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Role of the neural crest in development of the trabeculae and branchial arches in embryonic sea lamprey, *Petromyzon marinus* (L)

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Summary

Lamprey embryos were obtained by artificial fertilization to ascertain the contributions made by the neural crest to the head skeleton. Early-neurula-stage embryos of *Petromyzon marinus* were subjected to neural crest extirpation along the anterior half from one of seven zones, raised to a larval stage at which control larvae exhibit well-developed skeletons and analysed by light microscopy for any abnormalities to the cranial and visceral skeleton. The removal of premigratory neural crest at the level of the anterior prosencephalon (zone I) and at the level of somites 6 to 8 (zone VII) had no effect on skeletal development. However, the extirpation of neural crest from the intervening regions was positively correlated with deletions/reductions to the trabeculae (basicranial elements) and to the branchial arches (viscerocranial elements). Alterations to the trabeculae (16/27 cases, or 59 %) occurred only after extirpation of zones II–V (corresponding to the posterior prosencephalon to midrhombencephalon) while alterations to the branchial arches (21/28 cases, or 75 %) occurred only after removal of neural crest from zones III–VI (corresponding to the mesencephalon to the level of the fifth somite). Furthermore, the first three branchial arches were correlated in a majority of cases with neural crest from zone III, the next two arches with zones IV, V and VI and the last two arches with zone VI. Organs that develop within or adjacent to the area of neural crest extirpation such as the brain, notochord and lateral mesodermal derivatives were not affected. Parachordals were never altered by the operations nor were there any discernible changes to developing mucocartilage or to the prechondrogenic otic capsule. The contributions of the neural crest to the petromyzonid head skeleton described herein are compared with the roles of neural crest in the development of cranial and visceral skeletal elements in other vertebrates. The importance of these findings to the current hypothesis of the phylogeny of the vertebrate skeleton and the central role of the neural crest in vertebrate cephalization is discussed.

Key words: neural crest, lamprey, skeleton, *Petromyzon marinus*, branchial arch, trabecula.

Introduction

The currently well-accepted belief of neural crest participation in the development and evolution of the head skeletons of all vertebrates, (Le Douarin, 1982; Gans & Northcutt, 1983; Northcutt & Gans, 1983; Hall, 1987) is actually based experimentally on very thorough, very conclusive, yet very restricted, information principally from two classes of vertebrates; the birds (Johnston, 1966; Le Lièvre, 1971*a*,*b*, 1974, 1978; Le Lièvre & Le Douarin, 1975; Noden, 1983) and the amphibians (Chibon, 1966, 1967). The exceptions include two experimental studies of neural crest involvement in the development of the head skeleton in lampreys (Newth, 1951, 1956; Langille & Hall, 1986) and one in turtles (Toerien, 1965). There is a great need to explore more fully the role of the neural crest in the development of the head skeletons of those vertebrates belonging to the most primitive class, the Agnatha, which lie in the phylogenetic hierarchy between the protochordates and the gnathostome vertebrates, especially in the light of the

hypothesis that gives the neural crest a central role in the origin and evolution of the vertebrate head skeleton (Gans & Northcutt, 1983; Northcutt & Gans, 1983).

At present, any experimental investigation of the role of the neural crest in agnathan skeletal development must be restricted to the petromyzonids (lampreys), as these are the only group for which embryos can be obtained on a yearly basis (Langille & Hall, 1987*a*). In contrast, there is no known method for obtaining myxinoid (hagfish) embryos by artificial fertilization nor has it been possible, with rare exceptions, to collect myxinoid embryos from the wild.

As stated above, experimental information on cranial neural crest skeletal derivatives in lampreys is restricted to two studies. In the first study, Newth (1956) found deletions or reductions to the branchial arches of *Lampetra fluviatilis* and *L. planeri* larvae after removing neural crest from the corresponding embryos. Newth found no other changes to the petromyzonid head skeleton, even the trabeculae of the anterior neurocranium, which some authors (especially Damas, 1944) had argued were derived from ectomesenchyme, based on morphological observations. Newth's 1956 paper was actually the second series of experiments on lamprey neural crest. In an earlier paper (1951), Newth had found that the ablation of neural crest from lamprey embryos was correlated with (1) the failure of cranial and spinal dorsal root ganglia to develop and (2) a decrease in pigmentation. Yet although evidence was uncovered suggesting that petromyzonid neural crest gave rise to ectomesenchyme, the extirpations failed to produce any alteration of the skeleton and homoplastic and xenoplastic transplantation (the latter into urodele embryos) failed to produce any evidence of cartilage condensations (Newth, 1951). One of the difficulties with this early study was a high incidence of mortality among the operated embryos such that Newth was only able to analyse 18 % of the postoperative larvae. A further problem and one of the main reasons cited by Newth (1956) for repeating the experiments was that regulation by the neural crest might have occurred; the regions removed were small and migration back into the region might have occurred and produced a normal skeleton. Regulation or compensation is indeed a problem with extirpation experiments (Weston, 1970), but would such a regulatory event replace skeletogenic neural crest but not melanogenic or gangliogenic crest? This problem again surfaces in Newth's second series of experiments when, after extirpation of the posterior half of the cranial neural crest, branchial arches were absent but, in some instances, dorsal root ganglia of the branchial region, which, by his earlier experiments, should have been missing, were in fact present (Newth, 1951, 1956). As Newth used 'late-neurula-stage' embryos (1951) migration of the crest, which begins relatively far forward and proceeds posteriorly in all vertebrates studied to date (Le Douarin, 1982; Newgreen & Erickson, 1986), may well have already begun prior to removal of the crest. This possibility coupled with the inherent problem of regulation after neural crest extirpation may have affected the results. Further study of lamprey neural crest is thus warranted.

The present study is a continuation of the only other report of experimental data on lamprey neural crest skeletal derivatives, which confirmed the role of the cranial neural crest in the development of the branchial arches in lampreys (Langille & Hall, 1986). These initial data gave some indication of possible neural crest involvement in the development of the anterior neurocranial elements, the trabeculae, but the data were very sparse. The present study was thus undertaken to determine if the trabeculae of lampreys are indeed neural crest derived and also to locate more precisely the regions of the cranial neural crest that contribute to specific skeletal elements, so as to facilitate comparison with other vertebrates.

Materials and methods

Animals

Adult anadromous sea lamprey, *Petromyzon marinus* (Linnaeus), were caught at the beginning of their upstream migration from a fish ladder operated by the Department of Fisheries and Oceans Canada, on the LaHave river at New Germany, Nova Scotia. The lamprey were removed from the ladder by dip-net fishing and transported back to the laboratory at Dalhousie University.

Upon arrival at the laboratory, the lamprey were transferred immediately to either fibreglass flow-through tanks with running fresh cold water or to self-contained fibreglass tanks fitted with filter pumps (Fluval filter, Hagen Rolf Inc., Scarborough, Ontario) in a temperature-controlled room. The temperature within all these tanks was between 7–10°C at the outset.

The lamprey to be bred were separated by sex and placed in fresh water in either fibreglass flow-through tanks or in a self-contained, temperature-regulated water system, the Living Stream (Fridgid Units Inc., Toledo, Ohio). The animals were acclimated to water temperatures of between 16 and 20°C and were fully matured after several weeks under these conditions (Langille & Hall, 1987*a*).

Artificial fertilization and embryo rearing

Eggs and sperm were obtained from lamprey by the method of Langille & Hall (1987*a*) after anaesthetization with 0·05 % tricaine methanesulphonate buffered with 0·03 % aqueous $NaHCO_3$, pH 7 (after Robinson & Scadding, 1983). Briefly, the eggs were removed first by manual stripping and placed in shallow dechlorinated water or in 10 % Holtfreter's solution at egg densities low enough to

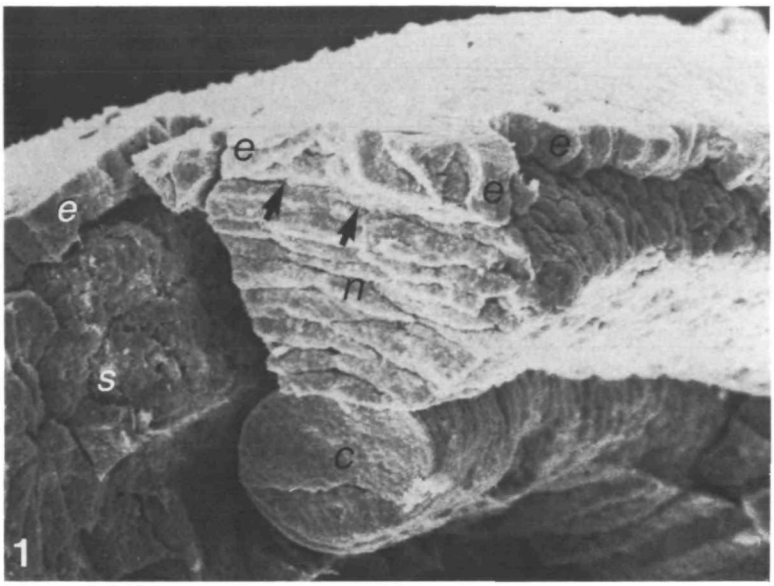

Fig. 1. Scanning electron micrograph of a cross section through a stage-12 (early) lamprey embryo revealing the neural rod (*n*), notochord (*c*), lateral mesoderm (*s*) and ectoderm (*e*). Neural crest cells (arrows) lining the dorsal surface of the neural rod are identified by their rounded shape relative to the cuboidal shape of the ectoderm and flat, elongated neural rod cells. ×1150.

prevent multiple layering of the eggs. Milt from one or two males was expressed over the eggs after which the mixtures of eggs and sperm were then swirled and allowed to sit for 1 h at a temperature of between 16 and 21 °C before being decanted and replaced with either fresh dechlorinated water or 10 % Holtfreter's solution (Holtfreter, 1935). The resultant embryos were then reared to larva in plastic tubs with loose-fitting lids in 4–6 cm of the original solution (changed every 3–4 days to prevent fungal buildup) at temperatures of 15, 18 or 21 °C in darkness or dim light.

Extirpation of neural crest from lamprey embryos

Lamprey embryos that had reached stage 12 (Piavis, 1961, 1971; Langille & Hall, 1986), the stage at which the neural crest appears, were washed in several changes of sterile saline and placed in full-strength Holtfreter's solution. The fertilization membrane (or chorion), a mucopolysaccharide layer external to the embryo, was then removed with fine forceps to expose the embryo (termed dechorionization hereafter).

Neural crest and small dorsal portions of the neural rod (Figs 1, 2) were removed with sharpened tungsten needles from one of seven 250 μm zones (Fig. 3) along the dorsal half of each embryo. As the neural rod is positioned slightly above the axial mesoderm, removal of the neural crest was easily effected with little or no damage to the mesodermal tissues (Figs 1, 2A). Controls consisted of sham operations performed on embryos at the same stage as those that received the neural crest ablations. Sham operations involved cutting and reflecting back the surface ectoderm only, carefully avoiding damaging the neural crest, then allowing the wound to heal. To check the extent of tissue removal, several embryos were fixed in neutral-buffered formal saline immediately after the extirpation of neural crest and prepared for light microscopy by the methods described below for larvae (Fig. 2B).

Normal unoperated, but dechorionized, embryos were also allowed to develop under the same postoperative conditions as both the operated and sham-operated embryos. Incubation of the postoperative, sham-operated control and normal control embryos was accomplished in full-strength Holtfreter's solution in the dark, at 21 °C for 14 days. By this time, the embryos had reached stage 17 (the larval burrowing stage; Piavis, 1961, 1971) during which normal embryos display extensive development of the head skeleton (Fig. 4). Some control embryos were fixed in neutral-buffered formal saline and prepared for light microscopy by the methods described below, for comparison with the operated embryos (Fig. 2A). As the tissues are partially obscured by the ubiquitous yolk platelets, additional control embryos were prepared for analysis by scanning electron microscopy (Fig. 1). These latter embryos were fixed for 2 h in 2 % glutaraldehyde in phosphate buffer, washed in the same buffer and sectioned with tungsten needles after which the embryos were postfixed for 1 h in 2 % OsO_4 (aq), dehydrated in a series of ethanols and critical-point dried.

Skeletal analysis and histology

Operated, sham-operated and normal control lamprey larvae were analysed initially at the gross level and compared with normal burrowing-stage larvae to check for any effects the ablations might have had on the development of nonskeletal tissues. Larvae were then fixed in neutral-buffered formal saline and processed for light microscopy by the following procedure. The specimens were dehydrated through a graded series of ethanols (EtOH), cleared in Histoclear (National Diagnostics, Somerville, NJ) and embedded in Paraplast (Fisher Scientific, Montreal, Quebec). 5 μm serial sections were cut on a rotary microtome, mounted on glass slides and stained with haematoxylin, alcian blue and eosin. The sections from operated specimens were then analysed for the skeletal elements present as well as for the general morphology of the major organs/tissues of the head and branchial region and the findings compared with the skeletons of normal specimens. The presence or absence of elements was then correlated

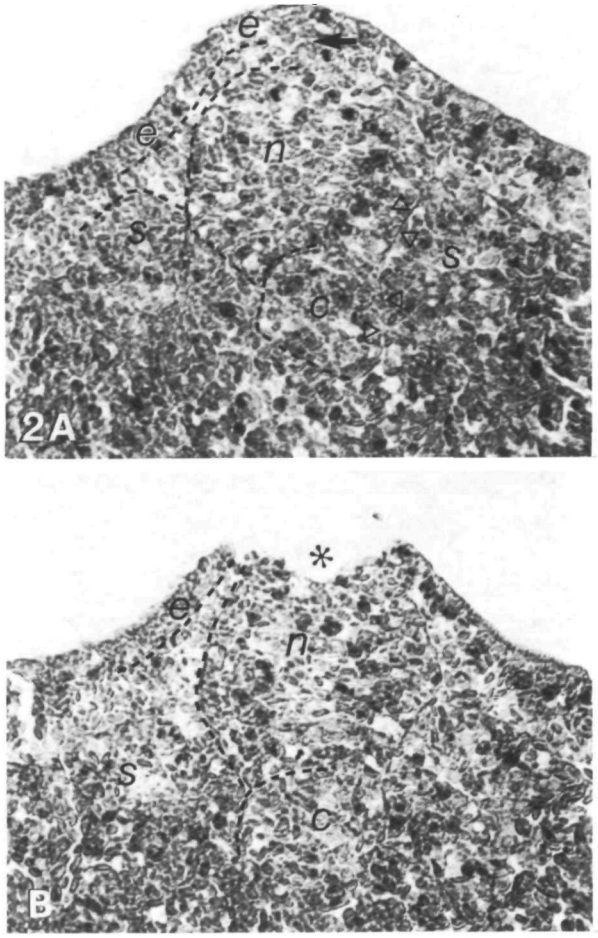

Fig. 2. Light micrographs of cross sections through a stage-12 (early) lamprey embryo at the level of the future mesencephalon (A) before and (B) after the removal of neural crest. Ubiquitous yolk platelets partly obscure the outline of differentiating tissues. In A, dotted lines on the left side delineate the boundaries between the notochord (*c*), lateral mesoderm (*s*), neural rod (*n*), ectoderm (*e*) and neural crest cells (arrow). Open arrowheads on the right side help identify some of the boundaries of the tissues described above. In B, which has been labelled in a similar manner, note how the operation has damaged the neural rod dorsally with the removal of neural crest (asterisk) but no damage to the lateral mesoderm has occurred. ×270.

with the zone that had been operated on to remove neural crest or been sham operated.

Results

Anatomical analysis

External anatomical analysis of the larvae that reached stage 17 revealed, with few exceptions, that they were well developed. Some of the larvae including the postoperatives, post-sham operatives and controls displayed a slight crook or twist to their

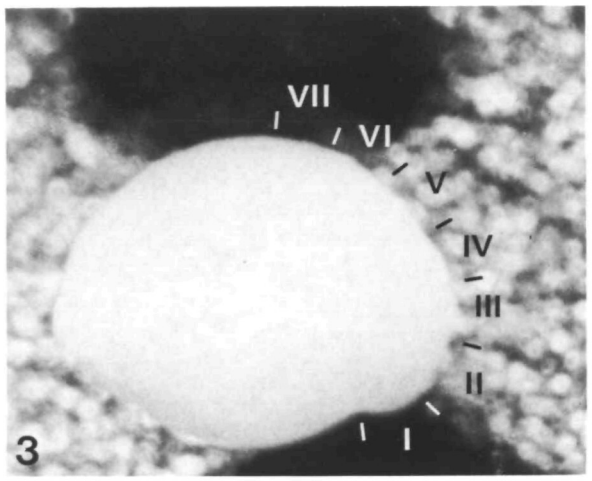

Fig. 3. A stage-12 lamprey embryo with the zones (I–VII, each 250 μm) of neural crest removal superimposed over top. Lateral view, anterior to the lower right. ×36.

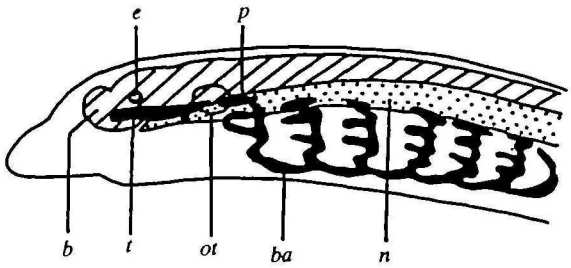

Fig. 4. Diagram of the cranial and visceral skeleton of a stage-17 (burrowing stage) larval lamprey, lateral view. Abbreviations; anterior end of brain (*b*), branchial arches (*ba*), eye (*e*), notochord (*n*), otic capsule (*ot*), parachordal (*p*), trabecula (*t*). ×14. (after deBeer, 1937).

bodies from one half to two thirds the way along their length, but this did not affect their swimming abilities. Apart from this one abnormality the control and post-sham-operated embryos appeared normal.

The postoperative larvae did, however, display additional changes to their external anatomy. A significant reduction of head pigmentation was observed in some postoperative larvae relative to controls, but only in those that received an ablation to a zone other than zone I. 26 out of 42 (62 %) of larvae with neural crest removed from a zone between and including II to VII, demonstrated a reduction in head pigmentation relative to controls. Of these, 22 or 52 % were also correlated with a reduction or deletion of the trabeculae and/or branchial arch elements. The largest number of larvae to demonstrate head pigment reduction were those with ablations to zone II (8 of 42). Pigmentation was not selectively deleted from any one part of the head, but represented an overall 'dilution' of pigment relative to

controls. This reduction of pigment, suggestive of neural crest involvement in the production of melanocytes, was also observed by Newth (1951, 1956). The development of pigment cells from neural crest has been well documented in all other vertebrates studied (Hörstadius, 1950; Weston, 1970; Le Douarin, 1982).

The only other abnormalities to the head region observed in operated larvae included a shortening of the rostrum, observed in three larvae, all of which had undergone ablation of zone I, and compression of the pharyngeal region observed in four larvae after having either zones III or VI ablated.

Histological analysis

The skeleton of the ammocoete (larval lamprey) at stage 17 (Fig. 4) has been found to be composed of (1) a pair of anterior parachordals or trabeculae (including the basitrabecular process and hereafter termed trabeculae) and a pair of (posterior) parachordals which together comprise the neurocranium, (2) a viscerocranium of branchial arches which are all fused to form a 'branchial basket', (3) a pair of otic capsules (which are not yet chondrified) and (4) a connective tissue unique to the ammocoete, mucocartilage (deBeer, 1937; Johnels, 1948; Hardisty, 1978, 1981). Although mucocartilage had formed no definite structures at this stage, it could nevertheless be differentiated from the rest of the connective tissue of the head by its positive staining with alcian blue.

The control and post-sham-operative larvae that had received only a disruption to the ectoderm, displayed no alterations of skeletal elements or other tissues when compared with normal specimens.

Of the 103 experimental embryos that survived longer than 24 h after the operation, 48 (46 %) attained stage 17 and were analysed for abnormalities to the skeletal elements. The only abnormalities to these larvae at the microscopic level were alterations to their skeletal elements. The other cranial organs such as the brain and spinal cord appeared normal.

The results of the analysis for skeletal abnormalities of the larvae that were subjected to neural crest extirpation are summarized in Table 1. These consisted entirely of reductions or deletions to the trabeculae and branchial arches (Fig. 5A–C). In all cases, the parachordals and otic capsules were found to be intact and mucocartilage, which is abundant throughout the head, showed no obvious differences from that of the control specimens.

Ablations to zones I, at the level of the anterior prosencephalon, and VII, at the level of somites 6–8, caused no alterations to either the branchial arches or trabeculae (Table 1). Only the removal of neural crest from zones II to V, from the level of the posterior prosencephalon to the midrhombencephalon, affected the development of the trabeculae

Table 1. *Deletions/reductions of trabeculae and branchial arches from the skeletons of lamprey larva after extirpation of neural crest from one of seven 250 μm serial zones beginning at the rostrum of the neural tube (see Fig. 3)*

Zone removed	Total no. of ablations	No. (and %) of larvae with skeletal reductions/deletions	
		Trabeculae	Branchial arches
I	6	0 (0)	0 (0)
II	9	7 (78)	0 (0)
III	10	5 (50)	7 (70)
IV	4	1 (25)	3 (75)
V	4	3 (75)	4 (100)
VI	10	0 (0)	7 (70)
VII	5	0 (0)	0 (0)

(Table 1). Neural crest ablations affecting the development of the branchial arches were restricted to those from zone III, the level of the mesencephalon, to zone VI, the level of the fifth somite.

A positive correlation was found between the anteroposterior position of the neural crest zone removed and the corresponding arches affected when the data of branchial arch reduction/deletion were analysed by breaking the branchial arches up into three regions, anterior (arches 1–3), mid (arches 4 and 5) and posterior (arches 6 and 7), as in Table 2.

Ablations of zone III (anterior mesencephalic) affected the development of the branchial arches in the anterior region in 71 % of the cases and the branchial arches in the mid region in 28 % of the cases while not affecting the posterior two arches at all.

Ablations of zone IV (posterior mesencephalic–anterior rhombencephalic) and V (rhombencephalic) causes a shift towards greater numbers of deletions/reductions of the middle two arches. When the data from zones IV and V are added together, the number of ablations found to affect the development of the arches in the mid region rose to 57 % (4 out of 7) while the number affecting the branchial arches in the anterior region fell to 43 % (3 out of 7). The development of the branchial arches in the posterior region was affected in only one instance after the removal of neural crest from the more posterior of these two zones (V).

Ablation of neural crest from the most-posterior zone observed to affect the branchial arch skeleton, number VI, had the greatest effect on the development of the two arches in the posterior region (71 % of the defects). The removal of this zone of neural crest had substantially less effect on the two arches in the mid region and affected the anterior arches in only one larva which lacked branchial arches altogether.

Discussion

Neural crest involvement in trabecular development

The findings of the present study provide clear evidence for neural crest involvement in trabecular development. Of the embryos that had neural crest extirpated from one of zones II–V, 16 of 27 (59 %, calculated from Table 1) either did not develop trabeculae or developed reduced trabeculae. These results corroborated the contention of Damas (1944) that the trabeculae do receive contributions from the neural crest, although whether the trabeculae are composed entirely of neural crest, as was stated by Damas, or contain additional cells derived from mesoderm, as is the case for urodeles (Chibon, 1966, 1967), could not be fully corroborated by the indirect evidence obtainable from an extirpation study such as the present one.

The trabeculae of the lamprey were derived from anteriorly positioned cranial neural crest, which follows the pattern (Fig. 6) of an anterior position for the neural crest which contributes to the trabeculae in the teleost, *Oryzias latipes* (Langille & Hall, 1987*b*), in urodeles (Hörstadius & Sellman, 1946; Chibon, 1966, 1967) and in birds (Le Lièvre & Le Douarin, 1975; Le Lièvre, 1978).

The evidence of neural crest involvement in the production of the trabeculae contradicts the observations of Johnels (1948) who, based on histological analysis of normal development, stated that, with the

Table 2. *Correlation between neural crest zone ablated and specific branchial arches affected*

Zone removed	Anterior (1–3)	Mid (4 & 5)	Posterior (6 & 7)	Total no.
I	0	0	0	0
II	0	0	0	0
III	5 (71 %)	2 (28 %)	0	7
IV	1 (33 %)	2 (66 %)	0	3
V	2 (50 %)	2 (50 %)	1 (25 %)	4
VI	1 (14 %)*	4 (51 %)	5 (71 %)	7
VII	0	0	0	0

* The only larva to display a defect of the anterior branchial arches after removal of the neural crest from zone VI displayed a total absence of all branchial arches.

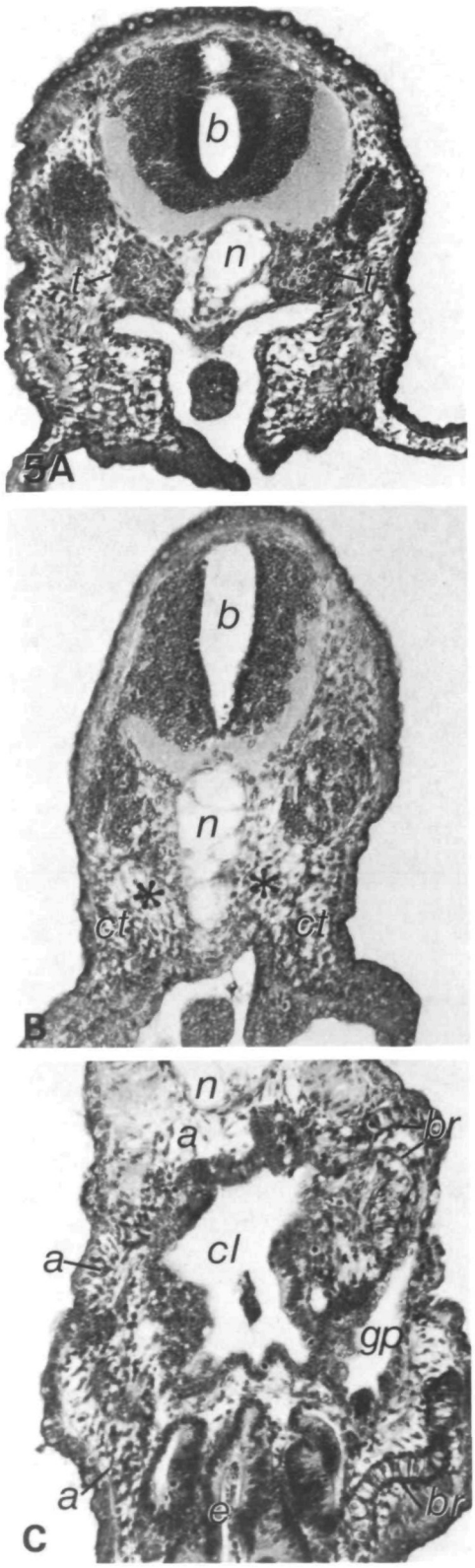

Fig. 5. Cross sections of burrowing stage larval lampreys. (A) Section through the head of a control larval lamprey reveals trabeculae (*t*) in their normal position on either side of the notochord (*n*) below the brain (*b*), anterior to the otic capsule. In B, after the ablation of neural crest from zone II, note the absence of trabeculae (asterisks) in the head of a larval lamprey. The rest of the organs and tissues appear unchanged; brain (b); notochord (n); connective tissue (*ct*). (C) After the ablation of neural crest from zone III, development of the visceral skeleton has been altered. A portion of the fourth branchial arch is visible (*br*) upper and lower right, but there is no sign of any of the third arch elements which would be located at points *a* (left side) in a normal larva. Again other organs, e.g. endostyle (*e*), notochord (*n*), appear unchanged. *cl*, central lumen of pharynx; *gp*, gill pouch. ×211.

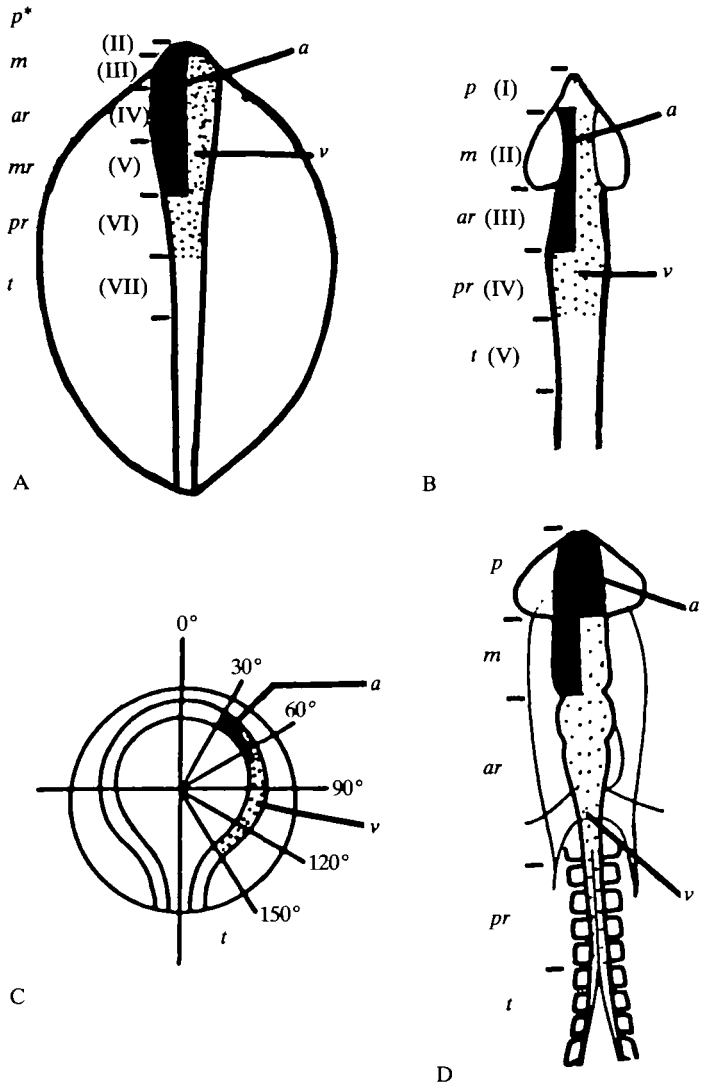

Fig. 6. The location along the embryonic axis of neurula stage embryos of the neural crest that gives rise to anterior neurocranial elements (shaded) and viscerocranial elements (stippled) in A lampreys, B teleosts, C urodeles, and D birds. Abbreviations for the specific regions of neural crest: *p*, prosencephalon (asterisk on lamprey embryo signifies this portion is not visible); *m*, mesencephalon; (*ar, mr* or *pr*) anterior, mid or posterior rhombencephalon; *t*, trunk. I–VII in brackets in A and B represents the zones of neural crest removed from the lamprey embryos in the present study and medaka embryos (after Langille & Hall, 1987*b*). Zones 0°–150° represent the regions of neural crest extirpated from urodele embryos by Chibon (1967). (A) From the present study; (B) from Langille & Hall (1987*b*); (C) redrawn from Chibon (1967); (D) redrawn from Le Lièvre & Le Douarin (1975).

possible exception of the trabecular commissure, all of the basicranial elements of lampreys were of mesodermal origin. Neural crest extirpation experiments carried out by Newth (1956) failed to demonstrate any neural crest involvement in the basicranial elements of the lamprey. However, as Newth claims to have operated on late neurula embryos (unlike the present study which used early- to mid-neurula-stage embryos), trabeculae-forming neural crest may well have already begun migration and thus would not have been extirpated. A number of authors using the technique of neural crest extirpation on amphibians and chicks noted that some crest cells migrate very precociously and may be missed if the extirpations are not performed early enough (Weston, 1963, 1970; Chibon, 1966; Johnston, 1966). Furthermore, if the migration of lamprey neural crest begins anteriorly and proceeds posteriorly as in all other vertebrates studied to date (see Le Douarin, 1982; Newgreen & Erikson, 1986 for a complete list), this mode of migration would then provide the reason that Newth, using late-neurula-stage lamprey embryos, was able to detect the contributions to the branchial arches from more-posterior cranial neural crest while failing to detect the involvement of more-anterior cranial neural crest in the development of trabeculae.

Neural crest involvement in branchial (visceral) arch development

Removal of neural crest from zones III–VI resulted in reductions/deletions to the branchial arches in 75 % of the cases (21 of 28 embryos, calculated from Table 1), confirming earlier experimental (Newth, 1956) and observational (Damas, 1944; Johnels, 1948) studies indicating the involvement of neural crest in the development of the petromyzonid visceral arch skeleton.

Results from the present study provide a better delineation of the neural crest responsible for branchial arch development than previous studies and, in addition, demonstrate that the anterior–posterior position of the neural crest cells along the neural rod, relates directly to the anterior–posterior position of the particular branchial arch(es) to which the neural crest contributes cells. The region of neural crest that contributed to the development of the branchial (visceral) arches of lampreys (zones III–VI), was found to extend from the level of the mesencephalon to approximately the fifth somite. The position of neural crest that contributes cells to the viscerocranium of the lamprey is similar to the posterior location of cranial neural crest (Fig. 6) that has been identified as contributing to the branchial arch skeleton in urodeles (Hörstadius & Sellman, 1946; Chibon, 1966, 1967), in the hyoid portion of the visceral arches in the turtle (Toerien, 1965) and particularly with the visceral-arch-producing neural crest regions identified in the teleost, *Oryzias latipes* (Langille & Hall, 1987*b*) and in the chick (Le Lièvre & Le Douarin, 1975; Le Lièvre, 1978), which have

been specifically localized to crest from the level of the mesencephalon to the fifth somite.

The overlap observed between the regions of lamprey neural crest which, in the present study, were found to contribute to both the trabeculae and the visceral arches were also observed to some degree in the chick (Le Lièvre & Le Douarin, 1975; Le Lièvre, 1978) and in the teleost, *Oryzias latipes* (Langille & Hall, 1987*b*). In the lamprey, the relatively large degree of overlap observed, comprising zones III, IV and V (posterior mesencephalon to midrhombencephalon), could be due to several factors. These include the specific geometry of the skeleton in lamprey, the fact that the branchial arches of lampreys presumably include visceral arch 1 and 2, the mandibular and hyoid arches of the gnathostomes, and the limits of the ablation technique to remove precisely each particular zone from every embryo, when the only landmark available was the anterior end of the 'head' from which each zone was a measured distance.

At this point, the other shortcomings of the ablation technique should be pointed out. Several authors have provided evidence that the neural tube, medullary plate, lateral mesoderm or nonextirpated regions of neural crest can compensate to form the neural crest derivatives lost to extirpation (Twitty, 1949; Lehman & Youngs, 1952; Bodenstein, 1952; Niu, 1954; Chibon, 1966; McKee & Ferguson, 1984).

With the limitations of the extirpation technique in mind, the success rates obtained in the present study, namely that in the majority of cases removal of neural crest from zones II–V resulted in the absence of trabeculae and removal of zones III–VI resulted in the absence of branchial arches, provide definite evidence of neural crest contributions to the development of these skeletal structures. Thus, experimental evidence is now available on the neural crest involvement in chondrocraniogenesis in lampreys (Newth, 1956; Langille & Hall, 1986, present study), teleosts (Langille & Hall, 1987*b*), amphibians (Chibon, 1966, 1967), reptiles (Toerien, 1965) and birds (Johnston, 1966; Le Lièvre, 1971*a*,*b*, 1974, 1978; Le Lièvre & Le Douarin, 1975; Noden, 1975, 1978, 1983). In all cases studied to date, including the present investigation, the anterior–posterior position of the neural crest along the neural tube is highly correlated with the position of the skeletal elements to which cells of the crest will contribute (Fig. 6).

Other skeletal elements of larval lampreys

The head skeleton of the larval lamprey, in addition to the trabeculae and branchial arches, is composed of parachordals, otic and nasal capsules, and mucocartilage (Fig. 7).

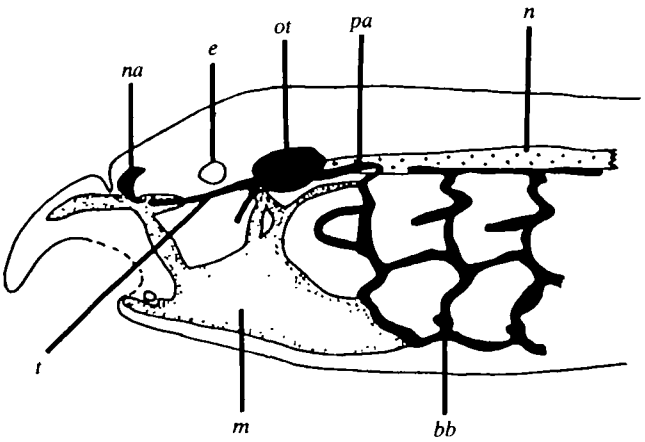

Fig. 7. Diagram of a portion of the cranial and visceral skeleton of a larval lamprey upon completion of larval development, lateral view. Abbreviations; first three arches of the branchial basket (*bb*), mucocartilage (*m*), nasal capsule (*na*), notochord (*n*), otic capsule (*ot*), parachordal (*pa*), trabecula (*t*). ×14. (after Hardisty, 1981).

The parachordals have in all observational and experimental studies of vertebrate head skeletal development been found to be derived largely, if not completely, from mesodermal mesenchyme (see deBeer, 1937; Weston, 1970; Hall, 1978). The two most recent observational analysis of petromyzonid skeletogenesis (Damas, 1941; Johnels, 1948) both claimed these elements as mesodermal derivatives, thus the lack of any evidence for neural crest contributions to these structures in the skeleton of lamprey from the present study and that of Newth (1956) is consistent with the earlier observations.

With regard to the otic capsule, however, the failure to find any neural crest involvement in this structure from extirpation studies cannot be taken as an indication that these structures receive no neural crest contributions. Studies utilizing chick–quail chimaeras (summarized in Le Lièvre, 1978; Le Douarin, 1982; Noden, 1975, 1983) have shown that, at least in the avian cranial skeleton, the auditory capsule receives both neural crest and mesodermal contributions. If this is the case in lampreys, then compensation for the missing neural crest mesenchyme by the remaining mesodermal mesenchyme could easily lead to normal otic capsule development after the removal of neural crest.

Because of its relatively late differentiation, well after the stage to which larval lamprey were raised, neither Newth (1956) nor the present study discovered anything about the origin of the nasal capsule.

Mucocartilage, a fibrous connective tissue (Hardisty, 1981) and unique component of the larval lamprey skeleton, was stated by Damas (1949) to be

derived from neural crest. A small percentage of cells derived from the mucocartilage during metamorphosis persists to form adult cranial cartilage elements (Armstrong, Wright & Youson, 1985). Thus uncovering the tissue origins of the mucocartilage could also uncover the tissue origins of a significant part of the adult skeleton.

Neither Newth (1956) nor the present study provides any evidence to support Damas' claim (1944) of a neural crest origin for the mucocartilage. This tissue occupies a very large area of the head running roughly from a dorsoanterior position to a ventral position in between the branchial arches (Hardisty, 1978, 1981). Thus, it could receive compensational contributions from adjacent tissues during extirpation experiments. Definitive evidence for the origin of mucocartilage will also have to wait until experiments using tissue markers are successfully employed with lamprey embryos.

Phylogenetic implications of neural crest involvement in petromyzonid skeletogenesis

The implication of the neural crest of lampreys in the development of the basicranial trabeculae and visceral arches is very important phylogenetically. This evidence suggests that a homology exists between these petromyzonid elements and the trabeculae and visceral arches of gnathostome vertebrates which are also neural crest derivatives (Hörstadius, 1950; Weston, 1970; Hall, 1978; Le Douarin, 1982) and this is consistent with the hypothesis for a central role of the neural crest in the origin and evolution of the earliest vertebrates due to its significant contributions to the head skeleton, one of the principal vertebrate characteristics (Gans & Northcutt, 1983; Northcutt & Gans, 1983).

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