Review

Evolution of rapid nerve conduction☆

Ann M. Castelfranco, Daniel K. Hartline*

Békésy Laboratory of Neurobiology Pacific Biosciences Research Center University of Hawai‘i at Manoa, 1993 East-West Rd, Honolulu, HI 96822, United States

A B S T R A C T

Rapid conduction of nerve impulses is a priority for organisms needing to react quickly to events in their environment. While myelin may be viewed as the crowning innovation bringing about rapid conduction, the evolution of rapid communication mechanisms, including those refined and enhanced in the evolution of myelin, has much deeper roots. In this review, a sequence is traced starting with diffusional communication, followed by transport-facilitated communication, the rise of electrical signaling modalities, the invention of voltage-gated channels and “all-or-none” impulses, the emergence of elongate nerve axons specialized for communication and their fine-tuning to enhance impulse conduction speeds. Finally within the evolution of myelin itself, several innovations have arisen and have been interactively refined for speed enhancement, including the addition and sealing of layers, their limitation by space availability, and the optimization of key parameters: channel density, lengths of exposed nodes and lengths of internodes. We finish by suggesting several design principles that appear to govern the evolution of rapid conduction.

This article is part of a Special Issue entitled SI: Myelin Evolution.

© 2016 Published by Elsevier B.V.

Contents

1. Introduction ..................................................... 12
2. Selective advantages of rapid conduction ..................................................... 12
3. Non-regenerative signal conduction mechanisms ............................................. 13
   3.1. Chemical signaling by diffusion ..................................................... 13
   3.2. Faster signaling by chemical transport ............................................. 13
   3.3. Voltage-based passive signaling ..................................................... 15
4. Regenerative voltage-based conduction mechanisms ....................................... 16
   4.1. Signal conduction in multicellular organisms ....................................... 17
   4.2. Impulse conduction in plants ..................................................... 17

☆Dedicated to the memory of David Colman who was the inspiration for our long-standing interest in myelin evolution.
*Corresponding author.
E-mail address: danh@hawaii.edu (D.K. Hartline).

http://dx.doi.org/10.1016/j.brainres.2016.02.015
0006-8993/© 2016 Published by Elsevier B.V.
1. **Introduction**

The most rapid internal signal transmission in living organisms is provided by large myelinated nerve fibers. Wrapped in multiple layers of condensed membrane interrupted at intervals by nodes of intricate construction and conducting up to speeds over 200 m s\(^{-1}\), the morphological as well as the physiological differences between myelinated and non-myelinated nerve fibers could hardly be greater. This contrast is vividly illustrated in Fig. 1 with electron micrographs comparing fibers from the same nerve trunk in two closely-related calanoid copepods, one myelinate (Fig. 1A) and one amyelinate (Fig. 1B). It is less obvious why and how the complex structures on the right diverged from their simpler antecedents on the left. Three questions need to be addressed: First, what are the adaptive values of signal conduction speed that drive its evolution? Second, what innovations (“adaptations”) had to be evolved, and in what sequence, to elevate conduction speed above slower earlier-evolved values while retaining overall fitness? Third, are there common rules that guide designs for rapid conduction – patterns that reveal themselves through convergent evolution? In considering these it should be kept in mind that there are usually costs to every evolutionary innovation that must be weighed against the benefits. Ancestral species had to rely on indirect evidence. Clues to the evolutionary progression of conduction speed are found in present-day “basal” taxa that retain putatively ancestral characters (see Fig. 2). To these may be added evidence from developmental observations and especially, from our perspective, quantitative modeling.

2. **Selective advantages of rapid conduction**

The ultimate cause of an evolutionary advance is the adaptive value or selective advantage of the evolved trait. It is usually taken for granted that the speed of signal conduction has adaptive value, and the faster the better. Several such advantages might be considered: (1) Timely reaction to predatory attack is perhaps the most often-cited and obvious advantage (e.g. Hartline and Colman, 2007). The faster an organism can transmit the sensory information on such an attack to the central nervous system (CNS), integrate it and deliver motor impulses to appropriate muscles, the more likely its evasive behavior will be effective. (2) Similarly, rapid conduction decreases reaction times in launching and coordinating predatory attacks when opportunity arises suddenly and/or when precise timing is critical (Zalc and Colman, 2000). (3) It improves precision in temporal discrimination, since it reduces the impact of natural variations in conduction rates or path distances (Hartline, 2008; Wang et al., 2008). (4) Speed provides smaller temporal dispersions in transmitting information back and forth between the CNS and spatially-dispersed sensors and effectors. It can thus, for example, serve to better synchronize contraction of a
distributed muscle sheet as in cephalopod mantle (Pumphrey and Young, 1938). (5) Having a range of conduction speeds in a communication network allows designs that provide timely temporal coordination. Signals in a more rapidly-conducting pathway can be programmed to arrive ahead of those simultaneously originated in a more slowly-conducting one for example to achieve preemptive timing-overrides of slower-conducting circuits (Eaton et al., 1995). Synchronization of spike arrival times can be effected in pathways of different lengths by using faster conduction in the longer paths (Carr and MacLeod, 2010; Mellon, 2010). (6) Faster conduction allows faster computation and hence more computational sophistication in a given time interval, which thus allows evolution of more complex nervous systems (Wang et al., 2008). (7) It facilitates evolution of larger body sizes, since faster speeds are needed to keep in timely communication with increasingly distant parts (Hartline 2008).

In what follows, we trace a phylogenetically-guided sequence of speed-enhancing innovations, from improvements over chemical communication speeds through the invention of electrical signaling, to channels with integrated voltage sensors for impulse generation, then to core-conductor axons capable of reaching more distant points and optimized for speed, and finally to the different forms of the complex insulation of myelin.

3. Non-regenerative signal conduction mechanisms

3.1. Chemical signaling by diffusion

The small size of basal single-celled organisms allows rapid communication to be accomplished with slower communication modalities, and these appear to have been the first to evolve. In the Bacteria, Archaea and unicellular Eukaryota, communication is often by chemical diffusion and reaction (Na+, H+, Mg2+, signal proteins, etc.) as in the control of flagellar movement (Blair, 2003) and turning responses in chemotactic or phototactic bacteria (Zusman et al., 2007) and motile algae (Jékely, 2009). While the speed of diffusional communication in cells of bacterial size (μm) involves timescales of milliseconds for key small signaling molecules such as calcium, this timescale lengthens with the square of distance, which is a rapidly-increasing disadvantage as cells enlarge. Diffusion-dependent signaling still remains an important modality in higher organisms, as in the spread of calcium waves in astrocytes (Table 1; see below), at synapses and in parasynaptic (retrograde) transmission (Fitzsimonds and Poo, 1998), but the key to diffusional speed is employing small molecules over short distances. The need to circumvent this diffusional constraint as an organism evolves toward larger and more complex morphologies is likely to have provided the evolutionary impetus for the early emergence of faster conduction mechanisms.

3.2. Faster signaling by chemical transport

Chemical communication can be speeded through adaptations for actively transporting the signal (or effector) molecules. These include “advective” bulk transport of the fluid in which the chemical signal is embedded, as in cyclosis (cytoplasmic streaming) in certain plant cells (Wayne, 1994) and unicellular organisms, and carrier-based transport as in axonal transport in animals with nervous systems (Fitzsimonds and Poo, 1998) and circulatory-system-based communication using hormones. The relative effectiveness of transport vs. diffusional chemical conduction can be represented by the “Péclet number”, \( Pe = \frac{T_{\text{diffusion}}}{T_{\text{transport}} = UL/D} \), where \( U \) is the transportation velocity, \( L \) is the size of the communication dimension (e.g. cell length) and \( D \) is Fick’s diffusion coefficient (Verchot-Lubicz and Goldstein, 2010). \( Pe \approx 1 \) is the dividing line between diffusional-dominated and transport-dominated signaling. With \( D \) for

Fig. 1 – Cross-sections through homologous nerves in amyelinate and myelinate copepods. Transmission electron micrographs of cross sections through the antennular nerve of (A) adult amyelinate Pleuromamma xiphias; note the numerous mitochondria (mt; cf (B)), indicative of the greater metabolic cost of activity in non-myelinated axons; (B) adult myelinate Undinula vulgaris. Virtually every axon in U. vulgaris is myelinated to some extent, the sheath being thicker in larger axons. Mitochondria are scarce. Scale bar: 5 μm. Abbreviations: ax: axon core; mt: mitochondrion; my: myelin. From Lenz et al. (2000), with permission.
Fig. 2 – Speed-related phylogenies. (A) Cladogram showing phylogenetic relations currently recognized among the various taxa mentioned in the text (simplified). The eukaryotes, which include the plants (Plantae), multicellular animals (Metazoa), and Protozoa (here represented by ciliates) and fungi (not shown) are distinguished from the prokaryotes (Bacteria and Archea) in possessing nuclei and mitochondria. The Chlorophyta are single and multicellular green algae while the Angiospermae are the flowering vascular plants. Among the multicellular animals mentioned, both the radially-symmetric Radiata (ctenophores or comb jellies; cnidarians, including jellyfish) and the bilaterally-symmetric Bilateria (all other animal groups) have nervous systems (sponges, lacking this character, are not shown). (B) Simplified cladogram of myelinate (underlined; red in electronic publication) and closely related amyelinate bilaterian taxa (blue), each with members possessing giant axons in escape circuits. The Hyperoartia are lampreys while the Gnathostomata are the remaining vertebrate clades (fish and higher including extinct placoderms but not ostracoderms). The Stomatopoda of the crustacean class Malacostraca are mantis shrimp while the Natantia are decapod shrimp. The order Calanoida of the crustacean subclass Copepoda contains two major divisions of several superfamilies each, the Amyelinata lacking myelin in the nervous system and the Myelinata possessing it. Finally, the Polychaeta are marine worms while the Oligochaeta are earthworms and their kin, both in the phylum Annelida. Side panels: (B1), (B2), (B3) transmission electron micrographs of myelin from nerve fibers of, respectively, a caridean shrimp (Palaemonetes vulgaris), a dendrobranchiate shrimp (Litopenaeus vannamei), and an earthworm (Lumbricus terrestris). Abbreviations: ax: axon; des: desmosome-like structure; MGF: axoplasm of medial giant fiber; ms: microtubular sheath; mt: mitochondrion; my: myelin; sc: Schwann cell. Micrographs from: (B1) Heuser and Doggenweiler (1966) with permission; (B2) image by M. Orcine and D. Hartline, reproduced from www.podbase.net with permission; (B3) Günther (1976) with permission.
small molecules $\sim 10^{-6}$ cm$^2$ s$^{-1}$ (100 $\mu$m$^2$ s$^{-1}$) and intracellular transport processes reaching up to 100 $\mu$m s$^{-1}$ (Table 1), diffusion can dominate in organisms below $\sim 1$ $\mu$m (bacterial size). However, even for small molecules traveling at the slow rates of axonal transport (1 $\mu$m s$^{-1}$ when in motion: Roy et al., 2007) over distances of centimeters, Pe is much greater than 1, and transport becomes by comparison a "rapid conduction" modality (e.g. Mignot et al., 2007).

### 3.3. Voltage-based passive signaling

Transport-based communication even as high as 100 $\mu$m s$^{-1}$ will only suffice for organisms as large as small protozoans (ciliates is not found in prokaryotes, although other transport processes are: Zusman et al., 2007). Given that many predators can react in a matter of milliseconds, a faster means of communication is needed for organisms larger than ca. 10 $\mu$m (e.g. Hartline 2015). This need for speed was satisfied by the invention of voltage-based signaling. Voltage perturbations, even passively ("electrotonically") conducted, spread much more rapidly along cell membranes than chemical diffusion or transport (Table 1). Various protozoans have achieved this by employing membrane-localized electrical signaling in the control of their locomotion. In the 100–200 $\mu$m-long protozoan ciliate, Paramecium, a mechanical stimulus applied posteriorly activates a non-regenerative hyperpolarizing potassium current with a concomitant speeding of the forward propulsion by the cilia (Naitoh and Eckert, 1973; review: Martinac et al., 2008). The use of electrical signaling enhances the protozoan’s capability for responding rapidly and correctly along its entire elongate ciliated body to predatory attack from behind. While electrical signals are intrinsically rapidly-conducting, they are also intrinsically slowed by the capacitance of the lipid membranes upon which they depend for the separation of charge needed to sustain a voltage signal. They also lack the richness that chemical signaling offers. For evolution to optimize an electrical modality of signaling, it must adjust the parameter controlling the speed of conduction in myelinated fibers, e.g. myelinated Mauthner cell (70 $\mu$m, Günther, 1976), to a median giant (50 $\mu$m, Pumphey and Young, 1938) to an unmyelinated giant (100 $\mu$m, Govind and Lang, 1976) to an unmyelinated giant (100 $\mu$m, Kusano, 1966) to an unmyelinated giant (100 $\mu$m, Ochs, 1965).
of conventional spikes has the disadvantage of requiring intensity to be encoded in spike timing (e.g. frequency), which provides only intermittent rather than continuous information transfer. Passive graded signal conduction in electrotonically short processes, as for example in many dendritic trees, permits continuous but still timely spatial and temporal integration of local information prior to the spike generation used for long-distance communication. Vertebrate photoreceptors, bipolar and horizontal cells operate rapidly over short distances without spikes entirely, and even long-distance voltage-based signaling can be achieved without spikes, in neurons with elevated membrane resistance (Mirolli, 1979).

4. Regenerative voltage-based conduction mechanisms

As cells evolve more elongated forms, electrical communication between increasingly distant points becomes difficult with passive electrical processes, owing to electrotonic decrement. The pressure to maintain timely communication over increasing distances is a likely selective force promoting the evolution of regenerative signaling. This solution is based on coupling a voltage sensor directly or indirectly to an ion channel. The coupling comes in two forms, a “graded” form in which a voltage perturbation of extrinsic origin is augmented by intrinsic mechanisms without being self-sustaining, and a self-sustaining action potential, typically of an “all-or-none” character. As an early example, also from Paramecium, the same mechanical stimulus that elicits a non- (or weakly) regenerative voltage signal when applied to the posterior end, when applied to the anterior end, elicits a regenerative (albeit not all-or-none) calcium action potential in the cilia, which causes ciliary reversal (Eckert et al., 1972; Naitoh et al., 1972; review: Eckert and Brehm, 1979). This enables the protozoan to respond within milliseconds by rapidly backing away from collisions with objects in a manner again coordinated along its entire body. Calcium diffusion by itself from end to end of this 100 μm organism would take ~10 s, and transport-based signaling ~1 s. Although most reported

---

Fig. 3 - Diversity of action potentials. Representative voltage responses of various excitable tissues from non-bilaterian taxa. (A) Intracellular recording of a response of a Paramecium to an abrupt mechanical stimulus (S) to the anterior end. The initial rapid depolarization is the receptor potential, which triggers a regenerative calcium potential (from Eckert et al., 1972, with permission). (B) Two examples of exumbrellar epithelial potentials recorded with extracellular suction electrodes from a scyphozoan, Chelophyes, following electrical stimulation (S), of which the deflection labeled P1 is the most consistent (from Mackie, 1965, with permission). (C) Electrical response of an unspeciﬁed plant to electrical stimulus at the apex (top) recorded extracorporally with 4 electrodes spaced along the stem. Note the inter-electrode time delay. Time, amplitude and distance scales not given. From Fromm and Lautner (2007), with permission. (D) and (E) Intracellularly-recorded action potentials from giant axons of Aglantha digitale during a fast swim (D) and an endogenously-generated slow swim (E). Two recording sites (labeled “A” and “B” on records) spaced respectively 2.5 and 6.9 mm apart. Calculated conduction velocity of the two spike types respectively 1.4 m s⁻¹ and 0.3 m s⁻¹. From Mackie and Meech (1985) with permission.
regenerative spike-producing mechanisms depend on depolarizing (positive-going) membrane mechanisms, hyperpolarization-activated regenerative hyperpolarization does occur: It can be produced in *Paramecium* in a potassium-free medium (Eckert and Brehm, 1979), in Necturus inner segments amplifying the negative-going primary receptor potential (Werblin, 1975) and mediating rapid relaxation in Ascaris oesophageal muscle (del Castillo and Morales, 1967) among others (Grundfest, 1961).

### 4.1. Signal conduction in multicellular organisms

With the evolution of multicellularity, the need arose to coordinate activity among multiple individual cells by extending impulse conduction to intercellular communication. The most widely-used solution has been the evolution of special channels directly communicating between adjacent cells. Such communication channels probably evolved initially to pass small signaling molecules of different kinds. Throughout much of the animal kingdom this communication mode is provided by gap junctions, which are broadly produced by the pannexin family of proteins (also connexins in chordates and plasmodesmata in plants) (White et al., 1995). Such systems evolved in basal metazoans (Mackie, 1965) and, with a few notable exceptions, are nearly ubiquitous between cells in physical contact, whether or not dependent on electrical signaling. Gap junctions in vertebrate brain have recently come into focus as the means by which waves of ATP-activated calcium entry into astrocytic networks spread. While still only partially understood, these slow-propagating waves are limited by the central role played by chemical diffusion (Table 1; e.g. Charles, 1998; Stout et al., 2002). However, the same gap junctions enable communication through the flow of ionic current and hence electrical signals. In such a role, they become “electrotonic connections” or “electrical synapses” according to the time-scale of signals being passed. Gap-junction-mediated spread of electrical signals among epithelial cells has been identified as the modality for producing coordinated swimming behavior in certain basal organisms to have employed the voltage-regenerative modality of conduction to communicate over their length (e.g. Findlay, 1959). For producing the inward currents of regenerative impulses, neuroscientists are used to thinking solely in terms of Na\(^+\) and Ca\(^{2+}\), both cations, as charge carriers, with voltage-gated cation-selective channels as their conduits. However, the stonewort alga Nitella (and the related Chara) respond to predator-induced injury with chloride-based action potentials to communicate along the several centimeter-long cell to halt protoplasmic streaming and minimize damaging cytoplasmic loss (Wayne, 1993). The exit of Cl\(^-\) through chloride/anion-selective channels provides the main depolarizing inward current sustaining this action potential. The signal propagates at rates comparable to those for calcium spikes in cnidarian epithelia and axons (Table 1). Thus at some level, choice of ionic species has little intrinsic impact on impulse speed. The ubiquity of calcium channels as charge carriers in many cells may be largely owed to the role for calcium established early in evolutionary history as a diffusion-conducted messenger within cells. Voltage changes originally produced as a byproduct of receptor or stimulus-based calcium permeability changes may have facilitated the emergence of faster voltage-gated processes.

Nor is there an intrinsic necessity for voltage signals to be depolarizing (positive-going), as the regenerative hyperpolarizations in *Paramecium*, Ascaris, and vertebrate rods illustrate (§4). The few cases of regenerative hyperpolarization that have been investigated identify potassium ions as the primary charge carrier (del Castillo and Morales, 1967; Eckert and Brehm, 1979; Werblin, 1975). While a few voltage-based signals involve conductance decreases (e.g. vertebrate photoreceptors, Werblin 1975), the lengthening of overall membrane time constant makes this an unlikely mode for the most rapid communication.
4.4. Molecular substrates

Accompanying the evolutionary changes in the morphological and physiological traits that underlie more rapid conduction is of course much molecular evolution, most of which we will not review (see reviews in this issue by Inouye and Kirschner (2016), Li and Richardson (2016) and Zalc (2016)). However the evolution, diversification, and activation mechanisms of inward current channel proteins are particularly relevant to speed issues. Inward current activation for “rapid” conduction in the relatively basal alga, Nitella is a two-step process: depolarization gates the entry of calcium ions from extracellular (or tonoplast) sources, and the calcium ions in turn activate chloride channels internally, which produces the depolarization needed for regeneration of the signal (Wayne, 1994). The chloride channels do not themselves contain a voltage sensor. This two-step process makes it intrinsically slower than one employing an integral voltage sensor (Castelfranco, 1988). Before the current picture emerged for vertebrate nerve, a two-step conduction process was once proposed involving local acetylcholine release followed by short-distance diffusion and receptor binding (Nachmanson, 1961; Ritchie, 1967). Single-step activation of voltage through an intrinsic voltage sensor does not in itself assure rapid channel kinetics. When expressed in mammalian cell lines, the bacterial sodium channel, which contains such a sensor, is much slower to activate than eukaryote sodium channels (NaV; Ren et al., 2001). Further, the closely-related (some have suggested ancestral to NaV) calcium channels for all of their diversity in the service of a variety of functions, are slow compared with NaVs. It has been suggested that this ability to utilize rapidly-conducting electrical signals, in particular the voltage-sensitive variety, arose during evolution from a non-voltage-sensitive ancestral ion-channel molecule consisting of two trans-membrane segments (S5 and S6) connected by an ion-selective pore-lining “p-loop”. To this was then added a voltage-sensor complex consisting of four trans-membrane segments (S1–S4) (Moran et al., 2015). This strategy has been retained (or convergently evolved) and elaborated upon (as with the joining of four such subunits to form calcium and sodium-selective channels) to produce the principal voltage-gated channels of more rapidly-conducting metazoan nerve.

Fig. 4 – “Intrinsic” conduction velocities for various nerve fibers vs. fiber diameter. Lines indicate general relations over a range of diameters, modified from Bullock and Horridge (1965) by adjusting to a standard temperature of 20 °C using a Q10 of 1.8 (Chapman and Pankhurst, 1967) and an internal and external ionic conductivity of a squid axon (35.4 Ω cm). Thus $v_{\text{meas}} = 1.8^{\text{temp}/10} \times \sqrt{(35.4/R_{\text{axoplasm}})}$ where $T$ is the temperature in °C for the measured velocity $v_{\text{meas}}$ and $R_{\text{axoplasm}}$ is the specific resistance of the axoplasm, if available, or the extracellular medium if otherwise. Specific labeled points or lines from the following sources: Squid: Hartline and Young (1936) cited in Pumphrey and Young (1938); Penaeus and Macrobachium: Kusano (1966). Lobster MG and LG (medial and lateral giants): Govind and Lang (1976); Hydromedusa: Mackie and Meech (1985).

5. Origins of the nervous system

Multicellular structures for rapid conduction have evolved independently in the lateral giant axons of decapods (Payton et al., 1969) and the medial giants of earthworms (Oesterle and Barth, 1981). In these, the lengths of active portions of each cell, joined end-to-end, are electrotonically short (<100 axon diameters), with strong electrical coupling between cells provided by gap junctions. Generally, however, the multicellular cell-to-cell “bucket brigade” approach to signal conduction tends to be slow, as it typically involves circuitries with high access resistances and capacitances (e.g. Lieberman et al., 1973). Also, electrical synapses, while more rapid than chemical ones, still produce a synaptic delay. Such delays may be acceptable or inevitable for some communication, especially local, but pressure for timely signaling to remote parts has led to evolution of elongate cellular processes and their accompanying transport mechanisms, resulting in long thin filiform cells specialized for communication: “neurons”.

5.1. Metazoan electrical communication

Surviving examples of the neuronal communication system that arose early are found in ctenophores and cnidarians. Their nervous systems produce regenerative impulses capable of traveling long distance through interconnected networks of axons from sites of input to effector organs (Satterlie, 2015). The communication is, however, generally slower than in nerve fibers of more advanced groups (hydrozoan points in Fig. 4). Part of the explanation for this appears to be the inheritance from protist ancestors of relatively slow voltage-gated channels. The particularly slow ones are permeable to calcium rather than sodium, but even axons with sodium-gated channels fall below the curve for more advanced groups in conduction speed for a given diameter (Meech and Mackie, 1993). Indeed, the employment of
calcium as a charge carrier in long-distance axonal conduction is rare, even among cnidarians. Sodium is by far the dominant charge carrier in rapidly-conducted metazoan action potentials, probably in part, at least, due to its adaptability to more rapid gating (see discussion in Meech, 2004). Nevertheless, the early evolution of a voltage-gated calcium channel molecule is a major advance in communication, since it has allowed linking of four previously separate subunits into a single molecule capable of forming a channel on its own, the different portions of which can be tailored to different functions, such as inactivation, within the same molecule (Moran et al., 2015).

6. Cables as substrates for rapid propagation of regenerative signals

6.1. Theoretical considerations: the Huxley function

The application of core-conductor theory (Kelvin, 1855; Fig. 5) to passive signal spread in axons by Rushton (1951) and others was followed by the research of Hodgkin and Huxley (1952) on the biophysics of action potential propagation along such cables. It is with these advances that additional factors affecting conduction speed became apparent. Huxley (1959) analyzed solutions of the Hodgkin–Huxley (HH) equations as a general case of a wave traveling along a uniform cable at constant velocity and showed that the velocity, \( v \), can be represented as the product of two factors dependent on the five basic parameters describing the active cable:

\[
\nu = a^{1/2}f(\beta), \quad \text{for } a = k d / (R C) \quad \text{and} \quad \beta = \gamma / (k C),
\]

where \( k \) is a scaling parameter for the rate constants of the active channels, \( d \) is the diameter of the axon, \( R \) is the specific resistance of axoplasm, \( C \) is the specific membrane capacitance of the axon, and \( \gamma \) is a scaling parameter for the conductance density of the active channels. The “Huxley function”, \( f(\beta) \), is a unitless empirically-determined function, which is plotted, normalized to squid axon parameters (indicated by the subscript “0”), in Fig. 6A (see caption for details). Multiplying \( f(\beta) \) by \( a^{1/2} \) for any set of values of the five parameters yields a predicted velocity for that set. Three such (normalized) plots are superimposed in Fig. 6B: varying the activation rate constant factor (\( k_0 \); broken line), varying the membrane capacitance factor (\( C_0 \); dotted line) and varying peak channel conductance factor (\( \gamma_0 \); solid line). Note that the latter line coincides with the \( f(\beta) \) line, since conductance does not contribute to \( a \). Thus the analysis predicts a maximum of \( f(\beta) = 1.16 \) for \( \beta = 6.5 \), which gives a peak in the velocity vs. conductance relation (Fig. 6B). Hodgkin (1975) and others have recognized that the sodium channel density in the squid giant axon may have evolved to maximize conduction velocity. However, the maximum for the Huxley function predicts a channel density much greater than that actually observed (Castelfranco and Hartline, 2015), and even carefully refined models of the squid action potential that include voltage-dependent gating currents predict an optimal sodium channel density significantly larger than the densities observed in nature (Sangrey et al., 2004). A solution was proposed by Crotty et al. (2006), who considered the metabolic cost of conduction as well as velocity in the calculation of optimal channel densities. They found that for a given conduction velocity and axon diameter, the squid densities minimize the metabolic cost of the action potential. Thus the adaptive impetus to evolve faster conduction is tempered by adaptive forces from other sources, and these must be quantitatively assessed before a clear model for the evolution of rapid conduction can be established with confidence.

6.2. Enhancing cable conduction: rate kinetics

In addition to optimizing channel densities, another way to increase conduction speed is to speed the kinetics of the
active channels. The Huxley formulation shows, and model runs and experimental manipulations confirm, that conduction velocity of the squid axon can be increased by increasing the rate constants (principally the opening rate for sodium; increasing temperature is one way to increase channel rate kinetics uniformly). However, the increase in speed obtained is only modest, as the $f(\beta)$ curve is nearly flat for axons with squid parameters, so conduction speed only increases with the square root of the rate-constant factor.

6.3. Enhancing cable conduction: $r_1$-based mechanisms

Basal metazoans invented a third mechanism for speeding communication, exploiting the fact that a lowered internal core resistance, $r_c$, of an axon enhances the electrotonic spread of current and hence increases the velocity of nerve impulses. Two strategies have evolved over time for doing this: increasing the inner diameter of the axon core and decreasing the specific resistance of the electrolyte contained in that core.

6.3.1. Axon diameter

Enlarging axon diameter operates on the principle that internal axial resistance decreases rapidly as the square of diameter. For unmyelinated fibers, impulse propagation velocity thus increases with diameter, usually with close to a square root dependence (Fig. 4; Pumphrey and Young, 1938; but for deviations, see e.g. Hoffmeister et al. (1991) and Govind and Lang (1976)). In terms of evolution, this should be a relatively easy route to rapid conduction, since the mechanisms for regulating axonal diameter already exist for the developmental program. The principle is taken to an extreme in the evolution of “giant axons” several times larger than other large axons in a nervous system. Such giant axons occur throughout the Metazoa, often associated with escape or other rapid reactions (see e.g. Bullock (1984) for an overview). The most basal phylum in which giant fibers have been described is Cnidaria. Studies by Mackie and Meech (1985) found two types of swimming behavior in the hydrozoan Aglantha, both mediated by propagating all-or-none spikes in giant (40 $\mu$m) axons. One is a slow swim mediated by slow calcium spikes (Fig. 3E); the other a fast escape swim elicited

Fig. 6 – Huxley function, $f(\beta)$. (A) Log-log plot of the empirically-derived function normalized to squid axon parameters as a function of the parameter $\beta = \gamma_0/(k_0 C_0)$ where the subscripts "0" indicate normalization with respect to squid values: $\gamma_0$ is the scaling factor for ionic conductances ($g_{Na} = 0.12$ S cm$^{-2}$; $g_K = 0.036$ S cm$^{-2}$; $g_{leak} = 0.0003$ S cm$^{-2}$); $k_0$ is the scaling factor for conductance rate constants $\alpha$ and $\beta$ for $m$, $n$ and $h$ in the Hodgkin–Huxley (HH) model relative to their value at 6.3 °C (Hodgkin and Huxley, 1952) and $C_0$ is the specific capacitance of the axolemma relative to 1 $\mu$F cm$^{-2}$ for squid. Symbols are points for particular model runs of a uniform cable having HH channels and electrical constants normalized with respect to those in the HH squid model. Dotted line is a best-fit parabola through the points having the equation log $f(\beta) = 0.0155673 + 0.14659 \log(\beta) - 0.11828 \log^2(\beta)$ and from which the curves in (B) were computed. (B) The Huxley development predicts a conduction velocity, $v_0$, relative the that of the squid axon (12.3 m s$^{-1}$) given by $v_0 = a^{1/2} f(\beta)$, where $a = k_0 d_0/(\rho_0 C_0)$, $d_0$ is the axon diameter relative to that of the HH squid unmyelinated axon (476 $\mu$m) and $\rho_0$ is the specific conductance of axoplasm relative to the squid value (35.4 $\Omega$ cm). The panel shows log–log plots of normalized velocity, $v_0$, as the normalized parameters $k_0$ (coarse broken line), $\gamma_0$ (solid line) and $C_0$ (dotted line) are varied from their standard value of 1.0 (abscissa). Decreasing $C_0$ or increasing $k_0$ from this value increases conduction velocity, but log–log sensitivity is greater for $C_0$ by a factor of two at this starting level. The effect of decreasing $C_0$ plateaus at ~8 $\times$ squid values while increasing $k_0$ plateaus at ~2 $\times$ without altering other parameters (indeed conduction fails at the right edge of the graph). Increasing $\gamma_0$ above 1.0 increases conduction velocity of axons but this reaches a maximum with $\gamma_0 > 1\%$ above the normal squid value. Sensitivity to $k_0$ is even less for initial $\beta < 1$ (fine-broken line labeled $f_{100} = 0.1$). Reprinted with permission from www.pbrc.hawaii.edu/~danh/MyelinEvolution/.
by faster-conducting sodium spikes (Fig. 3D; Table 1). Remarkably, both types of spikes, sodium and calcium, are carried in the same axon. The separation between the two systems is based on threshold and amplitude partitioning that allows the sodium spike and fast swim to be elicited only by strong stimulation evoking large depolarizations. Despite the size of these axons, propagation velocities for both spike types are well below the norm for unmyelinated axons of comparable diameter in more advanced taxa, as mentioned above (§5.1; Fig. 4; this and subsequent work reviewed by Meech (2004)). Giant-fiber-mediated escape systems are found in Reptantia (lobsters and crayfish) triggering the “tail-flip” response in this group (e.g. Johnson, 1924; Wiersma, 1947; Wine and Krasne, 1972). A similar system of giant fibers has been found in copepods, both myelinate and amyelinate (Lowe, 1935; Park, 1966) and is presumed to mediate their extremely rapid escape reactions (Lenz et al., 2000). Those of polychaetes have been extensively reviewed by Nicol (1948). In tube worms they mediate the rapid withdrawal reflex (e.g. Krasne, 1965). The large size of the giant axon in the sabellid, Mixicola, has facilitated study of ionic mechanisms of impulse conduction parallel to squid studies (e.g. Goldman and Schaaf, 1973; Goldman, 1991). Among insects the collection of giant fibers in the ventral nerve cord have been shown to underlie the rapid directional response of a cockroach to air puffs, such as would be produced by a predatory attack (Camhi and Tom, 1978). Fig. 7 shows an electron micrograph of a cross section through such a “giant” fiber in a predatory caterpillar, an inchworm relative that launches a rapid attack with the anteriorly-located grasping appendages, possibly mediated by this fiber. Scale bar: 2 μm. Image by J. Kong, reproduced from www.podbase.net with permission. All rights retained by the creator.

Fig. 7 – Giant fiber from the ventral nerve cord of a carnivorous caterpillar, Eupithecia orichloris. The caterpillar, a member of the geometrid family (inchworms), when contacted on posteriorly-located hairs by a small insect, launches a rapid attack with the anteriorly-located grasping appendages, possibly mediated by this fiber. Scale bar: 2 μm. Image by J. Kong, reproduced from www.podbase.net with permission. All rights retained by the creator.

by faster-conducting sodium spikes (Fig. 3D; Table 1). Remarkably, both types of spikes, sodium and calcium, are carried in the same axon. The separation between the two systems is based on threshold and amplitude partitioning that allows the sodium spike and fast swim to be elicited only by strong stimulation evoking large depolarizations. Despite the size of these axons, propagation velocities for both spike types are well below the norm for unmyelinated axons of comparable diameter in more advanced taxa, as mentioned above (§5.1; Fig. 4; this and subsequent work reviewed by Meech (2004)). Giant-fiber-mediated escape systems are found in Reptantia (lobsters and crayfish) triggering the “tail-flip” response in this group (e.g. Johnson, 1924; Wiersma, 1947; Wine and Krasne, 1972). A similar system of giant fibers has been found in copepods, both myelinate and amyelinate (Lowe, 1935; Park, 1966) and is presumed to mediate their extremely rapid escape reactions (Lenz et al., 2000). Those of polychaetes have been extensively reviewed by Nicol (1948). In tube worms they mediate the rapid withdrawal reflex (e.g. Krasne, 1965). The large size of the giant axon in the sabellid, Mixicola, has facilitated study of ionic mechanisms of impulse conduction parallel to squid studies (e.g. Goldman and Schaaf, 1973; Goldman, 1991). Among insects the collection of giant fibers in the ventral nerve cord have been shown to underlie the rapid directional response of a cockroach to air puffs, such as would be produced by a predatory attack (Camhi and Tom, 1978). Fig. 7 shows an electron micrograph of a cross section through such a “giant” fiber in a predatory caterpillar, an inchworm relative that launches a rapid attack with the anteriorly-located grasping appendages, possibly mediated by this fiber. Scale bar: 2 μm. Image by J. Kong, reproduced from www.podbase.net with permission. All rights retained by the creator.

6.3.2. Core specific conductivity

A method for reducing \( r_I \) that does not increase neuronal mass is to provide the core conductor with more highly-conducting medium. This has been the case for marine invertebrates, which, in order to maintain body fluids isotonic with the surrounding sea water, maintain a high axoplasmic ionic strength giving a low specific resistance (e.g. 35 Ω cm for squid giants: Hodgkin and Huxley, 1952). With much lower ionic strengths in vertebrates and non-marine invertebrates, axoplasmic specific resistances are typically 3-fold higher, so a marine invertebrate axon of a given size can conduct almost twice as fast (a correction for this has been made in the plots of Fig. 4). This principle has been carried even
further by penaeid shrimp, in which the heavy myelin sheath surrounds a large extracellular tube (Yeh et al., 1962; Xu and Terakawa, 1999; Figs. 2B2 and 8D). Instead of axoplasm, much of the interior of the tube is filled with fluid having conductivity close to that of sea water as the core conductor (23 Ω cm; Kusano, 1966), which in turn is predicted to increase conduction speed by 25% above that of squid axons of comparable diameter.

In principle, altering the external medium could have a similar effect on conduction, either by altering its conductivity or especially by increasing the electromotive driving force (EMF) on the inward current carriers, as has been shown by artificial manipulation (e.g. Katz, 1947; Hardy, 1973). While such a mechanism is utilized to amplify mechanoreceptive signals in the ear (Ochs, 1965), we are unaware of examples of its being used to specifically speed nerve impulse conduction. However, in a related phenomenon, the specialized perineurium of insects provides their nervous system with an assured supply of the necessary sodium ions required for reliable conduction speeds in the face of much lower, highly variable, and potentially disruptive ionic compositions of hemolymph (Treherne and Schofield, 1981).

7. Myelination

The final step in the evolution of increasing conduction speeds in nervous systems, and the one that frees axons from negative allometry, is the invention of myelin (Fig. 4). It is presently known in four widely-separated taxa: vertebrates, oligochaetes (earthworms), decapod shrimp and copepods (Roots, 1984; Davis et al., 1999; Schweigreiter et al., 2006; Hartline and Kong, 2008). While its form in invertebrates differs from that in vertebrates, its function, based on physiology and morphology, is identical. The myelinate taxa are separated phylogenetically by major amylinate taxa (Fig. 2B). Except for the two well-separated crustacean taxa, the last common ancestor of the myelinate groups appears to be a basal bilaterian, leading to the conclusion that it has emerged through evolutionary processes independently at least four times.

7.1. Myelinate taxa and myelin organization

Evidence for the emergence of myelin with the rise of the gnathostomes from ancestral craniates in the Ordovician is reviewed by Zalc (2016) in this issue. That for oligochaete myelin was first reported by Taylor (1940) and has been examined in detail at the electron microscopic level by Hama (1959) and Günther (1976) and extensively by Roots and her collaborators (e.g. Roots 1984, 2008). It consists of a spiral wrapping of 20–200 layers by a glial cell or cells (Fig. 2B3). It has a mixture of compact and less-compact regions with thin sheets of cytoplasm included in the latter. In the earthworm, myelination is confined to the giant axons, which conduct at speeds double those of unmyelinated axons of the same diameter and temperature (Eccles et al., 1932; Günther, 1976) (Fig. 4). No estimates for its emergence date have been published, to our knowledge. Myelin in decapod shrimp was reported by Friedländer (1889) thirty years after its first description in vertebrates by Virchow (1858, Rosenbluth, 1999) (extensively reviewed in a monograph by Xu and Terakawa, 2013). A Permian emergence for myelin in this group has been suggested (Lenz, 2012). Over a hundred
years after its description in shrimp, it was found to be ubiquitously distributed in another crustacean group, the more derived superfamilies of the copepod order Calanoidea (Davis et al., 1999; Lenz et al., 2000). Based on an analysis of changing selective forces, Bradford-Grieve (2002) has suggested an emergence for myelinate copepods of about 270 million years ago. A contrasting organization is afforded by these two crustacean taxa, consisting of concentrically rather than spirally-arranged membrane layers. Each layer encircles the fiber with few if any discernable connections between layers. In decapod shrimp, margins of each myelin layer abut where they meet, forming a "seam" built up from the enlarged and aligned marginal contacts (Heuser and Doggenweiler, 1966; Xu et al., 1994; Xu and Terakawa, 1999; Fig. 2B1,2). A concentric layering more easily insulates the inner axon than does a spiral one since tight seals are only needed at the margins of the layers, if any, to prevent short circuiting. In copepods, even marginal contacts are eliminated, the layers being formed from cisternae internally-deposited layer by layer against the inside of the nerve fiber with no breaks or gaps in fully-formed myelin (Wilson and Hartline, 2011b; Fig. 1B). The concentric geometry in shrimp and copepods reduces the need for compactness, which is potentially required in spirally-wrapped myelin to prevent current escape to the outside along spiral pathways between uncompacted layers. Thus crustacean myelin is sometimes compact and sometimes not, with little consequence for its insulating properties (Hartline, 2008). Permitting thin layers of cytoplasm within myelin layers likely facilitates supplying nutrients and other metabolic and signaling functions throughout the myelin layers and to the adjacent axon, as has been suggested for the Schmidt-Lanterman incisures and other intramyelinic cytoplasmic structures of vertebrate myelin (e.g. Raine, 1984; Nave, 2010). It increases the size of a myelinated fiber for a given amount of insulation, which can be a disadvantage where space is limited.

### 7.2. Node structure

Most mature myelins possess nodes, functionally equivalent to nodes of Ranvier in vertebrates. Vertebrates and caridean shrimp have circumferential or "annular" nodes, meaning nodes that encircle the axon core and provide a short cylindrical segment of axolemma with electrical access to the surrounding conducting medium (Fig. 8B). Earthworms and penaeid shrimp utilize a structure termed a "focal" or "fenestrated" node in the two groups respectively (Günther, 1976; Hsu and Terakawa, 1996). In these nodes, a small opening occurs in the myelin, giving a patch of axolemma access to the outside medium. In earthworms, an axoplasmic process penetrates the myelin to form a surface more or less even with the outer layers (Fig. 6C). In penaeids, a depression is carved in the myelin layers penetrating to the underlying axon (Fig. 8D). Copepods, possessing internal myelin, form abrupt "cliffs", where all myelin layers terminate in a line roughly perpendicular to the encircling axolemma. Their myelin in some cases only partially covers the inside of the fiber. In such axons conduction would be continuous (non-saltatory) but presumably fast (Wilson and Hartline, 2011b). The copepod architecture highlights the fact that nodes and saltatory conduction are not rigid requirements for conduction-speed increases by myelin.

### 7.3. Advantages and disadvantages

In addition to augmenting conduction speed, the evolution of myelin confers a few more advantages on myelinate species. At some trade-off cost to the extra speed that it promotes, it provides the possibility of a more compact nervous system. This has been singled out as particularly helpful for craniates, which encase their nervous systems in a heavy bony protection that is less costly in weight if its size is limited (Ochs, 1965). With a similar trade-off, regulating its parameters (thickness; node size and spacing) provides an alternative to regulating axon length and diameter as a method for controlling conduction time along a pathway for precise temporal coordination (Kimura and Itami, 2009; Pajevic et al., 2014; Seidl, 2014). An often-cited hypothesis for a myelin advantage is that by reducing the number of channels that need to open to produce an impulse, myelin decreases the number of ions that have to be pumped out after the impulse passes, potentially greatly reducing the energetic cost of activity (e.g. Rogart and Ritchie, 1977; Stiefel et al., 2013). By having nodes out of register spatially, myelin restricts the points for ephaptic interaction, allowing such to be better controlled than in unmyelinated axons (Hartline, 2008; Debanne et al., 2011). Myelin also potentially generates a thermal tolerance for the conduction safety factor, the failure temperature in simulated myelinated axons being significantly above that in unmyelinated ones (Moore et al., 1978; Castelfranco and Hartline, 2015). Myelin also comes with certain disadvantages, chief of which is nutritional. It requires a substantial amount of lipid-rich membrane and associated membrane proteins. In particular, protostomes (most invertebrates) don’t make their own cholesterol, a key ingredient of the membranes, which then has to be obtained from the diet. This may thus be particularly limiting in myelinate protostome taxa (Lenz, 2012). In addition, recent attention has turned to the cost of manufacturing and maintaining vertebrate myelin, which may turn out to offset its energetic savings (Harris and Attwell, 2012). Myelin also still occupies space (see §8.2), and as mentioned (§7.1), potentially complicates transport of substances across the sheath and to the invested axon. In evolution, features unrelated to speed interact with those promoting speed to approach an optimal mix.

### 8. Theoretical considerations: effects of myelin structure on conduction velocity

Conduction velocity is impacted by several morphological features of myelin. Of particular interest are those features that are common to more than one taxon in which myelin has evolved. By taking advantage of the underlying biophysical properties of nerve conduction, computational modeling and simulation provide a technique for examining the effects on conduction velocity of individual changes in the structure of the myelin sheath, allowing tests of hypotheses on myelin evolution that cannot be conducted as easily in natural
systems. While the approaches most often used for such studies involve varying one, or at most two, parameter(s) at a time, it should be kept in mind that it is unlikely that only one parameter at a time would be optimized by natural selection. Rather, the collection of sheath parameters that influence conduction velocity would be varied together. Nevertheless, this approach provides a way of evaluating whether a predicted evolutionary pathway could have proceeded without introducing traits that are disadvantageous to a functioning nervous system.

As a speed-enhancing mechanism, capacitance reduction is an effective alternative to reduction of longitudinal resistance (e.g. Eq. (1) above). The myelin sheath reduces the capacitance of the axon in several ways: in the multi-layered membrane coating investing the internodes; in the small size of the regions of exposed axolemma, reduced in some organisms to “focal” nodes, and in the wide internodal separation between the exposed nodal regions. Hence increasing the number of membrane layers in the sheath, elongating the ensheathed internodal segments and reducing the area of the nodes all have the effect of speeding the charging of the membrane and promoting increased conduction velocity.

8.1. Capacitance reduction through sealing glial shunts

Unmyelinated axons come nevertheless with a membranous glial sheath. This sheath fails to have much effect on conduction velocity because the 20 nm periaxonal gap in combination with a short mesaxon having a similar gap gives ion current flowing between axon and surrounding glial layer ready access to the external conducting spaces. Both calculations and measurements suggest this ensheatheent presents little impedance to escaping current (Frankenheuser and Hodgkin, 1956; Kuffler, 1967; Binstock et al., 1975). If the innermost glial cells were to completely seal the axon off from access to the external conducting spaces, the external longitudinal resistance, being then determined solely by the limited periaxonal space, would rise and impulse conduction would slow (e.g. Young et al., 2013b). If, however, gaps are left in the glial sealing (“protonodes”), at some point salatory conduction commences and even with just one or two unit membranes of capacitance-reduction, conduction speed increases (Castelfranco and Hartline, 2015). Relocation of ion channels to the protonodes then increases the molecular economy of the axon and the path to further evolution of myelin is open.

8.2. Capacitance reduction through multi-layering

Adding multiple layers of sealed membrane reduces the transverse capacitance and increases the transverse resistance of a sealed region (internode) of a cylindrical fiber in proportion to the number of layers. Simply increasing the thickness of the myelin sheath around an axonal core of fixed diameter without constraining the overall fiber diameter results in an increase in conduction velocity that saturates at large fiber diameters or thick sheaths provided other parameters such as node size and channel density are kept constant (Fig. 9A; Smith and Koles, 1970; Moore et al., 1978).

Even very small-diameter axons will experience an increase in conduction velocity if myelin is added with no other constraints, although the speed-up will saturate with fewer layers than for larger axonal cores. If instead fiber diameter is fixed, as for example with a constraint on available space (e.g. an enclosed and heavy cranium: Zalc and Colman, 2000), there is a thickness that maximizes conduction velocity (Rushton, 1951; Goldman and Albus, 1968). This observation has led to the concept of an optimal ratio of axon core
diameter to overall fiber diameter (including the sheath), termed the g-ratio. The optimal g-ratio of 0.6 predicted by Rushton agreed with physiological observations primarily taken from the vertebrate peripheral nervous system (Gasser and Grundfest, 1939; Waxman and Bennett, 1972). If the impact of increasing fiber diameter on the volume of the nervous system is included as an explicit constraint in the optimization, the optimal g-ratio is shifted toward larger values (i.e. thinner sheaths) the greater the cost of increasing nervous system size. This provides a possible explanation of the difference between the g-ratios observed in the vertebrate peripheral and central nervous systems (Chomiak and Hu, 2009). The g-ratio of 0.6–0.7 cannot be maintained for fibers less than the ca. 0.06 μm diameter set by the thickness of a single myelin lamella (∼12 nm in vertebrates, Raine, 1984; ∼9 nm in penaeid shrimp, Xu and Terakawa, 1999; ∼18 nm in copepods, Weatherby et al., 2000), which likely contributes to the observed lower limit on myelinated fiber diameter. Below this, a layer of myelin rapidly loses its speed-enhancing advantage. If one allows the possibility that active channel densities co-evolved with the addition of multiple layers of myelin, then the analysis based on Huxley’s (1959) relation (viz §6.1) predicts that if active conductance densities decrease in proportion to the decrease in capacitance, the conduction velocity will continue to increase until further myelin expansion is blocked by other physiological constraints (see Fig. 9B; Castelfranco and Hartline, 2015). The resistance of a layer of myelin also in principle affects the conduction velocity, although variation in myelin resistance between brain regions may have a relatively small effect on it (Bakiri et al., 2011).

8.3. Capacitance reduction by restriction of node size

Reducing the area of the axolemma exposed at the node is another mechanism for decreasing overall trans-fiber capacitance and hence one that likely impacts the evolution of conduction velocity. In general, it results in an increase in conduction velocity as long as the conductance density required to generate an impulse isn’t compromised (Moore et al., 1978; Young et al., 2013b; Castelfranco and Hartline, 2015). In a fully-myelinated fiber, a significant proportion of the conduction-slowing trans-fiber capacitance is localized at the node, relative to that of the internode. Thus, for equal specific capacitances of a unit membrane for myelin and axolemma, nodal and internodal capacitances will be equal for internodes that are n_m times the length of the flanking nodes, where n_m is the number of myelin unit membranes in the sheath (i.e. twice the number of lamellae). For example, a node 1 μm long has approximately the same capacitance as an internode of 0.1 mm with 50 myelin lamellae, which would correspond to a 30% lower conduction velocity than with an infinitesimally small node (Castelfranco and Hartline, 2015), and 10% the capacitance of a more standard 1 mm-long internode (6% lower velocity). Thus decreasing the size of an already small node can have a significant effect on conduction velocity. For vertebrates, these nodes have further reduced capacitance by decreasing the diameter of the axon core passing through the node and hence the exposed surface area (Robertson, 1959; Rydmark, 1981). In contrast, however, an expansion of surface area at nodes has been reported in caridean shrimp by Heuser and Doggenweiler (1966; confirmed in unpublished studies by M. L. Orcine and D. K. Hartline), which should slow the conduction owing to the increased capacitance. This expansion may have other benefits such as allowing a higher channel density at nodes.

Modeling studies have confirmed the basis on which small node size enhances conduction speed. Moore et al. (1978) found that the increase in conduction velocity due to a 50% reduction in the nodal specific capacitance was almost canceled out by a similar reduction in the nodal specific maximum conductances. Hence, reducing the nodal area in a way that decreased both the nodal capacitance and conductance had only a small effect on conduction velocity in their model. Another study has shown that if channel densities averaged over the whole fiber were kept constant, then as nodal area was reduced, predicted conduction velocity increased even with only a single layer of myelin (Castelfranco and Hartline, 2015). Halter and Clark (1993) found that conduction velocity was quite sensitive to nodal area in their model of a mammalian myelinated nerve fiber. In particular, for a range of fiber diameters, conduction velocity increased when the diameter of the axon core was constricted along the node and paranode while keeping the nodal length and specific permeabilities of the ionic channels fixed. This trend did not begin to reverse due to the associated increase in longitudinal axonal resistance until the radius was reduced to one sixth of the internodal radius. The nodal and paranodal radii that gave peak conduction velocities agreed well with measurements of cat myelinated fibers (Rydmark, 1981).

8.4. Capacitance reduction by extension of internode length

The relationship between internode length and conduction velocity is more complex, and depends on the other assumptions being made in the model (or on the details of the situation being studied). If the node size is assumed to be fixed, Rushton (1951) found that for the class of fibers satisfying the condition that dimensions scale in proportion to internode length, there is an optimal ratio of internode length to fiber diameter (∼100), which agreed with observations from physiological studies on mammalian nerves and modeling studies (Hursch, 1939; Goldman and Albus, 1968; Basser, 2004). Even myelinated fibers of penaeid shrimp have been observed to conform to this optimal ratio, the internode length being in the range from 70 to 100 times the fiber diameter (Hsu and Terakawa, 1996). Huxley and Stämpfli (1949) argued that near the maximum the dependence of conduction velocity on internode length might be nearly flat, with even relatively large reductions in internode length having but a small effect on conduction velocity. Brill et al. (1977) examined the effect of internode length on conduction velocity for a wide range of ratios of internode length to fiber diameter. By varying the internode length while holding all other parameters, including diameter, fixed, they found that conduction velocity increased rapidly with increasing internode length for short internodes, reached a broad maximum for intermediate lengths and finally slowed with conduction...
failing for very long lengths. This was confirmed in another study, which found that the optimal internode length (i.e. the length that corresponds to the maximum conduction velocity) depended on the thickness of the myelin sheath, with the optimal length shifting to longer internodes as the thickness increased (and capacitance dropped) (Castelfranco and Hartline, 2015). Another condition that affects the theoretical results is whether the models describe the myelin sheath as distinct from the axolemma, including current flow in the submyelin space as in “double cable” models (Blight, 1985; Halter and Clark, 1991; Hines and Shrager, 1991; McIntyre et al., 2002). Overall these detailed models show a relationship between conduction velocity and internode length similar to that described by Brill et al. (1977), but the predictions of the effect on conduction velocity of a specific change in internode length can differ (Lasiene et al., 2008; Young et al., 2013a; Young et al., 2013b). In particular, the optimal internode length depends on the amount of sealing around the margins of the myelin sheath at the nodes, with the peak conduction velocity occurring at shorter internodes the tighter the seal, as shown in Fig. 10. The shift to longer optimal internode lengths as the access resistance at the paranode is deceased reflects the compensatory effect of increasing longitudinal resistance by lengthening the path of the submyelin current from the node to the internode. This shift corresponds to a change in the shape of the conduction velocity versus internode length curve with the peak becoming broader and flatter as the seals are reduced. Hence, depending on the tightness of the sheath, a given change in the internode length might result in an increase or a decrease or little change in conduction velocity (see Fig. 10; Lasiene et al., 2008; Young et al., 2013a). Allowing other parameters such as node size to co-vary along with the internode length can also modify the effect on conduction velocity, e.g. if the node increases in proportion to the increase in the internode then there is no optimal internode length and the velocity declines with increasing internode length from an elevated “limiting” velocity (Castelfranco and Hartline, 2015).

While it is difficult in experimental preparations to separate the individual effects on conduction velocity of these capacitance reducing changes, there have been several studies that have succeeded in varying one property of the myelin sheath without changing others. The basic relationship between conduction velocity and internode length described by Brill et al. (1977) has been supported by physiological studies that have varied internode length without significant changes in axon diameter and myelin thickness. Mice expressing mutant forms of periaxin, a protein required for Schwann cell elongation, have shortened internodes with normal axon diameters and g-ratios. At three weeks, the average internode length in quadriceps nerve fibers in the mutants was 250 μm compared with ~450 μm in the wild type and the conduction velocity was reduced by more than 50% (Court et al., 2004; Wu et al., 2012). As the nerves and internodes lengthened this effect was reduced in mutants having a less severe phenotype: at 16 weeks the internode length was still significantly shorter than in the wild type (479 μm compared with the wild type average length of ~700 μm) but there was no significant difference in conduction velocity (Wu et al., 2012). In a study of limb lengthening in rabbits the internode length was increased ~35% above normal but there was no significant difference in conduction velocity, although the velocity was slightly reduced in the lengthened limbs (36.0 ± 1.6 m s⁻¹ compared with 39.2 ± 2.2 m s⁻¹ in the controls) (Simpson et al., 2013). Court et al. (2004) verified that the dependence of conduction velocity on internode length that they had observed was consistent with theoretical predictions by using a model based on that of McIntyre et al. (2002), with geometric parameters taken from their experimental measurements (Court et al., 2004; supplementary Fig. 3). In addition to showing the steep dependence of conduction velocity on internode lengths in their experimental range (250–500 μm), they found that there was only a small change in velocity (1 m s⁻¹ ~4%) for internode lengths between 750 μm and 1500 μm. Hence, these physiological studies confirm the theoretical prediction that if the normal internode length is close to the optimum then there is a broad range of internode lengths (both shorter and longer than normal) for which the conduction velocity is nearly normal (see Fig. 10, e.g. curves corresponding to δ₀ = 1 nm or 0.1 nm). Michaelov et al. (2004) found that mutant mice with reduced expression of Neuregulin-1, which regulates sheath thickness, had significantly thinner myelin (and larger g-ratios) in sciatic nerve axons with no significant change in

![Fig. 10](image-url) - Effect of sealing the margins of the myelin sheath (paranodes) on the relationship between conduction velocity and the spacing between the nodes. The amount of sealing in the 1 μm compartments flanking the nodes is indicated by δₚ with smaller values of δₚ corresponding to tighter seals (for details see Castelfranco and Hartline, 2015). The bottom curve (δₚ = 10, broken line with filled circles) has no additional sealing beyond the close apposition of the sheath along the internode. The top curve (solid line, filled triangles) with 10⁻¹⁰ MΩ cm⁻¹ seals adjacent to the nodes approaches the behavior of a model with lumped axolemma and myelin sheath at the internodes and no submyelin shunt currents. The simulated fiber had a 10 μm diameter axon core, 0.5 μm nodes, 1.0 μm paranodes, 100 myelin lamellae (g-ratio = 0.73) and HH sodium and potassium conductances at the nodes. As the node spacing increased, the internode length increased and the channel density was adjusted to keep the Huxley parameter β fixed at 2.0. The simulations were run at 6.3 °C using NEURON (Hines and Carnevale, 1997).
internode length nor axon size distribution, but the conduction velocity of the tail nerve was reduced from 34 ± 0.65 m s⁻¹ in the wild type to 26 ± 1.34 m s⁻¹ in the mutant. This supports the model predictions that thinner myelin sheaths result in decreased conduction velocity (Moore et al., 1978; Smith and Koles, 1970).

8.5. Sheath integrity and sealing of capacitance-shunting currents

The addition of an insulating myelin sheath, with just mesaxonal seals, is sufficient to increase nerve conduction velocity without the evolution of specialized paranodal structures to restrict the shunting of nodal currents under the myelin sheath and between the myelin lamellae (Hartline, 2008; Young et al., 2013b; Castelfranco and Hartline, 2015). However, with the exception of copepod myelinated fibers in which the stack of myelin lamellae is condensed against the axolemma, providing no access to the extracellular medium (Wilson and Hartline, 2011b), such specialized structures are required to fully exploit the speed enhancements properties of the sheath (see Fig. 10, increased seals are represented by smaller δ, values). In vertebrates, palaeonid and penaeid shrimp, the terminal loops of the myelin layers at the paranodes are attached to the axon by septate junctions, which contribute to the sealing function (Heuser and Doggenweiler, 1966; Rosenbluth, 1984; Hsu and Terakawa, 1996). Although the vertebrate paranodal junction serves to electrically isolate the node, the seal is far from tight, instead there is a narrow pathway along the transverse bands that runs the length of the paranode and allows slow diffusion of metabolites. This pathway is so narrow and the junction is so long that the resistance is sufficiently high relative to the extracellular current path to block most of the shunt current (Rosenbluth, 2009). The importance of blocking these shunt currents for rapid conduction velocity has been confirmed in modeling studies that use a double cable model to include the submyelin space along the internode (Hines and Shrager, 1991; Halter and Clark, 1993; Stephenova and Daskalova, 2005; Castelfranco and Hartline, 2015). The thicker the myelin sheath, the more critical the sealing around the nodes is for conduction velocity. For thin sheaths consisting of a few myelin layers (<10 lamellae for a 10 μm diameter axon core), the addition of seals at the paranode had little effect on conduction velocity (Castelfranco and Hartline, 2015). This implies that for fully developed myelin sheaths conduction velocity is quite sensitive to the disruption of the paranodal seals due to disease or injury. It also suggests that paranodal seals might have been relatively late in evolving, as the myelin itself became optimized. Another source of disruption of the myelin sheath is shunting between the myelin lamellae. Devaux and Gow (2008) found the tight junctions between myelin layers of the optic nerve were absent in Claudin 11-null mutant mice. Although the mutants had normal myelin sheaths in terms of g-ratio and internode length, the conduction velocity for small axons (~0.7 μm in diameter) was reduced by more than 50% while that of large diameter axons (~2.1 μm) was normal. The reduction of conduction velocity only for small diameter axons was confirmed by simulations of a model that included the tight junctions as a resistance in series with the axolemmal and myelin cables in a double cable model (Devaux and Gow, 2008; Gow and Devaux, 2008). This illustrates the principle that the conduction velocity of fibers with small axon core diameter is more susceptible to disruptions in the integrity of the myelin sheath and the shunting of currents between the sheath and the axolemma or through the sheath than that of fibers with large core diameters. It supports the speculation arising from other studies (e.g. Young et al., 2013b) that myelination might have evolved earlier in larger axons when its parameters were first being adjusted by evolution and then extended to smaller axons as the effectiveness of the sheath was improved.

8.6. Channel density and kinetics

Ionic channel densities must have co-evolved with the elongation of the myelin sheath resulting in enriched sodium channel densities in the nodes and decreased densities in the internodes with an overall reduction in the mean number of sodium channels per length of axon compared with an unmyelinated axon (Vanbick and Shrager, 1998; Hartline, 2008). In particular, increasing the ionic channel densities does not necessarily result in increased conduction velocity, as might be inferred from the Huxley relation described in §6.1. By adjusting densities to maintain the Huxley parameter, β, fixed at 6.5, we showed for a simple model of myelination that channel densities should decrease along with increases in sheath thickness in order to maintain maximal conduction velocity (Fig. 9B; Castelfranco and Hartline, 2015). This predicts that myelination should result in decreased overall channel densities – increased densities in the nodes but decreased total densities in the axon – and reduced metabolic costs of impulse conduction. Another prediction of this analysis is that sodium channel kinetics might co-evolve with myelination, since as myelination proceeds, the charging of distant membrane becomes more rapid and hence activation kinetics could become the limiting factor for conduction velocity. For example, Fig. 9A shows that increasing activation kinetics 3-fold, e.g. from k₈=1 to k₈=3, results in a greater relative increase in conduction velocity for thicker sheaths (e.g. 100 vs. 10 unit myelin membranes). Indeed activation of penaeid channels, which underlie action potentials that propagate even faster than those in vertebrates, is faster than vertebrate channels at the same temperature (130 vs. 150–180 μs; Terakawa and Hsu, 1991). Thus, myelination could provide selective pressure for the evolution of fast-activating sodium channels.

9. Evolution of myelin

Putting all of this together, what might the likely scenario be for the evolutionary sequence leading to rapid conduction in ultimately myelinate organisms? One key constraint, as mentioned in the introduction (§1), is the need to retain a functioning nervous system. How can this be assured? Several lines of reasoning point to the initiation of myelin evolution occurring in larger diameter axons: larger axons are less affected by imperfections in a newly-evolving sheath
since the access and shunting produced by an electrolyte-filled periaxonal shell of a given gap size are proportionately less the larger the axon. Further, in simulations in which a pre-formed sheath was tightened around an unmyelinated axon as a way of progressively increasing insulation, conduction velocity slowed a bit before speeding up with the onset of saltatory conduction. This slowdown would present a barrier to this route for myelin evolution. However the slowdown, and hence the barrier, was minimal for larger axons (Young et al., 2013b). The occurrence of giant axons in pre-myelin ancestors is likely in any event, since the selective forces promoting myelin evolution will have already led to the evolution of large diameters. Availability of such giant axons would then provide the opportunity for myelin to evolve under more permissive conditions. In fact as mentioned, in some organisms, such as earthworms, only the giant axons are myelinated (Günther, 1976; Hama, 1959). An evolving sheath might avert a slow-down with a “nodes first” route, arising initially as small patches of myelin membrane closely apposed to the axolemma, either externally from the attachment of a glial process (as at present-day nodes in most taxa) or by the tight apposition of an internal cisternal membrane (as in copepods: Wilson and Hartline, 2011b). For glial myelin, a “mesaxonal seal first” route would be an alternative possibility in which sheaths form by sealing (e.g. condensing) mesaxons over short longitudinal segments, the diverted external current being carried in a compensatory expansion of extracellular pathways around the submyelin space “isolated” thereby. In either case, Huxley’s relation (Eq. (1)) predicts a resulting speed gain, since the increase due to capacitance reduction will be greater than the loss from conductance decreases for parameters centered around squid axon values (Fig. 6). Furthermore, channel loss to patch isolation could be easily compensated in immediately adjacent membrane. Once such an isolated patch completely encircles an axon, conduction becomes technically saltatory. Strong selective pressure would then act to elongate or add layers to the myelin of the initial ensheathed patch. It is possible that lengthening and thickening of the sheath would occur in close succession, both since result in large increases in conduction velocity initially (Figs. 9A and 10). As a thicker sheath evolves the need for developing specialized seals at the margins of the nodes would become more pressing. Ultimately the node spacing, sheath thickness and blocking of shunt currents would co-evolve to produce a myelin sheath that supports conduction at nearly maximal velocity.

Not surprisingly these steps are consistent with the process of myelin development in many organisms (Carpenter and Bergland, 1957; Xu et al., 1994; Vabnick and Shrager, 1998; Wilson and Hartline, 2011a; Simons and Lyons, 2013; Snidero et al., 2014). In developing vertebrate nervous systems large diameter axons are the first to myelinate, in particular, the first axon myelinated in the zebrafish central nervous system is the very large diameter axon of the Mauthner-cell (Carpenter and Bergland, 1957; Almeida et al., 2011; Lee et al., 2012). The development of myelinated fibers in the rat sciatic nerve provides one example of the dramatic increase in conduction velocity that occurs with myelination (Fig. 11). At birth the fibers of the rat sciatic nerve are unmyelinated and impulses propagate slowly by continuous conduction (e.g. the fastest component of the compound action potential propagates at ~0.66 m s⁻¹ at room temperature; Vabnick and Shrager, 1998). During the first three postnatal weeks, conduction speed increases rapidly and propagation transitions from continuous to saltatory. Over the course of development, mammalian myelinated fibers undergo a 10-fold increase in diameter, a 6 to 8-fold increase in internodal length, and conduction velocity increases 10- to 100-fold (Vabnick and Shrager, 1998).

Fig. 11 – Increase in conduction velocity with myelination in a developing mammalian nerve. Conduction velocity measured by suction electrode recording from representative single fibers of the rat sciatic nerve at 37 °C (filled squares); curve is a sigmoidal fit to the data. Letters indicate stages in myelin development: “A” – fiber is unmyelinated at birth, continuous conduction of impulses; “B” – initiation of myelination, clusters of Na⁺ channels appear at borders of Schwann cells, node formation begins, “C” – formation of paranode; K⁺ channel clusters localize with paranode, expansion of myelin; “D” – saltatory conduction occurs in larger fibers; K⁺ channels cluster in juxtaparanode; “E” – sealing of axoglial junction at paranode and “F” – mature myelin (Vabnick and Shrager, 1998; Vabnick et al., 1999). The movement of delayed rectifier K⁺ channels out of the node and into the juxtaparanode during development is a mammalian adaptation that has not been reported in other taxa. Modified from Vabnick and Shrager (1998).

10. Conclusions

Factors contributing to the evolutionary increases in speed of impulse conduction in nerve are brought into better perspective through a comparison across a range of different signal-conduction modalities, in vertebrates, invertebrates and non-neuronal systems. These provide hypotheses relevant to the constraints on the evolutionary trajectories followed: (1) Rapid conduction is usually based on voltage-dependent mechanisms. An exception is provided by some plants that use pressure (e.g. Stahlberg et al., 2005), which illustrates the point raised earlier (§1) that once started along a path, the evolution of conduction speed is constrained by past history.
(2) The voltage-based mechanisms open aqueous-core ion channels in the membrane. (3) This leads to voltage-regulated facilitated diffusion of ions down concentration gradients. (4) The voltage-sensing mechanism must be intrinsic to the “voltage-gated” channel to achieve the most rapid activation. (5) Such activation leads to regenerative feedback producing “all-or-none” impulses, which are the key to long-distance rapid communication. (6) This endows the process with threshold characteristics and post-impulse refractoriness. (7) The most rapid conduction is realized in an electrical “core-conductor” system with a single elongate “cable” (usually an “axon”; cf penaeids), but a system of tubular elements connected by low-resistance junctions, as in giant axons of lobsters and earthworms and phloem cells of plants, can provide an acceptable alternative. (8) The core conductor is ideally cylindrical and unbranched. The velocity of impulses along the cylinder is maximized by: (9) adjusting the activation rate of the regenerative voltage-gated channels in the membranes; (10) optimizing channel density and distribution (e.g. increasing internodal trans-fiber resistance and tuning nodal conductance magnitudes); (11) increasing the EMF of the ionic battery driving the current; (12) reducing the capacitance of the fiber walls (increasing myelin thickness and decreasing nodal area); (13) optimizing the distribution of the capacitance reduction along the core conductor (node/internode geometry for node-possessing myelin); and (14) decreasing the resistance of the medium inside and outside of the core-conductor (e.g. enlarging diameter). All of these factors are susceptible to selective pressure, and their effects interact with each other and with other adaptive requirements and physical/chemical constraints (space availability; energetic, materials and other costs) so that optimization involves simultaneous (within evolutionary timescales) tuning of many factors. Changes in these parameters as an organism develops may provide a useful model for the evolutionary pathway they followed.

As we have noted before (Hartline, 2008), some of the more prominent features of vertebrate myelin are not crucial to the speed-enhancing properties of myelin. In particular: (1) compaction of layers contributes primarily to space conservation, but to speed only weakly (each successive layer of the thickening sheath, being of slightly larger surface area, makes a slightly smaller per-layer contribution to capacitance reduction). Compaction reduces trans-fiber shunts via the mesaxon and/or cytoplasmic paths in spiral myelin with few layers, but this contribution decreases with increasing numbers of layers as the mesaxon lengthens. (2) Spiral forms, characteristic of vertebrate myelin, are also of little (or even negative) value to speed. (3) Glial origins from which myelin arises in three of the four independently-evolved cases is similarly not essential to speed. Indeed, the internal generation of myelin by axoplasm-filled axons as core conductors for impulses. These cases show that axons, nodes and saltatory conduction are not the only evolutionary pathway to rapid conduction.

On the other hand, myelin has contributed to moderating the diminishing returns of increasing organismal size (cf. §6.3.1). Lacking myelin, communication speed from end-to-end of an organism scales as a power function of size with an exponent less than an isometric value of 1.0. For size on the order of that of a bacterium, diffusional speed scales (decreases) with an allometric $-1$ power of length. This restriction was overcome by the invention of electrical signaling, which, however, was constrained by decremental conduction. This limitation on long-distance electrotonic communication was broken by the invention of propagating all-or-none action potentials. Action potentials in unmyelinated cylinders were still impeded by the allometric dependence of conduction speed on the square root of diameter (i.e. an allometric $+1/2$ power scaling), but this limitation was finally surmounted by the invention of myelin with its linear, isometric, scaling. In addition, myelination gave options for potentially reducing energetic costs and for providing an additional avenue for adjusting time delays and timing precision, enabling evolution of compact nervous systems with complex high-speed energy-efficient computational capabilities (Wang et al., 2008). The limits myelin places on organism size may be few (e.g. whales and dinosaurs) – perhaps nutritional. While most current attention is focused on vertebrate myelin, it should be clear that vertebrates exhibit only one version of a solution to the speed problem. Indeed the speed record for impulse conduction is not held by a vertebrate. Understanding the designs used for the different versions contributes significantly to appreciating the vertebrate one and leads to a better understanding of the ways it could have evolved and may be affected by injury and disease.

Acknowledgments

We thank Monica Orcine and Jennifer Kong for participation in obtaining, and permission to reprint, the images in Figs. 2B2 and 7 respectively. We thank Tina Weatherby (Carvalho) and the PBRC Biological Electron Microscope Facility for providing the needed infrastructure and advice for these and Dr. Andrew Christie for obtaining the material for Fig. 2B2. We thank Dr. Petra Lenz for constructive comments on an earlier version of the MS and for insightful discussions on evolutionary processes. We are very grateful to the many investigators, cited or for lack of space, not, whose work underlies that reviewed here. We especially appreciate the permission to reprint their figures. Parts of this work were supported by National Science Foundation Grant IOS-0923692 and by the Idra Russell Cades Foundation, Honolulu HI.


