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The behaviour of growing axons invading developing chick wing buds with dorsoventral or anteroposterior axis reversed

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SUMMARY

The trajectories of motor axons innervating chick wings reversed about the DV or AP axis before axon invasion were analysed after retrograde filling by HRP injection into biceps or triceps muscles. Chick–quail chimaeras showed that the plane of reversal for flank operations was proximal to the confluence of the 14th, 15th, and 16th spinal roots as they form the plexus. The shoulder reversal plane was distal to the plexus.

In dorsoventral (DV) reversed wings at both shoulder or flank level, the motor axons do not alter their course as they enter the graft. They therefore innervate by passive deployment any target that they encounter. In anteroposterior (AP) reversed wings at both shoulder or flank level, the motor axons clearly corrected their position in the nerve tract after entering the graft and innervated appropriate targets. The innervation of appropriate targets in AP shoulder reversals shows that axons are sensitive to AP mismatch distal to the plexus. Since axons were displaced similar distances from their normal routes in flank DV and AP reversals, the difference in behaviour suggests that they respond to mismatch in the AP but not the DV axis.

INTRODUCTION

Axons normally connect with their targets in a predictable and spatially ordered way. One way of testing whether active guidance mechanisms are involved is to experimentally alter the topographic relationship between neurone and target before the connections are made. For example, when the early limb bud is rotated so that both dorsoventral (DV) and anteroposterior (AP) axes are reversed, motor axons show no evidence of pathway or target selection. Motor pools that normally innervate dorsal muscles instead innervate ventral muscles in the reversed wing and *vice versa* (Summerbell & Stirling, 1981). The same result is obtained after reversal of the DV axis alone. However, the situation is different after reversal of the AP axis of the limb. In the majority of such cases axons are able to reach their normal targets.

Following AP or DV reversals invading axons are displaced from their normal pathways by very different distances (Stirling & Summerbell, 1983). The aim of

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these experiments was to discover whether the failure of axons to find their appropriate targets in DV reversed wings is related to distance displaced from local guidance cues. This paper describes the routes taken by axons labelled retrogradely (after injection of selected upper arm muscles) with HRP into limbs following various reversals at different levels along the proximodistal (PD) axis. The results show that axons still fail to innervate appropriate targets even in proximal DV reversals where axons first encounter the reversed tissue at the plexus. We conclude that the difference in behaviour following AP or DV mismatch is due to a difference in the organization of the two axes. A similar conclusion has been reached by Bonhoeffer working on the retinotectal system (personal communication).

METHODS

Fertilized chick eggs from a mixed local flock (Needle Farm) were incubated to day 3 or 4. Pairs of eggs with embryos of similar ages (±1 stage, Hamburger & Hamilton, 1951) were selected. The wing buds from opposite sides of the two embryos were exchanged to produce reversal of either the dorsoventral axis (DV rev) or the anteroposterior axis (AP rev) as shown in Fig. 1. The incision was made using electrolytically sharpened tungsten needles. Polarity of the excised limb bud was preserved by inserting a 25 μm platinum pin at the anterior margin. Buds were held in place using this pin and a second pin inserted posteriorly. Shoulder reversals (aimed at a plane passing through the shoulder joint and distal to the plexus) were performed at stage 18–20. Flank reversals (aimed at a level proximal to the plexus) were performed at stage 16–18 and involved all the mesenchyme lateral to the somites (Fig. 1). For AP reversals it is usually necessary to remove the posterior limb organizer or zone of polarizing activity from the host stump to prevent mirror-image reduplication of the graft (Saunders, Gasseling & Gfeller, 1958; Amprino & Camosso, 1959). Only those limbs which showed no signs of reduplication were used.

HRP labelling (stage 33–35)

Six or seven days after the operation the embryos were bled, eviscerated and the ventral brachial spinal cord exposed. The triceps or biceps muscle was injected (see Summerbell & Stirling, 1981) with 20% w/v HRP in 5% aqueous Nonidet P90. Transport of HRP was allowed for 5–6 h in oxygenated Tyrodes solution at 30–35°C. The spinal cords were then fixed and embedded in albumen gelatin and transverse 60 μm frozen sections were stained using the benzidine–dihydrochloride blue reaction of Mesulam (1976).

Tracing axons in wings

Wings were fixed in extended (crucified) position, embedded in albumen gelatin, and were sectioned in a plane transverse to the humerus at 100 μm using a vibroslice (Campden Instruments). The sections were mounted on double gelatinized slides and air dried. They were reacted either with the cobalt-enhanced diaminobenzidine method (Adams, 1977) or using catechol and phenyldiamine (Perry, Henderson & Linden, 1983).

Reconstructions of the labelled axons were made from camera-lucida drawings of the sectioned material with the aid of a digitizing graphics pad and a visualization program developed by J. Green (Shepherd, Perkins, Green & Clark, 1984). The program allows the operator first to align the sections and then to view the result from different angles in stereo. This makes analysis of the paths taken by the axons much easier to interpret.

Additional methods

The trajectories of axons from individual nerve roots were followed in normal limbs after localized injections of HRP into the spinal cord or after filling selected roots with nickel chloride and staining with rubeanic acid. The latter method shows the distribution of a group of filled axons in whole cleared limbs (blue against clear background).

In some cases the cord was processed to demonstrate the labelled motor pools while the limbs were stained with a modified Bodian method (Lewis, Chevallier, Kieny & Wolpert, 1982) after HRP transport to show the pattern of nerves. It was necessary to make Bodian whole-mount preparations of some operated animals earlier than was suitable for HRP labelling so as to reveal details of plexus anatomy.

Position of graft/host junction

It was important to check that we had not cut axons in the flank grafts and also the position of the plane of reversal. Embryos were fixed immediately or 6 h after operating. They were

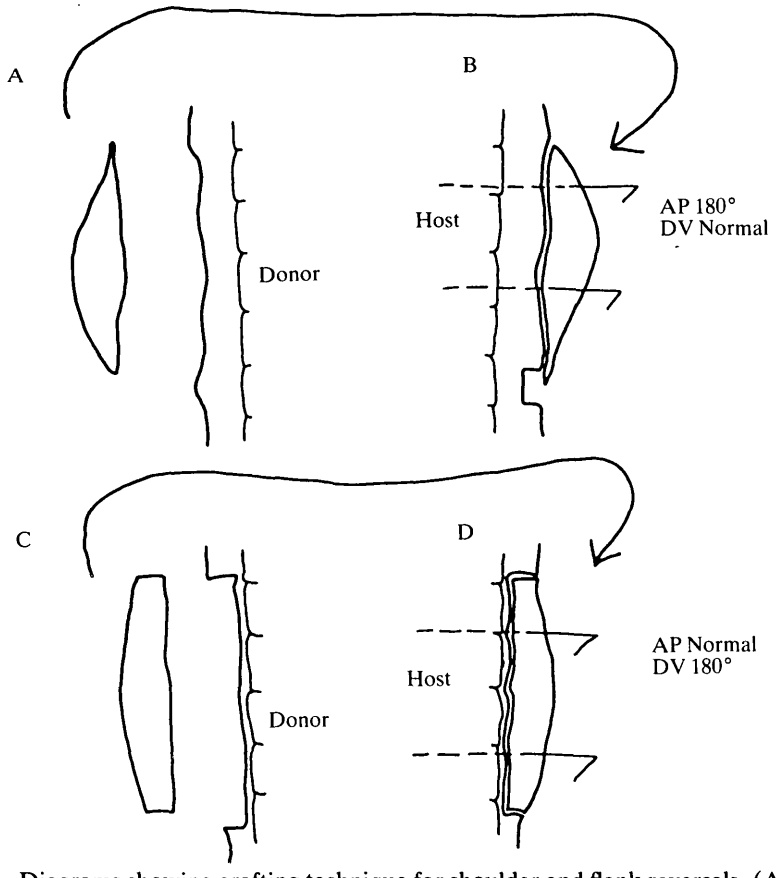

Fig. 1. Diagrams showing grafting technique for shoulder and flank reversals. (A) For shoulder reversals the left limb bud is removed from the donor at stage 19, it is then grafted onto the right side of the host (B) at the same stage with, for example, the AP axis reversed. The host ZPA had been previously removed. (C) For flank reversals, the left limb bud is removed from donor at stage 17, cutting as close to the myotomes as possible. It is then grafted (D), onto the right side of a similarly staged host with, for example, the DV axis reversed.

sectioned transversely and silver stained (Rager, Lausmann & Gallyas, 1979) to show the extent of axon invasion.

The position of the reversed flank grafts relative to the plexus was determined by injecting Indian ink at a level equivalent to the amputation plane, the embryo was then fixed at stage 26–27 and sectioned in the plane of the plexus (horizontal). In other cases quail embryos were used as donors. The chimaeric embryos were sectioned similarly then stained with Feulgen to show the nucleolar marker (Le Douarin, 1973).

RESULTS

Level of reversal in relation to axon outgrowth

At stage 18–20 axons can be seen in silver-stained preparations just below the myotome and proximal to the lateral plate mesenchyme and the base of the limb (Fig. 2A). The removal of the limb bud at this stage for shoulder reversals does not appear to cut axons, nor do the axons reach the cut surface before healing has taken place (within hours of the operation). The flank reversals were performed at stage 16–18 when axons can be seen contacting the medial part of the myotomes, here the amputation plane is quite close to the growing axon tips (Fig. 2B) (see also Hollyday, 1983, fig. 2).

The Indian ink markers and the chick–quail grafts demonstrated that the level of amputation in shoulder-reversed animals was distal to the plexus (Fig. 2C). The plane in flank reversals passed through a level proximal to the joining of roots to form the plexus (Fig. 2D).

Reconstruction of axon trajectories in normal animals

All three labelling methods (retro HRP, ortho HRP, nickel chloride) showed that groups of axons travel together to common targets. Orthograde labelling from cord or segmental nerve roots and also cleared whole-mount Bodian preparations show very little evidence for nerve crossing or sorting, instead axons maintain their relative positions in the nerves. Thus axons from root 14 supply the proximal

Fig. 2. (A) Transverse section of an embryo stained with the Rager silver method, showing the position of brachial axons 6 h following amputation of limb bud for shoulder reversal at stage 19. The leading edge of the axons can be seen (arrowed) beneath the somite (*so*). Calibration bar equals 100 μm. (B) A transverse section of a stage-17/-18 embryo stained as for 2A, showing the position of axons 6 h after amputation for flank reversal. The stained axon tips can be seen proximal to the end of the somite (*so*) some distance from the amputation plane. Calibration bar as in A. (C & D) Horizontal sections (distal to right) of chick host embryos with quail donor limbs after Feulgen staining to distinguish host from graft (quail cells have dark-staining nucleoli): border between chick host and quail graft, heavy dashed line; plexus outlined with fine dashed line; stars mark equivalent positions at confluence of nerve roots. (C) Quail donor limb grafted at shoulder level with no change of axes, the quail tissue is clearly distal to the confluence of R14, R15 and R16. (D) Quail donor limb grafted at flank level, here the quail tissue can be seen proximal to the confluence of R14, R15 and R16. Calibration bar equals 100 μm.

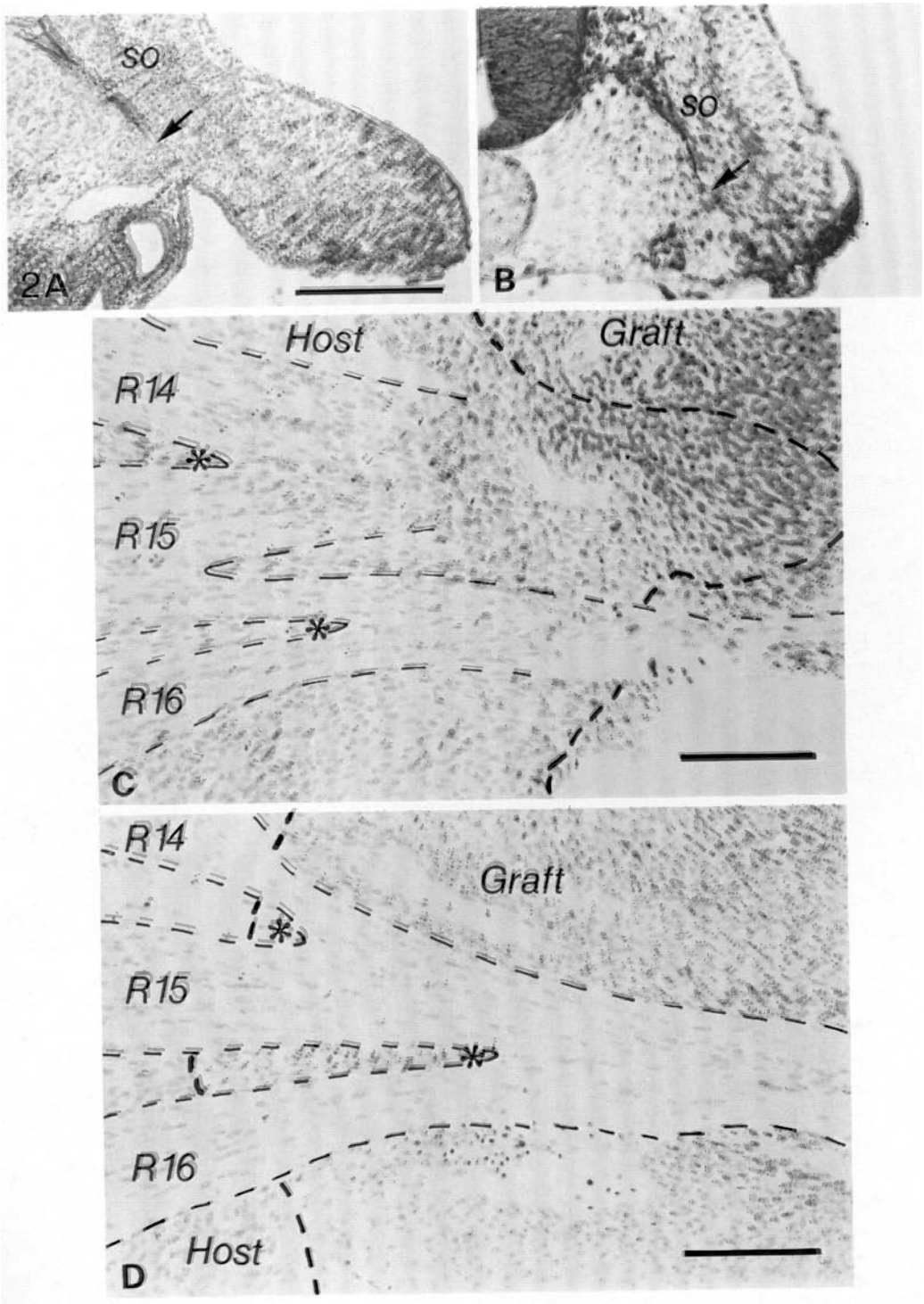

Fig. 2.

shoulder muscles and in the limb are found in the biceps nerve with a small contribution to the anterior radial (see Stirling & Summerbell, 1979), while the 16th nerve supplies posterior shoulder muscles and dorsal triceps and distal muscles of the limb. The pattern seen from the orthograde labelling is closely related to the position of entry of the axons in the plexus and the arrangement of branch points beyond it. The plexus structure is much simpler early in development (stage 26–28). Horizontal sections show that the main ventral supply to the anterior proximal wing clearly contains a larger contribution from rostral segments while the 15th and 16th roots innervate the postaxial and distal regions of the wing.

Typical trajectories of axons to biceps and triceps are shown on the unoperated sides in Figs 3, 6, 8. Axons to the biceps muscle from their rostral–medial motor pool (Fig. 3A) enter the limb occupying ventral sectors of the 14th and 15th segmental nerve roots (Fig. 3B). At the plexus they collect together and leave just distal to the division into dorsal (radial) and ventral (medioulnar) nerves, occupying the ventroanterior sector of the latter. The biceps motor nerve leaves the medioulnar from a ventral position (Fig. 3C). In a similar fashion axons to triceps muscle from their caudal–lateral motorpool (Fig. 8A,B,C) collect together at the plexus occupying a position (dorsoposterior) in the radial nerve in line with the position of exit of the triceps muscle nerve. The complete reconstructions of trajectories for normal biceps and triceps axons are shown for unoperated sides in Figs 4, 5, 7, 9. Yet again there is no evidence for gross rearrangement of axons to reach target muscles.

Dorsoventrally reversed limbs at shoulder level

Of twelve shoulder-reversed animals injected and processed for HRP histochemistry, eight clearly had inappropriately located motor pools with no evidence of axon correction, one had symmetrical pools but inadequate axon labelling and three had insufficient label to localize pools or analyse axon trajectories in the limbs.

Fig. 3. Position of cells and their axons on the unoperated (to the left, A,B,C) and operated sides (to the right, D,E,F) of an animal with DV reversed limb at shoulder level. The top pair of photographs show the position of labelled cells in the motor horn. Beneath them are transverse sections of the wings (we have orientated the photographs so that the host body axes of the two sides are the same: dorsal up and rostral [anterior] to the right) showing nerves with filled axons (arrows). Calibration bar for spinal cords equals 100 μm and limbs 100 μm. (A and D) Transverse sections of spinal cord showing the position of HRP-filled motoneurones at the level of the 15th segment following injection into biceps muscle on both sides, the labelled cells (arrowed) are dorsal medial on the unoperated side and lateral on the operated side. (B and C) On the unoperated side, axons from the medial motor pool enter the plexus ventrally to reach the biceps muscle. (E and F) On the operated side, axons from the lateral pool travel dorsally in the plexus to reach the dorsoposteriorly positioned biceps muscle in the reversed limb.

The cluster of labelled motoneurones following injection of biceps or triceps muscles in the reversed wing at stage 33–35 occupies a position characteristic of the pool of the antagonist muscle, i.e. is inappropriate. This means that cells innervating the muscle in the reversed limb are mismatched in both mediolateral and rostrocaudal axes. Thus injection of biceps on the operated side labelled cells situated laterally in caudal 15th and 16th segments rather than the normal medial position in more rostral 14th and 15th segments (Fig. 3D). This result is the same

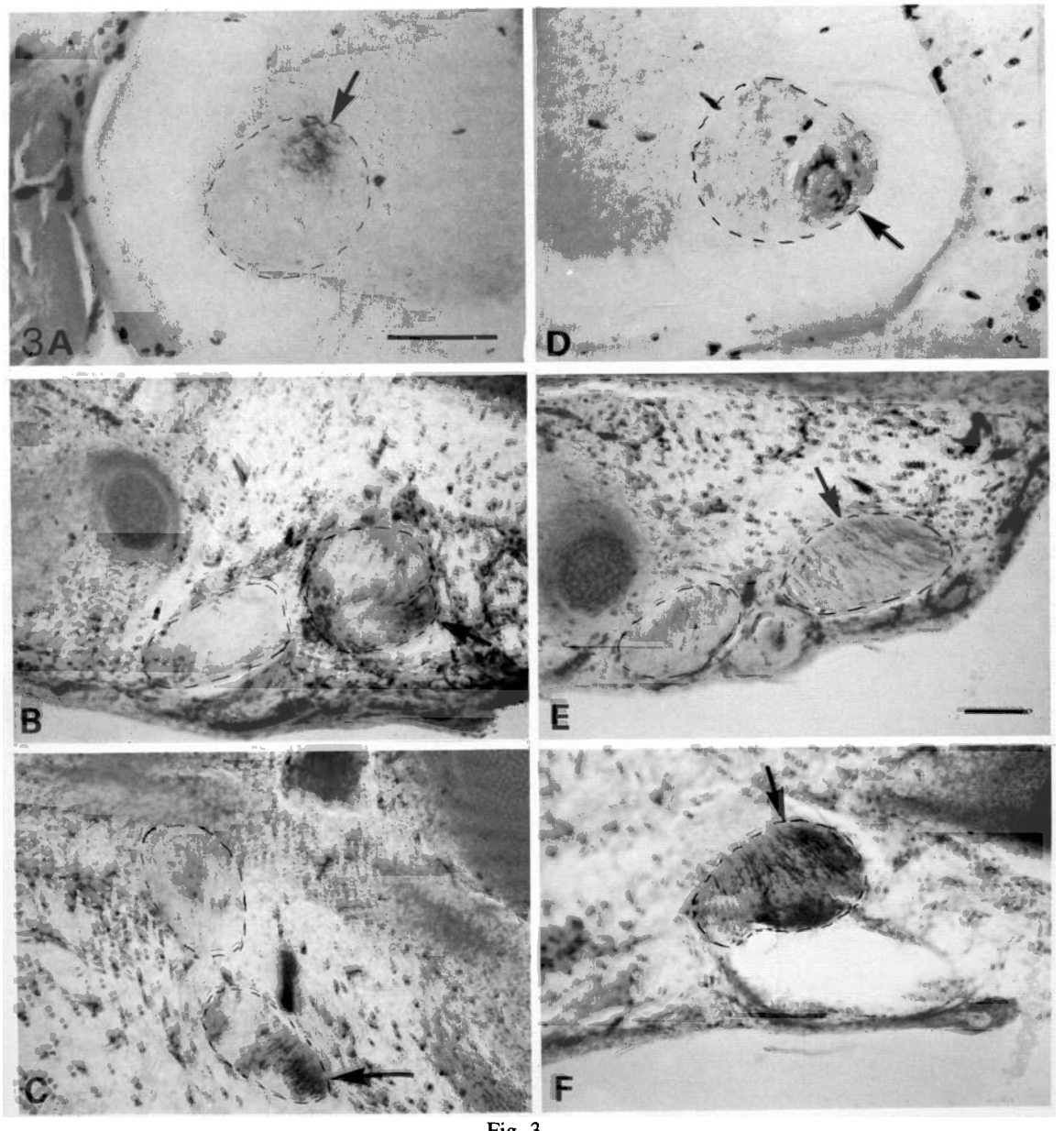

Fig. 3.

as that following 180° rotations (Stirling & Summerbell, 1979), i.e. no evidence of correction.

Fig. 3 shows axons labelled by biceps injection on operated and unoperated sides as seen in animals in which the labelled pool on the operated side was characteristic of the triceps muscle. The reconstruction in Fig. 4 shows that the axons to the biceps muscle on the operated side occupy a posterior as well as a dorsal position (relative to the host axes). During normal development biceps and triceps start as ventral and dorsal muscle masses respectively but tissue movements in the flank cause the limb to rotate so that they eventually occupy ventroanterior and dorsoposterior positions (Searls, 1983). In the operated limbs this rotation still takes place and is in the normal direction (relative to flank) so that biceps comes to lie dorsoposterior and triceps ventroanterior.

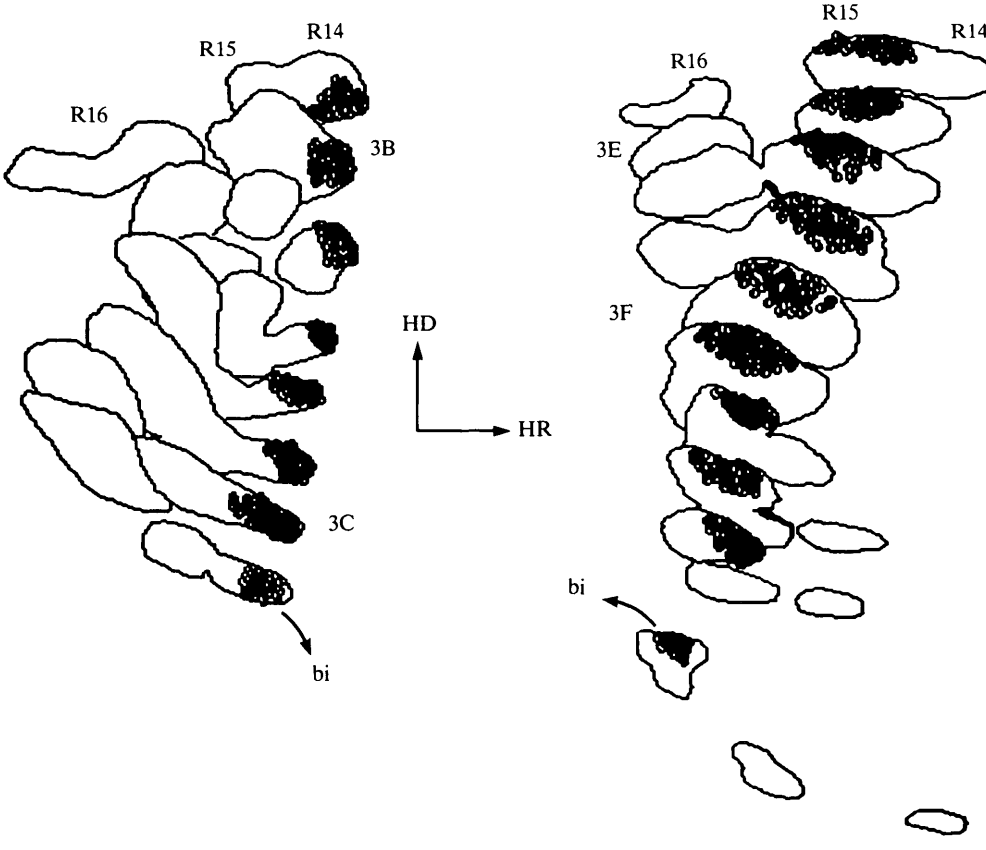

Fig. 4. Computer reconstruction from serial sections of the trajectories of filled axons (dots inside nerve perimeter) in the nerves from bilateral biceps (*bi*) injection on the unoperated and operated sides of the animal with DV shoulder reversal illustrated in Fig. 3. For all reconstructions, the unoperated side is to the left and operated side to the right of the pair, with host axes as for photographs, R14, R15 and R16 are the segmental roots. The levels at which photographs were taken for the corresponding figures are indicated. Host axes as for photographs: HD, host dorsal; HR, host rostral.

At no point is there any indication of a sudden change in position of the labelled axons as they travel from the host to the graft. A similar lack of course correction is seen after injection into ventral and anterior triceps muscles in DV reversed wings. Here the axons occupying the ventral (medioulnar) nerve in the host from medial motoneurones in segments 14 and 15 run into the radial nerve in the graft.

DV reversed limbs at flank level

When the flank reversal operations were done as described in the methods, the reversal plane was just proximal to the confluence of the three segmental nerve roots and the plexus was slightly abnormal. In all ten cases (five biceps, five triceps) axons showed no evidence of correction. Axons labelled from injection

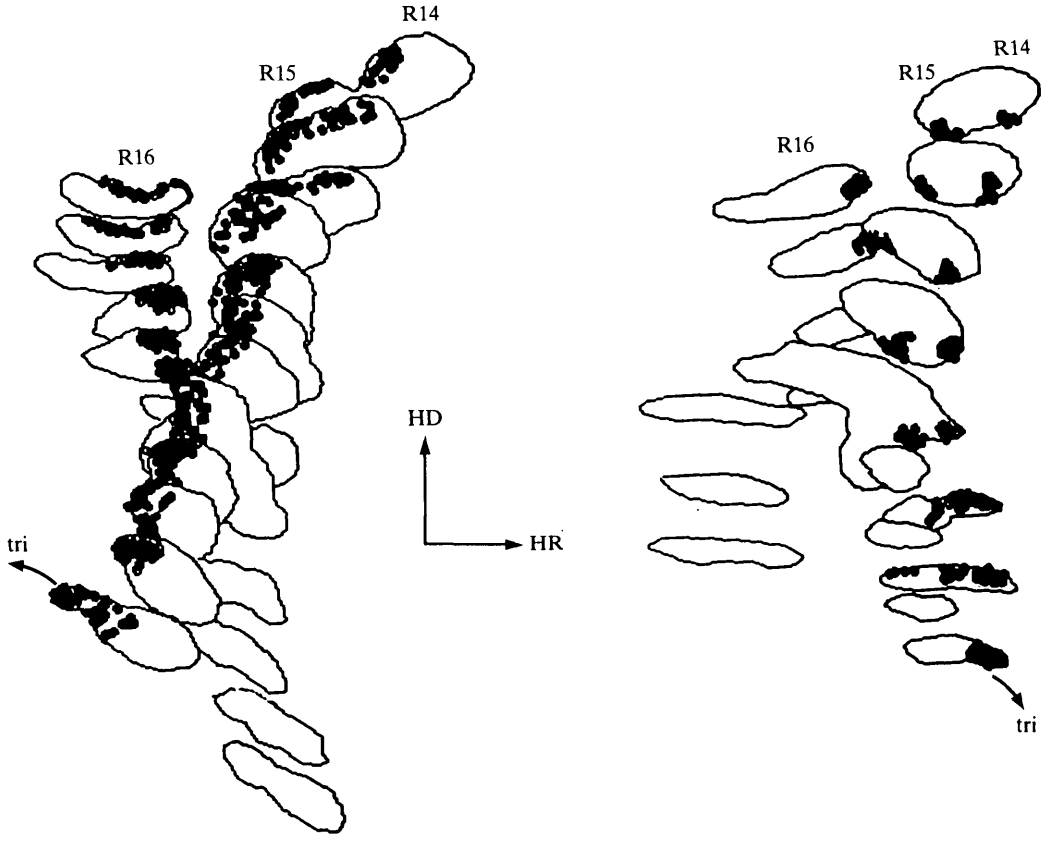

Fig. 5. Reconstruction of trajectories of filled axons from bilateral injection of triceps (*tri*) in an animal with DV flank reversal. Conventions as in Fig. 4 on the unoperated side the axons from their lateral position in the motor horn of segments 15 and 16 maintain their dorsoposterior position through the plexus to innervate the triceps muscle. On the operated side, axons from the medial motoneurone pool travel through the plexus occupying a ventroanterior position to innervate the ventroanteriorly positioned triceps muscle in the reversed limb.

into reversed triceps clearly entered the nerve roots in ventral position and labelled innappropriate medial motoneurones in the cord (Fig. 5), and *vice versa*.

Whole-mount preparation

The anatomy of the graft/host junction is clearest in whole-mount preparations. Both nickel chloride fills and Bodian whole mounts show some twisting of the plexus at the junction, but host ventral (medioulnar) nerves run straight into the 'dorsal' (radial) nerve of the graft and *vice versa*. These whole mounts also show that the triceps muscle lies ventroanteriorly relative to the host axes, while grafted biceps lies dorsoposteriorly, as had been seen in the sectioned material described above.

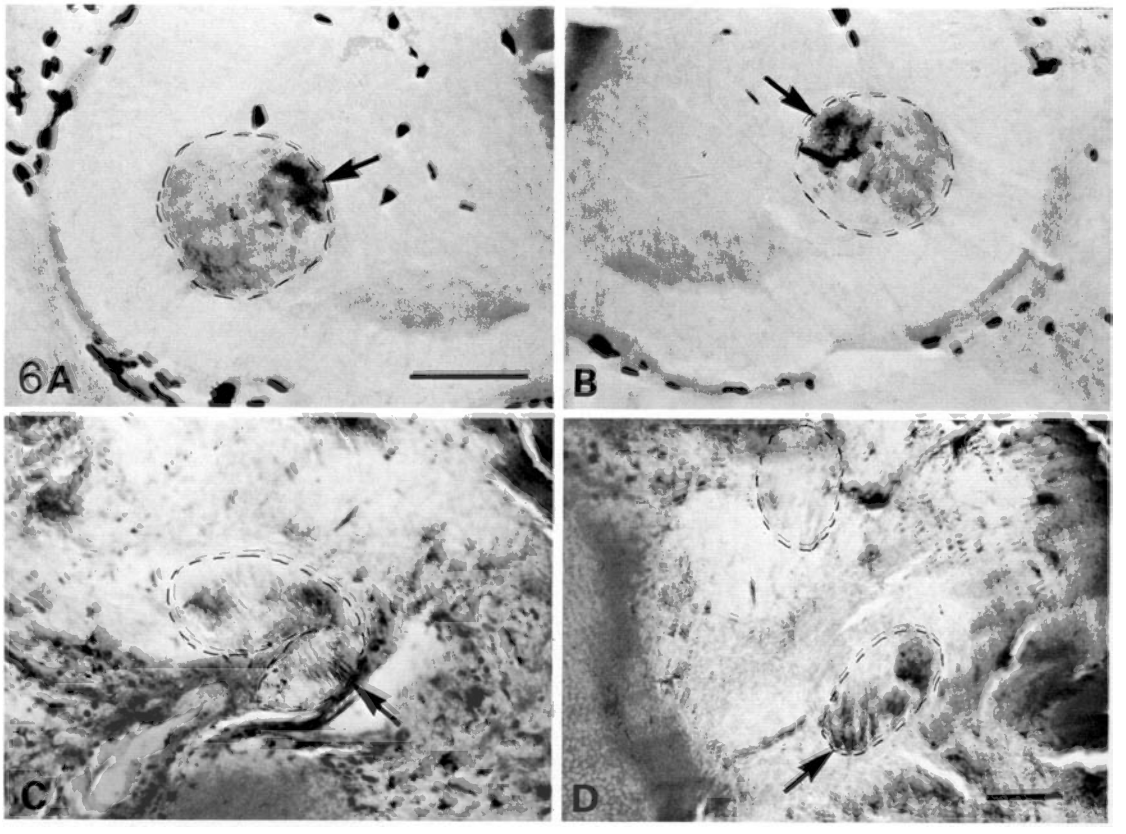

Fig. 6. The labelled cells and axons from bilateral biceps injection in an animal with AP reversed limb, conventions as in Fig. 3. (A and B) Heavily labelled cells (arrowed) occupying the same position in the medial motor horn at segment 14 on both sides, note some faintly labelled lateral cells on both unoperated and operated sides. (C) Transverse section of operated side at plexus in host, with filled axons in ventroanterior sector from rostral medial motor pool. (D) Further distal in the reversed graft the labelled axons have changed their position relative to the host axes, to reach the reversed biceps muscle.

Anteroposterior axis reversals

Reversal of the anteroposterior axis of the limb sometimes resulted in duplication despite the precautions detailed in the methods. Only limbs showing well-muscled reversed limbs without mirror-image reduplication were analysed.

AP reversed limbs at shoulder level

Of twelve such animals, eight had good evidence of correction, two showed abnormal pool labelling with no axon correction and two were not well enough labelled. Reconstruction of axon trajectories are shown in Figs 7, 9. The disruption of the nerve pattern in these reversals is clearly more devastating than

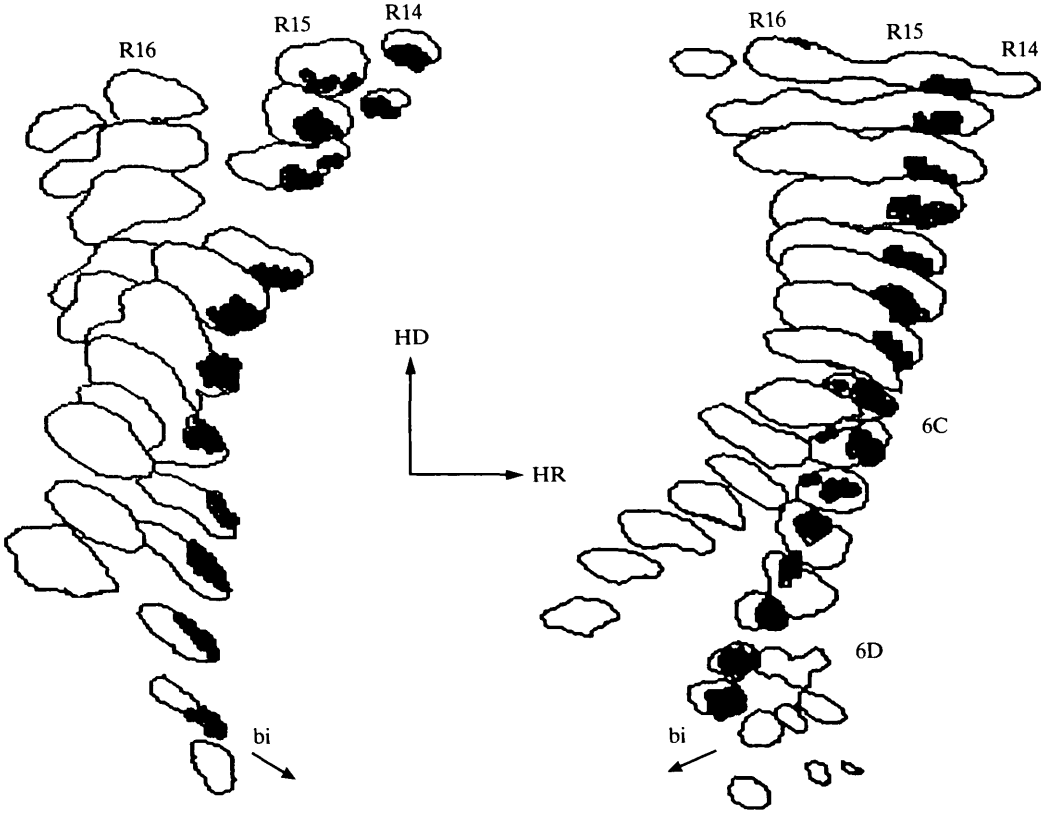

Fig. 7. Computer reconstruction of the trajectories of the filled axons from bilateral biceps injection in the animal with AP shoulder level reversal illustrated in Fig. 6. Conventions as in Fig. 4. The axons from the rostral medial pool enter the plexus in the host tissue in the ventroanterior sector, but clearly move posteriorly relative to the host axes on the operated side to reach the biceps in the reversed limb. No such movement is seen on the unoperated side, where axons in the ventroanterior sector travel straight to the ventroanterior biceps muscle.

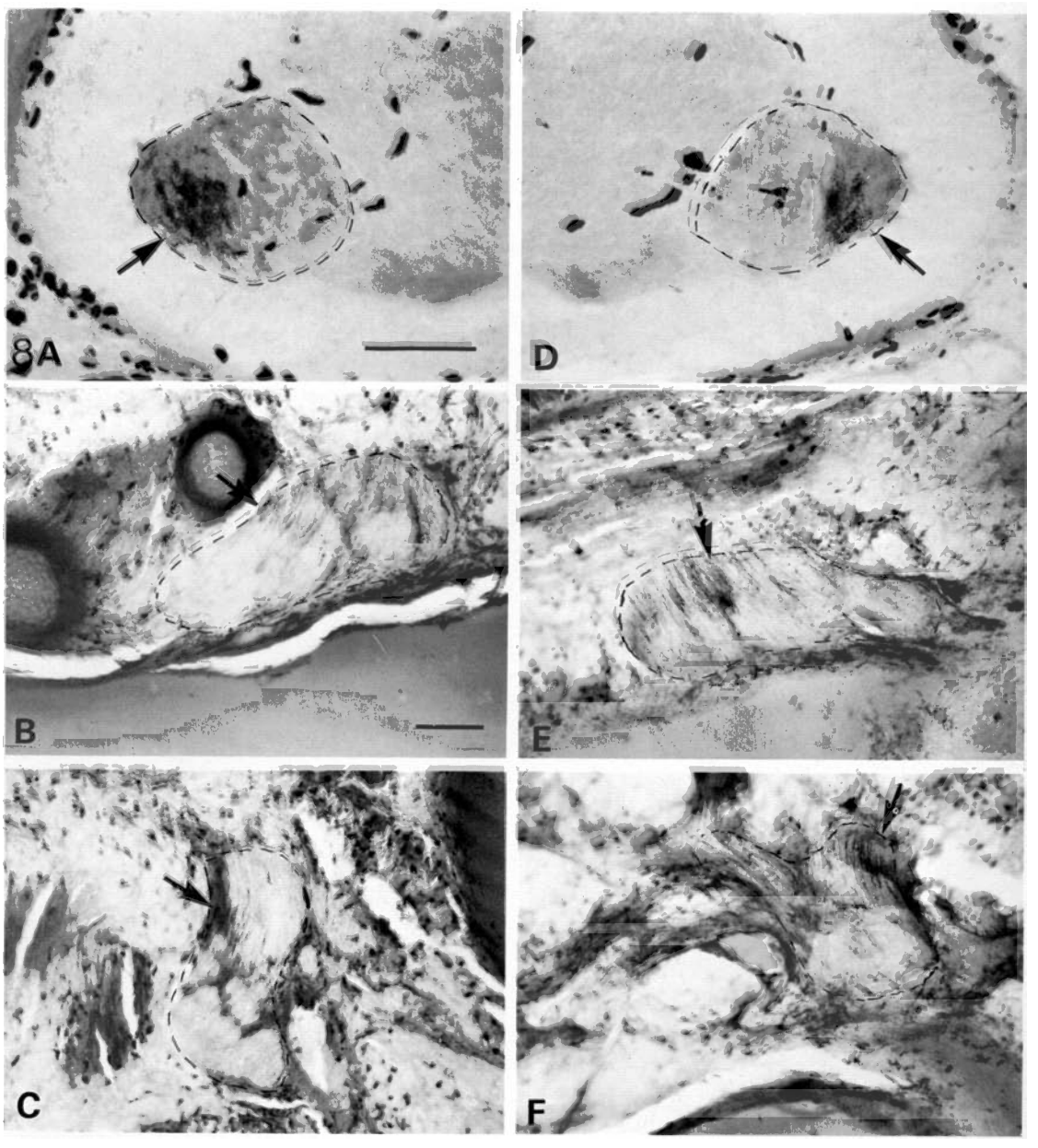

Fig. 8. Labelled motoneurones and filled axons from bilateral triceps (*tri*) injection in an animal with AP shoulder-reversed limb, conventions as for Fig. 3. (A and D) Labelled cells (arrows) occupying symmetrical lateral positions at segment 16 on the unoperated and operated sides. (B and C) Filled axons from the caudolateral pool on the unoperated side travel dorsally in the plexus to enter the triceps nerve. (E and F) On the operated side filled axons from the caudolateral pool enter the plexus as on the unoperated side but then change their position in the radial nerve to reach the anteriorly situated triceps in the reversed limb.

that following dorsoventral reversal mainly because the nerves normally enter the limb posteriorly and this entry point has been moved following AP reversal at the shoulder. One can see that despite (or perhaps because of) this disruption the labelled axons change their position relative to the host axes at the level of the plexus. Biceps axons from the appropriately located medial motor pool on the operated side (Fig. 6B) occupy the ventral anterior plexus (Fig. 6C) and then cross to innervate the posteriorly (relative to host axes) situated biceps (Fig. 6D). Triceps axons from the caudal lateral pool (Fig. 8D), occupy posterior dorsal positions proximal to the plexus (Fig. 8E) and then cross in the plexus to innervate the anteriorly (relative to host axes) situated biceps (Fig. 8F).

AP reversed limbs at flank level

The pattern of localization of motor pools and nerve trajectories in seven proximal reversals was similar to that seen in shoulder reversals. Good connection

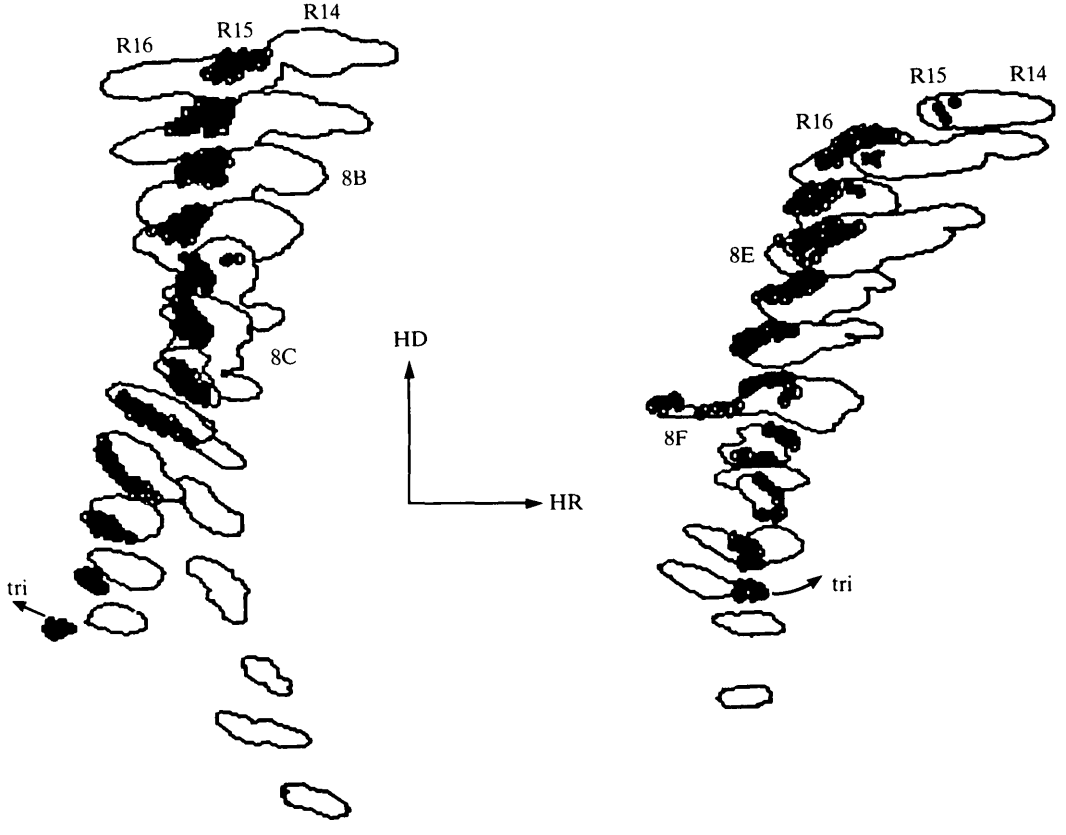

Fig. 9. Computer reconstruction of filled axon trajectories from bilateral triceps injection in the animal with AP shoulder reversal illustrated in Fig. 8. Triceps axons from lateral pools enter the plexus dorsoposteriorly but change their position in the plexus to reach the dorsoanteriorly positioned triceps in the reversed limb. Conventions as in Fig. 4.

between graft and host was more often seen after proximal reversals and all seven cases showed appropriate motor pool localization.

Whole mount preparations

The innervation of the operated wings was obviously very delayed; in normal stage-27 embryos axons were visible at the mid-humerus level, but on the operated side they were not seen beyond the plexus. At this stage in most operated limbs the biceps muscle still lay ventral, by stage 33 the limb had completed natural torsion bringing biceps to its final ventroposterior position. However, it is clear that for root 14 axons to reach the posterior biceps they would need to cross over other axons (Fig. 10) to achieve symmetrical labelling of biceps pools in segments 14 and 15.

DISCUSSION

Recently, three main hypotheses have been advanced explaining how axons from particular regions within the motor horns innervate particular target muscles in the limbs.

(A) *Active selection* (e.g. Cajal, 1910; Sperry, 1963; Lance-Jones & Landmesser, 1980; Landmesser, 1980) proposes that each motoneurone bears a specific label which matches that carried by its target in the periphery. Axons respond selectively to cues in the periphery and actively seek out their appropriate predetermined targets.

(B) *Random outgrowth and cell death* also invokes a predetermined match between neurone and target (e.g. Prestige, 1967; Lamb, 1977, 1984; Pettigrew, Lindeman & Bennet, 1979). The initial outgrowth of the axons is random so that any axon may reach and connect with any target. During the period of cataclysmic

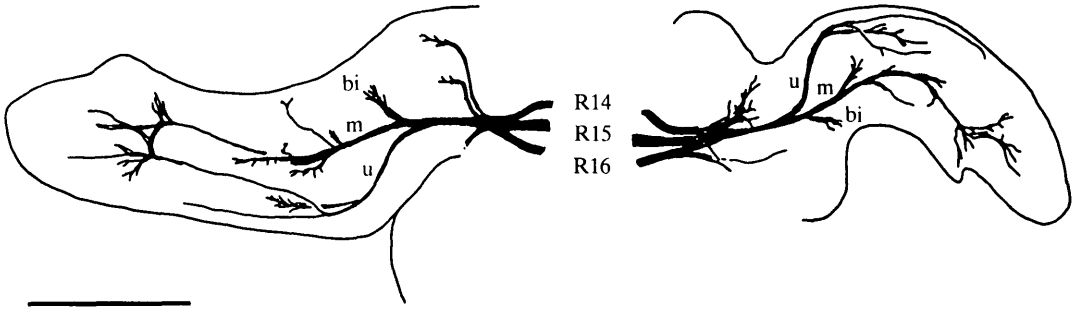

Fig. 10. Camera-lucida drawings of Bodian-stained wings of a stage-28 embryo with AP shoulder reversal on the right. Rostral host is up, the biceps nerve (*bi*) on the unoperated side clearly leaves the medioulnar nerve (*m* and *u*) rostrally and we know that it contains axons from roots R14 and R15. On the operated side the biceps nerve leaves caudally, axons from R14 and R15 must change their position in the plexus to supply the biceps muscle in the reversed wing. Calibration bar equals 1 mm.

cell death between stage 29–35, neurones that have contacted inappropriate targets die.

(C) *Passive deployment* does not feature predetermined matching between cell and target (e.g. His, 1887; Weiss, 1941; Horder, 1978; Lewis, 1978; Stirling & Summerbell, 1979, 1983; Summerbell & Stirling, 1982). Instead axons are seen as growing into the limb maintaining their neighbour relations in the bundle so that axons from neighbouring neurones tend to reach the same target in the limb, therefore showing apparent specificity.

It seems that all of these mechanisms may be involved in producing the normal pattern of innervation (Summerbell & Stirling, 1983), and these experiments were designed to see if the ability of axons to correct is related to the distance displaced from familiar pathways. The results show that axons respond differently depending on the axis of reversal of the limb bud even when the distance displaced is similar. There is little evidence for selective axon growth responses in a grafted limb reversed about the DV axis, but in the majority of cases muscles are ultimately innervated by appropriate motoneurones following reversal of the AP axis. *There must therefore be active mechanisms.* The following discussion explores how the difference might be related to the distance that axons are displaced from familiar guidance cues, or to the presence or absence of positional cues in the bud.

Familiar and foreign guidance cues

DV reversals at shoulder level are distal to the division at the plexus into dorsal (radial) and ventral (medioulnar) nerve trunks. Axons from the medial motor horn, occupying the ventral nerve 'track' in the host therefore enter the dorsal nerve 'track' in the graft. They can recognize that this is a 'track' and therefore follow it but the specific signals defining the local muscle branches are unfamiliar and they therefore passively enter nearest branches and innervate inappropriate extensor muscles by passive deployment. Following AP reversals at the shoulder, the axons from medial motor horn occupy the ventral nerve track in the host and enter the ventral nerve track in the graft and are only displaced within the confines of their appropriate nerve. Although displaced from their normal position within the track they can recognize the local cues and respond to them so as to correct their position within the track. They therefore enter appropriate branches and innervate their natural targets.

This hypothesis explains the results of operations at shoulder level for AP and DV reversals and for 180° limb rotations (both axes reversed) of wing and leg (Stirling & Summerbell, 1980; Summerbell & Stirling, 1981; Whitelaw & Hollyday, 1983). It also accounts for the spinal cord AP reversal experiments of Lance-Jones and Landmesser. They found that after reversal of three iliosacral segments some axons tended to connect to appropriate targets but that when the motoneurones were displaced greater distances all axons made inappropriate connections obeying passive deployment rules.

DV reversals proximal to the plexus should result in axons being displaced only within their roots and therefore not far from familiar cues, they should therefore correct. AP reversals at this level should initially be displaced into the track of a different root, but where the roots join at the plexus all axons will be within range of familiar cues and they too should correct. However, we found no evidence for correction in ten proximal DV reversals while seven proximal AP reversals reliably connected with appropriate muscles. Fig. 2 shows that the level of flank reversals was just proximal to the confluence of the three segmental roots where one would expect the displacement within the two axes to be similar. Ferguson (1983) has described appropriate innervation of proximal DV-reversed hind limbs. In these experiments the graft was made in the flank at stage 15/16 but resulted in a poor survival rate and many abnormal or missing limbs. Laing (1984) has also described normal motor pools following proximal rotations of both axes of the forelimb. His transplantation technique appears to be a coelomic graft and also frequently resulted in poorly innervated abnormal wings, and there was no analysis of axon trajectories. In both sets of experiments, the plexus was highly abnormal. Both authors concluded that the axons could still respond to local guidance cues. While we are critical of their techniques it is possible that the difference between our and their results is because our flank reversals are not sufficiently proximal.

Both Lance-Jones & Landmesser (1981) and Whitelaw & Hollyday (1983) have suggested that the plexus may be a uniquely important region carrying the cues that axons utilize to change position so that they enter the limbs in their appropriate track. If this were true one would not expect any corrections in shoulder reversals which are distal to the plexus (Fig. 2C). We suggest that this region is special only in the sense that it is the region that is familiar to the greatest number of axons. The formation of *de novo* pathways to nearby appropriate muscles (Lance-Jones & Landmesser, 1981) might reflect attempts by axons to reach their appropriate targets at more distal locations. Responses of axons to AP and DV reversals at various points along the limb will give useful information on this point. Preliminary (unpublished) observations on AP duplicated limbs suggests that axons distal to the plexus innervate duplicated forearm muscle appropriately, but we have no trajectory data yet.

Signal

The difference in response to the two types of reversal may depend on the presence of environmental cues to which the axons are responding. The chick limb is frequently seen as being organized as a Cartesian coordinate system in which the control of pattern across three major orthogonal axes is separate (Wolpert, 1969). The AP axis is known to be labile to a late stage of development with pattern-controlling factors active throughout the period of innervation (Summerbell, 1974, 1979). Control of the DV axis is less well characterized but it seems likely that the pattern is fixed from an early stage well prior to innervation and there is little

evidence for pattern-controlling factors at the stage of innervation (Summerbell & Honig, 1982).

This suggests that the difference in response to the two types of reversal may be dependent on the presence or absence of the pattern-controlling signals. The AP pattern-controlling signal is active during innervation and the axons may use it to correct mismatches. There is no DV signal and the axons cannot correct for mismatches in this axis. A similar difference between two axes has been observed in the retinotectal system by Bonhoeffer (personal communication). While this modification of the hypothesis explains most of the data it has difficulties with those cases in which DV reversals led to appropriate innervation of forearm muscles. However this may not be indicative of axon selection since the axons are fortuitously passing over their appropriate uninnervated muscle by an unusual route that is not a normal nerve track (Summerbell & Stirling, 1982).

Lance-Jones (personal communication) has recently examined specificity in the DV axis by manufacturing double dorsal limbs. She found that axons from the ventral motor pool innervated the ventrally situated dorsal character muscles with no evidence of selection, whereas we find that double AP limbs receive appropriate innervation to the reduplicated muscles. The axons select their appropriate muscles in these cases far distal to the plexus.

CONCLUSION

Axons clearly can respond to displacement of their targets in the AP axis, they may respond either at the plexus or distal to it. Axons are unable to respond to reversal of the DV axis even when the reversal is proximal to the plexus. Even though the axons in both cases pass through the plexus and the displacement from the normal path is very short the axons fail to correct the DV mismatch. The pattern of innervation of the limb is primarily a product of the axons affinity for the tracks determined by the limb tissues, any axon normally prefers any track to no track. Once in a track axons can respond to local cues but we have evidence for effective cues only for the AP axis. Recent work has aimed at distinguishing between the three hypotheses listed at the start of this discussion. It is increasingly obvious that all three are likely to play a role in the development of the peripheral innervation pattern.

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