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THE DEVELOPMENT OF THE SEGMENTAL PATTERN OF SKIN SENSORY INNERVATION IN EMBRYONIC CHICK HIND LIMB

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SUMMARY

1. The development of dermatomes in the chick hind limb was investigated with both electrophysiological recording from and horseradish peroxidase (HRP) labelling of neurones in lumbosacral dorsal root ganglia (d.r.g.s). The embryonic stages studied spanned the period before and after cell death.

2. In mature embryos, after the bulk of cell death, physiological mapping showed that the location of the dermatome of each d.r.g. is consistent from embryo to embryo. HRP studies showed that axons from each d.r.g. project through the limb to the skin via a characteristic set of nerve trunks. Both the dermatomes and axonal projection pathways of adjacent d.r.g.s partially overlap one another, producing an orderly progression in the location of dermatomes and projection pathways along and within the limb, respectively.

3. In younger embryos, before cell death, the location and amount of overlap of dermatomes, as well as the axonal projection pathways, are similar to the mature pattern. D.r.g.s that innervate distal skin on the shank and foot in mature embryos do not project out cutaneous nerve trunks in the thigh or contact nearby skin on the thigh at earlier stages.

4. Axons from a single d.r.g. initially contact the skin at one or more characteristic spots; the dermatomes then enlarge, adding fine axonal branches.

5. Carbon-marking experiments showed that there are no large distal migrations of skin on the limb during the stages studied.

6. Together these findings show that dermatomes on the chick hind limb do not develop by skin sensory axons simply growing to the nearest available skin, nor are axons towed to their final location by skin movements. Moreover, dermatomes are not shaped by cell death and the elimination of random or excessive axonal projections in the limb or the skin. It appears that skin sensory axons from each d.r.g. grow directly to their target skin along a defined set of pathways and establish their dermatome precisely at its characteristic location.

INTRODUCTION

In adult vertebrates sensory innervation of the skin is organized segmentally; cutaneous afferents from each dorsal root ganglion (d.r.g.) innervate a discrete region of skin, referred to as a dermatome. The dermatome of each d.r.g. has a characteristic

shape and location among individuals of a species (e.g. Sherrington, 1893; Kaiser, 1924; Cole, Lesswing & Cole, 1968; Aguilar, Bisby, Cooper & Diamond, 1973; Kukulinsky & Brown, 1979; but see Fletcher & Kitchell, 1966; Dykes & Terzis, 1981). Typically, dermatomes of adjacent d.r.g.s partially overlap one another, have a more complex shape on the limb than on the trunk (Kaiser, 1924; Keegan & Garrett, 1948) and do not necessarily overlie the muscles innervated by the same spinal segment (Sherrington, 1893; Dykes & Terzis, 1981).

The developmental origin of this segmental pattern of skin sensory innervation has been the subject of speculation for nearly a century (Sherrington, 1893; Harrison, 1899; Keegan & Garrett, 1948; Cole *et al.* 1968; Dykes & Terzis, 1981; Diamond, 1982), but there are few experimental studies on the development of dermatomes. Recently Honig (1982) has shown that from their earliest outgrowth skin sensory axons from the three most rostral lumbosacral d.r.g.s in the chick project through the hind limb along precise pathways. She did not, however, investigate the fate of the afferents as they ramified within the skin to establish their dermatome.

In the experiments reported here I have confirmed and extended Honig's (1982) observations by studying the development of the dermatomes of all eight lumbosacral d.r.g.s that supply the hind limb in chick embryos to determine the precision with which skin sensory innervation *per se* is established. The results of these experiments show that cell death does not play a significant role in shaping developing dermatomes. It appears that afferents from each d.r.g. grow directly to their appropriate skin region along a defined set of pathways, and establish their dermatome precisely at its characteristic location. Some of the results of the present investigation have been published in a preliminary report (Scott, 1981).

METHODS

General. Embryos were from white Leghorn eggs incubated at 38 °C in a forced-draft incubator. All experiments were performed in isolated hind limb–d.r.g. preparations in Tyrode solution of the following composition (mM): NaCl, 139; KCl, 3; $NaHCO_3$, 17; $MgCl_2$, 1; $CaCl_2$, 3; dextrose, 12; bubbled with 95 % O_2–5 % CO_2, pH 7·0. Embryos were staged according to Hamburger & Hamilton (1951), decapitated, eviscerated, and cut in half along the dorsal mid line, and the spinal cord and ventral roots were removed. Lumbosacral dorsal roots and d.r.g.s were freed from the surrounding connective tissue and left attached to the isolated hind limb (Fig. 1). It was usually necessary to sever the small dorsal nerve branches that supply skin on the back; this should not affect the results presented here, since these nerves do not innervate skin on the leg.

Physiology. The dermatome of each d.r.g. was mapped physiologically by recording extracellularly from the entire dorsal root with a suction electrode while the skin was brushed with a hand-held single bristle or stimulated electrically with a concentric electrode (tip diameter of 200 μm for stage (St.) 28–29 embryos and 300 μm for older embryos; 0·1–0·5 ms pulses) (Fig. 1). Evoked activity was recorded, amplified, and displayed on an oscilloscope as well as being relayed through an audiomonitor. The area of skin in which stimulation evoked action potentials in each dorsal root was recorded on a representative drawing of the leg, using the joints and underlying bones as reference points; this area of skin defined the dermatome of the d.r.g. All physiological experiments were carried out at room temperature.

Histology. For tracing axonal pathways a d.r.g. was pressure-injected with 40–70 % horseradish peroxidase (HRP) (Grade I, Boehringer-Mannheim) dissolved in 1 % lysolecithin. To maximize labelling the ganglion was injected three to five times during an 8 h period, and each time an attempt was made to damage many cells by making multiple penetrations (see also Honig, 1982). In a few embryos HRP labelling was accomplished by placing the severed dorsal roots in a polyethelene cuff, filled with approximately 150 % HRP in 1 % lysolecithin (Frank, Harris & Kennedy, 1980). For

embryos older than St.35 the skin was removed prior to labelling with HRP, but was left intact on younger embryos. HRP-treated preparations were either maintained in oxygenated Tyrode at 26–28 °C for 18–24 h or at 4 °C for 4 d; tissue preservation was better in the latter. The skin was then removed (see below) and the legs and d.r.g.s fixed in 2% glutaraldehyde in 0·1 M-phosphate buffer (pH 7·2) for 4 h at room temperature. Subsequently preparations were washed with 0·1 M-Tris

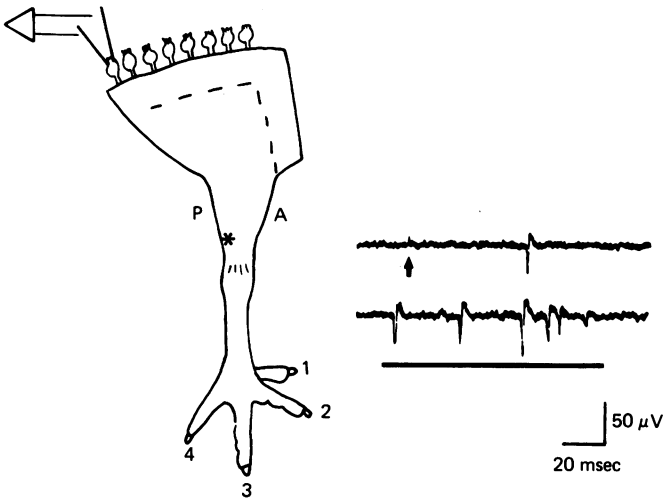

Fig. 1. Diagram of the hind limb–d.r.g. preparation (left). On the right are examples of action potentials recorded extracellularly from the 8th lumbosacral dorsal root of a St. 40 embryo when the skin was stimulated electrically (top trace) or brushed (bottom trace) on the spot marked with an asterisk. Arrow indicates stimulus artifact. Horizontal bar indicates approximate duration of brushing. A, anterior; P, posterior.

(pH 7·2), reacted with diaminobenzidine, dehydrated and embedded in paraffin as described by Landmesser (1978*a*). Serial 8–12 μm sections were cut through the d.r.g.s and leg, and stained with cresyl violet. In some embryos nerve pathways, including HRP-labelled axons, were reconstructed from camera lucida drawings of representative sections.

To delineate dermatomes morphologically, just prior to fixation small carbon particles were placed on the skin over the femur and tibia to serve as landmarks. The skin was then cut down the anterior or posterior margin of the leg, peeled off, flattened between two polyethylene sheets and fixed in 2% glutaraldehyde for 1 h. HRP was reacted with either tetramethyl benzidine (Mesulam, 1978) or benzidine dihydrochloride (Mesulam, 1976), as if the skin were a single histological section. The skin with labelled axons was mounted on a cover-slip with 0·5% gelatin, dried overnight, cleared, and viewed as a whole-mount.

Skin marking. To test for skin migration during limb development a small hole was cut in the eggshell above St. 27–28 embryos. Under sterile conditions the membranes over the embryo were opened and a small carbon particle was placed on the hind limb. After the location of the carbon was recorded on a drawing of the limb, the hole was sealed with a coverslip and melted paraffin, and the egg returned to the incubator until the embryo developed to St. 36–38. At that time the egg was re-opened and the location of the carbon particle was again recorded.

RESULTS

Physiological mapping of dermatomes

The hind limb of the chick usually receives sensory innervation from the first eight lumbosacral dorsal root ganglia (d.r.g.s) (LS 1–8 or segments 23–30); occasionally the

last thoracic and/or the 9th lumbosacral d.r.g. also contribute innervation (see Fig. 7). Embryos in which the lumbar plexus was unusual, including those with an obvious contribution from LS 9, were not used in physiological experiments.

The mature pattern of dermatomes. St. 40 embryos were used for physiological determination of the mature pattern of dermatomes since by this stage the cutaneous nerve supply in chicks is 'stabilized' and has assumed its adult pattern (Saxod & Verna, 1979), and the period of cell death should be over. While the timing and magnitude of cell death have not been examined in detail in lumbosacral d.r.g.s in the chick (Hamburger & Levi-Montalcini, 1949), in brachial d.r.g.s cell death begins at about St. 29, peaks at St. 34–35, and is complete by St. 38 (Hamburger, Brunso-Bechtold & Yip, 1981). A slightly later onset, peak and end of the period of cell death might be expected for the more caudal lumbosacral d.r.g.s studied here (Hamburger & Levi-Montalcini, 1949).

Dermatomes were mapped in St. 40 embryos by recording extracellularly from the dorsal roots while the skin was brushed with a single bristle; occasionally the skin was also stimulated electrically. Since specialized receptors have not yet differentiated by St. 40 (Saxod, 1978) no attempt was made to distinguish among different sensory modalities. Fig. 2 shows sample recordings obtained from dorsal root LS 2 and LS 7 when the skin was brushed in different regions.

In physiological experiments such as these it is important that the types of stimulation and recording used allow full and accurate determination of the dermatomes. Several lines of evidence suggest that the methods used here were adequate. In two St. 40 embryos dermatomes were mapped both by brushing the skin and by stimulating it electrically. Fields mapped with electrical stimulation overlapped completely those determined by brushing, but were usually slightly larger. The electrical stimulus never evoked activity from regions of skin far outside the fields mapped by brushing. The observed coincidence of dermatomes mapped with two such diverse techniques provides some measure of confidence that each method of stimulation activated most of the skin sensory nerve endings.

In some species a small fraction of d.r.g. axons travel to the periphery via the ventral roots (Coggeshall, 1980). If this were the case for skin afferents in chicks, recording from dorsal roots alone could underestimate the size of the dermatome. To test for d.r.g. axons in the ventral roots, in two St. 39 embryos the ventral roots were cut near the cord and placed in suction electrodes in the recording configuration used for dorsal roots. No action potentials were recorded when the skin was brushed, although large compound action potentials were evoked by stimulating the spinal nerves distal to the d.r.g.s. Thus, few, if any, skin sensory axons travel to the periphery in the ventral roots, and it should be adequate to record only from the dorsal roots.

The size and location of dermatomes in St. 40 embryos mapped physiologically were consistent from chick to chick, as shown in the summary maps of Fig. 3. Dermatomes of adjacent d.r.g.s partially overlapped one another, producing an orderly progression in the location of dermatomes along the limb. For example, the dermatomes of lumbosacral d.r.g.s 1–3 overlapped extensively to innervate skin on the anterior thigh, knee, and shank. However, the dermatome of d.r.g. 2 usually extended farther distally than that of d.r.g. 1 but never extended past the shank and onto the foot; the dermatome of d.r.g. 3 was shifted even farther distally, having only a small representation on the thigh, but occasionally reaching the toes. Most axons from d.r.g.s 4–6 bypassed the thigh entirely to innervate skin on the distal shank and foot; d.r.g. 4 generally innervated more anterior parts of the shank and foot than d.r.g. 6. D.r.g.s 7 and 8 innervated the posterior thigh and shank, never innervating

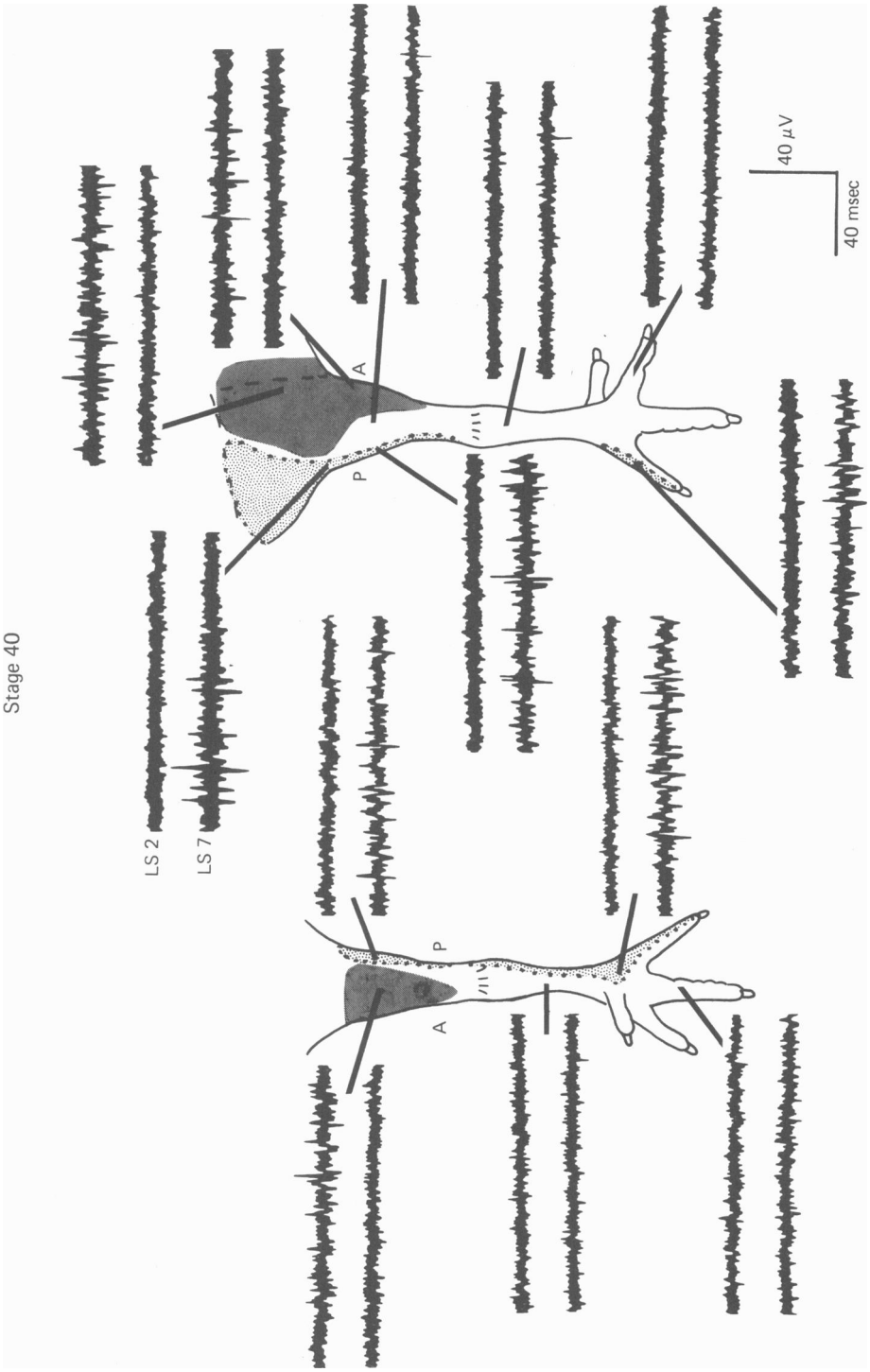

Fig. 2. Sample recordings obtained from lumbosacral dorsal roots 2 (top traces) and 7 (bottom traces) when the skin of a St. 40 embryo was brushed in different regions. The drawings show the medial (left) and lateral (right) view of the same leg. Shaded and stippled areas indicate the location of the dermatome of d.r.g.s 2 and 7, respectively.

skin preaxial to the femur; axons from d.r.g. 7 but never from d.r.g. 8 often extended onto the most posterior toe (toe 4).

While the dermatomes of adjacent d.r.g.s overlapped extensively, the dermatomes of widely separated d.r.g.s often abutted at a common border with little overlap. For example, the dermatomes of d.r.g.s 1 and 2 overlapped for 66 (±29, S.D.; $n = 4$) and 53 (±21) % of their respective areas. In contrast, the dermatomes of d.r.g.s 2 and 7 overlapped for only 1 % (±3; $n = 7$).

Development of dermatomes. Similar experiments were performed in progressively younger embryos: St. 37–38, St. 33–34, St. 31 and St. 29. In St. 34 and younger embryos responses evoked by brushing were often weak, making it difficult to define the dermatome borders. Thus in these young embryos dermatomes were mapped by electrical stimulation only; in each embryo 90–150 spots were sampled for each d.r.g. It appeared that the electrical stimulation activated only the sensory nerve endings and not the underlying nerve trunks, for muscle contraction and limb movement were not evoked by stimuli applied to the skin. Dermatome mapping in St. 28 embryos gave inconsistent results; brushing the skin did not elicit detectable impulses and in some preparations electrical stimulation was also not effective. It is not clear whether failure to activate impulses was due to a genuine inexcitability of the immature innervation or resulted from damaging the extremely fragile dorsal roots. In contrast, brushing the skin of St. 29 embryos activated impulses in sensory axons of some d.r.g.s, and electrical stimulation evoked consistent and robust responses (Fig. 4). Therefore, St. 29 embryos, near the onset of cell death, were the youngest in which dermatomes were mapped in detail.

The location and amount of overlap of dermatomes mapped at all stages were similar to the mature pattern, with few indications of random innervation or innervation extending beyond the mature dermatome borders (Fig. 5). As in mature embryos d.r.g.s 1–3 always innervated skin on the anterior thigh, knee and shank, and never innervated skin on any part of the foot. Most axons from d.r.g.s 4–6 bypassed the thigh entirely to innervate the distal shank and foot. D.r.g.s 7 and 8 innervated the posterior thigh and shank, and never innervated skin preaxial to the femur. At all stages the dermatomes of d.r.g.s 2 and 7 abutted at a common border, overlapping maximally at St. 33–34 for 7 (±6, $n = 5$) and 10 (±7) % of their respective areas. This small increase in overlap may not be real, since electrical stimulation, which delineates slightly larger dermatomes, was used to map St. 33–34 dermatomes, while St. 40 dermatomes were mapped by brushing.

There were two apparent differences between dermatomes in St. 29 and St. 40 embryos. First, d.r.g. 3 innervated the toes less frequently in St. 40 embryos. However, it seems unlikely that this dermatome actually shifted location during development since the proportion of embryos in which d.r.g. 3 innervated the toes varied and did not decrease consistently between St. 29 and St. 40. Secondly, d.r.g.s 4–6 innervated the proximal, posterior shank less frequently in the older embryos, perhaps due to an expansion of skin in this region as the shank elongated (see below).

Skin movements

Although axons from lumbosacral d.r.g.s 4–6 never functionally innervate skin on the thigh, the possibility existed that earlier in development these axons contact

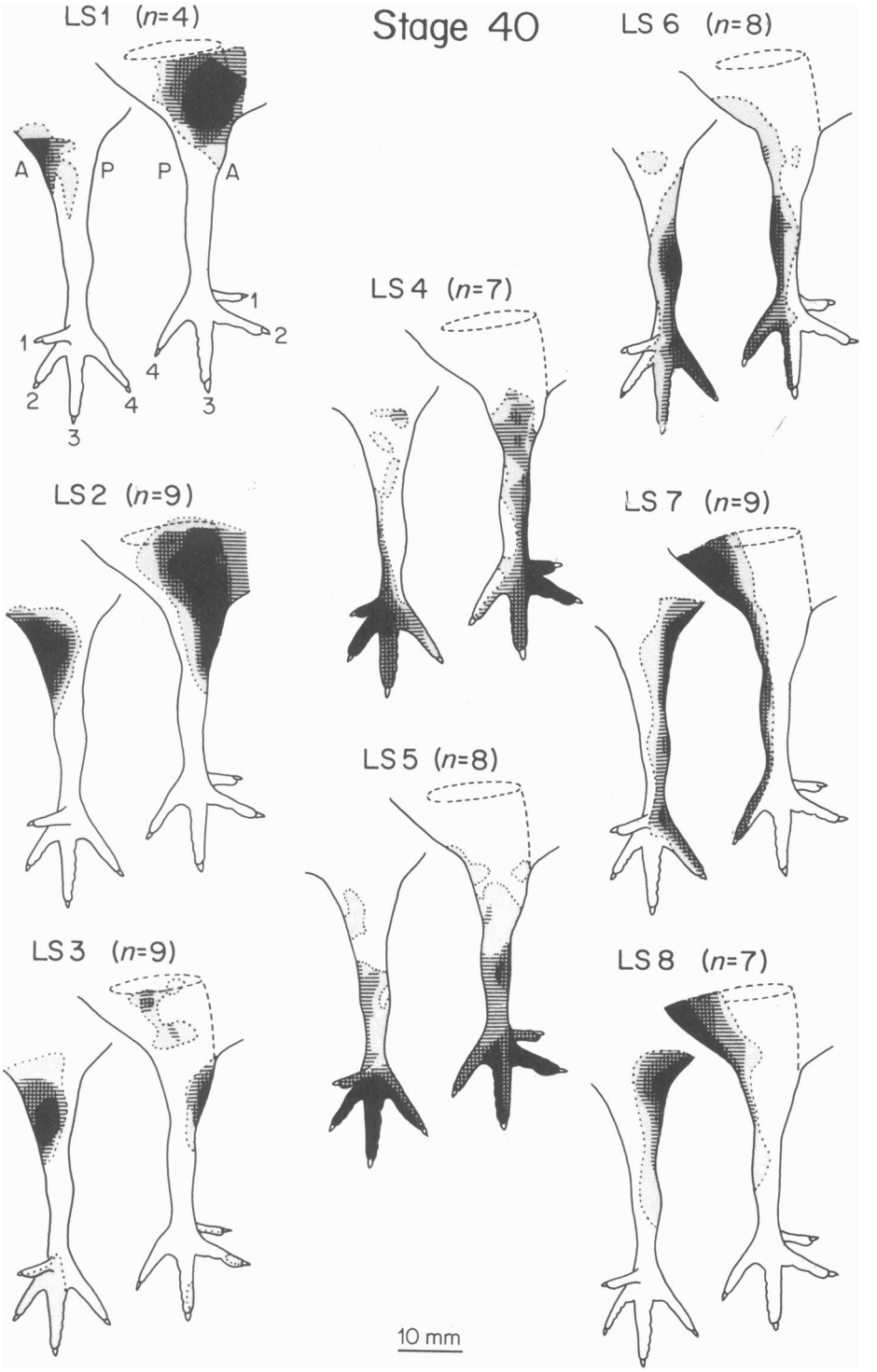

Fig. 3. Summary maps of dermatomes determined by brushing the skin of St. 40 embryos. Each pair of legs shows the location of the dermatome of one d.r.g. on the medial (left) and lateral (right) skin of the same leg. Dermatomes are shaded to indicate the percentage of embryos having innervation in each region: stippled, 10–25%; striped, 26–55%; hatched, 56–80%, filled, 81–100%. For each d.r.g. the number of embryos sampled is given in the parentheses.

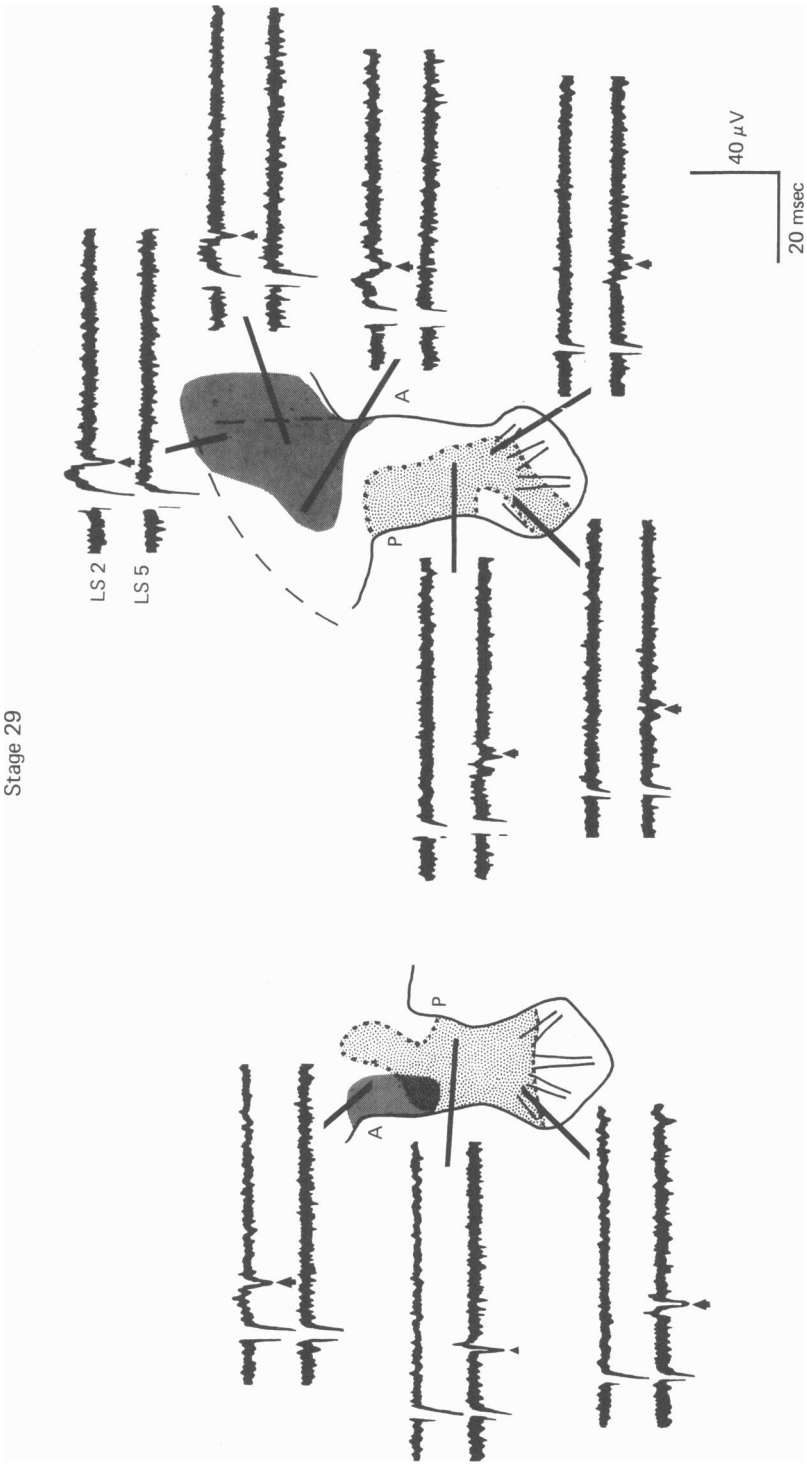

Fig. 4. Sample recordings obtained from lumbosacral dorsal roots 2 (top traces) and 5 (bottom traces) when the skin of a St. 29 embryo was stimulated electrically in different regions. Legs are shown in the same orientation as in Figs. 2 and 3. Shaded and stippled areas indicate the location of the dermatome of d.r.g.s 2 and 5, respectively. Arrows indicate compound action potentials.

nearby thigh skin and are then towed to their distal fields on the shank and foot by skin movements as the limb develops (Harrison, 1899).

This possibility was eliminated by carbon marking experiments (see Methods). As shown in Fig. 6 there were no massive distal skin movements as the leg elongated and changed shape. For example, particles placed on skin covering the knee in St. 27–28 embryos remained over the knee in older embryos, showing that the same skin covered this region throughout the period studied.

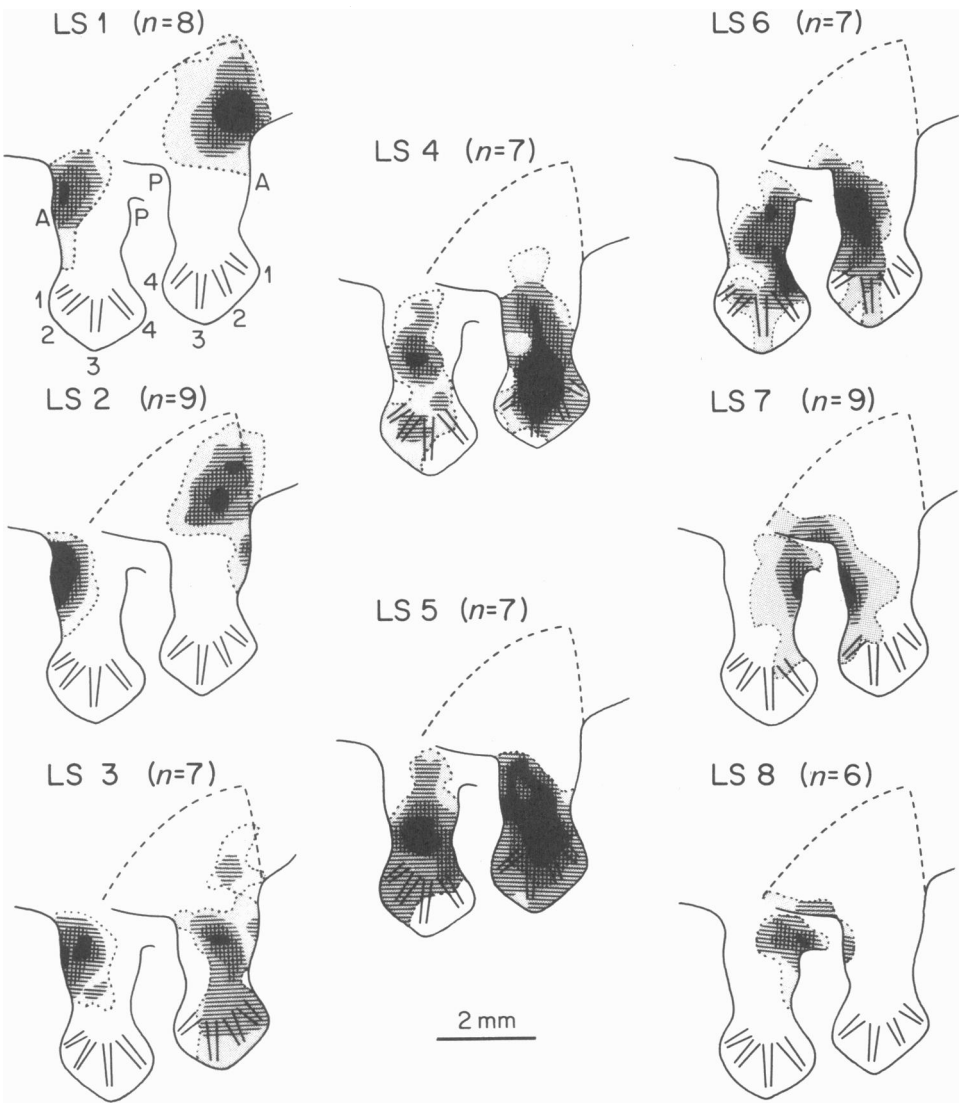

Fig. 5. Summary maps of dermatomes determined by electrical stimulation in St. 29 embryos. Orientation of the legs and shading patterns are the same as in Fig. 3.

There were, however, several types of small skin movements that could affect dermatome development. First, it appeared that as the limb grew and added new skin, regions that were initially adjacent moved slightly apart. Secondly, skin of the posterior edge of the limb apparently expanded distally as the leg elongated; particles initially placed in this region were spread out distally in older embryos. If sensory

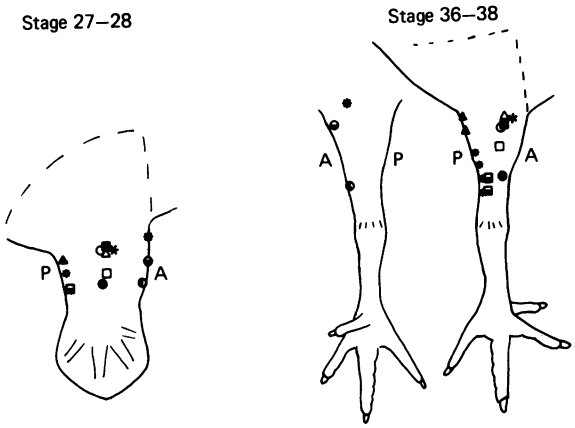

Fig. 6. Summary of carbon marking experiments. Each symbol represents the location of a carbon particle placed on the lateral surface of the leg of one St. 27–28 embryo (left) and subsequently identified when the embryo had developed to St. 36–38 (right). Both the medial (left) and lateral (right) view are shown for the St. 36–38 leg. No large skin movements were detected between St. 27–28 and St. 36–38.

nerves in this region also spread distally, then skin movements could be responsible for the decreased innervation from d.r.g.s 4–6 on the proximal posterior shank in older embryos. Thirdly, there was a slight rotation of skin about the limb axis in older embryos. This movement is probably more apparent than real, since it was impossible to place limbs of older embryos in exactly the same orientation as the younger ones.

None of the observed skin movements was large enough to account for the distal location of the dermatomes of d.r.g.s 4–6 by the towing of axons that initially contacted proximal skin. It appears that axons from each d.r.g. establish their dermatome in the appropriate skin from the outset and continue to innervate essentially the same skin throughout embryonic development.

Projection pathways

The physiological techniques used above are not suitable for examining the initial outgrowth of d.r.g. axons before functional innervation is established. The possibility existed that axons grow out randomly, but only axons that contact appropriate skin survive and become functional. Such errors in axonal projection would not be detected with physiological techniques. To test for early projection errors the pathways of axons from each d.r.g. were determined for St. 26–38 embryos by labelling each d.r.g. with the marker horseradish peroxidase (HRP) (see Methods). This technique necessarily labels muscle afferents as well as skin afferents, but only the cutaneous pathways will be described.

There are technical limitations to such labelling studies. HRP injections seldom labelled all of the neurones in a d.r.g., so projections made by only a few axons might possibly have been overlooked. However, it is unlikely that incomplete labelling would preclude the detection of projections made by a substantial number of axons; since the neurones that project out each nerve trunk are widely distributed throughout the d.r.g. (Honig, 1982), any injection should label at least some axons in each major projection.

Mature projection pattern. St. 36½–38, rather than St. 40, embryos were used to study the mature pattern of axonal projections because the older, larger embryos deteriorated before the HRP had travelled far enough peripherally. Pathways were determined in three to seven mature embryos for each d.r.g. Skin sensory axons from each d.r.g. projected through the thigh via a characteristic set of cutaneous nerve trunks as well as via the mixed nerves, n. fibularis and n. tibialis (Fig. 7). Within each cutaneous nerve trunk labelled axons from one d.r.g. tended to stay together in a tight cluster, distinct from the unlabelled axons from other d.r.g.s. Although the contribution of d.r.g.s to each nerve trunk was not studied quantitatively the general impression was that of a continuum of projection pathways in the limb; axons from the most rostral d.r.g.s travelled in the most anterior nerve trunks, while axons from more caudal d.r.g.s travelled in more posterior nerve trunks. For example, d.r.g. 1 sent most of its skin sensory axons out the most anterior cutaneous nerve in the thigh, the cutaneous femoralis lateralis, and sent fewer axons out the slightly more posterior cutaneous femoralis medialis. In contrast, d.r.g. 3 had a larger projection in the cutaneous femoralis medialis than in the cutaneous femoralis lateralis. Similar observations have previously been reported by Honig (1982). The majority of axons from d.r.g.s 4–6 travelled to the periphery via n. fibularis and n. tibialis, with axons from d.r.g. 4 lying anterior to those from d.r.g. 6. The most caudal ganglia, d.r.g.s 7 and 8, sent their major projections out the most posterior nerves, the cutaneous plantaris surae and cutaneous femoralis caudalis, while d.r.g. 7 also sent axons down the posterior part of n. tibialis.

Early projection patterns. The projection pathways of each d.r.g. were examined prior to cell death in four to nine St. 26–28 embryos, as well as in several St. 29–36 embryos and were similar at all stages. Axons from the most rostral d.r.g.s reached the skin by about St. 27; at this stage the most posterior cutaneous nerves had not yet formed into compact nerve trunks. For each d.r.g. the projection pathways as well as the relative distribution of labelled axons among these pathways were similar to those observed after the bulk of cell death (Fig. 7) and at intermediate stages (see also Honig, 1982). Even at the earliest stages before the cutaneous nerves were distinct, labelled axons were found only in the region of the limb in which their characteristic nerve trunks would soon develop.

Pettigrew, Lindeman & Bennett (1979) have reported that in the chick the wing initially receives innervation from adjacent non-brachial segments, which is lost during the period of cell death (but see Oppenheim, 1981). To test the possibility that extra segments may initially contribute skin sensory innervation to the hind limb the last thoracic (T 7) and 9th lumbosacral d.r.g.s were injected with HRP in five St. 26–27 embryos. Labelled axons from only one (LS 9) of the ten injected ganglia entered the limb. This is not convincing evidence for an initial diffuse projection since

both T 7 and LS 9 occasionally supply innervation to the hind limb in mature embryos, as mentioned above.

Together, these HRP studies indicate that skin afferents from each d.r.g. grow directly to their target skin along a defined set of pathways from the outset without making numerous projection errors. There are few, if any, misrouted skin sensory axons that fail to become functional or that are eliminated by cell death.

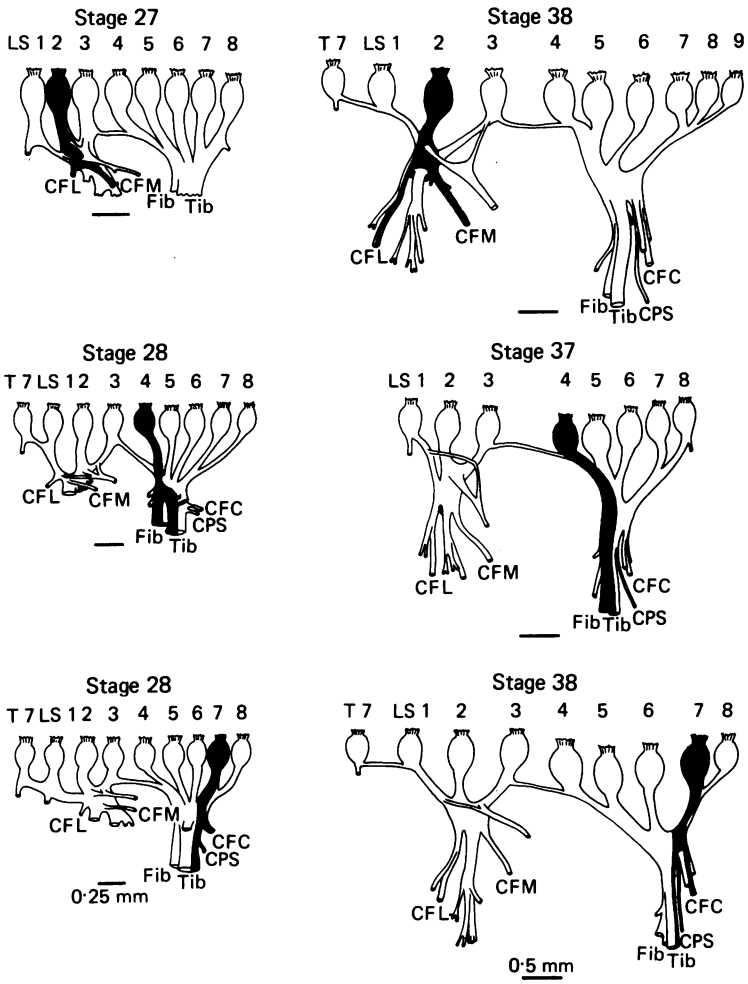

Fig. 7. Diagrams of the projection pathways of axons from lumbosacral d.r.g. 2 (top), d.r.g. 4 (middle), and d.r.g. 7 (bottom) before (left) and after (right) the bulk of cell death. In each example, a single d.r.g. was injected with HRP, the limb sectioned, and the axonal pathways reconstructed. Shading shows the location HRP-labelled axons; for simplicity labelled muscle afferents (except those in n. fibularis and n. tibialis) have been omitted. CFL, cutaneous femoralis lateralis; CFM, cutaneous fermoralis medialis; CFC, cutaneous femoralis caudilis; CPS, cutaneous plantaris surae; Fib, n. fibularis; Tib, n. tibialis.

Dermatomes viewed morphologically

In some embryos the HRP travelled far enough in the periphery to allow the dermatome to be viewed directly in a whole-mount preparation of the skin. HRP labelling of sensory axons in the skin was somewhat capricious and seldom worked in embryos older than St. 31. Successful preparations showed that sensory axons reached the skin at about St. 27. Axons from a single d.r.g. invaded the skin at one or more characteristic spots, which were often widely separated (arrows, Fig. 8). After

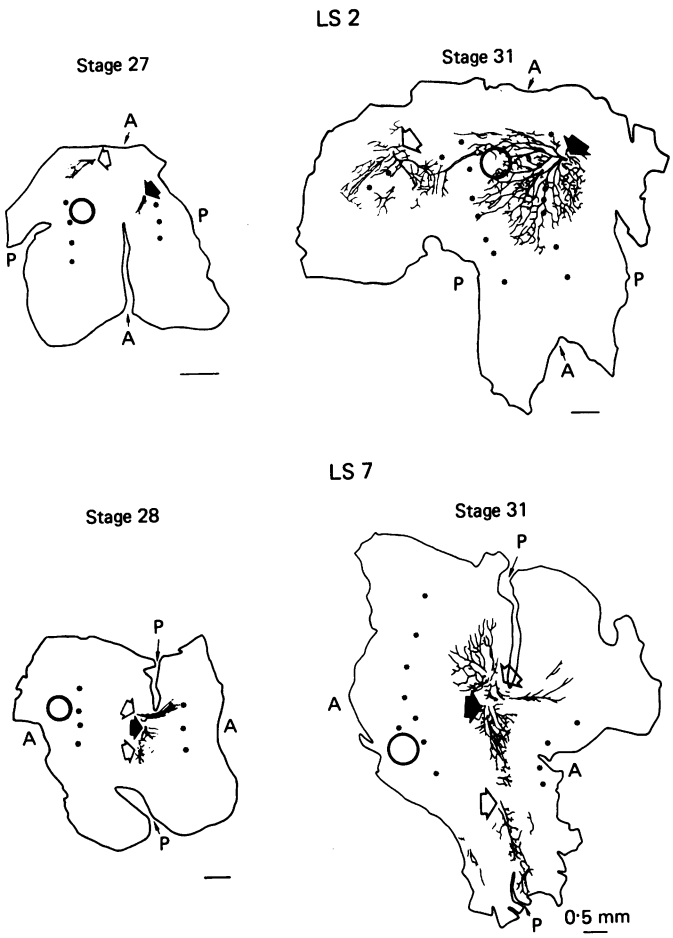

Fig. 8. Camera lucida drawings of HRP-labelled axons from lumbosacral d.r.g. 2 (top) and d.r.g. 7 (bottom) in whole mounts of skin. In the top figures the skin was cut down the posterior edge of the leg, removed and opened out flat; the two edges designated 'P' were joined in the embryo and the central region of skin designated 'A' covered the anterior part of the leg. In the bottom figures the skin was cut down the anterior edge of the leg; the two edges designated 'A' were joined in the embryo and the central region of skin designated 'P' covered the posterior part of the leg. Stars show the location of carbon particles placed on skin over the femur and tibia to serve as landmarks; circle shows the approximate location of the knee; large arrows indicate the points at which the nerve trunks invade the skin.

this initial contact the dermatomes then enlarged, added fine axonal branches. Thus, the dermatome of each d.r.g. usually developed from several arborizations that enlarged and merged together. The dermatomes seen morphologically were about the same size, shape and location as those mapped physiologically at the same stage, and labelled axons were never observed to travel long distances in the skin outside the usual dermatome area.

DISCUSSION

Segmentation of d.r.g.s. In studies that involve dermatome mapping the cells within a d.r.g. are treated as a unique, discrete neurone pool. However, the d.r.g. may instead be an arbitrary subdivision of a continuous column of cells. During development d.r.g.s arise from the unsegmented neural crest, with segmentation into d.r.g.s being imposed by the somites (Detwiler, 1934), and the apportioning of cells into d.r.g.s can be quite variable (Ygge, Aldskogius & Grant, 1981). The gradual shift of both the axonal projection pathways (see also Honig, 1982) and the location of dermatomes of adjacent d.r.g.s seen here and by others (see below) suggests that the d.r.g. cells are indeed a continuum. There is also a progressive shift in the location of the skin fields of adjacent rootlets within a single dermatome (Kuhn, 1953). Furthermore, in some species the entire dermatome pattern may be shifted rostrally or caudally by one or more spinal segments in different individuals (Fletcher & Kitchell, 1966; Dykes & Terzis, 1981). Despite this reservation it is convenient to consider the neurones within a d.r.g. as a discrete unit.

Location of mature dermatomes. The segmental pattern of dermatomes determined here for the hind limb of mature chick embryos is similar to the limb dermatome pattern in other species. As in most species the dermatomes of adjacent d.r.g.s partially overlap one another, while dermatomes of more widely separated d.r.g.s may abut at a common border. There is an orderly progression in the location of dermatomes along the limb (Sherrington, 1893; Kaiser, 1924; Aguilar *et al.* 1973; Dykes & Terzis, 1981), and some of the distal dermatomes are not continuous with the dorsal mid line (Sherrington, 1893; Kaiser, 1924; Kukulinsky & Brown, 1979; Dykes & Terzis, 1981; but see Keegan & Garret, 1948; Fletcher & Kitchell, 1966). The establishment of this complex dermatome pattern poses several interesting developmental problems.

Development of sensory function. Although the dermatome pattern mapped in St. 40 embryos was taken to be the mature pattern, sensory innervation is not truly mature at this stage; most skin sensory axons are not yet myelinated (Saxod & Verna, 1979) and specialized receptors have not yet developed (Saxod, 1978). Nevertheless, the skin sensory innervation is clearly functional by St. 29, when action potentials can be evoked by brushing the skin and when touch-evoked reflexes first appear (Hamburger & Levi-Montalcini, 1949). Precocious onset of skin sensory function has also been reported in other species (reviewed in Bradley & Mistretta, 1975), suggesting that the specialized 'receptors', which appear late in development are not required for mechanosensitivity *per se*, but may instead determine the response properties of the sensory neurones (Mendelson & Lowenstein, 1964; Kasprzak, Tapper & Craig, 1970; Gottschaldt & Vahle-Hinz, 1981).

Development of dermatomes

Dermatome development can be considered to occur in two consecutive stages. Skin sensory axons must first grow through the limb to the skin, and then must ramify within the skin to establish the dermatome. These two steps are not necessarily controlled by the same mechanisms. The basic finding reported here is that skin sensory axons accomplish both steps rather precisely, making few projection errors either within the limb (see also Honig, 1982) or skin. These findings, while not actually elucidating the means by which axons find their way through the limb or skin, rule out several possible mechanisms by which dermatomes could be established.

Unlikely mechanisms for dermatome development. First, it is clear that dermatomes are not shaped by cell death and the elimination of random or excessive projections of skin sensory axons. The location of dermatomes and the axonal projections (see also Honig, 1982) are the same before and after the peak of cell death. Similarly, cell death appears to be of little importance in shaping the motor projection pattern in the chick hind limb; motor axons grow to and innervate the appropriate muscles from the outset (Landmesser & Morris, 1975: Landmesser, 1978*b*).

Furthermore, the results presented here show that axons do not grow to the nearest available skin in a strict rostrocaudal order, with axons being towed distally, away from the dorsal midline, by skin movements, as originally proposed by Harrison (1899). Earlier observations by Taylor (1943) in the frog cast doubt on this mechanism; he noted that some axons grew for long distances in the limb before contacting the periphery. The present study provides experimental evidence that axons that innervate distal skin in mature embryos do indeed bypass nearby skin on the thigh from the outset, and demonstrates directly that skin does not migrate distally on the limb after being contacted by sensory axons. Skin movements could, however, influence dermatome development in other ways. Some authors have suggested that each d.r.g. innervates skin derived embryologically from the same segmental level (Cole *et al.* 1968), implying that skin from lumbosacral segments 4–6 migrates to the shank and foot early in development, before innervation is established (see also Amprino & Camosso, 1958). Furthermore, small skin movements must occur as the limb grows and new skin is added. Verna & Saxod (1979) have shown that migrating mesenchymal cells *in vitro* can affect the orientation of neurite growth by dragging along attached neurites. *In vivo* a similar phenomenon could increase the space between sensory nerve endings in the skin and enlarge the dermatome as the limb grows.

Possible mechanisms for dermatome development. Skin sensory axons from all eight hind limb d.r.g.s grow through the limb along the correct pathways from the outset (see also Honig, 1982). Honig (1982) has recently reviewed the possible mechanisms that could guide or direct the growth of skin sensory axons through the limb (see also Landmesser, 1981). Since the results presented here do not allow further distinction among the proposed mechanisms, this aspect of dermatome development will not be discussed in more detail.

Upon reaching the skin, sensory axons ramify within the correct region of skin and do not grow extensively into inappropriate regions. The variability in overlap of dermatomes at different embryonic stages is small, and at all stages each dermatome

occupies a characteristic location with respect to various landmarks on the limb. The mechanisms that determine the boundaries of dermatomes are unknown. One possibility is that developing sensory axons are specifically matched with or constrained to grow within a particular region of skin, perhaps the skin derived from the same segmental level (Cole *et al.* 1968). Such constraints on axon growth once innervation is established are well-documented in other species; in young rats and adult salamanders intact touch sensory axons sprout readily only within skin in a restricted 'domain' (Diamond, 1982).

Alternatively, the dermatome borders may be established by competition between ingrowing axons. This mechanism appears to be important in the development of limb dermatomes in the frog, since removal of limb d.r.g.s in tadpoles allows axons of the adjacent non-limb d.r.g.s to grow into skin on the limb and establish functional innervation (Miner, 1956; Frank & Westerfield, 1979; Davis & Constantine-Paton, 1981). That dermatomes from several d.r.g.s overlap in the chick does not preclude competitive interactions from having a role in establishing the dermatome pattern. Dermatomes that overlap extensively are derived from axons that travel together in a single nerve trunk, such as the cutaneous femoralis lateralis. Competition among axons within one nerve trunk may distribute the endings uniformly within the dermatome, as suggested by Ramón y Cajal (1919), rather than set up the dermatome borders. Axons travelling in different nerve trunks invade the skin at widely separated points; as the fields of the various nerve trunks enlarge they will eventually meet and may then compete for skin. Such competition could set up the boundary between the dermatomes that abut, such as those of d.r.g.s 2 and 7, without requiring any selective matching between sensory nerves and skin.

An additional factor that could influence the dermatome boundaries is the dermis. Feathered skin on the thigh is bounded by regions of nearly featherless skin, the aptera, which separate skin on the thigh from skin on the back, trunk, and shank. Development of dermis in such unfeathered areas lags behind dermis development in feathered regions (Sengel, 1976). Since the dermis is the first layer of skin contacted by ingrowing sensory axons and since dermis appears to possess positional or morphogenetic information (Carlson, 1975; Sengel, 1976), these limb aptera might limit growth of sensory axons in the skin. Some of the skin fields do indeed seem to follow these boundaries, and although quantitative studies were not performed here, the general impression was that these unfeathered regions were only poorly innervated. Investigations are currently underway to distinguish which, if any, of these mechanisms determines the dermatome boundaries.

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