REVIEW / SYNTHÈSE

The nervous system of amphioxus: structure, development, and evolutionary significance[1]

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Abstract: Amphioxus neuroanatomy is important not just in its own right but also for the insights it provides regarding the evolutionary origin and basic organization of the vertebrate nervous system. This review summarizes the overall layout of the central nervous system (CNS), peripheral nerves, and nerve plexuses in amphioxus, and what is currently known of their histology and cell types, with special attention to new information on the anterior nerve cord. The intercalated region (IR) is of special functional and evolutionary interest. It extends caudally to the end of somite 4, traditionally considered the limit of the brain-like region of the amphioxus CNS, and is notable for the presence of a number of migrated cell groups. Unlike most other neurons in the cord, these migrated cells detach from the ventricular lumen and move into the adjacent neuropile, much as developing neurons do in vertebrates. The larval nervous system is also considered, as there is a wealth of new data on the organization and cell types of the anterior nerve cord in young larvae, based on detailed electron microscopical analyses and nerve tracing studies, and an emerging consensus regarding how this region relates to the vertebrate brain. Much less is known about the intervening period of the life history, i.e., the period between the young larva and the adult, but a great deal of neural development must occur during this time to generate a fully mature nervous system. It is especially interesting that the vertebrate counterparts of at least some postembryonic events of amphioxus neurogenesis occur, in vertebrates, in the embryo. The implication is that the whole of the postembryonic phase of neural development in amphioxus needs to be considered when making phylogenetic comparisons. Yet this is a period about which almost nothing is known. Considering this, plus the number of new molecular and immunocytochemical techniques now available to researchers, there is no shortage of worthwhile research topics using amphioxus, of whatever stage, as a subject.

Résumé : La neuroanatomie de l'amphioxus est importante, non seulement en elle-même, mais parce qu'elle offre des perspectives sur l'origine évolutive et l'organisation de base du système nerveux des vertébrés. Notre rétrospective résume la disposition générale du système nerveux central (CNS), des nerfs périphériques et des plexus nerveux chez l'amphioxus et fait le point sur les connaissances sur l'histologie et les types cellulaires, et en particulier sur les découvertes récentes sur la corde nerveuse antérieure. La région intercalée (RI) est d'intérêt particulier des points de vue fonctionnel et évolutif. Elle s'étend vers l'arrière jusqu'à la fin du somite 4 qui est traditionnellement considéré la limite de la portion ressemblant à un cerveau du CNS de l'amphioxus et elle est remarquable par la présence de plusieurs groupes de cellules migrantes. Contrairement à la plupart des autres neurones de la corde, ces cellules se détachent de la lumière ventriculaire et envahissent le neuropile, comme le font les neurones en développement chez les vertébrés. Nous considérons aussi le système nerveux des larves, puisqu'il y a une abondance de données nouvelles sur l'organisation et les types cellulaires de la corde nerveuse antérieure chez les jeunes larves basées sur des analyses détaillées au microscope électronique et des études de traçage des nerfs et parce qu'un consensus sur la relation entre cette région et le cerveau des vertébrés est en train de se former. La période intermédiaire du cycle biologique, c'est-à-dire entre la jeune larve et l'adulte, est beaucoup moins connue; il doit cependant s'y compléter beaucoup de développement nerveux pour produire le système nerveux complètement mature. Il est particulièrement intéressant de noter que des phénomènes correspondant à au moins quelques événements de la neurogenèse de l'amphioxus se répètent dans l'embryon dans vertébrés. Cela implique qu'il faut tenir compte de l'ensemble de la phase post-embryonnaire du développement neural chez l'amphioxus afin de faire des comparaisons phylogénétiques. C'est néanmoins une période dont

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on connaît à peu près rien. Dans ces conditions, le nombre de nouvelles techniques moléculaires et immunocytochimiques disponibles pour la recherche fait qu'il ne manque pas de sujets de recherche de valeur sur l'amphioxus à tous les stades de son cycle.

[Traduit par la Rédaction]

Introduction

The common ancestor of present-day vertebrates and the invertebrate cephalochordates has long been extinct, but if one had to choose a living species likely to resemble it most closely, amphioxus (*Branchiostoma* spp.; lancelets) would be the organism of choice (Presley et al. 1996; Holland 2000). As such, amphioxus is of key importance to investigations into vertebrate origins and characteristic features of vertebrate organization. The nervous system is of special interest because its basic plan is highly conserved among vertebrates, yet almost nothing is known about how that plan originated. One has little recourse except to conduct investigations of amphioxus and other protochordates. This was the rationale behind the anatomical studies of amphioxus carried out in the late 19th century, principally on *Branchiostoma lanceolatum* (Pallas, 1774), the European species, by leading comparative zoologists of the day. There has been something of a hiatus in the 20th century, in part because of concerns that the apparent simplicity of amphioxus is due to its being degenerate and derived, rather than primitive, but also because of diminishing returns from studies using conventional light microscopical methods. Much of the original literature is in German, including major studies by Rohde (1887), Retzius (1891), Dogiel (1903), and Franz (1923) and a comprehensive review by Franz (1927). Bone (1959, 1960*a*, 1961) provides a comprehensive summary of previous work for non-German speakers, along with much new information from his own research. The most recent reviews on amphioxus are by Ruppert (1997), for the general anatomy of the animal as a whole, and Nieuwenhuys (1998), for the nervous system.

Why then review the nervous system yet again? The answer has two parts. First, the nervous system of amphioxus is surprisingly peculiar from a vertebrate perspective, so it is not a trivial matter to get a good conceptual feel for its organization. Just as one might have more than one tourist guide when visiting an unfamiliar city for the first time (each preferably with a good set of maps), there is a distinct benefit in having more than one review to consult regarding amphioxus neuroanatomy, preferably with good illustrations, as each will inevitably have a slightly different perspective. Second, and more importantly, a whole new tool kit of molecular and immunocytochemical techniques is now being employed to reexamine the structure and development of phylogenetically interesting animals, including amphioxus. New studies of the amphioxus nervous system now appear on a regular basis, and gene expression studies frequently also focus on the nervous system. Given our very limited understanding of amphioxus neural structure and function, these new results are often difficult to interpret, typically raising more questions than they answer. A reexamination of what we do know, as of 2004, is therefore not out of place. What is perhaps more important, however, is the way the new results highlight major gaps in our knowledge — for example, the postembryonic events that turn a larval nerve cord into an adult one (see below). Thus, as well as a summary of facts, we try here to provide insights into areas of emerging interest and highlight issues where we think major advances can be expected in the future. We hope, by this means, to stimulate new thinking about the anatomy and functional organization of the amphioxus nervous system, and better inform research into the origin of vertebrates and their nervous system.

In the account that follows, the adult nervous system is dealt with first, followed by a separate section on the larval nervous system, specifically that of young larvae 12–14 days old. Separate treatment is necessary because the nature of the data is so different for the two stages, being largely conventional anatomical descriptions based on light microscopy and immunocytochemistry in the case of the adult, and cellular-level details from electron microscopical (EM) reconstructions in the case of the larva. Because our knowledge of the intervening period of development is incomplete, it is not always clear how structures and cell types in the nervous system of young larvae relate to those in the adult, and it is consequently impossible to make the two sections as seamlessly complementary as one ideally might like.

Anatomical overview

General appearance

The central nervous system (CNS) of adult lancelets consists of a tubular nerve cord, located directly above the notochord, that extends most of the length of the body (Fig. 1). Rostrally, the notochord extends forward of the nerve cord, to almost the tip of the body, hence the name for this taxon: Cephalochordata. The nerve cord itself begins a few hundred micrometres caudal to the anteriormost coelomic cavity, which supports the rostral fin, and just forward, by a few dozen micrometres, of the first myomere. Caudally, the nerve cord reaches to the tip of the caudal fin where, just above the caudal end of the notochord, it forms a terminal enlargement of variable form, the caudal ampulla (Retzius 1891; Franz 1923).

Unlike the situation in vertebrates, the lancelet nerve cord displays few externally visible landmarks except for the above-mentioned ampulla and a transient anterior swelling (the cerebral vesicle) in young larvae. There are thus no neuromeres or other external indications of segmental arrangement other than the serially repeated exit points for the dorsal nerves. Internally, however, pronounced cytoarchitectural differences can be identified along the rostrocaudal axis, both in the adult and in larvae. These can be used to define a series of major regions along the nerve cord, and some of these can be further subdivided. Because of the absence of external landmarks, the boundaries of these subdivisions and their relation to the peripheral nervous structures

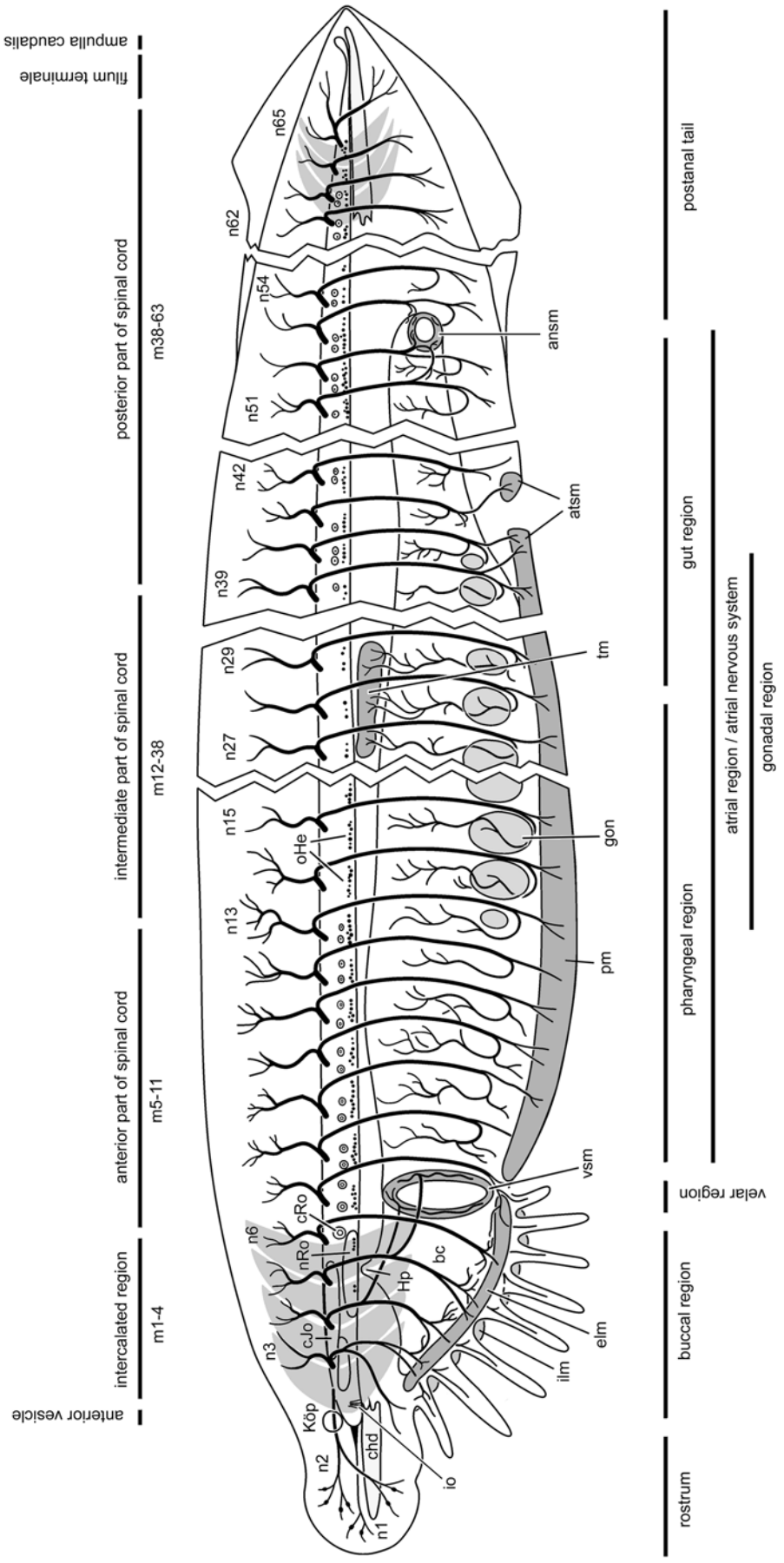


Fig. 1. A highly schematic lateral view of a lancelet (*Brachiostoma* sp.) with 63 myomeres (m1 to m63), showing the general organization of the central and peripheral nervous systems in relation to peripheral structures. The first four and last three members of the myomere series are highlighted (light shading) to clarify the relationship between myomere number and nerve number. Regions occupied by major blocks of non-myomeric muscle are indicated by darker shading. Selected body regions are cut away, as indicated by the jagged lines. Abbreviations and their corresponding terms are listed in Appendix A.

are best described with reference to the myomeres, of which, on average, there are 63 pairs in *B. lanceolatum* (Poss and Boschung 1996). This poses some problems if one is trying to compare CNS architecture throughout development because (*i*) though it is likely, it is not known for certain whether the myomeres are permanently fixed in position in relation to the nerve cord, and (*ii*) myomeres expand both lengthwise and dorsoventrally during development, which, combined with their chevron shape, means there is an increasing zone of overlap between them that varies depending on position along the dorsoventral axis. (*iii*) The left and right rows of myomeres in the adult are not aligned; the first larval somites, however, from which the myomeres develop, are. In the neurula stage, the left row of somites (as well as the left row of dorsal nerves and neuromuscular contact zones) starts to shift anteriorly (Conklin 1932). To make things even more complicated, this "left forward shift" is less pronounced in the region of the first five somites (myomeres). Thus, in the adult, the plane defined by the rostral tips of the first myomeres is almost perpendicular to the long axis of the body, whereas the tips of increasingly more posterior myomeres define increasingly more oblique planes. Approximately at the level of the sixth pair of myomeres, the "full" oblique angle of about 45° (i.e., the typical "half-myomere staggering") is reached and maintained throughout the caudal part of the body. In the present review, we shall use the transverse planes defined by the tips of the left row of myomeres as landmarks. Past accounts, however, have not always described the location of CNS landmarks in a sufficiently precise way to overcome these problems.

The peripheral nervous system (PNS) consists of a set of peripheral plexuses[3] and a segmental series of intermyomeric dorsal nerves (= dorsal roots or "true" nerves, as opposed to the apparent ventral roots, which are in fact muscle processes rather than nerves). The dorsal nerves are entirely devoid of ganglia; in other words, amphioxus has no counterparts of the vertebrate dorsal root ganglia. Peripheral nerves in amphioxus instead consist solely of axons, which derive from both central and peripheral neurons, and a few glial-like support cells. The peripheral neurons reside in various peripheral plexuses, which are especially well developed around the gut, and in sense organs and the skin. The nerves themselves issue from the dorsolateral margins of the nerve cord, their proximal parts being located in the myosepta, i.e., they pass between the myomeres. Owing to the left-right asymmetry of the myomeres (the left row is displaced a half-segment forward of the right one), the nerves are staggered left to right. The pattern of peripheral innervation for a typical nerve of the trunk is shown in Fig. 2; for details see the section (below) on the spinal cord. Innervation patterns in the rostral and oral region are somewhat different; Fig. 3 shows this in detail, while cytological details and cell types as seen in typical transverse sections of the nerve cord are shown in Figs. 5 and 6.

Anterior nerve cord

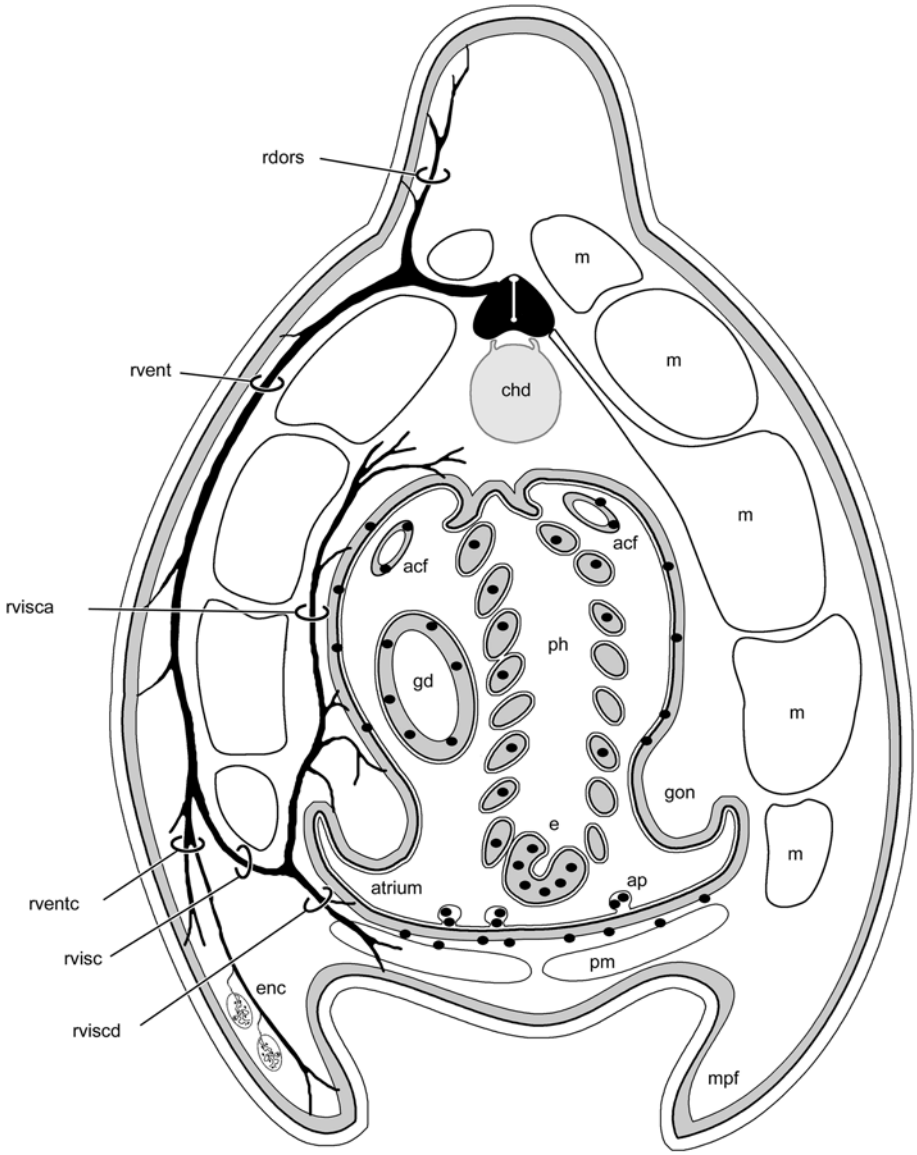
The tip of the adult nerve cord rostral to myomere 1 is referred to as the anterior vesicle (= cerebral vesicle (Willey 1894), *Gehirn* (Edinger 1906), archencephalon (von Kupffer 1906), and *Stirnbläschen* (Franz 1927)). The central canal is expanded in this region and is roughly circular in cross section. It opens on the left to the outside via Kölliker's pit (Kölliker 1843), a remnant of the anterior neuropore (Willey 1894). Two pairs of nerves emerge from the anterior vesicle to supply the rostral region. By convention these are numbered 1 and 2 (e.g., Dogiel 1903; Kutchin 1913; Nieuwenhuys 1998), but are referred to also as the rostral and anterodorsal nerves, respectively (e.g., Lacalli 2002*b*). They differ from remaining members of the dorsal nerve series in several respects. First, because of the absence of myomeres in the rostrum, both nerve pairs travel forward in the connective tissue sheath surrounding the nerve cord and notochord, whereas the rest of the dorsal nerves pass through the myosepta. Second, the various branches of nerves 1 and 2 carry small bulbous clusters of cells, the corpuscles of de Quatrefages (Fig. 3), putative mechanosensory organs consisting of primary sensory cells enclosed in a capsule (Baatrup 1982) that occur nowhere else in the body. Nerve 1, the rostral nerve, is unusual also in that it enters the nerve cord ventrally rather than dorsally. It has nevertheless become customary to include it with the rest of the dorsal nerves (e.g., Dogiel 1903; Kutchin 1913; Nieuwenhuys 1998). We follow this convention here, though it has the disadvantage that nerve and myomere numbers then no longer match; i.e., a given nerve n would emerge from between myomeres $n - 1$ and $n - 2$ (see Fig. 1).

The region of the adult nerve cord extending from the anterior vesicle through the first four myomeres is recognized as distinctive enough to require a special designation. It has been referred to variously as a deuterencephalon (von Kupfer 1906), hindbrain (Fritzsch 1996; Holland 1996), or caudal brain region (Nieuwenhuys 1998), but was regarded as a part of the spinal cord (*Rückenmarksteil*) by Franz (1923). Most recently, Ekhart et al. (2003) have coined the term intercalated region (IR) so as to avoid the implications of homology associated with the older terms. The IR as defined by Ekhart et al. extends from the infundibular organ (Boeke 1902, 1908), which produces Reissner's fiber (Obermüller-Wilén 1976), to the first giant cell of Rohde (Rohde 1887). It contains three subdivisions (anterior, intermediate, and posterior), described below, and is characterized by the presence of a number of cell types and groupings not seen elsewhere in the spinal cord. The most conspicuous of these are the large dorsal cells of Joseph (Joseph 1904), which are putative photoreceptors (see Figs. 6, 7). The IR gives rise to nerves 3–6, which, like all the dorsal nerves, send branches to the corresponding component of the subepidermal nerve plexus in the skin. These nerves also connect with two peripheral nerve rings (or plexuses) associated with the buccal region (Fig. 3), namely the buccal plexus, which innervates the buccal cavity, cirri, and associated muscles (the internal and external labial muscles, Franz 1927), and with the velar plexus, which innervates the tentacles and sphincter muscle of the velum.

An important non-neural structure located in this region is Hatschek's pit (Hatschek 1884), which lies ventral to the IR at the junction of myomeres 3 and 4. This is actually a part

[3] With respect to the common usage in the English-speaking world, we will not use the latin plural (plexūs).

Fig. 2. A highly schematic transverse section of a lancelet at the level of myomere 26, at the junction of the pharynx and gut. The notochord is lightly shaded; darker shading indicates regions occupied by various peripheral plexuses. The nerve cord and one dorsal nerve are shown in black; black dots indicate schematically where nerve cells associated with the atrial plexus would lie. Abbreviations and their corresponding terms are listed in Appendix A.

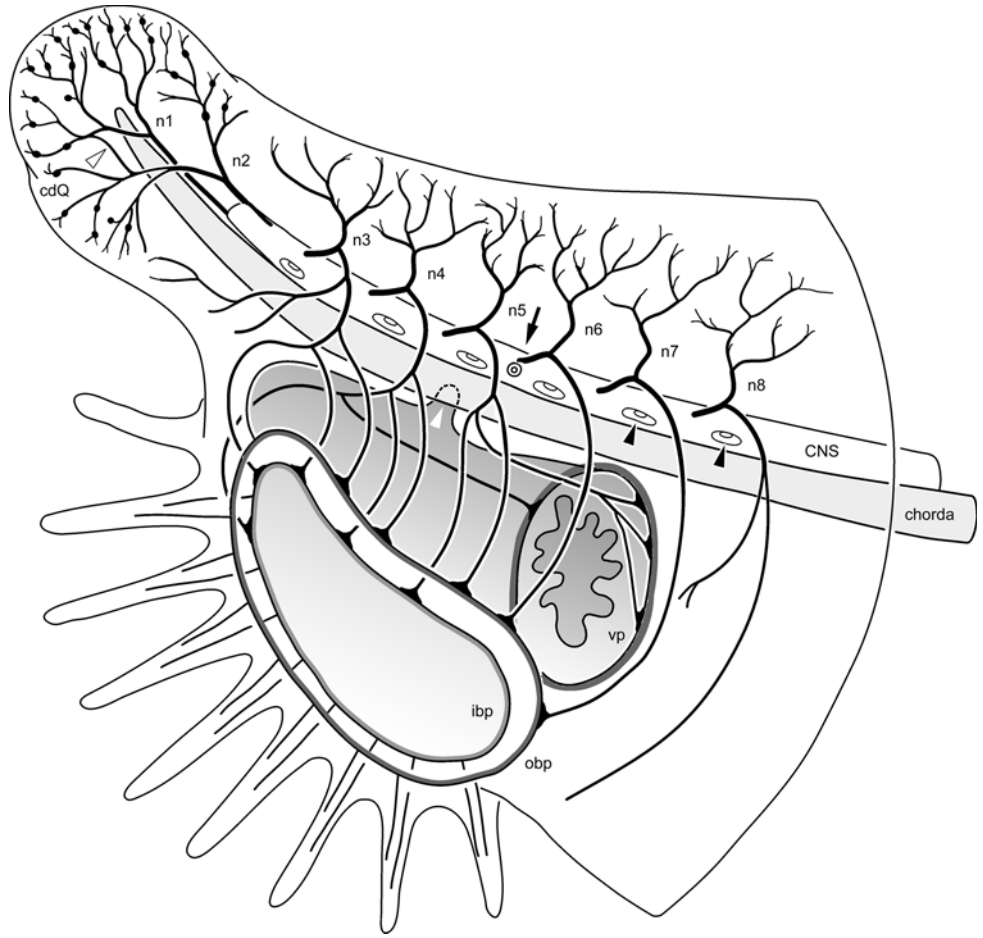


of the ciliated wheel organ of Müller (Müller 1844; Franz 1927), located in the roof of the buccal cavity (Figs. 1, 3, 7C). In some lancelet species the pit forms a direct contact with the base of the nerve cord (see below).

From both the adult anatomy and gene expression data it is clear that the anterior vesicle and IR together form a CNS region with a number of features in common with the vertebrate brain: they form the anterior part of the neuraxis, their nerves supply the region associated with the buccal cavity, and cytoarchitectural specializations occur that are not present in more caudal regions of the nerve cord. There is strong evidence that these anterior regions also contain homologs of neural centers and sense organs that occur in the brain of craniates, as discussed more fully in the last section. It is nevertheless difficult to determine the caudal extent of this brain-like region with any precision and define it in a way that applies equally to both larvae and adults. In adults, the first Rohde cell is usually assumed to mark the posterior end of the brain, but this is based more on convenience (the cell is very easy to recognize) than on evidence, since there are other internal landmarks and discontinuities that could be used just as well. On the other hand, determining the exact extent of the brain in craniates can be problematic, as the boundary between spinal cord and brain, as well as the number of cranial nerves, can vary from species to species (Nieuwenhuys 1998).

One way to define the brain is as that part of the CNS enclosed in the cranium, but this is obviously not a useful criterion when applied to acraniates. If, instead, the vertebrate brain is defined in terms of its peripheral nervous connections, which supply the full extent of the pharyngeal region, then the caudal end of the brain of lancelets would come to lie somewhere in the middle of the body. In larval lancelets, expression domains of *Otx* and various *Hox* genes indicate

Fig. 3. A ventrolateral view of the rostrum and oral region of a lancelet, showing the first eight dorsal nerves (n1 to n8), but only those on the left side. The buccal and velar plexuses supplying the buccal chamber that extends from the base of the cirri to the velum are shaded. Also shown are the first cell of Rohde (arrow), Hatschek's pit (white arrowhead), an anastomosis between nerves 1 and 2 (open arrowhead), and two of the neuromuscular contact zones (black arrowheads), for myomeres 5 and 6 in this instance. Note that each contact zone consists of a small dorsal and a larger ventral compartment. Abbreviations and their corresponding terms are listed in Appendix A.



that an equivalent of the vertebrate mid-hindbrain boundary may be present somewhere in the front half of somite 2, with a hindbrain-like region extending thereafter to about somite 7 or 8 (Shimeld and Holland 2005). This would move the junction between brain and spinal cord to a position three or four myomeres caudal to the first Rohde cell. The nerve cord in the neurula also extends from somite 1 to somite 8, which has led to the suggestion that the entire neurula is roughly equivalent in axial extent to the vertebrate head (Gilland and Baker 1993). This would make the nerve cord in the neurula coextensive with the vertebrate brain, with most of its length being hindbrain-like in character. While this may be true, the details of what it really means to be "hindbrain-like" remain to be worked out (Jackman and Kimmel 2002; Mazet and Shimeld 2002), so we must conclude that the exact caudal boundary of the brain in lancelets cannot currently be determined with any certainty.

Spinal cord, dorsal nerves, and associated peripheral structures

The largest region of the CNS is commonly referred to as the spinal cord, in recognition of its general similarity to that structure in vertebrates. Using the first Rohde cell as the landmark that divides it from the anterior CNS in the adult, the spinal cord stretches from the fifth myomere to the last, and thus gives rise to most of the dorsal nerves. Up to nerve 53, which innervates the anal sphincter muscle, the dorsal nerves connect the spinal cord to the atrial nervous system, the most extensive and complex component of the PNS. Figure 2 shows how the nerves and plexuses are arranged in the transverse plane. Once a typical nerve leaves the nerve cord, it enters the intermyotomal septum and passes laterally along it, dividing into a dorsal and a ventral ramus as it leaves. The rami travel under the skin lateral to the muscle block (note that in vertebrates the corresponding nerve instead lies medial to the trunk musculature), dorsally in the case of the dorsal ramus and ventrally in the case of the ventral ramus, to supply fibers to the subepidermal nerve plexus underlying the skin of the dorsal fin (dorsal ramus) or the subepidermal plexus beneath the skin on the flanks (ventral ramus). At the ventral margin of the myomere, the ventral ramus divides into a ventral cutaneous ramus, which supplies the skin of the metapleural folds, and a visceral ramus, which turns medially towards the wall of the atrial cavity, where it again divides into an ascending and a descending branch. The atrial epithelium and the epithelia of all organs

that either flank it (e.g., gonads and pterygeal muscle) or are located within it (pharynx and endostyle, the gut and its diverticulum) are underlain by a massive system of nerve cells and fibers collectively termed the atrial nervous system (Bone 1961). The descending branch of the visceral ramus contributes to the gonadal and pterygeal portions of that system and, in addition, contributes motor fibers to the cross-striated pterygeal (= transverse) muscle. The ascending branch of the visceral ramus turns dorsally and gives off fibers to the plexus covering the gonads and the lateral walls of the atrium. It then turns medially and reaches the denticulate ligament, which tethers the pharynx and the gut to the roof of the atrium, and by this means it reaches the nerve plexus of the pharynx, gill bars, endostyle, and gut plus diverticulum.

On the basis of its internal appearance, the adult spinal cord can be subdivided into three major regions (this account follows Franz 1923, 1927). The anterior part of the cord, at the level of myomeres 5–11, is characterized by the presence of large Rohde cells with descending axons and by a high density of pigmented, photoreceptive organs of Hesse (Hesse 1898). Peripherally, nerves connect this part of the cord with the most caudal parts of the buccal nerve plexus, via nerve 7, while the more posterior nerves in this region all connect with the atrial nervous system (Bone 1961; see Figs. 1, 2).

The intermediate part of the spinal cord, from myomeres 12 to 38, lacks Rohde cells, and the density of Hesse organs is reduced here. Peripherally, nerves from this region also connect to the atrial nervous system. Its rostrocaudal extent coincides roughly with that of the gonads, the caudal limit being approximately at the level of the atriopore, whose cross-striated sphincter is innervated by nerves 40 and 41 (Kutchin 1913). The transition between pharynx and gut is located approximately at the level of myomere 26 or 27. The atriocolemic funnels, enigmatic organs that may be sensory in nature, are located just above this point. They are surrounded by the cross-striated trapezius muscles (Franz 1927; Bone 1961), which are innervated by the ascending visceral branches of nerves 27–29.

The posterior part of the spinal cord extends from the atriopore, at the level of myomeres 38–40, to the last myomere, typically number 63 in *B. lanceolatum* (Poss and Boschung 1996). The number of Hesse organs increases again in this region and the Rohde cells reappear. Nerves 51–53 supply the anal sphincter muscle (Kutchin 1913). Nerves caudal to this point lack visceral rami (Franz 1927). The last nerve (nerve 65 in an animal with 63 myomeres) leaves the spinal cord at the caudal border of the last myomere and innervates the skin adjacent to the tail fin.

Terminal filament and caudal ampulla

Still more caudally, the spinal cord tapers into a thin ependymal tubule, the terminal filament (filum terminale), which connects the cord to a caudal ampulla just above the posterior tip of the notochord. Reissner's fiber, which is produced in the infundibular organ, extends into that caudal ampulla, where it is apparently phagocytosed by specialized cells in the walls of the ampulla (Obermüller-Wilén and Olsson 1974). There are numerous nerve fibers of unknown origin along the ventral and lateral margins of the caudal ampulla. These contain dense core vesicles, which implies that this structure may function as an endocrine organ comparable to the urophysis of anamniotic vertebrates (Ruiz and Anadón 1991*a*).

The adult PNS

Peripheral plexuses

The system of peripheral plexuses in amphioxus is unusual in a number of respects. It supplies a fine meshwork of free nerve endings that run through the entire epidermis (Dogiel 1903; Kutchin 1913; Franz 1923, 1927; Welsch 1968*b*, see Fig. 2) such that every epidermal cell is apparently in contact with them (Lele et al. 1958). The free nerve endings probably arise from populations of intramedullary sensory neurons, of which there are two types, according to Bone (1960*a*, 1961): the dorsal bipolar or Retzius bipolar cell and the dorsal root cell (see Fig. 4). The precise source of the free nerve endings has not been determined with certainty, however.

The epithelia that line the buccal and atrial cavities, as well as the organs embedded within them, are also supplied with an extensive set of neural plexuses collectively known as the atrial nervous system. Though the system is more or less continuous, it is usually subdivided on the basis of the organs it innervates; i.e., there are buccal, velar, gonadal, parietal, pterygeal, pharyngeal, and endostylar subdivisions, and so on (Bone 1961). While the various plexuses carry motor fibers innervating muscles associated with the atrium, they also contain the cell bodies and fibers of a large number of peripheral neurons (Figs. 2, 4). It has been argued (e.g., Boeke 1935) that the plexuses are homologs of the autonomic nervous system of craniates, but this is almost certainly false. Enteric neurons in vertebrates develop from migratory neural crest cells, a category of embryonic cells that is entirely absent in amphioxus so far as can be determined (Baker and Bronner-Fraser 1997). This implies by default that neurons in the peripheral plexuses in amphioxus arise locally, in situ, though Lacalli (2004) has pointed out that the embryo is so small at the time that neuronal precursors are probably first deployed that an origin close to the neural plate, similar to that of placodes and the neural crest, cannot be ruled out. It is also true, however, that the neurotransmitters identified to date in amphioxus peripheral neurons differ from those released by autonomic neurons in vertebrates. Specifically, neither acetylcholine (Flood 1974) nor catecholamines (Moret et al. 2004) occur in peripheral neurons, at least in the atrial nervous system. FMRFamide, however, does occur (Bone et al. 1996), and this is a transmitter restricted mainly to the CNS in vertebrates. In addition, many of the peripheral neurons in amphioxus send axons into the nerve cord via the dorsal nerves (Holmes 1953; Bone 1961). This implies a sensory function, rather than a motor one.

Based on these unique features, Bone et al. (1996) concluded that there were no obvious homologies between the PNS of amphioxus and that of vertebrates. In general terms this conclusion appears justified. Nevertheless, in rats, centripetal projections are known from peptidergic peripheral neurons (called rectospinal neurons) resident in the walls of the anus (Dörffler-Melly and Neuhuber 1988). These are ev-

idently not simply displaced dorsal root ganglion cells, but constitute instead a novel class of vertebrate enteric neurons (Neuhuber et al. 1993). They could conceivably be a relic of a primitive mode of visceral innervation related to that in amphioxus. Conversely, they could be a derived feature of no phylogenetic significance.

The velar and buccal plexuses deserve special attention owing to their curious asymmetry (Dogiel 1903; Kutchin 1913; Franz 1923, 1927). As shown in Fig. 3, there are two buccal plexuses, an outer and an inner one, and a single velar plexus (Bone 1961). The velar plexus derives developmentally from the oral plexus that encircles the larval mouth as it moves ventrally and caudally during metamorphosis (Franz 1923; Kaji et al. 2001; the term "oral" should thus be avoided when referring to neural structures associated with the cirri because they are really preoral, buccal structures). As with other parts of the atrial nervous system, the buccal and velar plexuses combine sensory components (Bone 1961) with motor ones; the latter innervate the labial and velar muscles. The connection to the nerve cord via nerves 1–7 (1–8 according to Dogiel 1903; Kutchin 1913) is highly asymmetrical. Nerves 3 and 4 on the left side are exceptional in having contralateral branches that connect with the inner buccal plexus on the right side. In addition, a subsidiary branch from the contralateral branch of the left nerve 4 connects to the right side of the velar plexus, while its left side connects to nerve 5 by means of a caudal branch from that nerve. This is all a consequence of the fact that the larval mouth develops initially on the left side and is innervated entirely by nerves emerging from the left side of the nerve cord (Lacalli et al. 1999). The initial connections are then retained during subsequent development, so the nerves are dragged along as the mouth is repositioned.

Peripheral sensory cells and organs

Lancelets have an assortment of sensory cells and organs located both inside the nerve cord and outside it. The former are dealt with in relevant sections dealing with the adult and larval CNS; they include the various photoreceptor systems, which are all intramedullary, and Kölliker's pit, for which an olfactory function has been suggested. Hatschek's pit, located in the roof of the buccal cavity (Fig. 3), is not, strictly speaking, part of the nervous system, but is considered here because of its close association with it.

Multicellular organs

The peripheral tissues are well supplied with solitary sensory cells and free nerve endings but harbor only a few multicellular structures to which a sensory function can be attributed. Four examples are considered here. (1) The atriocolemic funnels, first described by Lankester (1875), consist of paired conical recesses in the dorsal surface of the atrial cavity that project anteriorly into the subchordal coelom (Figs. 1, 2; see Willey 1894; Franz 1927). Both the funnels and the surrounding striated trapezius muscle are densely innervated by a branch from the ascending visceral ramus of nerve 27 (Holmes 1953; Bone 1961) and, to a lesser degree, by neighboring dorsal nerves (Franz 1927). The fibers may be chiefly involved in the innervation of the trapezius muscle, but the atriocoelomic funnels themselves contain many uni- and multipolar neurons (Bone (1961) distinguished three types) whose axons enter visceral rami; from there they appear to travel to the nerve cord. No function has yet been ascribed to the funnels, however. (2) The atrial papillae of Müller (1844) were initially thought to be excretory in nature (as renal papillae, Willey 1894). The papillae are located in the floor of the atrial cavity (Fig. 2) and are concentrated in the vicinity of the atriopore. They consist of longitudinal strips of tall, densely packed cells, many of which appear to be flagellated primary sensory cells (Bone 1961; Bone et al. 1996; Ruppert 1997). Again, their function is unknown. (3) The encapsulated endings of Fusari (1889) (see also Bone 1960*b*) are formed by free nerve endings surrounded by clusters of cell nuclei. They are located in the lateral walls of the metapleural folds (Figs. 2, 4) and may be mechanoreceptive. (4) The corpuscles of de Quatrefages (de Quatrefages 1845) are located in the connective tissue of the rostrum along the branches of the first and second nerves. They are typically located at branch points, mainly at distal branches just proximal to the nerves' entry into the subepidermal plexus (Dogiel 1903; Baatrup 1982; Fig. 3). The corpuscles consist of support cells and peripheral neurons enclosed in a capsule of connective tissue (Franz 1923). The neurons bear a pair of cilia that project into a small central cavity and give rise to axons that enter the branches of the adjacent nerves (Baatrup 1982). The corpuscles are assumed to be mechanoreceptors sensitive to the deformation of the rostral connective tissue.

Solitary receptors

Solitary receptors are widely distributed over the entire epidermis but are most common in the region of the rostrum, buccal cirri, and tail (Dogiel 1903; Franz 1923; Bone 1960*b*; Stokes and Holland 1995*a*; Holland and Yu 2002). They form small clusters in some instances (*Sinnesknospen*, Franz 1923; Schulte and Riehl 1977), especially along the buccal cirri. The most common receptor cell types are referred to by convention as types I and II (Schulte and Riehl 1977; Bone and Best 1978). Type I cells are primary sensory neurons with an apical circlet of microvilli, a single cilium, and a basal neurite. There are several subtypes, but all are probably mechanosensors (Baatrup 1981; Lacalli and Hou 1999). Their axons project to the CNS via the dorsal nerves; once there, they travel along the cord in two fiber tracts, dorsal and subdorsal in the terminology of Holland and Yu (2002), which may correspond to the somatosensory and viscerosensory tracts of Bone (1960*a*; see Fig. 4). The central axons of type I cells reach considerable lengths, so an axon entering the CNS via the first nerve can typically project caudally to mid-spinal levels, at least in larvae (Holland and Yu 2002). Little is known about the neurotransmitters released by peripheral neurons, but there is evidence that at least some type I cells are GABAergic (Anadón et al. 1998).

Type II receptors (Fig. 4) are secondary sensory cells with synaptic terminals borne on short basal processes, usually three per cell (Stokes and Holland 1995*a*; Lacalli and Hou 1999). Apically, they have a modified nonmotile cilium surrounded by a collar of branched microvilli. This extensive elaboration of the apical surface suggests a chemoreceptive function, but essentially nothing is known for certain about chemoreception in amphioxus, either in terms of structures or physiology (Lacalli 2004). The synaptic targets of type II

cells are not known, but most likely they are fibers belonging to intramedullary Retzius bipolar cells (Holland and Yu 2002).

Additional sensory cell types reported from larvae may well be present in adults as well, but perhaps have simply not yet been observed. Most notable are the (multi)ciliary spines along the oral margin in larvae, each consisting of a bundle of stiff cilia, one from each cell that contributes to the spine (Lacalli et al. 1999). These cells are secondary sense cells that synapse with local interneurons resident in the oral plexus, whose axons then travel to the CNS. It would be interesting to know whether this arrangement persists to the adult. A second way of stiffening cilia is by altering their internal support, and this is seen in ciliary spine cells (Lacalli and Hou 1999). Ciliary spine cells are solitary sensory cells present in small numbers on the larval rostrum, and possibly elsewhere, in which the ciliary axoneme is replaced with a lamellar matrix. Again, these cells are assumed to be mechanoreceptors.

As described above, there also are solitary sensory cells in most parts of the atrial nervous system. Bone (1961) described various types of multi- and unipolar cells that send neurites into the CNS (see Fig. 4). It is not clear, however, whether such cells are actually primary sensory cells or whether they participate in complicated synaptic chains of receptors, interneurons, and projection neurons, similar to those described for the larval oral plexus.

Hatschek's pit

Hatschek's pit (corresponding to the preoral pit of the larva) is the central element of the wheel organ, a system of ciliated ridges with an accessory feeding function, located in the roof of the buccal cavity (Figs. 1, 3; see Ruppert 1997 for a detailed description). As is the case with many other structures, Hatschek's pit is asymmetrical: the bottom of the pit comes to lie to the right of the notochord and points towards the base of the CNS (Fig. 7C). In some species of lancelets (Gorbman 1999; Gorbman et al. 1999) it may even be in contact with the CNS, a situation strongly reminiscent of the hypothalamus–pituitary relationship in vertebrates. Consequently, Hatschek's pit has been regarded as a good candidate for homology with Rathke's pouch or the adenohyophysis of vertebrates (e.g., Tjoa and Welsch 1974; Nozaki and Gorbman 1992). As discussed elsewhere in this issue (Sherwood et al. 2005), this hypothesis does not receive much support from an analysis of the secretions by Hatschek's pit (the evidence for the presence of typical adenohypohyseal hormones is somewhat inconclusive); on the other hand, pituitary-specific transcription factors are expressed in Hatschek's pit during development (Candiani and Pestarino 1998).

There are further complications, however. First, the zone of contact between Hatschek's pit and the CNS in amphioxus is displaced about two myomeres caudal to where it should be if it were an exact homolog of the vertebrate pituitary, since — according to the gene expression pattens in the larva — the amphioxus homolog of the forebrain would be adjacent to myomere 1 (Shimeld and Holland 2005). The junction of myomeres 3 and 4 lies, instead, in a region of the CNS that expresses *AmphiHox* genes during early larval development. Thus, this region is a more likely candidate for homology with the vertebrate hindbrain.

Second, the development of Hatschek's pit differs from that of the adenohypophysis of vertebrates, which is classically supposed to be of ectodermal (placodal) origin. Hatschek's pit, on the other hand, arises from the left anterior diverticulum of the endodermal embryonic foregut (see Conklin 1932; Stach 2000). This diverticulum then opens to the exterior by fusing with a preoral ectodermal invagination, thus forming the larval preoral pit. This pit is initially located at the level of somite 1, but there is no zone of contact with the nerve cord at this stage. The preoral pit then shifts caudally at metamorphosis, along with the whole assemblage of oral and preoral structures, and finally develops into Hatschek's pit at the boundary between myomeres 3 and 4.

Thus, despite claims that the adenohypophysis also develops from the endodermal foregut in certain craniates (i.e., in myxinoids, Gorbman and Tamarin 1985), both the development of Hatschek's pit and its position in adult amphioxus are sufficiently different from the development and position of the adenohypophysis in typical vertebrates to warrant some caution in interpreting the relationship between the two structures. Nevertheless, it is noteworthy that the preoral pit differentiates very early in amphioxus, so that it appears to be functional by the time the larvae begin to feed. That function is unknown, but feeding is the main larval activity besides swimming at this early stage, which implies for the preoral pit a role in either feeding or some related aspect of metabolic processing. Jacobs and Gates (2003) have suggested that the ancestral adenohypophysis may have been an external sense organ that acted on the internal physiology of the animal via some form of non-neural signaling. If the preoral pit is indeed an adenohypophyseal homolog that acts in this way, its involvement in feeding and metabolism in amphioxus would provide a rationale for the central role of its vertebrate counterpart in the control of metabolism and growth.

The adult CNS

General histological appearance

Most parts of the nerve cord are roughly triangular in transverse section, with curved sides and a concave base that rests on the notochord (Figs. 4, 5). Only the anterior vesicle and the caudal ampulla are more or less circular in cross section. The shape of the central canal (= ventricle, ventricular system) varies from region to region. However, with the exception of the anterior vesicle and the caudal ampulla, it generally has the form of a vertical slit expanded slightly at the top and bottom. The ventral expansion, which runs caudally from the level of the infundibular cells, houses Reissner's fiber. The dorsal expansion is variable, being pronounced in some regions and absent in others, and may be filled with fluid or with cellular processes. In the slit-like part of the central canal (= intermediate zone in some accounts), the opposing walls of the nerve cord are closely apposed and the canal itself is almost obliterated. Translumenal processes cross from both sides in this zone, and the remaining open space contains cilia that arise from both ependymal cells and neurons.

Fig. 4. A highly schematic transverse section of amphioxus through the central nervous system and the top portion of the notochord, showing various sensory and motor cell types and the composition of the dorsal nerves. The grey spot in the ventral expansion of the central canal is Reissner's fiber. Abbreviations and their corresponding terms are listed in Appendix A.

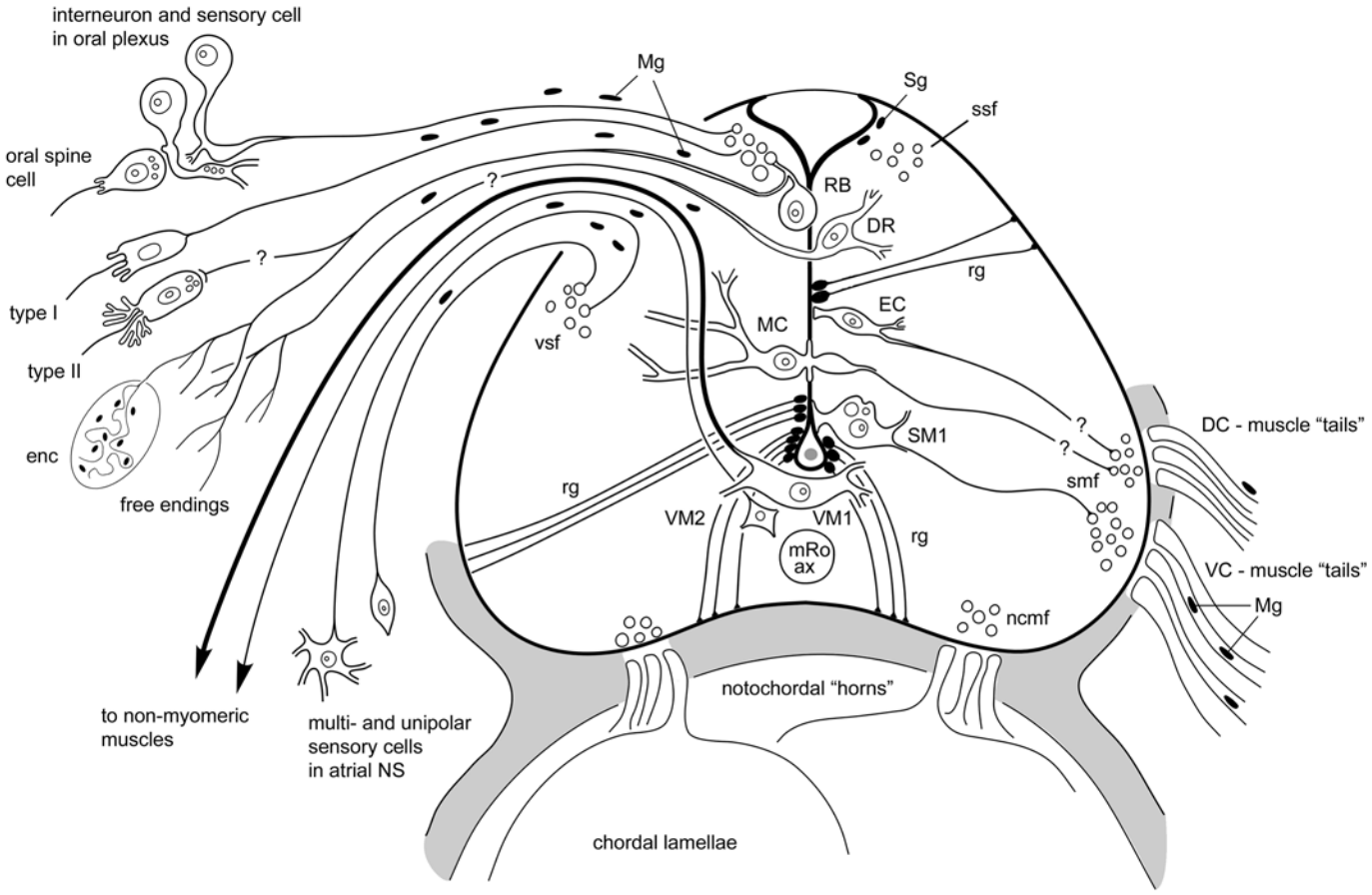
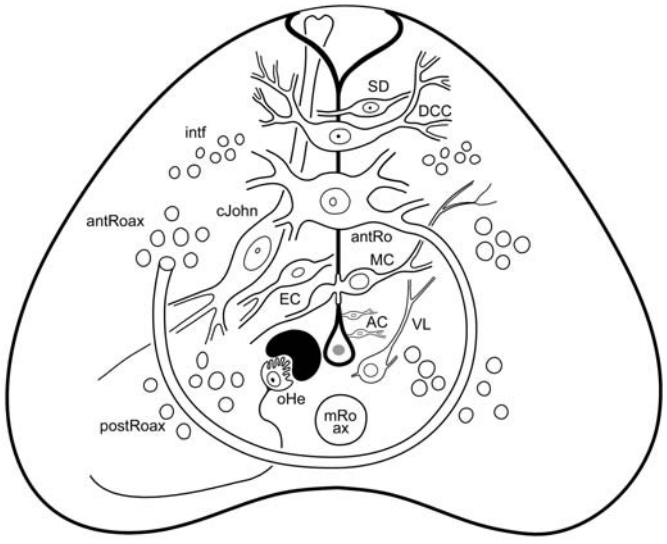


Fig. 5. A highly schematic transverse section of amphioxus through the anterior spinal cord, showing various types of interneurons and their characteristic arrangement. The grey spot in the ventral expansion of the central canal is Reissner's fiber. Abbreviations and their corresponding terms are listed in Appendix A.



The cell bodies of most of the neurons are located close to the central canal (Figs. 4–7), and most have an apical process that connects the cell to the ventricular surface. In terms of vertebrate neuroanatomy, the neurons thus form a dense periventricular layer and most, if not all, of the neurons in this layer are of the cerebrospinal fluid contacting type. Populations of cells variously termed translumenal neurons (Lacalli and Kelly 2003*a*) or commissural cells (e.g., Franz 1923; Bone 1960*a*) form a special subcategory of cerebrospinal fluid contacting cells. Not only do they contact the ventricular surface, but parts of their somata — apical processes in some instances or the whole cell body in others — lie transversely across the ventricle. Such cells occur in large numbers in most regions of the CNS (Figs. 4, 5, 6D) and include the largest cells in the cord, i.e., the cells of Rohde (see below and Fig. 5), along with other large neurons. Migrated neurons, i.e., those whose cell bodies are entirely detached from the ventricular surface, are scarce and restricted to the IR of the anterior nerve cord (see below).

The amphioxus CNS is not vascularized, and the axonal and dendritic processes of its neurons are not myelinated or otherwise enveloped by glial cells (Meves 1973). Nevertheless, various types of glial cells do occur in the nerve cord (Bone 1960*a*; see Fig. 4). Müller's glia (Müller 1900) consists of groups of very small cells with intensely staining nuclei that are clustered in the vicinity of the dorsal nerves and

in their peripheral branches. Some workers (e.g., Johnston 1905) have interpreted these as dorsal root ganglion cells; however, as noted by Franz (1927), they also occur between the muscle tails, i.e., the false ventral roots (Lele et al. 1958), and they lack neurites. Bone (1960*a*) consequently concluded that they were Schwann cell analogues. In some parts of the peripheral nerves, as well as the muscle tails, these cells form incomplete partial sheaths around axons and muscle tails (Flood 1966, 1974; also see Fig. 8B in Schulte and Riehl 1977). This may be a local phenomenon, however, rather than being widespread throughout the PNS.

A second type of glial cell with either short processes or no processes is Schneider's glia (Schneider 1879; Bone 1960*a*), which lines the walls of the dorsal expansion of the central canal. A third type of glia, the radial glia (= ependymal glia, Bone 1960*a*), lines the ventricular walls. These glia send long, fiber-filled processes through the white matter to the connective tissue sheath surrounding the nerve cord, and there they expand to form terminal endfeet. This forms essentially an outer limiting membrane around the neural components of the nerve cord and provides the mechanical support necessary to maintain the nerve cord's shape. Several other glial cell types have been reported from the larval nerve cord (Lacalli and Kelly 2002), including one, the axial glia, that appears to have a transient function in axonal guidance. These cells arise adjacent to the primary motoneurons, which suggests they could be related to vertebrate oligodendrocytes, which also develop from a restricted zone adjacent to the region where the primary motoneurons arise. We suggest, therefore, that the myelination function may have evolved secondarily in a cell line that functioned first in axonal guidance.

Selected cell types

Cells of Rohde

The cells of Rohde (*kollossale Ganglienzellen* = Rohde cells, not to be confused with the nucleus of Rohde, a ventral cluster of neurons in the anterior IR) are the largest neurons in the nerve cord. As described most thoroughly by Rohde (1887), they are translumenal cells with a large soma across the central canal and dendrites that ramify in the white matter on both sides of the nerve cord. The anteriormost Rohde cell marks the rostral boundary of the spinal cord. Its cell body is always located in the vicinity of the left sixth nerve (i.e., at the boundary of myomeres 4 and 5; see Figs. 1, 3, 7). Its giant axon (*kollossale Faser*, Rohde 1887), which arises from the left side of the cell, turns ventrally and is the most conspicuous structure in the ventral midline of the spinal cord (Fig. 6). It projects caudally to the level of the last myomere (Franz 1923). Much of the input to the dendrites of the first Rohde cell appears to be via gap junctions (Ruiz and Anadón 1989), as only a few synapses to them have yet been found. The giant axon displays numerous en-passant synapses (Ruiz and Anadón 1989); processes of somatomotor cells (see below) might be postsynaptic to the giant axon (Castro et al. 2004).

The remaining Rohde cells (Fig. 5) are much smaller than the first and form separate anterior and posterior groups (Fig. 1). The anterior group, excluding the first cell, consists of roughly 15 cells in the region of myomeres 5–11. Their cell bodies are in line with the transverse planes defined by the staggered left and right dorsal nerves. Cell size decreases progressively caudally, so that the most caudal of the anterior Rohde cells that can be identified with any certainty lies at the level of the right 13th nerve (Franz 1923). The cells give rise to thick axons alternating to the left and right sides in the anterior series of Rohde cells. The axons turn ventrally, cross the midline, and then travel caudally in a lateral fiber bundle (Fig. 5).

Rohde cells reappear at the level of myomere 38, adjacent to nerve 39. The posterior Rohde cells are, on average, much smaller than those in the anterior group, and they are not aligned with the dorsal nerves. There are between 14 (Rohde 1887) and 18 (Franz 1923) posterior Rohde cells, the most caudal one being at the level of myomere 60. The posterior cells have mainly ascending axons that travel in a ventrolateral tract (Franz 1923, see Fig. 5).

As in the case of the first and largest Rohde cell, almost nothing is known about the synaptic relationships and the neurochemistry of the rest of the Rohde cell series. It has, however, recently been shown that all the Rohde cells, including their axons, are immunopositive for progesterone (Takeda et al. 2003). The significance of this finding is entirely unclear. The most one can currently say is that the Rohde cells, though conspicuous, remain enigmatic.

Organs of Hesse

The organs of Hesse (= Hesse organs, dorsal ocelli) are composite photoreceptors (Hesse 1898) located in the ventral part of the periventricular grey (Figs. 5, 7). Each organ consists of a single rhabdomeric photoreceptor cell whose microvilli are enveloped by a cup-shaped pigment cell (Eakin and Westfall 1962; Ruiz and Anadón 1991*c*; see Fig. 5). The receptor cells are primary sensory cells, i.e., each has an axon (Franz 1923), and these project to the ventrolateral part of the spinal cord (Guthrie 1975). The first organ to form in development is unusual in consisting of three cells — two photoreceptors and one pigment cell — and the former in this case are known to innervate the dorsal compartment (DC) motoneurons (Lacalli 2002*a*). It is not known whether this is the case also for the rest of the series, but it does suggest that Hesse organs have something to do with controlling activities that depend on slow undulations of the body, as opposed to the fast ones used for escape swimming. Hesse organs may not express *Pax6* during development — certainly the first does not (Glardon et al. 1998) — but they do express S-antigen (arrestin), a protein typical of the phototransduction cascade in many animals (Mirshahi et al. 1985), and serotonin is present in the receptor cells (Candiani et al. 2001).

The first Hesse organ to develop is located adjacent to myomere 5, but the anteriormost in the adult lies at the boundary of myomeres 3 and 4; it is located in the posterior part of the IR. Hesse organs become more numerous as one moves caudally into the spinal cord, and they have a tendency to cluster opposite the centers of adjacent myomeres (Franz 1923) so as to form a segmental pattern. Moving further along the spinal cord, they first decrease in number towards the center of the body and then increase again more caudally. The orientation of the pigment cups is not random. Instead, groups of Hesse organs in certain regions of the spi-

nal cord point in particular directions. Franz (1923) undertook the tedious work of documenting this; for details, refer to his paper.

Motoneurons, with remarks on muscle innervation patterns

The innervation of the myomeric muscles in amphioxus is highly unusual. The myomeres consist of cross-striated muscles arranged in a series of chevron-shaped blocks along the flank. The muscle cells each send long processes (= muscle tails) towards the ventrolateral margins of the nerve cord. These processes were formely interpreted, mistakenly, as ventral nerve roots. Their true nature was first recognized by Flood (1966): the muscle processes are the sites where the synapses from motoneurons are received; the axons and synaptic terminals remain entirely confined within the nerve cord; and transmitter release occurs across the basal lamina (Figs. 3, 4). The synaptic zones are serially repeated, one for each myomere, and are staggered left to right in a manner similar to that of the myomeres. Amphioxus thus lacks any counterpart to the ventral nerve roots of vertebrates (Schneider 1879; Flood 1966, 1968).

The synaptic zones in each segment consist of two distinct domains, the ventral and dorsal synaptic compartments (Figs. 3, 4). Both utilize acetylcholine as a transmitter (Flood 1974). The ventral synaptic compartments are where the deep, anaerobic, fast muscle cells receive their innervation. The presynaptic motoneurons involved belong to a class of cells that Bone (1960*a*) called somatomotor (SM) cells; they may therefore also be called ventral compartment motoneurons. They are found in the ventral parts of the grey matter and have a tendency to cluster opposite the synaptic contact zones, and each has a broad apical process connecting it to the ventricular cavity. Some have internal vacuoles, and this character, together with size and positional differences, has been used to define several subtypes (Bone 1960*a*; only one such type, the SM1 cell, is shown in Fig. 4). The axons of the SM cells project laterally into the bundle of somatomotor fibers adjacent to the synaptic zone of the ventral compartment.

The dorsal compartment is where the superficial, aerobic, slow muscle cells of the myomeres receive their innervation. The DC motoneurons are known from larvae (Lacalli and Kelly 1999; Lacalli 2002*a*) but have not yet been identified with certainty in adults. From the larval data, however, it seems that the whole of the DC innervation along the nerve cord may derive from motoneurons located in the anterior cord at the level of somites 2–6 (see below). This is approximately equivalent to the zone fated to become the IR of the anterior cord, which extends from myotomes 2 to 4. Tracing experiments by Fritzsch (1996) and Ekhart et al. (2003) have revealed ventrally located cells with descending projections in the adult in this region, but it is not clear what types of cells these are, or even whether they are motoneurons or interneurons. It is also possible that local commissural cells contribute to the presynapses of the dorsal compartments (Fig. 4). The Edinger (EC) and mid-commissural (MC) cells of Bone (1960*a*) send axons into the bundle of somatomotor fibers adjacent to the synaptic zone of the dorsal compartments. Bone (1960*a*) regarded the entire DC system as a somatic sensory system; hence, he classified the EC and MC cells as afferent cells. It is further possible that the adult dorsal compartment is innervated by a subset of the somatic motoneuron series, i.e., one or the other of the SM cell types (Castro et al. 2004).

Unlike the notochord of any other chordate, the notochord of lancelets is itself a muscle, i.e., it consists mainly of specialized striated muscle cells referred to as notochordal lamellae (Welsch 1968*c*; Flood 1970). The notochordal muscle cells contact the nerve cord by means of processes that emerge from the cells dorsally, in small bundles (notochordal horns, Flood 1970), and pierce the connective tissue sheath separating the notochord and CNS (Fig. 4). There are many thousands of these horns, roughly one every 50 μm (Flood 1970), arranged in two rows to the left and right of the midline. Their contacts with the base of the CNS are specialized as postsynaptic swellings that are apposed to presynaptic terminals inside the CNS. Thus, there are serially (but not segmentally) repeated neurochordal synaptic contacts at the base of the nerve cord. These are thought to be cholinergic (Flood 1970), but their source within the nerve cord has not been identified. Lacalli (2004) has made a tentative suggestion that they originate from sensory cells located in the rostrum, but this remains to be proven.

The remaining muscles of the body, besides the myomeric muscles and the notochord, are innervated by a visceromotor (VM) system of cells and fibers. The neurons are distinctive in appearance and location (Bone 1960*a*), as they lie immediately beneath the ventral expansion of the central canal and dorsal to the medial axon of the giant Rohde cell (Fig. 4). This places them ventral to the SM neurons (cf. Figs. 4, 7), a situation that differs from that in vertebrates, where the VM neurons are dorsal to SM neurons (e.g., Nieuwenhuys 1998). There are both large (VM1, one per segment) and small (VM2, many per segment) VM cells in adult amphioxus. They are multipolar, and axons from both types leave the CNS in the dorsal nerves; similar cells are found also in larvae (Lacalli and Kelly 2002). Candiani et al. (2001) reported that the cell bodies of VM2 cells contain serotonin, but VM axonal terminals are cholinergic (Flood 1974) and are found in various muscles (e.g., the pterygeal muscle) associated with the atrium. Though referred to as visceral muscles, most of them are cross-striated (Franz 1927; Ruppert 1997) rather than smooth. There is therefore some question whether the VM system of lancelets is really comparable to the visceral muscles of vertebrates. A better comparison may be, instead, with the branchiomotor nerves and muscles of vertebrates, as suggested by Fritzsch and Northcutt (1993).

Intramedullary sensory neurons

As mentioned above, lancelets do not have dorsal root ganglia. Instead, they have intramedullary sensory cells comparable to the Rohon-Beard cells found in the larval nerve cord of anamniotic vertebrates (Fritzsch and Northcutt 1993). Bone (1960*a*) distinguished two major classes, the dorsal bipolar or Retzius bipolar (RB) cell and the dorsal root (DR) cell. There are two subclasses of the former and three of the latter. RB cells are situated in the most dorsal part of the periventricular grey and have both ascending and descending fibers. The fibers are a major component of the longitudinal somatosensory tracts, and the peripheral RB

Fig. 6. A schematic lateral view of the rostral end of the nerve cord of a lancelet, including the anterior vesicle plus the anterior and intermediate parts of the intercalated region (A), with cresyl violet-stained transverse sections (B–E) to show arrangement of cells in the transverse plane at the levels indicated in A. The diagram shows, projected onto the midsagittal plane, the various cells and (or) groups of cells that have been identified either by cytoarchitectural criteria, immunocytochemistry, or tracing experiments. Thin arrows indicate the general direction of axonal projections for selected examples; their exact targets are not shown because they are largely unknown. Abbreviations and their corresponding terms are listed in Appendix A.

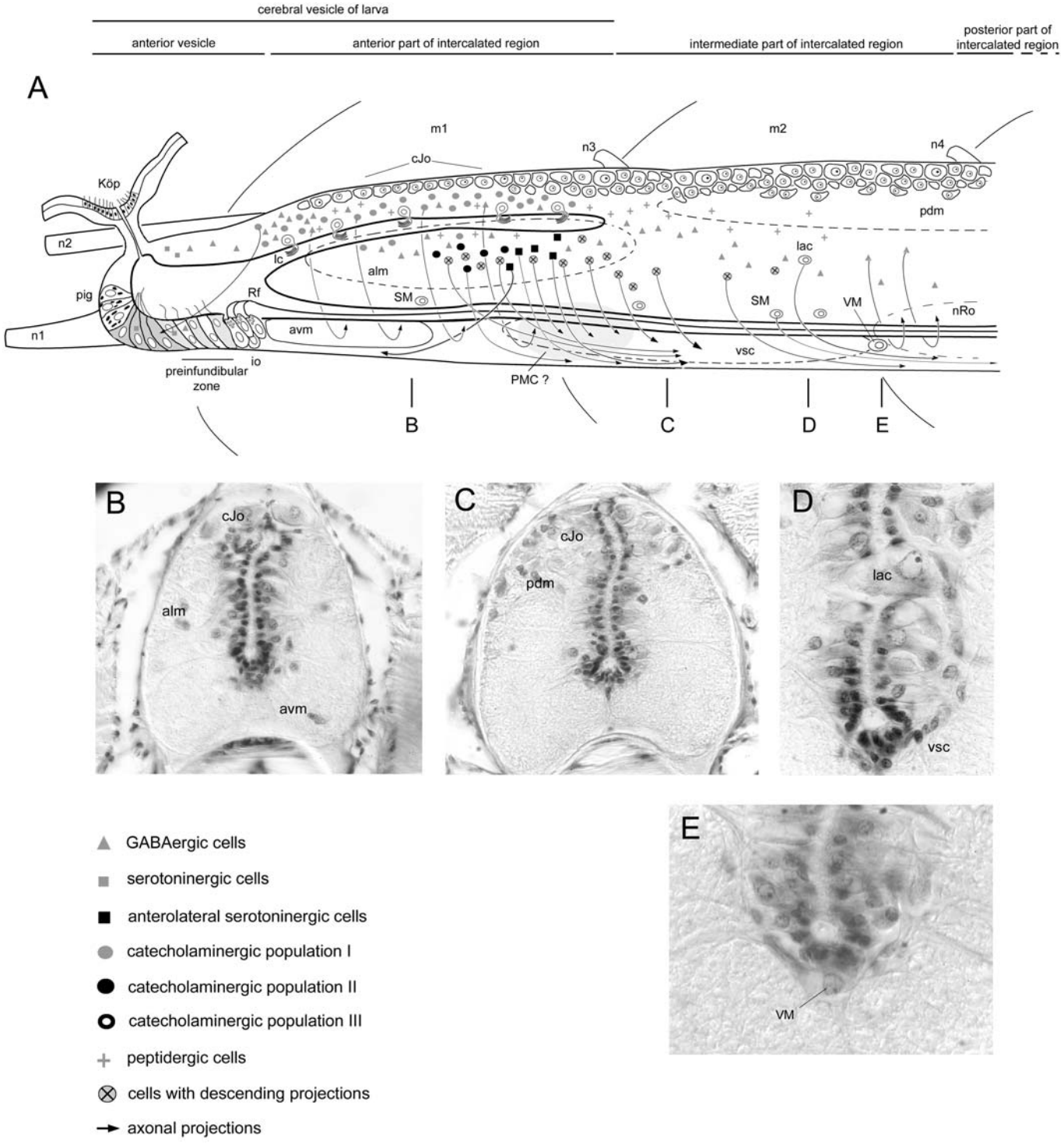
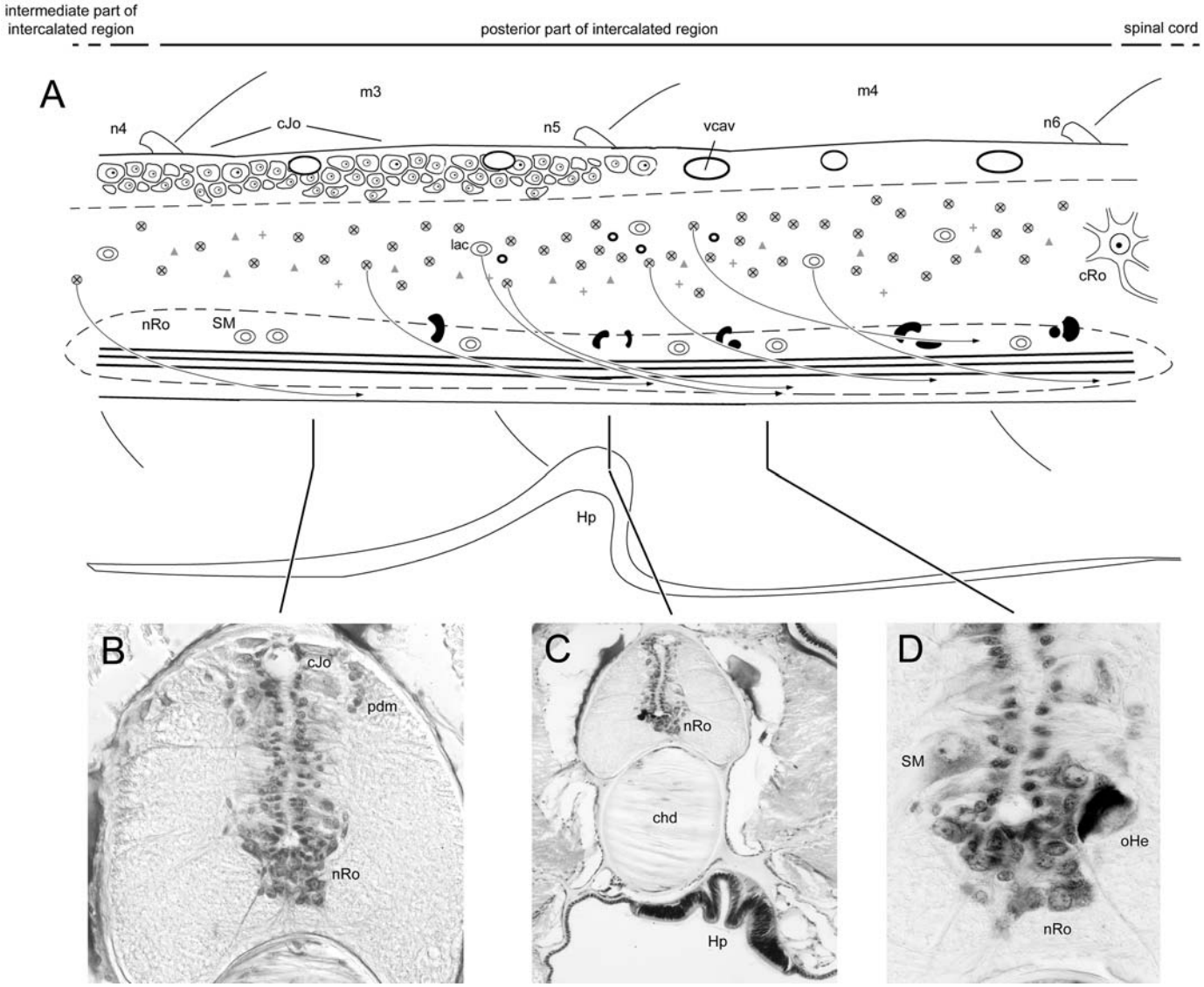


Fig. 7. A schematic lateral view of the region just behind that shown in Fig. 6, to show the posterior part of the intercalated region (A) and cresyl violet-stained transverse sections (B–D) at the levels indicated in A. Refer to Fig. 6 for details and an explanation of symbols. The black structures in the ventral part of the nerve cord are the pigment cells belonging to the Hesse organs.



processes originate as branches from them and enter the dorsal nerve. There are fewer DR cells and most of them have translumenal processes (Fig. 4).

As to function, the usual assumption is that the RB and DR axons are the most probable sources of receptive sensory fibers in the various peripheral plexuses and the skin. Presumably some branch and terminate as free endings, while others are postsynaptic to secondary receptor cells, wherever those occur. This is a logical supposition, and there is circumstantial evidence on the innervation of the larval rostrum that tends to support it (Lacalli 2002*b*, 2004). It has in practice, however, been impossible to prove. The necessary tracing studies have not yet been performed, for example, so we have no detailed information on the peripheral connections of either RB or DR cells, nor is it certain that their processes actually travel any distance outside the cord. There is recent immunocytochemical evidence that some DR cells contain γ-aminobutyric acid (GABA) (Anadón et al. 1998), but otherwise nothing is known about their transmitters.

Interneurons

The middle (i.e., intermediate) zone of the periventricular grey (Fig. 5) is occupied by a variety of cells that Bone (1960*a*) regarded as interneurons. These include the Rohde cells and, more dorsally, translumenal dorsal commissural cells and small dorsal cells. The latter are related to the sensory system in that they send small processes into somatosensory tracts. Bone (1960*a*) thought that both types might contribute axons to the dorsal nerves in some cases. The EC and MC cells are found more ventrally. As mentioned above, they may have something to do with the DC motor system. The MC cells are translumenal neurons of an unusual type: the cell body itself lies across the central canal, and short expansions project directly into the canal itself (Figs. 4, 5).

GABA, neuropeptide Y, and several other neuropeptides have been detected in various cells loosely classified as interneurons (Uemura et al. 1994; Anadón et al. 1998; Castro et al. 2003), but this clearly represents a very diverse assemblage of disparate cell types about which very little is known.

Three other interneuronal cell types deserve mention. First, the cells of Johnston (Bone 1960*a*) occur segmentally between the dorsal nerves, one on each side, and have a long process that projects to the dorsal expansion of the central canal and contacts the fluid it contains (Fig. 5). Their function is entirely unknown. Second, a novel class of interneurons, Anadón's cells, has been identified in the vicinity of the ventral expansion of the central canal (Anadón et al. 1998; see Fig. 5). These are very small GABAergic cells interspersed between the cell bodies of SM and VM neurons (cf. Figs. 4, 5). Anadón et al. (1998) have suggested that they might be comparable to the inhibitory Renshaw cells of vertebrates. Notably, these small cerebrospinal fluid contacting cells do not seem to be identical to a third group of interneurons, the ventral longitudinal cells of Bone (Bone 1960*a*; Fig. 5). The cell bodies of this third group are also found between those of the motor neurons (cf. Figs. 4, 5) but are much larger than those of Anadón's cells and are detached from the ventricle. They have dorsal, ascending, and descending processes, and Bone (1960*a*) thought that they also coordinate the activity of the motor neurons, in particular that of the VM cells.

The anterior nerve cord in detail

The region anterior to the first cell of Rohde has attracted less attention in cytological studies than the spinal cord, though the two differ, as noted by Bone (1960*a*), in terms of both their cell types and their general organization. What is clearly lacking is a descriptive study comparable to Bone's comprehensive analysis of the spinal cord, cited so extensively above. The closest is that of Ekhart et al. (2003), but this is focused more on cell grouping, cytoarchitecture, and general morphology, while providing much less information on individual cell types.

Anterior vesicle

The anterior vesicle (Fig. 6) corresponds to the anterior part of the larval cerebral vesicle (= anterior CV; see below and Fig. 8). The anterior vesicle has a central ventricular space (= central canal) but, unlike other regions of the nerve cord, this is relatively wide and lacks the translumenal cell processes found elsewhere in the cord. Dorsally, on the left-hand side, there is a remnant of the anterior neuropore in the form of a ciliated pit. This is Kölliker's pit (Kölliker 1843; Franz 1923), which has been ascribed an olfactory function, though without direct evidence. In fact, the neuropore remains an open channel, even in the adult (Vallet et al. 1985). However, it is so narrow and clogged with cilia that one can question how effectively it acts as a connection between the central canal and the outside. The outer pit lacks obvious receptor cells, nor are there any nerve fibers in evidence to connect it to the anterior vesicle (Edinger 1906; Tjoa and Welsch 1974). This tends to reinforce the conclusion that although Kolliker's pit may contain some specialized cell types, it is not a sense organ.

The caudal end of the anterior vesicle is marked dorsally by the beginning of the axial columns of Joseph cells, as well as scattered lamellar cells, and ventrally by the infundibular organ. The infundibular organ consists of several rows of columnar cells that secrete Reissner's fiber. It is though to correspond to the flexural and subcommissural organs of vertebrates (Obermüller-Wilén 1976; see Nieuwenhuys 1998 for details).

The walls of the anterior vesicle are formed by closely packed, ciliated epithelial cells, among which are the pigment cells of the frontal eye. While this region has been examined in larvae in great detail at the EM level (see below), the only EM study of the adult anterior vesicle is that of Meves (1973), which is much less complete. Meves observed rows of densely stained cells (Fig. 6), immediately ventral and caudal to the pigment cells of the frontal eye, that may correspond to the receptors and neurons of the larval frontal eye. More caudally, in the preinfundibular zone, i.e., between the frontal eye and the infundibular organ, she found two types of cells, the more densely stained of which had basal processes. These may correspond to some of the larval preinfundibular projection neurons described by Lacalli and Kelly (2000), including the cells of the balance organ. These authors found evidence for the latter structure in newly metamorphosed juveniles, so it may persist to the adult stage.

Whether by light microscopy (e.g., Edinger 1906; Franz 1923; Ekhart et al. 2003) or EM (Meves 1973), it is difficult to discern much about the neuronal and glial cells of the anterior vesicle, since most cells are small and rather densely stained and have few visible distinguishing features. Franz (1923, 1927) therefore concluded that the entire anterior vesicle consisted only of glial cells. However, GABAergic (Anadón et al. 1998) and serotoninergic neurons (Moret et al. 2004) have since been identified in this region in adult specimens, and since the same region in the larva is well supplied with neurons, it must continue to function in a neural capacity throughout the animal's life, though what it does in the adult is not evident.

The first nerve of the dorsal nerve series (= nerve 1, rostral nerve) enters the anterior vesicle ventrally at its rostral margin. The nerve's fibers then spread out to form a thin veil of white matter that covers the lateral and ventrolateral aspects of the anterior vesicle. Peripherally, this first pair of nerves traverses the top of the notochord, enclosed within the connective tissue sheath that surrounds the latter. Near the tip of the notochord, the nerves exit the sheath and ramify underneath the skin, so the innervation is very much restricted to just the tip of the rostrum. This is an important point, because if there are any as yet undescribed sensory cell types or subtypes that occur only at the tip of the rostrum, it is very likely that they would enter the cord by means of the first nerve, which is exceptional anyway for not being, strictly speaking, a dorsal nerve, so its CNS targets may also differ from those of the rest of the dorsal nerve series. Among known fibers in the first nerve are axons from peripheral primary sensory cells, mainly type I mechanoreceptors, and centrally derived axons from RB cells and possibly other CNS cells.

The second nerve of the dorsal series (= nerve 2, anterodorsal nerve) enters the CNS dorsally, at the junction

between the anterior vesicle and the IR. Unlike the first nerve, the second is not associated with the notochord, but turns laterally and enters the subepidermal connective tissue. The second nerve ramifies extensively in the more caudal parts of the rostrum, and several anastomoses are formed with the branches of the first nerve (Franz 1923, Fig. 3). The second nerve probably carries fiber types similar to those of the rostral nerves.

The peripheral branches of both the first and the second nerves bear numerous small swellings, the corpuscles of de Quatrefages described above. The centripetal axons derived from these enter the distal branches of both nerves 1 and 2, but it is not known whether they actually enter the cord in both nerves or only one. Some of the carbocyanine dye (DiI) tracing data have been interpreted as indicating that the corpuscles connect to the CNS mainly via nerve 2 (Fritzsch 1996; B. Fritzsch, personal communication), which is intriguing but requires confirmation. The central targets of axons from the corpuscles are unknown.

The intercalated region (IR)

The region between the caudal end of the anterior vesicle and the first giant Rohde cell has sometimes been regarded as a part of the spinal cord (e.g., Franz 1927), but it clearly has unique features that justify its being regarded as a specialized part of the CNS (see also Bone 1960*a*; Fritzsch 1996). The most prominent of such features are the dorsal Joseph cells, present throughout most of this zone, and a conspicuous ventral group of cells, the nucleus of Rohde, in the posterior part of the IR. The IR is not uniform along its length but instead can be subdivided into three parts on cytoarchitectural grounds. The description of these that follows is based mainly on Ekhart et al. (2003) and some more recent unpublished findings (H. Wicht, personal observations).

Anterior IR

The anterior part of the IR is located adjacent to the first myomere (Fig. 6) and probably corresponds to the posterior part of the larval cerebral vesicle. As in more posterior parts of the CNS, the central part of the ventricle is slit-like and traversed by cellular processes from translumenal neurons. Ventrally, a small expansion of the ventricular cavity houses Reissner's fiber. Dorsally, there is another expansion partly filled with processes belonging to lamellar cells (Meves 1973; Ekhart et al. 2003), a form of ciliary photoreceptor (Ruiz and Anadón 1991*b*), though a compact lamellar body like that seen in the larva is no longer present. The dorsal aspect of the anterior part of the IR is capped by the rhabdomeric Joseph cells (Welsch 1968*a*; Ruiz and Anadón 1991*b*, 1991*c*). These increase in number in a rostrocaudal direction. As with the Hesse organs, Joseph cells do not express *Pax6* during development but do contain a rhodopsin-like protein (Watanabe and Yoshida 1986), so they are almost certainly photoreceptors. The ventral and lateral aspects of the anterior IR are covered by a relatively thick layer of white matter.

Neurons and glial cells are easily distinguished in this region. The neuronal cell bodies are mostly located in the periventricular grey close to the central ventricular slit. Compared with neurons in more posterior regions of the CNS, these cells are relatively small. In the ventral part of the periventricular grey, however, just above the ventral expansion of the central canal, there are individual large cells that resemble the somatic motoneurons (SM cells) described by Bone (1960*a*) from spinal cord. Based on the larval data (e.g., Lacalli and Kelly 2003*b*), several classes of ventral interneurons with descending axons would also be expected to reside in this area.

Immunocytochemical studies by Holland and Holland (1993), Uemura et al. (1994), Anadón et al. (1998), Castro et al. (2003), and Moret et al. (2004), and tracing studies by Fritzsch (1996) and Ekhart et al. (2003), all show that the periventricular grey of the anterior IR contains distinctive cell types and groupings besides those evident in standard histological preparations. First, in the periventricular grey, there are numerous translumenal cells whose axons project to the spinal cord (Ekhart et al. 2003). Then, just ventral to the Joseph cells and surrounding the dorsal expansion of the central canal, there are bilateral, longitudinal bands of neurons immunoreactive for urotensin and FMRFamide (Uemura et al. 1994), GABA (Anadón et al. 1998), neuropeptide Y (Castro et al. 2003), and catecholamines (the catecholaminergic population I of Moret et al. (2004)). Axons from some of these catecholamine-containing cells travel anteriorly into the anterior vesicle, but fibers from most of the other cell types are directed ventrally into the lateral and ventral neuropile, where they form a dense commissure or plexus beneath the central canal.

Unlike the situation elsewhere in the CNS, there are groups of neurons in the anterior IR that detach from the ependymal layer and migrate into the white matter. In standard sections of the anterior IR they are seen in the ventral midline, below the ventral expansion of the central canal (the anteroventral migrated (avm) group, Fig. 6B), and bilaterally in the dorsal and lateral parts of the white matter (the anterolateral migrated (alm) group, Fig. 6B; see Ekhart et al. 2003 for details). There is no information on the nature of the avm cells, but recently published data, as well as some personal observations of immunocytochemical preparations of *B. lanceolatum*, have yielded interesting details on the alm cell group. Firstly, the more posterior alm cells (slightly rostral to the junction of myomeres 1 and 2; black squares in Fig. 6) seem to correspond to the anterolateral serotoninergic cells of Holland and Holland (1993) that were also observed by Moret et al. (2004). Slightly more anterior (black circles in Fig. 6) is another group of immunocytochemically identifiable cells within the alm group. This is the catecholaminergic population II of Moret et al. (2004). There is some uncertainty about the exact positions of these two cell groups, however. Moret et al. (2004) place them adjacent to the rostral half of the second myomere. In an independent immunocytochemical study, H. Wicht (unpublished data) localized them more anteriorly, adjacent to myomere 1 and thus within the confines of the alm group (see Fig. 6). Wicht's study did confirm, however, that both the catecholaminergic population II and the anterolateral sertoninergic neurons have long descending projections to the spinal cord. In retrograde tracing experiments, Fritzsch (1996) found pairs of labelled cells in late larvae that may correspond to the anterolateral serotoninergic cells, even though he did not specify their exact position, but Ekhart et al. (2003), in a similar study in adults, did not find such cells. Assuming the

latter result is a false negative, the cells and projections appear to be real; it is only their exact axial position that is a matter of some uncertainty.

Intermediate IR

The intermediate IR (Fig. 6) is located adjacent to myomere 2, i.e., between dorsal nerves 3 and 4. It is characterized by having several layers of Joseph cells. The dorsal expansion of the central canal is lacking in this region, and the canal has instead the shape of an inverted keyhole. The lateral and ventral migrated cell groups that occur in the anterior part of the IR are also absent. Instead, there is another such group (the posterodorsal migrated (pdm) group, Fig. 6C) located just ventral to the Joseph cells. Cells in this group probably correspond to the B cells described by Bone (1959, 1960*a*), which represent the anteriormost cluster of somatosensory RB cells found elsewhere along the nerve cord. A group of small, ventral spindle-shaped cells (vsc in Fig. 6) is found directly beneath the ventral expansion of the central canal. A number of large cells, presumably motoneurons of one kind or another, are found in the ventral part of the periventricular grey, and some of these send descending projections to the spinal cord (Ekhart et al. 2003). There also is a single large VM neuron in the ventral midline just posterior to the ventral spindle-shaped cell group (H. Wicht, unpublished data; see Fig. 6E). Bone (1960*a*) claimed that VM neurons were present only in the spinal cord, but there must be at least enough in the IR to supply dorsal nerves 3–7, which innervate the labial muscles and other buccal structures. In addition, there are some very large translumenal neurons in this region (lac, Fig. 6D) at approximately mid-level in the periventricular grey. They probably correspond to the giant translumenal cells described by Lacalli and Kelly (2003*b*) from late-stage larvae and the largest of the FMRFamide-positive cells of Uemura et al. (1994). Axons from at least some of the large translumenal neurons project to the spinal cord (Ekhart et al. 2003).

The periventricular grey of the intermediate IR also contains neurons positive for GABA (Anadón et al. 1998) and peptides (Uemura et al. 1994), but the pattern differs from that further forward. Specifically, neurons of corresponding type are shifted more ventrally compared with the anterior part of the IR.

The periventricular grey in this region also contains translumenal neurons with descending projections (Ekhart et al. 2003). These decrease in frequency towards the middle and caudal parts of the intermediate IR. From studies on larvae (see below), a major locomotory control center, the primary motor center (PMC), is expected to reside somewhere in this region, beginning roughly at the anterior tip of myomere 2 (Fig. 6, shaded region). There is no obvious adult counterpart to the PMC at this location, but even if the larval neurons persist to the adult, there are relatively few of them and they could easily be overlooked.

Posterior IR

The posterior IR (Fig. 7) is located adjacent to myomeres 3 and 4, between dorsal nerves 4 and 6. Its most conspicuous feature is the nucleus of Rohde (Ekhart et al. 2003, first described by Rohde in 1887). This is an agglomeration of relatively large cells with intensely staining cytoplasm (Figs. 7B, 7D) that surround the ventral expansion of the central canal. The rostrocaudal extent of Rohde's nucleus coincides with that of the columnar epithelium of the wheel organ and Hatschek's pit, located in the roof of the buccal cavity. The tip of Hatschek's pit extends to the side of the notochord and projects towards the base of the CNS at the junction of myomeres 3 and 4 (Fig. 7C). Above, we have discussed the evidence for homology between Hatschek's pit and the vertebrate adenohypophysis. In light of this hypothesis, it is of course tempting to speculate that Rohde's nucleus is the equivalent of the neurosecretory hypothalamic cell groups of vertebrates; however, as in the case of the adenohypophyis, the evidence is so far not very convincing. The intensely staining cytoplasm and the large amounts of Nissl substance within it actually point towards an intense secretory activity; however, none of the neuropeptides typical of vertebrate hypothalamic endocrine cells have been observed within the confines of the nucleus so far. Similarly, nothing is known about the axonal projections of these cells, so it is not clear whether they are the source of the fibers that, from circumstantial evidence, appear to innervate Hatschek's pit (Tjoa and Welsch 1974).

The posterior part of the IR is also where the rostralmost Hesse organs occur, while dorsally the Joseph cells vanish, roughly at the boundary between myomeres 3 and 4. The dorsal part of the central canal displays some isolated, bubble-shaped expansions, but these are filled with fluid rather than cell processes, and their functional significance is not clear.

The posterior IR contains only a few migrated cells. The column of pdm cells that originates farther forward (see Fig. 7) continues through this region into the spinal cord. However, roughly coincident with the last of the Joseph cells, the pdm columns move medially so as to effectively merge with the periventricular grey.

The periventricular grey of the posterior IR, flanking the slit-like part of the central canal, contains numerous translumenal neurons, many of whose axons descend to the spinal cord, including several especially large examples (Ekhart et al. 2003). In the ventral part of the periventricular grey, immediately dorsal to the nucleus of Rohde (Fig. 7), there are a few large neurons of the motoneuron series, some of which have descending projections (Ekhart et al. 2003). Serotonin-containing neurons are absent in this region (Moret et al. 2004), but a relatively large number of GABAergic and peptidergic cells (Uemura et al. 1994; Anadón et al. 1998; Castro et al. 2003) do occur. In addition, there are four relatively large catecholaminergic cells (population III of Moret et al. 2004) with translumenal processes in the vicinity of the roots of the fifth dorsal nerves.

The larval nervous system

Early development in amphioxus resembles that of a typical marine invertebrate: the egg is small, ca. 120 µm in diameter, and hatches after gastrulation as a free-swimming, but nonfeeding, ciliated larva. This stage undergoes neurulation and elongates to produce a more typical chordate-type larva, roughly 1.3 mm in length, with a notochord, somites, dorsal nerve cord, and one pharyngeal

slit, the first of the series. The larvae then feed and grow in the plankton until, after about 30 days under optimal conditions, they metamorphose to juveniles ca. 5 mm long. Excellent surveys of the morphological changes that occur during development have been published by Hirakow and Kajita (1990, 1991, 1994) and Stokes and Holland (1995*a*). Most of the recent larval research has been done on different species than those used for classical anatomical studies of the adult, notably *Branchiostoma floridae* (Hubbs, 1922) in North America and *Branchiostoma belcheri* (Gray, 1847) in Asia. The neuroanatomical differences between species appear minimal, though the differences observed in the extent of the Joseph cell columns (H. Uemura, personal communication) indicate that some caution is required when comparing data across species.

Amphioxus larvae are well known for their asymmetry, the mouth being initially on the left side and the pharyngeal slits on the right. This arrangement necessitates a major repositioning of structures at metamorphosis, especially of the mouth, which shifts caudally over a distance of several somites, and the pharyngeal slits. This is a dramatic process, achieved largely through differential growth, and has attracted a good deal of attention in the past. What is perhaps less appreciated is the magnitude of the size increase that occurs during the larval phase, for both the body as a whole and the internal structures as well. The nerve cord, for example, increases from an initial diameter of 15 μm to ca. 100 μm at metamorphosis. By then, there is substantially more sensory input, so the dorsal roots are larger and the cord has a greater diversity of neuronal cell types and much more neuropile, implying greater integrative capacity. Correlated with this, the animal's behavior becomes much more complex. A great deal of neural development thus takes place during the larval phase, and the nervous system consequently looks quite different, even at a fairly gross anatomical level, depending on the stage examined. Reconstructing the developmental events responsible for generating a characteristic adult neuroanatomy thus requires that a range of stages be examined, spanning a period of weeks, but this has seldom been achieved in practice. Our knowledge of neural development is therefore somewhat fragmentary, and only provisional conclusions can be drawn in many instances.

The first detailed description of larval neuroanatomy was that of Bone (1959), who made the prescient remark that larvae are likely to be more revealing about phylogenetic issues than the adult. Bone's study, and much subsequent larval work, concentrated on rather late stages, since these are better subjects for most staining techniques and are easier to handle than young larvae. The young stages are nevertheless the most important ones in terms of understanding early patterns of neural differentiation and tract formation and for comparison with the rapidly increasing body of gene expression data (Holland and Holland 1999; Shimeld and Holland 2005). Recent relevant work on young larvae includes studies of peripheral innervation by means of whole mount immunostaining and DiI tracing (Yasui et al. 1998; Kaji et al. 2001; Holland and Yu 2002), as well as a detailed study of the internal microanatomy of the anterior nerve cord at the EM level, using serial sections and three-dimensional reconstruction (e.g., Lacalli et al. 1994; Lacalli 1996, 2002*a*, 2002*b*; Lacalli and Kelly 1999, 2000, 2003*a*, 2003*b*). The latter study, in particular, has revealed much new information of interest, from both a neuroanatomical and an evolutionary perspective, and is the main focus of the account that follows.

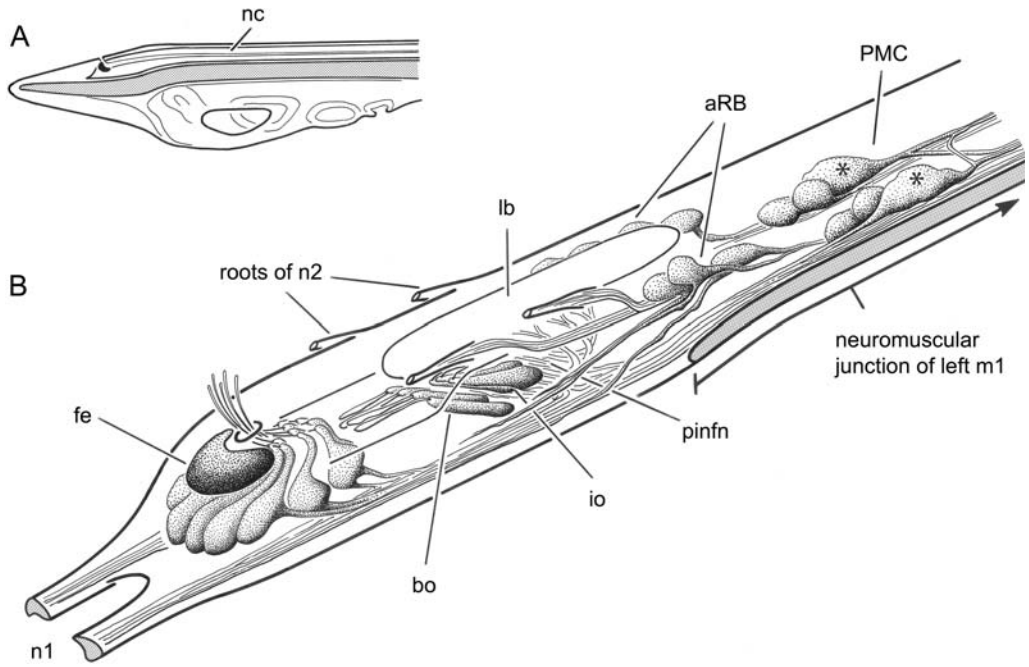
Anterior nerve cord

Several distinctive subdomains can be recognized in the anterior nerve cord. Externally, a slightly bulbous anterior zone, the cerebral vesicle (CV), is easily distinguished from the rest of the cord. The transition occurs slightly forward of the boundary between somites 1 and 2. Internally, however, the main anatomical landmark is the transition in the shape of the central canal of the cord that occurs at a level coincident with the ventral cluster of infundibular cells (Lacalli et al. 1994; Lacalli 1996; see Fig. 8). Forward of this point is the anterior CV, essentially the larval equivalent of the adult anterior vesicle. Here the central canal is cylindrical and lacks a floor plate, and most cilia project forward, some even escaping out the neuropore. The principal landmarks of the anterior CV are the frontal eye, located at the anterior tip of the cord, and a putative balance organ (Lacalli and Kelly 2000), positioned just in front of the infundibular cells. Most of the neurons are ventral, columnar, and closely packed, especially in the immediate preinfundibular region, and have caudally projecting neurites.

The posterior part of the CV (probably corresponding to the anterior part of the IR of adults) begins at the infundibular cells. The floor plate begins here, the central canal narrows to a slit shaped like an inverted keyhole, so that only the ventral portion remains open, and the cilia of the ependyma and floor plate project backwards. The dorsal part of the cord in this zone is occupied by an ovoid mass of ciliary lamellae, which together constitute the lamellar body. The corresponding ventral part of the cord is taken up first by a postinfundibular (= tegmental) neuropile and, behind this, the primary motor center (PMC), containing the anteriormost motoneurons and sets of interneurons with caudal projections that are involved in locomotory control (Lacalli 1996; Lacalli and Kelly 2003*b*). The transition from this region to a type of organization more typical of the rest of the nerve cord, whatever that entails, appears to be a gradual one, with no obvious landmarks (e.g., no Rohde cells) to indicate where it occurs. However, the caudal limit of the lamellar body, which extends almost to the boundary between somites 1 and 2 in late-stage larvae, and the similar extent of *Otx* expression suggest that there is something distinctive about the nerve cord to about the level of the boundary between somites 1 and 2 or slightly beyond.

Despite the usefulness of the infundibular cells as anatomical markers, there is no obvious transition in terms of neuronal cell type at this point. Instead, cells of essentially anterior character are found from the preinfundibular region to the beginning of the PMC. "Anterior" here refers to cells with irregular basal neurites that form repeated varicosities containing mixed vesicle types and few, if any, synapses. These are features that are generally associated with slow transmission, often involving neuropeptides (Burns and Augustine 1995). Beginning in the PMC, most of the neurons have well-defined axons and separate dendritic structures, either arbors or spines (both occur), and synaptic junctions, often with clear vesicles, predominate. This implies fast

Fig. 8. (A) Side view of the head of an amphioxus larva showing the position of the nerve cord (nc) in relation to the notochord (shaded). The head is highly asymmetric, with the mouth (heavy outline) on the left side and the pharyngeal (gill) slits (lighter outlines) on the right. (B) Oblique dorsal view of the anterior nerve cord showing its main landmarks and selected cells. Asterisks indicate the third pair of large paired neurons (LPN3s), which are putative locomotory pacemaker neurons. Landmarks include the frontal eye (fe), infundibular organ (io), lamellar body (lb), and primary motor center (PMC); the zone of neuromuscular junctions is shaded. This view extends to just beyond the boundary between somites 1 and 2, roughly the extent of the cerebral vesicle as anatomically defined and coextensive with the zone of *Otx* expression. The io marks the junction between the anterior and posterior parts of the cerebral vesicle. The two regions differ in the shape of the central canal and the direction in which most cilia project into it. Other abbreviations and their corresponding terms are listed in Appendix A. See text for further details. Modified from Lacalli (1996).



transmission and aminergic or amino acid transmitters, which is perhaps logical for neurons directly involved in the locomotory control circuits.

The very fact that distinctive regions can be recognized within the anterior cord, however this is defined anatomically, raises the question of how the subdivision of the anterior cord is controlled at the molecular level. The question is addressed in detail elsewhere in this symposium (Shimeld and Holland 2005), but a few remarks are useful here. Genes such as *Otx*, expressed throughout most of the CV, presumably define the character of that zone by some means, though how is still poorly understood. But this does not explain how specific structures within each zone are specified; for example, the frontal eye and infundibular cells. Signalling from boundaries is one possibility, and the anterior boundary of the neural plate (ANB) is a good candidate, since it is now recognized as an important signalling center in vertebrates (Grove 2002). A similar role for the ANB in amphioxus would help explain the organization of the frontal eye, in which cells of like type are precisely oriented in the transverse plane, parallel to the ANB. The infundibular region is a second possible candidate, not least because cells of similar type, including subtypes among the various preinfundibular, parainfundibular, and tegmental neurons, occur at roughly equal distances in both directions from this site. Any gene expressed early in a pattern centered in this region is then of potential interest. *FoxD* is one such gene (Yu et al. 2002), and further research may turn up more, since we are still at a very early stage in understanding how regional subdivision of amphioxus CNS is controlled.

Frontal eye

The frontal eye consists of a pigment cup, oriented so it opens dorsally, and four rows of neurons. The first two rows consist of simple sensory neurons, 6 in the first row and 10 in the second, with cilia that project out the neuropore, and basal axons. The two rows differ in terms of the extent and type of their varicosities, but both project to the ventrolateral tracts and continue through the anterior CV, but probably not much further. Their close association with the pigment cup indicates that these cells are probably photoreceptors, though this has not been tested experimentally. Behind the putative photoreceptors are the other two rows of neurons. The first of these rows (row 3) consists of 6 cells with multiple processes, typically short, by means of which the cells form multiple points of contact with each other. In the fourth row, only the two most medial neurons show a close association with the frontal eye. They have basal neurites that communicate synaptically via two routes (one anterior, one at the level of the postinfundibular neuropile) with the dendrites of the third pair of large paired neurons (LPN3 cells), which are key components of the locomotory control center (see below). This arrangement of photoreceptors and neurons has been compared to the vertebrate retina by Lacalli (1996), who suggested possible homology between cells in rows 3 and 4 and retinal amacrine and bipolar cells, respectively. A

further point of similarity between the frontal eye and the paired eyes of vertebrates is that both develop at the anterior margin of the neural plate in what is essentially a ventromedial position. Also, projections in both cases are to regions caudal to the infundibulum, to roughly midbrain level. The argument for homology is thus reasonably strong. However, while the vertebrate retina has a two-dimensional array of photoreceptors, cells in the frontal eye form strictly one-dimensional files, and there is no evidence that this is a secondarily degenerate condition, i.e., that amphioxus ever had an image-forming eye.

Behavioral experiments show that amphioxus larvae can orient to light while suspended and feeding at the water surface, probably by modulating ciliary beat on the body surface (Stokes and Holland 1995*b*). The frontal eye is implicated in this, though an appropriate neurociliary effector pathway has yet to be demonstrated. As the larva grows, the pigment spot enlarges somewhat, but the complement of photoreceptors and neurons appears to change very little. The function of the frontal eye in the adult is not known. Further information on this and other amphioxus photoreceptor systems can be found in Ruppert (1997) and Lacalli (2004).

Infundibular region

The infundibular cells are secretory cells rather than neurons, but they lie within a ventral mass of about 80 closely packed neurons that resemble primary sensory cells. There are a variety of subtypes among these. The most distinctive (14 cells) have expanded, club-shaped cilia, which suggests they may function to detect displacement, i.e., as a balance organ (Lacalli and Kelly 2000). Like axons of many of the surrounding cells, axons from this putative balance organ project to the postinfundibular neuropile and terminate in large varicosities. Among the other neuronal subtypes in this region are three classes of preinfundibular projection neurons (PPN1–3) with mixed clear and dense-core vesicles and comparatively short axons (Lacalli and Kelly 2003*b*); four cells, the PPN2s, have clear vesicles and axons that travel at least to somite 7 and possibly farther (Lacalli 2002*a*). In general, from the paucity of synaptic specializations within the postinfundibular neuropile, it appears that paracrine release is the predominant mode of transmission, suggesting that this region is mainly a modulatory center.

The lamellar body is the second major contributor to the postinfundibular neuropile. Each of its cells has a single large axon that travels down the side of the cord to the neuropile, where a tangled mass of subsidiary branches is formed (Lacalli et al. 1994). The lamellar body is generally accepted as a homolog of the vertebrate pineal organ, which suggests that either the cells themselves or their downstream targets in the neuropile generate a circadian rhythm. There is no direct experimental evidence for such rhythms in either adults or larvae, but the larvae have diurnal patterns of vertical migration in the plankton under some conditions (Wickstead and Bone 1959), which implies the presence of a circadian clock.

Primary motor center

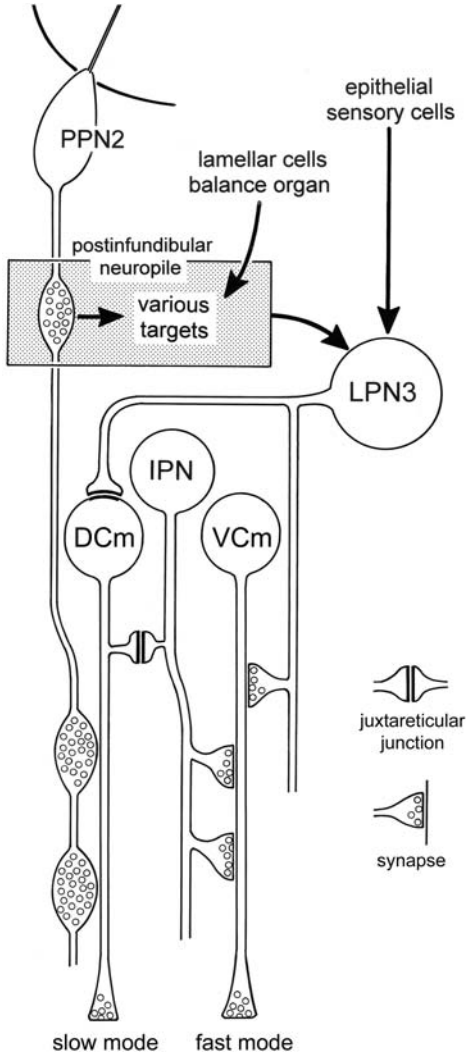
The PMC contains the anteriormost motoneurons in the cord and a number of large premotor interneurons. These cell types occur elsewhere in the cord, but not in such a large cluster. The important cells, from an organizational standpoint, are three pairs of large paired neurons (LPNs). These are extensively innervated by sensory inputs, both directly by primary sensory cells in the periphery and by synapses from the anteriormost RB cells (= B cells of Bone 1959, aRB cells in Fig. 8). The third pair, the LPN3s, are the most important and are cross-innervated in a bilaterally symmetrical fashion, an indication that they may be mutually inhibitory and hence capable of pacemaker function (Lacalli 1996; Lacalli and Kelly 2003*b*). Their output is to ventral compartment (VC, or fast) motoneurons via synapses and to DC (slow) motoneurons via an unusual class of intercellular junctions (juxtareticular junctions; see Lacalli 2002*a*).

The LPN3s are thus the best candidates for neurons exerting a direct controlling influence over both fast and slow swimming, which appear to have a similar neuromuscular basis in amphioxus and vertebrates (Bone 1989). Fast or escape swimming occurs in response to sensory inputs, which are a massive and redundant input to the VC system. The VC system also receives synaptic input from fibers in the postinfundibular neuropile and may be subject to additional paracrine input as well, via fibers passing through the neuropile, all of which provides an opportunity to modulate the response to sensory stimuli. In contrast, the slow system, which drives vertical migration, is almost devoid of synaptic input. Besides its link via junctions to the LPN3s, this pathway seems to be mainly under the control of the PPN2s mentioned above, a class of preinfundibular projection neurons that make repeated junctional contacts with the axons of the DC motoneurons. What this means in functional terms is not clear, but the circuitry (Fig. 9) suggests a switching device of some kind. Perhaps the escape response is suppressed during migration, which might itself be under circadian control. To assess such proposals, however, much more information is needed on the nature of the various types of preinfundibular neurons than is currently available.

VC motoneurons in larvae resemble the somatic motoneurons (SM cells) reported from the adult in overall morphology (cf. Lacalli and Kelly 1999; Bone 1959, 1960*a*). They are distributed rather irregularly in the anterior cord, with roughly equal numbers on each of the two sides, but there is no sign of bilateral pairing. They receive synapses on dendritic spines of varying length, located all along the axon, which confirms the supposition that the thin collaterals reported from adult motoneurons (Bone 1960*a*; Castro et al. 2004) are dendrites. It is useful to note that as the cord grows, and its neuropile expands, early dendrites would have to lengthen to maintain their original connections. Spines in the adult cord will thus be longer than those in the larval cord, and the longest spines are the earliest, and presumably most important, functional connections. Since the longest spines in the larva are postsynaptic to LPNs, this interpretation supports the central role proposed for these cells in initiating swimming.

It is not known whether the larval motoneurons persist through to the adult stage or whether the larval cells are replaced at some point in development. Lacalli (2000) has argued for the former, based on the measured lengths of the motoneuron apices. These are axially elongated by an amount that roughly matches the axial expansion of the somites during development.

Fig. 9. Schematic diagram of the main locomotory control circuits in the anterior cord in young amphioxus larvae. The LPN3s (third pair of large paired neurons) are central control neurons that probably act as pacemakers. They receive external sensory inputs via several pathways and communicate with the two classes of motoneurons (DCm and VCm) by synapses or junctions as shown. There is extensive additional synaptic input (not shown) to the VC (fast) system, but almost none to the DC (slow) system except for junctions with a single class of preinfundibular neurons (type 2 preinfundibular projection neurons, PPN2s) and, more caudally (not shown), input from the dorsal ocelli. The function of the ipsilateral projection neurons (IPNs) is not clear, but they appear to provide some kind of link between the two systems. The postinfundibular (= tegmental) neuropile is a paracrine center, and the specific interactions among its components are not clear from the morphological data. Modified from Lacalli (2002*a*).



DC motoneurons differ from VC motoneurons in being restricted to the anterior part of the cord, specifically somites 2–6. This restriction was first inferred from EM data, which showed that while axons project both rostrally and caudally from the last two members of the series, located in somites 4 and 5, none travel forward from more caudal segments (Lacalli and Kelly 1999). Confirming this, the amphioxus homolog of the estrogen-related receptor gene (*ERR*) selectively marks the same cells, revealing six pairs in the anterior somites and none more caudally (Bardet et al. 2005). Various molecular data support the idea of a segmental or otherwise periodic repeat in the arrangement of cell types in the cord at the level of somites 2–7 (Jackman and Kimmel 2002; Mazet and Shimeld 2002), which is essentially the amphioxus homolog of the hindbrain. The DC motoneurons evidently form a compressed series, with more than one pair per segment. The true nature of patterning in this part of the anterior cord is still, therefore, not clear. It may be that some cell types show a strictly repeating segmental pattern, while others are more loosely controlled, or there may be several quasi-segmental patterns superimposed over one another. See Shimeld and Holland (2005) for further discussion.

In contrast to the detailed information now published on the microanatomy of the anterior cord, nothing comparable is yet available for more caudal regions. Swimming behavior changes as the larva grows, from a phased side-to-side bending of the whole body in very young stages to what looks like a propagated wave of contractions (Stokes 1997). The latter implies a locomotory signal propagated from segment to segment, more like the situation in vertebrates. One interpretation is that the pacemaker circuits identified in the anterior cord of young larvae are involved in initiating locomotory contractions, but these are probably propagated through the more caudal segments by a series of local pacemakers. Regardless of details, it seems clear that the control circuits described from the anterior cord of young larvae cannot account fully for the complexity and dynamics of behavior in older larvae.

Peripheral sensory cells and nerves

This section is brief, as a recent review by Lacalli (2004) covers most aspects of the larval sensory system and includes a summary of what is known of the early circuitry. As in the adult, the surface epithelium in the larva is supplied with sensory cells of various types. The first evident functional response of the larva is to mechanical stimulation, and this correlates with the early appearance of primary type I sensory neurons in the rostrum and tail. Axons from these enter the cord at each end and travel long distances within it (Holland and Yu 2002), usually in the ventrolateral tracts, where they make repeated synapses with ventral interneurons involved in locomotory control (Lacalli 2002*b*, 2004). Those located at the tip of the rostrum enter the cord via the paired rostral nerves, which are substantial (ca. 25–30 fibers) at a time when the dorsal nerves consist of, at most, a few fibers. As the larva grows, primary sensory neurons differentiate over much of the body surface (Stokes and Holland 1995*a*; Holland and Yu 2002), and the dorsal roots become much larger as their fibers grow into the cord. In contrast to the rostral and caudal fibers, those entering via dorsal nerves pass into the expanding dorsal tract, which, by the late larval phase, has subdivided along most of the length of the cord into separate dorsal and subdorsal tracts that run in parallel (Holland and Yu 2002).

Other neuronal cell types identified in the epidermal tissues are (*i*) structures in the rostrum at the neurula stage identified as growth cones by Yasui et al. (1998) because of

their apparently transitory nature, but which may be (see Lacalli 2003*a*) cell bodies of pioneering rostral neurons that differentiate early; (*ii*) neurons associated with the various peripheral plexuses that synapse peripherally, including two types (intrinsic and extrinsic neurons) in the oral nerve plexus (Lacalli et al. 1999); (*iii*) additional, more specialized, type I sensory cells, including a variant with a modified, spine-like cilium (Lacalli and Hou 1999); (*iv*) type II sensory neurons, putative chemoreceptors with a collar of branched microvilli and basal synapses to peripheral nerves, which develop as the larva matures (Stokes and Holland 1995*a*; Lacalli and Hou 1999); and (*v*) ventral pit cells, which lie in rows along the developing metapleural folds (Stokes and Holland 1995*a*) and are present also in late larvae, though the evidence that these are neurons is equivocal. Further research is likely to reveal additional types and define subtypes among those already described. The above conclusion regarding the adult — that the overall organization of the peripheral system is complex and its function is poorly understood — applies equally to the larva.

Postembryonic growth: generating an adult nerve cord from the larval one

As the amphioxus larva grows, the various fiber tracts and regions of neuropile increase in size, expanding the cord both ventrally and laterally. The addition of new neurons, however, occurs mainly through proliferation and differentiation of the dorsal two thirds of the ventricular layer, a region chiefly occupied by the intermediate zone (not to be confused with other uses of this term in reference to subdivisions of the nerve cord along its longitudinal axis), which, in the transverse plane, is defined by the presence of populations of various types of translumenal neurons (Fig. 10; see Lacalli 2002*b*). In some cases the translumenal processes are large enough to bridge to the neuropile on the opposite side, but more commonly they are small and only just cross the central canal. The largest belong to the larval giant cells, of which there are five forward of the first Rohde cell (Bone 1959); these probably correspond to the large translumenal neurons described above from the adult IR. The larval cells have ipsilateral axons but otherwise resemble Rohde cells, which have medial or contralateral axons. Bone (1960*a*) suggests an internuncial function for cells of this general type, and tracings of some of the more abundant small translumenal neurons in larvae tend to confirm this (Lacalli and Kelly 2003*a*). The development of the intermediate zone, in fact, parallels the increase in peripheral input via dorsal nerve roots, which itself is a consequence of the increase in the surface area of the body and the number of peripheral sensory cells. Young larvae have very simple sensorimotor circuits, but it is unlikely that these would cope well with vastly increased input, presumably excitatory in nature, if it is not filtered or modulated in some way. This is the problem of gain control, which is common to developing neural systems (e.g., see Priebe and Ferster 2002). The additional level of processing inserted during larval development between the sensory input and the motor output is evidently a way of solving this problem.

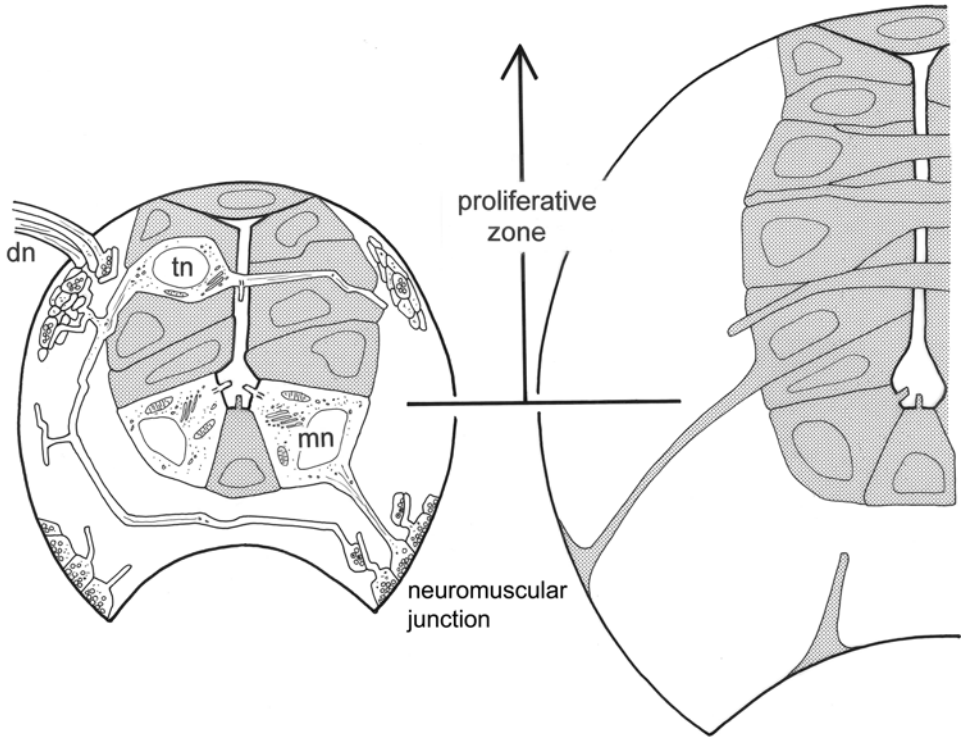
Except for the addition of radial glial cells with fibers that bridge to the top of the notochord (e.g., as in Fig. 4), the ventral part of the ependymal region, where the central canal remains open, changes very little throughout the larval phase (Lacalli and Kelly 2002). The progressive increase in radial glial cell numbers does appear to have a consequence, however, because the apices of motoneurons in the juvenile and the adult open into the central canal more dorsally than in the early larva, i.e., slightly above the ventral expansion rather than adjacent to the floor plate. Unless there is a wholesale replacement of larval neurons by juvenile and adult ones, which seems unlikely, this is best explained by a passive displacement of early neurons up the sides of the central canal as more ventrally positioned glial precursors proliferate.

In summary, the neurons in the amphioxus nerve cord appear to differentiate along most of the cord's length in a ventral to dorsal sequence. This can be interpreted in various ways. If ontogeny were, in fact, no more than a recapitulation of phylogeny, it would mean that the ventral locomotory circuits were evolutionarily older than the dorsal modulatory ones. While it is certainly true that some dorsal neurons are highly specialized, and may well be late-evolving cell types, this can be true of all dorsal cells only if there was an ancestral form that swam but lacked any way of modulating its locomotion in response to peripheral sensory input. This is highly unlikely. A better explanation is that the hatching stage in amphioxus has been secondarily reduced in size, to at least some degree, during evolution. As the hatching larva evolved ways to utilize the resources of the egg most effectively, the differentiation of the essential parts of the locomotory circuits was accelerated at the expense of everything else. In this interpretation, the early differentiation of the ventral circuits is a clear indication of their crucial importance to the hatching larva, whereas the dorsal modulatory pathways are evidently less important, such that their development can be delayed.

There are a number of specialized cell groupings at the anterior end of the adult nerve cord that are not present in early larvae, including various types of migrated cells described above from the adult IR. Judging from the time that the anterolateral serotoninergic cells first appear (Holland and Holland 1993), these cell groupings probably develop in the late larval phase or during metamorphosis. Despite the proliferative activity this entails, the anteriormost region fails to thicken as much as the rest of the cord, so the CV progressively disappears as an externally recognizable zone. Of the late-developing cell groups, the dorsal (population I) dopamine-containing cells reported by Moret et al. (2004) are especially noteworthy. These are as dorsal and anterior as one can get in the nerve cord, which is precisely where a telencephalic homolog would be predicted to form if amphioxus had one. For this and other reasons, Lacalli (2004) suggested that the population I cells may represent a primitive version of the olfactory bulb. Regardless of whether this is eventually confirmed, the key point is to recognize that these cells, like the Joseph cells and other anterior migrated cell clusters, are all late-developing centers that probably act in a modulatory way on established circuits. It should be possible, in principle, to determine what functions such cells perform by correlating their time of appearance during development with changes in behavior. This could prove a useful experimental strategy in future.

The existence of migrated cell groups of various types in

Fig. 10. The nerve cord in transverse section, at about the level of somite 2, in early- and late-stage amphioxus larvae. The locomotory control system in young larvae (left) depends on sets of motoneurons (mn) and caudally projecting interneurons, all of which are ventral, adjacent to the floor plate. Later development (right) mainly involves proliferation and expansion of the intermediate and dorsal parts of the cord, which results in increasing numbers of interneurons (tn, since most have translumenal processes) and complex circuitry as increasing numbers of peripheral sensory fibers enter the cord via dorsal nerves (dn). Thus, there is a clear directional bias in the way the cord differentiates: ventral first, dorsal later. Modified from Lacalli (2004).

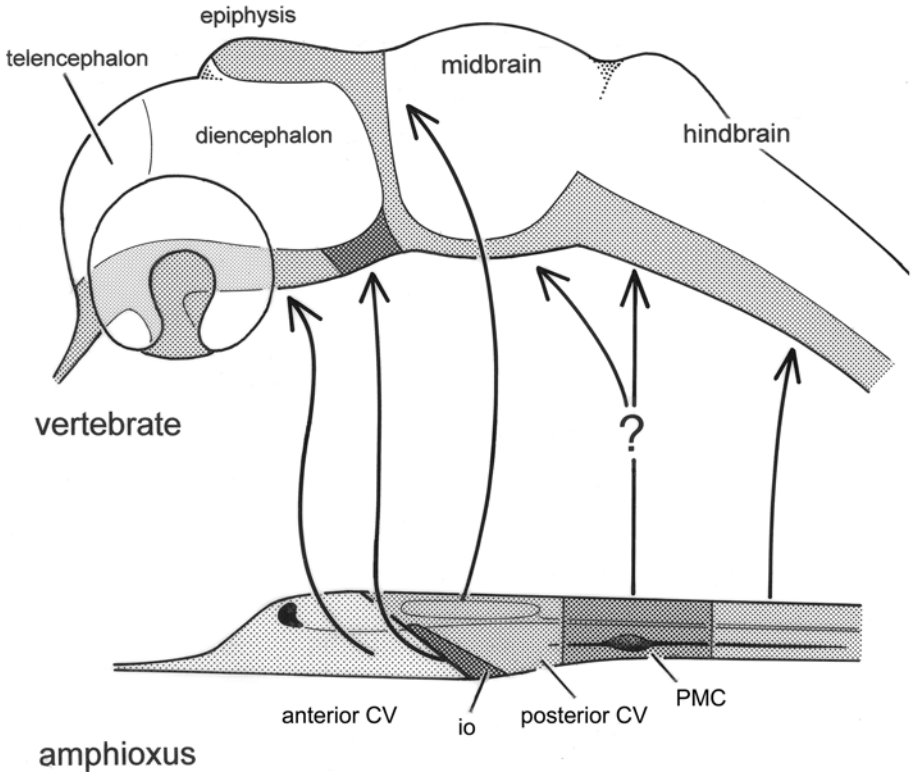


the anterior nerve cord raises an interpretive problem that deserves some attention. Elsewhere in the cord, the majority of neurons remain attached to the ventricular surface, so their site of origin with reference to the dorsoventral axis of the nerve cord is clear by inspection. Where this is not the case, e.g., for the anterior migrated cell groups, the final position of the cells with respect to the dorsoventral axis, whether they reside close to the periventricular layer or deep in the neuropile, may be secondarily altered. This can happen in two ways: either the cells themselves migrate dorsoventrally or the expanding ventricular layer leaves them behind. Thus, a migrated cell or group of such cells might be quite dorsal in terms of its point of origin in the ventricular layer, but could ultimately occupy a position well below the dorsalmost cells of the mature nerve cord. The time element in development therefore needs to be explicitly considered in such cases. This is an important issue, particularly in cases where one is trying to identify possible homologs of vertebrate CNS neurons, as we do in the section below. For example, in earlier attempts to deal with the different subcategories of RB cells (e.g., Lacalli 1996), the term "tectal cell" was applied to the anterior group (aRB cells in Fig. 8, equivalent to the A cells of Bone 1959). The term was chosen in part, and perhaps unfortunately, because it seemed that the dorsalmost cells in a region judged to be midbrain-like on the basis of molecular criteria could, in the course of evolution, have generated major new dorsal structures, including the optic tectum. However, cells in the dorsal part of the amphioxus ventricular layer in young larvae have only just begun to proliferate and generate neurons, so it is not obvious whether the late progeny of these cells are more or less dorsal in character than early ones. How this all relates to the appearance of entirely new dorsal structures and brain regions during the early stages of vertebrate evolution remains to be determined.

Comparison with vertebrates

Amphioxus is now generally accepted as the best available model for the immediate invertebrate ancestor of vertebrates (Holland 2000). As such, it is of key importance to investigations into vertebrate origins and characteristic features of vertebrate organization. The PNS, in particular, has been the subject of much comparative analysis in the past. Amphioxus differs from vertebrates in its reliance on peripherally derived sensory neurons and extensive peripheral plexuses, which are largely replaced in vertebrates by products of the neural crest and placodes. The reason for this transition and the relation (i.e., possible homology) between the component cell types is currently a matter of some interest, but many key questions remain unanswered (Lacalli 2004). At the anatomical level, it is the arrangement and innervation patterns of the dorsal nerves that have received the most attention, mainly because of the clues these provide concerning the segmental structure of the vertebrate head. The key issue here is the relation between the serially repeating units in the hindbrain (i.e., rhombomeres), pharynx (gills and gill arches), and paraxial mesoderm, and how

Fig. 11. A proposal, consistent with both anatomical and molecular data, for how the anterior nerve cord in amphioxus larvae maps to the vertebrate brain. Most of the former finds its closest counterpart, and probable homologs, in the ventral region (variously shaded) of the latter, with the exception of the lamellar body, a pineal homolog. Caudal to somite 2, the amphioxus nerve cord becomes more hindbrain-like in character, according to the molecular data, but the exact point of the transition is uncertain (indicated by the question mark), as there is no obvious corresponding anatomical transition. Abbreviations and their corresponding terms are listed in Appendix A. Modified from Lacalli (1996).

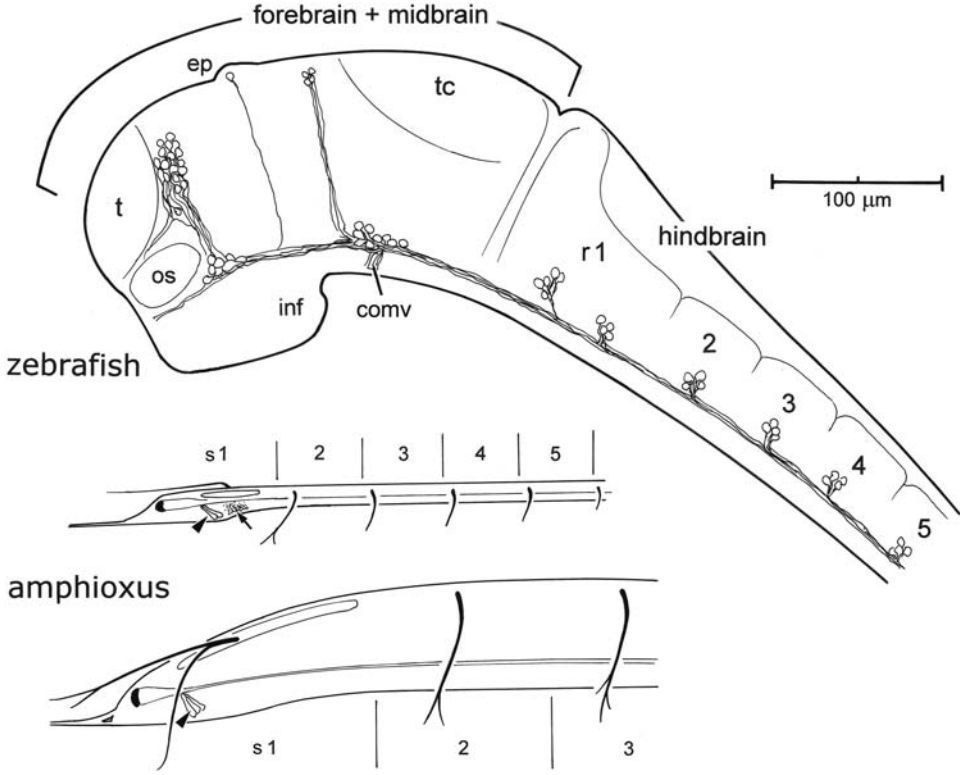


these all relate to the somite series. Do they, for example, reflect a single underlying pattern of repeats, or are they independent patterns secondarily superimposed on one another? To date, the contribution of amphioxus to this debate has proven less than enlightening (e.g., see Northcutt 2001), perhaps because patterns of peripheral innervation are less conservative than one would ideally like. In addition, however, the peculiarities of larval growth and metamorphosis in amphioxus, especially the caudal shift of the mouth and oral apparatus, ensure that the spatial relation between the nerve cord and peripheral structures in the head is not only different than that in vertebrates, but also undergoes developmental changes that have no vertebrate counterpart.

More recently, the wealth of data on patterns of gene expression during development has renewed efforts to identify regional homologies between the vertebrate brain and the anterior nerve cord of amphioxus. Here the molecular and anatomical data are largely in agreement, and a comparatively consistent story is emerging. The presence of a pineal homolog (the lamellar body) and a comparable infundibular region has long been accepted as evidence that the CV is basically a primitive counterpart of the diencephalon (Olsson 1986). Patterns of *Otx*, *FoxB*, and *Hox* gene expression indicate the presence of regions homologous with the forebrain + midbrain to about the level of the boundary between somites 1 and 2 (Holland and Holland 1999; Shimeld and Holland 2005). This places the PMC, with its pacemaker neurons, at roughly midbrain level. Then, somewhere adjacent to somite 2, a zone begins that is hindbrain-like in character, in which gene expression occurs in a segmental or quasi-segmental pattern of repeats (Jackman and Kimmel 2002; Mazet and Shimeld 2002). The microanatomy shows, however, that except for the lamellar body, it is only the ventral structures that are represented in the larval brain of amphioxus (Fig. 11). There are zones similar in organization to the ventral diencephalon, extending from the preoptic area to the hypothalamus and infundibulum, the floor of the midbrain, roughly equivalent to the tegmentum, and the anterior end of the reticulospinal system. Further, the arrangement of longitudinal fiber tracts is similar in vertebrates and amphioxus larvae (cf. Hjorth and Key 2002; Lacalli et al. 1994), and both develop an early ventral connection between the two sides of the cord in the immediate postinfundibular region, namely the ventral commissure in vertebrates and the postinfundibular neuropile in amphioxus larvae.

In addition, what is so far inferred concerning the functions performed in the anterior nerve cord in amphioxus larvae also indicates similarities to the vertebrate brain. Basically, a variety of modulatory inputs, including signals from sensory neurons located in the hypothalamus or its amphioxus equivalent, converge on a ventral locomotory control center (tegmentum and reticulospinal system in vertebrates, PMC in amphioxus), and this initiates a locomotory response. The ancestral plan one infers from this would thus have a series of basal centers and connecting tracts that activate swimming under the control of external sensory inputs, as well as

Fig. 12. Differences in scale between the anterior nerve cord of amphioxus at two stages (14-day-old larva, middle diagram, and newly metamorphosed juvenile, bottom diagram) and an embryonic vertebrate brain (zebrafish at 24 h, top diagram). For the zebrafish, cell clusters involved in early tract formation are shown. Arrowheads indicate the locations of the infundibular cells in amphioxus; the small arrow points to the postinfundibular (tegmental) neuropile. Based on a previous analysis of size differences; see Lacalli (2004). See text for discussion and see Appendix A for abbreviations and their corresponding terms.



internal homeostatic signals, and would incorporate a switching center to coordinate escape swimming with other basic activities, e.g., migratory swimming and feeding.

In contrast, there is no evidence in amphioxus for homologs of any of the dorsal brain structures involved in vertebrate sensory processing except for the pineal organ. A second possible exception is the olfactory bulb, which Lacalli (2004) has argued may be represented in rudimentary form in the advanced-stage larva of amphioxus (and presumably also the adult), but this remains to be proven. The significance of the Joseph cells, if any, in relation to dorsal visual centers in vertebrates, remains unresolved. The various major centers involved in processing inputs from the vertebrate organs of special sense therefore appear to be absent in amphioxus, again with the possible exception of the pineal organ and olfactory bulb. This is presumably because the sense organs themselves, and the corresponding CNS processing centers, evolved as vertebrates evolved, after their divergence from more basal chordate lineages.

A more detailed look at the nerve cord reveals that the arrangement of cell types across the dorsoventral axis has a number of recognizably vertebrate-like features: there is a floor plate, and motoneurons are ventral, while sensory interneurons are dorsal. There are also enough similarities between the RB cells in amphioxus and the Rohon-Beard cells of vertebrates to suggest homology (Fritzsch and Northcutt 1993). The anatomical evidence for a relation between Rohde cells and the various types of reticulospinal giant cells found in vertebrates is somewhat less convincing, however. Dorsal expansion of the cord is a noticeable feature of CNS development during the late larval phase; in contrast, the ventral components of the cord probably change very little. This reflects the need for a vastly increased capacity for sensory integration as the larva grows and approaches metamorphosis. Comparable events in vertebrate development occur in the embryo, when proliferation expands the cord dorsally and somatosensory reflex circuits complete their differentiation. These changes correlate with increased egg and embryo size and a prolonged period of embryogenesis in vertebrates, which allows for a much larger and better developed nervous system at hatching (see Fig. 12), possibly in response to the increased predatory pressures vertebrates (and their hatchlings) experienced during their early evolution. This is an important point, because it adds a developmental dimension to previous ideas about CNS evolution. Though many of the events of neurogenesis in vertebrate embryos have probably always been embryonic, it is likely that there are others that evolved as additions to an already functioning CNS in an active animal. These would have been incorporated into the embryo only secondarily, so their mechanism of formation and the way in which the existing circuitry accommodates new inputs should reflect this in some way.

By way of example, consider the development in amphioxus of midbrain-level dopaminergic and serotoninergic neurons reported by Moret et al. (2004). Neurons utilizing these transmitters are present at a roughly comparable location in vertebrates and contribute to several functionally important

modulatory systems. Why did dopaminergic neurons evolve in this particular location, and what can one infer about their original function? If they were not originally formed in the embryo, at what point in the life history did they first appear, and why? These questions are raised here not because we have answers, but to illustrate the following general point: when constructing evolutionary scenarios, one always needs to be thinking in terms of a sequence of changing life histories. From our analysis, this will be especially true in the case of innovations in CNS organization and structures that develop comparatively late in embryogenesis.

In the more immediate term, future work on the amphioxus nervous system might usefully be directed at refining our understanding of the molecular differences between neuronal subclasses, in terms of both gene expression patterns and neurotransmitters. Work of this type is now in progress in a number of laboratories, but much remains to be done. The amphioxus CNS is well suited for such studies because it is small and compact enough that a thorough inventory of cell types is a feasible objective in the larva at least, if not in the adult. A disadvantage is that many aspects of amphioxus neural organization are so peculiar that the conventional wisdom as to how a neural system ought to operate is sometimes more a hindrance than a help. On the evolutionary side, there are questions that can be addressed concerning the cell types and circuits in the ventral part of the anterior nerve cord, and here there may be direct homologs in the ventral brainstem centers of vertebrates. In contrast, amphioxus will likely provide very little in the way of useful clues regarding the origin and basic cell types of dorsal structures in the vertebrate brain, as these evidently evolved largely after lancelets and vertebrates diverged. A final point, especially relevant to the subject of this issue, is the relation to hemichordates. Here, amphioxus offers a possible bridge between the highly centralized system of vertebrates and the diffuse one of hemichordates (Lacalli 2003*b*). One would hope to find intimations of the latter in amphioxus sufficient to indicate whether the hemichordate system is derived or whether it retains at least some important ancestral features.

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Appendix A

List of abbreviations used in figures.

AC	Anadón's GABAergic cells
acf	atriocoelomic funnels
alm	anterolateral migrated cell group
ansm	anal sphincter muscle
antRo	anterior Rohde cell
antRoax	anterior descending Rohde axons
ap	atrial papillae
aRB	anterior Retzius bipolar cells
atsm	atriopore sphincter muscle
avm	anteroventral migrated cell group
bc	buccal cavity
bo	balance organ
cdQ	corpuscles of de Quatrefages
chd	notochord
cJo	Joseph cells
cJohn	Johnston cell
CNS	central nervous system
comv	ventral commissure
cRo	Rohde cell
CV	(larval) cerebral vesicle
DC	dorsal compartment
DCC	dorsal commissural cell
DCm	(larval) dorsal compartment motoneuron
DR	dorsal root cell
e	endostyle
EC	Edinger cell
elm	external labial muscle
enc	encapsulated nerve endings
ep	epiphysis / pineal organ
gd	gut diverticulum
gon	gonad
Hp	Hatschek's pit
ibp	inner buccal plexus
ilm	inner labial muscle
inf	infundibulum
intf	internuncial fiber bundle
io	infundibular organ
Köp	Kölliker's pit
lac	large translumenal (= commissural) cell
lc	lamellar cells
m1, m2, m3…	myomere 1, 2, 3…
MC	mid-commissural cell
Mg	Müller's glia (Schwann cell analogues)
mpf	metapleural folds
mRoax	median descending Rohde axon
n1, n2, n3…	dorsal nerve 1, 2, 3…
ncmf	notochordal motor fibers/tract
nRo	nucleus of Rohde
NS	nervous system
obp	outer buccal plexus
oHe	organ of Hesse
os	optic stalk
pdm	posterodorsal migrated cell group
ph	pharynx
pig	pigment cells of frontal eye
pinfn	postinfundibular neuropile
pm	pterygeal muscle
PMC	primary motor center
postRoax	posterior ascending Rohde axons
r1, r2, r3…	rhombomere 1, 2, 3…
RB	Retzius bipolar cell
rdors	dorsal ramus
Rf	Reissner's fiber
rg	radial glia
rvent	ventral ramus
rventc	ventral ramus, ventral cutaneous branch
rvisc	visceral ramus
rvisca	visceral ramus, ascending branch
rviscd	visceral ramus, descending branch
s1, s2, s3…	somite 1, 2, 3…
SD	small dorsal cell
Sg	Schneider's glia
SM	somatomotor cells
SM1	somatomotor cell type 1
smf	somatomotor fibers/tract
ssf	somatosensory fibers/tract
t	telencephalon
tc	optic tectum
tm	trapezius muscle of atriocoelomic funnel
VC	ventral compartment
vcav	ventricular cavity
VCm	(larval) ventral compartment motoneuron
VL	ventral longitudinal cell
VM	visceromotor cell
VM1, VM2	visceromotor cell type 1, 2
vp	velar plexus
vsc	ventral spindle-shaped cell group
vsf	viscerosensory fibers/tract
vsm	velar sphincter muscle