Bioelectricity in Living Organisms

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Biomolecular recognition is approached within the establishment of coherent synchronizations among signaling players, whose physical nature can be equated to oscillators tending to the coherent synchronization of their vibrational modes. Cytoskeletal elements are now emerging as senders and receivers of physical signals, “shaping” biological identity from the cellular to the tissue/organ levels.

Keywords: physical energies ; mechanical vibration ; electric fields ; electromagnetic radiation

1. From Pioneering Studies to the New Course

The term bioelectricity points at the capability of living cells, tissues, and organisms to endogenously generate electric fields, with the potential to affect biological/functional dynamics. This field of enquiry grew progressively and has spread since the first studies, published in 1791 by Luigi Galvani in “De Viribus Electricitatis in motu musculari”, and Galvani’s subsequent discovery that a twitch can be elicited by placing a muscle in contact with a deviating cut sciatic nerve without the supply of metal electricity [1][2][3][4]. A fundamental advancement in the field of bioelectricity was achieved by the work of Emil du Bois-Remond, who demonstrated macroscopic electricity in frogs, fish, and human tissues, thus discovering the action potentials [5][6], and conclusively demonstrating the injury potential and current [7], for which Galvani himself had previously unknowingly provided evidence [4].

Following these pioneering studies, a fundamental breakthrough in the history of bioelectricity was marked by the relentless work of Harold Saxton Burr in the early 20th century (from 1916 up to the late 1950s). His studies were published in extremely relevant journals, including the Proceedings of National Academy of Sciences USA and Science. Burr developed an accurate millivoltmeter [8], and was able to trace and characterize the field properties of a developing frog’s egg [9]. In these studies, Burr performed some six thousand determinations on fifty frogs’ eggs, prior to the development of the primary axis of the embryo, as seen in the appearance of the medullary plate, recording potential differences in the electric pattern between the vertex of the terminal pole and four equidistant points on the equator of the egg [9]. The characterization of the electric field properties along the embryo development provided the first evidence that the primary axis of the organism came to lie in the plane of the greatest voltage drop from the vertex. In other worlds, Burr was able to predict, from the voltage pattern, where the head of the organism would develop, coming to the conclusion “that the electric pattern is primary and in some measure at least determines the morphological pattern” [9]. Burr also conducted studies on the electrodynamic patterns in a wide variety of plants, spanning from the growth correlates of electromotive forces in maize seeds [10] to the effect of a severe storm on the electric properties of a tree and the earth [11]. His rigorous methods, coupled with a visionary and eclectic personality, made Dr. Burr conceive that all living forms rely upon the existence of electrodynamic fields [12]. In this study, Burr declared his intention of “searching for the explanation of the phenomena, not in the currents alone but also in the surrounding medium”, drawing his attention to the field physics, rather than to the particle physics, being aware of the fact that “field physics centers theory and experimentation upon the medium in which the system as a whole is embedded and upon its structure” [12]. For these purposes, Burr designed a “vacuum-tube microvoltmeter” with a high degree of sensitivity and stability. This tool allowed him to explore the electric properties of a wide variety of living forms, with contacts between the instrument and the living organism made through silver–silver chloride electrodes immersed in physiological salt solution, with the entire apparatus being shielded and grounded at appropriate points, so that the recorded deflections of the galvanometer would have provided an accurate picture of the voltage differences in the explored living system. Remarkably, in his experiments, Burr showed that voltage gradients between the head and tail of Amblystoma or chick embryos could be determined with considerable certainty, not only when contact was made directly with the organism’s surface, but even when the electrodes were up to 2 mm away from the embryo surface [12]. With the same technique, Burr could show that the salamander embryo, revolving between the tips of a pair of capillary electrodes as a result of ciliary action, produced defined oscillations in the galvanometer as the developing head passed in a sequence under electrode pair of the system [12]. These findings gave the first evidence that, under the explored conditions, the embryo was acting as an AC generator of very-low frequencies, a phenomenon that could only be explained on the assumption that an electric field was existing and acting in the embryo. In his studies, Burr provided seminal discoveries, ranging from the first dissection of the response of slime mold to electric stimuli [13], to the discovery of defined bioelectric patterns during human ovulation [14]. Through the cooperation of a patient subjected to a laparotomy, Burr was able to perform a continuous recording of voltage differences intervening between the symphysis pubis and the vagina for 57 h, showing the feasibility of using bioelectric field assessment to determine with certainty and accuracy the time of ovulation in an intact human being [14]. Within this context, Dr. Burr also dissected the electrical signatures emerging from human diseased states, such as the electric correlates from nerve injury [15][16]. Dr. Burr addressed the electric features of cancer-susceptible mice to explore whether changes in voltage measurements may occur during the onset and development of a malignant tissue [17][18]. The results of the experiment consistently showed that twenty-four to twenty-eight hours after tumor implantation, changes were observed in the voltage gradients. This differential increased steadily and quite smoothly to reach a maximum of approximately five millivolts on or about the eleventh day. The analysis of bioelectric fields in the course of malignancies was also extended to human beings. In collaboration with Dr. Luis Langman, the approach of recording voltage gradients between the symphysis pubis and the vagina was exploited to assess whether marked changes in these gradients may reveal an early onset of malignancies [19][20]. In case of anomalous recordings, Langman offered the woman a laparotomy to confirm his suspicions. The technique proved astonishingly effective, since out of the 102 cases in which a significant shift in voltage recording was observed, 95 were confirmed to have malignancies [19][20]. While the exact malignancy location was variable form one patient to another, the cancers were often discovered before the patient had experienced suspicious symptoms. On the whole, the results from these studies led Dr. Burr to hypothesize the existence of “Fields of Life, or L-Fields”.

2. A Visionary Perspective Awaiting Future Developments

In addition to reporting further citations of studies from the monumental scientific production of Dr. Burr, some excerpts taken from his “Blueprint for Immortality, The Electric Patterns of Life”, first published in 1972 [21], will help us to appreciate his visionary and pioneering contribution to the field of bioelectricity and, more generally, to the advancement of science and the chance of novel cures for suffering people.

“Electro-dynamic fields are invisible and intangible; and it is hard to visualize them. But a crude analogy may help to show what the fields of life—L-fields for short—do and why they are so important. Most people who have taken high school science will remember that if iron filings are scattered on a card held over a magnet, they will arrange themselves in the pattern of the ‘lines of force’ of the magnet’s field. And if the filings are thrown away and fresh ones scattered on the card, the new filings will assume the same pattern as the old. Something like this happens in the human body. Its molecules and cells are constantly being torn apart and rebuilt with fresh material from the food we eat. But, thanks to the controlling L-fields, the new molecules and cells are rebuilt as before and arrange themselves in the same pattern as the old ones” [21].

Burr had a clear vision of the needs for novel tools and strategies to investigate novel fields and falsify or confirm a remarkable paradigm shift, as the chance of reinterpreting cellular and organ dynamics through the hypothesis that field physics, more than particle physics, may help with navigating the largely unexplored territory of morphogenetic signaling under normal and diseased states.

(“Until modern instruments revealed the existence of the controlling L-fields, biologists were at a loss to explain how our bodies ‘keep in shape’ through ceaseless metabolism and changes of material. Now the mystery has been solved: the electro-dynamic field of the body serves as a matrix or mould which preserves the ‘shape’ or arrangement of any material poured into it, however often the material may be changed” [21]).

The feelings of Dr. Burr, while discovering this new landscape, are well expressed in other excerpts from his work:

(“In the growth and development of every living system there is obviously some kind of control of the processes. As a distinguished zoologist once said, “The growth and development of any living system would appear to be controlled by someone sitting ‘on the organism’ and directing its whole living process. The Field theory suggested that it should be possible to determine the polarity and direction of the flow of energy transformations in the living system. The organism, as a whole, depends on such directives for its continued existence; so also does atypical growth” [21]).

Burr had also provocatively hypothesized that living organisms possessed a global bioelectric field orchestrating and/or emerging from more localized fields and acting as a sort of electrodynamic representation of smaller-scale components (organs, tissues, and cells) of the whole body itself, thus setting the basis for future studies aiming at verifying or falsifying such a hypothesis.

A major outcome from Dr. Burr's work was laying the basis for a transdisciplinary effort in science, fostering the needs for cooperation among committed "researchers" in apparently different disciplines, such as the arts, philosophy, and religion, always aiming at taking a glimpse from the merging of different viewpoints.

This transdisciplinary endeavor is now being propelled by the merging of cellular and developmental biology studies with the most advanced applications in computer science and artificial intelligence (AI), coupled with the development and availability of novel probes designed for the 3D imaging of electric microcurrents at both the single-cell and tissue levels.

3. Novel Evidence Supporting a Morphogenetic Code

Addressing the intracellular electric fluxes by the aid of a patch or voltage clamp merely allows for the assessment of electric dynamics at the cellular membrane level, which only accounts for about 0.1% of the cell volume. The availability of E-PEBBLEs (photonic explorer for biomedical use with biologically localized embedding), nanosized voltmeters that can diffuse throughout the entire cell volume, is currently providing a novel landscape based upon the 3D profiling of electric fields in living cells and tissues [22][23]. E-PEBBLEs encompass *di-4-ANEPPS*, a fast-responding, *ratiometric*, voltage-sensitive probe, revealing electric field changes as a shift in its fluorescence spectrum emission [22][23]. Notably, E-PEBBLEs revealed the existence of intracellular electric fields other than those traversing the cell membrane, but rather originating inside cells and exhibiting diffusing characteristics through the cytosol and beyond the cellular boundaries [22][23]. These observations are in agreement with the findings discussed herein, indicating that microtubules and microfilaments behave as electrically charged, oscillating circuitries amenable for both intra- and intercellular connectivity. A significant boost in the analysis of bioelectricity, conceived as cell processes involving ions or ion fluxes, has been afforded by the synthesis and availability of fluorescence voltage reporters, including DiBAC4 and CC2-DMPE [24][25]. These dyes, differently from classical electrode-based electrophysiological tools that are constrained to single-cell measurements, can be used in cultured cells, monitoring multicellular areas and volumes, with the chance for monitoring mobile targets and performing measurements over long periods of time. A detailed comprehensive description of the use and characteristics of fluorescent voltage reporters is available elsewhere [24][25]. The use of DiBAC4 and CC2-DMPE, in combination with confocal microscopy analysis, has clearly shown that: (i) membrane potential controls adipogenic and osteogenic differentiation of mesenchymal stem cells [26][27]; (ii) developing neurons form transient nanotubes facilitating electrical coupling and calcium signaling with distant astrocytes [28][29], affording neural-to-glial communication and developmental signaling through a physical form of bioelectronic circuitry; (iii) bioelectric signaling via potassium channels operates as a crucial mechanism in craniofacial patterning [30]; and (iv) altered ion fluxes hamper skeletal morphogenesis, as occurs in defined channelopathies [31].

Taken together, these findings corroborate the notion of a bioelectric memory, modeling the onset of shapes and functions in amphibian embryos and mammalian cells [32][33], prompting consideration for the capability of endogenous bioelectrical networks, such as those associated with microtubular proteins, to store non-genetic patterning information during development and regeneration [34][35].

A major perspective is now emerging from these studies—that endogenous voltage potentials and the microenvironment entail bioelectric signals whose complexity may span from revealing, inducing, and even normalizing cancer [36][37], with the perspective that bioelectric signaling may be part of reprogrammable circuits underlying embryogenesis, regeneration, and cancer [38][39][40][41].

References

1. Clarke, E.; Jacyna, L.S. Nineteenth-Century Origins of Neuroscientific Concepts; University of California Press: Berkeley, CA, USA, 1987.
2. Caneva, K.L.; Pera, M.; Mandelbaum, J. The Ambiguous Frog: The Galvani-Volta Controversy on Animal Electricity. J. Interdiscip. Hist. 1993, 23, 796.
3. Piccolino, M.; Bresadola, M. Shocking Frogs: Galvani, Volta, and the Electric Origins of Neuroscience; Oxford University Press: New York, NY, USA, 2013.
4. McCaig, C.D.; Rajnicek, A.M.; Song, B.; Zhao, M. Controlling Cell Behavior Electrically: Current Views and Future Potential. Physiol. Rev. 2005, 85, 943–978.

5. Du Bois-Reymond, E. Untersuchungen über thierische Elektricität . Ann. Phys. Chem. 1848, 151, 463–464.
6. Schuetze, S.M. The discovery of the action potential. Trends Neurosci. 1983, 6, 164–168.
7. Finkelstein, G. Emil du Bois-Reymond: Neuroscience, Self, and Society in Nineteenth-Century Germany; The MIT Press: Cambridge, MA, USA; London, UK, 2013.
8. Burr, H.S.; Mauro, A. Millivoltmeters. Yale J. Biol. Med. 1949, 21, 249–253.
9. Burr, H.S. Field Properties of the Developing Frog's Egg. Proc. Natl. Acad. Sci. USA 1941, 27, 276–281.
10. Nelson, O.E.; Burr, H.S. Growth Correlates of Electromotive Forces in Maize Seeds. Proc. Natl. Acad. Sci. USA 1946, 32, 73–84.
11. Burr, H.S. Effect of a Severe Storm on Electric Properties of a Tree and the Earth. Science 1956, 124, 1204–1205.
12. Burr, H.S.; Northrop, F.S.C. Evidence for the Existence of an Electro-Dynamic Field in Living Organisms. Proc. Natl. Acad. Sci. USA 1939, 25, 284–288.
13. Burr, H.S.; Seifriz, W. Response of the Slime Mold to Electric Stimulus. Science 1955, 122, 1020–1021.
14. Burr, H.S.; Musselman, L.K.; Barton, D.S.; Kelly, N.B. A bio-electric record of human ovulation. Science 1937, 86, 312.
15. Grenell, R.G.; Burr, H.S. Electrical Correlates of Peripheral Nerve Injury: A Preliminary Note. Science 1946, 103, 48–49.
16. Grenell, R.G.; Burr, H.S. Surface potentials and peripheral nerve regeneration. Fed. Proc. 1947, 6, 117.
17. Burr, H.S.; Strong, L.C.; Smith, G.M. Bio-Electric Correlates of Methylcolanthrene-Induced Tumors in Mice. Yale J. Biol. Med. 1938, 10, 539–544.
18. Burr, H.S. Changes in the Field Properties of Mice with Transplanted Tumors. Yale J. Biol. Med. 1941, 13, 783–788.
19. Langman, L.; Burr, H.S. Electrometric Studies in Women with Malignancy of Cervix Uteri. Science 1947, 105, 209–210.
20. Langman, L.; Burr, H. A technique to aid in the detection of malignancy of the female genital tract. Am. J. Obstet. Gynecol. 1949, 57, 274–281.
21. Burr, H.S. Blueprint for Immortality, the Electric Patterns of Life; Neville Spearman Limited: London, UK, 1972.
22. Tyner, K.M.; Kopelman, R.; Philbert, M.A. "Nanosized voltmeter" enables cellular-wide electric field mapping. Biophys. J. 2007, 93, 1163–1174.
23. Lee, Y.-E.K.; Smith, R.; Kopelman, R. Nanoparticle PEBBLE Sensors in Live Cells and In Vivo. Annu. Rev. Anal. Chem. 2009, 2, 57–76.
24. Adams, D.S.; Levin, M. General Principles for Measuring Resting Membrane Potential and Ion Concentration Using Fluorescent Bioelectricity Reporters. Cold Spring Harb. Protoc. 2012, 2012, 385–397.
25. Adams, D.S.; Levin, M. Measuring Resting Membrane Potential Using the Fluorescent Voltage Reporters DiBAC4(3) and CC2-DMPE. Cold Spring Harb. Protoc. 2012, 2012, 459–464.
26. Sundelacruz, S.; Levin, M.; Kaplan, D.L. Membrane Potential Controls Adipogenic and Osteogenic Differentiation of Mesenchymal Stem Cells. PLoS ONE 2008, 3, e3737.
27. Sundelacruz, S.; Moody, A.T.; Levin, M.; Kaplan, D.L. Membrane Potential Depolarization Alters Calcium Flux and Phosphate Signaling During Osteogenic Differentiation of Human Mesenchymal Stem Cells. Bioelectricity 2019, 1, 56–66.
28. Wang, X.; Bukoreshtliev, N.V.; Gerdes, H.-H. Developing Neurons Form Transient Nanotubes Facilitating Electrical Coupling and Calcium Signaling with Distant Astrocytes. PLoS ONE 2012, 7, e47429.
29. Wang, X.; Gerdes, H.-H. Long-distance electrical coupling via tunneling nanotubes. Biochim. Biophys. Acta (BBA) Biomembr. 2012, 1818, 2082–2086.
30. Adams, D.; Uzel, S.G.M.; Akagi, J.; Wlodkowic, D.; Andreeva, V.; Yelick, P.C.; Devitt-Lee, A.; Pare, J.-F.; Levin, M. Bioelectric signalling via potassium channels: A mechanism for craniofacial dysmorphogenesis in KCNJ2-associated Andersen-Tawil Syndrome. J. Physiol. 2016, 594, 3245–3270.
31. Leddy, H.A.; McNulty, A.L.; Lee, S.H.; Rothfusz, N.E.; Gloss, B.; Kirby, M.L.; Hutson, M.R.; Cohn, D.H.; Guilak, F.; Liedtke, W. Follistatin in chondrocytes: The link between TRPV4 channelopathies and skeletal malformations. FASEB J. 2014, 28, 2525–2537.
32. Levin, M. The Biophysics of Regenerative Repair Suggests New Perspectives on Biological Causation. BioEssays 2020, 42, e1900146.
33. Law, R.; Levin, M. Bioelectric memory: Modeling resting potential bistability in amphibian embryos and mammalian cells. Theor. Biol. Med. Model. 2015, 12, 22.

34. Tseng, A.; Levin, M. Cracking the bioelectric code: Probing endogenous ionic controls of pattern formation. Commun. Integr. Biol. 2013, 6, e22595.

35. Levin, M. Endogenous bioelectrical networks store non-genetic patterning information during development and regeneration. J. Physiol. 2014, 592, 2295–2305.

36. Chernet, B.; Levin, M. Endogenous Voltage Potentials and the Microenvironment: Bioelectric Signals that Reveal, Induce and Normalize Cancer. J. Clin. Exp. Oncol. 2013, Suppl. 1, S1–002.

37. Levin, M. Morphogenetic fields in embryogenesis, regeneration, and cancer: Non-local control of complex patterning. Biosystems 2012, 109, 243–261.

38. Levin, M. Bioelectric signaling: Reprogrammable circuits underlying embryogenesis, regeneration, and cancer. Cell 2021, 184, 1971–1989.

39. Chernet, B.T.; Fields, C.; Levin, M. Long-range gap junctional signaling controls oncogene-mediated tumorigenesis in Xenopus laevis embryos. Front. Physiol. 2015, 5, 519.

40. Tuszynski, J.; Tilli, T.M.; Levin, M. Ion Channel and Neurotransmitter Modulators as Electroceutical Approaches to the Control of Cancer. Curr. Pharm. Des. 2017, 23, 4827–4841.

41. Lobikin, M.; Chernet, B.; Lobo, D.; Levin, M. Resting potential, oncogene-induced tumorigenesis, and metastasis: The bioelectric basis of cancer in vivo. Phys. Biol. 2012, 9, 065002.