition to the axons of the primary motoneurons, other sources of guidance information are available to the growth cones of the later-developing neurons that enable them to extend towards their targets in the absence of the pioneer axons. By identifying the cues that play a role in growth cone guidance and then manipulating different combinations of these cues, we hope to be able to determine the specific contributions different types of guidance cues make to pathway navigation.

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