Polychaete nervous systems: Ground pattern and variations—cLS microscopy and the importance of novel characteristics in phylogenetic analysis

Monika C. M. Müller[1]

Zoologie, Fachbereich Biologie/Chemie, Universität Osnabrück, D-49069 Osnabrück, Germany

Synopsis In Annelida, as well as in other invertebrate taxa, the nervous system is considered to be a very conservative organ system. Immunohistochemical investigations [use of anti-5-HT (serotonin), FMRFamide, and acetylated α-tubulin antibodies] in combination with laser scanning microscopy enable more detailed reanalyses of known structures and detection of new characteristics that are useful for phylogenetic analyses. One hypothesis enabled by such studies is outlined for the evolution of arrangements of the dorsal circumesophageal roots in polychaetes and oligochaetes. These roots are not a unique feature of polychaetes; they also occur in oligochaetes. According to the Articulata hypothesis of metazoan relationships, the specific structure of the rope-ladder-like nervous system is, among others, an autapomorphic characteristic that unifies Annelida and Arthropoda. Recent studies applying the techniques mentioned here, however, demonstrate that the annelidan *bauchmark* (central nervous system of the trunk), in contrast to the arthropod pattern, is highly variable in terms of the number and position of connectives and the number of commissures per segment. The variability of the neuronal architecture as well as a hypothesis on how it evolved will be introduced with the aid of regeneration and developmental studies. Furthermore, it is shown that hitherto unknown nerves are present in the peripheral nervous system.

Introduction

Neuroanatomical studies as well as studies of other organ systems (for example, musculature; Müller and Schmidt-Rhaesa 2003; Müller and others 2004; Hooge and Tyler 2006) have been revitalized by immunohistochemical methods in combination with confocal laser scanning microscopy (cLSM), as evidenced by the increasing number of papers in this field. The advantages of the technique are obvious: complex organ systems can be studied in great detail in whole mounts; entire systems or subsystems can be stained specifically; time-consuming histological or ultrathin sectioning is, at least for some purposes, obsolete; the structures can be 3-dimensionally reconstructed and displayed; and it is possible to process a larger number of specimens in a reasonable time, as is necessary for developmental or regeneration studies. For example, joint application of immunohistochemistry and cLSM enabled a convincing demonstration of the segmented nature of Myzostomidae (Müller and Westheide 2000), exposure of the true segmentation pattern obliterated by secondary annulation in Dinophilidae (Müller and Westheide 2002), and detection of new structural characteristics in Gnathostomulida (Müller and Sterrer 2004).

The method is limited by the need for preparations to be small and transparent (otherwise investigations have to be preceded by sectioning) and resolution is not beyond that of light microscopy. The latter point indicates that cLSM does not replace but complements other methods, especially transmission electron microscopy (Müller and others 2004). With cLSM new structures can be detected and the area of interest can be defined in order to minimize sectioning for transmission electron microscopy, whereby specific characteristics can be analyzed on a subcellular level (Purschke and Müller 1996).

The method is further limited by the availability of appropriate antibodies. For neuroanatomical studies in invertebrates, antibodies directed against 5-HT, FMRFamide, and acetylated α-tubulin are an accepted standard. In particular acetylated α-tubulin is sufficient to reconstruct entire nervous systems. The drawback of this antibody is that sensory and locomotory cilia are also stained, which is a barrier to analyzing developmental stages. On the other hand, such "wrongly"

stained structures can be used as a "morphological atlas" or to identify other valuable organs of phylogenetic interest (Müller 1999, 2002; Worsaae and Müller 2004).

Investigation of the nervous system is important because it is regarded as a deeply conservative system (Bullock and Horridge 1965; Orrhage 1974; Rouse and Fauchald 1997) and therefore as very valuable for phylogenetic analyses among higher taxa. The best example is the rope-ladder-like nervous system, which may be seen as a synapomorphy unifying Annelida and Arthropoda as the taxon Articulata (Scholtz 2002). However, recent molecular investigations have significantly reduced confidence in the monophyly of the Articulata. Instead, the Arthropoda have been united with other molting taxa (Ecdysozoa; Eernisse and others 1992; Aguinaldo and others 1997), forming a distinct monophylum from the annelids along with mollusks and sipunculans. These form the Lophotrochozoa (Rota and others 2001; Halanych 2004; Purschke and Müller 2006). The recognition of Ecdysozoa and Lophotrochozoa implies that a rope-ladder nervous system has either developed twice or once but at an earlier time, before the separation of these 2 lineages. In order to assess these possibilities and to reach a well-supported hypothesis, the degree of difference and likeness between the 2 systems must be identified. The new techniques allow comparison on the single nerve-cell level and even, in combination with injection methods, recognition of distinct branching patterns, thus revealing a great number of new characteristics of high reliability.

A starting point for the overarching investigations is the evaluation of variation and resemblance within a taxon. In the Annelida, particularly among polychaetes (which may not be a monophyletic group), the nervous system is more variable than previously documented. Here it is demonstrated that developmental and regeneration studies are definitely required to gain information on the fundamental organization, or the ground pattern, of the nervous system.

In what follows, cephalic structures and the ventral nerve cord of the central nervous system are presented separately, and then information is presented for the peripheral nervous system. Unfortunately information about the stomatogastric nervous system is still limited, and the available data do not allow definition of a more general pattern (Orrhage and Müller 2005).

The cephalic nervous system

The cerebral commissures and the circumesophageal connectives, rather than the overall architecture of the polychaete brain (Orrhage and Müller 2005), will be
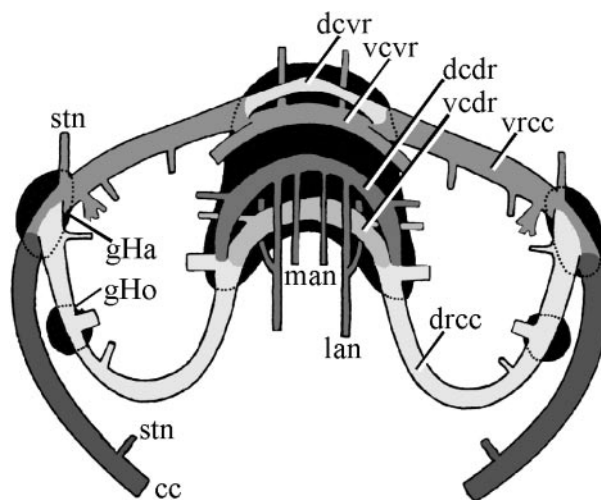

Fig. 1 Schematic drawing of the polychaete supraesophageal ganglion and associated nerves. The circumesophageal connectives (cc) are basally simple; anteriorly they split into a dorsal (drcc) and a ventral (vrcc) connective root. Each root splits again and gives rise to 2 commissures that traverse the brain: dorsal (dcdr) and ventral (vcdr) commissure of the dorsal root; dorsal (dcvr) and ventral (vcvr) commissure of the ventral root. The cerebral and 2 further ganglia are indicated in black. Hamaker's ganglia (gHa) are located at the bases of the 2 roots; Homlgren's ganglia (gHo) lie further toward the brain. If present, the median antenna is solely innervated from nerves (man) branching off from the dcdr; the lateral antennae are innervated from nerves (lan) originating from the dcdr and additionally the cerebral neuropile. Nerves innervating the palps branch off from both roots and all 4 commissures. stn, stomatogastric nerves. *Source*: synopsis of Orrhages's studies (1964–1999), modified from Orrhage (1995, 1999).

discussed. In a synoptic illustration, Orrhage (1995) indicated the nerves that innervate the prostomial appendages. His popular drawing (Fig. 1) also shows that the basically simple circumesophageal connective of each side splits into a dorsal and a ventral root. Each root splits again, and the nerves interconnect with the adjacent fibers from the other side, forming 4 commissures. This distinctive design is unique to polychaete annelids and has been regarded as an apomorphy for the taxon as well as part of the cephalic ground pattern (Purschke and others 2000). It is, however, not present in every species investigated, and the absence of the 4 commissures and the dorsal roots casts doubt on the assumed ground pattern. The structures in question, however, might be present but just undetected.

The 4 prominent cerebral commissures were first detected by Rhode (1887), confirmed by Gustafson (1930), and reported by Orrhage in 26 out of the 32 families he investigated (Orrhage 1995; Orrhage and Müller 2005, and literature therein). In regenerating

specimens of *Dorvillea bermudensis* the 4 commissures are clearly visible only within a narrow time frame; later, formation of a neuronal plexus between the commissures obliterates this pattern (Müller and Henning 2004). In most studies, adult specimens have been investigated; thus the absence of (or simply the inability to identify) all 4 commissures might be due to a similar ontogeny of these structures. These findings indicate the necessity of investigating the developmental stages of species, particularly those in which the commissures are "absent."

The dorsal circumesophageal roots are variable: in some taxa they are so short that they have been overlooked (Onuphiidae, Glyceridae; Manaranche 1966; von Haffner 1995a, 1995b), whereas in others they are really missing (Müller and Westheide 2000; Orrhage and Müller 2005). Tracing regeneration of the anterior nervous system in several polychaetes, Müller and coauthors (Kreischer and Müller 2000; Müller and Berenzen 2002; Müller and others 2003; Müller and Henning 2004) determined that the circumesophageal connectives are initially paired structures (Fig. 2A) that form a partly single connective during differentiation by a fusion that proceeds from the ventral cord toward the brain (Fig. 2B). In *D. bermudensis*, where the dorsal roots are clearly visible, the fusion stops half way (Müller and Henning 2004), whereas in *Eurythoe complanata* both roots fuse more or less totally (Müller and others 2003), leaving only short dorsal roots behind (Gustafson 1930; Orrhage 1990). These findings led Müller and coauthors to the assumption that different arrangements simply reflect different degrees of fusion, with the dorsal roots appearing to be absent in the most extreme cases. This hypothesis was tested for the Clitellata, from which dorsal roots are entirely missing—a characteristic that was thought to distinguish the taxon from polychaetes (Purschke and others 2000). In fact, early stages of stolonizing *Stylaria lacustris* and anteriorly regenerating *Enchytraeus fragmentosus* (Fig. 2D) do possess dorsal and ventral roots (Müller 2004a). During differentiation the roots fuse completely, leaving no traces of the dorsal roots in adults.

Since regeneration takes place in proximity to (Kubo and others 1996) and possibly under the control of differentiated tissue, this may be a unique pattern of development and morphogenesis. Thus the statement that the presence of paired circumesophageal connectives is a general design for polychaetes rather than being exclusive to regeneration has to be substantiated by developmental studies. Therefore, neurogenesis was traced in polychaete species. In *Scoloplos armiger* (Müller 1999, 2005) and *Parpionosyllis minuta* (personal observation, M.C.M.M. and A. Berenzen)

paired connectives are formed by a double scaffolding: axons of an anterior subsystem growing backward and axons of a posterior subsystem growing forward. They meet each other at the transition from peristomium to trunk. From here the forward-growing nerves form the future dorsal roots; they use the existing axons of the anterior system (ventral roots) as a guiding line and grow in parallel to them toward the brain. Neurogenetic studies of oligochaetes (Hessling and Westheide 1999; Yoshida-Noro and others 2000) do not confirm the presence of 2 circumesophageal roots. Further developmental studies are needed to exclude the possibility that these structures were simply not observed in the oligochaetes, and regeneration studies are needed to verify those reported findings.

Unintended fusion during regeneration of fragments produced chimeric specimens of the polychaete *D. bermudensis*, enabling valuable observations (Müller 2004b). Neuroregeneration in anteriorly fused individuals demonstrated that the circumesophageal roots kept their identity: in all cases dorsal and ventral roots fused with the correct counterpart, even though this belonged to another organism. At the location of fusion, the new brain developed, suggesting that contact of the roots initialized its formation. Subsequent to the first stereotypic phase, including formation of the 4 cerebral commissures, brain differentiation exhibits extreme plasticity. Outgrowing nerves innervate the correct target, but sometimes in the other animal (Fig. 2C); in some cases 1 organ is aberrantly innervated by 2 nerves. Most extraordinary is the creation of "auxiliary" nerves that conserve the typical architecture and enable innervation of inappropriate developed appendages at the same time. According to Orrhage (1995, 1999) antennae are always innervated from nerves emanating from the dorsal root commissures (Fig. 1). In chimeric *D. bermudensis* the dorsal roots of 2 individuals are interconnected via auxiliary nerves, from which the antennal nerves branch off (Fig. 2C, yellow arrows). This seems to indicate that the morphological target attracts the nerve rather than that the nerve initiates formation of the structure.

The ventral nerve cord

According to the conventional view, the rope-ladder-like nervous system with 2 ganglia per segment linked within 1 segment via commissures and with the neighboring segments via 2 connectives is a synapomorphy for Annelida and Arthropoda. Polychaete nervous systems, however, display a great variety of connective systems: the adult ventral nerve cord may possess 5 (for example, Dinophilidae, Fig. 2F), 3 (for example, Histriobdellidae, Fig. 2G), 2 (for example, Hesionidae, Fig. 2H) or only 1 (for example,

Nerillidae, Oweniidae within polychaetes; also oligochaetes) connective. If 5 such longitudinal fiber cords are present, they are named ventromedian (1), paramedian (2) and main (2) nerves.

The formation of the nervous system from 1 anterior and 1 posterior subsystem underlies the laying down of the first 4 connectives. From the peristomium–trunk transition posteriorly growing nerves form the future paramedian nerves; they use the existing neurits of the posterior system (main nerves) as guiding lines and grow between them and parallel to them, toward the posterior end of the animal (Fig. 2E, growth cones; Orrhage and Müller 2005). The ventromedian nerve is established later. It is assumed that it originates from the circumesophageal connectives or from 5-HT-containing nerve cells located at the transition between
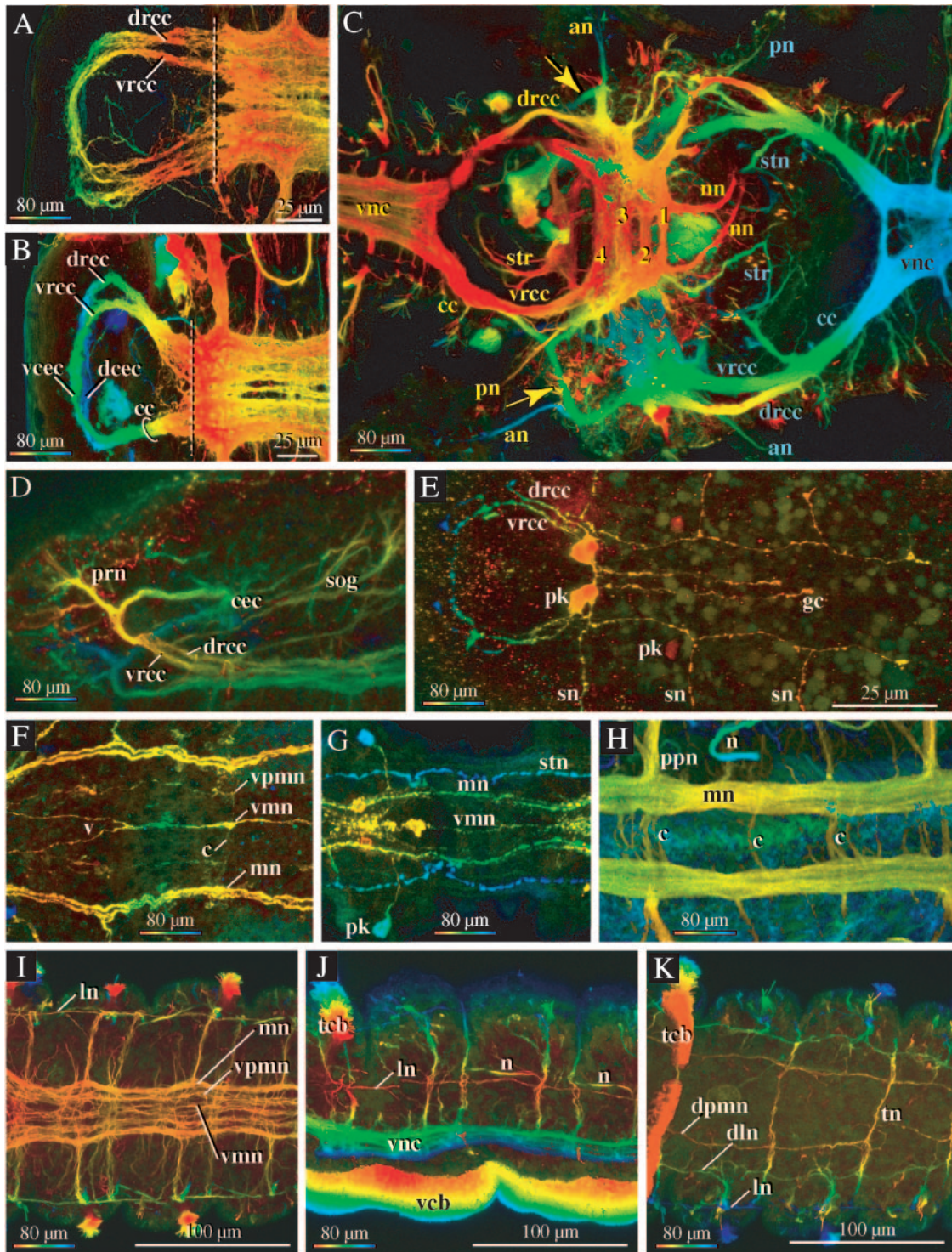
these connectives and the ventral cord. The serotonergic cells are the first cells visible in the *bauchmark* (ventral cord) and they remain the most prominent ones. Within the Annelida, Hirudinea (Sawyer 1986) and 7 out of 28 polychaete species investigated (Orrhage and Müller 2005) possess the median nerve. In adult oligochaetes the median nerve is invisible; it was, however, reported in the regenerating nerve cord in *E. fragmentosus* (Müller 2004a). In the phylogenetic tree presented by Rouse and Fauchald (1997) the characteristic "median nerve" is widely distributed, leading to the assumption that it belongs to the annelid ground pattern. Because it is also present in arthropods, however, it must be regarded as a plesiomorphic characteristic for the taxon Annelida. Secondary loss of the nerve during ontogeny is described for *Nereis virens* by Ushakova and Yevdonin (1985). Although the median nerve is involved in innervation of the intestine in hirudineans (Bullock and Horridge 1965), nothing is known about its function in the remaining annelids and arthropods. One can guess that it might be involved in innervation of the ventromedian longitudinal muscles, but such a function requires clarification (Purschke and Müller 2006).

Paramedian nerves are present in Dinophilidae (Donworth 1986; Beniash and others 1992; Müller and Westheide 1997), Saccocirridae (Kotikova 1973; Müller 1999), Protodrilidae, Ctenodrilidae, and Magelonidae (Müller 1999).

The entire set of all 5 connectives has hitherto been reported only for polychaete larvae (Dorvilleidae: Müller and Westheide 2002; Myzostomidae: Eeckhaut and others 2003; Orbiniidae: Müller 2005; Fig. 2I) and adult Dinophilidae (Müller and Westheide 1997, 2002). In the larvae this architecture is only transient, because the paramedian and the main nerves fuse and thus produce a trineuralian nerve cord in the adults. This fusion is also visible at the posterior end of the adult animals, where differentiation follows a posterior–anterior gradient. Only in Dinophilidae is the arrangement visible in adults, confirming their progenetic origin; persistence of the larval design is an apomorphic characteristic for the taxon.

The number of commissures varies among species (Fig. 2F–H; Müller 1999). The commissures can be numerous without a distinct pattern (for example, Protodrilidae, Saccocirridae) or few, down to 1, in a specific segmental arrangement (for example, 1 main and 2 subordinate commissures in Dinophilidae; Müller and Westheide 2002). As a result of fusion, the number of commissures can also vary within an individual (for example, *Pisione remota*), with a decreasing number anteriorly (Müller 1999).

The peripheral nervous system

The peripheral nervous system comprises (1) the epidermal plexus at different locations, (2) segmental nerves branching off from the connectives, and (3) longitudinal nerves branching off from the brain.

The number of segmental (side) nerves that branch off from the connectives and extend laterally varies among species (Fig. 2H and I) and possibly also during ontogeny, with the number decreasing during development as a result of amalgamation. The

Fig. 2 Tubulinergic (A–D, H–K), serotonergic (E) and FMRFamide-like (F, G) neuronal subsets of various annelids. The images are cLSM color-coded maximum-intensity-pixel images (red, peripheral; blue, central structures). The colored bar indicates the depth of the stack. (A–C) *D. bermudensis*. (A, B) Anteriorly regenerating nervous system; the amputation site is indicated by the dotted line. (A) After 143 h the dorsal (drcc) and the ventral (vrcc) roots are clearly separated. (B) After 192 h both roots are fused basally, forming a simple connective (cc, circle). Ventral (vcec) and dorsal (dcec) cerebral commissures are visible. (C) Chimeric individuals, anteriorly fused. Yellow letters indicate structures of the left animal; light blue, structures of the right animal. Nerves innervating antennae (an) and palps (pn) are present; partly they branch off from additional structures (yellow arrow). The left animal shows 4 cerebral commissures (1–4) in atypical arrangement. Nuchal nerves (nn) of the left animal innervate nuchal organs of the right animal. The ventral cord (vnc), stomatogastric nerves (stn), and ring (str) are present; the latter 2 are deformed. (D) Regenerating brain of *E. fragmentosus*. Two roots are visible. Nerves branching off from the single cerebral commissure (cec) form the brain rudiment (sog). prn, prostomial nerves. (E) *Parapionosyllis minuta*, embryo. Dorsal and ventral roots form the circumesophageal connectives. The ventral cord consist of 4 connectives; the median ones are growing backwards, indicated by growth cones (gc). pk, perikarya; sn, segmental nerves. (F–H) Variations of the polychaete ventral nerve cord. (F) *Dinophilus gardineri*, pentaneuralian cord with single median (vmn) and paired paramedian (vpmn) and main (mn) nerves. The commissure (c) is hardly visible. The median nerve branches anteriorly in a V shape (v). (G) *Histriobdella homari*, trineuralian cord. The peripheral blue connectives belong to the stomatogastric nervous system (stn). (H) *Microphthalmus sczelkowii*, dineuralian cord. The parapodial nerves (ppn) are prominent; nephridia (n) are visible because of their cilia. (I–K) *S. armiger*. (I) Ventral view with pentaneuralian cord and lateral nerves (ln). (J) Lateral view with lateral nerves (ln). Ventral (vcb) and transverse (tcb) ciliary bands serve as orientation marks. (K) Dorsal view with dorsal paramedian (dpmn) and dorsolateral (dln) nerves, interconnected by transverse nerves (tn). (A, B) modified from Müller and Henning (2004); (C) modified from Müller (2004b); (D) modified from Müller (2004a); (E) M.C.M.M. and A. Berenzen, unpublished data; (F–H) M.C.M.M., unpublished data; (I–K) modified from Orrhage and Müller (2005).

parapodial nerves (special segmental nerves) show a conservative branching pattern (Myzostomidae: Müller and Westheide 2000); for the other nerves exact data are missing. Some segmental nerve pairs interconnect at the dorsal side and innervate ciliary bands, when present. In some species the segmental nerves form a regular grid in combination with the longitudinal nerves (Müller and Westheide 2002).

The occurrence of 1 pair of lateral nerves was initially observed in Amphinomidae. To indicate that they possess 4 longitudinal nerves (2 in the ventral cord, 2 laterally) the taxon was called "Tetraneura" to contrast with "Dineura," which lacked the lateral nerves (Storch 1913). However, it has turned out that lateral nerves (Fig. 2I and J) are present in almost all annelidan subtaxa; in polychaetes they are missing in only 3 out of 28 species investigated (Müller 1999; Orrhage and Müller 2005). Through the application of immunohistochemistry in combination with cLSM more longitudinal nerves were found in the peripheral nervous system (Müller 1999; Müller and Westheide 2002); as many as 17 fibers were found in *Saccocirrus papillocercus* (Müller 1999), evenly distributed around the trunk. Apart from the single dorsomedian nerve, all other fibers occur pairwise, and 1 pair of dorsolateral fibers exists in nearly every species investigated (Fig. 2K). It is not possible to establish homologies of the nerve pairs between species at the moment because the nerves occur in different numbers or, even if they can be compared numerically, they occupy different positions. Information about the structures they innervate is required to resolve this problem.

Nevertheless, it can be hypothesized that a regular grid of such longitudinal and perpendicular (side nerve) fibers is the best design to innervate a cylindrical body.

Conclusions

The most recent information led me to hypothesize that the ground pattern (Fig. 3, central image) of the annelid nervous system comprises (1) primarily paired circumesophageal connectives with similar dorsal and ventral roots, with corresponding roots interconnected via 1 dorsal and 1 ventral commissure (4 cerebral commissures); (2) a ventral nerve cord with primarily 5 connectives: 1 unpaired median and paired paramedian and paired main connectives; (3) numerous commissures per segment; (4) numerous segmental nerves per segment; (5) a peripheral nervous system with several nerves that, apart from the dorsomedian one, occur in pairs.

The variations present in recently investigated species can be derived from this ground pattern (Fig. 3).
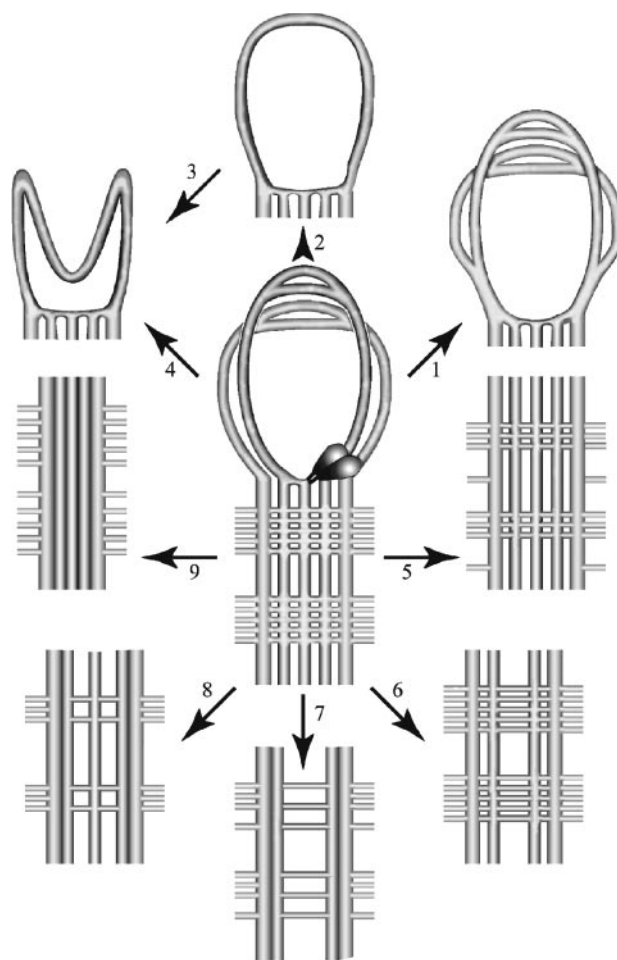

Fig. 3 Schematic drawing of a hypothetical polychaete ground pattern and its subsequent reformation. The center shows the ground pattern with paired circumesophageal connectives, 4 cerebral commissures, 5 connectives, and numerous commissures in the ventral nerve cord. (1) Partial fusion of the ventral and dorsal esophageal root forms the typical polychaetous architecture (Fig. 1). (2) Complete fusion of the roots results in a simple connective throughout. (3, 4) Complete fusion and backward shift of the brain leads to the oligochaetous arrangement. (5) Through neoteny the Dinophilidae retain a pentaneuralian ventral cord. (6) Absence of the median nerve leads to a tetraneuralian cord. (7) Absence of the median nerve and fusion of the 2 peripheral nerve pairs leads to the dineuralian cord. (8) Formation of the median nerve and fusion of the peripheral nerves forms a trineuralian cord. (9) Medial relocation and fusion of all connectives forms a unineuralian cord, present in oligochaetes and some polychaetes.

At the anterior end such variations include (1) the typical "polychaete" cephalic nervous system (Fig. 1) produced by partial fusion of the paired connectives, leaving dorsal roots of different lengths; (2) formation of simple circumesophageal connectives by complete fusion, as present in some polychaetes; (3) hypothetical
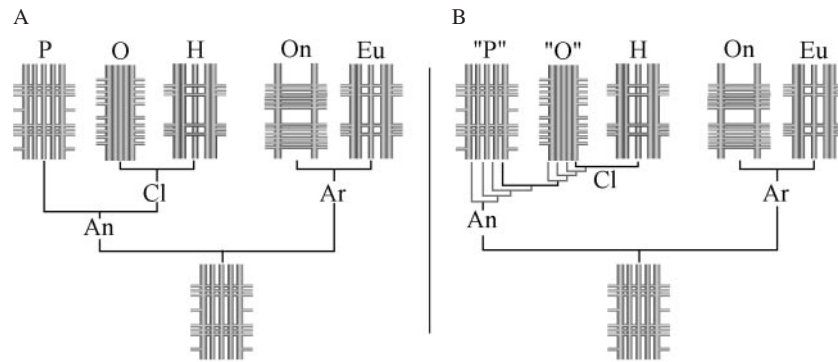
Fig. 4 Schematic visualization of the ventral nervous system present in various subtaxa of the Articulata. (A) Phylogenetic relationships with monophyletic Polychaeta and Oligochaeta. (B) Phylogenetic relationships with paraphyletic (indicated by quotation marks) "Polychaeta" and "Oligochaeta"; modified from Westheide (1997). An, Annelida; Ar, Arthropoda; Cl, Clitellata; Eu, Euarthropoda; H, Hirudinea; O, Oligochaeta; "O," paraphyletic Oligochaeta; On, Onychophora; P, Polychaeta; "P," paraphyletic Polychaeta.

transition from "polychaete" to "oligochaete" design; (4) formation of simple connectives by complete fusion and backward shift of the supraesophageal ganglion, as present in oligochaetes. In terms of the ventral nerve cord, (5) conservation of the larval architecture of the ventral nerve cord with 5 connectives is the result of progenesis in Dinophilidae; because it is unique for this taxon, it represents an autapomorphy. Other variations are (6) formation of a tetraneuralian ventral nerve cord through secondary loss of the median nerve (hypothetical); (7) formation of a dineuralian ventral nerve cord via fusion of the 2 outer nerve pairs (observed) and secondary loss of the median nerve (hypothetical); (8) formation of a trineuralian ventral nerve cord via fusion of the paramedian and main nerves and persistence of the median nerve (observed in the genera *Ophryotrocha*, *Scoloplos*, and *Myzostoma*); (9) formation of a unineuralian cord by medial shift of all connectives, as seen in oligochaetes and some polychaetes (for example, Nerillidae, Oweniidae).

Paraphyletic relationships in polychaetes and oligochaetes (Fig. 4B; Westheide 1997) do not alter the hypothesis. In such a scenario, however, condensation of the ventral nerve cord had to occur in several oligochaete lines, which might be explained by functional pressure, such as burrowing in firm substrate.

The high degree of diversity of the annelidan nervous system may seem to weaken the power of the rope-ladder-like nervous system to act as an apomorphic characteristic for a taxon comprising Annelida and Arthropoda. It might also seem to fit an interpretation that this type of nervous system has evolved independently in annelids and arthropods. However, another scenario is also possible (Fig. 4). The nerve cord of the Articulata stem species might have been less condensed than previously thought; instead of 2, it may have had 5 connectives, and instead of 2, it may have had several commissures per segment. As explained above—and, at least to a certain extent, this is observable in recently investigated species—this loose arrangement was transformed into the various designs within the Annelida, up to the "typical" rope-ladder visible in hirudineans, but including the median nerve (Fig. 3), which is often ignored. In principle the same scenario is possible for Arthropoda; evidence to support this might include the loose arrangement of the ventral nerve cord with 2 widely separated connectives and numerous commissures per segment in Onychophora (Fig. 4). Neurogenesis and, if possible, neuroregeneration in taxa regarded as occupying a basal position within the Arthropoda, other Ecdysozoa, and Lophotrochozoa should be investigated in this respect.

Although current opinion seems to favor a Lophotrochozoa hypothesis rather than an Articulata hypothesis, future studies should focus on detailed comparisons among the annelidan and arthropod nervous systems and those of their "new relatives" (for example, mollusks).

Tanja Paulus, and Sindy Göbel for their valuable work, and Prof. Dr. Wilfried Westheide for inspiring discussions.

References

Aguinaldo AMA, Turbeville JM, Linford LS, Rivera MC, Garey JR, Raff RA, Lake JA. 1997. Evidence for a clade of nematodes, arthropods and other moulting animals. Nature 387:489–93.

Bullock TH, Horridge GA. 1965. Structure and function in the nervous system of invertebrates. Volume 1. London: W.H. Freeman and Company.

Beniash EA, Yerlikova NN, Yevdonin LA. 1992. Some characteristics of the *Dinophilus vorticoides* anatomy of the nervous system. In: Bushinskaja GN, editor. Polychaeta and their ecological significance: explorations of the fauna of the seas. Volume 43(51). St. Petersburg: Russian Academy of Sciences. p. 5–9.

Donworth PJ. 1986. A reappraisal and the validation of the species *Dinophilus taeniatus* Harmer, 1889 and the taxonomically significant features in monomorphic dinophilids (Annelida Polychaeta). Zool Anz 216:32–8.

Eeckhaut I, Fievez L, Müller MCM. 2003. Development of *Myzostoma cirriferum* (Myzostomida). J Morphol 258: 269–83.

Eernisse DJ, Albert JS, Anderson FE. 1992. Annelida and Arthropoda are not sister taxa: a phylogenetic analysis of spiralean metazoan morphology. Syst Biol 41:305–30.

Gustafson G. 1930. Anatomische Studien über die Polychaeten-Familien Amphinomidae und Euphrosynidae. Zoologiska Bidrag fran Uppsala 12:305–471.

Halanych KM. 2004. The new view of animal phylogeny. Ann Rev Ecol Evol Syst 35:229–56.

Hessling R, Westheide W. 1999. CLSM analysis if development and structure of the central nervous system of *Enchytraeus crypticus* ("Oligochaeta," Enchytraeidae). Zoomorphology 119:37–47.

Hooge MD, Tyler S. 2006. Concordance of molecular and morphological data: the example of Acoela. Integr Comp Biol 46:118–24.

Kotikova EA. 1973. New data concerning the nervous system of Archiannelida [in Russian]. Zool Zh 52:1611–5.

Kreischer S, Müller MCM. 2000. Development of neuronal structures in stolons of *Autolytus prolifera* (Polychaeta). Zoology 93(1):58.

Kubo T, Arai T, Kawasaki K, Natori S. 1996. Insect lectins and epimorphosis. Trends Glycosci Glycotechnol 8:357–64.

Manaranche R. 1966. Anatomie du ganglion cèrèbroide de *Glycera convoluta* Keferstein (Annèlide Polychète), avec quelques remarques sur certains organes prostomiaux. Cah Biol Mar 7:259–80.

Müller MC. 1999. Das Nervensystem der Polychaeten: immunhistochemische Untersuchungen an ausgewählten Taxa. [PhD thesis]. Osnabrück, Germany: Universität Osnabrück.

Müller MCM. 2002. *Aristonerilla*: a new nerillid genus (Annelida: Polychaeta) with description of *Aristonerilla (Micronerilla) brevis* comb. nov. from a seawater aquarium. Cah Biol Mar 43:131–9.

Müller MCM. 2004a. Nerve development, growth and differentiation during regeneration in *Enchytraeus fragmentosus* and *Stylaria lacustris* (Oligochaeta). Dev Growth Differ 46:471–8.

Müller MCM. 2004b. Immunohistochemical analysis of nervous system regeneration in chimeric individuals of *Dorvillea bermudensis* (Polychaeta, Dorvilleidae). Dev Growth Differ 46:131–8.

Müller MCM. 2006. Neurogenesis in *Scoloplos armiger* (Polychaeta; Orbiniidae): evidence for paired oesophageal connectives and a pentaneural ventral cord. J Comp Neurol (in press).

Müller MCM, Berenzen A. 2002. Asexuelle Fortpflanzung bei Anneliden (Ringelwürmern): Teil III. Regeneration des Nervensystems am Beispiel von *Eurythoe complanata*. Mikrokosmos 91:108–14.

Müller MCM, Berenzen A, Westheide W. 2003. Regeneration experiments in *Eurythoe complanata* ("Polychaeta," Amphinomidae): reconfiguration of the nervous system and its function for regeneration. Zoomorphology 122:95–103.

Müller MCM, Henning L. 2004. Neurogenesis during regeneration in *Dorvillea bermudensis* (Dorvilleidae): formation of the polychaete cephalic ground pattern. J Comp Neurol 471:49–58.

Müller MCM, Jochmann R, Schmidt-Rhaesa A. 2004. The musculature of horsehair worms (*Gordius aquaticus*, *Paragordius varius*, Nematomorpha): F-actin staining and reconstruction by cLSM and TEM. Zoomorphology 123:45–54.

Müller MCM, Schmidt-Rhaesa A. 2003. Reconstruction of the muscle system in *Antygomonas* sp. (Kinorhyncha, Cyclorhagida) by phalloidin labelling and cLSM. J Morphol 256:103–10.

Müller MCM, Sterrer W. 2004. Musculature and nervous system of *Gnathostomula peregrina* (Gnathostomulida) shown by phalloidin labelling, immunhistochemistry, and cLSM, and their phylogenetic significance. Zoomorphology 123:169–77.

Müller MCM, Westheide W. 1997. The nervous system of apodous polychaetes: orthogonal structures of the nervous system in juvenile stages and progenetic species. Verh Dtsch Zool Ges 90(1):209.

Müller MCM, Westheide W. 2000. Structure of the nervous system of *Myzostoma cirriferum* (Annelida) as revealed by immunohistochemistry and cLSM analyses. J Morphol 245:87–98.

Müller MCM, Westheide W. 2002. Comparative analysis of the nervous systems in presumptive progenetic dinophilid and dorvilleid polychaetes (Annelida) by immunohistochemistry and cLSM. Acta Zool (Stockh) 83:33–48..

Orrhage L. 1974. Über die Anatomie, Histologie und Verwandtschaft der Apistobranchidae (Polychaeta Sedentaria) nebst Bemerkungen über die systematische Stellung der Archianneliden. Z Morph Ökol Tiere 79:1–45.

Orrhage L. 1990. On the microanatomy of the supraoesophageal ganglion of some amphinomids (Polychaeta, Errantia), with further discussion of the innervation and homologues of the polychaete palps. Acta Zool (Stockh) 71:45–59.

Orrhage L. 1995. On the innervation and homologues of the anterior end appendages of the Eunicea (Polychaeta), with a tentative outline of the fundamental constitution of the cephalic nervous system of the polychaetes. Acta Zool (Stockh) 76:229–48.

Orrhage L. 1999. On the morphological value of the glycerid-goniadid prostomium and its appendages (Polychaeta). Acta Zool (Stockh) 80:251–64.

Orrhage L, Müller MCM. 2005. Morphology of the nervous system of Polychaeta (Annelida). Hydrobiologia 535/536: 79–111.

Purschke G, Hessling R, Westheide W. 2000. The phylogenetic position of the Clitellata and the Echiura: on the problematic evaluation of absent characters. J Zool Syst Evol Res 38:165–73.

Purschke G, Müller MCM. 1996. Structure of prostomial photoreceptor-like sense organs in *Protodriloides* species (Polychaeta, Protodrilida). Cah Biol Mar 37:205–19.

Purschke G, Müller MCM. 2006. Evolution of the body wall musculature. Integr Comp Biol (in press).

Rhode E. 1887. Histologische Untersuchungen über das Nervensystem der Chaetopoden. Zool Beitr 2:1–81.

Rota E, Martin P, Erséus C. 2001. Soil-dwelling polychaetes: enigmatic as ever? Some hints on their phylogenetic relationships as suggested by a maximum parsimony analysis of 18S rRNA gene sequences Contrib Zool 70:127–38.

Rouse GW, Fauchald K. 1997. Cladistics and polychaetes. Zool Scr 26:139–204.

Sawyer RT. 1986. Leech biology and behaviour. Volume 1. Oxford: Clarendon Press.

Scholtz G. 2002. The Articulata hypothesis—or what is a segment? Org Divers Evol 2:197–215.

Storch O. 1913. Vergleichend-anatomische Polychätenstudien. Sitzungsbericht der Akademischen Wissenschaften Wien 122:877–988.

Ushakova OO, Yevdonin LA. 1985. Architectonic of larval nervous system in *Nereis virens* Sars (Polychaeta, Nereidae). Untersuchungen der Meeresfauna 34:128–31.

Von Haffner K. 1959a. Über den Bau und den Zusammenhang der wichtigsten Organe des Kopfendes von *Hyalinoecia tubicola* Malmgren (Polychaeta, Eunicidae, Onuphiidae), mit Berücksichtigung der Gattung *Eunice*. Zool Jahrb AbtAnat Ontogenie Tiere 77:133–92.

Von Haffner K. 1959b. Untersuchungen und Gedanken über das Gehirn und Kopfende von Polychaeten und ein Vergleich mit den Arthropoden. Mitteilung aus dem Zoologischen Staatsinstitut u. Zoologischen Museum Hamburg 37:1–29.

Westheide W. 1997. The direction of evolution within the Polychaeta. J Nat Hist 31:1–15.

Worsaae K, Müller MCM. 2004. Nephridial and gonoduct distribution patterns in Nerillidae (Annelida: Polychaeta)—examined by tubulin staining and cLSM. J Morphol 261:259–69.

Yoshida-Noro C, Myohara M, Kobari F, Tochinai S. 2000. Nervous system dynamics during fragmentation and regeneration in *Enchytraeus japonensis* (Oligochaeta, Annelida). Devel Genes Evol 210:311–19.