The early development of the primary sensory neurones in an amphibian embryo: a scanning electron microscope study

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SUMMARY

We have described the development of the primary sensory system of the trunk region of *Xenopus laevis* embryos from larval stages 21 to 32. The system is based upon Rohon-Beard and extramedullary cells, which have central axons forming a dorsolateral spinal tract and peripheral neurites which innervate the skin. The pioneer axons of the central tract grow along the outer surface of the cord at stage 22. These pioneer axons may be used by secondary axons as a growth substrate. As the tract forms it is covered by the radially expanding distal processes, 'end feet', of the ependymal cells of the cord. Cell bodies of the extramedullary cells bulge out of the cord surface, and are first seen between the newly segmented myotomes, at stage 24. Peripheral neurites from these extramedullary cells grow out laterally from the cord. The Rohon-Beard cells, located within the cord, produce similar peripheral neurites which grow laterally with the extramedullary cell neurites, using them as a substrate. The neurites form bundles which coincide with the intermyotomes and are periodically spaced. The growth cones of these neurites contact the outer surface of the myotomes and proceed ventrally, first on the myotomes and then on the basal lamina of the skin. 'Pioneer' neurites are used by later neurites as a growth substrate, but not to the exclusion of all other substrates. The neurites form a plexus on the skin's basal lamina and contact the underlying epidermal cells through holes in the basal lamina. These holes occur in positions over the intercellular boundaries of the epidermal cells.

INTRODUCTION

The primary sensory system of embryonic and early larval *Xenopus* is based upon Rohon-Beard cells, the somas of which lie dorsally within the spinal cord (Nieuwkoop & Faber, 1956; Hughes, 1957; Muntz, 1964; Roberts & Hayes, 1977; Roberts & Clarke, 1982). Peripheral neurites from Rohon-Beard cells exit from the spinal cord to innervate the skin (Hughes, 1957; Muntz, 1964; Roberts & Hayes, 1977). On the skin’s basal lamina they form a nerve plexus, from which processes penetrate between the cells of the epidermis (Roberts & Hayes, 1977). A second class of neurones which produce peripheral neurites in the trunk region of early *Xenopus* embryos has been described (Hughes, 1957; Muntz, 1964).

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These extramedullary neurones are large cells lying in a dorsolateral position just outside the cord. Both the Rohon-Beard cells and the extramedullary cells have ascending and descending axons in the dorsolateral tracts of the spinal cord (Hughes, 1957; Muntz, 1964). The ascending axons project to the hindbrain (Roberts & Clarke, 1982).

We have used the scanning electron microscope to follow the outgrowth of the pioneer neurites and axons from the Rohon-Beard and extramedullary cells. The growth of the peripheral neurites, through the myotomes and onto the skin which they innervate, has been followed. On the basis of this detailed account we consider possible factors which could influence the pathway followed by these neurites. The character and behaviour of the growth cones of these neurites are considered in the companion paper (Roberts & Taylor, 1983).

METHODS

Embryos were obtained by induced breeding in adult pairs of *Xenopus* after injection with chorionic gonadotrophin. The embryos were removed from their egg membranes and staged according to the normal tables of Nieuwkoop & Faber (1956). Entire embryos were fixed for periods of less than 24 h in 5 % glutaraldehyde in 0·05 M-cacodylate buffer at pH 7·3. After fixation, embryos were washed in 0·05 M-cacodylate for at least 5 h.

The skin was removed from the embryos in a bath of cacodylate buffer using fine pins to tease away the skin. Myotomes were dissected after skin removal using fine pins.

Preparations of skin, myotome and embryo were dehydrated through an ethanol series and then through acetone. The preparations were critical-point dried using CO₂ in a Polaron Critical Point Dryer, mounted on aluminium stubs, and sputter coated with gold. All microscopy was done on a Cambridge Stereo-scan S4 scanning electron microscope.

RESULTS

1. *The development of the primary sensory system of the trunk*

In embryos of *Xenopus* a strong rostrocaudal gradient of development exists. As a result, sensory neurites emerge later more caudally. In our account we follow the development of the more precocious rostral segments but may illustrate this using photographs drawn from more caudal segments of older embryos.

We have examined embryos from stages 21–32 of Nieuwkoop & Faber (1956). All embryos were skinned and in many the myotomes were removed to expose the lateral aspect of the spinal cord.
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Stages 21 to 22

Much of the spinal cord is concealed by unsegmented paraxial mesoderm. The exposed spinal cord shows no evidence of any surface features suggesting the presence of a spinal tract.

Stages 22 to 23

The most rostral paraxial mesoderm has segmented to form eight myotome blocks on each side of the spinal cord. Caudal to these there is a continuous band of mesoderm lying lateral to the cord. In transverse section the cord is not rounded but has two distinct ridges at the level of the dorsal edge of the paraxial mesoderm (Fig. 1, Fig. 7 in Schroeder, 1970). When viewed with the S.E.M. large rounded structures are partly visible on the dorsal aspect of these ridges (Fig. 2). Some of these bodies have longitudinal processes which extend along the cord surface (Fig. 2). We suggest that these bodies are neurone somas and that the processes are their central axons. The axons are only found in the region of the ridges in the dorsolateral quadrant of the spinal cord and are generally oriented rostrocaudally although slight meandering does occur. The axons, which are exposed on the cord surface during their growth, occur on the outer surface of the ependymal cells.

Stage 24

Longitudinal axons arising from neurones are visible on the surface of the midtrunk region of the spinal cord. In rostral regions there are increasing numbers of axons joining the ‘pioneers’ seen at stage 22. A tract can be discerned in these rostral regions which becomes increasingly obvious as development proceeds (Fig. 3). Where the axons are fully exposed on the cord surface they can be seen to fasciculate but will separate and form new fascicles in an apparently random manner. Occasionally growth cones are seen growing along the ‘pioneer axons’ (Fig. 4). These growth cones are small compared to the growth cones of the peripheral neurites of Rohon-Beard cells, and produce few long filopodia, except along the axon bundle upon which they are growing. The possible preference of axons for each other is also shown by the close association of the axons within axon bundles (Fig. 5). Anchoring filopodia from axons wrap around adjacent axons to form coherent fascicles.

As the tract increases in size, it is concealed by a sheet of thin cellular processes produced by the ependymal cells of the spinal cord. The distal parts of the ependymal cells, the ‘end feet’, expand concentrically to form a thin cellular plate (Nordlander & Singer, 1982a,b). As this expansion of the end feet proceeds the component axons of the dorsolateral tract are enclosed, incorporating them into the spinal cord (Fig. 6). The expansion of the ependymal end feet only occurs in the dorsolateral spinal cord in the region of the tract axons. When the
Figs 1–5.
expansion is completed S.E.M. observation of the developing tract is no longer possible.

By late stage 25, in the rostral parts of the cord, at the level of the dorsal limits of the myotomes, processes start to grow away from the spinal cord to the periphery (Figs 7 and 9). These can be seen to be of two types; large neurites (0.7 μm–1.5 μm diameter) which usually have enlarged proximal regions protruding from the cord surface (Fig. 8), and finer neurites (0.7 μm diameter) which grow out directly from the dorsal tract (Fig. 16).

These outgrowths are considered to be sensory neurites on the basis of the following criteria:

(i) The cell bodies have a similar morphology and position to the cell bodies of sensory neurones identified in other studies (Coghill, 1914; Dushane, 1934; Hughes, 1957; Roberts & Hayes, 1977; Lamborghini, Revenaugh & Spitzer, 1979).

(ii) Processes from these cell bodies form a dorsolateral spinal tract, in the position of the sensory tract described by other workers (Hughes, 1957; Schroeder, 1970; Hayes, 1974; Roberts & Hayes, 1977; Katz & Lasek, 1978, 1979; Roberts & Clarke, 1982).

(iii) Processes from this dorsolateral tract grow out over the myotomes onto the skin's basal lamina. No evidence has been found that these processes grow to any other targets, e.g. muscle (Roberts & Hayes, 1977).

(iv) There is no evidence of any further large scale outgrowth of neurites onto the basal lamina at later stages, so the neurites which form the plexus at this stage are those which have been shown to conduct sensory impulses from free nerve endings in the skin (Roberts & Hayes, 1977).

The finer neurites usually fasciculate with the larger neurites as they leave the

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Fig. 1. A drawing of a transverse section from a stage-25 embryo at mid-trunk level, caudal to the most recently segmented myotome. Arrows indicate the lateral ridges. (sk) skin, (M) myotomal tissue, (No) notochord.

Fig. 2. A stage-23 embryo viewed from dorsolateral, i.e. from above the spinal cord lateral ridge. A large cell body (1) which has a caudally growing axon arising from it, lies in the central photograph. Three other cell bodies (2, 3, 4) are also visible positioned on the lateral ridge. r, rostral, d, dorsal. ×2000.

Fig. 3. The spinal cord viewed laterally, has been exposed by removing the myotomes. Areas of dorsal tract (DT), can be seen in a restricted region of the spinal cord surface. The axons of the tract are not covered by basal lamina. Two neurite outgrowths (►), which have been detached from the myotomes, arise dorsolaterally from the cord. Parts of three ventral roots and exposed ventral tract (VT) are also visible. (N), notochord, (sc), spinal cord, (nc), neural crest cell. ×540. Stage 25/26.

Fig. 4. Two growth cones (►), growing rostrally along a 'pioneer' axon, which is lying on top of the unexpanded end feet of ependymal cells. Dorsal to this a bundle of axons is partly concealed from view. ×2400. Stage 24.

Fig. 5. An exposed region of dorsal tract in a stage-26 embryo showing an axon bundle lying over an expanding ependymal cell process (1). A neighbouring bundle is being concealed by a second ependymal cell process (2). ×3000.
Figs 6–9.
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cord (Fig. 8). Most of the outgrowths arise at the intermyotomes and are oriented perpendicular to the axis of the cord.

Stages 24 to 25

Ependymal cell end feet have concealed the rostral regions of the tract. The groups of neurites leaving the cord at midtrunk level are well established and can be seen on the outer surface of the myotomes (Fig. 11). Here the outgrowth fascicles usually separate, the neurites diverging and refasciculating as they course around the outer myotome surface (Fig. 11). The neurites are characterized by their large numbers of fine filopodia which appear to anchor them to the myotome surface. Their growth cones are large and flattened, commonly producing filopodia which appear to be in close contact with the myotome surface (Figs 10 and 11, and see Roberts & Taylor, 1982b). These growth cones may be complex if they are formed from two or more growth cones at the end of a neurite fascicle (Fig. 9).

In caudal regions, neurites have begun to grow out from the cord where the myotomes have segmented.

During later parts of this developmental stage neurites have grown from the outer surface of the myotomes onto the rostral part of the skin's basal lamina. Small, broken, terminal parts of growth cones, often with part of the neurite attached to them are found adhering to the basal lamina (Fig. 14). These growth cones have microspikes which extend over the surrounding basal lamina. There does not appear to be any directional bias to this microspike production. These growth cones must adhere strongly to the basal lamina as they appear relatively undisturbed by peeling off the skin.

Stages 25 to 26

Myotome segregation and the outgrowth of neurites proceed in more caudal

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Fig. 6. A lateral view of exposed spinal tract, clearly showing the expansion of ependymal cell end feet to cover the exposed tract. ×1800. Stage 26.

Fig. 7. A dorsal outgrowth from the cord with two growth cones still intact after removal of the myotomes on which they were growing. Ventral to the outgrowth is an exposed region of longitudinal tract. ×2175. Stage 25/26.

Fig. 8. Two outgrowths at an intermyotome. The myotome rostral to the outgrowth has been removed to expose the outgrowth on the more caudal myotome’s surface. A large process arising from cell body (1), is being used by secondary neurites as a growth substrate. Distally the large neurite has been detached. Caudal to this group of neurites, two finer neurites with large flat growth cones (*), are emerging together from the cord. c, caudal, d, dorsal. ×900. Stage 24.

Fig. 9. A bundle of neurites leaving the spinal cord (sc), at mid-trunk level and growing onto the myotomes (m). A flat complex growth cone leads this outgrowth. Note that the distance between the spinal cord and the myotomes is not great and could easily be spanned by a filopodial extension from a growth cone on the cord surface. c, caudal, 1, lateral. ×2450. Stage 24.
regions of the embryo. Most of the dorsal tract is covered by ependymal cell processes. The myotomes have expanded dorsally and have concealed most of the dorsolateral spinal cord. Neurite bundles now appear to arise from between the myotome blocks. It is no longer obvious that these neurites initially grew away from the cord at the dorsal limits of the intermyotome. Where the myotomes have been removed, the periodic nature of the bundles of outgrowing neurites is very clear (Figs 12 and 13). Some of the cell bodies which were just visible on the cord surface at earlier stages have now migrated outwards (Figs 12 and 15) and in some cases have left the cord altogether (Figs 12 and 17). The peripheral neurites from these cell bodies can be traced to growth cones on the basal lamina of the skin (Fig. 18), while their proximal processes enter the dorsal tracts (Fig. 15). Commonly these cell bodies form the main bulk of the outgrowth at each intermyotome (Fig. 16). Increasing numbers of fine neurites leave the dorsal tracts and are associated with the larger neurites, protruding cell bodies, and existing fasicles. These fine neurites consolidate the outgrowths so that distinct fascicles are apparent at each intermyotome.

On the basal lamina of the skin more growth cones have established contact in the rostral and midtrunk regions of the embryo. After their initial lack of
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Fig. 12. Traced from a photomontage. A stage-24 embryo viewed from dorsal with the left myotomes dissected away to reveal the dorsal and lateral spinal cord (stippled). Clumps of extramedullary cell bodies are shown and are periodically spaced along the cord surface. Arrow heads indicate outgrowths not at an intermyotome.

Fig. 13. Traced from a photomontage. Dorsolateral view of a stage-27 embryo, with the myotomes removed from the left hand side. The neurite outgrowths are evenly spaced along the cord and are separated by a distance which closely correlates with the width of the myotomes on the opposite side of the cord. (stipple, spinal cord, arrows, outgrowths not at intermyotomes.) r, rostral.

orientation the growth cones start to produce microspikes in a predominantly ventral direction. These growth cones are less elaborate than those which were found on the myotomes. They have fewer lamellipodia and fine filopodia than before, a feature that may be related to the changed growth substrate (Fig. 19, and see Roberts & Taylor, 1983). A plexus of neurites extends caudally and ventrally, which becomes more complex as secondary neurites join it on leaving the outer myotome surface. On reaching the basal lamina of the skin these neurites often leave their outgrowth fascicles and can be seen crossing other neurites and forming new fascicles of varying length as they grow ventrally (Fig. 20). The orientation of the neurites in the plexus has been considered elsewhere (Roberts & Taylor, 1982).

Stages 26 to 27

The migration of cell bodies from the cord continues. Clumps of cell bodies are spaced periodically along the dorsolateral surface of the cord (Figs 13 and 16).

As the number of neurites increases on the basal lamina of the skin, a plexus of crossing, fasciculating and diverging fibres forms (Fig. 20). There appears to be one major wave of outgrowth of Rohon-Beard cell peripheral neurites (see Roberts & Taylor, 1983). In the ventral regions the growth cones are smaller than those seen in the dorsal areas at earlier stages, and tend to produce fewer
filopodia. Individual growth cones can be seen to have grown into holes in the basal lamina (Fig. 21). These holes correspond to the intercellular boundaries of the overlying epidermal cells, and are not thought to be an artifact of the preparation. With the technique we have used it was not possible to follow the path of the growth cones beneath the basal lamina, so we are not able to trace the establishment of contacts with the epidermal cells.

Stages 28 to 32

The dorsolateral tract has now been enclosed by ependymal cells and the cord surface has a more orderly appearance. At the intermyotomes, cell bodies and the dorsal neurite fascicles protrude from the cord surface (Fig. 22). The surface of the cord becomes obscured by neural crest cells which have begun to migrate from the dorsal spinal cord. These cells make observation of the cord surface difficult.

On the basal lamina of the skin the plexus covers the mid-trunk region and has extended to the ventral extremities of the embryo. Growth cones are rarely seen in the established plexus but do occur in the caudoventral regions. Neurites have formed their connections with the epidermal cells (Roberts & Hayes, 1977). The clarity of the plexus begins to be lost as a collagen matrix forms, the fibrils of which are indistinguishable from the neurite filopodia (Fig. 23). This collagen matrix will later form the basal lamella of the skin (Overton, 1979).

II. Periodicity of neurite outgrowth from the spinal cord

The outgrowth of neurites from the spinal cord follows the segmentation of the myotomes. Where the myotomes have been dissected away to allow observation

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Fig. 14. A growth cone on the basal lamina of the skin from a neurite which has just changed substrate, (myotome to skin). The neurite has been broken off (►), during skin sheet preparation but the growth cone remains relatively undisturbed. It is noticeable that there is no strong directional trend in the production of filopodia or micropodia by the growth cone. ×4200. Stage 26.

Fig. 15. A cell body protruding from the dorsolateral cord of a stage-24 embryo. Proximal processes (►), can be seen entering the cord, and a single distal neurite (*), forms part of the neurite bundle at the tip of the cell body. ×1560.

Fig. 16. A cluster of partly visible cell bodies in the dorsolateral region of the cord. Fine neurites are found on the cell bodies and form a fascicle of peripherally growing neurites. ×1700. Stage 32.

Fig. 17. A cell body lying on the basal lamina of the skin having migrated completely away from the cord. A proximal process from the cell body (top), has been cut during skin removal. A fine neurite (►a), has grown over the left side of the cell body and has fasciculated with the neurite arising from the cell body (►b). The fine neurite has branched (►c), and a small growth cone (►d), can be seen on a colateral fascicle of the main fascicle (►e). ×2000. Stage 25.

Fig. 18. The growth cone of the cell body in Fig. 17, found in a mid-dorsoventral position on the skin’s basal lamina. ×5400. d, dorsal.
Figs 19–23.
of the spinal cord, the outgrowths are seen to emerge from the dorsolateral cord surface at fairly regularly spaced intervals. In caudal regions the separation of the outgrowths is less than in rostral regions. This was related to the matched reduction in the length of the myotome blocks in more caudal regions, which are at an earlier stage of development.
To investigate this feature of the neurite outgrowth measurements were taken of the distance of each outgrowth from the nearest intermyotome. This measurement was divided by the distance between intermyotomes and expressed as a percentage. In this way myotomes of different ages (and widths), could be compared. The results were plotted as a histogram to show the number of outgrowths in each 5% band of deviation from the intermyotome (Fig. 24). All measurements were based upon photomontages of embryos viewed in the S.E.M. The data shows that most of the outgrowths (104) occur at the intermyotome position and that the rest are randomly distributed (58). These figures take no account of the numbers of neurites in each outgrowth fascicle which would accentuate the periodicity even more as the outgrowths at the intermyotome always consist of many neurites, while those between are single neurites or very small fascicles.

DISCUSSION

The Rohon-Beard cells of *Xenopus* embryos are particularly suited to S.E.M. observation since relatively simple dissection reveals most of their peripheral and, in early stages of development, central processes. The growing neurites of these cells can be seen on easily identified substrates like the spinal cord and dermatome, which at these stages of development are uncluttered by migrating neural crest cells or by extensive connective tissue layers. While there are artifacts of preparation (see below) this technique offers resolution of the finest processes of growing neurites and gives a very full picture of neurite shape in relation to the morphology of the substrate. Our observations of sequential stages of development in fixed tissue allows us to build a much more complete picture than any other available technique would permit. We have used this emerging picture to suggest mechanisms which may influence this development. These suggestions help to direct attention firstly to possible experimental testing and secondly to situations where other more subtle influences may be at work.

Central axons

Axons are first seen with T.E.M. in *Xenopus* spinal cord in a ventrolateral position at stage 20 (Hayes & Roberts, 1973). In the S.E.M. longitudinal axons first appear on the dorsolateral cord surface at stage 22 to 23, growing longitudinally either rostrally or caudally. Other axons fasciculate with these first axons to form a shallow tract which is visible in places on the cord's surface, and can be quite extensive. T.E.M. observations of *Xenopus* embryos have also shown axon and growth cone profiles at the surface of the spinal cord immediately under a thin basal lamina (Roberts & Hayes, 1974; Nordlander & Singer, 1982a,b; Hayes, personal communication). At the growing edge of ribbon optic nerve in Cichlid fish, axons and growth cones are present in a similar position (Scholes, 1982). T.E.M. sections usually show a basal lamina, 40 to 130 nm thick (Egar & Singer, 1972; Hayes & Roberts, 1973; Singer, Nordlander & Egar, 1979a,b;
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Nordlander et al. 1981; Nordlander & Singer, 1982a,b). At the magnifications we have used in the S.E.M. we have seen only occasional signs of this lamina bridging gaps between ependymal cell profiles. The most reasonable explanation at this point is that this thin layer has collapsed during drying to conform to the shapes of the underlying cells and has been torn where cells have shrunk and separated during dehydration. What in the S.E.M. we see as naked cell surfaces would therefore in vivo be covered by a thin basal lamina, which would form a substrate for axon growth and ependymal cell end-feet expansion at the surface of the spinal cord. However, the extent and nature of the basal lamina around the spinal cord at these early stages remains uncertain and clearly requires further exploration in view of our results.

Axonal guidance

We have seen no indication for any clear longitudinal orientation in ependymal cells or the spaces between them. For Rohon-Beard axons at the surface of the cord it therefore seems unlikely that ‘spaces precede axons’ and direct growth longitudinally as has been suggested by Nordlander & Singer (1982a). However two other mechanical limitations could encourage longitudinal axon growth. Initial Rohon-Beard axons occur on two dorsolateral ridges along the spinal cord (Fig. 1). The straightest path for growth is therefore longitudinal, along the inside surface of these ridges. Dunn & Heath, (1976), have shown that the orientation of movement in cultural fibroblasts relates to the radius of curvature of the substrate, a similar mechanism could operate for neurites in vivo. A second limitation to growth towards the dorsal midline of the cord could be the presence of a continuous row of Rohon-Beard somas and an absence of ependymal cells (Hughes, 1957; Roberts & Clarke, 1982; Roberts, unpublished observations). Since the embryos are elongating during the initial axon outgrowth period, stretch could influence the direction of growth cone extension and neurite alignment within the tracts (see Bray, 1979).

If initial axon growth is longitudinal then two further factors could contribute to the growth of longitudinal tracts. The first is fibre following or fasciculation by growth cones which we have seen frequently on the cord surface (e.g. Fig. 4) and also in peripheral neurites (e.g. Fig. 8; Roberts & Taylor, 1983). The second factor is the enclosure of tracts at the cord surface by ependymal cell processes (e.g. Fig. 6). Once regions of tract are enclosed by ependymal cell processes, tunnels will be formed so the growth of later axons could be guided by the tunnel walls as well as by fibre following (c.f. Singer, Nordlander & Egar, 1979).

Outgrowth from the spinal cord

Our principle observations are that following myotome segmentation, large neurite processes are seen emerging from the dorsolateral region of the cord often from protruding somas. The majority of these outgrowths occur at the
intermyotome and peripherally, their large growth cones contact the myotome surface. However initial outgrowth from the cord can also occur at other positions. The large outgrowths at intermyotomes provide a clear, physical bridge from the neural tubes to the newly segmented myotomes. At later stages, we see more neurites on the large outgrowths at the intermyotomes which extend onto the myotomes (e.g. Fig. 8). By these later stages, many somas giving rise to the large initial neurites have migrated out of the cord at the intermyotomes.

We would like to raise three points on the basis of these observations. Firstly, the association of outgrowth from Rohon-Beard cells with the intermyotome position immediately following segmentation of the myotomes, which is very clear from the developmental gradient along single embryos (Figs 12 and 13), suggests a causal relationship between the two events. This should be open to experimental analysis by interference with normal myotome development (e.g. Lehman, 1927; Detwiler, 1932, 1934). Since peripheral neurites can also form at other locations, influences at the intermyotome are not necessary for neurone outgrowth. Secondly, since later outgrowths are often seen on the initial large outgrowths, it is possible that Rohon-Beard cell axons growing along the spinal cord use these first outgrowths as a substrate and reach the myotome surface by fibre following or fasciculation. There are clearly other possibilities since this option is not available to the first neurites which grow out from the cord. Thirdly, associated with the major neurite outgrowth at the intermyotomes there appears to be a migration of cell somas from the spinal cord. Such cells have been called extramedullary cells (Hughes, 1957; Muntz, 1964; Lamborghini, 1980). The migration of these cells from the usual position of Rohon-Beard cells within the cord, and the fact that they are indistinguishable from Rohon-Beard cells except for the position of their cell soma, suggests that they are a subset of Rohon-Beard cells with extramedullary somas. In many cases, it is these extramedullary cells whose peripheral processes first contact the myotome surface at the intermyotomes.

Peripheral neurites

The neurites grow over the myotomes first laterally and then ventrally. Contact with the skin's basal lamina occurs as they begin to grow ventrally. At some point in this process the growth cones change substrate. On myotome preparations the growth cones are only found on dorsal myotome. On skin sheet preparations the first growth cones appear at midmyotome level and show relatively few damaged filopodia on the surface that was contacting the myotomes. In trying to postulate a mechanism for this substrate change we have made measurements of growth cones on these two substrates (Roberts & Taylor, 1983). Since growth cones are less flattened, and have fewer filopodia and lamellipodia on skin basal lamina, we conclude that they are moving against an adhesion gradient, assuming Letourneau's (1979), observations of cultured neurites are valid for our in vivo system.
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The growth on the basal lamina has been shown to be predominantly ventrally oriented (Roberts & Taylor, 1983). There is no evidence of any obviously oriented structure within the basal lamina which neurites are responding to. After dissection of the skin sheets we can see the pattern of grooves and ridges impressed upon the skin by the myotomes and intermyotomes, but these do not appear to influence neurite orientation. With the S.E.M. the only clue which is visible for a structure promoting ventral growth is the neurite response to the myotome surface. The myotomes may direct growth on the basal lamina by providing an initially oriented pattern of growth. Having started to grow ventrally by following the curvature of the myotomes the growth cones may continue in this direction unless affected by neurite–neurite interactions (Roberts & Taylor, 1983).

This study provides a description of the formation of a relatively simple nerve plexus. We have used our observations to suggest possible mechanisms which may influence parts of the development of this system. However, only by experimental investigation of these mechanisms can the relevance of our suggestions be tested.

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