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Sensory nerve routes in chick wing buds deprived of motor innervation

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SUMMARY

To what extent do motor and sensory nerve fibres depend on one another for guidance during the development of peripheral nerve patterns? This question has been examined by looking at the paths taken by sensory nerve fibres growing into the embryonic chick wing in the absence of motor axons. The precursors of the motoneurones were destroyed by irradiating the appropriate part of the neural tube with a focused beam of ultraviolet light, before axons had grown out. The limb nerve patterns seen 5 to 7 days later revealed that sensory fibres followed the usual paths of main nerve trunks and formed cutaneous nerve branches in an almost normal way. However, the sensory fibres did not take the paths where muscle nerve branches are normally seen. Apparently, sensory axons for the most part do not depend on motor axons for guidance, except in the case of proprioceptive fibres, which require guidance from motor axons over the final steps of their path into muscle.

INTRODUCTION

The developing nerve pattern in an embryonic chick wing is determined, both in its spatial layout and in the timing of its formation, by the limb tissues through which the nerves grow (Swanson & Lewis, 1982; Lewis, Al-Ghaith, Swanson & Khan, 1983; Tosney & Landmesser, 1984, 1985*a*). The limb can be described as providing 'public highways' for nerve outgrowth: 'highways' in the sense that the nerve fibres appear to be restricted to grow only along these routes, with side branches appearing at intervals channelling axons off to specific targets (muscles or areas of skin); and 'public' in the sense that the same (or almost the same) routes are followed even by foreign nerves in experimentally displaced limbs (in the chick: Hamburger, 1939; Narayanan, 1964; Hollyday, 1981; Summerbell & Stirling, 1982; Whitelaw & Hollyday, 1983*b*; and in amphibians: Braus, 1905; Harrison, 1907; Hamburger, 1939; Piatt, 1956).

These conclusions are, however, drawn from studies of limbs with a mixed innervation, comprising both motor and sensory fibres. Such observations leave unanswered several questions regarding the individual behaviour and possible interactions of the two types of nerve fibre. To what extent are motor and sensory axons selective for different branches of the nerve pattern? How will the one type of axon behave in the absence of the other? To what extent is the pattern of nerve

Key words: limb innervation, chick limb bud, axonal guidance, motoneurone, sensory nerve, growth cone.

branches formed by the one set of fibres dependent on the other? Are the pioneer fibres that make the initial selection of routes necessarily sensory or necessarily motor, or can both types act in this role?

These questions can be tackled by making embryos in which either the motor or the sensory neurones have been eliminated, and examining the pattern of the residual innervation. Thus Landmesser & Honig (1982) and Landmesser, O'Donovan & Honig (1983) have briefly reported that sensory fibres fail to project to muscles in the chick hindlimb when motoneurones are absent; hence they argue that sensory neurones do depend on motoneurones for guidance. In addition, Tosney & Landmesser (1985*b*) note differences of growth cone morphology between motor and sensory fibres which seem to support this view. On the other hand, cutaneous nerves are seen in the limbs without motor innervation (Landmesser & Honig, 1982), and when dorsal root ganglia are implanted in a limb bud, sensory axons do grow out from them, even when no motor innervation is present (Swanson, 1985).

In this paper, we too focus on the nerve patterns formed by sensory nerve fibres growing (from their normal origin) into a chick limb in the absence of motor axons. We have studied the wing, rather than the leg, and have analysed the results in silver-stained whole mounts, so as to get a picture of the total pattern in three dimensions. This makes it easy to see clearly which nerves are present as well as which nerves are missing. Our results confirm and extend the above observations, but we would state our conclusions with a different emphasis. We find that the sensory fibres follow the paths of the normal main nerve trunks and branch off to innervate patches of skin in an almost normal fashion, implying that the growth cones of sensory nerve fibres can act as pioneer growth cones, correctly responsive to the usual influences that restrict routes of nerve outgrowth to a standard highway system. Moreover, the sensory nerve fibres are revealed as selective as between paths leading only to muscle (which they do not take) and paths leading to skin (which they do take). The fact that the sensory fibres do not form muscle nerve branches in the absence of motor axons indicates that motor axons are required as part of the guidance system, but only for proprioceptive fibres, and only over the last stages of their journey towards muscles.

MATERIALS AND METHODS

Fertilized chick eggs (White Leghorn or White Leghorn × Rhode Island Red) from Needle Farm, Elstree, Herts.) were incubated at 38 ± 1 °C and windowed at 2–2·5 days (stages 12 to 16 of Hamburger & Hamilton (1951)). The vitelline and amniotic membranes were torn to expose the embryo. To gain access to the ventral part of the neural tube, a sharpened tungsten needle was carefully run along the dorsal seam of the tube from the level of (about) somite 10 to beyond the level of the most recently formed somite block (somite 15 at stage 12, somite 20 at stage 16). This is the same method as was used, we understand, by Landmesser *et al.* (1983) (L. T. Landmesser, personal communication). After a couple of minutes, the two halves of the neural tube had separated, opening like the pages of a book to expose the inner faces that line the neural canal. In younger embryos, and at the caudal end of the neural tube in older embryos, this opening was symmetrical, so that both faces could easily be seen. At the more rostral levels

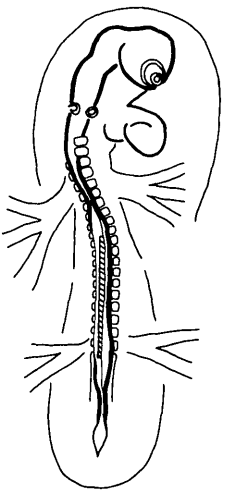


Fig. 1. *Camera-lucida* drawing of a 20-somite embryo, showing its appearance shortly after the dorsal seam of the neural tube has been slit open to expose the inner faces that line the neural canal. The extent of tissue that is irradiated is indicated by cross-hatching. Scale bar, 1 mm.

of the cords of older embryos, the flexure of the embryos meant that only the left-hand face was clearly visible.

To destroy the precursors of the motoneurones, a narrow beam of ultraviolet light was then focused (as described below) onto the ventral half of the exposed luminal face of the neural tube on the left side of the embryo (Fig. 1). From a series of test exposures, an exposure of 5 to 10 s was found to be optimal for destroying the neuroepithelium destined to give rise to motoneurones while avoiding damage to adjacent tissues.

After irradiation, a few drops of Hepes-buffered Hanks balanced-salt solution with penicillin (50–100 i.u. ml^{-1}) and streptomycin (50–100 $\mu g\,ml^{-1}$) were added to prevent infection before the eggs were resealed and returned to the incubator. The embryos were fixed after a further 5 to 7 days when they had reached stage 30–35. The fixed embryos were silver stained as whole mounts, dehydrated and cleared in methyl salicylate, as described previously (Lewis, 1978; Lewis, Chevallier, Kieny & Wolpert, 1981). Drawings of stained limbs were made using a *camera lucida* attached to a Zeiss stereomicroscope. The nomenclature for muscles follows that of Sullivan (1962). Some of the limbs were subsequently embedded in Araldite, serially sectioned at 5–10 μm and stained with toluidine blue.

The trunks of some of the specimens, after silver staining, were embedded in wax in the usual way, serially sectioned at 10 μm, and stained either with haematoxylin and eosin or with cresyl violet. The outlines of the brachial motor columns and adjacent dorsal root ganglia were traced in every tenth section with a *camera lucida.* The areas of the tracings were measured with an Apple Graphics Tablet and summed to give an estimate of the relative volumes of these structures on the control and irradiated sides.

U.v. irradiation apparatus

As a source of ultraviolet irradiation, we used a high-pressure mercury-vapour discharge lamp (250 W, type ME/D with quartz window, from Thorn Lighting, for most of our operations; subsequently, for the last few operations in the series, a 200 W lamp, type HBO 200 W/4, from Osram). The light from the lamp was projected through quartz light-gathering lenses onto the aperture of a field stop of adjustable dimensions, and a pair of front-surface aluminized mirrors, arranged as in a Newtonian reflecting telescope, then focused an image of the illuminated field-stop aperture onto the embryo, as shown diagrammatically in Fig. 2. Apart from the use of reflecting optics, the principles are the same as those of Koehler illumination by a conventional

microscope condenser. The size and shape of the patch of tissue irradiated could be precisely controlled by adjusting the size and shape of the field-stop aperture; the exact positioning of the beam on the embryo was adjusted by means of a micromanipulator, on which the two mirrors were mounted. A yellow glass filter was inserted in the light path (between the quartz lens and the field-stop aperture) while the beam was being aligned and adjusted. This filter cut out wavelengths shorter than about 500 nm, while transmitting visible light harmless to the cells. U.v. irradiation was then delivered for a measured length of time by removing the filter from the light path. Using the manufacturers' data sheets, we estimate that the total power incident on the embryo was 40–80 mW mm^{-2}, of which 10–20 mW mm^{-2} was in the far u.v. range, from 250–350 nm.

RESULTS

The ventral part of the left-hand side of the neural tube, containing the precursors of the motoneurones that normally innervate the left wing, was irradiated with a destructive dose of u.v. light in a total of 155 embryos at 2–2·5 days of incubation. Of these, 106 survived to stage 30–35 and were silver stained as whole mounts. The left and right wings of these embryos were systematically scored as to the presence or absence of each of the 27 muscle nerve branches and 14 cutaneous nerve branches that are reproducibly found in a mature normal wing (see Fig. 3). The trunks of a subset of the specimens were embedded in wax and sectioned to assess what damage the u.v. irradiation had done to the spinal cord and dorsal root ganglia.

Effects on the pattern of nerve branches

The effects of the irradiation, as revealed by reduction of the innervation of the left wing as compared with the right, were variable, presumably because of inaccuracies in the alignment of the beam or variability in the susceptibility of the

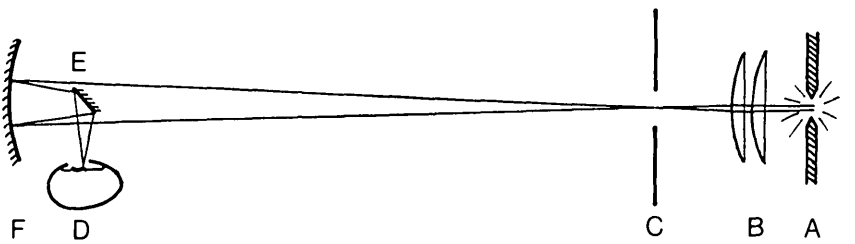


Fig. 2. Schematic diagram of the ultraviolet irradiation apparatus (not drawn to scale). A is a high-pressure mercury vapour discharge lamp. B is a set of quartz lenses, projecting the light of the lamp through the field stop aperture C. The field stop aperture is adjustable in diameter and shape. D is the embryo in the egg. E is a front-surface aluminized plane mirror, measuring 10 × 7 mm^2, at 45° to the optic axis. F is a front-surface aluminized concave mirror, of focal length 8·0 cm. The mirrors E and F jointly project a sharply focused image of the illuminated aperture, C, onto the embryo. The distance CF was 80 cm, so that the image on the embryo was 1/9 × the (linear) size of the aperture C. Because of the danger of explosion, the lamp was enclosed in a metal housing with a thick flat quartz window (not shown). While the beam was being aligned on the embryo, a yellow glass filter was interposed between B and C to cut out the ultraviolet part of the lamp output; this filter was then removed for the irradiation of the neural tube.

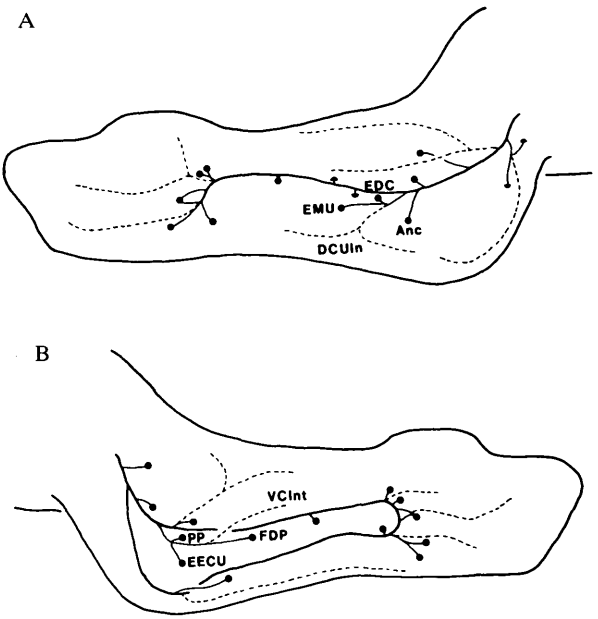


Fig. 3. Schematic diagrams of the patterns of innervation, as seen at stage 32, of the dorsal (A) and ventral (B) sides of a chick wing. Major mixed nerve trunks and individual muscle nerve branches are indicated by solid lines and filled circles, respectively, and the major (that is, regularly identifiable) cutaneous nerve branches are indicated by broken lines. The branches singled out for labelling are those specifically referred to in the text.

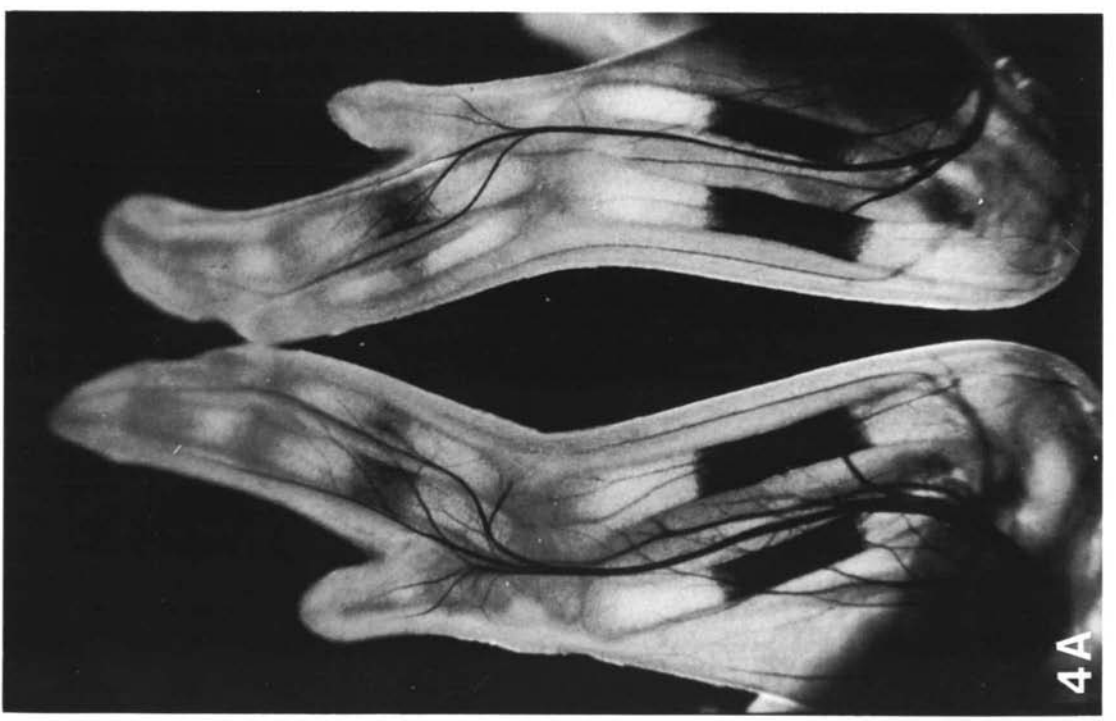
tissue to u.v. In some specimens, the wing nerve pattern appeared normal; in others, only one or two muscle nerve branches were missing; and in yet others, there was a drastic reduction in the number of muscle nerve branches; cutaneous nerve branches were also sometimes missing. The descriptions and analyses to be given below are based on a selection of twenty-six successfully stained specimens representing opposite extremes of the range of variation. Thus, sixteen specimens were selected as 'severely affected' on the criterion that the motor innervation was severely reduced, to the point where not more than 4 muscle nerve branches (out of a possible 13) were visible on the dorsal side of the limb, and not more than 4 (out of a possible 14) on the ventral side (hence in all not more than 8 muscle nerve branches were visible, out of a possible 27). For comparison with these, ten specimens were selected as 'relatively unaffected' on the criterion that the motor innervation was reduced only slightly or not at all, so that the total number of muscle nerve branches on the irradiated side did not fall short of the number on the control side by more than 3. In all the selected specimens, the innervation of the control wing appeared normal (though the number of nerve branches visible was somewhat variable, according to the stage at fixation and the quality of the staining – see below). All the selected wings were examined also between crossed polarizing filters to check the musculature (Lewis *et al.* 1981): it was normal in all cases.

The first major point to be emphasized is that, although certain nerve branches were often missing on the irradiated side, the branches that were present were confined to the normal paths (see Figs 3,4,5). This fact made it possible to identify according to the normal nomenclature the branches that were present and to match each one with its counterpart in the control wing.

The embryos of greatest interest are those in which the u.v. irradiation had had a marked effect, i.e. the set of sixteen 'severely affected'. Since in the upper arm, forearm and hand of a normal mature wing one can regularly identify a total of 27 muscles nerve branches and 14 cutaneous nerve branches, we had potentially a total of 432 (= 27×16) muscles nerve branches and 224 (= 14×16) cutaneous nerve branches to consider in this set of specimens. However, some of the embryos were too young for certain of the nerve branches to have yet become visible on the control side (Swanson & Lewis, 1982; Swanson, 1983) and in some regions of some of the wings the staining was too weak to allow a judgement as to whether a particular nerve branch was present or absent. Any nerve branch that was unscoreable for either of these reasons was omitted from the comparison of the irradiated and control sides (and it should be noted that we never saw a stained nerve branch on the irradiated side when none had been seen on the control side). This left a total of 324 muscle nerve branches that were visible in the control wings and could be scored as present or absent in the experimental wings. Of this total, only 63 (19 %) were present in the experimental wings. Counting the cutaneous nerve branches in the same way, we found that out of a total of 213 visible in the control wings, 126 (59 %) were present in the experimental ones. The corresponding figures for the set of 10 'relatively unaffected' specimens were 96 % (for the muscle nerve branches) and 100 % (for the cutaneous nerve branches).

Evidently, although the irradiation procedure interfered with the development of some of the cutaneous nerve branches, the muscle nerve branches were affected to a much greater extent. The fact that in some cases the muscle nerve branches were almost entirely eliminated, while the cutaneous nerve branches were almost all present, implies that sensory axons in isolation selectively avoid paths into muscles. It appears also that the sensory axons are able to navigate along the normal routes to their cutaneous targets independently of motor axons. (A class of possible exceptions to this latter rule is described below.) These conclusions, however, rest on the assumption that the u.v. irradiation had achieved its intended effect of destroying the motoneurones in the spinal cord, while sparing the sensory ganglia. We must therefore consider the evidence that this was so.

Fig. 4. The innervation of the dorsal side of an experimental wing with its contralateral control, as seen (A) in a dark-field photograph, and (B) in a *camera-lucida* drawing. The control wing is on the left. The dorsal muscle nerve branches visible under the microscope are labelled in (B), following the terminology of Swanson & Lewis (1982); all but one of those present on the control side are missing on the irradiated side, whereas the cutaneous innervation on the irradiated side (dotted lines) is nearly normal. The slight deformity of the experimental wing is probably due to muscle atrophy. Both wings fixed at stage 35. Scale bar, 1 mm.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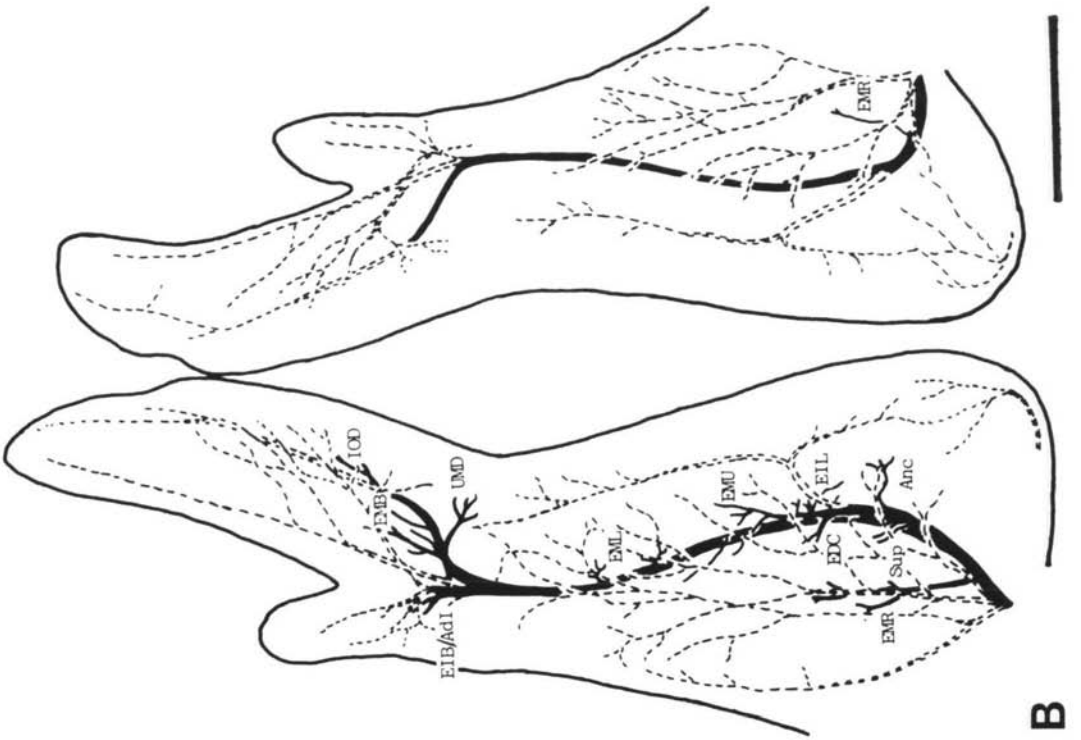
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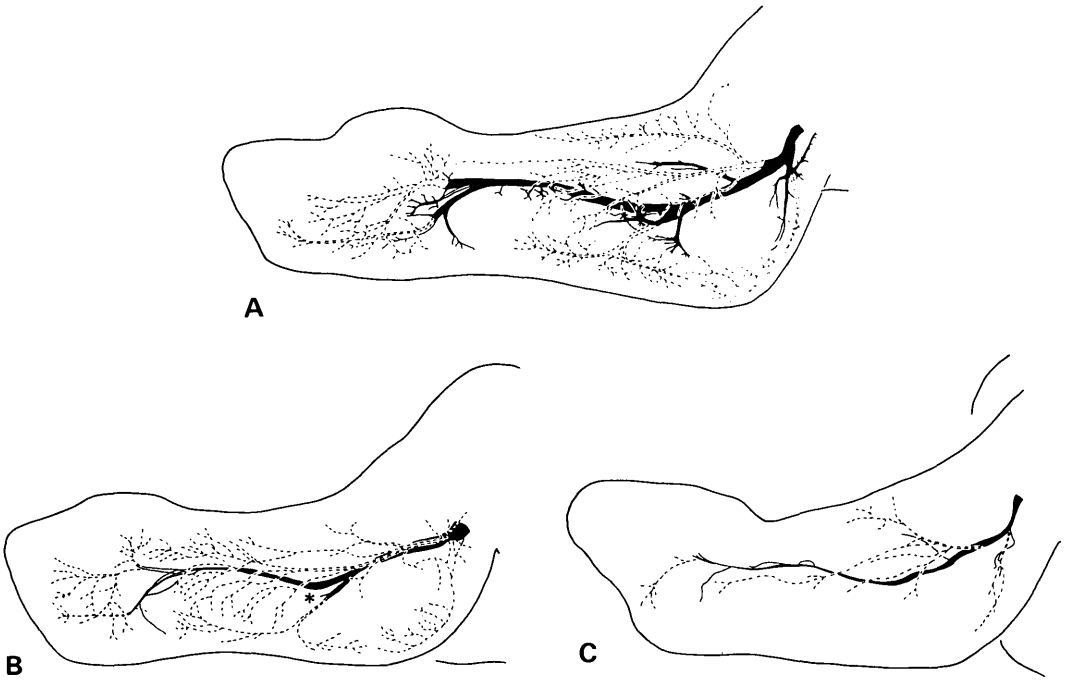


Fig. 5. *Camera-lucida* drawings of the dorsal nerve patterns in silver-stained left wings at stage 32 to illustrate the range of results of the ultraviolet irradiation. A is a normal control wing. B,C are wings of irradiated specimens belonging to the 'severely affected' group (see text). B shows practically normal cutaneous innervation (dotted lines) but only one small muscle nerve branch, EMU (asterisk). In C, the cutaneous innervation as well as the motor has been reduced; presumably the irradiation did some unintended damage to the developing dorsal root ganglia. Nevertheless, the main nerve trunk follows its normal course, and those cutaneous branches that are present originate at the normal points and follow normal directions. Scale bar, 1 mm.

Effects on the neural tube

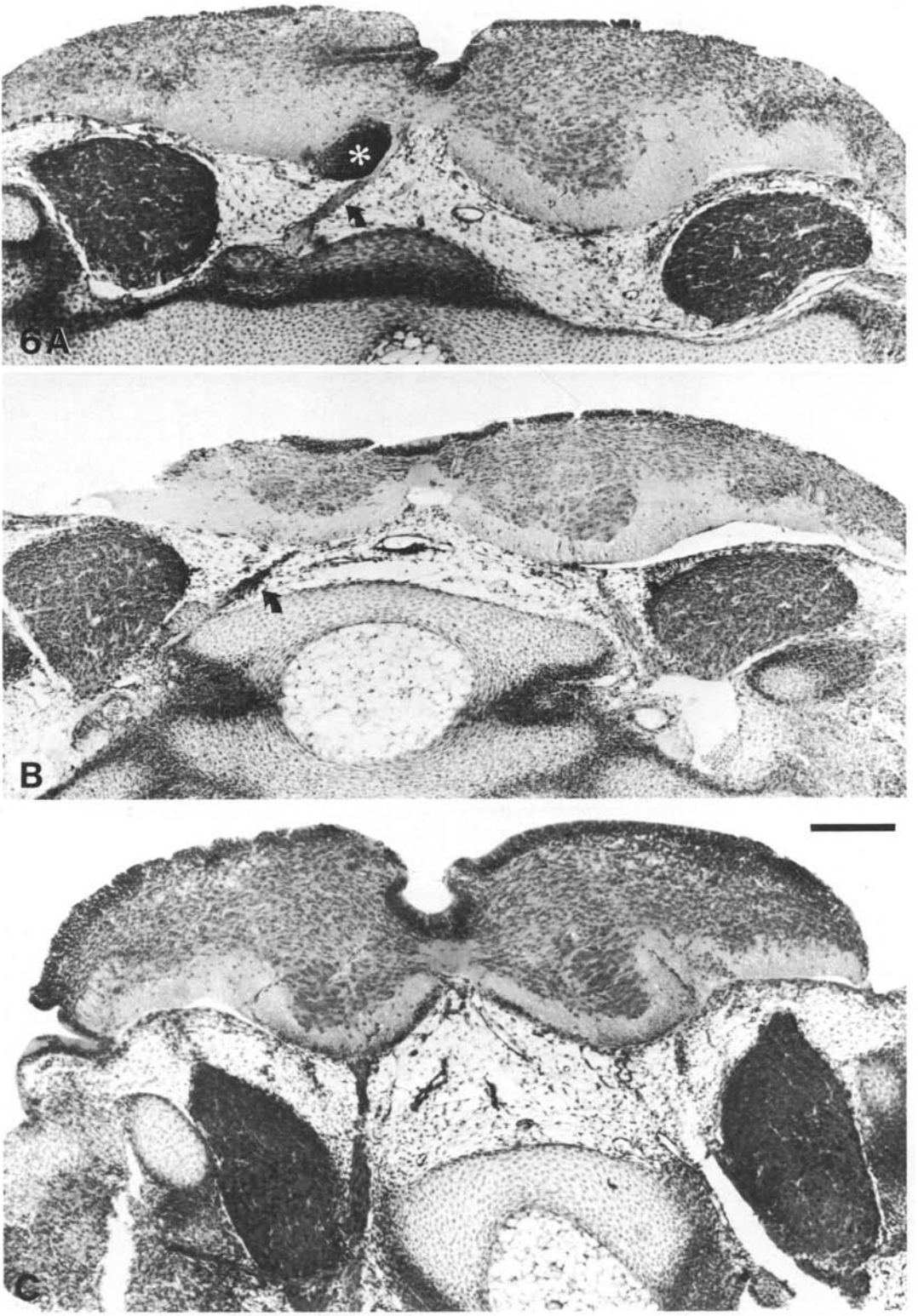
To check that the absence of muscle nerve branches in the limb was indeed a reflection of the absence of motoneurones, we embedded the trunks of silver-stained embryos and cut serial cross sections through the vertebral column and neural tube. In almost all cases, the neural tube was found to have remained open following the surgery done at the time of u.v. irradiation. Of the twenty-six specimens selected on the criteria given above for detailed analysis, twenty-four yielded adequate sets of serial sections of the neural tube. Fig. 6 illustrates three typical cases; two from the group where there was severe reduction of the number of muscle nerve branches (Fig. 6A,B), and one from the relatively unaffected group (Fig. 6C,D). In all three, the neural tube has remained open. In the specimen with the relatively unaffected wing nerve pattern (Fig. 6C,D), the lateral motor columns, containing motoneurones that innervate the wings, are seen to be present and of practically normal size on both the irradiated and the control side, as are the dorsal root ganglia. But, as expected, in the specimens with 'severely affected' wing nerve patterns, the lateral motor column on the irradiated side

appears to be missing (Fig. 6A) or at least greatly reduced in size (Fig. 6B). By contrast, in these same specimens, the sensory ganglion on the irradiated side is clearly present, and of nearly normal size. Fig. 6A,B shows also a feature observed in several of the sets of spinal cord sections from this group. There appears to be a diminutive ventral root leaving the cord from the irradiated side. This might perhaps account for the fact that the wings of both specimens still had a few thin muscle nerve branches in the wing on that side. In Fig. 6B the axons in the root may come from residual motoneurones; in Fig. 6A, on the other hand, the ventral motor pools seem to have been destroyed completely on the irradiated side, and the root leaves the spinal cord from a much more medial position than normal. In fact it lies medial to a large haematoma (also a common feature) which sits near the region where most of the u.v. irradiation damage would be expected. The source of the fibres in this root is unknown; it seems unlikely that they belong to residual motoneurones on the irradiated side. They may be aberrant central axons that should normally have remained within the spinal cord, or even motor axons from the intact contralateral motoneurone pools.

For each of our sets of sections, we estimated the numbers of motor and sensory neurones on the irradiated and control sides. Motoneurones in a normal embryonic spinal cord are easily identified by their location, their large size, and their strongly stained cytoplasm. However, in many of our specimens, because of the prior silver-staining process, and because of the distortions caused by the operations on the neural tube, it was difficult to be certain of the exact boundaries of the lateral motor columns, and often hard to decide whether an individual cell was or was not a motoneurone. Therefore, rather than laboriously count cells of uncertain identity, we estimated the surviving numbers of motoneurones on the irradiated side, as compared with the control side, simply by measuring the apparent cross-sectional areas of the lateral motor columns, judging their outlines as best we could, in each of a series of sections spaced 100 μm apart. By summing these areas for the irradiated side, and dividing by the corresponding sum for the control side, we got a rough value for the volume of the lateral motor column, and hence for the number of brachial motoneurones, on the irradiated side, expressed as a percentage of the corresponding quantity on the control side. At these embryonic stages there does not appear to be any significant inflammation or gliosis to confuse the issue; but we grant that our estimates are crude. The same procedure was used to assess the relative numbers of dorsal root ganglion cells in the brachial region on the irradiated side as a percentage of the value for the control side.

The mean values obtained are shown in Table 1. It can be seen that, in the set of specimens lacking most nerve branches, there has indeed been a selective destruction of motoneurones while the sensory neurones have been largely (though not entirely) spared.

Since destruction of the brachial motor column was often associated with some damage to the dorsal root ganglia, further statistical analysis is needed to assess the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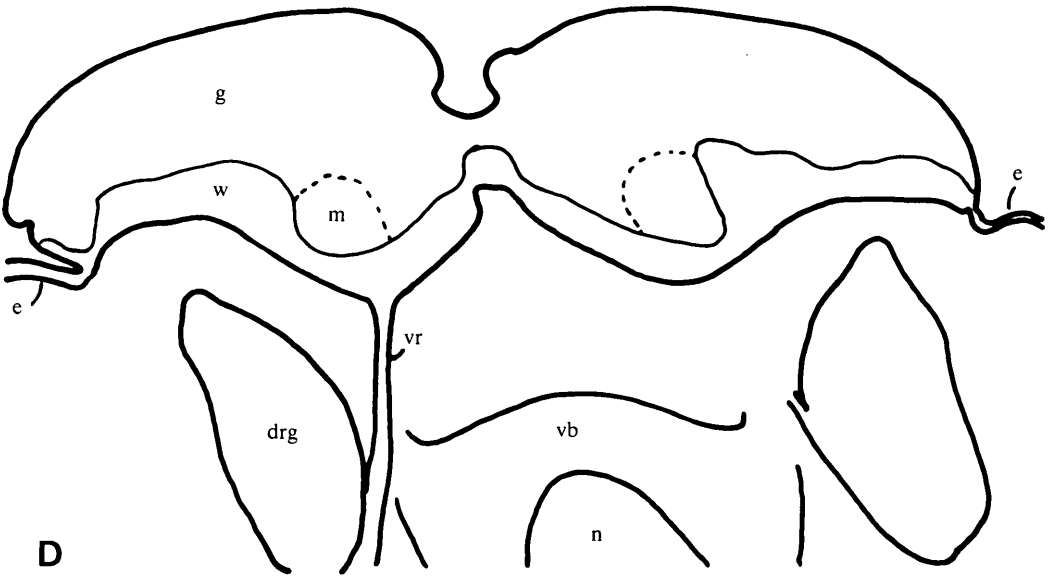

Fig. 6. Cross sections of three spinal cords (A,B,C) that had been irradiated seven days earlier, silver-stained and then embedded and sectioned; D is a tracing from photograph (C) to serve as a key to the structures. In all three cases, the neural tubes have remained open. The lateral motor column on the left side has been destroyed completely in A, greatly reduced in B, and only slightly reduced in C. A,B belong to the 'severely affected' set of specimens, while C is one of those 'relatively unaffected', as judged from the innervation of the wing. In all three specimens, the free surface (ependyma) of the open neural tubes has been slightly damaged, presumably through handling during the two histological processes. In both specimens the dorsal root ganglia appear unaffected by the irradiation. The arrow in A,B points to a residual ventral rootlet (see text); the asterisk marks a haematoma (see text). The apparent absence of a ventral root on the control side is merely an accident of the plane of section. Labelling in D: *e*, ectoderm; *g*, grey matter of spinal cord; *w*, white matter; *m*, lateral motor column; *drg*, dorsal root ganglion; *vr*, ventral root; *vb*, body of vertebra; *n*, notochord. Haematoxylin and eosin stain, 10 μm sections. Scale bar, 100 μm.

causes of the elimination of muscle nerve branches. Was this truly a consequence of the damage to the motor column, and independent of the degree of damage to the dorsal root ganglia? An answer can be obtained by calculating the partial linear regression of the number of muscle nerve branches, regarded as the dependent variable, on the volume of the brachial motor column and on the volume of the dorsal root ganglia, regarded as the independent variables (Bailey, 1959). That is, we fit the data to a linear regression of the form

$$n_m = a + b_m V_m + b_s V_s$$

where n_m is the number of muscle nerve branches on the irradiated side, expressed as a percentage of the number on the control side, V_m and V_s are the corresponding percentage volumes of brachial motor column and sensory ganglia, and a, b_m and b_s are constants to be determined; b_m and b_s are the partial regression

Table 1. *Numbers of muscle and cutaneous nerve branches and volumes of brachial motor columns and sensory ganglia*

	Severely affected specimens (n = 14)	Relatively unaffected specimens (n = 10)
Muscle nerve branches (%)	19 (± 3)	96 (±2)
Brachial motor column (%)	14 (± 5)	88 (± 6)
Cutaneous nerve branches (%)	57 (± 6)	100 (± 1)
Dorsal root ganglia (%)	58 (± 6)	101 (± 2)

The values given are means (± S.E.M.) over the percentage values for the irradiated side as compared with the control side.

coefficients of n_m on V_m and V_s, respectively. We find, for our total set of twenty-four histologically analysed specimens:

$$b_m = 0{\cdot}81 \text{ with a standard error of } \pm 0{\cdot}15$$
$$b_s = 0{\cdot}11 \text{ with a standard error of } \pm 0{\cdot}22$$

Thus the number of muscle nerve branches shows a strong and highly significant dependence on the volume of the brachial motor column, but no significant dependence on the volume of the sensory ganglia. (See Bailey (1959) for details of the method of calculation.)

Two possible exceptions to the general rule: cutaneous nerve branches closely associated with muscle nerve branches

In general, as stated above, cutaneous nerve branches developed normally even in the absence of adjacent muscle nerve branches. There were, however, two cutaneous nerve branches for which it seemed that perhaps this might not be true; these were the dorsal cutaneous nerve (DC Uln) that supplies the ulnar border of the wing, and the ventral cutaneous nerve (VC Int) that supplies the intermediate region of the forearm (see Fig. 3). The fibres forming each of these cutaneous nerves branch off from the main nerve trunks in company with fibres destined for adjacent muscles; that is, they diverge from a main nerve trunk as part of a mixed nerve, which sends branches to muscles as well as to the skin. For both DC Uln and VC Int, but only for these two components of the wing nerve pattern, we noted a possibly significant correlation between absence of the cutaneous nerve branch and absence of the associated muscle nerve branches (to muscles Anc, EMU and EDC in the case of DC Uln (see Fig. 5); to PP, EECU and FDP in the case of VC Int (not illustrated)). Thus out of the sixteen experimental wings, there were only three where DC Uln was present in the apparent absence of any of its associated muscle nerve branches; in the remaining thirteen cases, either DC Uln and one or more of the muscle nerve branches were jointly present (ten cases) or they were jointly absent (three cases). On the ventral side of the limb the staining was usually less good, and the innervation in general more scanty, so that it was

harder to draw firm conclusions; but there were no specimens in which VC Int was clearly present in the absence of its associated muscle nerve branches, as against five cases in which VC Int and one or more of these muscle nerve branches were both clearly present and five cases in which they all were apparently absent. This suggests that the guidance of sensory nerve fibres along the routes of DC Uln and VC Int may depend on the presence of the motor axons that normally form the associated muscle nerve branches. It should be emphasized, however, that there were three clear cases where DC Uln was present even though all the associated muscle nerve branches appeared to be absent.

Incidental observations relevant to motoneurone specificity

Our specimens also, incidentally, provide some evidence bearing on the question of motoneurone specificity (reviewed by Hollyday, 1980; Landmesser, 1980). In effect, we have unintentionally repeated on the wing, in a partial and haphazard way, the experiments of Lance-Jones & Landmesser (1980) on the leg, in which they deleted a portion of the neural tube and examined the pattern of connections between the surviving motoneurones and the limb muscles. Like them, we found no tendency for a few remaining motor axons to become distributed over the muscles in a simple proximodistal sequence. For example, the most proximal muscle of the dorsal or ventral series (triceps dorsally, biceps ventrally) was often (six cases out of thirty-two) uninnervated in wings in which more distal muscles of the series were innervated, as one might expect if neuronal specificity governs the formation of neuromuscular connections and causes motor axons to bypass inappropriate targets.

DISCUSSION

From our results, it appears that sensory neurites can grow out into the embryo chick wing in the absence of motor axons. The fibres follow the limb's normal nerve pathways to form branches innervating the skin, diverging from the main nerve trunks at the normal branch points. In the absence of motor axons the sensory fibres do not innervate muscles.

Taylor (1944) did an experiment similar to ours in the frog, *Rana pipiens*, with similar results. He removed the entire spinal cord of young embryos to eliminate the sources of the hindlimb motor innervation, leaving the spinal ganglia on either side intact. When the nerve anatomies of the legs were later examined, the main nerve trunks and cutaneous nerve branches resembled those of a normal leg, but branches to individual muscles were missing. Taylor found in addition a suggestion, as we have, that certain cutaneous nerve branches depend on the presence of certain associated muscle nerve branches for their development, even though they do not innervate muscles themselves. In two frogs (out of five) described in detail, a certain cutaneous nerve branch in the thigh, *n. femoris medialis,* was missing in the limbs deprived of motor innervation. In normal limbs, the branch was found to grow to the skin from the distal end of a muscle nerve branch,

n. profundus posterior. Taylor concluded that the cutaneous branch in question was dependent on the presence of the accompanying muscle nerve fibres to act as a bridge through developing muscle tissue, in order to reach the skin.

The observations most directly comparable with our own are those reported briefly by Landmesser & Honig (1982) and Landmesser, O'Donovan & Honig (1983). They destroyed the motoneurones at lumbosacral levels in chick embryos at stages 16–20, and fixed the adjacent legs for analysis at stages 30–32. They found, as we also have found, that muscle nerve branches were absent or greatly reduced, although at least some cutaneous nerves were present.

Muscle nerve branches do normally include sensory nerve fibres, even at early stages (Honig, 1982). These sensory fibres evidently depend on motor axons to get to their targets, and cannot make their way to muscles by themselves. Tosney & Landmesser (1985*b*) back up this conclusion with a further intriguing observation. They find that the growth cones of the two types of fibre differ in shape, the sensory ones appearing small and simple (like growth cones in other systems following an established fascicle (e.g. Lopresti, Macagno & Levinthal, 1973)), in contrast to the larger and more complex growth cones of the motor axons. This difference is especially evident in 'decision regions' (in the neighbourhood of the plexus and of branch points). The suggested interpretation, therefore, is that motor axons are the pathfinders, providing a bridge or track along which sensory fibres are guided by fasciculation.

We would, however, emphasize that by no means all outgrowth of sensory fibres is dependent on motor fibres in this way. Our observations show that sensory fibres generally follow practically normal paths to the skin even when the motor axons are largely or completely absent: these cutaneous fibres can act as pioneers and respond correctly in their own right to guidance cues provided by the surrounding limb tissues. This point is particularly clearly demonstrated by our whole-mount staining method, which directly displays the pattern of nerves in three dimensions. Three caveats must be stated, however. First, as described in Results above, there may perhaps be some exceptions, where motor axons are required to enable the sensory fibres to travel their normal routes to certain patches of skin. Second, it is possible, in principle, that the few residual motor axons in our specimens may have played a crucial part and that if all motor axons had been totally absent we would have seen a different pattern; but this seems to us unlikely, especially since dorsal root ganglia implanted in a wing bud that is totally devoid of motor innervation send out axons through the limb tissues and these axons display a tendency to follow the normal highways (Swanson, 1985). The third caveat concerns the specificity of the sensory projections: our observations, while showing that sensory peripheral nerves follow the normal routes, do not allow us to say whether the fibres in those nerves derive from the same dorsal root ganglia as in a normal animal, where the motoneurones have not been destroyed (see Honig, 1982; Scott, 1982). Nor can we say whether the fibres growing into cutaneous branches in our limbs lacking muscle nerve branches are ones that would normally all project to the skin, or whether they are a mixture of normal cutaneous fibres and rerouted muscle sensory fibres. Landmesser,

O'Donovan & Honig (1983) reported that the cutaneous nerve branches remaining in their experiments after motoneurone extirpation were thicker than those in the control leg, and suggested that muscle sensory fibres, not being able to project to their normal targets, were diverted to the skin. We did not notice any such effect in our specimens, though that may simply reflect the limitations of our silver-staining technique.

Our observations imply that developing sensory and motor axons have different pathway selectivity, in that they form different sets of branches of the peripheral nerve pattern. Any theory that seeks to explain how axons are guided through the limb must accommodate this fact. Rutishauser, Grumet & Edelman (1983) have found that the homophilic neural cell adhesion molecule, *N*-CAM, is present on developing muscle cells and mediates an interaction in tissue culture that causes axons from embryonic spinal cord cells, also displaying *N*-CAM, to adhere to them and grow over them, forming the tissue-culture analogue of muscle nerve branches. One might be tempted to suggest on this basis that *N*-CAM is *the* molecule that causes growth cones of motor axons *in ovo* to turn aside from a main nerve trunk to innervate adjacent muscle. But the present results imply that such a theory would be at best simplistic: for *N*-CAM is present on sensory as well as motor neurites (Rutishauser *et al.* 1978) and yet they do not, in the absence of motor axons, form muscle nerve branches.

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