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Phil. Trans. R. Soc. Lond. B 1998 **353**, 1943-1967
doi: 10.1098/rstb.1998.0347

THE ROYAL SOCIETY

The developing dorsal ganglion of the salp *Thalia democratica*, and the nature of the ancestral chordate brain

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The development of the dorsal ganglion of the salp, *Thalia democratica*, is described from electron microscope reconstructions up to the stage of central neuropile formation. The central nervous system (CNS) rudiment is initially tubular with an open central canal. Early developmental events include: (i) the formation of a thick dorsal mantle of neuroblasts from which paired dorsal paraxial neuropiles arise; (ii) the differentiation of clusters of primary motor neurons along the ventral margin of the mantle; and (iii) the development from the latter of a series of peripheral nerves. The dorsal paraxial neuropiles ultimately connect to the large central neuropile, which develops later. Direct contact between neuroblasts and muscle appears to be involved in the development of some anterior nerves. The caudal nerves responsible for innervating more distant targets in the posterior part of the body develop without such contacts, which suggests that a different patterning mechanism may be employed in this part of the neuromuscular system.

The results are compared with patterns of brain organization in other chordates. Because the salp CNS is symmetrical and generally less reduced than that of ascidian larvae, it is more easily compared with the CNS of amphioxus and vertebrates. The dorsal paraxial centres in the salp resemble the dorsolateral tectal centres in amphioxus in both position and organization; the central neuropile in salps likewise resembles the translumenal system in amphioxus. The neurons themselves are similar in that many of their neurites appear to be derived from the apical surface instead of the basal surface of the cell. Such neurons, with extensively developed apical neurites, may represent a new cell type that evolved in the earliest chordates in conjunction with the formation of translumenal or intralumenal integrative centres. In comparing the salp ganglion with vertebrates, we suggest that the main core of the ganglion is most like the mes–metencephalic region of the vertebrate brain, i.e. the zone occupied by the midbrain, isthmus, and anterior hindbrain. Counterparts of more anterior regions (forebrain) and posterior ones (segmented hindbrain) appear to be absent in salps, but are found in other tunicates, suggesting that evolution has acted quite differently on the main subdivisions of the CNS in different types of tunicates.

Keywords: *Thalia democratica*; tunicates; CNS organization; chordate evolution; salps

Received 30 April 1997 *Accepted* 30 June 1997

1. INTRODUCTION

The brain is remarkably similar in overall organization among vertebrates, its main subdivisions (forebrain, midbrain and hindbrain) being identifiable in even the most primitive representatives of the group. Regions homologous with both the forebrain and hindbrain have been identified also in amphioxus, an invertebrate chordate, by using both molecular markers (Holland & Garcia-Fernàndez 1996; Holland *et al.* 1996; Williams & Holland 1996) and structural landmarks (Lacalli *et al.* 1994; Lacalli 1996*a*), and the zone between these has some of the attributes of the vertebrate midbrain (Lacalli 1996*a*). This suggests that much of the basic plan of the anterior part of the nerve cord, from which the vertebrate brain is evidently derived, was established by the time the common ancestor of amphioxus and vertebrates had evolved. Much less is known, however, about how that basic plan originated.

Tunicates, i.e. appendicularians, ascidians, doliolids, salps and pyrosomes, are generally accepted as being even more primitive than amphioxus, and are arguably the surviving group closest to the ancestral chordate (Bone 1960; Gans 1989; Gee 1996). The CNS in tunicates with a pelagic larva (appendicularians, ascidians and doliolids) typically begins as a tubular rudiment, as in other chordates, but in all tunicates except appendicularians, this develops into a compact ganglion with a central neuropile that is superficially quite different from the CNS in either amphioxius or vertebrates. There is now a wealth of evidence for conserved patterns of neural organization within otherwise diverse taxa (e.g. among arthropods; Edwards & Palka 1991; Whitington 1996), and for conservation of developmental control mechanisms, even between phyla that are only distantly related (e.g. between vertebrates and arthropods; Finkelstein & Boncinelli 1994; Manak & Scott 1994; Simpson 1995). Similarities in the underlying basic plan of the CNS within any one phylum are therefore to be expected despite differences in anatomy. By using modern techniques, it should be possible to clarify the relationship between the tunicate ganglion and the CNS in other chordates and, specifically, to identify counterparts of the forebrain, midbrain and hindbrain, if they exist.

This paper concerns the development of the dorsal ganglion in a salp. Salps are pelagic tunicates known especially for their complex life histories. They have an alternation of generations in which the sexually produced individual (the solitary stage) reproduces asexually by budding off a chain of blastozooids, which are usually then released as aggregates. Each member of the aggregate in turn reproduces sexually, typically by producing a single egg that is fertilized internally and develops directly into a small adult within the parent atrium, nourished by a placenta. Embryonic development therefore takes place within a protected environment, and a free-swimming larval stage is absent. The rudiment of the CNS develops directly to form the dorsal ganglion, which is essentially the 'brain' of the organism, i.e. it is the only concentrated neural centre. The ganglion (figure 1) is globular with a central neuropile, a surrounding rind of neuronal cell bodies, and a series of radiating peripheral nerves. As there are no obvious subdivisions along its anteroposterior axis, however, it cannot easily be compared with brains that are axially subdivided, as in vertebrates. The object of this study was to see whether an electron microscope (EM) study of ganglion development would reveal landmarks and/or substructure not previously reported, and so provide the basis for a more meaningful comparison with other chordates.

Past developmental research on tunicates has concentrated on ascidians, which are generally easier to study than pelagic forms. Salps were chosen here instead of ascidians because the larval nervous system in the latter is asymmetrical, and the adult ganglion is represented by only a small rudiment. This is probably a derived condition, and makes the preservation of a significant number of primitive features less likely. The salp ganglion, in contrast, is symmetrical and develops from a rudiment of some size, so there is at least the possibility that it has been subject to less modification during evolution than the ascidian CNS. Among other tunicates, the appendicularian CNS is reduced both in size and cell number (Brien 1948; Olsson *et al.* 1990), as indeed are the organisms themselves; the embryo in pyrosomes (the cyathozooid; Godeaux 1957, 1990) is so modified that it lacks a functional nervous system altogether, which leaves doliolids as the only group besides salps with a substantial and well-developed ganglionic rudiment in the embryo. Later in the life cycle of doliolids, pyrosomes, salps and colonial ascidians, neurogenesis occurs again, indeed repeatedly, as the ganglion of each blastozooid is formed. This does not, however, mean that the process of embryonic neurogenesis is reiterated without modification. In fact, CNS development in blastozooids appears to differ in various respects from that occurring in the embryo. In salps, for example, the embryonic ganglion develops from an ovoid, self-contained rudiment, whereas the blastozooid ganglion buds from an elongate tubular structure resembling a neural tube (Berrill 1950*b*). The blastozooid ganglion also incorporates a neural vesicle derived from genital tissue (Sawicki 1966), a curious phenomenon that has no counterpart, so far as is known, in the embryo.

Our observations on the development of the embryonic ganglion in *Thalia* are described below in §§ 3 and 4. The ganglion is symmetrical and shows a significant degree of internal differentiation into regions that resemble, to a degree, CNS structures in other chordates. The significance of the observations, both in relation to other chordates and to prechordate dipleurula larvae, is discussed in §§ 5 and 6.

2. METHODS

Specimens of *T. democratica* were collected by plankton tows in the Bay of Villefranche, France in Spring, 1987 and 1990. The zooids are small and reasonably well suited to detailed EM analysis. Embryos were isolated from the aggregate stage and fixed in 3% glutaraldehyde in 1% $K_2Cr_2O_7$ (pH 7.4) containing 0.7 NaCl, and embedded in Spurr's resin according to the methods of Holland (1988). Section series were prepared from seven specimens, covering a range of stages of CNS development. EM sections were taken typically at 0.5 µm intervals through regions of interest; they were stained

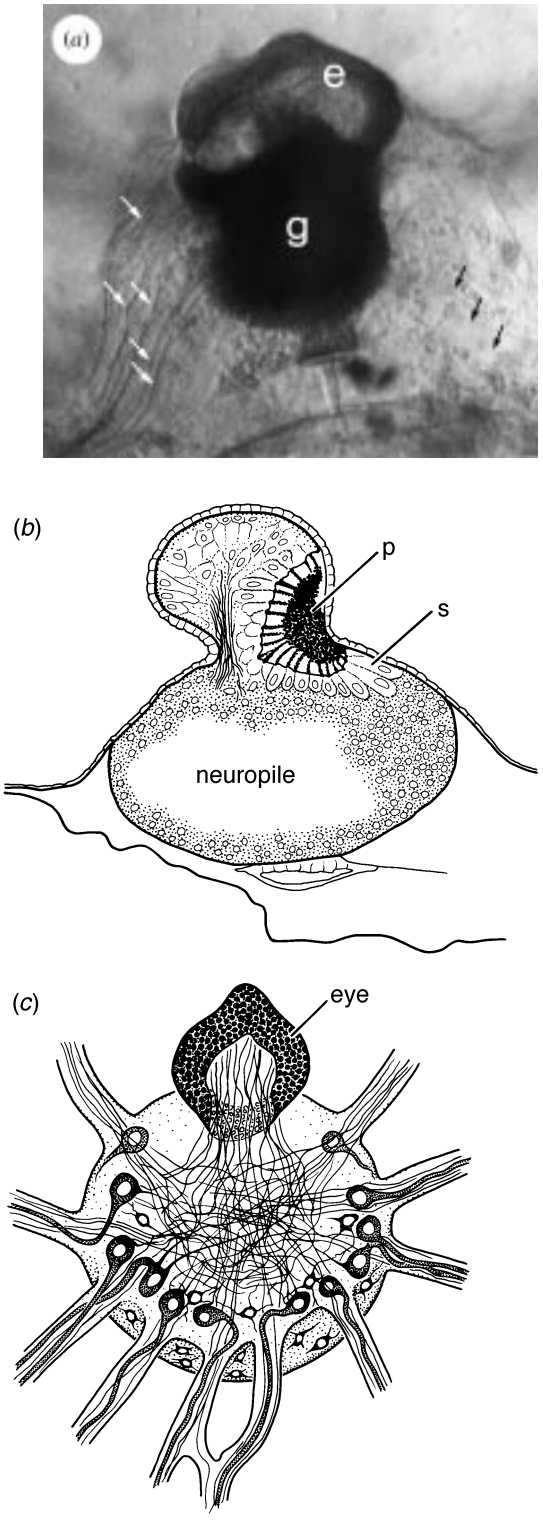

Figure 1. Some representative salp ganglia. (*a*) The dorsal ganglion of an advanced-stage embryo of *Cyclosalpa affinis* from a stained whole-mount. The solitary stage in this species has a large, horseshoe-shaped cerebral eye (e) positioned directly above (dorsal to) the main body of the ganglion (g). The open end of the horseshoe points forward (to the left in the figure); both rostral (white arrows) and caudal (black arrows) nerves can be seen in this view, radiating from the underside of the ganglion. (*b*) A mid-sagittal section through the ganglion of the solitary stage of *Thalia democratica*, from Metcalf (1893). The eye is much smaller than in *Cyclosalpa*. This section cuts the closed (posterior) end of the horseshoe; pigment (p) and sensory cells (s) are shown, along with a nerve tract linking the eye to the neuropile. (*c*) A schematic diagram showing the arrangement of large motor neurons and selected smaller cells and fibres in the ganglion of the aggregate stage of *Salpa maxima*, after Fedele (1933). Neurobiological studies typically concentrate on the aggregate stage. Solitary stage ganglia have been less studied, but their internal organization is assumed to be similar.

with uranyl acetate and lead citrate using standard methods. A serial EM series was obtained through the anterior half of the developing ganglion of one of the late neural tube (t3) stages, and was used for reconstructing individual neurons. Muscle patterns were reconstructed from series of 0.5 µm light microscope sections. Methods for reconstruction and imaging are described by Lacalli *et al.* (1994). Volume estimates from reconstructions have about a 10% uncertainty because of variable section thickness, lost sections, and our inability to acurately guage the degree of shrinkage during fixation.

Specimens had to be staged by relative size rather than age, as the size–age relation of growing embryos has not been precisely determined. However, if the embryos grow at the same rate as the young feeding stages (a minimum 10% increase in length per hour under optimal conditions (Heron 1972)), the time of development for the early to late neural tube stages examined here would be about 6 h from t1 to t2, and likewise for t2 to t3. On the basis of body size alone, there would be a difference of 2 h between t3 and the ganglion stage described here. The much greater degree of tissue differentiation in the latter suggests that this is probably an underestimate, however.

3. RESULTS

Early development in salps is unusual for chordates in that gastrulation and neurulation do not occur in a conventional fashion. Instead, the blastomeres become dispersed during early embryogenesis within a conical mass of follicle cells called the blastophore. As the follicle cells degenerate, the blastomeres coalesce and form the tissue and organ rudiments directly (Brien 1928; Sutton 1960). By the time this happens in *T. democratica*, the embryo is about 200 µm long with a ventral placental mass and a posterior eleoblast (figure 2*a*). The former is a nutritive organ of largely maternal origin (Bone *et al.* 1985); the latter is a derivative of the embryonic mesoderm that is thought to serve as a metabolic reserve and source of blood cells (Brien 1928). The rudiment of the CNS is visible first as a solid mass of cells lying immediately above the anterior part of the pharynx. A canal opens in the centre of the rudiment by the time the embryo has a body length, exclusive of the eleoblast, of 250 µm. The open canal persists until the embryo has grown to about 600 µm, by which time the CNS rudiment has taken on the more globular shape of the definitive ganglion and begins to project above the dorsal surface of the body. A central neropile then forms, so that the neural canal is obliterated by a mass of nerve fibres. The period of development during which the canal remains open will be referred to here as the neural tube, or 'tube' phase. We focus on three embryos representing early, middle and late stages of this period of development (t1, t2 and t3, respectively; see figures 2 and 3). The

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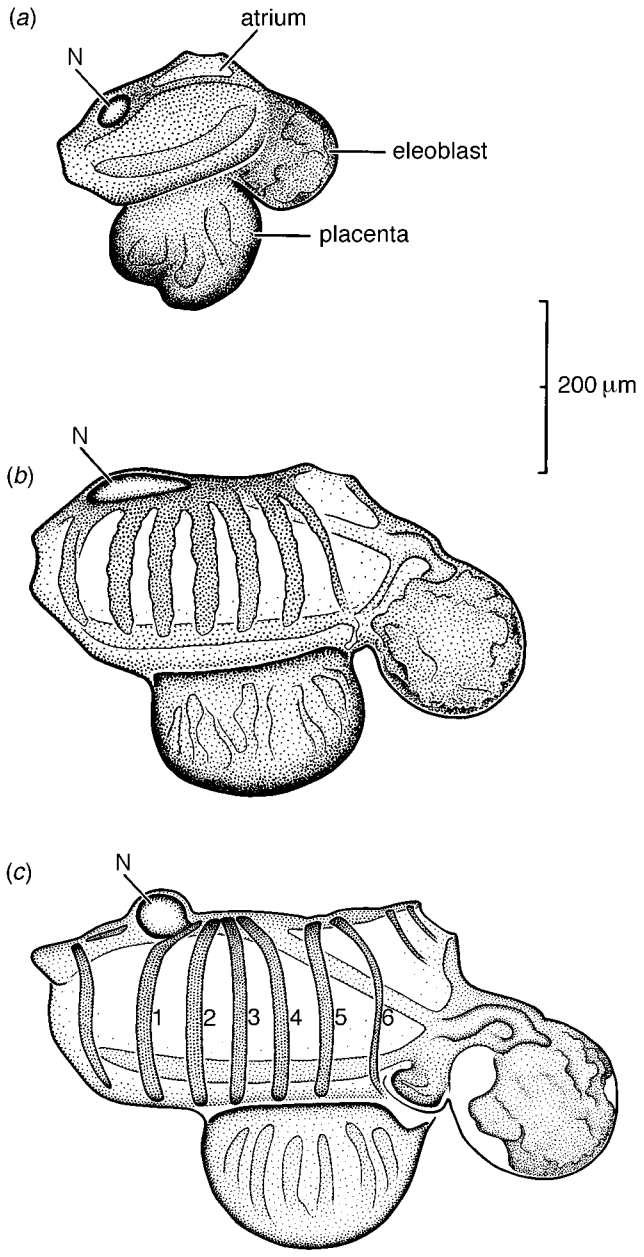

Figure 2. The embryo of *T. democratica* at three stages roughly corresponding to the neural tube stages (t1–t3) described in the text. The sequence shows the progressive development of the definitive pattern of muscle bands (numbered) and the neural rudiment (N).

'ganglion' stage described here is from an embryo only slightly larger than the oldest tube stage, with a body length of about 800 μm. The ganglion is clearly not fully developed at this stage, but shows significant changes from the earlier condition. What we lack in this study are the intermediate stages between the late tube and the early ganglion to show the initial phase of neuropile formation. Consequently, events during this period, and the precise relationship between the neural canal and the neuropile, can only be inferred.

(a) *Early development: the neural tube phase*

Figures 3 and 4 show the changes that occur during this period in the external form of the neural rudiment (figure 3*a*), the extent of its lumen (figure 3*b*), and the arrangement of cells in transverse section (figure 4). The rudiment consists initially of a single-layered epithelial tube (t1) that lengthens and broadens as the embryo grows (t2), and then swells and contracts to form a compact ovoid mass (t3). The anterior end of the tube at t1 contacts the pharyngeal epithelium and, in fact, the two tissues are fused and indistinguishable at this point. The cells in this region are subsequently drawn out to form the rudiment of a ciliated duct, the neural (or neurohypophyseal) duct. The duct develops its own internal canal that is at first separate from the rest of the neural canal. The two connect secondarily (figure 3*b*), and an open channel is thereby formed connecting the pharynx with the inside of the neural tube. The inner surface of the duct is ciliated at this stage. The duct later separates from the ganglion as the latter differentiates, so its connection to the neural canal is broken. In the ganglion stage examined here (figure 3*c*), the top surface of the duct was still attached to the front of the ganglion, but its bottom surface was not, leaving small gaps connecting the lumen with the surrounding space. The mature duct is heavily ciliated (figure 3*d*), and its cells are multiciliate. Cells facing the rest of the neural canal (i.e. the neuroepithelium proper) lack cilia. Each, however, has a single basal body and rootlet at its apical surface, which shows that the cells are essentially uniciliate even though ciliary axonemes fail to form.

By the middle phase of neural tube development (t2), the tube is dorsoventrally flattened, and layers of cells have begun to accumulate basal to the neuroepithelium to form a mantle that extends over the dorsal and lateral surface of the neural tube (figures 4*b*,*c* and 5*a*). We assume that the mantle is made up of postmitotic cells that have migrated outward from the neuroepithelium, as occurs in other chordates, but we cannot be sure. All but one of the mitotic figures encountered in sections were located in the neuroepithelial layer, and many mantle cells by t2 showed some signs of differentiation (e.g. neurite formation). The presence of proliferative centres elsewhere than in the neuroepithelium cannot be ruled out, however. The one mitotic figure found outside this layer was located near the ventral midline, in a cluster of cells belonging to a midventral cord of tissue that joins the neural duct further forward. These cells remain undifferentiated during the stages examined (they are visible as a midventral knot of cells in figure 4*b*, and at the very base of the ganglion in figure 7). Rather than being neurons, they may be a floor plate derivative, as discussed in § 3b.

Judging from the increase in the volume of the neural rudiment, from t1 ($8.5 \times 10^4\ \mu m^3$) to t2 ($3.75 \times 10^5\ \mu m^3$), and the decrease in average cell volume (from 220 to $80\ \mu m^3$), cell number increases roughy 12-fold during this period, to about 4500 cells. The mantle layer is best developed laterally and dorsolaterally near the front of the neural tube (figure 5*a*), and cell differentiation appears to be more advanced in this region. The presence of a medial crease along the top of the CNS rudiment at the front suggests that dorsolateral proliferation exceeds that in the medial plane.

From later stages, it is evident that the primary motor neurons develop from among the large cells located along

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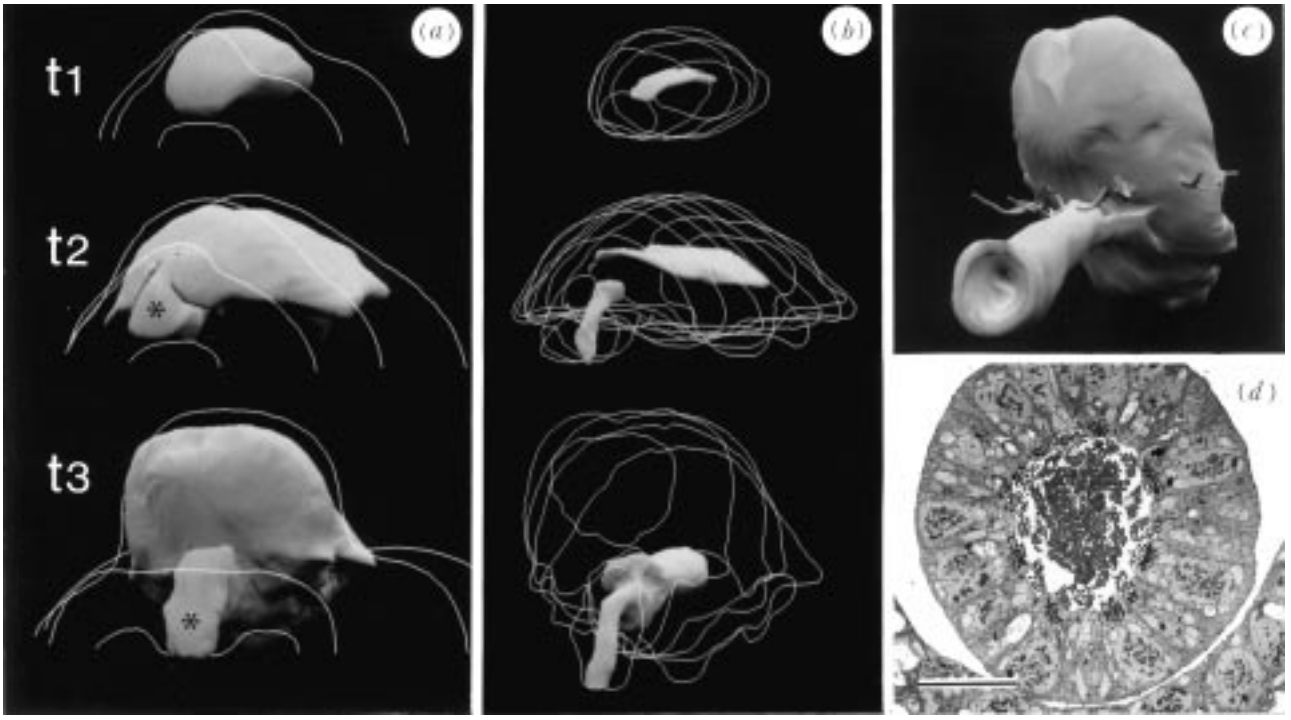

Figure 3. (*a*) Reconstructions of the developing neural rudiment at stages t1–t3, with selected body contours (35-μm intervals) and the inner surface of the pharynx (lower contour) where it meets the developing neural duct (*). (*b*) The same reconstruction series as in (*a*) showing the outer surface of the rudiment in contour (12-μm intervals) with, inside these, the surface of the neural canal and the lumen of the neural duct. The canal and duct form separately, but connect secondarily by t3. (*c*) A reconstruction of the ganglion stage described in § 3b. The duct has expanded, and separates from the ganglion so that it no longer connects internally to the central canal which, by this stage, has been obliterated by the developing neuropile. (*d*) Transverse section through the duct at the ganglion stage, showing the dense mass of cilia that fill its lumen. Scale bar, 10 μm.

the lower margin of the outermost layer of the mantle. We cannot be certain of precisely where, along the dorsoventral axis of the neural tube, these neurons originate. They are clearly more ventral than the cells of the dorsolateral mantle, however, which means that they are ventral in origin in relative terms at least.

The second phase of neural tube development, from t2 to t3, involves a change in shape, as the comparatively short neural rudiment becomes even shorter and more globular. Mitotic figures were still encountered at t2, but the t2 stage is probably near the end of the proliferative phase. Tissue volume increased only slightly from t2 to t3 (to *ca.* $4.0 \times 10^5\ \mu m^3$), and the cells were smaller on average ($60–65\ \mu m^3$). The t3 stage thus contains about 6400 cells, an increase of only 30% from t2. Also, by t3, the peripheral nerves that supply adjacent muscles have formed. We have not traced the t3 neuronal clusters and fibres in detail, but most of the nerves identified in the ganglion stage, which was examined in detail, appear to be present in rudimentary form at t3, as short tracts of two to three fibres (figures 6 and 13). There are pairs of rostral and lateral nerves, and a number of individual posteriorly projecting fibres that are almost certainly pioneers for the caudal nerves described here.

One aspect of neuronal differentiation in lower chordates deserves special attention. This is the relationship between the pattern of neurite outgrowth and the apicobasal polarity of the cells. The axons of many types of invertebrate neurons develop from the basal surface of the cell, i.e. they arise from the basolateral compartment of the cell surface. Motor neurons in amphioxus larvae are an example: they retain their apical connection to the neural canal, so it is quite clear that their axons are basal structures, whereas their dendrites typically arise from somewhere near the base of the axon. There are other instances in amphioxus where cells produce neurites from their apical surface while retaining their connection to the neural canal, so the neurites themselves project into or across the canal and form a kind of plexus (Lacalli & West 1993). Furthermore, in other cases, the cells first migrate away from the neural canal, and then develop neurites whose apical origin can be inferred from their proximity to the ciliary basal body, which is retained. Examples are the tectal cells described by Lacalli (1996*a*). With this last example in mind, an attempt was made in this study to see whether neuronal cell polarity could be monitored in *Thalia* by examining the t3 and ganglion stages for a consistent relationship between centriole position and sites of neurite outgrowth.

For cells in the dorsal and lateral parts of the CNS in both stages, considerable variability was observed in both cell morphology and degree of differentiation. Most of the cells that had differentiated were multipolar, with several to many short processes, typically of similar size, so that no one process could be identified as an axon. The processes arose from diverse points on the cell surface, and no obvious patterns were discernable. Basal bodies (some still attached to the cell membrane, figure 5*b*) and centrioles (figure 5*c*) were found near sites where several processes originated in some cells, but off to the side of

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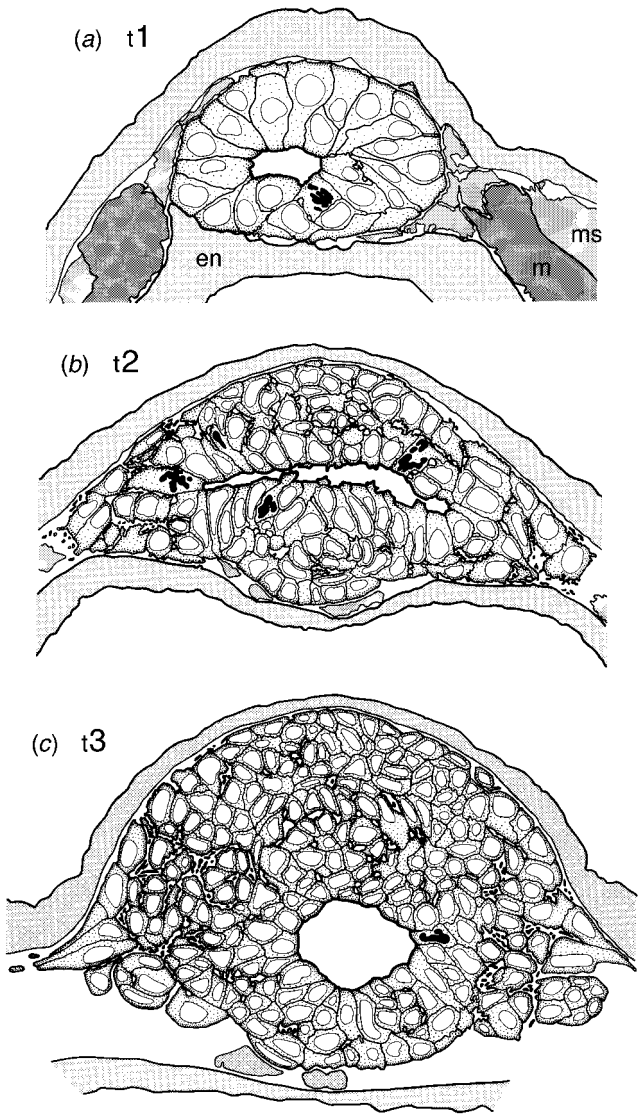

Figure 4. Transverse sections through the neural tube about midway along its length at stages t1–t3. Cell outlines show the layered structure and the degree of fibre development. Surrounding tissue includes differentiating muscle bands (m), loose mesenchyme (ms), and the pharyngeal epithelium (en); mitotic figures are solid. The developing mantle, consisting of differentiating neuroblasts, is visible in (*b*) and (*c*), but is more obvious in more anterior sections, e.g. in figure 5*a*.

the cell, away from such sites, in others. Some cells had two centriole-like dense bodies, often separated by some distance. Ventrally, below the neuropile, it was more usual to find centrioles facing the lumen at the base of the cell's largest neurite, which then projected directly into the neuropile. We conclude, therefore, that at least some of the neurites entering the neuropile are apical processes, but it is by no means certain that all, or even most, are. Some of the variability in these observations could be accounted for if the correlation between sites of neurite outgrowth and centriole position were a transient characteristic of a particular stage of differentiation. If the relationship persisted only through early phases of neurite outgrowth, for example, it would be seen in only a fraction of the cells in any one specimen, as neurons in different parts of the ganglion are clearly at different stages of differentiation.

The situation is clearer in the case of the primary motor neurons. A total of five examples were examined, and each had either one or two centrioles or dense centriole-shaped structures. Remnants of rootlets were attached to some of these. In each case, the centrioles were some distance from the base of the axon, usually on the opposite side of the cell, despite the presence of microtubule arrays in the axon itself. Dendritic zones were also on the side opposite the axon, often near one or the other of the centrioles (figure 6*c–f*). Our conclusion is that the axons of primary motor neurons arise from the basal surface of the cell. Dendrites form on the opposite surface, nearer the centriole.

(b) *Ganglion structure*

Figures 7–9 show the one ganglion stage examined in detail. By this stage, the central canal was completely obliterated and replaced by a fibrous neuropile comprising neurites arising from the cells arranged around it. There is, in addition, a pair of small dorsolateral neuropiles running parallel to the ganglion axis, referred to here as the paraxial neuropiles. Fibre tracts link the latter with the central neuropile at two points: near the centre, where tracts from each side converge towards the midsagittal plane, and near the back, where the junction is more lateral in position. The paraxial neuropiles are not linked directly to each other. The only other identifiable fibres tracts were two small ones originating from cells located immediately in front of the paraxial neuropiles, connecting first to the latter, and then running down to the anterior cluster of motor neurons as shown in figure 9. These two nerves are the front 'horns' in the neuropile reconstructions (e.g. figure 8*d*). There are large regions containing only undifferentiated cells below the neuropile and at the back of the ganglion at this stage.

As all of the cells in the midventral line that face the central canal produce neurites, the ganglion lacks an identifiable floor plate of the kind found in nerve cords in more advanced chordates. We note, however, the presence of a ventral cord of cells (* in figure 7) extending along the lower surface of the ganglion that connects with the upper surface of the neural duct. The cells do not differentiate as neurons so far as we could determine. They could instead be a floor plate derivative, but one that separates early from the neuroepithelium rather than remaining part of it. This interpretation is supported by the recent finding that genes specific to floor plate are expressed in the ascidian larval nerve cord (Corbo *et al.* 1997).

No specific signs of eye differentiation were apparent in the ganglion at the stage examined. According to Metcalf (1918), the cerebral eye develops from a dorsal, horseshoe-shaped rudiment, lying immediately above the zone occupied, in our specimens, by the paraxial neuropiles. There is, in fact, a roughly horseshoe-shaped mass of undifferentiated cells in this location (** in figure 7) that we interpret as being presumptive eye tissue. The crease visible at the dorsal midline in figures 7 and 8 then runs down the centre of the horseshoe, between its forward-projecting arms. If this interpretation is correct, it would mean that the eye is essentially a dorsal structure, not an anterior one. The anterior mantle tissue in the developing salp ganglion is quite thick, however,

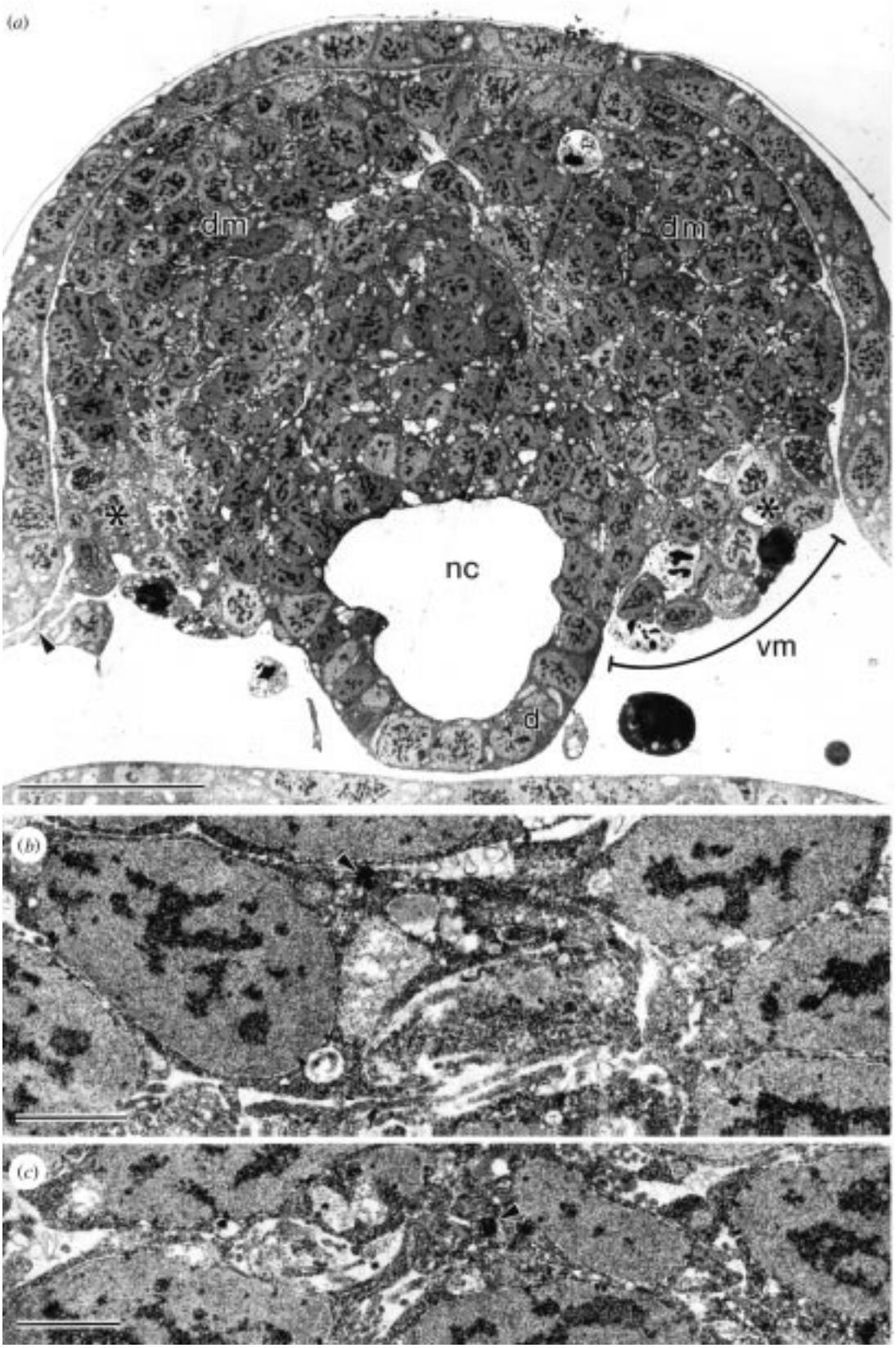

Figure 5. (*a*) Transverse sections through the neural tube at t3 near the connection between the duct (d) and the neural canal (nc), anterior to the section shown in figure 4*c*. Paired dorsolateral masses of mantle cells (dm) are evident; the primary motor neurons (clustered near *) are found along the ventral margin of the mantle (vm), and give rise to peripheral nerves (the arrow indicates one of these). Scale bar, 20 µm. (*b*, *c*) Details of t3 dorsal neurons with multiple cell processes typical of those found throughout the developing dorsolateral parts of the mantle. Arrows indicate a basal body, minus cilium, at the cell surface in (*b*), and a centriole in (*c*). Scale bar, 2 µm.

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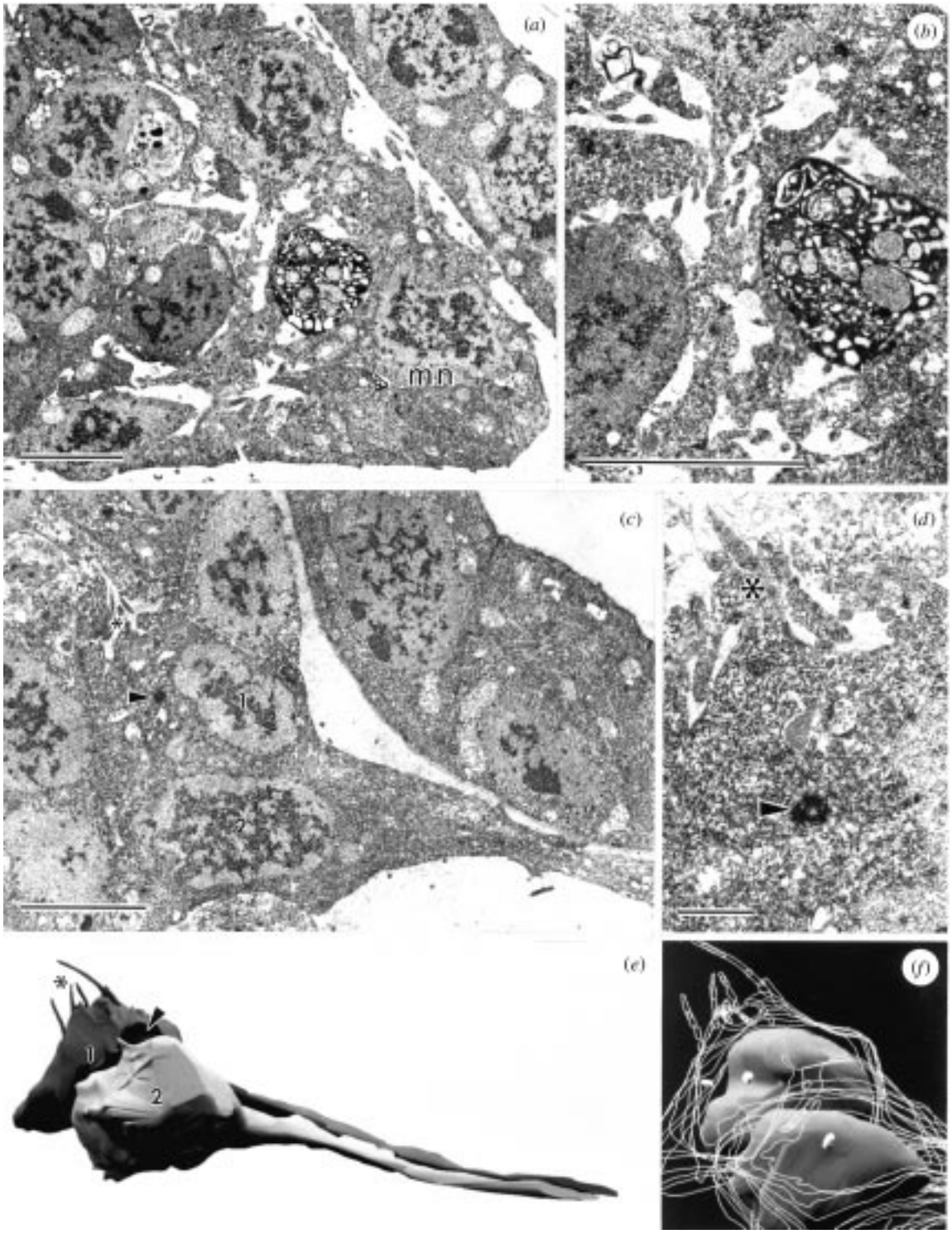

Figure 6. Differentiation of motor neurons in the anterior (C1 and C2) clusters. (*a*) The ventrolateral part of the mantle at t2; the primary motor neurons (mn) develop from cells in the surface layer. (*b*) A detail of (*a*) showing the early neurites just medial to the primary motor neurons. (*c*) The region shown in (*a*) at t3 showing the motor neurons responsible for one of the lateral peripheral nerves; detail in (*d*), reconstructions in (*e*) and (*f*). A total of three motor neurons contribute; two of these (numbered) are shown. The third cell lies forward of the other two; its axon passes between cells 1 and 2 at the point indicated by the arrow in (*e*), and then travels out of the ganglion beside the other two axons. The dendritic zone of cell 1 is marked (*), and the position of the centrioles in cells 1 and 2 are shown in (*f*); their proximity to the dendritic zone is evident in (*d*). Scale bar, 5 µm in (*a*, *b*, *c*); scale bar, 1 µm in (*d*).

and cell rearrangement and differential growth could displace tissue rudiments secondarily within it. We therefore cannot rule out the possibility that the eye rudiment is of anterior origin, but has secondarily expanded across the dorsal surface during development. The issue is important in terms of relating the salp eye to photoreceptors in other tunicates (§ 5a), and in assessing the nature of the neural duct (§ 4c).

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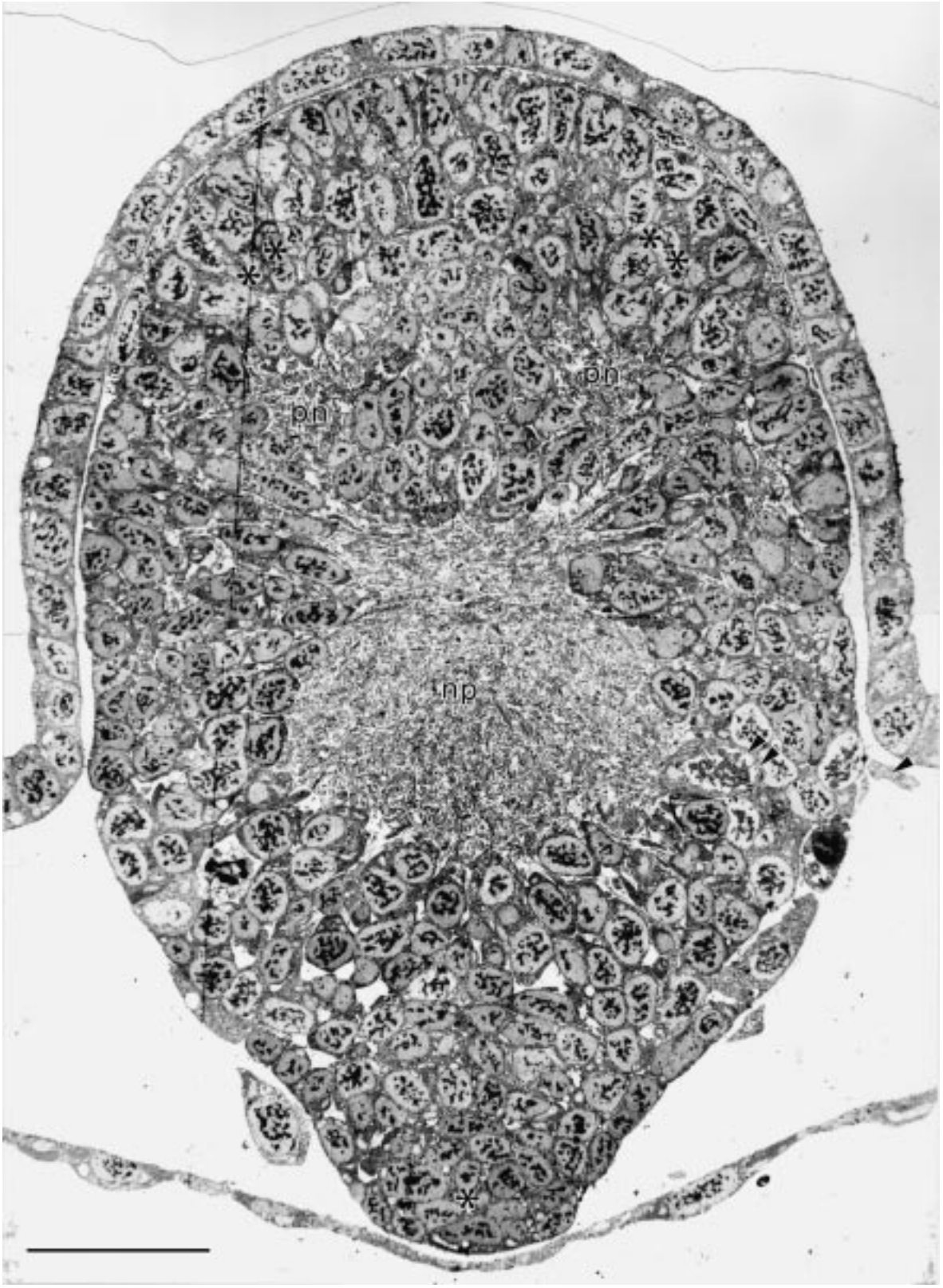

Figure 7. Transverse section through the ganglion in figure 3*c* to show the two dorsal paraxial neuropiles (pn), and the large central neuropile (np) formed by fibres projecting inward from both lateral and ventrally positioned cells. Based on past studies, the compact masses of cells (**) located just above each paraxial neuropile belong to the eye rudiment. The ventral cluster of cells marked with a single * belongs to a strand of tissue that runs along the underside of the ganglion. This may be a floor plate homologue, but if so, it detaches early from the neuroepithelium. The section is taken near the back of the C1 clusters of motor neurons, and includes one large C1 cell (double arrows) that contributes directly to the neuropile. Part of nerve 4 (arrow) is also indicated. Scale bar, 20 μm.

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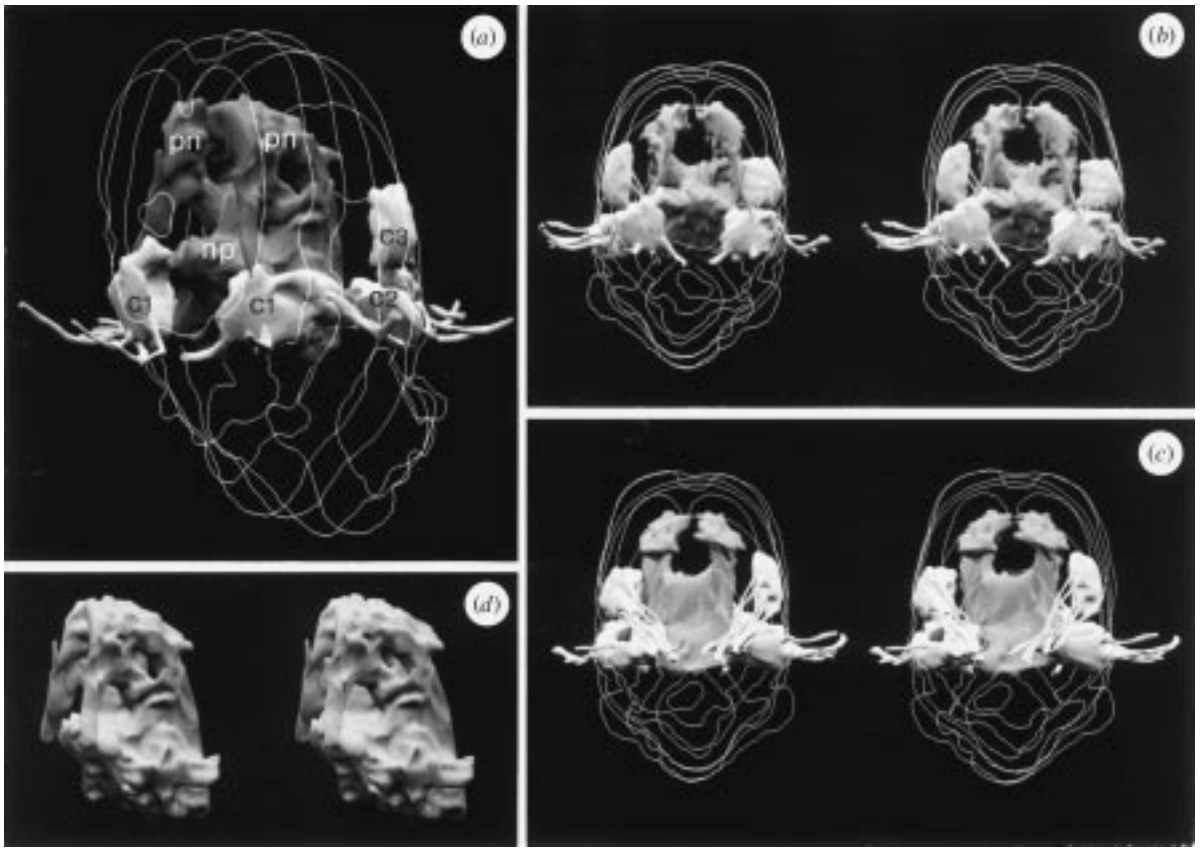

Figure 8. (*a*) Reconstructions of the ganglion in oblique front view. The figure shows the central neuropile (np), the dorsal paraxial neuropiles (pn), and the surrounding clusters of motor neurons (C1–C3) from which the peripheral nerves arise. Surface coutours are at 8 μm intervals. (*b*, *c*) Stereo front and back views of the same reconstruction. (*d*) Stereo side view of the neuropile alone.

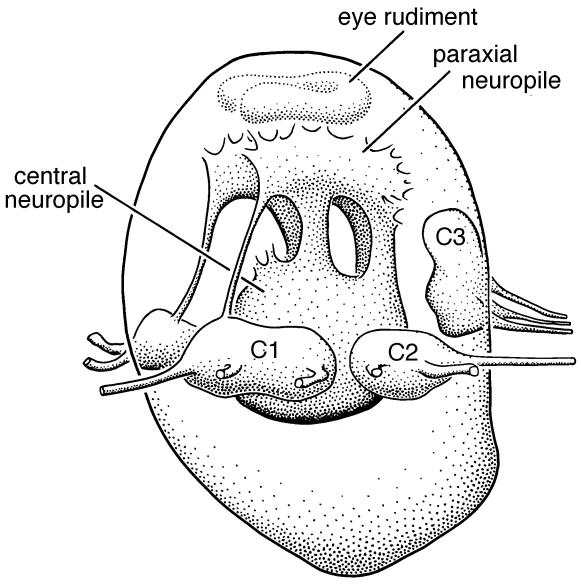

Figure 9. Summary diagram of the ganglion, for comparison with figure 7; shows the neuron clusters (C1–C3), the paraxial and central neuropiles, and the position of the cells that will later form the cerebral eye.

The peripheral nerves that radiate from the ganglion originate from three paired clusters of cells with large cell bodies (C1–C3 in the figures). The most anterior pair (C1) is located at the front of the ganglion in the equatorial plane. A total of three anteriorly directed nerves arise from it on each side, along with, on the right side, an unpaired medial nerve. The middle clusters (C2) lie near the back of the ganglion, also in the equatorial plane, and their nerves project mainly to the sides. Each member of the third pair (C3) is positioned above and slightly behind C2, with its ventral margin very close to the posterior margin of C2. The C3 clusters differ somewhat from C1 and C2 in the way the cells are organized and in the appearance of their nerves. Neuronal cell bodies in C1 and C2 are buried in the ganglion and make direct contact, at their inner surfaces, with the central neuropile or parts thereof (cf. figure 7). Their axons emerge from the ganglion in distinct bundles, and supply the muscles located mainly rostral and lateral to the ganglion, although posterior branches from C2 also supply the muscle bands immediately behind the ganglion (figure 13). In contrast, cells in C3 are arranged along the side of the ganglion (figure 10*a*,*b*), and their axons travel a short distance caudally before combining to form three to four loosely organized bundles on each side (figure 10*c*,*d*). The number of fibres in each bundle varies, fibres appear to cross between bundles (the reconstructions are simplified in this respect; they show the main bundles, but not individual fibres), and fibre numbers also increase with distance from the ganglion. This suggests that the fibres may branch within each nerve, or, possibly, that differentiating sensory fibres are already growing into the ganglion using existing tracts as pathways.

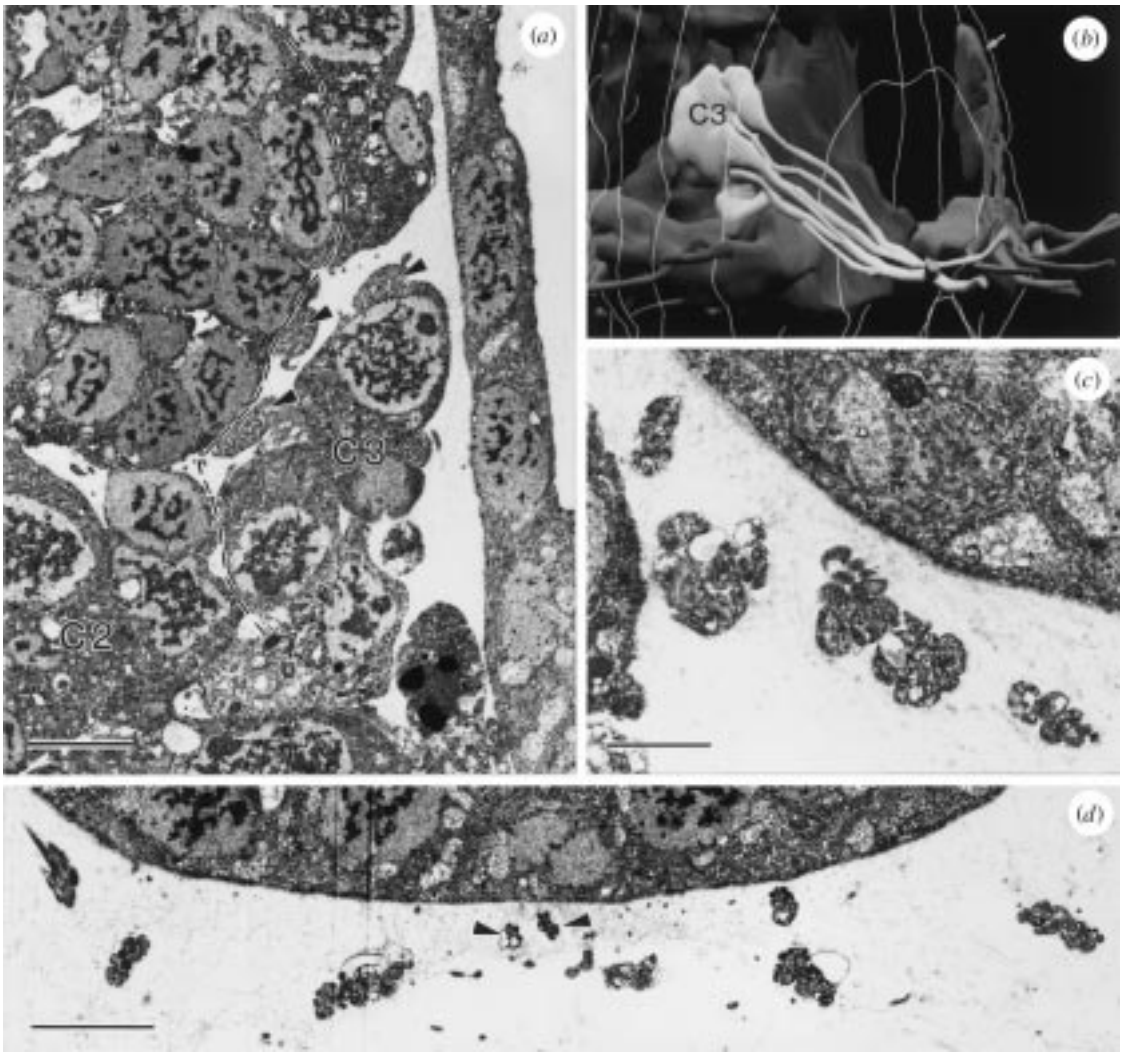

Figure 10. The caudal nerves at the ganglion stage. (*a*) A transverse section through the back of the ganglion showing the peripheral location of the neuronal cell bodies belonging to C3 on the left side. The probable limit to C3 is shown with the dashed line, C2 is adjacent, and arrows indicate proximal parts of individual axons arising from cells in C3. Scale bar, 5 µm. (*b*) Detail from the reconstruction in figure 8 to show the caudal cells. The fibres are simplified: most are bundles of several individual fibres, and these cross at times from one bundle to another, indicating that the nerves are rather loosely organized. The extreme flattening of the C3 cluster as a whole can be seen on the right side (arrow). (*c*) A section near the very back of the ganglion on one side; shows bundles of caudal nerves derived from the cells in (*a*). Scale bar, 2 µm. (*d*) A section taken behind the ganglion, centred on the dorsal midline. Arrows indicate the two small medial nerves derived from the dorsal-most cells in C3. Scale bar, 5 µm.

The C3 clusters also differs from other clusters in having no direct access to the neuropile at the stage examined. Instead, they are separated from the neuropile by several layers of undifferentiated cells. Assuming the C3 cells eventually add to the neuropile as they differentiate, a link could be formed between the neuropile and C3, but if so, this is a comparatively late event. The dorsal position of the C3 clusters is also noteworthy, although it may be largely a secondary consequence of changes in cell position that occur as the neural tube shortens. At t2, the large neuroblasts we presume to be the primary motor cell precursors lie along the two sides of the neural tube, and all occupy approximately the same equatorial position. As the neural tube shortens, the more caudally positioned of these cells are evidently drawn forward and upward, so they form a compact mass above the equator of the ganglion, while cells behind them and deeper in the mantle are pulled forward to from the posterior ventral 'tail' of the ganglion visible in the reconstructions.

(c) *Muscle position and innervation*

The muscle bands that encircle the salp body are numbered, by convention, in an anteroposterior sequence starting with the first band that extends to the dorsal midline. Partial bands further forward are referred to as intermediate muscles. Applying this rule across species leads to inconsistencies, however, as the dorsoventral extent of the anterior-most bands differs between species and also changes during development (see, for example, Metcalf 1918). For *Thalia*, we number the muscle bands beginning with the first pair that converges behind the ganglion (figures 11 and 13). In the older literature, this muscle is considered to be intermediate in character.

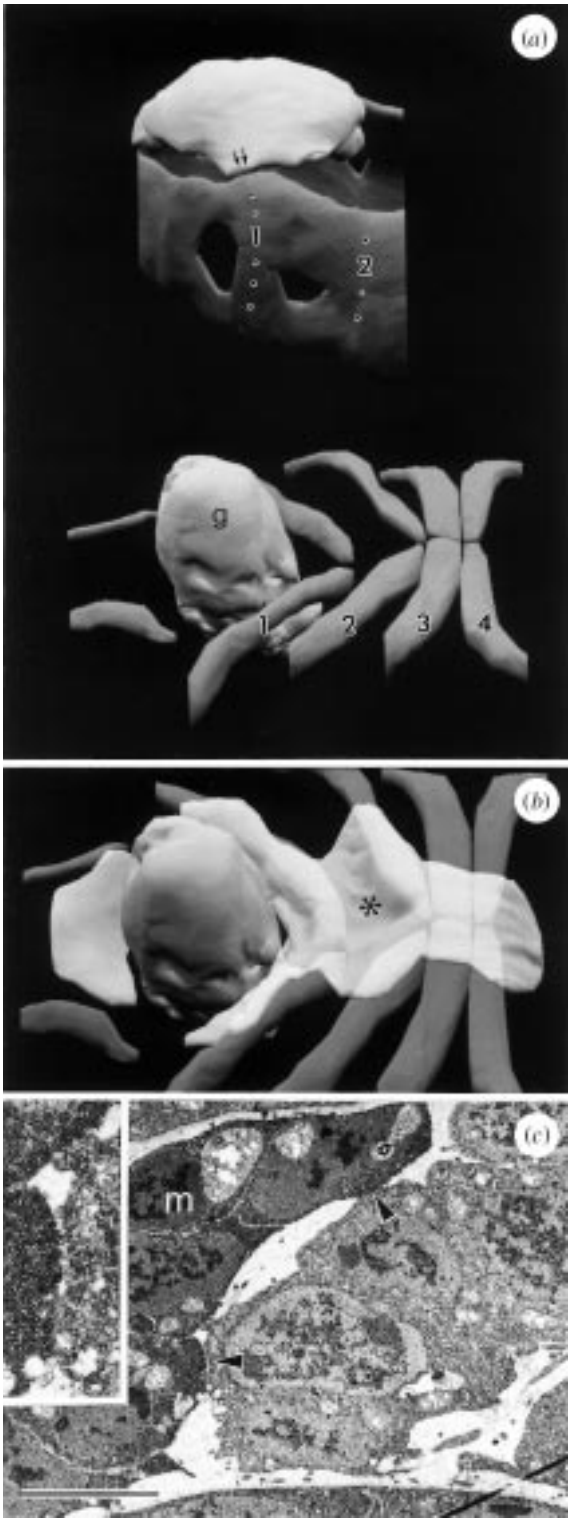

Figure 11. Development of the dorsal body muscles in reconstruction. (*a*) A comparison of the mesodermal mantle at t2 (top), in which the developing muscle bands are embedded, and the final position of the bands themselves (numbered) at the ganglion (g) stage (bottom), to the same scale. Note the relative change in registry between the neural tube and the mesodermal elements: the muscle pattern expands as the embryo grows while the neural tube undergoes a pronounced shortening. (*b*) The muscle pattern at the ganglion stage overlaid with the thickened zone of epithelium (*) where muscle attachments occur. (*c*) Section showing contacts (arrows) at t2 between the neural tube and muscle (m) at the point indicated in (*a*) by double arrows. Scale bar, 5 µm.

The muscle bands develop from an initially unsegmented sheet of lateral mesoderm (Berrill 1950*b*). They are visible as distinct bands by t2, but narrow and become better defined as development proceeds. The anterior bands develop first as paired rudiments that are brought together secondarily at the dorsal midline (figure 11*a*). The more posterior bands develop as a single unit with no obvious gap at the dorsal midline, which has led to disagreement over whether they derive from single or paired rudiments (Brooks 1893). The posterior bands in *Thalia* are clearly paired, although the gap between the two halves of the band is very small (figure 12*c*). The entire muscle series evidently therefore begins as a set of bilaterally paired rudiments, and dorsal convergence and fusion occurs secondarily.

The presence of obvious serial repeats in the mesoderm raises the question of whether the salp CNS also incorporates serially repeating elements and, if so, how these relate to the mesodermal pattern. To deal adequately with this issue means taking account of the changing positional relationship between the neural tube and the mesoderm. The problem is that the muscle bands shift their position relative to the neural tube as the body grows. The neural tube not only fails to keep up, but actually shrinks somewhat. The farther away a particular muscle is from the ganglion, the more pronounced this effect is, so it is quite obvious in the posterior muscle bands (cf. figure 2), but is noticeable also in the anterior bands. For example, near the time the bands first begin to develop, the dorsal margins of bands 1 and 2 are in register (i.e. aligned) with the middle and posterior parts, respectively, of the neural tube (top, figure 11*a*). By the ganglion stage (bottom, figure 11*a*), both bands meet at the dorsal midline behind the ganglion, the dorsal part of each band having been shifted backwards as the ganglion itself is compressed forward. There appear to be several reasons for this. The dorsal epithelium thickens progressively (figure 11*b*), presumably because the tissue itself is contracting locally, and this draws the muscle bands attached to it towards the dorsal midline and backwards, away from the ganglion. Actual fusion is prevented in the case of band 1 by a keel of very thick tissue that develops just behind the ganglion (figure 12*a*). To a degree, therefore, one can envisage the dorsal margins of the muscles being towed some distance toward the midline by epithelial contraction. Considering the distances involved, however, it seems likely that some active movement of muscle rudiments over the inner surface of the epithelium would also be required. Zones of contact between the two tissues are observed (figure 12*b*) that could be involved in such movements.

The effect of shifting muscle position can also be seen in innervation patterns (figure 13). The ganglion stage has seven approximately symmetrical pairs of rostral and lateral nerves (nerves 1–7 in the figure), one anterior medial nerve on the right (nerve 0), and three pairs of caudal nerves. Nerves from C1 travel forward to the oral muscles (one innervates the dorsal horizontal muscle in passing), whereas band 1 is innervated by three nerves, with various branches, from C2. The posterior-most of these pass over band 1 and, through branches, supply more lateral parts of bands 2 through 4. The caudal nerves from C3 appear to bypass bands 2–4 altogether, and travel on to bands 5 and 6, the atrial muscles, and

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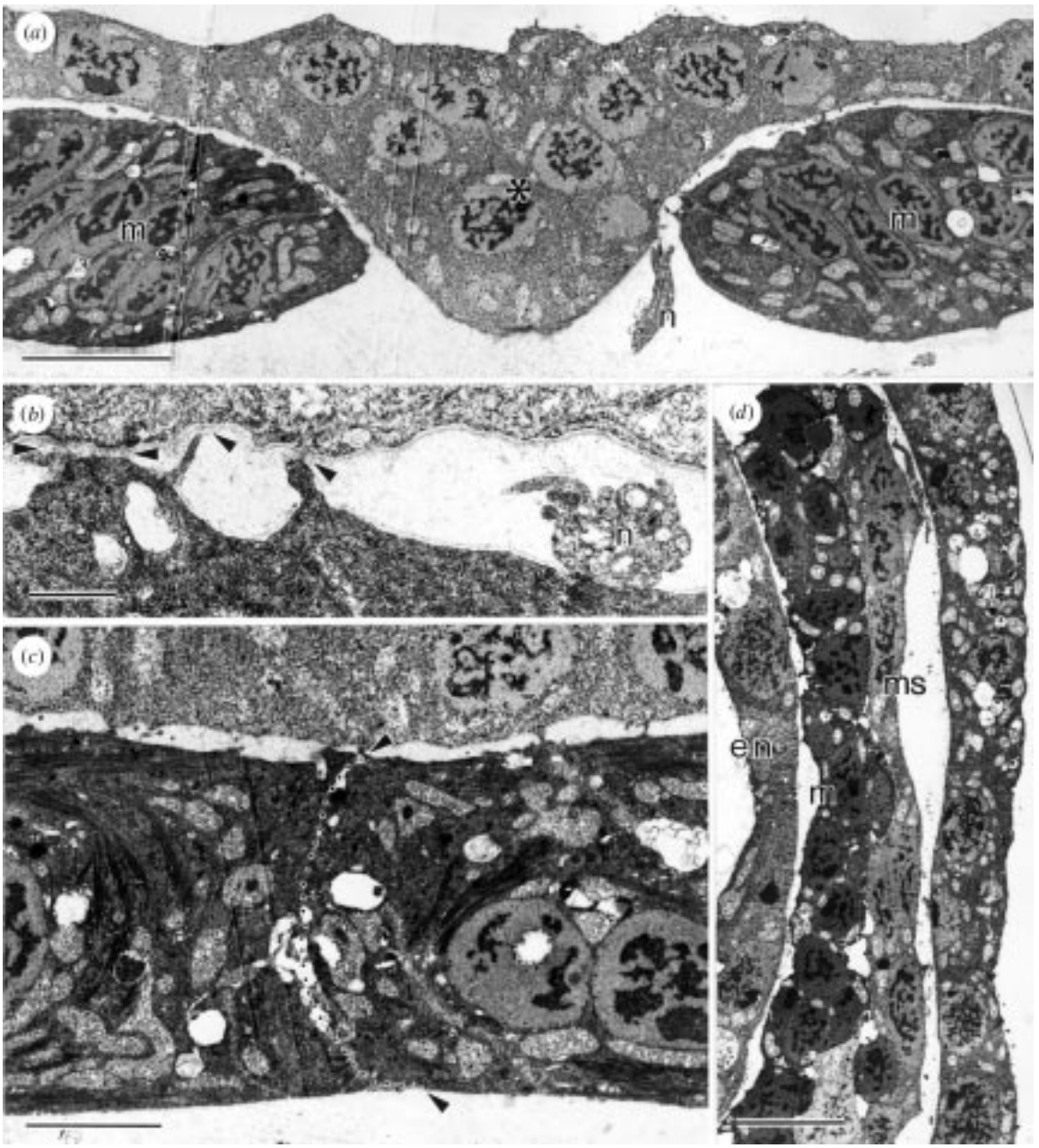

Figure 12. The muscles bands, each of which consists of a bundle of long multinucleate muscle fibres. (*a*) The convergence of the two halves of band 1 (m) at the dorsal midline at the ganglion stage; they are prevented from meeting by the keel-like midline thickening of the epithelium (*). Scale bar, 10 µm. (*b*) Detail of mucle attachments (arrows) to the dorsal epithelium adjacent to the midline keel; nerve (n). Scale bar, 1 µm. (*c*) Band 6, which is joined mid-dorsally at this same stage; the dotted line (between arrows) indicates the junction between the left and right halves of the band to show that they are, in fact, separate muscles. Scale bar, 5 µm. (*d*) An anterior band at t2, before its full dorsal extent is established. Shows the sheet of mesenchyme (ms) that overlies the differentiating muscle (m) adjacent to the pharynx (en). Contacts link the mesenchyme to the ectoderm; these may be involved in positioning the mesodermal mantle during this phase of its development. Scale bar, 10 µm.

other posterior structures, probably including the visceral organs.

We lack some intervening stages, but from the specimens available, the innervation appears to develop as follows (summarized in figure 14): at t2, the developing muscle bands are visible as cords of densely staining cells lying adjacent to the pharyngeal epithelium on either side (figure 12*d*). The sheet of mesenchyme and muscle has a well-defined upper (dorsal) margin that approaches the developing neural tube closely only along part of its length, specifically, along the anterior and middle part of the neural tube. There are regions of direct contact (figure 11*c*) adjacent to band 1 (figure 11*a*, top), which at t2 is orientated roughly perpendicular to the antero-posterior axis. Both in front and behind this region the muscle sheet is separated by a gap from the neural tube, and this is especially large posteriorly. A similar gap is also seen at younger stages, before the muscle bands have begun to develop. The intervening space at these stages is filled with a loose mesenchyme, but this disperses so that, by t2, the space between the neural tube and muscle sheet is largely empty. This is of some importance

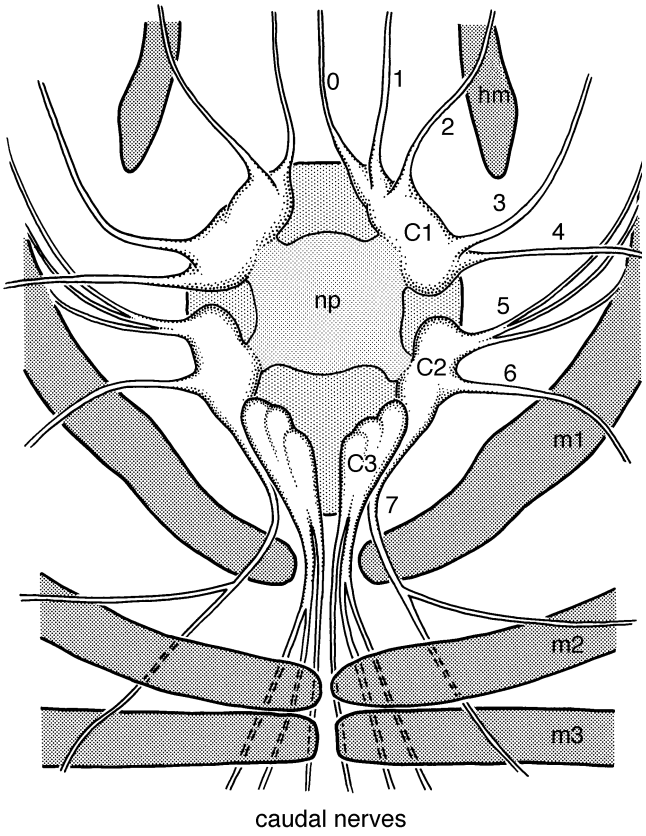

Figure 13. Diagram showing the nerves (numbered, with the medial anterior nerve as 0) and muscle bands (shaded and numbered) at the ganglion stage, somewhat simplified. Most of nerves shown (excepting small branches) were found also in the late tube stage (t3).

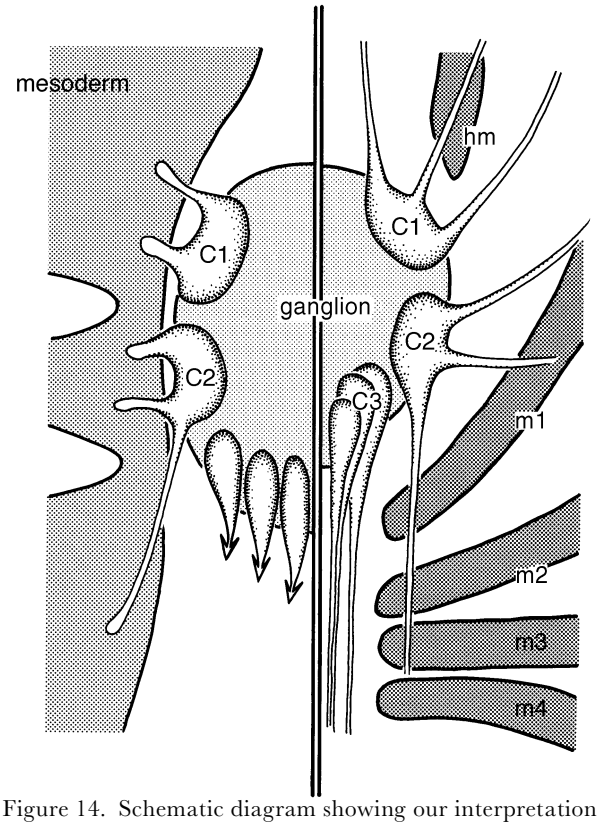

Figure 14. Schematic diagram showing our interpretation of how nerve–muscle registry changes between t2 (left), when the innervation pattern is first being established, and the t3–ganglion stage (right). The muscle bands are displaced, the effect being most pronounced at the dorsal midline, and the nerves are towed along with the muscles. The caudal nerves initiate their growth after t2, but we do not know precisely when. They are portrayed schematically as originating from a row of three groups of cells. The cells involved lie along the back of the ganglionic rudiment, and may be arranged in groups in this fashion, but we are uncertain of the details.

because, from our observations, t2 appears to be about the stage that nerves first grow out from the neural tube. It is logical to suppose that the targets of these very early nerves would, in each instance, be the closest mesodermal derivatives. This is easy to see in the case of the C2 nerves innervating band 1, where there is clearly direct contact between developing neural and mesodermal cells. The only nerve from C2 that does not supply band 1 (nerve 7) nevertheless begins by crossing over band 1 on its way to more posterior muscle bands. In fact it is typical for salp nerves to cross several muscle bands, innervating each in passing (Fedele 1933; Bone & Ryan 1973). There may be a comparable stage of contact also between C1 and adjacent muscle precursors, but if so, we have missed it. The distance between C1 and the anterior part of the mesodermal sheet, which later breaks up to form a complicated set of oral muscles, is not very great, however. For C1 and C2, therefore, either the target muscles themselves or the mesodermal rudiment from which they develop are very close to the sites of initial nerve outgrowth.

The same cannot to be said of C3. There are no signs of the caudal nerves at t2, but they are visible as small fibres by t3. This suggests they grow out comparatively late, but are established before the anterior muscle bands have fully converged on the dorsal midline. If the caudal nerves grow out at t2 or later, they presumably must pioneer pathways to distant targets without direct mesodermal contact to provide either patterning or guidance cues, because the mesenchyme is largely dispersed by then.

4. CONCLUSION AND GENERAL REMARKS

(a) *The dorsal ganglion*

This account traces the development of the CNS of a salp from its early tubular stage, through periods of cell proliferation and differentiation, to the formation of a compact ganglion with a large internal neuropile. Early events include the elongation of the tubular rudiment and the formation of the neural duct. A thick dorsal mantle of neuroblasts then develops; clusters of motor neurons differentiate around the equatorial margin of the mantle, the peripheral nerves develop, and a pair of dorsal paraxial neuropiles are established within the mantle. Later events include the shortening of the rudiment as a whole, which gives the ganglion its definitive ovoid shape, and the inward growth of neurites to form a central neuropile in place of the original neural canal. The primary motor neurons and cells in the dorsolateral parts of the mantle thus begin their development, and form nerves and internal plexes, considerably before the rest of the ganglion differentiates, and well before there is any sign of a central neuropile. As the paraxial neuropiles lie immediately below the zone in which the cerebral eye develops, their role may be to provide a pathway from the eye to the central neuropile. This seems to be the pattern in

other salp species, especially those that have a large eye, as their suboptic neuropile is effectively continuous with the main neuropile (Metcalf 1893, 1918).

Tunicate ganglia, including those of salps, are generally described as being globular structures with very little in the way of internal subdivision or axial patterning. While the ganglion in *Thalia* is severely truncated in comparison with more typical neural tubes, our results show that it nevertheless has patterns of dorsoventral differentiation that resemble those found in nerve cords of more advanced chordates, i.e. in vertebrates and amphioxus. Recent molecular evidence indicates that this is also true of the larval nerve cord in ascidians (Corbo *et al.* 1997; Wada *et al.* 1997). In advanced chordates generally, the first motor neurons to develop are ventral, whereas interneurons are predominantly dorsal. Primary motor neurons in *Thalia* are also ventral, at least in relative terms, as they develop along the lower margin of the outer layer of the mantle tissue. The dorsal part of the mantle, in contrast, consists of a loose mass of cells, most of which probably become interneurons, and which form local neuropiles. The large central neuropile, which forms later, is a typical feature of tunicate ganglia generally. Although vertebrates lack such neuropiles, amphioxus has a translumenal fibre system, discussed more fully in § 5b, that is similar in some respects.

Unlike nerve cords in other chordates, the salp CNS shows no obvious signs of being subdivided along the anteroposterior axis. However, because of the comparatively loose organization of the caudal motor neurons clusters (C3) and their separation from the neuropile, we will treat the caudal part of the ganglion as a distinct region for comparative purposes (cf. § 5a).

(b) *Neural and mesodermal patterning: the registry problem*

The muscle bands in salps are arranged in a regular series along the length of the body, although the body is not segmental in any strict sense of the term. From a comparative perspective, it would be very useful to know how salp embryos manage to establish appropriate innervatation to a repeating series of muscle elements. Basically, what one wants to know is how and where the serial pattern originates, and how development in the mesoderm and nervous system are coordinated. Unfortunately, nothing is known of gene expression in salps, and the embryos are not amenable to the kinds of experimental manipulations normally used to answer such questions. The development of the ganglion does, however, suggest to us that the anterior and posterior parts of the ganglion may be patterned in slightly different ways. The main clusters of motor neurons in the anterior and middle parts of the ganglion (in Cl and C2) innervate anterior muscles associated with the oral complex and anterior part of the pharynx. The clusters develop roughly in register with the muscle rudiments they ultimately innervate, and in close proximity or direct contact with them. This means that patterning events in the two tissues could, in principle, be coordinated by a contact-mediated signalling mechanism of some kind.

The posterior region of the salp body, including much of the rest of the pharynx and all of the atrial and visceral musculature, is controlled by caudal nerves originating from paired clusters (C3) located at the back of the ganglion. Explaining how these establish appropriate innervation patterns is more difficult because the muscles rudiments lie some distance from the CNS at the time the nerve fibres first grow out. We cannot rule out the possibility that the pattern is specified much earlier when the body as a whole is smaller and the tissue rudiments much closer together. It seems unlikely that the posterior mesoderm is ever in register with the ganglion rudiment, however, because the atrial rudiment occupies the posterior middorsal region from quite early in development, and the posterior part of the mesodermal mantle develops adjacent to it.

We conclude, therefore, that the demands placed on the neuromuscular patterning system in the anterior part of the body may differ from those acting more posteriorly. Specifically, there may be less need for an intrinsic patterning mechanism in the anterior part of the neural tube, compared with the posterior part, if patterning cues are imposed on the former by adjacent tissues.

(c) *The neural duct*

The neural tube shortens prior to neuropile formation, and this coincides with the period during which the lumen of the neural (neurohypophyseal) duct connects to the neural canal. The duct is an enigmatic structure that forms early in embryogenesis in tunicates. In ascidians it develops later into the neural gland, which remains connected to the pharynx. The situation in salps is more complicated because the mature solitary stage lacks a typical neural gland. Instead, it produces a second set of neural ducts that are unrelated to the embryonic duct (Metcalf 1918). The embryonic duct nevertheless does appear to be homologous to the ascidian neurohypophyseal duct and neural gland system.

The term 'neurohypophyseal' is used to imply homology with the vertebrate pituitary, but there are problems with this idea. Ruppert (1990) has noted that the orifice of the duct could be treated as either a coelomopore or neuropore. In principle, because coelomic cavities in deuterostomes develop from the anterior endoderm, a duct arising from pharyngeal tissue could be thought of as belonging to a coelomic rudiment that has failed to complete its separation from the endoderm. Its orifice would therefore be essentially a coelomopore. On the other hand, any extension from the anterior neural tube that connects at some point to either ectoderm or endoderm, as the duct does, could be a secondarily displaced neuropore.

From our salp data we cannot rule out the possibility that the duct is derived in some way from the neuropore, but this does not seem very likely. When the duct opens into the neural canal, it does so by fusing to what is then the underside of the neural tube, so the point at which the duct and the neural tube meet is definitely neither dorsal nor anterior at that stage. When the duct rudiment is first evident, however, it appears to be developing from the very front of the neural tube. Because proliferating mantle cells then grow down over the front of the neural tube as well, it is possible that original anterior pole of the neural tube is shifted ventrally, so that the duct ultimately connects at a point that has been secondarily

displaced. This would mean, however, that the cerebral eye ultimately forms a considerable distance away from what would have originally been the anterior pole of the neural tube. Therefore, either the eye is not itself a very anterior structure or, conversely, the anterior-most cells of the neural tube have been rearranged so as to separate the neuropore from any nearby photoreceptor fields.

Considering the alternatives, it seems more likely that the duct is a coelomic derivative. This accords more closely with the probable function of the duct and the neural gland that develops from it. Despite evidence for hormone secretion (Goodbody 1974), the neural gland seems to be mainly a hydrostatic organ that controls fluid flow into the blood spaces of the tunicate (Ruppert 1990). This would make the embryonic neural duct in salps and the neural duct–gland system in other tunicates the functional counterpart of the pore canal–hydropore complex in dipleurula-type larvae of echinoderms and hemichordates (Ruppert & Balser 1990), which derives from the larval coelom. The pore canal in dipleurula larvae connects to the dorsal (aboral) surface of the body. Garstang (1894) has proposed that the aboral epithelium is rolled into the neural tube in chordates. If this is correct, the hydropore homologue in chordates would be predicted to open immediately behind the infundibular region in the floor of the neural tube. A duct connecting this site to the pharynx could then be accounted for as a derivative of the pore canal in the dipleurula. Although the duct does appear to develop from the neural rudiment, if it actually connects to the floor plate, it would be essentially a piece of modified somatic ectoderm rather than neural tissue, as usually supposed.

Published accounts of salp development show the connection between the pharynx and neural tube, but do not trace its mode of formation in detail. Based on our specimens, the cells of the duct originate from a region of the neural tube where the fusion of neural and pharyngeal tissue makes the two indistinguishable from one another. The duct itself could therefore combine cells of both pharyngeal and neural tube origin. The lumen of the duct connects first to the pharynx, and only secondarily to the neural canal. By the time the neural tube is beginning to shorten, the duct is ciliated and presumably functional. In fact, if it were capable of generating an inwardly directed fluid flow at this stage, it would provide a mechanism for driving the shape change the ganglion experiences during this period, i.e. by pumping fluid into the neural canal. This could explain why the epithelial lining of the neural canal remains intact until comparatively late in development, and also why neuropile formation is delayed until after the ganglion has achieved its final dimensions and globular shape.

5. COMPARISON WITH OTHER CHORDATES

(a) *Other tunicates*

There are various theories as to how tunicates evolved, summarized by Holland (1992). The ideas of Garstang (1928) and Berrill (1950*a*) have been especially influential until recently; both argued that the ancestral tunicate was essentially similar to modern ascidians, i.e. it was sedentary rather than pelagic, and had a tadpole larva. Recent evidence, however, from data on gamete morphology (Holland *et al.* 1988) and 18S RNA sequences (Wada & Satoh 1994) indicates that the pelagic appendicularians diverged earlier from the tunicate lineage than ascidians. Even so, the ancestral tunicate may not have resembled modern appendicularians very closely, as the latter have adapted to an unusual and very specialized mode of feeding involving a disposable mucus filter or 'house'. If appendicularians are inadequate models for the ancestral tunicate, it is not clear that ascidians are any better. The problem becomes apparent when comparing CNS organization among the different tunicate groups, because it is then difficult to accept the tadpole CNS as being other than specialized and rather modified, at least in comparison with salps. Figure 15 illustrates this by comparing the *Thalia* ganglion (figure 15*b*) with the CNS of selected ascidian tadpole larvae. A similar case can be made by comparing the salp and appendicularian CNS, but we will focus here on the better-known ascidians.

Among ascidians, the main features of the larval CNS are best seen in solitary forms like *Ciona* (figure 15*a*), because the rudiment of the adult ganglion has not yet developed. Here the CNS includes an expanded anterior sensory vesicle and a smaller posterior visceral ganglion, connected to the nerve cord, which extends along the tail. The sensory vesicle contains a ventral otolith, formed from a single cell, and an ocellus with separate pigment and receptor cells, and lens cells in some species. A similar arrangement occurs in the larvae of compound ascidians (figure 15*b*), although some styelids (e.g. *Botryllus*) have a photoreceptor only, with a pigment cup that receives thick projections derived from the cilia of adjacent sensory cells (figure 15*c*). This unusual receptor has been called a 'photolith' and is thought to derive from the otolith (Torrence 1980). It is clearly not a modified ocellus. Cells with bulbous ciliary projections have also been described from tadpole larvae (e.g. ciliary bulb cells in *Ciona*, figure 15*a*). These cells are variously interpreted as pressure or motion detectors (Katz 1983; Nicol & Meinertzhagen 1991), and may be related to the sensory projections of the photolith.

Tracts of fibres run from tissue at the back of the ascidian sensory vesicle through the visceral ganglion. The visceral ganglion itself contains a number of large cell bodies that probably contribute to the nerve tracts. Nicol & Meinertzhagen (1991) counted 18 such cells in *Ciona*, although details of the neuronal circuitry in the larva have not been worked out. The portion of the nerve cord extending into the tail is composed of ependyma, and lacks neurons (Crowther & Whittaker 1992), although sensory neurons are found in the adjacent epithelium (Torrence & Cloney 1982).

In the larvae of compound ascidians, there is typically some precocious differentiation of adult structures, including the rudiment of the ganglion. Figure 15 shows *Amaroucium* and *Botryllus*, but a good deal is also known about *Distaplia* (Torrence & Cloney 1982, 1983). The ganglionic rudiment in these species develops from the left side of the neural tube between the sensory vesicle and the visceral ganglion, but remains largely undifferentiated until after metamorphosis. Ganglion differentiation and neuropile formation has not been examined in detail, and it is not known whether there are subdomains within the developing ganglion in any way comparable

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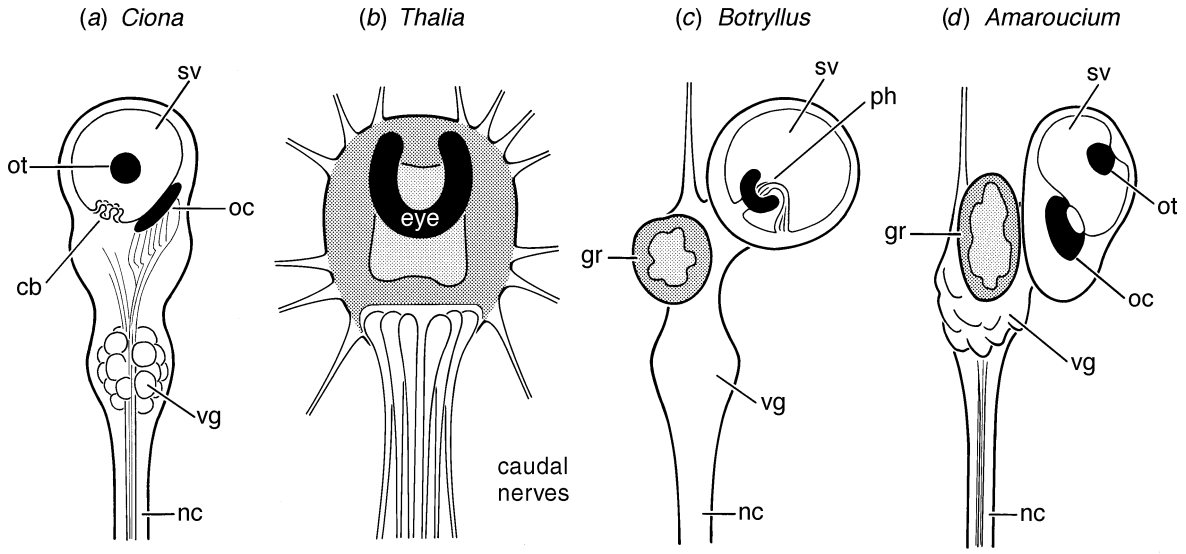

Figure 15. A comparison of CNS organization in tunicate embryos and larvae: (*a*) the larval CNS in *Ciona*, a solitary ascidian, based on Nicol & Meinertzhagen (1991). *Ciona* has an anterior sensory vesicle (sv) containing an otolith (ot), ocellus (os), and clusters of ciliary bulb cells (cb). This connects posteriorly to the visceral ganglion (vg) and thence to the nerve cord (nc); there is no obvious ganglionic rudiment at this stage. (*b*) The *Thalia* ganglion, as described here, for comparison. The main neuropile region is shaded to indicate its essential similarity to undifferentiated ganglion rudiment (gr) in the other examples. The cerebral eye in salps is basically dorsal in position. *Thalia* otherwise lacks anterior sense organs or any obvious homologue of the sensory vesicle. (*c*) The larval CNS in *Botryllus*, a compound ascidian, from Grave & Woodbridge (1924). The sensory vesicle contains a single sense organ, the photolith (ph). The ganglionic rudiment is situated between the sensory vesicle and the visceral ganglion. (*d*) Larval CNS in *Amaroucium*, another compound ascidian, from Grave (1921). *Amaroucium* has the same complement of sense organs as *Ciona*, i.e. an otolith and an ocellus.

to those reported here for *Thalia*. Nor is the fate of the visceral ganglion in ascidian larvae fully understood. It is considered to be a larval structure and, in fact, is referred to as the 'larval' ganglion in some accounts. It is said to disperse or be destroyed at metamorphosis (see, for example, Scott 1952), which would be expected if it were responsible only for nerves supplying the tail. The posterior part of the adult body, including the visceral organs, is innervated by a single visceral nerve originating from the posterior part of the ganglion. There is also a visceral nerve plexus supplying the gonads and gonoducts (Mackie 1995). This part of the nervous system is notoriously difficult to follow (cf. Goodbody 1974), and it is quite possible that the visceral innervation includes retained elements of the larval visceral ganglion. This is an important point, as the visceral ganglion expresses Hox genes (Katsuyama *et al.* 1995; Satoh *et al.* 1996), which suggests it is a homologue of vertebrate hindbrain. It is consistent with our understanding of the relationships between body plans in chordates (Gilland & Baker 1993; Kuratani 1997) to expect nerves from the hindbrain, or its homologue, to innervate visceral organs in the adult.

A comparison of the CNS of ascidians with that of *Thalia* suggests that the neuropile of the latter, together with the cells immediately surrounding it (shaded in figure 15) is the probable homologue of the ganglionic rudiment (also shaded) in the ascidian tadpole. The main sensory structures (sensory vesicle in ascidians, cerebral eye in salps) lie either forward of this zone (ascidians) or dorsal to it (salps). We do not know whether the dorsal position of the salp eye reflects its site of origin in the neural tube, or is secondary. If the latter, it could instead be essentially an anterior structure, in which case it could be a homologue of one of the structures contained within the ascidian sensory vesicle. The ocellus is the most likely candidate. Receptor cells in tadpole ocelli have arrays of ciliary lamellae (Eakin & Kuda 1971; Barnes 1974), but some also have surface microvilli. Both are considered typical structural specializations to be expected in cells that have adapted for photoreception by elaborating the cell membrane at or near the base of the cilium (Vanfleteren & Coomans 1976; Vanfleteren 1982). Photoreceptors in the salp eyes so far examined have microvillar arrays (Gorman *et al.* 1971), and these could conceivably be more developed versions of ocellar microvilli. If, however, the salp eye is truely a dorsal structure, not an anterior one, it is more likely to be related to the one other dorsal microvillar photoreceptor system known from lower chordates, the Joseph cells of amphioxus, as discussed in § 5b. If this is the case, it would mean that the cerebral eye in salps has no homologue among other tunicate photoreceptor systems and, further, that salps have no obvious counterpart of the ascidian sensory vesicle or any of the sense organs it contains.

The cells responsible for caudal or visceral innervation lie behind the main body of the ganglion (salps) or the rudiment thereof (ascidians). The precise nature of the caudal cells in *Thalia*, and their relationship to the tadpole visceral ganglion is problematic. Caudal neurons in the *Thalia* ganglion are similar to the visceral ganglion cells in position, as they lie behind the main part of the developing neuropile, and both innervate posterior structures. Beyond this, however, there is nothing very specific to suggest that the caudal cells and the visceral ganglion are true homologues. They may simply be the most

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posterior motor neurons in the two ganglia, co-opted by default to innervate posterior structures. As Hox genes are now known to be expressed in the ascidian visceral ganglion, it may in future be possible to show a more convincing link between these two structures.

If we accept conventional ideas concerning tunicate relationships, that salps are derived by modification of an ascidian-like ancestor with a tadpole larva, the main features of the salp ganglion described here can be readily explained as examples of selective loss of CNS structures. Specifically, salps have apparently lost the main anterior structures involved in sensory reception (the sensory vesicle and its sense organs), along with the posterior ones required for larval locomotion (the nerve cord and some or all of the visceral ganglion). What is retained is the large central part of the ganglion. Whether the organization of the salp ganglion differs in any essential way from that in other tunicates is difficult to assess, because ganglion development is not well studied in the other groups. What we do know of ascidians is that the developing CNS, in contrast with *Thalia*, is highly asymmetrical owing to the segregation of the larval and adult components. On the one hand, there is an accelerated differentiation of the larval locomotory system required for precocious motility; on the other, the adult CNS is suppressed and develops as a segregated rudiment on one side only. Comparable structures in *Thalia* are more fully integrated into a single symmetrical entity. Whereas it is possible that the ascidian condition is primitive, and that symmetry has been re-established secondarily in salps, it is far easier to see the ascidian CNS as secondarily modified in this respect. A symmetrical arrangement, with the neuropile aligned with the midline of the cord, e.g. as in amphioxus, is closer to what one would normally assume to be the ancestral condition. This suggests that if salps once had a tadpole larva, it was much less specialized than the tadpole larvae of modern ascidians.

There is a second alternative, as there is both molecular and morphological data to support the idea that salps and ascidians are sister groups (Holland *et al.* 1988; Wada & Satoh 1994). Both could therefore have arisen from a common pelagic ancestor, as suggested by Wada & Satoh (1994), but there is currently no evidence (e.g. no fossil record) to indicate whether salps arose before ascidians or vice versa, or which resembles the ancestral form more closely. If salps are closer to the ancestral form, it would be easier to explain the apparent similarities between CNS structure in salps, amphioxus and more primitive dipleurula larvae discussed in § 5b and § 6.

(b) *Amphioxus*

The cerebral vesicle, i.e. the slightly enlarged anteriormost part of the amphioxus nerve cord, has long been thought to be homologous to all or part of the vertebrate brain. Recent structural and molecular studies of amphioxus embryos and young larvae have clarified the situation. The anterior part of the cerebral vesicle is now known to express amphioxus homologues of two genes characteristic of vertebrate forebrain, *Dll* and *Otx* (Holland *et al.* 1996; Williams & Holland 1996), and this same region contains structures resembling those found in the diencephalon (Lacalli *et al.* 1994; Lacalli 1996*a*). Further, homologues of Hox1 and Hox3 are expressed in the nerve cord in a similar pattern to that in vertebrates, which provides quite strong evidence that the amphioxus nerve cord caudal to somite 3 is homologous to the vertebrate nerve cord caudal to rhombomere 3 in the hindbrain (Holland *et al.* 1992; Holland & Garcia-Fernàndez 1996). The difficulty comes with the region between the supposed forebrain and hindbrain homologues in amphioxus, which effectively constitutes a 'middle zone' extending from roughly the middle of somite 1, i.e. from immediately behind the infundibular cells, through some or all of somite 2. The posterior part of the cerebral vesicle and the primary motor centre, both regions defined on structural criteria by Lacalli *et al.* (1994), occupy the front part of this middle zone, but precisely where it grades into something more like the segmental hindbrain is not yet clear. However, if there are homologues of the midbrain and/or anterior hindbrain in amphioxus, they would presumably lie somewhere in this middle zone.

There are clear parallels between the anterior part of the cerebral vesicle in amphioxus and the sensory vesicle in ascidian larvae. Both, for example, express homologues of the vertebrate gene *Otx* (Wada *et al.* 1996; Williams & Holland 1996). Characteristic structures in this zone in amphioxus larvae include the front part of the dorsal lamellar body, which extends forward to immediately behind the neuropore, the frontal eye, and a cluster of cells with specialized club-shaped cilia interpreted by Lacalli (1996*a*) as a balance organ. The most striking similarity is between the lamellar body in amphioxus and the ascidian ocellus, which have nearly identical ciliary lamellae (cf. Barnes 1974; Lacalli *et al.* 1994). The amphioxus frontal eye is not at all convincing as an ocellus homologus, as its putative receptor cells are simple ciliated cells lacking both microvilli and lamellae. Instead, the frontal eye is structurally closer to the botryllid photolith, which has a pigment cup into which is inserted a bundle of densely staining cilium-like processes (Grave & Woodbridge 1924; Torrence 1980). As the supposed balance organ in amphioxus also has specialized cilia, it is possible that the otolith, the photolith, the frontal eye, and the balance organ are all somehow related. All are located on the floor of the anterior nerve cord (for the otolith, this position is secondary in some instances; see Willey 1894), whereas the ascidian ocellus is generally lateral or dorsolateral in position, and develops on one side only. In fact, it is comparatively easy to imagine the ascidian ocellus originating from an ancestral structure resembling the amphioxus lamellar body, but if so, the ocellus has been much reduced during subsequent evolution.

In summary, the anterior-most part of the nerve cord in both ascidians and amphioxus consists of a chamber containing an assortment of sensory receptors for light, motion, gravity or some combination of these. The sense organs themselves, although diverse, probably represent variations on at most three basic types of receptor cells. The precise homologies between these are uncertain at this stage, but the regional homology between the cerebral vesicle in amphioxus and the ascidian sensory vesicle is well supported by both structural and molecular evidence.

Comparing sensory structures between amphioxus and salps is more difficult. The cerebral eye in salp embryos is cup-shaped, as is the frontal eye in amphioxus, but the two differ in size, position and orientation. Also, receptor cells in the salp eye have characteristic arrays of microvilli (Gorman *et al.* 1971), whereas receptor cells in the amphioxus frontal eye have simple cilia and no microvilli. There are, however, two other photoreceptor systems in amphioxus larvae that need to be considered, the lamellar body and the Joseph cells. The former has ciliary lamellae rather than microvilli, but as ascidian ocelli can have both, it is still possible to argue that the lamellar body and the salp eye are homologues (see § 5a). Both the lamellar body and the salp eye are dorsal, and both develop from bilaterally paired files of cells, which form the two arms of the horseshoe in the salp eye, and the two sides of the lamellar body in amphioxus. The Joseph cell system is the last of the larval photoreceptor systems to differentiate, but it is well developed by metamorphosis. It also consists of paired rows of cells, located on either side of the lamellar body. They begin near the posterior end of the lamellar body and continue caudally for some distance. The cells themselves are microvillar: dense arrays of short microvilli typically cover the whole of one side of the cell (Welsch 1968; Watanabe & Yoshida 1986). In this respect, they are more like the cells described from the salp eye by Gorman and co-workers (1971) than any of the other anterior receptor cell systems in amphioxus.

A comparison of neuronal organization in salps and amphioxus reveals two features in the amphioxus nerve cord, the tectal structures associated with the anterior dorsolateral nerve tracts and the translumenal fibre system, that may have homologues in the salps.

The amphioxus 'tectum', as defined by Lacalli (1996*a*), is a small zone on either side of the lamellar body containing clusters of cells whose input, in part, is from the frontal eye, and whose output is to dorsolateral tracts carrying sensory input from the external surface of the rostrum. Rostral input to the dorsolateral tracts increases considerably in older larvae and juveniles, and associations develop with the overlying Joseph cells (T. C. Lacalli, unpublished data). It is not clear how the resulting, rather complicated system of tracts and neuropile relates to the vertebrate optic tectum, which is a much larger and anatomically better defined entity, but we will continue here to use the terms 'tectum' or 'tectal' for the amphioxus structures for want of a better alternative.

The translumenal fibre system in amphioxus develops progressively through the larval period, at a time when larval behaviour becomes much more complex and the pharyngeal and visceral innervation is established (Bone 1961). In the young larva, most of the early motor neurons and interneurons are ventral in position, and cluster near the lower margin of the neural canal. Above this point the two sides of the neural canal are closely apposed, so as to form an 'intermediate' zone in which the lumen is largely obliterated. This zone is initially quite small (figure 16*a*), but becomes much larger as development proceeds (figure 16*b*). Cells in the intermediate zone develop an extensive system of translumenal processes (figure 16*c*) that is especially well developed in the anterior cord beginning just behind the tectal region. The anterior Rohde cells are the best-known examples of cells with translumenal processes (Bone 1959). They are unusual for their large size, but many other cells in the anterior cord have similar processes, and these can be clearly shown to derive from the apical surface of the cell (Lacalli & West 1993). The function of the processes is not known, nor even whether they are afferent or efferent, but they could, in principle, provide a means of coordinating neuronal activities on opposite sides of the cord.

Both the tectal zone and the translumenal system in amphioxus are places where functional neurites are regularly formed at the apical surface of the cells. In the salp ganglion there are also two places where the cells have several neurites of which at least some are evidently apical in origin: in the dorsolateral mantle, and around the central neuropile (figure 17). The dorsolateral cells, like tectal cells, form a local neuropile, and both are adjacent to sites of photoreceptor input. The main neuropile forms symmetrically around the neural canal and develops later, as does the translumenal system. However, while we can be quite certain of the apical origin of translumenal processes in amphioxus, we are less certain about the neurites in the salp neuropile. Some are apical, but not necessarily all. Nevertheless, the massive growth of neurites either into or across the central canal looks remarkably similar in the two organisms.

We therefore suggest that the main ganglionic region in the salp may be the equivalent of the middle zone of the anterior nerve cord in amphioxus, i.e. the zone beginning at the tectum, near the back of the lamellar body in young larvae, and extending caudally at least through somite 1 and perhaps somewhat further. At some point in somite 2, the nerve cord must lose its anterior character and become more hindbrain-like, but we do not yet know where this occurs or what it entails. As the translumenal system does, however, continue through a number of somites in amphioxus, it is evidently more than just a feature of the anterior cord. The salp ganglion has no comparable caudal extent, so we have no basis for comparing it with amphioxus in this respect.

(c) *Vertebrates*

The vertebrate brain is divided into three regions: fore-, mid- and hindbrain, each derived from a morphologically identifiable region of the early neural tube. As discussed here, molecular and structural studies together support the idea that the anterior part of the cerebral vesicle in amphioxus, with its various sensory structures, is largely a forebrain homologue, and the same appears to be true of the sensory vesicle in ascidians. There is also evidence for a more caudal zone homologous with vertebrate hindbrain in both amphioxus (Holland *et al.* 1992) and ascidian tadpole larvae (Katsuyama *et al.* 1995; Satoh *et al.* 1996). The problem arises with the region between these, which in vertebrates would comprise the midbrain, rhombencephalic isthmus, and anterior hindbrain, specifically rhombomere 1, which is anomalous in not expressing Hox genes or being the source of any members of the cranial nerve series. The same mes–metencephalic region has been identified as a distinctive transitional zone on other

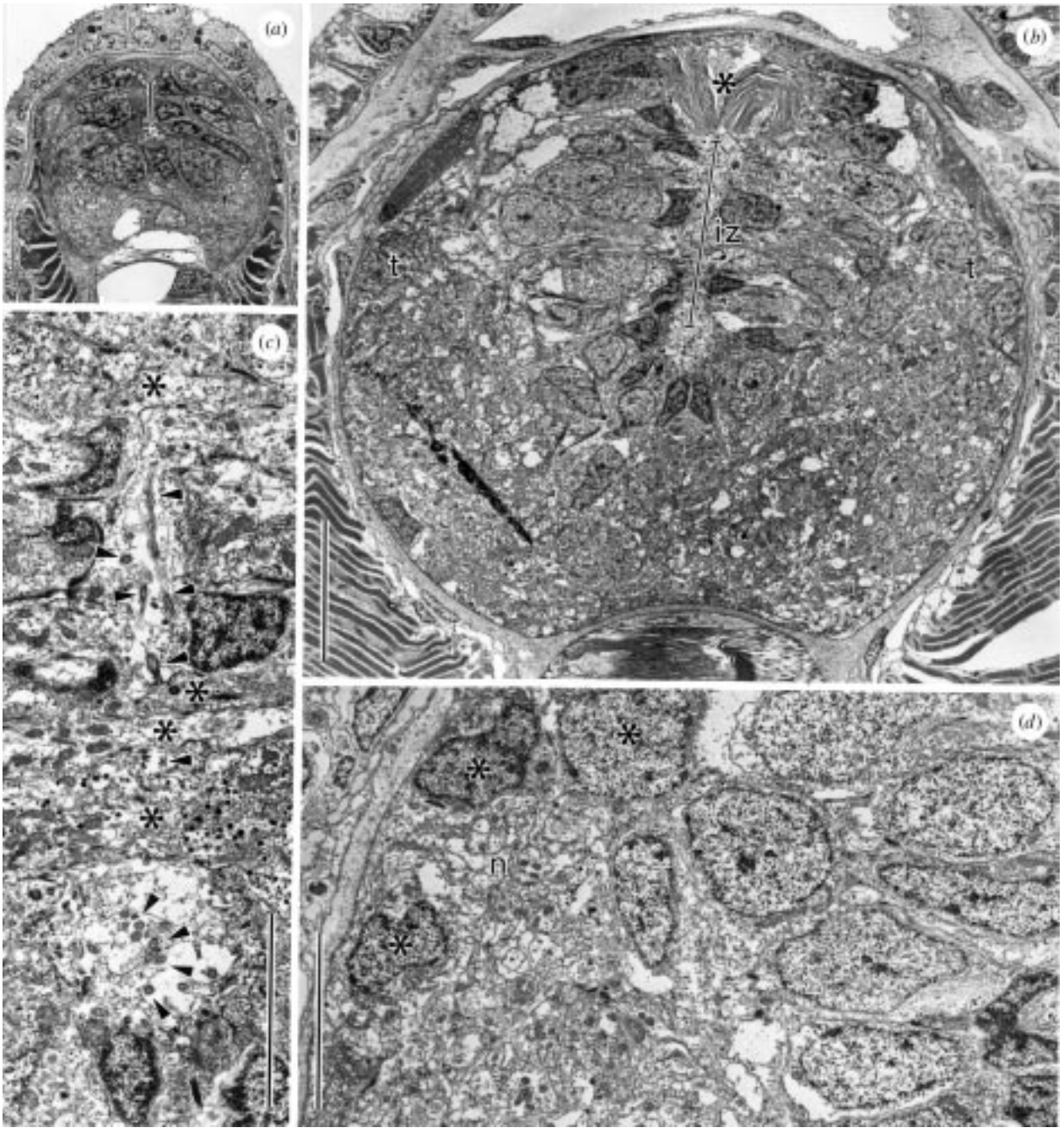

Figure 16. Selected features of the anterior nerve cord in amphioxus. (*a*) A transverse section through the nerve cord in a 12-day larva near the back of somite 1, for comparison with (*b*), which shows the same region in a newly metamorphosed juvenile. Both are to the same scale; note the much larger intermediate zone (bar, marked iz) in the juvenile. (*b*) The tectal region (t) and the lamellar body (*), which extends somewhat further back in the juvenile, are shown. Scale bar, 10 µm. (*c*) Detail of the intermediate zone in the juvenile. Ciliary profiles (arrows) are visible wherever the lumen of the nerve cord remains open. Between these are regions where large apical processes (*) from cells on both sides cross from one side of the cord to the other. Scale bar, 5 µm. (*d*) Tectal region on one side of the juvenile cord in the same region. Several tectal cells (*) are shown; these lie well to the side (the central canal is at the far right), just above and beside the dorsolateral nerve tracts (n). Scale bar, 5 µm.

criteria (Bally-Cuif & Bocinelli 1997) and, because its development is crucially dependent on signals emanating from the isthmus (Marin & Puelles 1994; Crossley *et al.* 1996), there is some justification for referring to it as an isthmus-dependent zone. A number of key developmental genes are expressed specifically in the isthmus, notably *engrailed*, which has proven a useful marker for this region.

Neither amphioxus nor salps have anything that could be morphologically identified as an isthmus, i.e. no region within the middle part of the brain that clearly subdivides it further into distinct anterior and posterior components. Instead, both organisms have a single middle zone exhibiting approximately uniform organization, with dorsolateral centres and local neuropiles, a central neuropile (in salps) or translumenal plexus (amphioxus), ventral clusters of motor neurons supplying adjacent muscles, and (in amphioxus) populations of caudally projecting motor and interneurons supplying the posterior

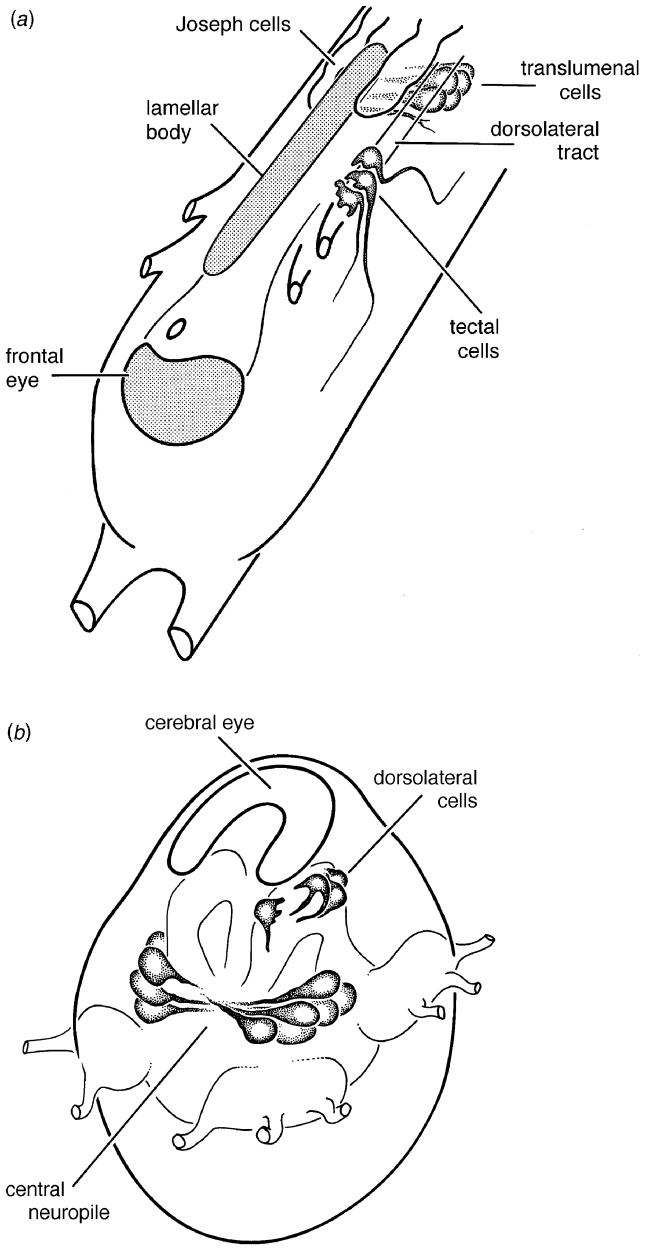

Figure 17. A summary diagram comparing tectal and translumenal organization in a newly metamorphosed amphioxus juvenile (*a*) and the salp ganglion (*b*); oblique front view, somewhat schematic, but approximately to the same scale. By this stage, amphioxus has three anterior photoreceptors, the frontal eye, the lamellar body and the Joseph cell system; the salp has one, the dorsal cerebral eye. We suggest the main classes of neurons contributing to anterior neuropiles and plexes in the two organisms are potentially homologous, as follows (selected cells are drawn): (i) the tectal cells in amphioxus, which lie over the dorsolateral nerve tracts, may be homologues of the dorsolaterally positioned cells responsible for the paraxial neuropiles in the salp; and (ii) cells with apical translumenal processes in amphioxus may be homologues of at least some of the cells involved in forming the central neuropile in the salp. The anterior-most motor neurons in the case of amphioxus would lie below and slightly caudal to the tectal cells. See text for further discussion.

part of the body. The region as a whole thus resembles the vertebrate midbrain, with its dorsal tectum, involvement with visual processing, ventral motor nuclei, and caudally projecting reticulospinal neurons.

We are not convinced that it is appropriate to treat the middle zone in salps and amphioxus as a direct counterpart of the vertebrate midbrain, however, because too little information is currently available on the relevant gene expression patterns within this region in chordates other than vertebrates. The amphioxus *engrailed* gene, for example, is expressed in the anterior nerve cord, but if it marks the isthmus homologue in this organism, it is too far forward to allow for a midbrain homologue of any size (Holland *et al.* 1997). There are two reasons to doubt this expression zone as a true homologue of the vertebrate midbrain–hindbrain boundary, however. First, the expression zone of *engrailed* in vertebrates is initially rather broad, extending forward nearly to the dien–mesencephalic boundary and, under certain conditions, *engrailed* can be expressed in vertebrate diencephalon (Bloch-Gallego *et al.* 1996). The region expressing *engrailed* in amphioxus could therefore also belong to the amphioxus diencephalon homologue. Second, the expression zones of other genes in the amphioxus cerebral vesicle are inconsistent with the supposition that amphioxus *engrailed* marks an isthmus homologue. The expression zone of amphioxus *Dll*, a diencephalic marker, extends slightly caudal to *engrailed* in the cerebral vesicle (Holland *et al.* 1996), whereas the amphioxus homologue of *Otx*, expressed in vertebrates from forebrain to the caudal limit of the midbrain (Shimamura *et al.* 1995), extends caudally in amphioxus embryos, at its time of maximal extent, to almost the end of somite 1 (Williams & Holland 1996; N. A. Williams, personal communication). This is considerably more caudal than *engrailed* expression in the anterior nerve cord, and would put the isthmus homologue, if amphioxus had one, at least as far back as the junction between somites 1 and 2.

Figure 18 shows our proposal for how the salp ganglion relates to the anterior CNS of amphioxus and vertebrates, taking all currently available structural and molecular data into account. The 'middle zone' in salps and amphioxus finds its closest apparent counterpart in vertebrates, in this scheme, in the mes–metencephalic region as a whole. As the middle zone in the salp case represents the bulk of the ganglion, including the central neuropile, what we are really suggesting is that there is an ancestral ganglionic zone that is equivalent to the region occupied, in vertebrates, by the midbrain and anterior hindbrain. The organizing activities of the isthmus could be equally ancient, or they could have arisen later, to control the diversification of the ancestral ganglion into separate mesencephalic and metencephalic parts.

A final point concerns the issue of apicobasal polarity of neurons in vertebrates. As discussed here, neurons with apical processes that penetrate into or across the neural canal appear to be an important feature of the CNS of invertebrate chordates, yet are absent in vertebrates. The arrangement may represent an early chordate solution to the problem of coordinating neural activities on the two sides of the cord. If so, it has been supplanted in vertebrates by other forms of organization. This may be owing to the inherent limitations of the design, as the integrating capacity of such a system would be limited by the size of the neural canal or, in the case of amphioxus, by the surface area of the lumenal interface. Vertebrates may, however, retain some vestige of this heritage, in the inverted polarity

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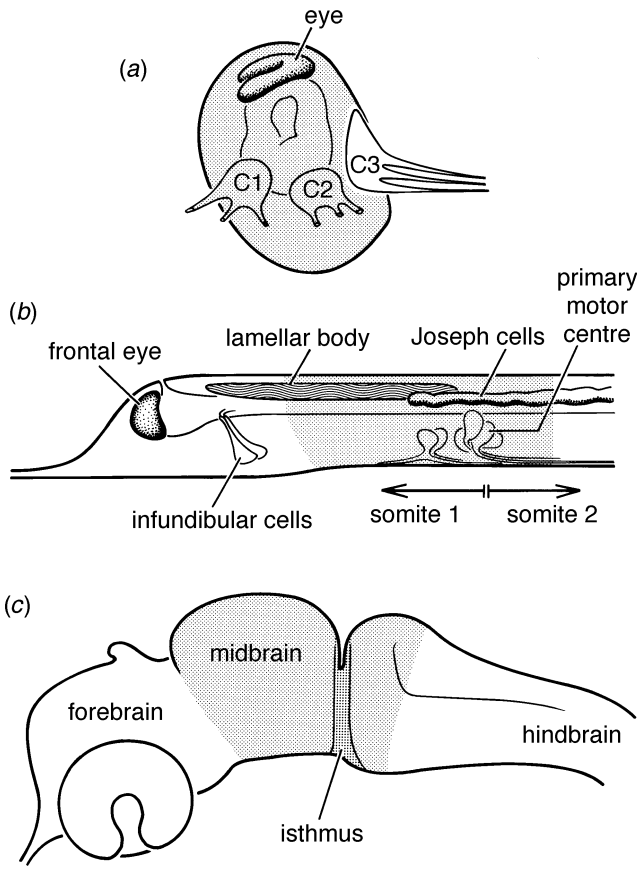

Figure 18. A comparison of the salp ganglion, the anterior nerve cord of amphioxus, and a vertebrate brain, to show the primitive ganglionic region we postulate is common to all three (shaded zone). In (*a*), the salp, the region in question is the main, central part of the ganglion with its neuropile. In (*b*), amphioxus, it would be the middle zone between the anterior cerebral vesicle and posterior, segmental parts of the nerve cord. It would therefore include all of the region adjacent to the back part of somite 1 and possibly some of somite 2, although its precise extent in either direction is uncertain. In young amphioxus larvae, this zone contains the large interneurons of the primary motor centre and the anterior-most of the primary motor neurons, as shown. The relative position and extent of the other structures is from data on late-stage larvae and newly metamorphosed juveniles. In vertebrates (*c*), the comparable zone is the isthmus-dependent region comprising the midbrain, isthmus, and the anterior part of the hindbrain. See text for further discussion.

of the neurons themselves. Neurites (including axons) in some classes of brain neurons appear to arise from the apical surface of the cell (Craig & Banker 1994; Prochiantz 1995), and CNS neurites generally have remarkable integrative properties that are not found in more primtive neurons (Yuste & Tank 1996; Svoboda *et al.* 1997). We conjecture that some of these properties may have originated in lower chordates, as nerve cells with apical neurites evolved to perform increasingly specialized and/or new functions. The apical cell surface is also extensively elaborated in the various photoreceptor cells described from lower chordates, which could be a further indication of the potential for generating new cell types from more primitive precursors by this means.

6. POSSIBLE ANTECEDENTS: NEURAL ORGANIZATION IN DIPLEURULA LARVAE

Garstang's 1894 auricularia hypothesis is one of the few plausible ideas currently available for explaining the origin of the chordate nerve cord. The hypothesis derives the neural tube from the ciliary bands of an ancestral dipleurula-type larva by having the latter fold together in a process akin to neurulation (Garstang 1894, 1928). Of the two relevant phyla, echinoderms and hemichordates, the larvae of the former are better known, but the larval nervous system is difficult to study in both groups and is still very poorly understood. In part, this is because it is diffuse, consisting of cells scattered along the length of the ciliary band, with a few local clusters of neurons, loosely called 'ganglia'. Of the latter, the most relevant to this account are those located just forward of the anterodorsal lobes in the starfish bipinnaria, the 'dorsal ganglia' (Nakajima 1988; Moss *et al.* 1994; see figure 19*c*). In sections, neurons in this region can be seen to be distributed across the width of the band (figure 19*a*), and most of them produce apical neurite-like processes similar to those described by Lacalli & West (1993) from other parts of the ciliary band system (figure 19*b*). Anterior concentrations of neurons resembling ganglia also occur in auricularia larvae (figure 19*d*,*e*), at about the same location in relation to the anterodorsal lobes, although the cells themselves are more diffusely distributed. In both the bipinnaria and auricularia therefore, folding the bands together would produce a local concentration of neurons with apical processes that could cross the gap between the two sides and form a zone of interdigitation. This would partly account for the type of ganglion organization seen in tunicates, with its characteristic central neuropile, as well as the translumenal system in amphioxus.

A related issue concerns the organization of the body musculature in dipleurula larvae and the relationship between the dorsal muscles and nearby neural centres. As the dorsal muscles in the bipinnaria are arranged in series, dorsal convergence followed by neurulation would generate an arrangement approximately equivalent to that seen in salps (Lacalli 1996*b*). In the bipinnaria, the first of the main muscle bands attaches behind the dorsal ganglia at the level of the anterodorsal ridges. As the ridges can give rise to multiple lobes in some species (e.g. *Luidia*), they could in principle generate attachment sites for more than one muscle band. They could each, in other words, be the site of one or more muscle organizing centres. There are other muscles further forward, in front of the ganglion region in the bipinnaria, but these are either apical muscles or are associated with the ventral preoral shield region, and are therefore not members of the dorsal series. Similarily, in salps, there is also a tendency for the main muscle bands to meet behind the ganglion. It is possible that the similar position of the dorsal nerve centre, in both dipleurula larvae and salps, in relation to the main organizing centre for the dorsal muscles, is due to homology, i.e. that it has been substantially conserved, despite the other major changes to body shape and organization that have occurred with evolution.

If one supposes that a key region of the early chordate brain originated from paired dorsal neural centres in a

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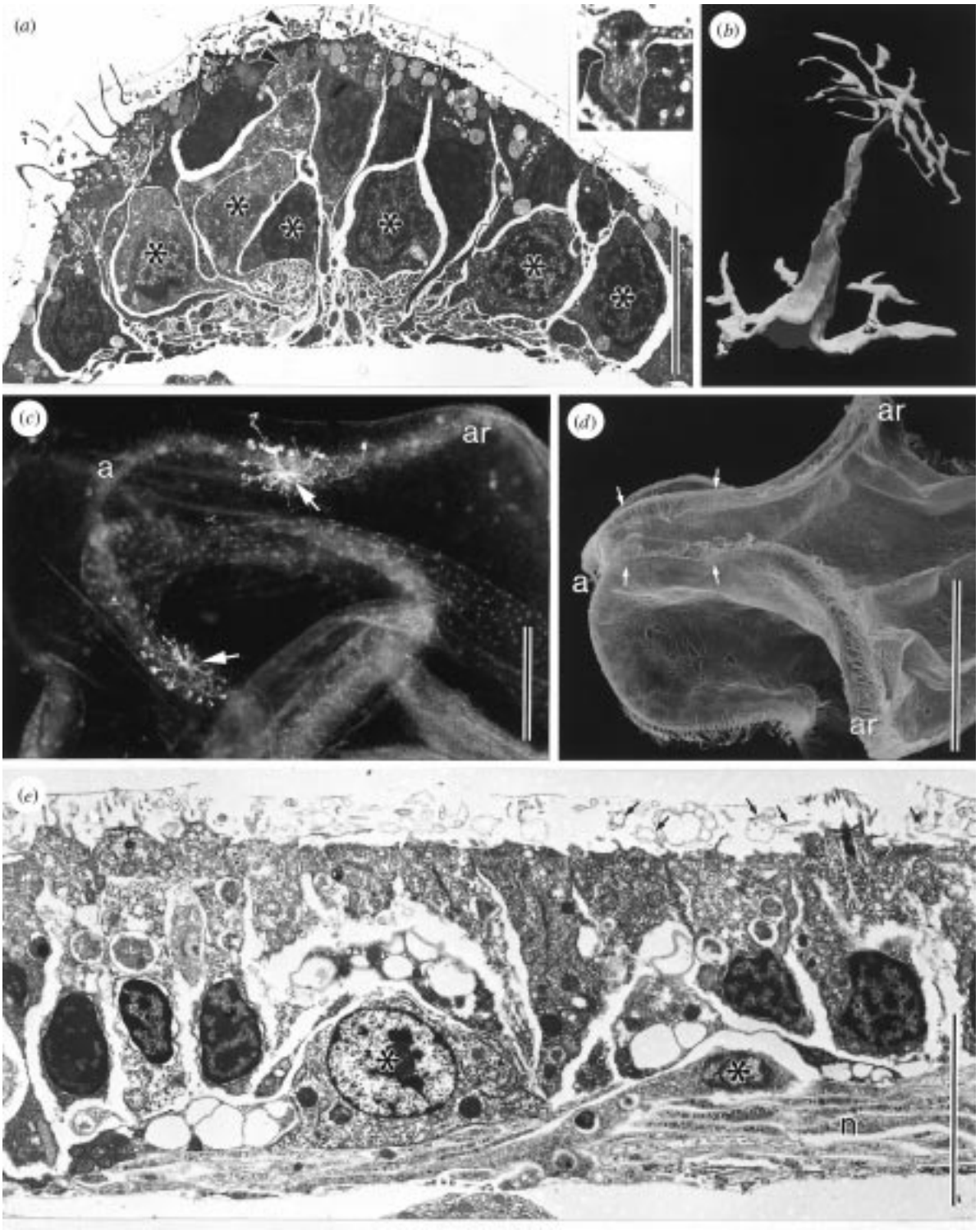

Figure 19. The anterior 'ganglionic' zone in selected echinoderm larvae. (*a*) A transverse section through the dorsal ciliary band in the region of the dorsal ganglion in a starfish bipinnaria larva (*Pisaster ochraceus*, for methods see Lacalli (1996*b*)). The section cuts part of a cluster of neurons (*) with apical processes that run across the surface of the ciliary band. Two parts of the apical region one of these cells are marked (arrows), and the apex of the same cell (inset) is included to show its junction with an apical process. Scale bar, 5 µm. (*b*) 3D reconstruction of a cell with this same type of morphology, a 'multipolar cell' from the preoral transverse band in *P. ochraceus* (data from Lacalli *et al.* (1990)), showing the branched system of apical processes more fully. (*c*) The anterior end of a *P. ochraceus* larva in dorsal view, anterior end to the left, immunostained for neuropeptide S1 to show the tight cluster of peptide-containing cells in the ganglionic region (arrows) between the anterodorsal ridges (ar) and the apex (a) of the larva. Photo courtesy of Claire Moss (see Moss *et al.* (1994) for details). Scale bar, 100 µm. (*d*) Oblique dorsal scanning electron microscope view of the anterior end of an auricularia larva (*Stichopus californicus*, see Lacalli (1993) for methods). The zone of ciliary band between the arrows on each side, behind the apex (a) and about as far forward of the anterodorsal ridge (ar) as in *Pisaster*, is especially rich in neurons. Scale bar, 100 µm. (*e*) Longitudinal section through the region of the band indicated by arrows in (*d*) to show the ciliary nerve (n), two neurons (*), and apical processes (arrows) belonging to neurons located in this region. Scale bar, 5 µm.

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dipleurula-type ancestor, it remains to explain the origin of the extended tubular part of the CNS, from which the hindbrain and spinal cord evolved. This is more difficult. Garstang's idea in its simplest form begins with an elongate dipleurula larva, so that folding up the bands generates the entire nerve cord. However, no reliable landmarks have yet been recognized in the posterior part of the dipleurula to support a detailed comparison with the extended nerve cord of tunicate larvae or adult appendicularians. Further structural and molecular work may yet identify hindbrain or caudal nerve cord homologues in dipleurula ciliary bands, but until then, from the evidence currently available, there is more of a basis for comparing the anterior parts of the CNS between dipleurula larvae, tunicates, and more advanced chordates.

Two recent papers provide additional information on tunicate larvae. Burighel & Cloney (1997) review the larval sensory organs. Wada *et al*. (1998) summarize current data on gene expression patterns in the larval CNS, and reach essentially similar conclusions to those discussed here regarding regional CNS homologies among chordates.

This work was supported by NSERC Canada. We thank S. Hou and S. Smith for technical assistance, and Q. Bone, J. E. A. Godeaux, and G. O. Mackie for comments and additional information on tunicates.

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