Development 113, 701-707 (1991)
Printed in Great Britain © The Company of Biologists Limited 1991

Early ablation of target muscles modulates the arborisation pattern of an identified embryonic *Drosophila* motor axon

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Summary

The *Drosophila* RP3 motor axon establishes a stereotypic arborisation along the adjoining edges of muscles 6 and 7 by the end of embryogenesis. The present study has examined the role of the target muscles in determining this axonal arborisation pattern. Target muscles were surgically ablated prior to the arrival of the RP3 axon. Following further development of the embryo in culture medium, the morphology of target-deprived RP3 motor axons was assayed by intracellular injection with the dye Lucifer Yellow. Axonal arborisations were formed on a variety of non-target muscles when muscles 6 and 7 were removed and these contacts were maintained into stage 16. The pattern of axonal arborisations over non-target muscles varied between preparations in terms of the number of muscles contacted, and the distribution of arborisations on individual muscles. Following removal of muscle 6, the RP3 motor axon frequently contacted muscle 7, and axonal arborisations were present along the distal edge of the muscle. In the absence of muscle 7, the RP3 axon often did not contact muscle 6 and when muscle 6 was contacted, the arborisation of RP3 was poorly developed. Axonal processes were retained on non-target muscles when only one target muscle was present. Therefore, the establishment of a stereotypic arborisation by the RP3 motor axon is apparently dependent on growth cone contact with both target muscles.

Key words: *Drosophila* embryo, *Drosophila* motoneuron, neuromuscular specificity, muscle ablation, insect embryo.

Introduction

The characteristic axon morphologies attained by the end of embryogenesis are the culmination of growth cone responses to pathway options and final targets. Indications of the underlying cellular mechanisms for axon pathfinding and target recognition have come from observing the behaviour of single neurons in the relatively simple insect nervous system. Studies in the locust embryo in particular have identified candidate axon guidance mechanisms within the central nervous system (Goodman *et al.* 1984; Raper *et al.* 1983*a*,*b*,*c*) and periphery (Bate, 1976; Bentley and Keshishian, 1982; Berlot and Goodman, 1984; Myers *et al.* 1990; Meier and Reichert, 1990; Whitington, 1989), and tested the role of several cellular cues with ablation experiments (Bentley and Caudy, 1983; Raper *et al.* 1983*a*,*b*,*c*; Bastiani and Goodman, 1986; Bastiani *et al.* 1986; du Lac *et al.* 1986; Whitington *et al.* 1982; Whitington and Seifert, 1982; Whitington and Seifert, 1984). Despite this wealth of information on axon guidance at the cellular level, the relatively intractable genetics of the locust has constrained the analysis of the molecular basis of axon pathfinding and target recognition.

In contrast, the embryo of the fruit fly *Drosophila melanogaster* is particularly attractive for genetic and molecular studies (Thomas and Crews, 1990). Recent studies have identified the cellular contacts made by embryonic *Drosophila* motor axon growth cones that may be important for defining axon trajectories and establishing arborisation patterns (Hartenstein, 1988; Johansen *et al.* 1989*a*; Sink and Whitington, 1991*b*). We are taking these descriptive cellular studies a step further with ablation experiments, which are designed to pinpoint the growth cone contacts that are critical for the development of stereotypic motor axon morphologies. The results from ablation experiments may also indicate the specificity of marker molecule(s) distribution along axon pathways and on target muscles.

During normal development, the RP3 axon in the *Drosophila* embryo (Sink and Whitington, 1991*b*), like leg-innervating motorneurons in the locust embryo (Myers *et al.* 1990), extends processes over non-target muscles before ultimately establishing a stereotypic arborisation over the target muscles. These observations raise the question of whether a signal from the target muscles causes the axon to retract inappropriate processes and stabilise the processes contacting the target muscles. In the present study, we have examined the role of the target muscles in the establishment of the embryonic *Drosophila* RP3 motor axon's stereotypic arborisation pattern (Johansen *et al.* 1989*a*; Sink and

Whitington, 1991*a*,*b*). Muscles were surgically ablated prior to the arrival of the axon, and following further development in embryo culture medium, axon behaviour was assayed by intracellular dye injection. Ablation of both target muscles resulted in variable arborisation patterns over non-target muscles. Results from the ablation of single target muscles indicate that RP3 growth cone contact with both target muscles prior to stage 16 is necessary for (a) the establishment of a consistent arborisation pattern by the middle of stage 16, and (b) the complete retraction of processes in contact with non-target muscles.

Materials and methods

Stock

Oregon-R wild-type embryos of *Drosophila melanogaster* were used in this study. Eggs were chemically dechorionated by agitation in a 25% commercial bleach solution. Embryos were examined under a dissecting microscope, and staged according to the morphological criteria of Campos-Ortega and Hartenstein (1985).

Dissections

Embryo dissections and culturing were carried out in modified M3 medium (Shields and Sang, 1978). In our version of M3 medium, equivalent quantities of L-alanine were substituted for the a- and b-alanine, choline-Cl was substituted for choline-HCl, and aspartic acid was omitted. Foetal calf serum (FCS) was omitted from the dissection solution to facilitate adhesion of the embryo to the slide.

Embryos were dissected on poly-L-lysine (Sigma)-coated glass slides under sterile FCS-free M3 medium held in a Vaseline dam. The anterior end of the egg was cut open, and the embryo was squeezed from the vitelline membrane. Embryos were dissected longitudinally along the dorsal midline using an electrolytically sharpened tungsten needle. The bodywall was gently flattened onto the slide, and the digestive system was removed to expose the central nervous system (CNS) and musculature. The FCS-free medium was replaced three times with sterile M3 medium supplemented with 10% FCS (Cytosystems Ltd, Australia).

Muscle ablations

Preparations were examined using a Leitz water immersion objective on a Zeiss photomicroscope equipped with Nomarski optics. Selected muscles in abdominal segments A4–A6 were surgically ablated with a 30–60 MΩ microelectrode pulled on a Brown-Flaming horizontal puller (Sutter Instrument Co., USA). The microelectrode was inserted in one end of the chosen muscle, then raised slightly, and manouvered towards the opposite end of the muscle and out of the segment. This procedure usually removed the selected muscle(s) as a whole. The embryo was then placed in an humidified chamber in a 25°C incubator for approximately 5 h.

Intracellular dye injection

For intracellular dye injections, the RP3 soma was identified in the manipulated hemisegment, and penetrated with a 30–60 MΩ microelectrode filled with a 5% Lucifer Yellow (LY) solution. The dye was iontophoretically injected by applying a 0.2 nA DC hyperpolarizing current for 20 s. As a control, an RP3 soma in an untreated, homologous abdominal segment was also intracellularly injected with LY.

Immunohistochemistry

For anti-LY immunohistochemical processing, preparations were fixed in 4% paraformaldehyde in Millonig's buffer for 15–20 min, washed in phosphate-buffered saline (PBS), and incubated overnight in a rabbit anti-LY antibody (raised in our laboratory) diluted 1:500 in PBS/0.4% Triton X-100/0.25% bovine serum albumin (PBT). The next day the preparations were washed in PBS and incubated for 24 h in HRP-conjugated goat anti-rabbit IgG antibody (Amersham) diluted 1:250 in PBT. Preparations were then washed in PBS, incubated for 1 h in 0.5% diamino-benzidine in PBS, and reacted with hydrogen peroxide to give a stable reaction product in the injected neurons. Following a final wash in PBS, the embryos were cleared and mounted in 100% glycerol.

All preparations were examined on a Zeiss photomicroscope, equipped with Nomarski optics, using a Zeiss Planapo 100× oil immersion objective. Injected neurons were drawn with the aid of a camera lucida.

Results

General comments

In normal, unoperated embryos the RP3 motor axon in abdominal segments A3–A7 has formed a stereotypic arborisation along the adjoining edges of muscles 6 and 7 by mid stage 16 (Sink and Whitington, 1991*a*,*b*), and appears to retain this arborization through larval development (Johansen *et al.* 1989*a*,*b*; Budnik *et al.* 1990) (Fig. 1A).

The culturing system that we have used appears to support relatively normal continued development of the embryo. The muscles of cultured embryos sometimes differed slightly in appearance from those in normal embryos, in that they were thinner at the insertion sites and thicker in the central third region. Also, in such embryos, the CNS appeared less cohesive in that the cells were not as tightly packed as in normal embryos. Despite these occasional differences, culturing *per se* does not appear to affect the development of the motorneurons, as the RP3 axons in the control segments successfully grew to, and arborised along, the same region of the target muscles as in normal, uncultured embryos (Fig. 1B). In some cases, RP3 neurons in several unoperated segments were injected in single cultured embryos and all showed normal arborisation patterns. In addition, the developmental sequence of RP3 axon growth in culture, determined by halting culturing at different times, was similar to that observed in non-cultured embryos (Sink and Whitington, 1991*b*). Fig. 2 shows schematically the region of musculature encountered by the RP3 axon during outgrowth. In some cultured embryos, the RP3 axon had not contacted muscles 6 and 7 in control segments, presumably as a result of aborted development. However, RP3 axonal arborisations to muscles other than 6 and 7 were only seen in unmanipulated segments when the musculature and/or the CNS was grossly abnormal in appearance. Such embryos were rejected.

Ablation of muscles 6 and 7

Muscles 6 and 7, the targets for motoneuron RP3, are

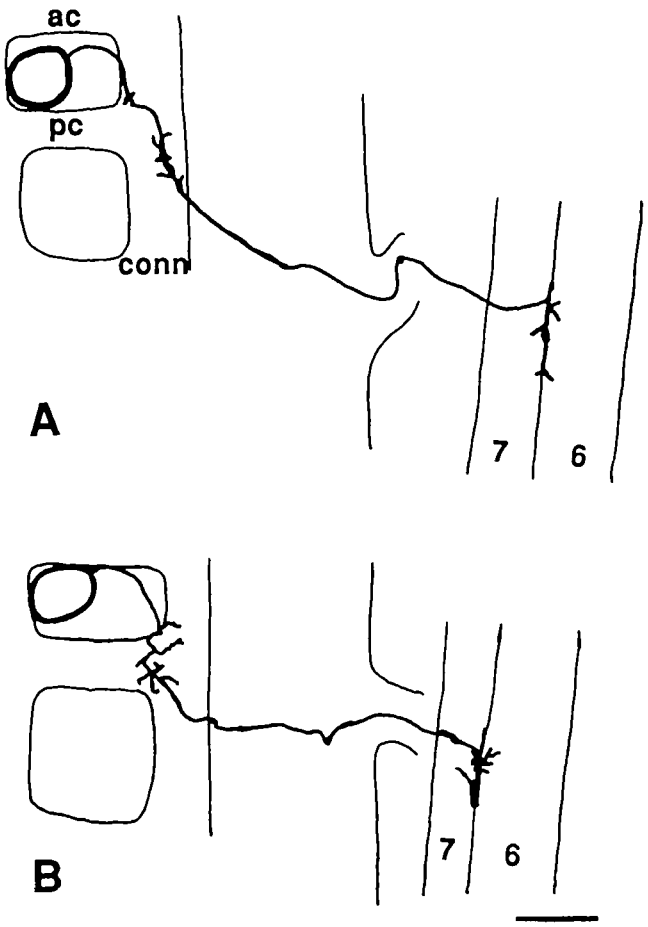

Fig. 1. Camera lucida drawings of LY-filled RP3 motorneurons. (A) RP3 from a late stage 16 embryo that has not undergone culturing, (B) RP3 neuron in an unoperated segment in an embryo which was removed from the egg at stage 15 then cultured for 5 h. conn, connective; ac, anterior commissure; pc, posterior commissure. Scale bar=10 μm.

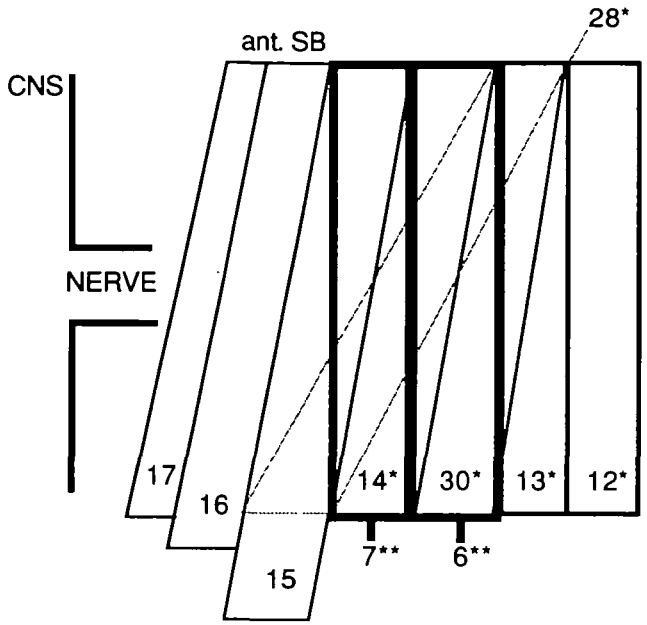

Fig. 2. Schematic diagram of the musculature in the right hemisegment encountered by an RP3 axon during outgrowth. Muscles 6 and 7 are internal muscles (bold lines); muscles 12, 13, 14, 15, 16 and 17 are intermediate muscles (unbroken lines); muscle 28 is an external muscle (broken lines). Double asterisks indicate target muscles; single asterisk indicates muscles contacted on the internal facing surface by RP3 processes during normal development. CNS, central nervous system; NERVE; segment nerve; ant. SB; anterior segmental boundary.

located in the most internal muscle layer. This location makes it possible to remove surgically these two muscles without causing obvious disruption to other muscles in the region. Muscle ablations were performed at early stage 15, at which time RP3's motor axon has just exited the CNS (Sink and Whitington, 1991*b*) and has, therefore, not yet contacted muscles 6/7. The following observations are based on 21 manipulations (in 20 embryos) in which both muscles 6 and 7 were removed in an abdominal segment, (generally A4–A6).

In the absence of both target muscles, the RP3 axon arborised on a number of muscles in the ventral muscle group (summarised in Table 1). Axonal arborisation patterns differed between embryos in terms of the number of muscles contacted, and the identity of the muscles contacted (Fig. 3). In eight preparations, the target-deprived RP3 axon extended beyond the region where the target muscles are normally situated (Fig. 3B,C,E,F). In two preparations, the RP3 axon grew anteriorly, and extended processes along and across the segmental boundary (Fig. 3A,D). In one preparation, the RP3 axon fasciculated with the intersegmental nerve, which extends to more distal muscles.

Intramuscular arborisations of target deprived RP3 axons differed between embryos. For example, the axonal arborisations present on muscle 14, the most frequently contacted non-target muscle (Table 1), varied in both number, and extent of muscle surface contacted. In some cases (Fig. 3B), the axon has processes only along the distal edge of muscle 14 whereas in others (Figs 3C,D and F) the processes spread across the entire internal surface of the muscle. (We define distal as being further removed from the CNS and internal as being furthest removed from the epidermis).

Ablation of muscle 6

In the absence of muscle 6, the RP3 axon grew into the periphery, and in 11 out of 14 cases (in 14 embryos) contacted muscle 7 (Fig. 4A,C,D,E,F). The pattern of axonal arborisations in contact with muscle 7 varied between embryos but in all cases was restricted to the distal third of the internal face of the muscle. In one embryo, a process was extended for approximately 10 μm anteriorly along the distal edge of muscle 7 (Fig. 4D). In other embryos, the arborisation of RP3 was less extensive (Fig. 4E,F).

Although most (78%) of RP3 axons in the treated

Table 1. *The frequency of contact of body wall muscles by the RP3 motorneuron after ablation of one or both of its target muscles M6 and M7*

Muscle ablated	Muscle contacted												Cross segment border
	6	7	8	12	13	14	15	16	28	30	26	17	
Both M6 and M7 (n=21)	–	–	2	1	4	14	14	10	6	3	3	3	2
M6 only (n=14)	–	11	1	0	5	11	5	0	3	0	0	0	1
M7 only (*n*=9)	4	–	0	2	4	5	2	3	6	0	1	0	1

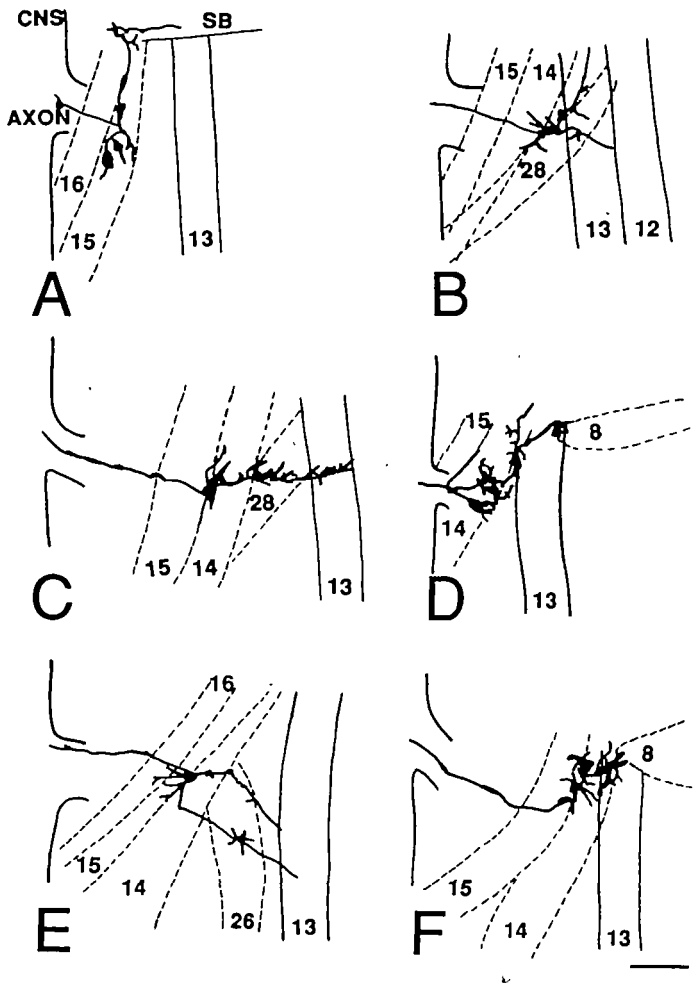

Fig. 3. Camera lucida drawings of RP3 motor axons in embryos after removal of both muscles 6 and 7 in the same hemisegment, followed by a 5 h culture period. In A the axon has extended a process anteriorly along the external surface of muscle 15, then distally along the segmental boundary (SB). In D a branch has extended anteriorly along muscle 14, then crossed the segmental boundary. Axons in B,C,D,E and F have extended distally beyond the region normally occupied by muscles 6 and 7. CNS, central nervous system; AXON, RP3 axon. Scale bar=10 μm.

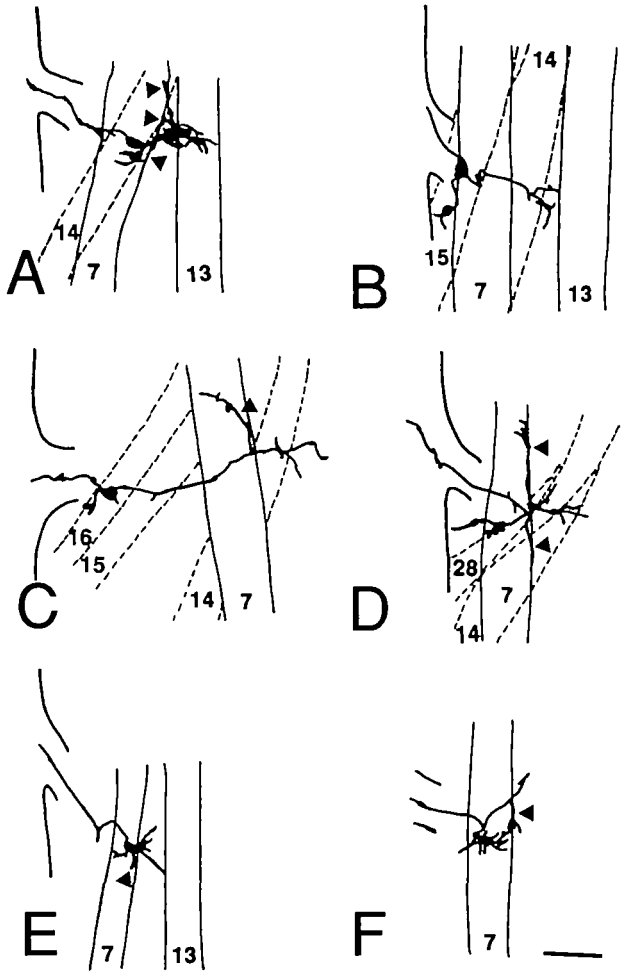

Fig. 4. Camera lucida drawings of RP3 axons in segments where muscle 6 had been removed. Arrowheads indicate the axonal arborisations that contact muscle 7. Scale bar=10 μm.

segments contacted muscle 7, in all cases the axon maintained some processes in contact with non-target muscles (Table 1). The most commonly contacted non-target muscle was muscle 14 (11/14 preparations).

The identity and number of non-target muscles contacted differed between embryos, as did the pattern of intramuscular arborisations on non-target muscles (e.g. compare muscle 15 in Fig. 4B and 4C). In one preparation, the RP3 axon crossed the anterior segmental boundary.

Ablation of muscle 7

In the absence of muscle 7, less than 50% (4/9) of the RP3 axons contacted muscle 6. Axonal contact with muscle 6, when it occurred, was minimal and was generally confined to the proximal edge of the muscle

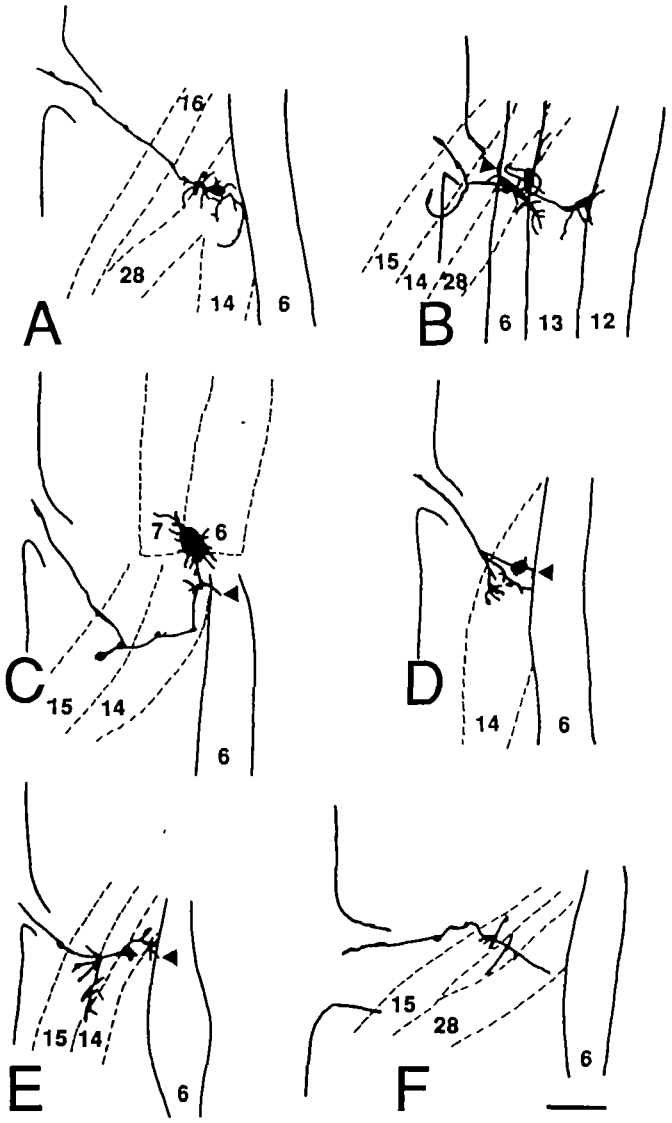

Fig. 5. Camera lucida drawings of RP3 axons in segments where muscle 7 had been removed. In A and F, the axon failed to contact muscle 6. In C the axon has crossed the anterior segmental boundary and terminated on muscles 6 and 7 in the next segment. Arrowheads indicate the axonal arborisations that contact muscle 6. Scale bar=10 μm.

(Fig. 5B,C,D,E). Arborisations over non-target muscles varied in both the number and the region of the muscle contacted.

Although the RP3 axon contacted muscle 6 in four preparations, the axon still retained processes on several non-target muscles in the ventral muscle group (Table 1). The most frequently contacted non-target muscles were muscles 28 and 14.

In one preparation, an RP3 axon had extended a process across the anterior segment boundary, and this terminated along the adjoining edges of muscles 6 and 7 in the next segment (Fig. 5C). In four preparations, the RP3 axons extended distally beyond muscle 6, and contacted muscle 13 (Fig. 5B), and in two preparations, muscle 12 (Fig. 5B).

Discussion

In the present study, the target muscles of the RP3 motoneuron were ablated prior to the arrival of the motor axon. Examination of the behaviour of target-deprived RP3 axons provides insights into the role of target muscles in (a) directing axon growth into the periphery; (b) the establishment of specific connections with the target muscles, including the retraction of processes contacting non-target muscles and; (c) the formation of a stereotypic intramuscular arborisation pattern.

Axon growth into the periphery

During normal development, the RP3 motor axon, once in the periphery, diverges from the segmental nerve onto the external surfaces of the ventral oblique muscles 16 and 15 (Johansen *et al.* 1989*a*; Sink and Whitington, 1991*b*). In the absence of both target muscles, the RP3 motor axon still diverged from the segmental nerve in this region, indicating that the axon is not guided from the nerve by chemotropic cues diffusing from the target muscle.

In a similar experiment in the locust embryo, ablation of the 133a muscle pioneer (MP) cell prior to the arrival of the D_f motor axon, resulted in the D_f axon failing to branch from the main leg nerve (Ball *et al.* 1985). While the response of this motoneuron to ablation of its target is different to that seen for the RP3 neuron in *Drosophila*, it does not necessarily argue for the existence of diffusible chemotropic cues from the target MP cell, since the D_f axon is within filopodial reach of the MP cell before it leaves the main leg nerve.

Establishment of specific connections with the target muscles and retraction of inappropriate branches

During normal development, motor axons in both locust and *Drosophila* embryos send processes in aberrant directions (Myers *et al.* 1990; Sink and Whitington, 1991*b*), pointing to the importance of selective axon retraction in generating specific neuromuscular connections. At least two different mechanisms could underlie this process. Under the first mechanism, motor axon contact with the target muscle causes a withdrawal of branches of that same neuron which are in contact with non-target muscles. According to the second mechanism, withdrawal of a branch on a non-target muscle is a result of a competitive interaction between that branch and axonal branches arising from the appropriate motoneuron for that muscle. If only the first mechanism is in operation, a motoneuron should fail to withdraw branches to inappropriate muscles if prevented from contacting its target muscle.

In the presence of both its target muscles, the *Drosophila* RP3 motor axon attains a stereotypic axon arborisation pattern by the middle of embryonic stage 16 (Sink and Whitington, 1991*a*,*b*). At the end of the culturing period, the embryo appears to be well into stage 16, as judged by the morphology of RP axons in unoperated segments. When both target muscles are

ablated before the RP3 motor axon has contacted these muscles, RP3 arborised over inappropriate muscles. The processes in contact with non-target muscles were in most cases judged to be axonal branches, rather than filopodia, since they were of non-uniform thickness and branched. Our results, therefore, suggest that contact by RP3 with its target muscles during normal development plays a decisive role in the retraction of processes extended by this neuron onto inappropriate muscles. Muscle ablation experiments on leech embryos (Baptista and Macagno, 1988; Loer and Kristan, 1989), also support the idea that contact with the target muscle is necessary for inappropriate process retraction.

When both of its target muscles were removed, the set of non-target muscles in the ventral muscle group over which RP3 arborised varied between individual operated embryos. Target-deprived motor axons in the flesh fly (Nässel *et al.* 1986), locust embryo (Whitington and Seifert, 1984), and leech embryo (Baptista and Macagno, 1988; Loer and Kristan, 1989), also have variable axonal arborisation patterns over non-target muscles. This suggests that invertebrate motor axons do not have a rigid hierarchy of choices for particular non-target muscles when target muscles are ablated. The variability in the pattern of non-target muscles contacted both in muscle-ablated and normal embryos may result from a random extension and initial adhesion by filopodia from the motoneuron growth cone.

In the presence of only one target muscle, either 6 or 7, the RP3 axon still retained processes on non-target muscles. This suggests that contact with both target muscles is necessary to cause retraction of all inappropriate processes. It is also possible that contact with a single muscle is sufficient for withdrawal of inappropriate branches but that development of axons deprived of one of their target muscles is delayed. Indeed, it has been shown recently for the embryonic leech S interneuron (McGlade-McCulloh and Muller, 1989), that there is a delay between contact with the axon target and the cessation of axon extension. Alternatively, one muscle may provide an insufficient number of synaptic contacts for the RP3 axon, which in turn extends onto other, non-target muscles in the region.

Certain RP3 projections which were occasionally observed in muscle ablated embryos (e.g. to muscles 8 and 26 and across the anterior segmental border), have not been seen at any stage of normal development (Johansen *et al.* 1989*a*; Sink and Whitington, 1991*b*). This shows, firstly, that the segmental border does not act as a barrier to motor axon growth, a finding made in earlier studies in the locust embryo (Whitington and Seifert, 1984). Second, it appears that contact with the target muscle normally causes the axon to stop growing and hence restrict axon growth to one segment and to a particular territory in that segment.

Although the target-deprived RP3 motor axon occasionally extended branches over the anterior segmental boundary, branches were never observed distal to muscle 12. Adhesive substrata have been identified as axon guiding mechanisms *in vitro* (Letourneau, 1975; Bonhoeffer and Huf, 1982; Hammarback *et al.* 1988) and *in vivo* (Berlot and Goodman, 1983; Caudy and Bentley, 1986). In the *Drosophila* embryo, an adhesive substratum that permits RP3 axon extension may be confined to this region of musculature. Alternatively, a substance may be present on the membranes distal to muscle 12 which inhibits advancement of the RP3 growth cone. Axon inhibition has been demonstrated *in vitro* for chick temporal retinal axons. These axons grow on membranes from the anterior tectum (Walter *et al.* 1987*a*), but actively avoid growing on membranes from the posterior tectum (Walter *et al.* 1987*b*).

In the absence of muscle 6, the RP3 motor axon frequently contacted muscle 7 (78%), whereas far fewer RP3 axons contacted muscle 6 after muscle 7 had been removed (<50%). A possible explanation of this result is that contact with muscles proximal to muscle 7 (e.g. muscles 16, 15 and 14) is generally sufficient to guide the axon of RP3 to that muscle, but that muscle 7 is required to guide the axon as far as muscle 6. Alternatively, muscle 7 may interact directly with muscle 6 to prepare it for innervation by RP3. In the absence of this interaction, RP3 may not be able to reliably contact or maintain an arborisation on muscle 6.

Intramuscular axonal branching

In normal embryos, RP3 forms a stereotypic arborisation along the distal and proximal faces of muscles 7 and 6, respectively. The arborisations present along muscle 7 in the absence of muscle 6 were variable in extent, but tended to be confined to the distal edge of the muscle, as in normal development. In addition, when RP3 made contact with muscle 6 in the absence of muscle 7, processes were confined to the proximal edge of the muscle 6, although the extent of contact never approached that seen in normal development. These observations speak against the hypothesis that the localisation of RP3's arborisation along the adjoining faces of muscles 6 and 7 depends upon maintained contact between those muscles.

We wish to thank G. Barrett and U. Munro for comments on earlier versions of the manuscript. This work has been supported by an Australian Postgraduate Research Award and a U.N.E. Internal Research Grant (H.S.) and an Australian Research Council Grant (P.M.W.).

References

Ball, E. E., Ho, R. K. and Goodman, C. S. (1985). Development of neuromuscular specificity in the grasshopper embryo: Guidance of motoneuron growth cones by muscle pioneers. *J. Neurosci.* **5**, 1808–1819.

Baptista, C. A. and Macagno, E. R. (1988). Modulation of the pattern of axonal projections of a leech motor neuron by ablation or transplantation of its target. *Neuron* **1**, 949–962.

Bastiani, M. J., du Lac, S. and Goodman, C. S. (1986). Guidance of neuronal growth cones in the grasshopper embryo. I. Recognition of a specific axonal pathway by the pCC neuron. *J. Neurosci.* **6**, 3518–3531.

Bastiani, M. J. and Goodman, C. S. (1986). Guidance of neuronal growth cones in the grasshopper embryo. III. Recognition of specific glial pathways. *J. Neurosci.* **6**, 3542–3551.

BATE, M. (1976). Pioneer neurones in an insect embryo. *Nature* **260**, 54–57.

BENTLEY, D. AND CAUDY, M. (1983). Pioneer axons lose directed growth after the selective killing of guidepost cells. *Nature* **304**, 62–65.

BENTLEY, D. AND KESHISHIAN, H. (1982). Pathfinding by peripheral pioneer neurons in grasshoppers. *Science* **218**, 1082–1088.

BERLOT, J. AND GOODMAN, C. S. (1984). Guidance of peripheral pioneer neurons in the grasshopper: an adhesive hierarchy of epithelial and neuronal surfaces. *Science* **223**, 293–295.

BONHOEFFER, F. AND HUF, J. (1982). *In vitro* experiments on axon guidance demonstrating an antero-posterior gradient on the tectum. *EMBO J.* **1**, 427–431.

BUDNIK, V., ZHONG, Y. AND WU, C. F. (1990). Morphological plasticity of motor axons in *Drosophila* mutants with altered excitability. *J. Neurosci.* **10**, 3754–3768.

CAMPOS-ORTEGA, J. A. AND HARTENSTEIN, V. (1985). *The Embryonic Development of* Drosophila melanogaster. Berlin: Springer-Verlag.

CAUDY, M. AND BENTLEY, D. (1986). Pioneer growth cone steering along a series of neuronal and non-neuronal cues of different affinities. *J. Neurosci.* **6**, 1781–1795.

DULAC, S., BASTIANI, M. J. AND GOODMAN, C. S. (1986). Guidance of neuronal growth cones in the grasshopper embryo. II. Recognition of a specific axonal pathway by the aCC neuron. *J. Neurosci.* **6**, 3532–3541.

GOODMAN, C. S., BASTIANI, M. J., DOE, C. Q., DU LAC, S., HELFAND, S. L., KUWADA, J. Y. AND THOMAS, J. B. (1984). Cell recognition during neuronal development. *Science* **225**, 1271–1279.

HAMMARBACK, J. A., MCCARTHY, J. B., PALM, S. L., FURCHT, L. T. AND LETOURNEAU, P. C. (1988). Growth cone guidance by substrate-bound laminin pathways is correlated with neuron-to-pathway adhesivity. *Devl Biol.* **126**, 29–39.

HARTENSTEIN, V. (1988). Development of *Drosophila* larval sensory organs: spatio-temporal pattern of sensory neurones, peripheral axonal pathways and sensilla differentiation. *Development* **102**, 869–886.

JACOBS, J. R. AND GOODMAN, C. S. (1989). Embryonic development of axon pathways in the *Drosophila* CNS. II. Behavior of pioneer growth cones. *J. Neurosci.* **9**, 2412–2422.

JOHANSEN, J., HALPERN, M. E., JOHANSEN, K. M. AND KESHISHIAN, H. (1989*b*). Stereotypic morphology of glutamatergic synapses on identified muscle cells of *Drosophila* larvae. *J. Neurosci.* **9**, 710–725.

JOHANSEN, J., HALPERN, M. E. AND KESHISHIAN, H. (1989*a*). Axonal guidance and the development of muscle fiber-specific innervation in *Drosophila* embryos. *J. Neurosci.* **9**, 4318–4332.

LETOURNEAU, P. C. (1975). Cell-to-substratum adhesion and guidance of axonal elongation. *Devl Biol.* **44**, 92–101.

LOER, C. M. AND KIRSTAN, W. B. (1989). Peripheral target choice by homologous neurons during embryogenesis of the medicinal leech. I. Segment-specific preferences of Retzius cells. *J. Neurosci.* **9**, 513–527.

MCGLADE-MCCULLOH, E. AND MULLER, K. J. (1989). Developing axons continue to grow at their tip after synapsing with their appropriate target. *Neuron* **2**, 1063–1068.

MEIER, T. AND REICHERT, H. (1990). Embryonic development and evolutionary origin of the Orthopteran auditory organs. *J. Neurobiol.* **21**, 592–610.

MYERS, C. M., WHITINGTON, P. M. AND BALL, E. E. (1990). Embryonic development of the innervation of the locust extensor tibiae muscle by identified neurons: formation and elimination of inappropriate axon branches. *Devl Biol.* **137**, 194–206.

NÄSSEL, D., HELGEE, A. AND SIVASUBRAMANIAN, P. (1986). Development of axon paths of motorneurons after removal of target muscles in a holometabolous insect. *Dev. Brain Res.* **26**, 211–219.

RAPER, J. A., BASTIANI, M. J. AND GOODMAN, C. S. (1983*a*). Pathfinding by neuronal growth cones in grasshopper embryos. I. Divergent choices made by the growth cones of sibling neurons. *J. Neurosci.* **3**, 20–30.

RAPER, J. A., BASTIANI, M. J. AND GOODMAN, C. S. (1983*b*). Pathfinding by neuronal growth cones in grasshopper embryos. II. Selective fasciculation onto specific axonal pathways. *J. Neurosci.* **6**, 31–41.

RAPER, J. A., BASTIANI, M. J. AND GOODMAN, C. S. (1983*c*). Guidance of neuronal growth cones: Selective fasciculation in the grasshopper embryo. *Cold Spring Harbor Symp. quant. Biol.* **48**, 587–598.

SHIELDS, G. AND SANG, J. H. (1970). Characteristics of five cell types appearing during *in vitro* culture of embryonic material from *Drosophila melanogaster*. *J. Embryol. exp. Morph.* **23**, 53–69.

SINK, H. AND WHITINGTON, P. M. (1991*a*). Location and connectivity of abdominal motoneurons in the embryo and larva of *Drosophila melanogaster*. *J. Neurobiol.* **22**, 298–311.

SINK, H. AND WHITINGTON, P. M. (1991*b*). Pathfinding in the central nervous system and periphery by identified embryonic *Drosophila* motor axons. *Development* **112**, 307–316.

THOMAS, J. B. AND CREWS, S. T. (1990). Molecular genetics of neuronal development in the *Drosophila* embryo. *Fedn Am. Soc. exp. Biol.* **4**, 2476–2482.

WHITINGTON, P. M. (1989). The early development of motor axon pathways in the locust embryo: the establishment of the segmental nerves in the thoracic ganglia. *Development* **105**, 715–721.

WHITINGTON, P. M., BATE, M., SEIFERT, E., RIDGE, K. AND GOODMAN, C. S. (1982). Survival and differentiation of identified embryonic neurons in the absence of their target muscles. *Science* **215**, 973–975.

WHITINGTON, P. M. AND SEIFERT, E. (1982). Axon growth from limb motoneurons in the locust embryo: the effect of target limb removal on the path taken out of the central nervous system. *Devl Biol.* **93**, 206–215.

WHITINGTON, P. M. AND SEIFERT, E. (1984). Axon growth from limb motoneurons in the locust embryo: the effect of target limb removal on the pattern of axon branching in the periphery. *Devl Biol.* **106**, 438–449.

(*Accepted 11 July 1991*)