The oral nerve plexus in amphioxus larvae: function, cell types and phylogenetic significance

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Serial electron microscope reconstructions were used to examine the organization and cell types of the nerve plexus that surrounds the mouth in amphioxus larvae. The plexus is involved in a rejection response that occurs during feeding: a number of oral spines project across the mouth, and debris impinging on them triggers a contraction of the gill slit and pharyngeal musculature that forces water through the mouth, dislodging the debris. The oral spine cells are secondary sense cells that synapse with neurites belonging to a class of peripheral interneurons intrinsic to the oral nerve plexus. These in turn synapse with a second class of peripheral neurons with large axons that we interpret as sensory cells and which probably transmit signals to the nerve cord. The intrinsic cells also appear to synapse with each other, implying that local integrative activities of some complexity occur in the oral plexus. In comparative terms, the intrinsic neurons most closely resemble the Merkel-like accessory cells of vertebrate taste buds, and we postulate a homology between oral spine cells and taste buds despite differences in function. There are also similarities between the amphioxus oral plexus and adoral nerves and ganglia of echinoderm larvae, suggesting homology of both the oral nerve plexus and the mouth itself between lower deuterostome phyla and chordates.

Keywords: oral nerves and sensory cells; amphioxus; peripheral nerve organization

1. INTRODUCTION

Amphioxus is a key organism from a phylogenetic perspective, since it is the best model available for showing what the immediate invertebrate ancestors of vertebrates were probably like (Gans 1989; Holland 1996). The head of amphioxus, although structurally much simpler than the vertebrate head, is of special interest. There is a surprising degree of homology between anterior parts of the amphioxus nerve cord and vertebrate brain (Lacalli *et al.* 1994; Holland 1996), and a number of rostral sensory structures and cell types may also have vertebrate homologues (Stokes & Holland 1995a; Northcutt 1996; Baker & Bronner-Fraser 1997; Lacalli & Hou 1999). However, amphioxus is generally thought to lack placodes and a neural crest, which are the embryonic source for such structures and cell types in vertebrates. Further, although the visceral organs, atrium and oral region of amphioxus are supplied with an extensive system of peripheral neurons and fibre tracts (Franz 1927; Bone 1961), the neurons themselves appear to be sensory rather than motor, so they are probably not homologues of the enteric neurons that innervate the visceral organs of vertebrates (Bone 1958, 1961; Bone *et al.* 1996). From the morphological data available, mainly from whole-mount tissue preparations, it is not clear that the axons of the amphioxus neurons enter the dorsal nerve cord in all instances. This suggests that at least some of the cells may

function locally, at the periphery. In this respect, peripheral neurons in amphioxus resemble those found in peripheral nerve tracts in related invertebrate phyla, e.g. echinoderms and hemichordates. Amphioxus is thus a potentially important source of information on how vertebrate patterns of neural organization evolved from simpler antecedents.

This study extends previous analyses of an electron microscope (EM) serial section series obtained through the anterior end of a 12.5-day-old amphioxus larva (Lacalli *et al.* 1994; Lacalli 1996; Lacalli & Kelly 1999). When the oral region of this specimen was examined, neuronal cell bodies and numerous synapses were found in the circumoral nerve, features that do not generally occur in peripheral nerves in advanced chordates or in the other anterior nerves of amphioxus. Here we report our conclusions on the cell types, function and organization of the circumoral nerve and discuss relevant phylogenetic issues.

2. METHODS

Larvae of *Branchiostoma floridae*, from Tampa Bay, Florida, were raised in the laboratory and maintained to metamorphosis as described by Holland & Holland (1993a). Feeding behaviour was observed in live larvae tethered by suction pipettes and videotaped using the methods of Gilmour (1989, 1996). The methods for EM fixation, serial sectioning and three-dimensional reconstruction are described by Lacalli *et al.* (1994), and this study relies on the same specimens, ranging in age from

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Figure 1. Side views at two planes of focus of the head region of a 14-day-old *Branchiostoma floridae* larva (*a*) at the level of the mouth (*m*) and pre-oral pit (*p*) and (*b*) through the pharynx to the endostyle (*en*), club-shaped gland (double arrows) and gill slits (*). The neuropore and frontal eye (arrow) lie at the anterior end of the nerve cord, which runs along the top of the notochord (*not*); metachronal waves can be seen around the edges of the gill slits. Scale bars, 100 μ m. (*c*–*g*) Video images of feeding larvae. (*c*) An oblique view of the mouth from above showing the row of oral spines along the upper lip. Scale bar, 20 μ m. (*d*, *e*) Side views of the mouth (*m*) from the left side, rostrum to the upper right, to show the normal posture of the papillar cilia (double arrows) during feeding. The oral spines lie in or close to the plane of the mouth; they are faintly visible (arrows) in (*e*). Scale bars, 50 μ m. (*f*) Top view of the head showing the mouth (*m*) and papillar cilia (arrow). Scale bar, 100 μ m. (*g*) Details of the larva in (*f*) to show the papillar cilia (arrows) in the erect position (top part) and during a recovery stroke (bottom part). Scale bar, 50 μ m.

six to 14 days, with section series from one eight-day-old larva (specimen 3 in previous accounts) and two 12.5-day-old larvae (specimens 4 and 6). The transmission electron microscope images are all shown as seen from the front, i.e. the left side of the specimen is to the right in each figure. Reconstructions are of specimen 4 and cover 650 sections, extending to the caudal end of somite 2, which includes roughly the front third of the mouth. Cells were traced from micrographs taken at three to four section intervals, with intervening sections included where necessary. Partial series of this type are a practical necessity for the peripheral nervous system, because a montage of micrographs is required to show all the nerves in any one section clearly. Data on the remainder of the oral region came from low-power studies of other specimens and are much less complete. Some assumptions are therefore required to interpret the results, as discussed below.

3. RESULTS

Our observations are on feeding larvae during the second week of development, up to the 14-day-old stage shown in figure 1*a*. The larval head is asymmetrical, with the mouth on the left side (figure 1*a*) and the gill slits on the right (figure 1*b*). The initial gill slits are in fact left gill slits; the right gill slits develop much later (Willey 1891; Stokes & Holland 1995*a*).

(*a*) Feeding behaviour and debris rejection

Larvae typically feed while suspended in the water column in an approximately vertical posture which is maintained by the beating of the body cilia (Stokes & Holland 1995*b*). Feeding currents are driven by the gill slit cilia, which beat vigorously in metachronal waves. The current generated by the gill slits draws water into the mouth from the side (Gilmour 1996), and the general body cilia appear to have little or no role in this process. Past reports have implicated both the pre-oral pit (Hatschek's pit) and the club-shaped gland as accessory feeding organs, with a role in providing mucus (Ruppert 1997). Because the pit is located some distance from incoming water currents and because the club-shaped gland appears to drive water inward rather than outward (Olsson 1983; Gilmour 1996), it seems doubtful that either is involved directly in particle capture in the pre-oral region. The mucus involved in food trapping in the pharynx appears instead to be largely the product of the endostyle, with a possible contribution from the mucus cells at the upper (inner) end of the club-shaped gland (figure 2; see Gilmour 1996).

There are two other ciliary organs that may have a role in feeding: (i) the oral papilla, which bears a tuft of long cilia that emerges just below the anterior margin of the mouth, and (ii) the oral spines, formed by clusters of

shorter, rigid cilia that project at regular intervals along the margin of the mouth.

The cells of the oral papilla are elongate, as described by Andersson & Olsson (1989) and in young larvae they extend some distance along the basement membrane beneath the otherwise rather thin epithelium. When feeding, their cilia project forward, as shown in figure 1*d,f*. The posture of the papillar cilia in non-feeding larvae is shown in figure 3*a*. Our observations confirm previous reports that the cilia beat irregularly and slowly, in groups of a few or altogether, moving down and across the front part of the mouth in a sweeping action (figure 1*g*) that typically takes approximately one-third of a second. Gilmour (1996) suggested a sensory role in initiating the cough response for these cells, but further observations are in better accord with there being ciliary effectors, not sensory cells. Neurites from the oral nerve pass beside the bases of some of them, but there is no sign of synaptic specializations as found elsewhere among amphioxus sensory cells by Lacalli & Hou (1999). The function of the oral papilla in feeding is consequently not clear to us at this time. The rostral papilla (rp in figure 3*a*), the cells of which were described by Lacalli (1996), is ultrastructurally similar to the oral papilla, and it may be an essentially equivalent structure. Its function is also not known.

The ciliary spines are composed of closely apposed cilia and are completely immobile. They are only faintly visible in single video frames, but are easily identified in continuous playback against a background of moving particles and water flow. They project slightly outwards across the mouth opening and are best seen in oblique view (figure 1*c*). Sections show that the cilia always emerge on the outer side of the lips, although this is more pronounced and the angle is greater on the lower lip (figure 4).

The function of the oral spines is clearly to initiate the larval 'cough' response, which acts to clear the mouth of debris. Coughing is a well-known response in adult amphioxus (Barrington 1965; Ruppert 1997) and its counterpart in the young larva is a rapid contraction of the gill slit and pharyngeal muscles (figure 2), coupled with an interruption to the beat of the gill cilia. This stops the feeding current, partially closes the gill slits and reduces the volume of the pharynx by a significant fraction. Much of the excess fluid is evidently discharged through the mouth and this dislodges the debris. We observed this response repeatedly in feeding larvae viewed from the side, but at first the cause was not apparent because the debris, which consisted of lumps of transparent mucus containing a few algal particles, was essentially invisible out of the focal plane. In top view, however, the entire sequence could be observed (figure 2): debris approached the mouth with the food current, encountered the oral spines, and this was followed within one-30th of a second by contraction of the gill slits and pharynx. Recovery of ciliary beat and relaxation of the muscles then required approximately one-third of a second. So far as we could determine, contact with the oral spines is thus directly responsible for the cough response.

(b) The dorsal nerves

Figure 3 shows the main peripheral nerves in the head region, numbered from the anterodorsal nerves of the

Figure 2. Frame-by-frame traces from a video sequence at intervals of one-30th of a second; one of three very similar events showing the approach and rejection of a lump of debris. On contact with the oral spines (frame 11), a muscular contraction occurs (heavy arrows) which reduces the pharyngeal volume and simultaneously propels the debris away from the mouth, after which it drifts out of the plane of focus. Dashed lines delimit the zone occupied by feeding currents as determined from particle streamlines.

cerebral vesicle. Yasui *et al.* (1998) document the early development of the anterior nerves. Here, the anterior-most nerves of the dorsal series are treated as a single pair (nerve 1). In fact, two anterodorsal nerves leave the cerebral vesicle on each side in young larvae (Lacalli 1996), but our data from older larvae suggests these initially tiny roots later combine. Published drawings showing two separate nerves on each side probably mistake the large proximal branch that develops later for a separate nerve.

The mouth lies below somites 3 and 4 on the left side and is innervated by the closest dorsal roots, nerves 3 and 4 (figure 3*a*). This pattern is retained through metamorphosis, when the mouth shifts to a medial and more caudal position (Van Wijhe 1919). In consequence, the adult mouth is innervated by nerves that cross from the left side of the oral hood to the velum (variously shown by Kutchin (1913) and Franz (1927); see also Ruppert 1997). The two largest of these appear to derive from dorsal roots 3 and 4, although published figures do not always show their source clearly.

In our eight-day-old larvae, nerves 3 and 4 on the left side each consist of three large fibres, one of which can be traced to a visceral motor neuron in each case, and three to four smaller cell processes that are not necessarily all neural. Our 12.5-day-old series does not extend to nerve 4, but nerve 3 on the left side is roughly the same size as in eight-day-old larvae, with three large fibres, one of which belongs to a visceral motor neuron located near the back of somite 2. We cannot rule out the possibility that all three of the large fibres in each nerve derive from motor neurons, making six motor neurons overall, but it is also possible that only one is motor and the other two are sensory. Our tracings of oral nerve fibres (§3(c)) suggest the latter is more likely.

Figure 3. (a) Summary drawing to show the positional relationships between the main structures, including the peripheral nerves, of the 12- to 14-day-old larval head. The data are from sections, and small nerve branches (i.e. with less than four to five fibres) are omitted. Somites (s) are numbered from the first myotome. The dorsal nerves (n) are numbered to give a corresponding series that excludes the paired rostral nerves that extend forward from the anterior end of the nerve cord (shaded) to the tip of the rostrum. Also shown are the pre-oral pit (p), rostral papilla (rp) and oral papilla (op). Our reconstructions cover the zone between arrows. (b) Summary of the main anatomical features in the reconstructions: the oral nerve, a selection of ciliary spine cells, the six neuronal cell bodies (extrinsic cells 1 and 2 and intrinsic cells 4–6) and the arrangement of the muscle cells. The latter is shown in a somewhat simplified and schematic fashion; some small fibres are omitted.

(c) *The oral muscles and their innervation*

One particular type of neurite was consistently found in the oral nerve near muscle fibres, containing clusters of clear vesicles (25–35 nm in diameter) adjacent to small dense zones of membrane (figure 5a). The basal lamina intervenes between such zones and the muscle cells, so these are not sites of direct contact with muscle cells even if they are neuromuscular junctions. However, this is also the situation in the myotome. There, the innervation of the dorsal compartment is cholinergic, the synaptic vesicles are very similar in size and general appearance to those found in the oral nerve, and the nerve terminals are also separated from the muscle cell by intervening basal lamina (Lacalli & Kelly 1999).

Most examples of putative neuromuscular junctions in our oral nerve reconstruction proved to belong to one fibre (m1 in figures 5a,b and 6b), which appeared to innervate a number of muscle cells. Several other small neurites were encountered and, although these may belong to a second fibre (m2 in figure 5a), we cannot rule out their being branches of m1. Other motor neurons may be present in the oral nerve, but, from our tracings of fibres with evident junctions, it seems likely that the majority of the muscles of the anterior oral region are innervated by one or two nerve fibres at most.

The difference between the shape of the mouth of feeding and non-feeding larvae depends on the develop-

mental stage and is not very pronounced after eight to nine days (cf. figure 1a with 1d,e). The upper and lower lips pinch together at the front of the mouth when feeding stops and this is clearly due to an action of the oral muscles. The latter radiate from a point directly in front of the mouth (figure 3b) and they attach to comparatively rigid support structures, including the basal lamina associated with Hatschek's nephridium, the notochord and the rostral coelom. This arrangement ensures that the force of contraction raises the floor of the pharynx and draws the upper and lower lips towards each other, i.e. contraction partially closes the mouth.

(d) *The oral nerve, its cells and fibres*

The oral nerve consists of approximately a dozen fibres (fewer in the lower lip, which develops more slowly) in the eight-day-old stage and 30 fibres (in both upper and lower lips) at 12.5 days. This would mean that the oral nerve in 12.5-day-old larvae contains more fibres than those that enter the nerve cord, so long as we assume that nerves 3 and 4 on the left side are as similar to one another in size at 12.5 days as they are at eight days. However, we lack the detailed data on nerve 4 for 12.5-day-old larvae which is needed to prove this. Nevertheless, the abundance of synaptic complexes in the oral nerve (figure 5a,b) clearly shows that at least some of the peripheral neurons function locally in the periphery,

Figure 4. (a) A sample section from the series used for reconstruction from a 12.5-day-old larva. This section passes through the pharynx (ph) at the level of the mouth (m) and shows the notochord (not), the internal and external openings of the club-shaped gland (asterisks), the position of the oral nerve (circled) in both upper and lower lips, the locations and angle of the oral spines (arrows) and the point of entry of nerve 3L into the nerve cord (arrowhead). Scale bar, 25 µm. (b) Detail of the upper lip to show the position of the oral nerve (n), including a small branch (arrowhead) supplying one of the two muscle bands (asterisks) and an emerging oral spine cilium (arrow). Cells along the inner side of the lip contain characteristic vesicles (v). Scale bar, 5 µm.

which would be expected if not all of the cells are centrally connected.

From sections of the limited portion of the oral plexus available, we identified six peripheral nerve cells positioned as shown in figure 3*b*. The cell bodies are all similar in appearance: they are larger than epithelial cells and have large nuclei and an adjacent zone of granular cytoplasm richly supplied with Golgi cisternae and mitochondria (figure 5*a*). The cells do not extend to the surface of the epithelium, but residual basal bodies were found in the cytoplasm in two cases, and there are apparently similar cells in the eight-day-old larvae that do extend to the surface.

Two of the cells in the 12.5-day-old stage lie some distance from the oral nerve; we refer to them here as extrinsic neurons. The principal neurite in each case, i.e. the axon, was large, of uniform diameter and contained scattered microtubules, mitochondria and clear vesicles *ca.* 50 nm in diameter. The axons were involved in synapse-like interactions with surrounding fibres within the oral nerve, being postsynaptic in most cases but not all. Examples in which extrinsic cells are apparently presynaptic to other fibres are shown in figure 5*a* and

examples of the reverse are shown in figure 5*b*. Out of the two extrinsic cells, one sent an unbranched axon along the upper branch of the oral nerve (cell 1, figure 6*c*), while the other branched to supply both upper and lower lips (cell 2, figure 6*d*) and a third large axon on the nerve indicated the probable presence of a third extrinsic cell located outside the section series. Given the size of these axons and the absence of any evidence that such fibres terminate within the oral nerve, it is reasonable to suppose that their main targets lie outside it. Bone (1961) identified cells with similar morphology elsewhere in the peripheral system that enter the cord, and extrinsic cells may do the same. If this is the case and if they reach the nerve cord by day 8 of development, they would account for three or perhaps four of the six large fibres that enter the cord via dorsal nerves at that stage.

The remaining four neurons lay next to the nerve with one surface embedded in it; we will refer to them as intrinsic neurons. Large neurites were traced from three of these cells (examples in figure 6*c,d*). All were unipolar; each had other small processes that extended around adjacent nerve fibres, but these did not appear to be functional neurites. The large neurites were far less regular

Figure 5. Details of the oral nerve and oral spine cells. (a) A tangential section through the oral nerve as it arcs in front of the mouth. The intrinsic neuron (asterisk), partially embedded in the nerve, is cell 3. The neurite running along the basal lamina (m1) is the motor fibre shown in figure 6*b*; part of a second, similar fibre (m2) is also visible. These contain clusters of small clear vesicles (double arrows). The axons (a) of known (numbered) or presumed (unnumbered) extrinsic cells are indicated and examples of their presumed synapses occur in this section (single arrows). The remaining large fibres are interpreted as belonging to intrinsic cells; some synapses are visible (arrowheads) and two of these belong to cell 3. (b) As in (a), more examples of synapses (arrowheads) between presumed intrinsic cell fibres and extrinsic cell axons. (c) Bases of several oral spine cells (asterisks) that partially encapsulate the nerve (n) and form synapse-like junctions (arrowheads) with adjacent neurites. (d) Detail of the base of an oral spine cell (asterisk) and its synapse-like junctions (arrowheads). (e) Cilium of an oral spine cell on the lower lip. Hoops of microtubules (arrowheads) encircle the apex of the cell. The lip itself is formed by the cell to the left; note its vesicles (v). (f) Cilium belonging to one of the lip cells. At this point the accessory centrioles reverse position (compare with *e*). (a, c) Scale bar, 2 μ m. (b, d-f) Scale bar, 1 μ m.

Figure 6. Reconstructions from sections. (a) An oblique front view of the reconstructed region in stereo. The oral nerve and its cells, the anterior-most group of ciliary spine cells and the cells of the oral papilla are seen through the semi-transparent body surface. (b) Side view of the oral nerve showing the approximate extent of the main groups of muscle fibres (dark structures) and the m1 motor neuron (arrowhead) seen through the semi-transparent oral nerve. (c, d) Side view of the oral nerve and selected neurons indicated by number.

than the axons of the extrinsic cells and were twisted and compressed by surrounding fibres. Even over the short distance they could be traced, there were regions containing vesicles and synaptic junctions (e.g. figure 5b), separated by regions with neither of these features. Branches were not observed, but similar nearby fibres did have lateral projections and blind-ending varicosities filled with a similar complement of vesicles. In fact, all the neurites in the nerve, except those belonging to the extrinsic cells and motor neurons, appeared to be of a similar type. They contained large numbers of clear vesicles (35–55 nm in diameter) and scattered dense-core vesicles (60–75 nm in diameter) and formed frequent synaptic junctions, in some instances with extrinsic cell

axons, but also with small dendrite-like processes that could be traced in several instances to fibres like themselves. Since we have no evidence to the contrary, we conclude that all of these fibres are of one type and that all belong to intrinsic neurons located in the section series or outside it. However, we cannot rule out the possibility that further work on the oral region may reveal additional types of neurons elsewhere in or around the oral plexus.

(e) Oral spine cells

Oral spines in both eight- and 12.5-day-old larvae arise from clusters of flask-shaped uniciliate cells that taper together at their apices. The cell apices emerge at the surface either as single or double rows and are reinforced by rings of microtubules (figure 5e). The apices project on the outside of the lip. The lip itself is formed by epithelial cells with apical vesicles (figure 4b) whose cilia are reversed in orientation (figure 5f) compared with both spine cells and nearby external epithelial cells. The lip edge is thus a point of transition between cells whose cilia beat inwards and cells whose cilia are orientated to beat outwards.

The bases of the ciliary spine cells (figure 5c,d) are filled with closely packed clear vesicles of 70–90 nm in diameter. Synapse-like junctions occur between the base of the cells and adjacent small dendrite-like cell processes. Two of the latter were traced to known intrinsic cells, while others belonged to unassigned intrinsic-type fibres. We conclude that the oral spine cells are secondary sensory cells that synapse mainly or exclusively with the intrinsic neurons.

4. DISCUSSION

(a) Summary and functional interpretation

The peripheral nerve plexuses of adult amphioxus have been the subject of repeated study (Franz 1927; Bone 1958, 1961), but the nerves associated with the velum and mouth are less accessible than those supplying the atrium and gut and are consequently less well known. The oral spines of amphioxus larvae have been noted by past workers (e.g. Willey 1891), but neither the spine cells nor the oral nerve have previously been examined in detail. This study was not intended as a comprehensive one, since only a few selected stages were examined and only the anterior margin of the mouth was examined in detail. It nevertheless provides enough data to distinguish characteristic cell types and allow informed conjecture concerning the nature of the cells and their probable functional relationships.

To summarize, the oral region is innervated by both centrally derived nerve fibres and local peripheral neurons. The only clearly identifiable central elements are the axons of motor neurons, which arise from the third and fourth dorsal roots on the left. We conclude from our fibre counts that there are probably only a few such fibres, perhaps only two, which innervate a much larger number of muscle cells. The peripheral neurons are of two types: intrinsic, i.e. embedded in the nerve, and extrinsic, lying some distance from it. We identify four cells of the former type and two of the latter. If their distribution is similar around the rest of the mouth, one might suppose there are perhaps 20 such cells overall, which, at one fibre each,

Figure 7. Our interpretation of the cell types in the oral region and the synaptic relationships between them. Representative examples of three fibre classes are shown: extrinsic neurons, intrinsic neurons, and a class of unassigned fibres essentially identical to the latter that we assume belong to intrinsic neurons located outside the part of the oral nerve that we have reconstructed. See text for details.

would account for a majority of the fibres within the oral nerve. It is possible that there are fewer cells and that their neurites travel more than once around the mouth, a pattern of fibre growth that is seen in the gut plexus (e.g. Bone 1961, fig. 10).

In general, the morphology of the extrinsic cells is consistent with there being sensory neurons the axons of which probably enter the nerve cord and terminate within it. The axons of the intrinsic neurons are clearly multifunctional. Their neurites bear both presynaptic and postsynaptic specializations, and fibre counts suggest that they may be largely restricted to the oral nerve itself. The intrinsic cells also appear to be the chief target of synapses formed by the oral spine cells which, from our data, are secondary sense cells. They are clearly mechanoreceptors, based both on their structure and the responses we observe when debris impinges on them during feeding. A similar response to mechanical stimulation of the oral region is observed in tunicates (Mackie *et al.* 1974; Bone *et al.* 1979), and both appendicularians (Olsson *et al.* 1990) and ascidians (Burighel & Cloney 1997) have oral mechanoreceptors that are evidently secondary sense cells. However, neither has a system of peripheral neurons in the oral region which is like that described here for amphioxus.

Keeping the limitations of this study in mind, we interpret the oral system in functional terms as shown in figure 7. The intrinsic cells receive input from the oral spine cells and communicate via synapses with the extrinsic cells. The latter probably function as sensory neurons, and we assume from their large axons that they enter the nerve cord. However, the possibility of direct peripheral connections to the gill slit muscles cannot be ruled out. The intrinsic cells also synapse with other fibres in the oral nerve and, for lack of an alternative, we suggest these belong to other intrinsic cells. The oral nerve plexus is rich in synapses, which indicates that

peripheral integrative activities of some kind undoubtedly occur as part of the rejection response.

(b) *Vertebrate homologues and invertebrate antecedents*

Despite the apparent simplicity and small size of the anterior nerve cord in amphioxus, there are clear regional and structural homologies with the vertebrate brain (Lacalli *et al.* 1994; Holland 1996). However, oral innervation in the two groups appears to be quite different. In vertebrates, effectors in the oral region are innervated by motor neurons arising in the brain, and the sensory neurons are derived from the dorsolateral placode series and neural crest (Northcutt 1996). Amphioxus lacks a neural crest so far as can be determined (Baker & Bronner-Fraser 1997) and, although the sources of neuronal cell bodies in the oral region are effectively mini-placodes, they could not conceivably be considered part of a dorsolateral series. The closest possible equivalent to the latter would have to be the ventral pit cells of the metapleural folds, which develop later near the end of the larval phase (Stokes & Holland 1995b). The only neural elements of peripheral origin that develop in the vicinity of the vertebrate mouth are taste buds and solitary chemosensory cells. Both of these are secondary sense cells, i.e. they synapse with peripheral nerves and lack axons themselves (Finger 1997). Experimentation shows that taste cells are derived locally and neither arise from identifiable placodes nor the neural crest (Barlow & Northcutt 1995). While we cannot be sure of the origin of receptor cells and neurons in the oral region in amphioxus, there is no evidence to date to indicate anything other than a local origin.

On this basis, we postulate that the mechanoreceptive oral spine cells in amphioxus may be homologues of vertebrate taste cells. While the functions of the two cell types are clearly different, a second feature supports the idea of homology, namely, the nature of the accessory cells with which each is associated. Taste cells in lower vertebrates are closely associated with a class of neuron-like cells located at the base of the taste bud, the serotonergic Merkel-like cell (Reutter & Witt 1993). Merkel-like cells in amphibian taste buds synapse with both sensory afferents and taste cells, and synapses with the latter are bidirectional (Delay & Roper 1988; Ewald & Roper 1994). Merkel-like cells evidently act as a kind of peripheral interneuron, providing positive feedback that modulates receptor cell sensitivity, stabilizing and amplifying the initial signal.

Serotonergic cells are not known to occur in the oral region in amphioxus larvae (Holland & Holland 1993b), but from the morphology and organization of the amphioxus oral nerve, it is possible to envisage intrinsic neurons functioning in a rather similar way to that proposed for vertebrate Merkel-like cells. Synapses between receptor cells and intrinsic neurons are not obviously reciprocal, but the extensive synaptic communication between these cells and surrounding fibres of a similar type may be an alternative way of achieving the same result.

There are also similarities between oral innervation in amphioxus and that of more primitive invertebrates. The ancestral chordate is generally assumed to resemble

modern hemichordates and/or echinoderms to at least some degree. However, the latter two phyla most resemble each other in the larval stage, which suggests the larva may be a better candidate for an ancestral chordate than the more specialized adult. A characteristic of such larvae is the presence of a well-developed plexus of nerves and clusters of cell bodies around the mouth. Neurotransmitters identified in such clusters, loosely called 'ganglia', include unspecified catecholamines, dopamine, serotonin and neuropeptides (Burke 1983; Burke *et al.* 1986; Bisgrove & Burke 1987; Thorndyke *et al.* 1992; Chee & Byrne 1997), and in several instances the oral nerves are continuous with a plexus that extends over the surface of the anterior gut.

Garstang (1928), a major proponent of the larval origin of chordates, derived the chordate dorsal nerve cord from the neurogenic ciliary bands of an ancestral larva essentially like that of modern echinoderms. A chief weakness of his idea is that there is no obvious counterpart of the larval adoral centres and visceral plexus in vertebrates. In other words, a significant portion of the ancestral nervous system must have disappeared as vertebrates evolved. Amphioxus may provide the missing link in this scenario, because it essentially has a vertebrate-like body plan, but it retains an oral and visceral innervation more like that of echinoderm larvae than of vertebrates. One can suppose, following Northcutt & Gans (1983), that with the evolution of the vertebrate head and neural crest, the monitoring and control of many visceral functions was centralized. Diffuse peripheral plexuses throughout the body were probably reduced or lost at this time, leaving little evidence of the ancestral condition except in organisms like amphioxus, which also retain a comparatively unmodified, pre-vertebrate head.

As a final point, our observations may help resolve a perennial question concerning the origin of the mouth in chordates. In amphioxus, besides the mouth and gill slits, connections between the endoderm and the exterior are formed by the club-shaped gland and the pre-oral pit. This and the curious position of the mouth have led to suggestions that the mouth may be secondarily evolved from a gill slit and, in addition, that the primitive mouth may be represented by the pre-oral pit (Van Wijhe 1919; also see Ruppert 1996). The similarities between the oral nerve in amphioxus and that of echinoderm larvae, and the absence of any such nerves in the pre-oral pit, provides comparatively strong evidence, in our view, that the amphioxus mouth and the mouth of more primitive deuterostomes are homologues.

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