Perspective: Do Macromolecules Play a Role in the Mechanisms of Nerve Stimulation and Nervous Transmission?

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Received 17 July 2015; accepted 5 August 2015; published online 28 September 2015
DOI: 10.1002/polb.23898

ABSTRACT: Fibrillar macromolecular networks are ubiquitous in biological systems, from cellular cytoskeletons to tissues such as muscle and tendon. The presence of such networks in neuronal tissue is known, for example, in the cytoskeleton and extracellular matrix in and around neuronal and glial cells, but their function is believed to be principally mechanical/structural in nature. However, there has long been speculation regarding a broader role for neuronal fibrillar macromolecules, which are anionic polyelectrolytes, specifically regarding their participation in nervous stimulation and transmission. This Perspective reviews literature that spans more than a century, including very recent work, and attempts to build a case for considering a multifunctional role for such macromolecules that includes participation in not only nervous activity but also in diverse phenomena including electric communication within and between cells and mechanisms of anesthetic action. Perhaps the creation and utilization of "artificial axons" is within reach with design rules coming at least in part from fundamental considerations of macromolecular science. © 2015 Wiley Periodicals, Inc. J. Polym. Sci., Part B: Polym. Phys. **2016**, *54*, 7–14

KEYWORDS: biomaterials; fibers; ion exchangers; polyelectrolytes

INTRODUCTION Biological cells are laden with fibers, from chromatin in the nucleus to the complex and dynamic cytoskeleton. Indeed, Chambers[1] noted long ago that "the physical properties which so fundamentally characterize protoplasm, such as elasticity, rigidity, and imbibition, exist only in virtue of its fibrous structure." Cells are, in fact, soft materials.[2] The cell with its cytoskeleton is perhaps rather well described as a viscoelastic, nanofibrous cation exchanger, the latter property being the result of fixed anions from ionized aspartic and glutamic acid units. The presence of polyelectrolytes in a crowded cellular environment can be considered, from a polymer physicist's perspective, as a "Coulomb soup,"[3] and complex intracellular phenomena that can result are only beginning to be studied in detail.

As pointed out by McNiven,[4] the structure–function relationships between the components of the nanofibrous cytoskeleton continue to be defined and understood. Furthermore, these key nanofibrous components of cytoskeleton, namely, actin, intermediate filaments, and microtubules, are connected through the cell membrane to the materials of the extracellular matrix such as collagen. Cellular assemblies in tissues and organs can thus be considered as a continuous fibrous network connecting nucleus to cytoskeleton to extracellular matrix to many (most?) other cells, with important physiological consequences that likely have yet to be revealed from this complex and quite literal web of interactions. It is therefore most interesting that Needham[5] presciently remarked in 1936 that "biology is largely the study of fibers."

Let us now briefly turn attention to irritability, a term used many decades ago to offer a contrast between living and nonliving systems. For example, according to Heilbrunn,[6] "Exposed to a sudden change in its environment, a living system typically shows a reaction or a response ... This characteristic of living material is called "irritability" and, perhaps more than any other vital characteristic, serves to distinguish living systems from non-living." Muscle and nerve combine to form the prototypical irritable system where a macroscopic effect, specifically motion in the form of muscle contraction, is observed. Muscle has been studied in much detail from a macromolecular perspective beginning decades ago,[7] and the sliding filament model of muscle contraction is well established. Nerves, on the other hand, have received much less attention in this regard, which is interesting given an admittedly tenuous premise by the author that muscle

This contribution is based on a lecture given as part of the celebration of the 50th anniversary of the founding of the Department of Macromolecular Science and Engineering at Case Western Reserve University in June 2013.

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and nerve might have evolved in concert. However, a significant body of literature, for example that of Tasaki[8,9] contained in two monographs and many papers (cf. refs. 10–13), suggests that fibrous, cation-exchanger macromolecules may play a key role in nervous stimulation and transmission. Others have also supported this view,[14] suggesting that the entire cell surface of an axon, namely, the cell membrane, sometimes called the axolemma, plus the immediately underlying cytoplasmic structure, referred to as the ectoplasm—a polyelectrolyte network and biological ion exchanger—is responsible for the nervous excitation process. Altogether, the neuronal membrane plus underlying macromolecular fiber system has been referred to as the axolemma–ectoplasm complex.

Thus, the main aim of this Perspective is to review these ideas and to extend them with a small refinement, albeit untested, of a mechanism for nervous excitation or stimulation and transmission which involves direct participation of macromolecules. Credit for promoting an awareness of Tasaki's and related work goes to Pollack,[15] who has also offered much original thinking about cellular phenomena from a macromolecular perspective and who stresses the importance of phase transitions in many biological systems.

NERVE STIMULATION AND NERVOUS TRANSMISSION

The seat of excitability of nerve has generally been believed to be the lipid membrane in which ion channels are activated transiently on stimulation, with signal propagation resulting from successive opening of voltage-gated channels as summarized in Figure 1.

The details of this process represent the foundation of the Hodgkin–Huxley (HH) model[17] and remains the principal basis for rationalizing nervous stimulation and transmission. Recently, however, the HH model has been criticized by Heimburg and Jackson[16(a)] as being confined to an electrical analysis without physicochemical considerations including heat release and absorption (during initiation and termination of a nerve signal pulse, respectively[18]) as well as mechanical changes (swelling and contraction, again during initiation and termination), as previously emphasized by Tasaki.[19] In an attempt to reconcile all of these points, Heimburg[16(b)] proposed that quantized conduction events coupled with reversible thermal and thickness changes can be explained by considering nervous transmission as a "pulse along the membrane" with the propagation of a soliton wave through the lipid bilayer being responsible for nervous transmission (Fig. 2).

Neither the classical HH nor the recent Heimburg models include macromolecules in the mechanism of nerve excitation and signal propagation. Is there a missing link?

MACROMOLECULES AND NERVE PHYSIOLOGY

As noted earlier, it has long been recognized that a macromolecular network exists just under the lipid bilayer outer membrane of nerve, and this macromolecular network or layer is sometimes referred to as the ectoplasm. This layer has the properties of a cation exchanger and suggests that the protein components are rich in anionic amino acids (e.g., glutamic and aspartic acids, substantially ionized at physiological pH).[20] As summarized by Tasaki,[21] "...the colloidal state of the surface layer of the nerve and muscle fiber is regulated by a change in the ratio of the concentration of Ca^{2+} ions to that of univalent cations. They proposed that the process of nerve excitation involves a rapid change from a compact, Ca^{2+}-rich state to a swollen, Ca-deficient state." It is notable that "They" in the sentence above refers to Loeb[22] and Höber,[23] who made such a proposal over a century ago. Tasaki also pointed out the significance of rapid switching due to a cooperative transition between the compact and swollen states, and this bistability is believed to be crucial to nervous excitation and transmission. Interestingly, modern books on the subject, compare ref. 24, note the presence of this fibrillar protein network but emphasize its role in material (e.g., membrane and secretory protein) transport and the mechanical role of dictating neuron shape. Components of the network include microtubules (outside diameter of about 25 nm), neurofilaments (about 10 nm in diameter), which are related to intermediate filaments in the cytoskeleton of other cell types, and actin-based microfilaments (3–5 nm in diameter). However, no consideration is apparently given to the possibility of a cortical layer of such materials actively participating in nerve stimulation and nervous transmission.

The structure of this fibrillar macromolecular layer has been studied in some detail [ref. 25(a,b); see also ref. 9, Chapter 9], and Figure 9.2 from Tasaki's book (ref. 9, p. 162) is reproduced below (Fig. 3). Interestingly, the nanofibers are aligned (within about 5° to 10°) along the long axis of the axon.

The finding that these macromolecular fibers are important for nerve stimulation and signal transmission is suggested by experiments wherein the axoplasm (gel inside the axon) is removed and the inside is perfused with appropriate salt solutions with a Ca^{2+}-containing solution available on the outside. Among the key observations are that (1) the

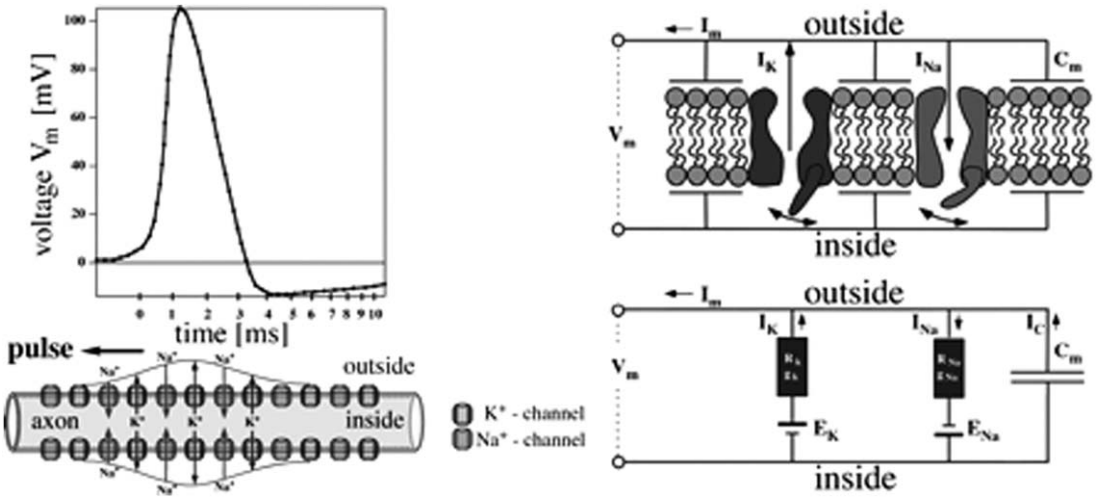

FIGURE 1 From Ref. 16b: "The basic elements of the Hodgkin-Huxley model. The nerve pulse from the original publication of Hodgkin and Huxley (top left) [17] is generated by the transient opening of sodium and potassium channels (bottom). Sodium and potassium ions flow through the channel proteins that are embedded in the membrane (top right). The corresponding electrical currents can be represented by an equivalent electrical circuit diagram (bottom right). Here, V_m is the observed membrane voltage. The quantities I are the ion currents, the E_{Na} and E_K are the Nernst potentials of sodium and potassium. C_m is the membrane capacitance. Adapted from [16e]."

axoplasm gel is apparently not necessary for nerve excitability; (2) Ca^{2+} is essential, but only if present on the outside of the axon, consistent with the old ion-exchange idea mentioned previously; and (3) excitability is lost if the inside of the axon is treated with a proteolytic enzyme, suggesting that degradation of the macromolecular fiber network is linked to loss of excitability.

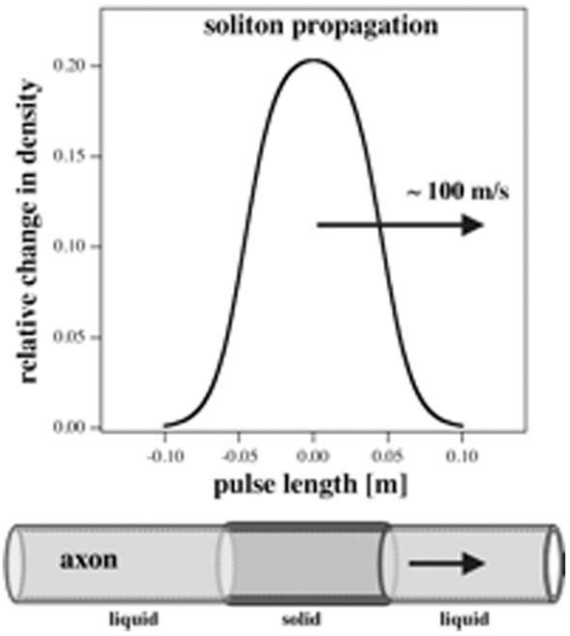

FIGURE 2 Solitons propagate with a velocity close to the lateral speed of sound of lipid membranes, that is, with about 100 m/s. This corresponds to the pulse velocity of myelinated nerves. See Ref. 16(b).

SYNTHETIC MACROMOLECULAR MIMICS

Tasaki coupled these collective observations with studies on synthetic, polyanionic gels to outline important ideas about the role of macromolecules in nervous transmission. Key observations with spheres or thick fibers of crosslinked poly(sodium acrylate) include volume shrinkage of the Na^+-form on titration with $CaCl_2$, with the transition sharpening in the presence of a bathing salt solution of appropriate concentration. Figure 4 shows a discontinuous volume change for a polyacrylate gel fiber immersed in 40 mM NaCl and titrated with $CaCl_2$.[11] The abrupt diameter reduction suggests a cooperative transition at a critical Ca^{2+} concentration.

The Ca^{2+}-contracted form of the synthetic poly(acrylate) gels was proposed to represent the resting ectoplasm of nerve, with the swollen, Na^+-form being the excited (stimulated) form. The potential importance of this phase transition in nervous transmission has been additionally emphasized by Pollack.[26]

Interestingly, Shashoua[27] demonstrated that bilayer complexes of synthetic, oppositely charged polyelectrolytes such as poly(acrylic acid-*co*-acrylamide) and poly(dimethylamino ethylacrylate) are capable of spontaneously generating transient electrical signals with d.c. fields that have time constants and amplitudes which are analogous to spike potentials of neuronal membranes. It is of particular interest that polyacrylate membranes exposed at one surface to calcium hydroxide also exhibited spike generation. Subsequently, Huang and Spangler[28] demonstrated oscillatory activity in poly(glutamic acid)/Ca^{2+} asymmetric membranes, and it was proposed that ion accumulation in the interfacial region alters poly(glutamic acid) conformation and hence ion permeability.[29]

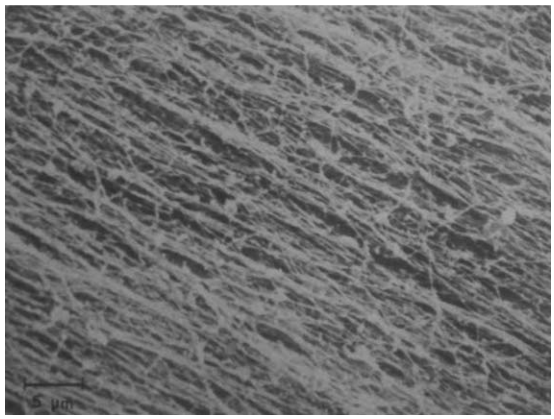

FIGURE 3 SEM of ectoplasm of squid axon (Fig. 9.2 from Ref. 9).

A PROPOSAL FOR THE INTERMEDIACY OF FIBRILLAR MACROMOLECULAR NETWORKS

Based on these many observations and hypotheses, we offer a proposal that builds on them and which is depicted schematically in Figure 5. Attention is focused on actin-like microfilaments, although other fibrillar macromolecular components may be important. A key premise is that the resting and excited states of nerve are represented by the Ca^{2+}-contracted and the swollen monovalent (Na^+/K^+)-forms, respectively, and that the ionic environment is such that facile switching is allowed between the two states. An excitation (electrical, mechanical, and thermal) destabilizes the resting form, leading to rapid exchange with monovalent ions, principally Na^+ and K^+. The finding that this exchange is exothermic in inanimate macromolecular ion exchangers[30] can account for the exotherm on stimulation, which is known to occur in nerve. In the model, two options for ion exchange include Ca^{2+}, leaving the polyanionic macromolecular layer and/or Cl^- entering to preserve electrical neutrality if the mobility of Ca^{2+} for escape from the layer is sufficiently slow. The "interphase" within the fiber between adjacent stimulated and resting zones is bistable, and the excited zone can move (toward the right in Figure 5, although that can occur in both directions) with creation of a new excited zone, whereas the previous zone relaxes back to the resting state. This would be an adiabatic process, consistent with expectations. As noted by Tasaki (Ref. 7, pp. 128–129), termination of an action potential is a spontaneous process. Therefore, for the free energy change ΔG to be negative at termination, the endothermic transition to the Ca^{2+} resting state must be accompanied by an increase in entropy, and it has been proposed that liberation of water molecules surrounding the expanded Na^+-form on Ca^{2+} binding is the source of the entropy gain in addition to the polyanion releasing two moles of Na^+ per mole of Ca^{2+} bound.

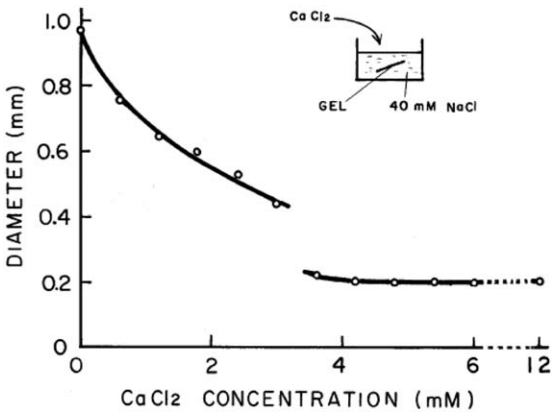

FIGURE 4 Discontinuous diameter change in a crosslinked polyacrylate gel fiber induced by $CaCl_2$ added to external 40 mM NaCl concentration plotted against $CaCl_2$ concentration.[11]

A key point in Figure 5 is that the lower impedance excitation zone advances axially via a series of closely coupled ion exchange events that occur radially. Thus, the excitation is propagated by a series of successive and very local macromolecular phase transitions. It is suggested here that such an excitation along a microfilament network can occur as propagating solitons. Channels and pumps in the plasma membrane could ensure that the [monovalent]/[divalent] balance is set correctly for a hair-trigger phase transition between resting and excited states. Moreover, as the ectoplasm is bonded to the underside of the membrane, there may be a synergistic coupling between radial ion exchange and the proposed signal propagation in the ectoplasm (e.g., a pulse along the nanofiber matrix) and Heimburg's "pulse along the membrane" model. It is interesting to speculate about the collapse of the ectoplasm to the resting state as the excitation propagates. Conversion back to the Ca^{2+}-compacted resting state may not be immediately reversible, with instead ion exchange leading to a more compact (more so than the original resting state) transient state that over a short time relaxes to the equilibrium resting state as a result of the kinetics of multistep ion-exchange processes and water redistribution around mobile ions and macromolecular fixed charges. Such a relaxation process may contribute to the well-known refractory period where some time must pass before an impulse can be triggered once again.

It would be of great interest to elucidate whether macromolecular phase transitions coupled with ion transport make significant contributions to the nerve signal propagation process versus ion transport directly along polyelectrolyte fiber surfaces. Perhaps both mechanisms can be operative depending on specific cell environment conditions. Toward that end, it must be noted that proposals for soliton transport in cytoskeletal proteins, with actin being a major component, are not new.[31,32] For example, it has been observed that actin filaments, considered as highly charged, one-dimensional polyelectrolytes, can have significant ionic movements along the fiber axis connected to "counter-anionic clouds," affording propagation of soliton-like, traveling ionic waves. It has been suggested these intracellular transmission lines may be the basis of a wide variety of physiological effects.[32]

We conclude this section with a connection to anesthetic action. Heimburg[16(b,c)] noted that the mechanism of action of

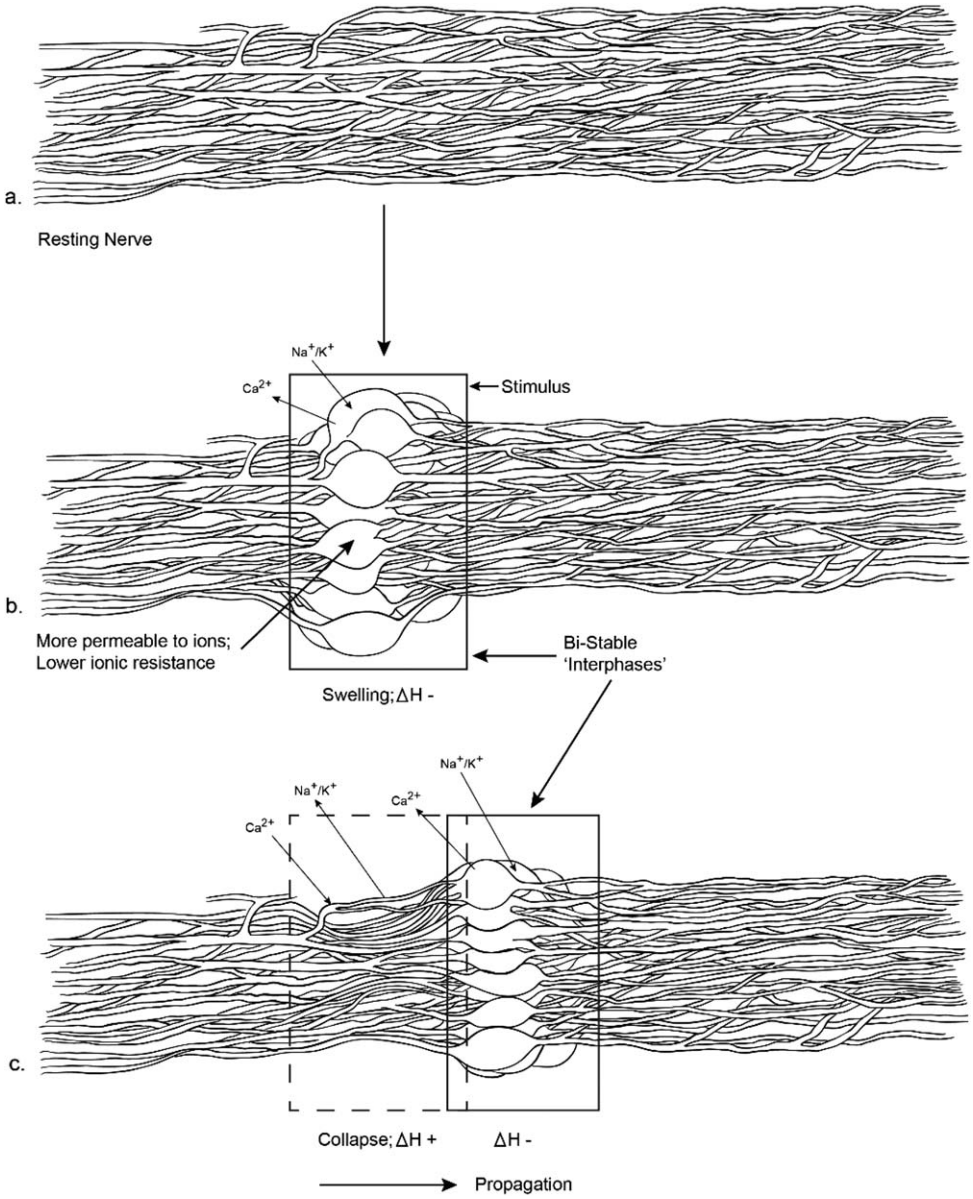

FIGURE 5 Proposed soliton-like mechanism of nervous transmission along ectoplasm fibers: (a) resting (Ca^{2+}-bound) state; (b) stimulated zone resulting in Ca^{2+}/monovalent ion exchange and local swelling; and (c) propagation of stimulation through an adjacent interphase region. The process can continue via successive steps in both directions. The degree of swelling is arbitrary.

anesthetics has remained somewhat of a mystery for at least 150 years and that much can be explained by the influence of anesthetics on the melting transitions of lipid bilayers which in turn can influence soliton wave propagation along the bilayer. However, a possible mechanism of anesthetic action could also be associated with the "two-state" ion-exchanging, polyanionic ectoplasm model discussed above. Specifically, any substance that could increase the stability of the resting (Ca^{2+}) form would render stimulation and transition to the swollen, monovalent-exchanged form more difficult. Many anesthetics are rather nonpolar but also contain dipoles and sites for hydrogen bonding, and many of these molecules may work in complex ways to stabilize multivalent ion-containing polyelectrolytes. Studies of the stabilities of synthetic polyelectrolyte systems in the presence of anesthetics may be a fruitful area of investigation.

As a final thought, it is interesting that plants, which lack formal neurons, are nevertheless affected by anesthetics.[33] Although plant roots (the "brains" of plants) contain membranes and ion channels which may be the locus on anesthetic action, they are also composed of a cytoskeletal network of macromolecular fibers (e.g., actin and microtubules). Perhaps the diminution of excitability in plant roots in the presence of anesthetics is at least partially due to the enhanced stabilization of the resting macromolecular ion complex in Figure 5, thereby raising the threshold for excitability.

TOWARD "ARTIFICIAL NERVES"

Although there is a robust research interest in artificial muscles, much less attention has been directed toward artificial nerves perhaps because of a lack of broad material design rules. A few are now offered based on the premise that a key seat of electrical activity in nerve, as outlined by Tasaki, is a nanofibrous network (ectoplasm) that can reversibly exchange monovalent and divalent metal ions, the latter most appropriately being Ca^{2+}. The nanofibers should principally be oriented longitudinally as part of a tubular construct and should contain fixed anions at a desired pH to allow for cation exchange. Importantly, the transition between a compact, Ca^{2+}-contracted form and a more swollen, monovalent cation-exchanged form should be very sharp such that small disturbances (electrical, thermal, and mechanical) of the stability of the Ca^{2+}-form can initiate ion exchange.

Much fundamental work on synthetic polyanionic gel beads and threads by Tasaki[9–12] and Horkay et al.[34,35] have laid the foundations for simple mimics of the properties noted above. One important conclusion from this work is that the volume shrinkage due to Ca^{2+} binding by sodium polyacrylate gels is not the result of tight ionic bridge formation but rather the result of an increase in the Flory–Huggins χ-parameter indicating that Ca^{2+} binding modulates the solvent quality of water thereby increasing chain–chain aggregation. Polyanionic gels also exhibit interesting electrical properties, including static potentials of negative several tens of millivolts which is curiously in the range of biological cell resting potentials.[36] The broad connection between ion exchanger properties and cell cytoplasm was suggested nearly a century ago by Loeb[37] and developed in more detail by Damadian[38] and Ling.[39]

As a first step toward extending ideas from bulk gels to fibrous constructs of synthetic polyanions, we recently reported[40] on the electrospinning and crosslinking of poly(acrylic acid) (PAA) tubular fiber constructs, with fibers rather randomly arranged, that show reversible length changes when the Na^{+}-form it titrated with $CaCl_2$ and then titrated with the chelator sodium citrate. The reversible length change transition is especially sharp if carried out with the tubes immersed in 150 mM NaCl. Work is in progress to fabricate highly aligned, crosslinked PAA fibers and to study electrical stimulation under conditions of bistability of monovalent–divalent cation exchange in an effort to test the general model outlined previously. It is hoped that at a minimum, efforts to design and fabricate artificial nerves will contribute to fundamental understanding of the behavior of polyelectrolytes and ion-exchanging nanofiber assemblies, aid in the design of improved interfaces that connect neurons with electrodes,[41] make important contributions to the development of smart materials systems and functional artificial neuron construction, and perhaps serve as crude models of the early versions of biological cell assemblages which were capable of exhibiting excitability and motion as well as bioelectric phenomena. It is likely that the potentially complex state of water around networks of polyelectrolyte fibers and attendant counterions will need to be considered for a more full understanding and exploitation of these ideas.[42] Toward that end, it is suggested that important clues lie in literature over many decades which must be acknowledged and critically reviewed.

CONCLUSIONS AND OUTLOOK

A key point of this Perspective has been advocacy of a consideration of nervous excitation and transmission in terms of roles that fibrillar macromolecular systems may play in this important area of physiology. Significant past work points to the intermediacy of macromolecules, and possible directions for the pursuit of "neuromimicking" materials is especially intriguing. Important information about diverse phenomena including electric communication within and between cells, mechanisms of anesthetic action, and cognition and memory,[43] as well as improved therapies for nerve repair and regeneration and mitigation of neurodegenerative and other diseases, may result from considerations of polyelectrolyte fibers as a possible component of the seat of electrical activity in nerves.

ACKNOWLEDGMENTS

The author thanks Dr. Ferenc Horkay of the National Institutes of Health and Dr. Murugappan Muthukumar of the University of Massachusetts, Amherst, for several stimulating discussions, Dr. Thomas Heimburg of the University of Copenhagen for kindly providing original artwork of Figures 1 and 2 from Ref. 16(b), and Zhongyang Li, a student in the Biomedical Art Program at the Cleveland Institute of Art, for the drawing of Figure 5. The support of the Joseph F. Toot, Jr., Professorship is also gratefully acknowledged.

REFERENCES AND NOTES

1 (a) R. Chambers, *Amer. Nat.* **1926**, *60*, 121–132; for detailed discussions, see also (b) W. Seifritz, Protoplasm; McGraw-Hill: New York, **1936**; (c) A. Frey-Wyssling, Submicroscopic Morphology of Protoplasm; Elsevier: Amsterdam, Netherlands, **1953**; (d) A. Frey-Wyssling, Macromolecules in Cell Structure; Harvard University Press: Cambridge, MA, **1957**.

2 See, for example, (a) D. E. Ingber, *J. Cell Sci.* **2003**, *116*, 1157–1173; (b) I. W. Hamley, V. Castelletto, *Angew. Chem. Int. Ed. Engl.* **2007**, *46*, 4442–4455; (c) D. A. Weitz, P. A. Janmey, *Proc. Natl. Acad. Sci. USA* **2008**, *105*, 1105–1106.

3 M. Muthukumar, Polymer Translocation; CRC Press: Boca Raton, FL, **2011**.

4 (a) M. A. McNiven, *Biol. Cell* **2003**, *94*, 555–556. McNiven reflects on the work of T. Keith Porter and the "microtrabecular lattice" concept. For a detailed review, see (b) J. Hauser, *Biol. Cell* **2003**, *94*, 561–596. For a discussion specifically concerning the interaction of axonal membrane systems and the "microtrabecular lattice," see (c) M. H. Ellisman, J. D. Lindsay, C. Wiley-Livingston, S. R. Levinson, In Structure and Function of Excitable Cells; D. C. Chang, I. Tasaki, W. J. Adelman, H. R. Leuchtag, Eds.; Plenum Press: New York, **1983**; Chapter 1, pp. 3–23.

5 J. Needham, Order and Life; Yale University Press: New Haven, **1936**; paperback edition: MIT Press, Cambridge, MA, **1968**, p. 152.

6 (a) L. V. Heilbrunn, General Physiology, 3rd ed.; W. B. Saunders: Philadelphia, **1952**, p. 564; Heilbrunn also wrote

extensively about the importance of calcium ions and physiological processes. See (b) L. V. Heilbrunn, The Dynamics of Living Protoplasm; Academic Press: New York, **1956**.

7 (a) A. Szent-Gyorgyi, Chemistry of Muscular Contraction, 2nd ed.; Academic Press: New York, **1951**; On muscle and motion: "(Muscle's) function is motion, one of the simplest and oldest signs of life which has always been looked upon by man as the criterion of life." Interestingly, some of the earliest work on artificial muscles involved polyelectrolytes. See, for example, (b) W. Kuhn, B. Hargitay, A. Katchalsky, H. Eisenberg, *Nature* **1950**, *165*, 514–516; (c) D. H. Walters, W. Kuhn, H. J. Kuhn, *Nature* **1961**, *189*, 381–383.

8 I. Tasaki, Nerve Excitation: A Macromolecular Approach; Charles C. Thomas: Springfield, IL, **1968**.

9 I. Tasaki, Physiology and Electrochemistry of Nerve Fibers; Academic Press: New York, **1982**; a PDF version can be found at http://stbb.nichd.nih.gov/pdf/tasaki/physiology-and-electrochemistry-of-nerve-fibers-Tasaki1982.pdf.

10 I. Tasaki, In Phase Transitions in Cell Biology; G. H. Pollack, W.-C. Chin, Eds.; Springer Science + Business Media B.V.: Berlin, **2008**; pp. 1–21.

11 I. Tasaki, *J. Theor. Biol.* **2002**, *218*, 497–505.

12 I. Tasaki, *J. Theor. Biol.* **2005**, *236*, 2–11.

13 I. Tasaki, P. M. Byrne, *Biopolymers* **1994**, *34*, 209–215.

14 (a) D. C. Chang, In Structure and Function of Excitable Cells; D. C. Chang, I. Tasaki, W. J. Adelman, H. R. Leuchtag, Eds.; Plenum Press: New York, **1983**; Chapter 11, pp. 227–254. See also Ref. 25 for detailed discussions of the axolemma–ectoplasm complex. Matsumoto was also a proponent of such a complex and its implications: (b) G. Matsumoto, *J. Theor. Biol.* **1984**, *107*, 649–666.

15 G. H. Pollack, Cells, Gels and the Engines of Life; Ebner and Sons: Seattle, WA, **2001**.

16 (a) T. Heimburg, A. D. Jackson, *Proc. Natl. Acad. Sci. USA* **2005**, *102*, 9790–9795; (b) T. Heimburg, *Phys. J.* **2009**, *8*, 33–39; Available at: http://arxiv.org/abs/1008.4279v1 [physics.bio-ph]; (c) T. Heimburg, Available at: arXiv:1409.2430v1 [physics.bio-ph]; **2014**. For an early discussion of solitons and action potentials, see (d) Y. Aizawa, Y. Shimatani, Y. Kobatake, *Prog. Theor. Phys.* **1975**, *53*, 305–314; (e) T. Heimburg, A. D. Jackson. *Biophys. Rev. Lett.* **2007**, *2*, 57–78.

17 A. L. Hodgkin, A. F. Huxley, *J. Physiol.* **1952**, *117*, 500–544.

18 Thermal effects are discussed in some detail in A. V. Hill, Chemical Wave Transmission in Nerve, Cambridge University Press: London, **1932**; Hill's book offers a critical assessment of the understanding of nervous transmission at that time.

19 Ref. 9, p. 267.

20 The polyanionic nature of the cortical layer likely plays a role in the mechanism of Golgi staining (metallic silver deposition; http://en.wikipedia.org/wiki/Golgi%27s_method) via reduction of Ag^+ exchanged at cation-binding sites.

21 Ref. 9, p. 257.

22 (a) J. Loeb, *Am. J. Physiol.* **1900**, *3*, 327–338; see also (b) J. Loeb, The Dynamics of Living Matter, Columbia University Press, New York, **1906**; Loeb was an early proponent of the connection between membrane potentials and Donnan equilibria.

23 (a) R. Höber, *Zentralbl. Physiol.* **1905**, *19*, 390–392. Höber also led the authorship of an important book: see (b) R. Höber, D. I. Hitchcock, J. B. Bateman, D. R. Goddard, W. O. Fenn, Physical Chemistry of Cells and Tissues; Blakiston: Philadelphia, **1945**.

24 (a) E. R. Kandel, J. H. Schwartz, T. M. Jessell, Essentials of Neural Science and Behavior; Appleton and Lange: Stamford, CT, **1995**; (b) J. E. Dowling, Creating Mind: How the Brain Works; W. W. Norton: New York, **1999**; (c) S. Ochs, A History of Nerve Functions; Cambridge University Press: London, **2004**; Chapter 8. The latter makes a passing reference to the view of Schultze in 1870 that the role of "fibrils" is to conduct an action potential and is perhaps the earliest suggestion of that possibility. See (d) M. Schultze, In Manual of Comparative and Human Histology; S. Stricker, Ed.; The New Sydenham Society London: London, 1870; Chapter 3, pp. 147–187. The volume is available as a free eBook at https://books.google.com/books?id=611JAAAAYAAJ.

25 (a) J. Metuzals, I. Tasaki, *J. Cell Biol.* **1978**, *78*, 597–621; (b) J. Metuzals, D. F. Clapin, I. Tasaki, In Structure and Function of Excitable Cells; D. C. Chang, I. Tasaki, W. J. Adelman, H. R. Leuchtag, Eds., Plenum Press: New York, **1983**; Chapter 3, pp. 53–75.

26 Ref. 15, Chapter 8.

27 V. E. Shashoua, *Nature* **1967**, *215*, 846–847.

28 L.-Y. M. Huang, R. A. Spangler, *J. Membr. Biol.* **1977**, *36*, 311–335; the membranes studied are asymmetric in that Ca^{2+} ions are complexed to only one face of the poly(glutamic acid) membrane, with the opposite face being partially ionized poly(glutamic acid). Such work does raise the question as to whether classical electrophysiological studies may at least on occasion be probing macromolecular transitions in the ectoplasm rather than, or in addition to, membrane channel protein characteristics.

29 A. Katchalsky, In The Neurosciences: A Study Program; G. C. Quarton, T. Melnechuk, F. O. Schmitt, Eds.; Rockefeller University Press: New York, **1967**; pp. 326–343. Shashoua [27] communicated his results to Katchalsky prior to publication, and the latter offered an analysis of the "firing" mechanism. In essence, Katchalsky proposed that salt accumulation at the interface between the two oppositely charged polyelectrolytes raises the conductance until a point is reached where the ionic strength is sufficient to cause collapse of the macromolecules and an increase in ion permeability, allowing the flushing of accumulated salt and restoring the initial membrane resistance. The interpretation of Huang and Spangler [28] builds on this idea. Significant opportunities may exist within the now-mature field of layer-by-layer polyelectrolyte complexes to probe electrical phenomena and neuronal-like behavior in detail.

30 D. S. Flett, P. Meares, *Trans. Faraday Soc.* **1966**, *62*, 1469–1481.

31 E. C. Lin, H. F. Cantiello, *Biophys. J.* **1993**, *65*, 1371–1378.

32 J. A. Tuszynski, S. Portet, J. M. Dixon, C. Luxford, H. F. Cantiello, *Biophys. J.* **2004**, *86*, 1890–1903.

33 (a) A. Grémiaux, K. Yokawa, S. Mancuso, and F. Balûska, *Plant Signal Behav.* **2014**, *9*, pii: e27886; (b) F. Balûska, *Plant Signal Behav.* **2010**, *5*, 87–89; see also (c) M. Pollan, *The New Yorker*, December 23, **2014**.

34 F. Horkay, I. Tasaki, P. J. Basser, *Biomacromolecules* **2001**, *2*, 195–199.

35 F. Horkay, I. Tasaki, P. J. Basser, *Biomacromolecules* **2000**, *1*, 84–90.

36 T. F. Shklyar, A. P. Safronov, I. S. Klyuzhin, G. Pollack, F. A. Blyakhman, *Biophysics* **2008**, *53*, 544–549.

37 J. Loeb, Proteins and the Theory of Colloidal Behavior; McGraw-Hill: New York, **1922**.

38 R. Damadian, *Ann. NY Acad. Sci.* **1973**, *204*, 211–248.

39 G. N. Ling, In Search of the Physical Basis of Life; Plenum Press: New York, **1984**; Chapter 14.

40 L. Meng, W. Klinkajon, P. K-hasuwan, S. Harkin, P. Supaphol, G. E. Wnek, *Polym. Int.* **2015**, *64*, 42–48.

41 Polyelectrolyte assemblies may have a key role in the new area of "iontronics," an emerging technology based on sophisticated control of ions as signal carriers to bridge solid-state electronics and biological systems. See H. Chun, T. D. Chung, *Annu. Rev. Anal. Chem.* **2015**, *8*, 441–462.

42 (a) Ref. 39, Chapter 9; see also (b) G. H. Pollack, The Fourth Phase of Water: Beyond Solid, Liquid and Vapor; Ebner and Sons: Seattle, WA, **2014**.

43 The long-standing belief that memory is stored in synapses is challenged by recent research. See (a) S. Cozier, *Sci. Am.*, in press. Available from: http://www.scientificamerican.com/article/could-memory-traces-exist-in-cell-bodies. Indeed, macromolecule-based memory storage has been a subject of attention for several decades. See, for example, (b) V. E. Shashoua, *Proc. Natl. Acad. Sci. USA* **1977**, *74*, 1743–1747.