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The natural history of neuroglia: an agenda for comparative studies

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Neuroglia, which are the non-neuronal cells of the nervous system, except for meninges and vasculature with its endothelium, blood vessel walls and relatively rare smooth muscle cells, have received much attention in mammalian investigations and occupy a voluminous literature. The term has come to be understood as embracing at least three diverse types of cells: astrocytes, oligodendroglia and microglia, each of which might be heterogeneous in some degree. Schwann cells ensheathing the myelinated axons in the PNS resemble oligodendrocytes in the CNS and might be considered a fourth cell type, distinct from the connective tissue elements of the sheaths of bundles of axons or nerves. Although no one would claim we know enough about neuroglia in the best studied animals, the fact I want to point out here is that we know far less about these classes of cells in non-mammalian vertebrates and invertebrates. My thesis is that we may be overlooking major clues to the understanding of their roles and functions that could come from relatively modest effort to uncover their natural history in animals with less complex brains among the lower vertebrates and invertebrates. The techniques and findings that have been worked out in laboratory mammals can be expected to facilitate greatly the discerning of commonalities and differences in less familiar animals.

The aim of this piece is to point out some of the more obvious questions that might be important in view of what we know from mammals. It is hardly a review, so few are the relevant papers except for those concerning axonal sheaths. It is actually an agenda which could be formulated as existence hypotheses.

I begin with the so-called lower vertebrates – the classes that evolved earlier and seem generally less complex – fishes, amphibians and reptiles. The reason for this is that either from strong inference or hints in histological papers on these taxa we believe that they possess cells in each of the four categories – astrocytes, oligodendroglia and microglia inside the CNS and Schwann cells in the PNS – that are not obviously different from those in mammals (Cohen *et al.*, 1968; Kuffler, 1967; Kuffler and Nichols, 1964; Kuffler and Nichols, 1966; Kuffler *et al.*, 1966; Oejand *et al.*, 1966). Thereafter, I will consider some representatives from the main invertebrate phyla, mainly arthropods and gastropods.

The strong inference is that taxa exhibiting myelin sheaths possess cell types equivalent to oligodendroglia and Schwann cells because they are known to be the main basis for myelin sheaths of mammals, the layers of which consist of the cell membranes of these cells spirally wrapped, one cells per internode between Ranvier nodes to a consistent, controlled thickness (French-Constant *et al.*, 2004; Michailov *et al.*, 2004). The controlled thickness, specific for the diameter of axon being wrapped and the controlled length of axon between Ranvier nodes,

are such specialized features that it seems safe to assume the responsible cell type is equivalent in different classes that share these features. Internodal length varies markedly and it is difficult to say whether this feature is similar in diverse fish, amphibia and reptiles. But the detailed architecture of nodes and of spirally wrapped axons can be seen in published electron micrographs of rays and sharks, bony fishes, frogs, salamanders, turtles and lizards, and other representatives of these classes.

A remarkable finding, however, is that no myelin sheath has been found in lampreys or hagfish (Bullock *et al.*, 1984). It appears that real myelin sheaths with Ranvier nodes were invented by a vertebrate ancestor of the elasmobranchs and holocephalans (chimaeras and ratfish) both of which display well developed, abundant myelination and, therefore, presumably oligodendroglia and Schwann cells. Equivalent glial cells or astrocytes and microglia have not been looked for carefully by either electron microscopy or histochemical markers to distinguish them from each other in the agnathan groups (lampreys and hagfishes). The sudden appearance of oligodendroglia and spirally wrapped myelin in the earliest gnathostomes is the most dramatic saltation so far known in the evolution of neuroglia and must have made a large difference in some aspect of behavior, most likely in reflex time for those responses involving long distance propagation. Zalc and Colman (Zalc and Colman, 2000) consider that this invention permitted the success of vertebrates. However, time saved in conduction by large axons and those employing saltatory conduction is usually not great compared to time lost in 'nuclear delays', based on the rise time of summated synaptic prepotentials leading to spike firing.

This is the place to site recent work on sheaths in certain shrimps (*Penaeus* and others) that achieve conduction velocities higher than the fastest mammalian axons ($>200 \text{ m s}^{-1}$). Several invertebrate taxa have many layered sheaths of their large neurites (I usually use this term instead of axon for invertebrates because, generally, their neurons lack a contrasting dendritic arbor); they are often called myelin sheaths but none has been shown to have Ranvier-type nodes and spirally wrapped, single-cell-per-internode sheaths as in jawed vertebrates. The earthworm, *Lumbricus*, has three central giant fibers, often 50–100 micrometers in diameter, surrounded by twenty or more layers of cell membranes with the cytoplasm pretty well squeezed out. Nuclei here and there indicate that we are not dealing with a single glial cell spirally wrapping the neurite but some modification of shingling by several cells and without Ranvier nodes where all layers of sheath end together. Conduction velocity is high ($20\text{--}45 \text{ m s}^{-1}$, which equals and exceeds the squid giant axons of many times larger diameter that lack a multilayered sheath), but there is no evidence of saltatory propagation. Shrimps (*Penaeus*, *Leander* and others, with much variation among species) apparently achieve their high conduction velocities (Holmes *et al.*, 1941; Kusano, 1965; Kusano and La Vail, 1971; Fan *et al.*, 1961) by saltatory conduction with functional nodes in each segmental ganglion, where branches emerge or synaptic contact is made with motor neurons. This remarkable case was not known to Bullock and Horridge (Bullock and Horridge, 1965), who compared conduction velocity and fiber and sheath diameter in many species (Holmes *et al.*, 1941), although one paper in Chinese (Fan *et al.*, 1961) had already appeared announcing the unprecedented velocity. Confirmation followed in several laboratories (Kusano, 1965; Huang and Yeh, 1963; Kusano and La Vail, 1971; Hsu *et al.*, 1975), and some aspects of ionic currents in the presumptive nodes and in the huge space between the axon and the sheath (Fig. 1) were reported by Terakawa and Hsu (Terakawa and Hsu, 1991). Some crustaceans (*Cancer*) have nodes that seem very much like the Ranvier nodes of vertebrates (McAlear *et al.*, 1958). Some copepods (*Calanus*) (Weatherby *et al.*, 2000) of the order of a millimeter in total body length, have well developed giant fibers with sheaths considered by the authors to be true myelin, although spiral wrapping by a single glial cell and nodes with all laminae ending together have not been demonstrated.

The state of understanding is clearly not satisfactory and new work is needed with the same methods on several species, probably requiring reconstruction from serial electron micrographs. Some authors of studies on invertebrates have distinguished the common glial cells that fill spaces between neurons or nerve fibers from those that contribute to tightly packed laminated sheaths that are suggestive of oligodendroglia of vertebrates (see pp. 901–903 of Bullock and Horridge, 1965). In electron micrographs, the cross sections of many neurites are seen to be completely within a profile belonging to a glial cell. Possibly these last two cell types are one and the same kind of cell. Many, perhaps most, glial cells are part of the sheath of the single axons or bundles of axons or the ganglion, connective, or nerve. Small, flat glial cells hug most neuron somata. Especially needed are studies of the proteins involved, where they come from and how they communicate with axons. The lipids also need to be explored; so far myelinated species all have galactosylceramide as the most crucial molecule for myelin to occur (anonymous manuscript reviewer, personal communication).

Returning to the main theme, it is the assumption and assertion here that there must have been evolution of some anatomical features and physiological traits of the neuroglial cells during evolution of vertebrates from the earliest fishes to the latest mammals, as well as in the transition from invertebrate ancestors to the early vertebrates. It is my strong hunch that finding these changes would improve our understanding and amend our perspective in significant ways. This essay is primarily a call for new research to distinguish the conservative features and traits from those that have changed – in which higher taxa differ.

Examples of the questions that our present perspective suggests are:

- For different vertebrate classes, what percentage of brain volume is occupied by identifiable glia (astrocytes, oligodendroglia including myelin and microglia), neurons and intercellular space? There is a general impression that more advanced taxa have many more glial cells per neuron than ‘lower’ taxa, but this author cannot find the evidentiary basis for this generalization and wonders whether there is any general difference between more and less-advanced invertebrates. The latter have survived for a long time but may have been limited in certain behavioral respects. Clearly, speed of response matters but is not all important because many groups have and many lack giant fibers. Fiber size is not the only or the best way to increase velocity of conduction, but saltation, which is better and saves space, occurs here and there in a few taxa. Assuming that types of glial cells can be distinguished reliably by eye in rapidly and well-fixed electron micrographs, they can be hand painted and computer measured for the respective areas with due consideration of the sampling problem and the comparison of homologous regions in different vertebrate classes. By following the Van Haareveld protocol of rapid freezing, a potentially significant comparison could be made of the volumes of the intercellular compartment in tissues of different premortem history.
- A second domain of functional questions might include the following: Compared with mammals, what features of the ionic milieu are the glia of non-mammalian vertebrates and invertebrates concerned with? Are they concerned with taking up potassium after neuronal activity? Are they involved in the tight potassium barrier between blood and extraneuronal space, known for insects? Are glia concerned with control of glutamate release? Is there a scaffolding function of a subset of glia that guides neuronal processes in their growth toward genetically specified pathways? Is there a subset concerned with repair and reconstitution after injury and local mistakes in connectivity? Do glia play a greater or lesser role in actual information processing, for example in controlling the release of modulators?

It seems quite possible, given the terrestrial insect blood–brain barrier and the relatively facile regenerative capacity of many taxa, that neuroglia in non-mammalian vertebrates and invertebrates might have quite different roles and significance from those in familiar laboratory animals. If so, we should learn about the differences in order to appreciate neural evolution. As in other areas of comparative study, from Harvey onwards, we can anticipate benefits for applied medicine from such basic investigations without being able to predict what they will be.

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Fig. 1.

(A) Diagrammatic cross sections of large axons from three kinds of animals, showing the myelin sheath in the vertebrate and the quasi-myelinated sheaths in two kinds of invertebrates. In the vertebrate type, all laminae spirally wrap from a single glial cell and end together at a low-resistance region that defines the ends of an internode. The prawn-type also represents the earthworm, crayfish and lobster, where more than one glial nucleus occurs and there is no Ranvier node. The Kuruma shrimp are species of *Penaeus* and several other genera where functional nodes at segmental sites of axon branches, and synapses with motor neurons, allow saltatory propagation via the large, confined space ('gap') under the high-resistance sheath. (B) Arrangement of isolated, ventral nerve cords of a lobster and a shrimp to compare their

conduction velocities. Stimulating electrodes are at one end and recording electrodes at the other, with ~55 mm of cords between vaseline barriers. Note segmental ganglia. **(C)** Responses from the foregoing preparation. Note that the lobster latency is >7 ms (~ 8 m s⁻¹) whereas the Kuruma-shrimp response has a latency of a fraction of a millisecond ($\sim >90$ m s⁻¹). **(D)** Diagram to show the functional nodes at branches. Not illustrated are the regions of synapses between them. Note that the wavelength of the spike is more than the whole length of the cord. (Modified from Kusano, 1965; Kusano and LaVail, 1971.)