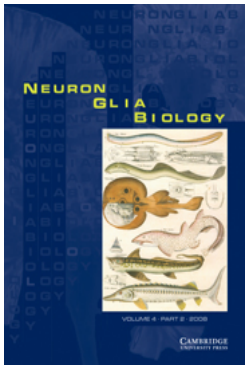

The phylogeny of invertebrates and the evolution of myelin

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The phylogeny of invertebrates and the evolution of myelin

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Current concepts of invertebrate phylogeny are reviewed. Annelida and Arthropoda, previously regarded as closely related, are now placed in separate clades. Myelin, a sheath of multiple layers of membranes around nerve axons, is found in members of the Annelida, Arthropoda and Chordata. The structure, composition and function of the sheaths in Annelida and Arthropoda are examined and evidence for the separate evolutionary origins of myelin in the three clades is presented. That myelin has arisen independently at least three times, namely in Annelids, Arthropodas and Chordates, provides a remarkable example of convergent evolution.

Keywords: Evolution, invertebrates, myelin, phylogeny

The advent of molecular and genetic analyses stimulated a renewed interest in phylogeny and led to the development of a new approach, cladistics, in which information from a variety of sources, 18S rDNA analyses, genomic analysis (e.g. *Hox* genes) and behaviour as well as the traditional morphological, anatomical and developmental characteristics, is used to assess the relationships between taxa. This has resulted not only in new insights and hypotheses but also to conflicting conclusions due to the difficulties in reconciling different methodologies and lack of data (see Graham, 2000; Jenner, 2004; Rousset *et al.*, 2007; Dunn *et al.*, 2008).

Uncertainty, for the most part, has centred on the reassignment of smaller groups. For example, a small group of interest in the present context, the Phoronida (horseshoe worms), has been placed in a clade including the Annelida. Another example is that the Pogonophora (beard worms), previously regarded as a separate phylum, are now considered to belong to the polychaete annelids. The relationships of other groups such as the Tardigrada (water bears) remain under discussion. However, the affinities of the large taxa, Mollusca (slugs, snails, oysters, squid, octopi), Annelida (bristleworms, earthworms, fresh water ringed worms, leeches) and Arthropoda (crustacea, insects, spiders, mites, scorpions) have also been questioned. It is these groups that are of prime interest in a discussion of the evolution of myelin. Previously the Annelida and Arthropoda were regarded as being closely related having arisen from a common annelid-like ancestor. However, the current view, originating from 18S rDNA studies by Aguinaldo *et al.* (1997), is that the Arthropoda and Annelida belong in separate clades.

A simplified rendering of the relationships between groups based on Dunn *et al.* (2008) is shown in Fig. 1. The salient points emerging from the publications cited above are that the Metazoa (multicellular animals) represent three major divergent lineages stemming from some kind of flagellate ancestor. One lineage is the Ctenophora (comb jellies, sea gooseberries), and the other the Cnidaria (sea anemones, jellyfishes) and Porifera (sponges). The third one includes all other phyla, which together are referred to as the Bilateria because of their bilateral symmetry. An early ancestral bilaterian species with certain traits gave rise to descendants that form the deuterostomes (second mouth) in which the blastopore becomes the anus and a second opening becomes the mouth. Arising from the same ancestor but diverging along a different path was the common ancestor of the protostomes (first mouth) in which the blastopore becomes the mouth and the anus breaks through as a second opening. Members of the deuterostome clade are the Echinodermata (sea urchins, starfishes, sea cucumbers) and the Chordata (all vertebrate animals and some other groups). The protostomes are now divided into two large clades, the lophotrochozoa and the ecdysozoa. Members of the lophotrochozoa have either a trochophore (toplike) larva or a feeding organ (lophophore) composed of a ring of ciliated tentacles, those of the ecdysozoa molt their cuticles or exoskeletons as they grow. Annelida and Mollusca are placed in the lophotochozoa and Arthropoda in the ecdysozoa. Annelida fall into two groups, the polychaeta (bristle worms) having many chaetae as the name implies and the clitellata that includes the oligochaeta (few chaetae) and the leeches. Several groups comprise the Arthropoda, the crustacea (shrimps, prawns, crabs, copepods), the insects, arachnida (spiders, scorpions, mites) and myriapoda (millipedes, centipedes).

Another issue to be addressed is the definition of myelin. I propose a functional definition that myelin is a sheath of multiple layers of membranes around nerve axons which increases conduction velocity. This definition allows comparison of the myelin sheaths found in disparate groups with the potential to lead to a better understanding of the role of individual components and their properties, e.g. topology of myelin proteins, necessary for their function.

OCCURRENCE AND STRUCTURE

In early light microscopical studies vertebrate nerve fibres with a sheath especially those revealed by staining with lipid stains such as osmic acid were described as medullated.

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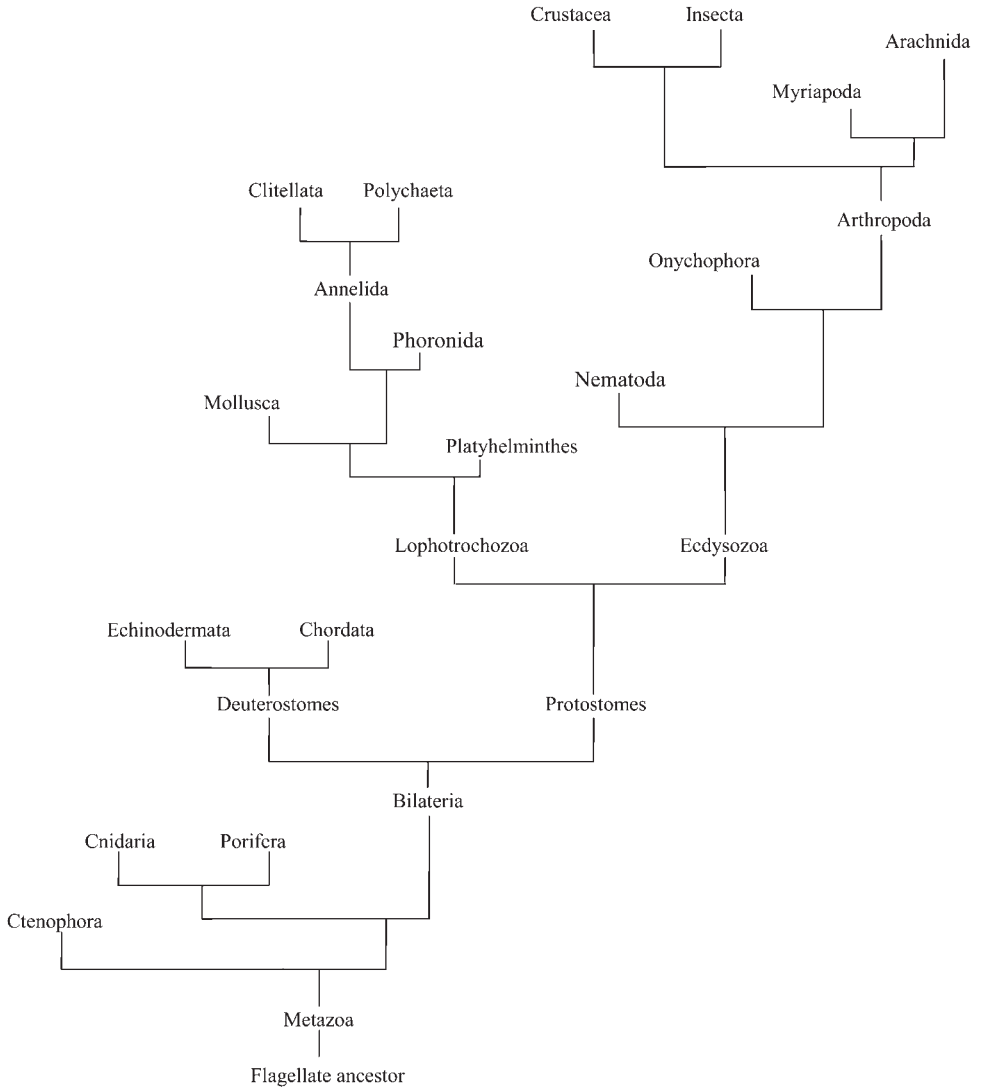

Fig. 1. Phylogenetic relationships.

Only with the advent of electron microscopy was the detailed structure of multiple layers of membranes revealed. Similarly, with invertebrates medullated nerve fibres were reported before the multiple membrane layers were described (Nicol, 1948). Nerve fibres with a sheath have been reported only in annelids, the closely related phoronids, and arthropods (see below).

Annelida and Phoronida

In the Polychaete annelids well-developed sheaths are present in members of three groups: Capitellidae (*Mastobranchus* sp.), Spionidae (*Prionospio steenstrupi*) and Maldanidae (*Clymene producta* and *Clymenella torquata*) (Nicol, 1948), but the ultrastructure of these sheaths has not been described. However, electron microscopical studies on *Ridgeia sp.* and *Riftia pachyptila* (Vestimentifera, now included in the polychaeta) show giant axons with sheaths of up to 50 lamellae. The degree of compaction is varied with layers of cytoplasm ranging from 50 nm to 2 μm (Jones and Gardiner, 1989).

Ultrastructural studies on the phoronids *Phoronis australis*, *P. hippocrepia* and *Phoronis psammophila* have shown that the giant axons of the trunk are wrapped by four to nine membrane lamellae in *P. australis* and *P. hippocrepia* and up to 20 lammellae in *P. psammophila.* An interesting variation is that in *P. australis* and *P. hippocrepia* the sheaths are only partial. The axons run closely apposed to the epidermal basal lamella and in these regions the sheath is absent (Fernández *et al.*, 1996).

The descriptions of myelin sheaths in the annelida are most complete for those found in oligochaetes especially those in the Lumbricidae (earthworms). The structure of these sheaths has been described in two species of earthworm, *Eisenia foetida* (Hama, 1959) and *Lumbricus terrestris* (Coggeshall, 1965; Günther, 1973, 1976; Roots and Lane, 1983), and a sludge worm, *Branchiura sowerbyi* (Zoran *et al.*, 1988). Drewes and Brinkhurst (1990) show an electron micrograph of giant fibres in a newly hatched fresh water ringed worm, *Lumbriculus variegatus*. No description was given but the sheath appears to be similar to that described for other oligochaetes.

In the earthworms the three dorsal giant axons, a median and two lateral ones, have thick sheaths (Fig. 2), which bear a striking resemblance to vertebrate myelin. Multiple glial cell processes from which most of the cytoplasm has been

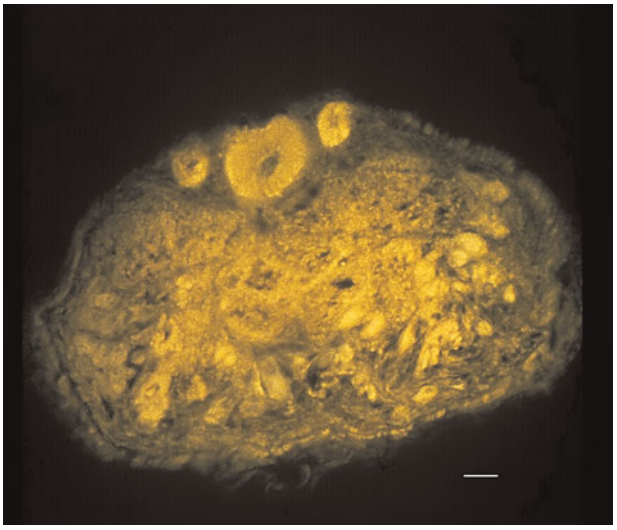

Fig. 2. Cryosection of earthworm *L. terrestris* nerve cord stained with tetracyline-HCl. The sheaths of the three dorsal giant fibres show intense fluorescence. Scale bar = 100 μm.

excluded spirally wrap the axons. The number of lamellae is variable from 15 to 30 and 2 to 15 in the median and lateral giant fibres, respectively, of *E. foetida*, to 60 to 200 in *L. terrestris* with the median fibre similarly being more heavily myelinated than the laterals. The spacing between membranes is highly variable. In the median giant fibre of *L. terrestris* in particular in some regions the cytoplasm is almost totally extruded. In other regions especially where the sheath is buckled forming redundant loops, considerably more cytoplasm is present in which there are large numbers of glial filaments. In these regions too adjacent lamellae are attached by desmosome-like structures, which run in register across the sheath (Fig. 3). Although these structures bear a close resemblance to desmosomes in thin-section electron micrographs, freeze-fracture replicas show that they lack the intramembraneous specialization characteristic of desmosomes (Roots and Lane, 1983). Furthermore, their protein composition is quite different lacking desmogloeins and being closer to that of adherens junctions (Pereyra and Roots, 1988). The glial cell nuclei are outside the sheath (Coggeshall, 1965).

Interruptions, functionally equivalent to vertebrate nodes of Ranvier but totally different in structure, occur in each segment. Two circular pores, 10–15 μm in diameter, pass through the sheath dorsally (Günther, 1973, 1976). These are referred to as focal or fenestration nodes (Fig. 4). Axoplasm protrudes through the pore to meet the fibrous collagen capsule above, and in the circular paranodal region the myelin lamellae are attached throughout most of their length by desmosome-like structures similar to those found in register across the sheath (Figs 5 and 6) (Roots, 1984).

Sheaths similar to those in earthworms are found around the paired lateral giant fibres of the sludge worm, *B. sowerbyi* (Tubificidae). There are about 50 lamellae with variable amounts of cytoplasm between them. As in earthworms there are redundant loops and swirls and stacks of desmosome-like structures run across the sheath. Desmosomal attachments are particularly common near the origin of the ventral collateral nerves. Dorsal nodes were not described (Zoran *et al.*, 1988).

Arthropoda

Myelin sheaths have been reported only in the Crustacea. They are found in malacostracan crustacea, several shrimps, prawns, crayfish and crabs (Holmes, 1942; McAlear *et al.*, 1958; Hama, 1966; Heuser and Doggenweiler, 1966; Kusano, 1966; Govind and Pearce, 1988; Cardone and Roots, 1991; Xu and Terakawa, 1999) and in three groups of copepods (Davis *et al.*, 1999; Lenz *et al.*, 2000; Weatherby *et al.*, 2000).

The sheaths differ radically from those of annelids consisting of concentric lamellae rather than being spirally wrapped. Moreover, there are two patterns found in the concentric sheaths. In the first, where the concentric laminae meet themselves a short seam, or mesaxon, is formed. In the prawn *Palaemonetes vulgaris* (Heuser and Doggenweiler, 1966) the arrangement of the seams in most sheaths is very regular with those of alternate laminae being found on opposite sides of the axon. In shrimps of the genus *Penaeus* the

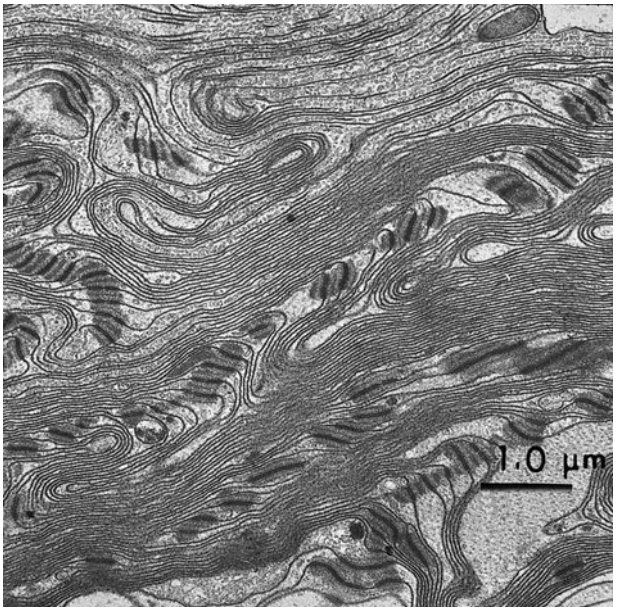

Fig. 3. Electron micrograph of part of the myelin sheath of the median giant fibre of the earthworm *L. terrestris*. Note the desmosome-like structures running in register across the sheath and the packets of cytoplasm retained between the layers.

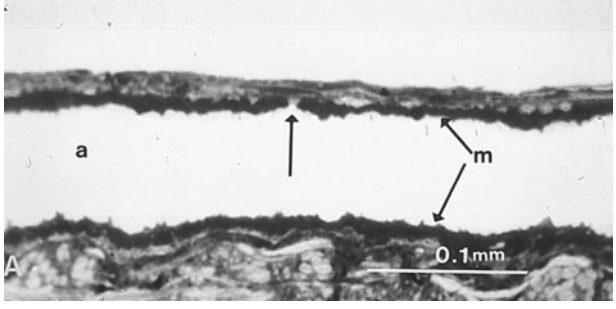

Fig. 4. Light micrograph of a toluidine-blue-stained longitudinal section through the median giant fibre of *L. terrestris*. The arrow indicates a dorsal node in the myelin sheath (m) round the axon (a). Reprinted from Roots (1984) with permission.

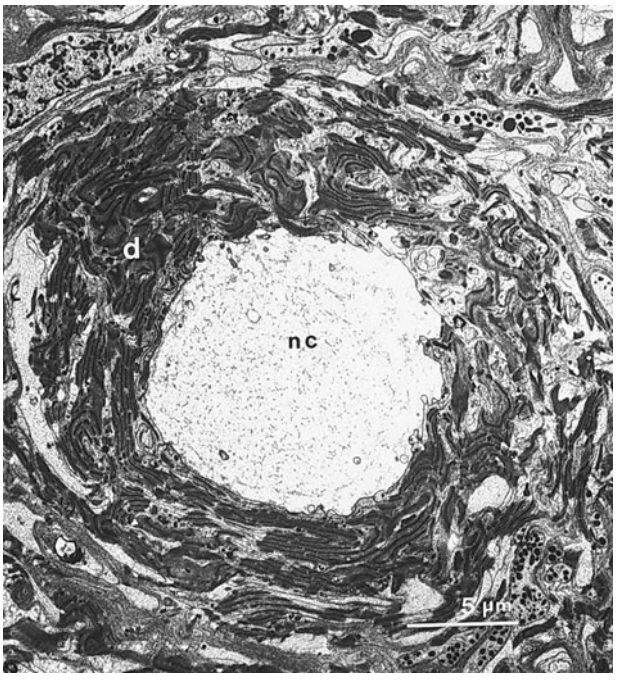

Fig. 5. Electron micrograph of a tangential section through a node in the sheath of the median giant fibre of *L. terrestris*. Note the numerous desmosome-like structures between the lamellae surrounding the node (d). nc, nodal cytoplasm. Reprinted from Roots (1984) with permission.

disposition of the seams is more varied. In small fibres usually there is only one seam in each lamina with the seams being arranged in a radial line. In larger fibres there may be two or three seams in a single lamina, indicating its origin from two or three glial cells (Xu and Terakawa, 1999). The second pattern is found in the copepods *Undinula vulgaris*, *Neocalanus gracilis* and *Euchaeta rimana* where the lamellae form complete circles without seams (Weatherby *et al.*, 2000). In the decapods glial cell nuclei are found between the axolemma and the sheath and in varying positions within the sheath (Heuser and Doggenweiler, 1966; Xu and Terakawa, 1999).

The number of lamellae varies from one or two to more than 50. Where the number of lamellae is low the sheaths are loosely wound as seen for example in the crayfish *Procambarus clarkii* (Fig. 7). In *P. vulgaris*, there are 10–50 laminae varying in thickness between 10 and 200 nm, the thicker ones being in the inner regions of the sheath. The glial processes generally contain cytoplasm although in the outermost layers of the thickest sheaths both it and the extracellular space between processes may be obliterated producing close membraneous appositions analogous to the major and intraperiod lines of vertebrate myelin. As in *L. terrestris* desmosome-like structures occur in register across the sheath (Heuser and Doggenweiler, 1966). In copepods there are up to 60 lamellae (Weatherby *et al.*, 2000).

The spacing between lamellae also varies between species. In prawns the periodicity is more than 20 nm (Heuser and Doggenweiler, 1966) but in *Penaeus* shrimps it is 8 or 9 nm (Xu and Terakawa, 1999). In copepods it varies between 3 and 30 nm with the compact laminae (fused membranes) being 18 nm thick (Weatherby *et al.*, 2000). Not only is periodicity species-dependent in both invertebrates and vertebrates, it is also affected by the processing of the tissue (see Roots, 1993). Low-angle X-ray diffraction of fresh nerve probably gives the most accurate results. The periodicity of fully compact myelin of *Penaeus setiferus* determined by X-ray diffraction is 16 nm, which is similar to that of peripheral myelin in teleosts (Blaurock, 1986).

Fenestration nodes similar to those in earthworms, but differing in detailed ultrastructure, are found in six species of shrimp in the genus *Penaeus*, and in a number of copepods (Hsu and Terakawa, 1996; Xu and Terakawa, 1999; Weatherby *et al.*, 2000). In *Penaeus* shrimps node diameter

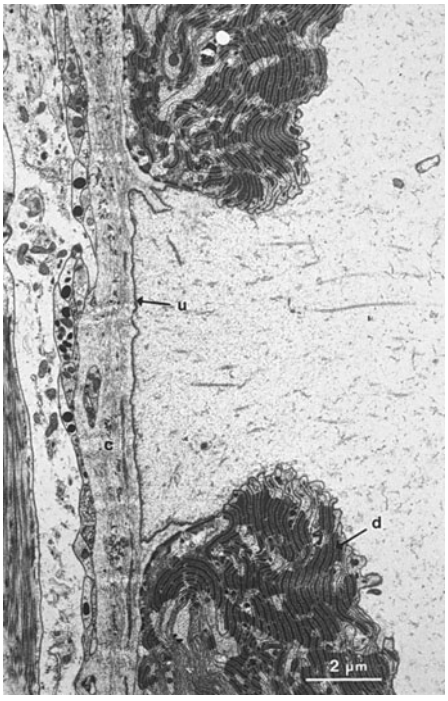

Fig. 6. Electron micrograph of a longitudinal section through the median giant fibre of *L. terrestris* passing through a node. The dorsal surface is on the left. The axonal cytoplasm protrudes through the node and abuts onto the collagenous sheath (c). Note the dense undercoating of the axolemma (u) and numerous desmosome-like structures (d) in the paranodal regions. Reprinted from Roots (1984) with permission.

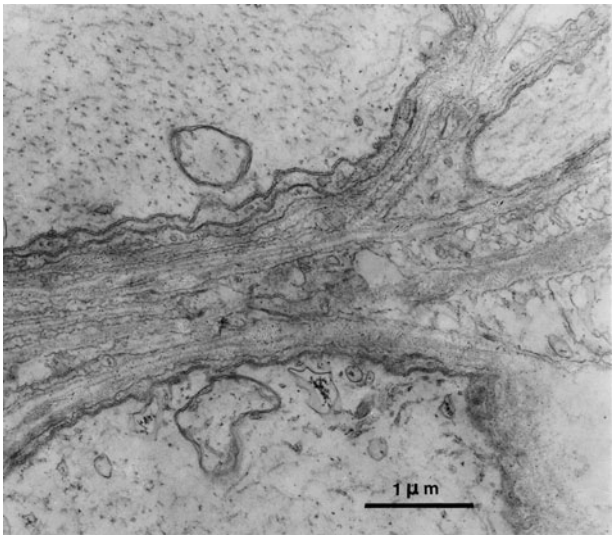

Fig. 7. Loosely wrapped myelin sheaths of two neighbouring axons in the ventral nerve cord of the crayfish *P. clarkii*. Reprinted from Roots (1995) with permission.

and internodal distance are both approximately proportional to fibre diameter. Node diameter varies between 5 and 50 μm and internodal distance between 3 and 12 mm (Xu and Terakawa, 1999).

In a number of decapod crustacea, shrimps (*Palaemon squilla*, *Palaemon serratus*, *Crangon* spp.), the prawn (*P. vulgaris*) and the crab (*Cancer irroratus*) and in a mysid, the opossum shrimp, *Mysis* sp., the nodes bear a striking resemblance to those of vertebrates, not only in general morphology (Retzius, 1890; Nageotte, 1916; Holmes *et al.*, 1941; Holmes, 1942) but also in the termination of the laminae on the axolemma with structures resembling septate desmosomes (McAlear *et al.*, 1958; Heuser and Doggenweiler, 1966). The internodal distance is generally shorter than in vertebrates (Holmes *et al.*, 1941). For a more detailed comparison of this type of crustacean node with vertebrate nodes, see Roots (1984).

Special mention must be made of the sheath of the crab, *C. irroratus*, which is exceptional in several respects and bears a striking resemblance to that of vertebrates. The glial cell nuclei are outside the sheath, the degree of compaction is similar to that of vertebrate compact myelin and structures resembling Schmidt–Lanterman incisures and nodes of Ranvier are present (McAlear *et al.*, 1958). This singular observation needs to be confirmed and extended to determine whether the sheath is wound concentrically or spirally.

Two other distinctive morphological features are found in shrimps of the genus *Penaeus*. One is a sheath of microtubule-packed glial cell processes closely apposed to the axon. This sheath, designated the microtubular sheath, is hypothesized to provide mechanical support to the axon (Xu and Terakawa, 1999). The other is a gel-filled space between the microtubular sheath (axon) and the myelin sheath. This submyelinic space effectively increases axon diameter, therefore increasing conduction velocity. Moreover, it is tightly sealed at the node regions, thereby permitting saltatory conduction and further increasing conduction velocity (Hsu and Terakawa, 1996; Xu and Terakawa, 1999).

ELECTROPHYSIOLOGY

Annelida

Conduction velocity and electrophysiological properties have been studied in the median giant fibre of *L. terrestris* (Günther, 1976). Conduction velocity is slower than would be expected for a vertebrate fibre of comparable size – see Table 1. A small diphasic positive/negative action potential travels along the length of the fibre except over the dorsal nodes where triphasic positive/negative/positive responses with large negative values occur. The big increase in the negative wave indicates pronounced current sinks in the node. Changes in the shape and amplitude of the longitudinal currents indicate a relatively high amount of leakage. High current losses may be explained by the high specific myelin capacitance of 0.28 μF/cm², which is about 100 times greater than the value for vertebrates, and a specific myelin resistance of 33 kΩ/cm², which is about five times smaller than the value for vertebrates. Thus the myelin sheath in *L. terrestris* is much less efficient than that of vertebrates. Although the dorsal nodes do mediate saltatory conduction the decrease in conduction time is small, only about 3.5 ms over a distance of 20 cm.

Table 1. Conduction velocities.

Nerve fibre	Diameter (μm)	Velocity (m/s)
Unmyelinated squid	500	20
Myelinated earthworm	90	30
Myelinated shrimp	120	90–219
Myelinated rat	4.5	59

The difference between earthworm and shrimp may be related to the efficiency of the nodes. In earthworm nodes reduce conduction time by only 3.5 m/s over a distance of 20 cm.

Increased current density through the nodes and blocking of the inward current during spike propagation by tetrodotoxin and local anaesthetics indicate that, as in vertebrates, sodium channels are concentrated at the nodes (Günther, 1976). However, channel density has not been estimated.

Arthropoda

Conduction in the median giant axons of the prawn, *Leander serratus*, is also slower than in vertebrate fibres of comparable size, 25 m/s for a 35 μm diameter fibre in prawn versus a 12 μm fibre in frog. In the giant fibres of shrimps of the genus *Penaeus* conduction velocities of 90–210 m/s have been recorded (Kusano, 1966; Kusano and LaVail, 1971; Terakawa and Hsu, 1991; Xu and Terakawa, 1999). Saltatory conduction occurs mediated not only by the fenestration nodes but also by functional nodes provided where branches arise and at the synapse area between the motor and giant fibres.

There is evidence that in the penaeid shrimps sodium channels are concentrated in both synaptic and fenestration nodes (Terakawa and Hsu, 1991; Hsu and Terakawa, 1996). A density of 1400–5000 channels/μm² has been estimated for synaptic nodal membranes (Terakawa and Hsu, 1991), and a much lower value of 530 channels/μm² for fenestrated nodal membranes (Hsu and Terakawa, 1996). For comparison, values given for squid giant axon vary from 100/μm² (Chandler and Meves, 1965) to 500/μm² (Levinson and Meves, 1975; Nonner *et al.*, 1975), and the value reported for rabbit unmyelinated C-fibre is 110/μm² (Ritchie *et al.*, 1976). However, considerable uncertainty exists regarding the density of sodium channels in the nodal membranes of penaeid shrimps. The estimates depend on assuming a value for the conductance of a single sodium channel. The value for rat nodes, 14 pS (Neumcke and Stämpfli, 1982), was used by both Terakawa and Hsu (1991) and Hsu and Terakawa (1996), but as Terakawa and Hsu (1991) point out, using the value for frog nodes (6.4 pS; Sigworth, 1980) would lead to a higher channel density for shrimp nodes. Another parameter for which there are only rough estimates is the area of nodal membrane involved (Terakawa and Hsu, 1991; Hsu and Terakawa, 1996). Clearly this is an area for future research.

COMPOSITION

Proteins

The composition of myelin membranes is known for only one annelid, the earthworm, *L. terrestris*, and one crustacean, the

pink shrimp, *Penaeus duorarum*. In both the protein components are very different from those of vertebrates. The protein pattern of earthworm myelin is relatively simple with 80 and 42 kDa proteins predominating and with 28–32 kDa proteins as minor components. There is no cross-reactivity with antibodies generated against mammalian myelin basic protein (MBP), proteolipid protein (PLP), myelin-associated glycoprotein (MAG) or 2′,3′-cyclic nucleotide phosphodiesterase (CNP) (Pereyra and Roots, 1988; Cardone and Roots, 1990). However, the presence of CNP in the glial cells of the tobacco hornworm, *Manduca sexta*, has been reported (Taylor *et al.*, 1976), and in the crab, *Ucides cordatus*, a monoclonal antibody to CNP binds to glial cells in the visual system (da Silva *et al.*, 2003). The significance of these findings awaits further research.

In the pink shrimp the pattern is slightly more complex. Four major proteins 21.5, 40, 78 and 85 kDa and four minor proteins 36, 41.5, 43 and 50 kDa are found in the sheath membranes. There is no cross-reactivity with mammalian MBP or PLP or with trout BP, 36K or IP1 antibodies (Okamura *et al.*, 1986; Waehneldt *et al.*, 1989). A monoclonal antibody generated to earthworm myelin-like membranes and showing cross-reactivity to 30–32 and 40 kDa proteins cross-reacts with 60–65, 42 and 40 kDa proteins in crayfish (*P. clarkii*) axon ensheathing membranes (Cardone and Roots, 1996). Thus, earthworm and crayfish membrane proteins have some antigenic epitopes in common. Until the amino acid sequences and structure of these proteins are known, the significance of this will remain obscure.

Lipids

Birefringence studies on the myelin sheath of earthworms showed that 60–70% of it is composed of lipids (Taylor, 1940). Isolated myelin membranes from earthworms have not been analysed for lipids. However, the lipid composition of whole nerve cords has been determined (Okamura *et al.*, 1985b). Cholesterol and phospholipids predominate with traces of glucocerebroside. Galactocerebroside, galactosulphatides and sphingomyelin were not detected. From these results it may be deduced that the myelin membranes contain cholesterol and phospholipids.

Myelin in the pink shrimp, *P. duorarum*, has a much higher lipid to protein ratio of 15:1. Cholesterol and phospholipids are the main components. Galactocerebroside and galactosulphatides were not found. A particularly interesting finding is the presence of substantial amounts of glucocerebroside in about the same proportion as galactocerebrosides in mammalian myelin. Sphingomyelin was found but its structure is different from that found in vertebrates (Okamura *et al.*, 1986). Findings are summarized in Table 2.

Table 2. Lipid composition.

	Vertebrata	Annelida	Crustacea
Cholesterol	+	+	+
Phospholipids	+	+	+
Galactocerebroside	+	−	−
Sulphatide	+	−	−
Glucocerebroside	Gadoid fish	trace	++++ +
Sphingomyelin	+	−	+diff structure
Gangliosides	+	−	−
Lipid:protein ratio	≃2:1	≃2:1	15:1

DISCUSSION AND CONCLUSIONS

The evolution of myelin is closely linked to the evolution of glial cells. Naked axons are common only in the coelenterates. In all other phyla glial lamellae, often overlapping, cover both axons and neuronal somata (Roots, 1978). It is thus easy to imagine how myelin sheaths with differing degrees of compaction may have evolved in crustacea, annelida and chordates. Nevertheless, it is a remarkable example of convergent evolution that myelin sheaths have evolved in such disparate clades.

Evidence for the separate evolutionary origins in the three clades lies in the information presented above. Annelids and chordates both have a spirally wound sheath with neighbouring lamellae attached by junctions, desmosomes and tight junctions in chordates and desmosome-like structures in annelids. The sheaths of crustacea are clearly different with concentrically arranged lamellae. In all three clades the sheaths have modifications, nodes, which permit saltatory conduction of nerve impulses. It is worth noting here that the ionic basis for the conduction of nerve impulses is universal as is the concentration of sodium channels in the nodal membranes. However, there have been some novel inventions in the crustacea. For example the submyelinic space in shrimps, which effectively increases axon diameter and therefore conduction velocity. A curious feature found in calanoid copepods is the development of the myelin as an internal organelle eventually forming concentric rings of membrane inside the axolemma (Wilson and Hartline, 2007, 2008).

The myelin proteins in the three clades are completely different. With the further separation between annelids and arthropods in current phylogenetic thinking, it is even more intriguing that proteins in earthworm myelin and crustacean glial cells have epitopes in common. Another curiosity is the presence of CNP in the glial cells of an insect, *M. sexta*, (Taylor *et al.*, 1976) and its possible presence in a crab (da Silva *et al.*, 2003). The function of CNP has been of particular interest since Drummond *et al.* (1962) first showed that the nervous system of vertebrates contained at least ten times more CNP than other tissues. The fact that the substrates, 2′,3′-cyclic nucleotides, for the enzymatic activity of CNP are not found in any tissue indicated that other functions needed to be sought (see Roots, 1981, for review). It has been suggested that in vertebrates CNP plays a role in stabilizing the myelin sheath through interactions with other proteins (Pereyra *et al.*, 1988). Thus, CNP appears to be a factor in the evolution of a compact highly efficient myelin sheath. It is now known that members of the 2′,3′-cyclic phosphodiesterase superfamily, to which CNP belongs, are involved in RNA metabolism and cellular signaling (Mazumder *et al.*, 2002). The 2′,3′-cyclic phosphodiesterases are almost universally present in all extant groups. Thus CNP is a member of a group of ancient proteins that have evolved to have multiple functions, not all of them as enzymes. Analyses of the crystal structure of individual members of the phosphodiesterase superfamily are throwing light on structure–function relationships and their evolution (Sakamoto *et al.*, 2005). It remains for further research to determine whether CNP

occurs in other invertebrates and to determine its role, if any, in their myelin sheaths.

Myelin across all three clades contains cholesterol and phospholipids. Virtually no cerebroside is found in annelids, whereas in crustacea glucocerebroside is present rather than the galactocerebroside found in deuterosomes. From their studies on a wide variety of species Okamura *et al.* (1985a) concluded that only glucocerebrosides are found in protostomes, whereas in deuterostomes galactocerebrosides and galactosulphatides predominate. Considerable amounts of glucocerebroside are found in gadoid fishes (Tamai *et al.*, 1992), indicating that deuterostomes retained the ability to synthesize glucocerebroside after acquiring the ability to synthesize galactosphingolipids.

A myelin sheath confers a number of advantages in the survival of invertebrates dependent upon startle reactions (earthworms), escape responses (crayfish, shrimp, copepods) and retraction of vulnerable organs (crab eyestalks) where high conduction velocity is crucial. See Bullock (1984), Schweigreiter *et al.* (2006), Roots and Gould (2007) for further discussion. Although increased axon diameter improves conduction velocity in squids (mollusca), which also depend on quick reactions, it is much less efficient and therefore evolutionarily less favourable than myelination.

In conclusion, the fact that annelids and arthropods are not considered now to be closely related only reinforces the conclusion reached previously (Roots, 1993) that myelin has arisen independently at least three times, namely in chordates, annelids and arthropods, providing a remarkable example of convergent evolution. A curious anomaly remains – myelin has not evolved in the mollusca.

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REFERENCES

Aguinaldo A.M., Turberville J.M., Linford L.S., Rivera M.C., Garey J.R., Raff R.A. *et al.* (1997) Evidence for a clade of nematodes, arthropods and other moulting animals. *Nature* 387, 489–493.

Blaurock A.E. (1986) X-ray and neutron diffraction by membranes: how great is the potential for determining the molecular interactions? In Watts A. and DePont J.J.H.H.M. (eds) *Progress in Protein–Lipid Interactions*. Elsevier Amsterdam, vol. 2, pp. 1–43.

Bullock T.H. (1984) Comparative neuroethology of startle rapid escape and giant-fiber mediated responses. In Eaton R.C. (ed.) *Neural Mechanisms of Startle Behavior*. Plenum Press, pp. 1–13.

Cardone B. and Roots B.I. (1990) Characterization of the myelin-like membranes in earthworm CNS using immunolabelling techniques. *Society for Neuroscience Abstracts* 16, 666.

Cardone B. and Roots B.I. (1991) Comparative studies of myelin-like membranes in annelids and arthropods. *Transactions of the American Society for Neurochemistry* 22, 148.

Cardone B. and Roots B.I. (1996) Monoclonal antibodies to proteins of the myelin-like sheath of earthworm giant axons show cross-reactivity to crayfish CNS glia: an immunogold electronmicroscopy study. *Neurochemical Research* 21, 505–510.

Chandler W.K. and Meves H. (1965) Voltage clamp experiments in internally perfused giant axons. *Journal of Physiology (London)* 180, 788–820.

Coggeshall R.E. (1965) A fine structural analysis of the ventral nerve cord and associated sheath of *Lumbricus terrestris* L. *Journal of Comparative Neurology* 125, 393–437.

Davis A.D., Weatherby T.M., Hartline D.K. and Lenz P.H. (1999) Myelin-like sheaths in copepod axons. *Nature* 398, 571.

Drewes C.P. and Brinkhurst R.O. (1990) Glial nerve fibres and rapid escape reflexes in newly hatched aquatic oligochaetes *Lumbriculus variegatus* (family Lumbriculidae). *Invertebrate Reproduction and Development* 17, 91–95.

Drummond G.I., Iyer N.T. and Keith J. (1962) Hydrolysis of ribonucleoside 2′3′-cyclic phosphates by a diesterase from brain. *Journal of Biological Chemistry* 237, 3535–3539.

Dunn C.W., Hejnol A., Matus D.Q., Pang K., Browne W.E., Smith S.A. *et al.* (2008) Broad phylogenomic sampling improves resolution of the animal tree of life. *Nature* 452, 745–749.

Fernández I., Pardos F., Benito J. and Roldán C. (1996) Ultrastructural observations on the phoronid nervous system. *Journal of Morphology* 230, 265–281.

Govind C.K. and Pearce J. (1988) Remodeling of nerves during claw reversal in adult snapping shrimps. *Journal of Comparative Neurology* 268, 121–130.

Graham A. (2000) Animal phylogeny: Root and branch surgery. *Current Biology* 10, R36–R38.

Günther J. (1973) A new type of 'node' in the myelin sheath of an invertebrate nerve fibre. *Experientia* 29, 1263–1265.

Günther J. (1976) Impulse conduction in the myelinated giant fibres of the earthworm. Structure and function of the dorsal nodes in the median giant fibre. *Journal of Comparative Neurology* 168, 505–531.

Hama K. (1959) Some observations on the fine structure of the giant nerve fibres of the earthworm *Eisenia foetida*. *Journal of Biophysical and Biochemical Cytology* 6, 61–66.

Hama K. (1966) The fine structure of the Schwann cell sheath of the nerve fiber in the shrimp (*Penaeus japonicus*). *Journal of Cell Biology* 31, 624–632.

Heuser J.E. and Doggenweiler C.F. (1966) The fine structural organization of nerve fibers, sheaths, and glial cells in the prawn *Palaemonetes vulgaris*. *Journal of Cell Biology* 30, 381–403.

Holmes W. (1942) The giant myelinated nerve fibres of the prawn. *Philosophical Transactions of the Royal Society London Series B* 231, 293–311.

Holmes W., Pumphrey R.J. and Young J.Z. (1941) The structure and conduction velocity of the medullated nerve fibres of prawns. *Journal of Experimental Biology* 18, 50–54.

Hsu K. and Terakawa S. (1996) Fenestration in the myelin sheath of nerve fibers of the shrimp: a novel node of excitation for saltatory conduction. *Journal of Neurobiology* 30, 397–409.

Jenner R.A. (2004) The scientific status of metazoan cladistics: why current research practice must change. *Zoologica Scripta* 33, 293–310.

Jones M. and Gardiner S. (1989) On the early development of the vestimentiferan tube worm *Ridgeia sp.* and observations on the nervous

system and trophosome of *Ridgeia sp.* and *Riftia pachyptila*. *Biological Bulletin* 177, 254–276.

Kusano K. (1966) Electrical activity and structural correlates of giant nerve fibers in *Kuruma* shrimp (*Penaeus japonicus*). *Journal of Cellular Physiology* 68, 301–383.

Kusano K. and LaVail M.M. (1971) Impulse conduction in the shrimp medullated giant fiber with special reference to the structure of functionally excitable areas. *Journal of Comparative Neurology* 142, 481–494.

Lenz P.H., Hartline D.K. and Davis A.D. (2000) The need for speed. I. Fast reactions and myelinated axons in copepods. *Journal of Comparative Physiology A* 186, 337–345.

Levinson S.R. and Meves H. (1975) The binding of tritiated tetrodotoxin to squid giant axons. *Philosophical Transactions of the Royal Society London Series B* 270, 349–352.

Mazumder R., Iyer L.M., Vasudevan S. and Aravind L. (2002) Detection of novel members, structure-function analysis and evolutionary classification of the 2H phosphoesterase superfamily. *Nucleic Acids Research* 30, 5229–5243.

McAlear J.H., Milburn N.S., Chapman G.B. (1958) The fine structure of Schwann cells, nodes of Ranvier and Schmidt–Lanterman incisures in the central nervous system of the crab *Cancer irroratus*. *Journal of Ultrastructural Research* 2, 171–176.

Nageotte J. (1916) Note sur les fibres a myéline et sur les étranglements de Ranvier chez certains crustacés. *Comptes rendus des scéances de la Société de biologie et de ses filiales Paris* 79, 259–263.

Neumcke B. and Stämpfli R. (1982) Sodium currents and sodium-current fluctuations in rat myelinated nerve fibres. *Journal of Physiology (London)* 329, 163–184.

Nicol J.A.C. (1948) The giant axons of annelids. *Quarterly Review of Biology* 23, 291–323.

Nonner W., Rojas E. and Stämpfli R. (1975) Gating currents in the node of Ranvier: voltage and time dependence. *Philosophical Transactions of the Royal Society London Series B* 270, 483–492.

Okamura N., Stoskopf M., Hendricks F. and Kishimoto Y. (1985a) Phylogenetic dichotomy of nerve glycerosphingolipids. *Proceedings of the National Academy of Sciences of the U.S.A.* 82, 6779–6782.

Okamura N., Stoskopf M., Yamaguchi H. and Kishimoto Y. (1985b) Lipid composition of the nervous system of earthworms (*Lumbricus terrestris*). *Journal of Neurochemistry* 45, 1875–1879.

Okamura N., Yamaguchi H., Stuskopf M., Kishimoto Y. and Saida T. (1986) Isolation and characterization of multilayered sheath membrane rich in glucocerebroside from shrimp ventral nerve. *Journal of Neurochemistry* 47, 1111–1116.

Pereyra P. and Roots B.I. (1988) Isolation and initial characterization of myelin-like membrane fractions from the nerve cord of earthworms (*Lumbricus terrestris* L.). *Neurochemical Research* 13, 893–901.

Pereyra P., Horvath E. and Braun P.E. (1988) Triton X-100 extractions of central nervous system myelin indicate a possible role for the minor myelin proteins in the stability of lamellae. *Neurochemical Research* 13, 583–595.

Retzius G. (1890) Zur Kenntniss des Nervensystems der Crustaceen. *Biologische Untersuchungen* [N.S.] 1, 1–50.

Ritchie J.M., Rogart R.B. and Strichartz G. (1976) A new method for labelling sagitoxin and its binding to non-myelinated fibres of the rabbit vagus, lobster walking and garfish olfactory nerves. *Journal of Physiology (London)* 261, 477–494.

Roots B.I. (1978) A phylogenetic approach to the anatomy of glia. In Schoffeniels E. Franck B. Hertz L. and Towers D.B. (eds) *Dynamic Properties of Glial Cells*. Pergamon Press, New York, pp. 45–54.

Roots B.I. (1981) Comparative studies on glial markers. *Journal of Experimental Biology* 95, 167–180.

Roots B.I. (1984) Evolutional aspects of the structure and function of the nodes of Ranvier. In Zagoren J.C. and Fedoroff S. (eds) *The Node of Ranvier*. Academic Press, Orlando, Fl, pp. 1–29.

Roots B.I. (1993) The evolution of myelin. In Malhotra S. (ed.) *Advances in Neural Science*, vol. 1, JAI Press Inc., Greenwich, CT, pp. 187–213.

Roots B.I. (1995) The evolution of myelinating cells. In Vernadakis A. and Roots B. (eds) *Neuron-Glia Interrelations During Phylogeny: I. Phylogeny and Ontogeny of Glial Cells*. Humana Press Inc., Totawa, New Jersey, pp. 223–248.

Roots B.I. and Lane N.J. (1983) Myelinating glia of earthworm giant axons: thermally-induced intramembranous changes. *Tissue & Cell* 15, 695–709.

Roots B.I. and Gould R.M. (2007) Evolution of myelinated systems. In Bullock T.H. Rubenstein J.L.R. and Kaas J.H. (eds) *Evolution of Nervous Systems Vol. 3 – Evolution of Nervous Systems in Non-Mammalian Vertebrates*. Elsevier Ltd, pp. 469–484.

Rousset V., Pleijel F., Rouse G.W., Erséus C. and Siddall M.E. (2007) A molecular phylogeny of annelids. *Cladistics* 23, 41–63.

Sakamoto Y., Tanaka N., Ichimiya T., Kurihara T. and Nakamura K.T. (2005) Crystal structure of the catalytic fragment of human brain 2′3′-cyclic-nucleotide 3′-phosphodiesterase. *Journal of Molecular Biology* 346, 789–800.

Schweigreiter R., Roots B.I., Bandtlow C.E. and Gould R.M. (2006) Understanding myelination through studying its evolution. *International Review of Neurobiology* 73, 219–273.

Sigworth F.J. (1980) The variance of sodium current fluctuations at the node of Ranvier. *Journal of Physiology (London)* 307, 97–129.

da Silva S.F., Bressan C.M., Cavalcante L.A. and Allodi S. (2003) Binding of an antibody against noncompact myelin protein to presumptive glial cells in the visual system of the crab *Ucides cordatus*. *Glia* 43, 292–298.

Tamai Y., Kojima A., Saito S., Takayama-Abe K. and Horichi H. (1992) Characteristic distribution of glycolipids in gadoid fish nerve tissues and its bearing on phylogeny. *Journal of Lipid Research* 33, 1351–1359.

Taylor D.P., Dyer K.A. and Newburgh R.W. (1976) Cyclic nucleotides in neuronal and glial enriched fractions from the nerve cord of *Manduca sexta*. *Journal of Insect Physiology* 22, 1303–1304.

Taylor G.W. (1940) The optical properties of the earthworm giant fiber sheath as related to fiber size. *Journal of Cellular and Comparative Physiology* 15, 363–371.

Terakawa S. and Hsu K. (1991) Ionic currents of the nodal membrane underlying the fastest saltatory conduction in myelinated giant nerve fibers of the shrimp *Penaeus japonicus*. *Journal of Neurobiology* 22, 342–352.

Waehneldt T.V., Malotka J., Kitamura S. and Kishimoto Y. (1989) Electrophoretic characterization and immunoblot analysis of the proteins from the myelin-like light membrane fraction of shrimp ventral nerve (*Penaeus duorarum*). *Comparative Biochemistry and Physiology* 92B, 369–374.

Weatherby T.M., Davis A.D., Hartline D.K. and Lenz P.H. (2000) The need for speed. II. Myelin in calanoid copepods. *Journal of Comparative Physiology A* 186, 347–357.

Wilson C. and Hartline D.K. (2007) The development of calanoid copepod myelin. Program No. 674.8. 2007 Neuroscience Meeting Planner. San Diego, CA: Society for Neuroscience 2007. Online.

Wilson C. and Hartline D.K. (2008) A non-glial source of myelin in copepods? Program No. 79.23. 2008 Neuroscience Meeting Planner. Washington, DC: Society for Neuroscience 2008. Online.

Xu K. and Terakawa S. (1999) Fenestration nodes and the wide submyelinic space form the basis for the unusually fast impulse conduction of shrimp myelinated axons. *Journal of Experimental Biology* 202, 1979–1989.

Zoran M.J., Drewes C.D., Fourtner C.R. and Siegel A.J. (1988) The lateral giant fibers of the Tubificid worm *Branchiura sowerbyi*: structural and functional asymmetry in a paired interneuronal system. *Journal of Comparative Neurology* 275, 76–86.

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