Alternative model of propagation of spikes along neurons

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Here a viable and never before investigated mechanism of propagation of spikes along neurons is proposed. In the following, velocity of propagation of small-amplitude pressure waves through the cytoplasmic interior of myelinated and unmyelinated axons of different diameters is theoretically estimated, and is found to generally agree with the action potential (AP) conduction velocities. This remarkable coincidence allows to surmise a model in which AP spread along axon is propelled not by straggling ionic currents as in the widely accepted local circuit theory, but by mechanoactivation of the membrane ion channels by a traveling pressure pulse. Hydraulic pulses propagating in the viscous axoplasm are calculated to decay over \( \sim 1 \) mm distances, and it is further hypothesized that it is the role of inflowing during the AP Ca\(^{2+}\) ions to activate the membrane skeletal protein network for a brief radial contraction amplifying the pressure pulse and preventing its decay.

The model correctly predicts that the AP conduction velocity should vary as the one-half power of axon diameter for large unmyelinated axons, and as the first power of the diameter for myelinated axons, provided that specific mechanical properties of axons are independent from diameter; that myelinization increases the conduction velocity; that the conduction velocity increases with the temperature. Unlike the local circuit theory, the model is able to qualitatively explain observed increase in the AP duration in axons of smaller diameters. Predictions of absolute AP conduction velocities are limited by the knowledge of relevant to propagation of pressure waves mechanical properties of axons, still, the velocities are predicted well for myelinated axons, while an agreement for unmyelinated axons requires 3 orders of magnitude higher resistance of axons to deformation increasing their diameter, compared to values deduced from published data on membrane area expansion moduli. Experimental test of the model is needed.

Depolarization of interior of an excitable cell to a critical level leads to spiking behavior of voltage across the cell membrane, or action potential (AP). The spike has been shown to arise from rapid changes in membrane ion-specific permeability, allowing flow of transmembrane ionic currents powered by electrochemical gradients [1]. Membrane permeability to ions was found to be regulated by voltage across membrane, and a self-regenerating scheme of time course of the AP was developed [2]. The rapid changes in membrane permeability were later shown to arise from brief activation of voltage-gated ion-selective protein channels embedded in the membrane [3]. These findings are supported by more than half a century of experiments and are a basis of modern research in neuroscience [3].

Phenomenon of propagation of the AP through excitable cells (as opposed to development of the AP at a given cell point) has been explained by the local circuit theory, which postulates that spread of ionic currents powered by AP-associated voltage spike across membrane depolarizes adjoining unexcited membrane and brings it to the critical level for excitation [2]. Prediction of velocity of AP conduction is an important test of this model, and can be found from solution of a second-order partial differential equation for time-dependent potential along the cell length, with parameters such as electrical permeability of cytoplasm and membrane, and membrane capacitance [2]. A simplified form of this equation was numerically solved in [2] for a squid giant axon model, with membrane permeability to Na\(^{+}\) and K\(^{+}\) parametrized as a function of voltage history across membrane, as deduced from voltage-clamp data. The conduction velocity was found to be 18.8 m/s for a 476 \( \mu \)m diameter axon at 18.5 \(^\circ\)C with 21.2 m/s experimental value.

The local circuit theory predicts that conduction velocity in unmyelinated nerve fibers should vary as the one-half power of axon diameter if axoplasmic resistivity and membrane electrical properties per unit area are constant, with experimental data fitting powers between 0.57 and 1 depending on the fiber class [1]. For myelinated fibers, the local circuit theory predicts that the velocity should vary as the first power of diameter, again with provisions of constant specific electrical properties, and with additional constraint of proportional scaling of axon diameter, external myelin diameter, distance between the nodes of Ranvier and membrane area at the nodes [1]. The first power relationship is well established for large myelinated fibers [1].

Experiments show that in smaller diameter axons of a related class, duration of the AP spike becomes longer, a fact completely unexplained by the local circuit theory [1]. Conduction velocity increases with the temperature, e.g., for cat vagus myelinated fibers \( Q_{10} \) (ratio of the velocity at one temperature to the velocity at a temperature 10 \(^\circ\)C lower) is 4.8 at 18 \(^\circ\)C, 2.5 at 28 \(^\circ\)C and 1.6 at 37 \(^\circ\)C [2], and for desheathed rabbit vagus unmyelinated fibers \( Q_{10} \) is 3.5 at 10 \(^\circ\)C, 2.1 at 20 \(^\circ\)C and 1.7 at 30 \(^\circ\)C [1]. These changes are explained by the local circuit theory only qualitatively, through increased rate of con-
formational changes in the membrane channel proteins at higher temperatures [1].

Overall, except for the numerical calculation of the conduction velocity for the squid giant axon model [2], agreement between local circuit theory predictions and measurements is close, but rather loose. Agreement in dependence of the velocity on axon diameter for unmyelinated fibers requires variation in specific electrical properties of axoplasm or membrane with diameter, while for similar agreement for myelinated fibers these are assumed to be constant with (experimentally not strictly satisfied) assumptions of geometrical scalings [1]. Temperature affects conduction velocity similarly in myelinated and unmyelinated fibers, while if due only to changes in activation rate of membrane channels, simple reasoning suggests that myelinated fibers would be affected much less, since their conduction velocity would change at the Ranvier nodes only. As already mentioned, longer duration of the AP in fibers of smaller diameters is not explained.

The purpose of this paper is to show that by making two assumptions, namely, that a hydraulic pulse propagating through axoplasm along axon length is able to mechanosensory ion channels for the influxing currents to depolarize membrane to the excitation level, and that at least one of influxing during the AP ion species, presumably Ca$^{2+}$, activates the membrane skeletal protein network for a brief contraction timed to amplify the propagating hydraulic pulse, one obtains a competing with the local circuit theory model of AP propagation, and that this model agrees reasonably well with experimental data on the AP conduction velocities.

The exact mechanisms of how a traveling hydraulic pulse may be amplified and how it might elicit membrane depolarization are uncertain and most appealing to author processes are discussed here. However, exact nature of these processes does not change basic model features and conclusions drawn, as long as these processes do amplify the hydraulic pulse and do excite the membrane upon arrival of the pulse. Just as in the local circuit theory, it is assumed that depolarization of a membrane segment to the critical for excitation level causes membrane voltage to spike due to activation of membrane voltage-gated ion channels. The distinction from the local circuit theory is that this initial depolarization is brought about by a propagating hydraulic pulse and not by the straggling ionic currents, and thus velocity of AP conduction is equal to velocity of propagation of the hydraulic pulse. It should be noted that correct prediction of AP shape by the Hodgkin-Huxley equations [2] does not immediately refute the present model, since local development of AP is the same in both mechanisms.

Although most of discussion here is concerned with the AP propagation in neurons, the model is easily generalized to non-neuronal excitable cells.

### I. MODEL FEATURES AND NECESSARY ASSUMPTIONS

Hydraulic pulses do propagate through viscous liquids enclosed in flexible tubes, a fact illustrated by blood pulse propagating through blood vessels. In addition to normal force on the tube wall exerted by the pulse, viscosity of the liquid introduces shear stress in the direction parallel to the axis of the tube [2]. These forces acting on an extensible axolemma should lead to a membrane stretch, which, according to the first assumption, alone or in combination with bending and compression of the membrane by the propagating pulse, should open enough ion channels to bring membrane voltage to the excitation level. Evidence is now mounting that many unrelated types of ion channels are (for unclear reasons) mechanosensitive [3], but still it is not here possible to quantitatively substantiate this assumption, partly because magnitude of the membrane deformations depends on an unknown pulse amplitude. Since many mechanosensory ion channels are activated by protein links anchoring channel proteins to extracellular structures and to the cytoskeleton [3], it is also reasonable to propose that perturbation of the cell interior cytoskeleton by the propagating hydraulic pulse could be directly mechanically coupled by the protein links to the channel proteins, for a fast and sensitive activation.

As will be shown below, pressure waves in axoplasm decrease e-fold in amplitude over $\sim 1$ mm distances, so that their sustained propagation in long axons would require amplification. It appears that forceful circumferential (and consequently radial) contraction of the membrane skeletal protein network, triggered by influxing during the AP Ca$^{2+}$ ions, is the most plausible mechanism of such amplification. Indeed, virtually every excitable cell has voltage-gated Ca$^{2+}$ channels in its membrane and, in fact, calcium is the basis of the AP in muscles of many invertebrates, in smooth muscles and in many gland cells of vertebrates [3]. At the same time, increase in free intracellular calcium is often associated with initiation of motion, from motility in freely moving cells and muscle contraction to synaptic vesicle release in synapses [3]. Influxing during the AP calcium would instantly many-fold increase its concentration in the membrane skeletal protein network near the inner membrane surface and that, coupled with calcium ability to quickly induce conformational changes in proteins, such as upon its binding to actin filaments in actin-myosin muscle complex, could provide for a fast contractile response necessary to amplify a quickly propagating pressure pulse. The well-known sodium impulse in this scheme could simply be the means to rapidly change transmembrane voltage by the sodium current and thus quickly open relatively sparse voltage-gated calcium channels.

The purpose of the voltage spike in this model is to activate voltage-gated calcium channels, but clearly the spike will create axoplasmic ionic currents that, just as in the local circuit theory, will depolarize adjacent unexcited regions.
cited membrane. This depolarization, then, will decrease number of mechanoactivated by the hydraulic pulse ion channels needed to bring the membrane to the excitation voltage; but if the straggling currents are able to excite adjacent membrane before arrival of the pressure pulse, AP will obviously spread according to the local circuit theory mechanism. Thus, it appears that the two mechanisms of propagation can be viewed as competing, and it is conceivable that different cells, depending on their particular electrical and mechanical properties, are able to realize one or the other mechanism.

Another argument in favor of pressure pulse amplification by structures adjacent to the membrane inner surface is the existence of (currently functionally unexplained) dense “undercoating” just beneath axon membrane in the axon hillock and in the nodes of Ranvier. Initiation of a hydraulic pulse in the axon hillock, as well as restoration of its amplitude in the nodes of Ranvier after passive propagation of the pulse through myelinated part of axon, would require extra effort, and it is reasonable to expect that features underlying pulse creation and amplification should be emphasized. Expression in these regions of the dense membrane undercoating might then suggest that structures adjacent to the membrane inner surface take part in creation and amplification of the pulse, possibly, as hypothesized above, by a forceful circumferential contraction of a protein network included in the undercoating. Another well-known feature of the nodes of Ranvier, high density of voltage-gated sodium channels, can be justified within the model by necessity to rapidly change transmembrane voltage by the sodium current to quickly open voltage-gated calcium channels, to rapidly change transmembrane voltage by the sodium channels, can be justified within the model by necessity to realize one or the other mechanism.

Let’s suppose that a cell has developed an ability to rapidly withdraw a part of its membrane to which an external negative pressure and/or stretch was applied. This reaction could be aimed at detachment from adhesion surfaces, or simply at preservation of shape of a freely moving cell in a dynamic mechanical environment. If such cell happens to have cylindrical shape, and an external stimulus causes rapid membrane withdrawal at a cell end, a wave of increased cytoplasmic pressure will be created and will propagate along the axis of the cell toward its other end. Since passive membrane movement is determined by pressure difference across it, circular membrane segments subjected to increased intracellular pressure in the pulse will move as if negative pressure was applied at the extracellular side; membrane stretch caused by the motion of the viscous cytoplasmin the pulse should also be indistinguishable from a membrane stretch exerted from outside the cell. If these stimuli are sufficient to elicit forceful inward retraction of the circular membrane segments, new cytoplasmin pressure waves will be created, which, if timed properly, will amplify the original hydraulic pulse carrying the signal of stimulation at one cell end to the other.

II. PASSIVE PROPAGATION OF HYDRAULIC PULSES THROUGH AXONS

For the case of small-amplitude harmonic waves in incompressible liquid enclosed in a thin-walled elastic tube, with the wave wavelength large compared to the radius of the tube, and the case of “large” liquid viscosity (defined below), speed of propagation of the waves $v_{inc}$ and decay length $L_{inc}$ (length over which amplitude of the waves decreases e-fold) are

$$v_{inc} = \left( \frac{Eh}{2\rho R} \right)^{\frac{1}{3}} R \left( \frac{\omega \rho}{\mu} \right)^{\frac{1}{3}} \frac{1}{(5 - 4\nu)^{\frac{1}{3}}},$$

$$L_{inc} = \frac{1}{\omega} \left( \frac{Eh}{2\rho R} \right)^{\frac{1}{3}} R \left( \frac{\omega \rho}{\mu} \right)^{\frac{1}{3}} \frac{1}{(5 - 4\nu)^{\frac{1}{3}}},$$

where $E$ is the Young’s modulus of the tube wall material, $h$ is the thickness of the tube wall, $R$ is the radius of the tube, $\rho$ is the liquid density, $\mu$ is the liquid viscosity, $\omega$ is the wave frequency, $\nu$ is the Poisson’s ratio of the tube wall material.

The condition of “large” viscosity (or small tube radius, or low frequency) is $R(\omega \rho / \mu)^{1/2} \ll 1$. That this condition holds well for axons of diameters at least up to $\sim 40 \mu$m can be seen from the following estimate. Measurements of macroscopic cytoplasm viscosity with $\sim 1$ $\mu$m objects freely diffusing or moving under $\sim 1$ pN forces yield $\mu \sim 0.2$ Pa s \([10, 11]\), while application of larger $\sim 1$ nN forces to objects of similar sizes yields $\mu \sim 210$ Pa s \([12]\). Duration of the pressure pulse should be close to that of the contraction that created and amplified it, which in turn should be determined by duration of influx of calcium ions, rate of dissipation of free intracellular calcium and time properties of the presumed process of contraction. Lacking detailed knowledge of the last two processes, it is here simply assumed that duration of the pressure pulse is equal to the AP duration, e.g. $\sim 0.34$ ms for large-diameter cat myelinated axons at $37.1 \, ^\circ C$ \([13]\), with corresponding $\omega \approx 2\pi / (2 \cdot 0.34 \, \text{ms}) \approx 9200 \, \text{rad/s}$. Taking $R = 20 \, \mu$m, $\rho = 1000 \, \text{kg/m}^3$, $\mu = 0.2$ Pa s, $\omega = 9200 \, \text{rad/s}$, yields $R(\omega \rho / \mu)^{1/2} \approx 0.14$, which should be smaller enough than 1 for the large viscosity limit to hold. Another condition used to derive (1) – (2),
of large wavelength $\lambda$ of the pressure waves compared to the radius of the tube, or $\lambda = 2\pi v/\omega \gg R$, as will be seen later, also holds well for axons.

For the case of small-amplitude harmonic waves in a compressible liquid enclosed in a rigid tube, and the above conditions of large viscosity $(R(\omega \rho/\mu)^{1/2} \ll 1)$ and large wavelength $(\lambda \gg R)$ [14],

$$v_{\text{comp}} = v_{\text{sound}} R \left( \frac{\omega \mu}{\mu} \right)^{\frac{1}{2}} \frac{1}{2}, \quad (3)$$

$$L_{\text{comp}} = \frac{1}{\omega} v_{\text{sound}} R \left( \frac{\omega \mu}{\mu} \right)^{\frac{1}{2}} \frac{1}{2}, \quad (4)$$

where $v_{\text{sound}}$ is the speed of sound in the liquid in the absence of viscosity.

It should be noted that in the absence of viscosity, speed of propagation of small-amplitude harmonic pressure waves in a compressible liquid enclosed in a thin-walled elastic tube is given by (e.g. [15])

$$v_{\mu=0} = \left( \frac{1}{\rho(k + \frac{2R}{Eh})} \right)^{\frac{1}{2}}, \quad (5)$$

where $k$ is the intrinsic bulk compressibility of the liquid, and $k + 2R/\rho$ is the compressibility of the liquid in the tube stemming from both the intrinsic compressibility of the liquid and distortibility of the walls. In the limit of rigid tube walls so that their distensibility can be ignored, i.e. when $k \gg 2R/\rho$, (5) reduces to $v_{\mu=0} = (1/\rho k)^{1/2}$, which is equal to the speed of sound in the liquid $v_{\text{sound}}$ (e.g. [15]) and the first factor in (3) and the second in (4).

In the opposing limit of $k \ll 2R/\rho$, i.e. when internal compressibility of the liquid can be ignored, (5) simplifies to $v_{\mu=0} = (\rho(2R)^{1/2})$, which is known as the Moens-Korteweg equation [16] and the first factor in (1) and the second in (2). It now can be seen that outcome of introduction of large liquid viscosity in the inviscid case is independent from whether compressibility of the liquid in the tube arises from distortibility of the walls, or from intrinsic compressibility of the liquid, except for an additional factor $2/\sqrt{4-4\nu}$ in (1) and (2), which takes into account movement of the tube wall in the direction of the tube axis by the viscous liquid [16]. In principle it is a straightforward, although cumbersome, task to modify equation of conservation of mass (3) in [16] to include intrinsic compressibility of the liquid in the approximate form $dp = kdp$, repeat derivations and obtain expressions for the wave speed and decay length for the case of a compressible viscous liquid in an elastic tube. Here, however, it is noted that the factor $2/\sqrt{4-4\nu}$ varies from 0.89 to 1.15 for physical values of Poisson’s ratio $0 < \nu < 0.5$, and is close enough to 1 to be neglected in the present analysis. Therefore, neglecting axial motion of the tube wall, (1) – (5) can be combined to obtain for the case of small-amplitude harmonic waves in a compressible liquid enclosed in a thin-walled elastic tube, with the conditions of large liquid viscosity and large wavelength:

$$v = \left( \frac{1}{\rho(k + \frac{2R}{Eh})} \right)^{\frac{1}{2}} R \left( \frac{\omega \mu}{\mu} \right)^{\frac{1}{2}} \frac{1}{2}, \quad (6)$$

$$L = \frac{1}{\omega} \left( \frac{1}{\rho(k + \frac{2R}{Eh})} \right)^{\frac{1}{2}} R \left( \frac{\omega \mu}{\mu} \right)^{\frac{1}{2}} \frac{1}{2}. \quad (7)$$

In general, when velocity of harmonic waves (phase velocity) $v$ depends on frequency of the waves $\omega$, velocity of propagation of a wave packet is equal to the group velocity $v_{gr}$, given by (e.g. [15])

$$v_{gr} = v + \omega \frac{dv}{d\omega}. \quad (8)$$

Application of (8) to (6) yields group velocity $v_{gr} = 1.5v$.

Derivations in [16] assumed isotropy of the tube wall material, while structure of lipid membranes suggests high anisotropy of their elastic properties in the transverse direction compared to the in-plane directions. By following the derivations, however, one can ascertain that terms corresponding to the transverse elasticity of the wall material have to be neglected. Then Young’s modulus $E$ and Poisson’s ratio $\nu$ in the formulas above represent membrane resistance to a tangential to the membrane stretch and shrinkage of perpendicular to stretch in-plane dimensions respectively, under assumption of in-plane isotropy of membrane elastic properties. Then, neglecting transverse stresses on the membrane, Hook’s law yields (e.g. [17]): $(\sigma_1 - \nu\sigma_2)/E$, $\sigma_2 = (\sigma_1 - \nu\sigma_2)/E$, where $\sigma_1$, $\sigma_2$ – relative orthogonal elongation of membrane subjected to orthogonal tangential stresses $\tau_1$, $\tau_2$. From the definition of elastic area expansion modulus $K$ (e.g. [18]): $(\tau_1 + \tau_2)/2 = K dA/A$, where $\tau_1$ and $\tau_2$ are orthogonal tensions in the membrane surface, $dA/A$ – fractional area change. Taking into account that $\tau_1 = \sigma_1 h$, $\tau_2 = \sigma_2 h$, and for small $\epsilon_1, \epsilon_2$: $dA/A = \epsilon_1 + \epsilon_2$, one can obtain:

$$Eh = 2K(1 - \nu). \quad (9)$$

III. NUMERICAL ESTIMATES AND COMPARISON OF MODEL PREDICTIONS TO EXPERIMENT

Inspection of formulas (6) – (8) in relation to propagation of pressure pulses through axons leads to the following statements: velocity of propagation $v$ for a given $\omega$, $\mu$, $k$, $R$ increases with increased membrane rigidity $Eh$; for a given $\omega$, $\mu$, $k$, $Eh$, dependence of the axon radius is intermediate between $R^{1/2}$ and $R$ and specifically depends on the product $Eh k$; for a limiting case of a soft membrane ($Eh \ll 2R/k$), $v \sim R^{1/2}$; for the opposing case of a rigid membrane ($Eh \gg 2R/k$), $v \sim R$. 


for an axon of fixed $R$, $Eh$ and $k$: $v \sim (\omega/\mu)^{1/2}$; decay length $L$ behaves similarly to $v$, except for the $L \sim \omega^{-1/2}$ dependence.

As has been mentioned earlier, the local circuit theory is unable to explain increased duration of the AP in the smaller diameter fibers of a related class [1]. Since in the model being presented membrane channels are activated not only by voltage across membrane (as in the local circuit theory), but also by the traveling pressure pulse, hydraulic pulses of longer duration should increase duration of the AP due to longer mechanostimulation of the membrane channels. Since lower frequency components of a pressure pulse decay less with distance (from (7)), increased damping in fibers of smaller diameters (again from (7)) in general means that propagating pressure pulses in these fibers will have lower frequency and longer duration, and, as mentioned above, longer duration of the AP.

From the above considerations it also follows that duration of a “membrane” AP in this model is predicted to be independent from the fiber diameter, and equal to values obtained with the local circuit theory, owing to absence of a propagating pressure pulse in this case. Duration of a propagated AP, however, is predicted to be longer than that for a membrane AP, and is predicted to increase with decreasing fiber diameter reflecting increased duration of the pressure pulse. The idea that the AP duration $\Delta T_{AP}$ adjusts to the duration of the pressure pulse $\Delta T_{pr}$ reinforces the approximate relation $\Delta T_{AP} \approx \Delta T_{pr}$, assumed earlier from converse arguments that duration of the pressure pulse should be close to that of influx of calcium ions.

### A. Myelinated axons

As follows from (6) – (8), the larger the stiffness of the axolemma, the larger the speed of propagation of the pressure waves and the lesser their decay with distance. From this perspective, myelinization of axons by Schwann or oligodendroglial cells tightly wrapping around axon in a spiral manner is aimed at increase of rigidity of the axon membrane. Successive layers of the same myelin cell are known to firmly adhere together by proteins from the cell surface glycoprotein family, which are in fact main protein constituents of myelin [13]. This adherence should be effective against unwinding of the spiral when subjected to increased pressure in the hydraulic pulse, and should transfer the distending load to lipids and proteins of all myelin layers. In order to be able to neglect distensibility of the myelin sheath compared to intrinsic compressibility of the axoplasm, “effective” Young’s modulus $E$ of the myelin should satisfy stemming from (6) approximate condition $E > 2R/(h \kappa)$. Taking $k$ as for saline water at 37 °C, $k = (\rho v_{\text{sound}}^2)^{-1} \approx 4.04 \times 10^{-10}$ Pa at 1.7 mm, and $R \approx h$ leads to the condition $E > 5 \times 10^9$ Pa. Area expansion modulus $K$ for plasma membranes is on the order of 0.4 N/m with membrane thickness of $h \approx 6$ nm [13], which corresponds to $Eh \approx 0.6$ N/m (from (9), $v$ taken as 1/4) and “effective” Young’s modulus of $E = Eh/h \approx 10$ Pa. Thus, if myelin sheath is as elastic as plasma membrane, it cannot be considered indistensible compared to the intrinsic compressibility of axoplasm. However, considering that lipid composition of myelin differs markedly from that of plasma membranes, in particular in that it contains significantly more cholesterol known to increase membrane rigidity [14 20], that the protein-rich major dense lines might also significantly contribute to myelin rigidity, and that in the model being presented increased myelin stiffness is beneficial for propagation of the pressure pulses and thus should be sought after by the cellular mechanisms, it is here assumed that circumferential distensibility of myelin sheath does not appreciably effect velocity of propagation of axoplasmic pressure waves. Then, also neglecting reduction of the propagation velocity in the nodes of Ranvier, the quotient $2R/(Eh)$ in (6) and (7) can be disregarded for myelinated axons.

Substituting in (6) compressibility of saline water $k = 4.04 \times 10^{-10}$ Pa$^{-1}$, viscosity $\mu = 0.2$ Pa s, frequency $\omega = 2\pi/(2 \cdot 0.6 \text{ ms}) \approx 5200 \text{ rad/s}$, axon radius $R = 1 \mu$m leads to the pressure pulse velocity $v_{pr} = 6$ m/s, which, given the approximate nature of the estimate, is in a good agreement with the experimental value of 10 m/s for the AP conduction velocity in cat myelinated axons of 1 μm radius at 38 °C [1]; the AP duration of 0.6 ms for the mentioned fibers was measured at 37.1 °C in [1]. Use of $\mu = 0.2$ Pa s in the estimate can be justified by considering that it should represent viscosity arising from small-amplitude cytoskeletal deformations [11 11] that are likely to accompany propagation of a small-amplitude pressure pulse, while larger values of $\mu = 210$ Pa s were obtained with forceful displacement of cytoplasmic objects that possibly disrupted the cytoskeleton or distorted it considerably [12]; still, given the large scatter of values for $\mu$ available in the literature, uncertainty in $\mu$ should represent a major contribution to overall error of the estimate.

From (7), e-fold amplitude decay length for the considered case is then $L \approx 1.2$ mm, and wavelength $\lambda = 2\pi v/\omega \approx 7.3$ mm.

### B. Unmyelinated axons

For unmyelinated axons of radius $R = 0.65$ μm, assuming membrane $Eh = 0.6$ N/m as before, and taking $\mu = 0.2$ Pa s, $k = 4.04 \times 10^{-10}$ Pa$^{-1}$, $\omega = 5200$ rad/s for the myelinated axons, (6) and (8) yield the pressure pulse velocity $v_{pr} = 0.053$ m/s, which is ~40 times lower compared to measurements of 2.3 m/s for the AP conduction velocity in cat unmyelinated nerve fibers of 0.65 μm radius at 38 °C [1], notwithstanding the low value for viscosity used. Taking into account agreement obtained with the same values of $\mu$, $k$, $\omega$ for myelinated axons, and that the main difference in mechanical properties
between myelinated and unmyelinated axons should be in the axolemmal resistance \( Eh \) to increases in axon diameter, one can conclude that in order for the model to predict correct AP conduction velocity for the unmyelinated axons, “effective” membrane \( Eh \) has to be \( \sim 1600 \) times larger than the value of 0.6 N/m used. This latter value represents resistance of lipid plasma membranes to area expansion. Additional resistance to increases in diameter of unmyelinated axons can come from deformation of adjacent glial cells; from deformation of the cytoskeleton tethering the membrane; from passive resistance of the membrane skeletal protein network to the radial deformation and from active contraction of the network during amplification of the hydraulic pulse. Since axial membrane elasticity does not appreciably influence velocity and decay length of the pressure waves, the mentioned above factors might selectively increase membrane resistance to the circumferential expansion, leaving axial rigidity at a lower value. From this perspective, the model predicts “effective” area expansion modulus for the deformation described \( \sim 3 \) orders of magnitude larger than for lipid membranes.

### C. Velocity dependence on axon diameter and temperature

From (6) – (8) it follows that for unmyelinated fibers of small diameters \((2R \ll Ehk)\), \( v_{gr} \sim R \), while in the opposing limit of large diameters \((2R \gg Ehk)\), \( v_{gr} \sim R^{1/2} \). In unmyelinated C-fibers (diameters \( 0.4 – 1.2 \) \( \mu m \)) of cat saphenous nerve AP conduction velocity \( v_{AP} \) is proportional to \( R \), in unmyelinated fibers \((1.6 – 20 \mu m)\) of locust and cockroach \( v_{AP} \sim R^{0.7} \sim R^{0.8} \), while in unmyelinated fibers \((2 – 520 \mu m)\) of cephaloid molluscs \( v_{AP} \sim R^{0.37} \). Thus, assuming that \( Ehk \) is on the order of \( 10 \mu m \) and is not significantly different for the mentioned fibers, predicted gradual change in dependence from \( R \) to \( R^{0.5} \) with increasing unmyelinated fiber diameter is very well matched by experiment.

For myelinated fibers, assuming \( Eh \gg 2R/k \), (6) – (8) give \( v_{gr} \sim R \) dependence, again closely matched by experimentally determined \( v_{AP} \sim R \). In the considered above limit of small unmyelinated fibers, condition \( 2R \ll Ehk \) is the same as for myelinated fibers, \( Eh \gg 2R/k \), which indicates that for these fibers myelination would not increase the speed of propagation or decay length. Very small diameter fibers are indeed usually unmyelinated.

The analyses above implicitly assumed that specific mechanical properties of axons, \( k, \mu, Eh \), are constant across fibers of varying diameters, or that their variation is small enough not to appreciably change obtained \( R \) dependences. The same condition was assumed for the central frequency \( \omega \) of the pressure wave packet. In cat saphenous myelinated fibers at 37.1 °C, AP duration \( \Delta T_{AP} \) changes from 0.6 ms to 0.34 ms with 8-fold increase in diameter. Corresponding change in \( \omega = 2\pi/(2 \cdot \Delta T_{AP}) \), from \( 5.2 \cdot 10^{3} \) to \( 9.2 \cdot 10^{3} \) rad/s, can be shown to introduce not more than an additional 0.14 power of \( R \) in the predicted dependences. A larger variation of \( \omega \) with \( R \), however, would have a greater impact on the predictions.

Assuming that \( Eh, k \) and \( R \) do not significantly depend on temperature \( T \), and that changes in \( \omega \) and \( \mu \) with \( T \) are independent from degree of fiber myelination, (6) – (8) predict that myelinated and unmyelinated fibers should be similarly affected by a temperature change, just as observed experimentally \[1\]; the predicted dependence is \( v_{AP} \sim (\omega(T)/\mu(T))^{1/2} \). Experimental data on \( \mu(T) \) is not readily available, however, for most liquids viscosity increases with decreasing temperature \[21\]. An increase in viscosity means (see (7)) augmented damping of pressure waves of all frequencies, and, just as in the case of fibers of smaller diameters considered earlier, duration of the AP can be argued to increase. AP duration indeed increases with decreasing temperature, e.g. with \( Q_{10} \) of 3.4 at 37 °C for cat vagus myelinated fibers \[13\], but this large variation could mainly stem from change in rate of activation of voltage-gated membrane channels \[1\]. According to \( v_{gr} \sim \omega^{1/2} \) and the assumed relation \( \omega = 2\pi/(2 \cdot \Delta T_{AP}) \), \( Q_{10} \) of 3.4 for the AP duration \( \Delta T_{AP} \) implies that \( Q_{10} \) for the pressure pulse propagation velocity is predicted to be \( 3.4^{1/2} \approx 1.8 \) without even considering \( \mu(T) \) dependence. Experimental value of \( Q_{10} = 1.6 \) for the AP conduction velocity in the cat vagus myelinated fibers at 37 °C \[3\] thus might indicate that viscosity of the axoplasm does not change appreciably in the \( 27 – 37 \) °C range.

It is also interesting to note that cooling-related removal of AP conduction block in damaged (e.g. by multiple sclerosis) myelinated axons \[22\] can be naturally explained within the model by increase in the decay length \( L \) with the decrease of \( \omega \sim (\Delta T_{AP})^{-1} \) at lower temperatures.

### D. Depletion of sodium and calcium concentrations

Depletion of sodium concentration around a squid giant axon leads to wider and slower propagating APs \[24\], in close agreement with the Hodgkin-Huxley equations \[24\]. However, longer duration of the AP in the presented model also decreases velocity of AP propagation, through relation \( v_{AP} \sim (\Delta T_{AP})^{-1/2} \); qualitative analysis of \( v_{AP} \) and \( \Delta T_{AP} \) from Fig. 4 in \[25\] does not exclude validity of this relation.

Conduction in axons in low external calcium is poor or blocked at turnon of cathodal current \[1\]. These axons, however, do respond better to anodal break excitation \[1\], which does provide higher gradient for \( Ca^{2+} \) to enter the axon. Of interest is whether axons propagate APs in extremely depleted external calcium. If they do, interesting is to know whether conduction velocity increases or decreases, the latter being predicted by the model, if conduction can switch to lower velocity...
local circuit mechanism. In the local circuit theory, conduction velocity with depleted external calcium should, in the first approximation, increase: decrease in free extracellular calcium significantly decreases thresholds of activation of both sodium and potassium channels [24]. Free intracellular calcium can activate polarizing calcium-dependent potassium currents [24]. Then, observed increase in the velocity would favor the local circuit theory, but, strictly speaking, would not disprove the model, since it is possible that the velocity given by the local circuit theory became larger than that predicted by the model after modification of channel gating, and the local circuit theory mechanism took over the propagation from the pressure wave. Data on the AP conduction velocities in axons with manipulated calcium concentrations is not readily available, since most experiments use voltage-clamp methods under these conditions; detailed analysis of these phenomena will be done in a subsequent work.

IV. DISCUSSION

It is very remarkable that transmission of hydraulic pulses through axoplasm, a process so disparate from passive spread of ionic currents, reasonably well reproduces all major features of AP conduction: changes in the velocity with membrane rigidity, with axon diameter, with the temperature and with AP duration; changes in the AP duration with fiber diameter. Accurate predictions of absolute AP conduction velocities by the model are limited by the knowledge of relevant to propagation of the pressure pulses cytoplasm viscosity, resistance of myelinated and unmyelinated axons to increases in diameter, cytoplasm compressibility, and by approximate nature of the estimate of duration of the hydraulic pulse. Still, estimates of the velocity for myelinated fibers, with an assumption of indistensibility of the myelin sheath, give values close to those observed experimentally: for unmyelinated fibers, although the velocity is underpredicted, the situation might change as the appropriate experimental data on membrane distensibility becomes available.

It also has been noted (Prof. M. Kardar, 2003, private communication) that the lipid membrane can be more realistically modeled as 2-dimensional liquid rather than elastic solid, with wave propagation velocity shaped by bending membrane rigidity rather than area expansion modulus. With the different treatment, which will be attempted in the future, predicted by the model AP velocity in unmyelinated axons might change dramatically.

On the other hand it is noteworthy that all fundamental ingredients necessary for realization of the described model are in place: mechanosensitivity of many membrane ion channels (e.g. [7]), low free calcium concentration in a resting cell, ubiquity of the calcium impulse and its trait to induce motion in filaments [3], and high filament content of the cell interior [3]. Whether or not these ingredients are present to a degree sufficient for the active propagation of the hydraulic pulses, and whether any cells in fact use the described mechanism for AP conduction, should be best settled by experiment.

A small axon swelling followed by a smaller shrinkage, both roughly synchronous with propagating APs, was demonstrated in crab and squid giant axons [25]. Beyond increased pressure inside axon, the swelling can be caused by variation of the voltage across membrane through the ensuing change in difference in surface tensions between the two membrane interfaces [26]. Axial motion of an axoplasmic object, such as a vesicle, observed simultaneously with the AP, should be less influenced by the voltage-dependent membrane movement and more sensitive to axoplasm disturbances induced by the hydraulic pulse. The model predicts that a swelling should precede the voltage spike, and, in fact, figures in [25] indicate that the swelling by several milliseconds does precede the voltage spike, but the authors’ conclusion was that cause of that might be in electronics delays; careful analysis of the delays in an experiment similar to that in [25] could provide another test of the model.

Knockout of a neurofilament subunit gene has been reported to ~ twice decrease conduction velocity in mice large myelinated axons, with axon diameter, AP amplitude, duration and shape preserved. Neurofilament proteins are the most abundant cytoskeletal element in large myelinated axons [27]. These data are favorable for the presented model, with the changes in conduction velocity explained by modification of cytoskeletal and membrane skeletal protein mechanics. The local circuit theory predicts that conduction velocity is independent from mechanical properties of axons. Somewhat contrary to that, it is known that cell pressurization does block AP conduction in at least in some axons [11].

Other tests can come from measurements of velocity of propagation of pressure pulses through axons; from precise measurements of mechanical properties of neurons and other excitable cells; from experiments on mechanical stimulation of AP in excitable cells; from measurements of the conduction velocity with depletion of free intracellular calcium, or with disruption of the membrane skeletal proteins; or from a setup aimed at direct detection of pressure waves accompanying APs.

The following circumstances can be recognized to indirectly support the model: in many non-neuronal excitable cells AP seems to be connected to mechanical motion [3]; neurons often have convex shape as if pressurized from inside; myelination appears to be the only feasible way to substantially increase mechanical resistance of axolemma, while resistance of the membrane to leak currents could in principle be increased by other means, e.g. decrease in number of leak channels; many freely moving cells have an ability to control membrane movement by forces of microfilaments located just beneath the plasma membrane [3]; the same ability is attributed to neurons, where the forces are believed to arise from interactions of actin, fodrin and other elements [3]. Finally, the main
components of the model, propagating hydraulic pulses and radially contracting tubes generating a directed axonal force on the tube contents, are exploited in cardiovascular, intestinal and other tubular systems in many organisms, which might even suggest an evolutionary link between the systems and excitable cells.

Central role of hydraulic pulses and pressure-generating processes in the model implies that pressure-related phenomena should play important roles in the function of those neurons and excitable cells, that would realize the model mechanisms: synaptic vesicle release in neurons might be aided by impact of the AP-associated hydraulic pulse on the vesicles or by contraction of the bell-shaped membrane skeletal protein network of the synapse, creating a directed push on the cytoplasm and the vesicles; depolarization of the axon hillock to the excitation level might be accomplished not only by passive spread of ionic currents from postsynaptic sites, but also by mechanoactivation of the membrane ion channels by cytoplasm displacement or pressurization; therefore, synaptic integration might involve relay of displacement or pressurization of cytoplasm through the dendritic tree to the axon hillock, and learning and memory in neurons might be partially reflected in strength and duration of \(Ca^{2+}\)-activated contraction of postsynaptic membrane skeletal proteins, or in another pressure-generating process: AP generated in a neuron might influence processes in the cell body, dendrites and contacting synapses by mechanical disturbances introduced by the back-propagating pressure pulse; AP-associated hydraulic pulses might participate in transport of substances in excitable cells. Other outcomes of the model are the hypotheses on the evolution of the signal transmission in cells, on reasons for mechanosensitivity of many unrelated types of ion channels, and on the physiological role of axonal calcium influx.

In the case the presented model is not experimentally confirmed, calculations in the paper show that velocity of propagation of \(\sim 1\) kHz pressure waves through axons can be close to the AP conduction velocities, and hence pressure waves generated by voltage-induced membrane movement during AP conduction \([26]\) might accumulate into a larger shock-like wave.

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