

Alexander G. Volkov *Editor*

Plant Electrophysiology

Methods and Cell Electrophysiology

 Springer

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Preface

Plant electrophysiology is the study of the electrochemical phenomena associated with biological cells and tissues in plants. It involves measurements of electrical potentials and currents on a wide variety of scales from single ion channels to whole plant tissues. Electrical properties of plant cells mostly derive from the electrochemical properties of their membranes. Electrophysiological study of plants includes measurements of the electrical activity of the phloem, xylem, plasmodesmata, stomata, and particularly the electrical signals, propagation along the plasma membrane. Action potentials are characteristic responses of excitation that can be induced by stimuli such as: applied pressure, chemical substances, thermal stimuli, electrical or magnetic stimuli, and mechanical stimuli.

There are two major divisions of electrophysiology: intracellular recording and extracellular recording.

The electrical phenomena in plants have attracted researchers since the eighteenth century and have been discussed in a variety of books (Baluška et al. 2006; Bertholon 1783; Bose 1907, 1913, 1918, 1926, 1928; Lemström 1902; Ksenzhek and Volkov 1998; Volkov 2006; Volta 1816). The identification and characterization of bioelectrochemical mechanisms for electrical signal transduction in plants would mark a significant step forward in understanding this under-explored area of plant physiology. Although plant mechanical and chemical sensing and corresponding responses are well known, membrane electrical potential changes in plant cells and the possible involvement of electrophysiology in transduction mediation of these sense-response patterns represent a new dimension of plant tissue and whole organism integrative communication. Plants continually gather information about their environment. Environmental changes elicit various biological responses. The cells, tissues, and organs of plants possess the ability to become excited under the influence of certain environmental factors. Plants synchronize their normal biological functions with their responses to the environment. The synchronization of internal functions, based on external events, is linked with the phenomenon of excitability in plant cells. The conduction of bioelectrochemical excitation is a fundamental property of living organisms.

Electrical impulses may arise as a result of stimulation. Once initiated, these impulses can propagate to adjacent excitable cells. The change in transmembrane potential can create a wave of depolarization which can affect the adjoining resting membrane. Action potentials in higher plants are the information carriers in intracellular and intercellular communication during environmental changes.

The conduction of bioelectrochemical excitation is a rapid method of long distance signal transmission between plant tissues and organs. Plants promptly respond to changes in luminous intensity, osmotic pressure, temperature, cutting, mechanical stimulation, water availability, wounding, and chemical compounds such as herbicides, plant growth stimulants, salts, and water potential. Once initiated, electrical impulses can propagate to adjacent excitable cells. The bioelectrochemical system in plants not only regulates stress responses, but photosynthetic processes as well. The generation of electrical gradients is a fundamental aspect of signal transduction.

The first volume entitled “Plant Electrophysiology—Methods and Cell Electrophysiology” consists of a historical introduction to plant electrophysiology and two parts. The first part introduces the different methods in plant electrophysiology. The chapters present methods of measuring the membrane potentials, ion fluxes, trans-membrane ion gradients, ion-selective microelectrode measurements, patch-clamp technique, multi-electrode array, electrochemical impedance spectroscopy, data acquisition, and electrostimulation methods. The second part deals with plant cell electrophysiology. It includes chapters on pH banding in Characean cells, effects of membrane excitation and cytoplasmic streaming on photosynthesis in *Chara*, functional characterization of plant ion channels, and mechanism of passive permeation of ions and molecules through plant membranes.

The second volume entitled “Plant Electrophysiology—Signaling and Responses” presents experimental results and theoretical interpretation of whole plant electrophysiology. The first three chapters describe electrophysiology of the Venus flytrap, including mechanisms of the trap closing and opening, morphing structures, and the effects of electrical signal transduction on photosynthesis and respiration. The Venus flytrap is a marvelous plant that has intrigued scientists since the times of Charles Darwin. This carnivorous plant is capable of very fast movements to catch insects. The mechanism of this movement has been debated for a long time. The [Chap. 4](#) describes the electrophysiology of the Telegraph plant. The role of ion channels in plant nyctinastic movement is discussed in [Chap. 5](#). Electrophysiology of plant-insect interactions can be found in [Chap. 6](#). Plants can sense mechanical, electrical and electromagnetic stimuli, gravity, temperature, direction of light, insect attack, chemicals and pollutants, pathogens, water balance, etc. [Chapter 7](#) shows how plants sense different environmental stresses and stimuli and how phytoactuators respond to them. This field has both theoretical and practical significance because these phytosensors and phytoactuators employ new principles of stimuli reception and signal transduction and play a very important role in the life of plants. [Chapters 8](#) and [9](#) analyze generation and transmission of electrical signals in plants. [Chapter 10](#) explores bioelectrochemical aspects of the plant-lunisolar gravitational relationship. The authors of [Chap. 11](#)

describe the higher plant as a hydraulic-electrochemical signal transducer. **Chapter 12** discusses properties of auxin-secreting plant synapses. The coordination of cellular physiology, organ development, life cycle phases and symbiotic interaction, as well as the triggering of a response to changes in the environment in plants depends on the exchange of molecules that function as messengers. **Chapter 13** presents an overview of the coupling between ligands binding to a receptor protein and subsequent ion flux changes. **Chapter 14** summarizes data on physiological techniques and basic concepts for investigation of Ca^{2+} -permeable cation channels in plant root cells.

All chapters are comprehensively referenced throughout.

Green plants are a unique canvas for studying signal transduction. Plant electrophysiology is the foundation of discovering and improving biosensors for monitoring the environment; detecting effects of pollutants, pesticides, and defoliants; monitoring climate changes; plant–insect interactions; agriculture; and directing and fast controlling of conditions influencing the harvest.

We thank the authors for the time they spent on this project and for teaching us about their work. I would like to thank our Acquisition Editor, Dr. Cristina Eckey, and our Production Editor, Dr. Ursula Gramm, for their friendly and courteous assistance.

Prof. Alexander George Volkov Ph.D.

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Contents

Part I Methods of Plant Electrophysiology

1	At the Roots of Plant Neurobiology	3
	V. A. Shepherd	
2	Plant Electrostimulation and Data Acquisition	45
	Emil Jovanov and Alexander G. Volkov	
3	Plant Response to Stress: Microelectrode Voltage-Clamp Studies	69
	François Bouteau and Daniel Tran	
4	Application of Non-invasive Microelectrode Flux Measurements in Plant Stress Physiology	91
	Sergey Shabala and Jayakumar Bose	
5	Intracellular Measurements of the Electrical Properties of Walled Cells	127
	Roger R. Lew	
6	Making Contact and Measuring Cellular Electrochemical Gradients	145
	Anthony J. Miller	
7	Studying Membrane Transport Processes by Non-invasive Microelectrodes: Basic Principles and Methods . . .	167
	Sergey Shabala, Lana Shabala and Ian Newman	

8	Multielectrode Array: A New Approach to Plant Electrophysiology	187
	Elisa Masi, Elisa Azzarello and Stefano Mancuso	
9	Electrochemical Impedance Spectroscopy	205
	E. Azzarello, E. Masi and S. Mancuso	
10	Patch Clamp Techniques for Plant Cells	225
	J. Theo M. Elzenga	
 Part II Cell Electrophysiology		
11	pH Banding in Charophyte Algae	247
	Mary J. Beilby and Mary A. Bisson	
12	Membrane Excitation and Cytoplasmic Streaming as Modulators of Photosynthesis and Proton Flows in Characean Cells.	273
	A. A. Bulychev	
13	Functional Characterization of Plant Ion Channels in Heterologous Expression Systems	301
	Yi Wang	
14	Mechanism of Passive Permeation of Ions and Molecules Through Plant Membranes	323
	Alexander G. Volkov, Veronica A. Murphy and Vladislav S. Markin	
	Index	359

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Part I
Methods of Plant Electrophysiology

Chapter 1

At the Roots of Plant Neurobiology

V. A. Shepherd

Abstract If biology throughout the nineteenth and twentieth centuries was dominated by the metaphor of the machine, the metaphor underlying twenty first century biology is that of the network or web. A rapid proliferation of molecular data coupled with increased computational power has revealed that gene regulation, protein interaction, the topology of metabolism and signal-transduction in and between cells, tissues, organs and organisms can all be described as robust, resilient and modular networks. Such small-world networks are characterised by rapid signal propagation, a capacity for computation and for synchronisation between the same, or different, hierarchic levels. Organelles, cells, tissues, organisms and ecosystems are not mere aggregations of components, but are hierarchies of interacting systems or modules, each possessing a degree of autonomy, and each a degree of interdependence. Into this metaphor of the network has emerged the discipline of integrative plant electrophysiology, called by its adherents, plant neurobiology. This field aims to understand how plants perceive, recall and process experience, coordinating behavioural responses via integrated information networks that include molecular, chemical and electrical levels of signalling. Integrative plant electrophysiology rejects the long standing view of plants as passive insensate automata that react to the environment with mechanical simplicity. The controversial use of the word ‘neurobiology’ as applied to plants signifies that long-distance electrical signals, such as action potentials, convey meaningful information from the site of initiation to a distant site, where the signal is interpreted and evaluated, and an adaptive behavioural

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response is mounted. Such inter-module communication is ‘nervous’ in the sense that it is adaptive, thereby implying capacities for memory, learning, anticipating the future and for generating novel responses. By itself a touch stimulus is meaningless, and by itself a behaviour (e.g. *Mimosa* leaf folding) is meaningless. Meaning lies in the network of processes that associate and integrate these events. Communication processes within, and between plants and associated organisms, can therefore be considered as biosemiotic, involving as they do the interpretation and evaluation of stimuli. This review traces historical aspects of the development of integrative plant electrophysiology and the methods that inform it, with a special emphasis on the work of Indian biophysicist Sir J. C. Bose (1858–1937), who, in an impressive body of published research, proposed that plants and animals share essentially similar fundamental physiological mechanisms. The first scientist to appreciate that responses in plants (e.g. leaf folding in the sensitive plant *Mimosa*) constitute behaviour reliant on integrative electrical signals; Bose argued further that all plants co-ordinate their movements and integrate their responses to the world through electrical signalling. Despite their sessile habits, plants are to be regarded as sensate, active, intelligent explorers of the world. Bose identified a fundamental physiological motif that interlinked measurable pulsations or oscillations in cellular electric potentials with oscillations in cell turgor pressure, cellular contractility and growth. All plants respond to the world and to other living things through adaptations of this pulsatile motif, an electromechanical pulse that underlies electro-osmotically enacted behaviour. J.C. Bose’s conclusions that all plants possess a nervous system, a form of intelligence, and a capacity for remembering and learning, were poorly received by prominent electrophysiologists of his time. Experiments devoted to plant responsiveness, inter-organism communication, kin-recognition, foraging, intelligence and learning as mediated by electrical signalling, are now published and debated in the mainstream literature as aspects of integrative plant electrophysiology.

1.1 Introduction: It’s a Small World

The two acts which seem to constitute the excitatory process, viz. excitation and response, are not continuous, but are joined together by a non-measurable link.... It is, in short, something which is involved in organism, for which the most proper designation is organismal. (Sir John Burdon-Sanderson 1904, cited by Haldane 1912).

In an emerging ‘biosemiotic’ understanding of life (Kauffman et al. 2008; Riofrio 2008) a living system processes, interprets and evaluates information that is meaningful in the sense that it is *about* the state of the external and internal worlds, requires interpretation by the system, and impels a response that propagates the organisation of the system. Thus, according to Kauffman et al. (2008), bio-information is semiotic or meaningful, and is therefore distinguishable from Shannon-type information, which, being non-semantic is inapplicable to biological systems.

As part of the propagating organization within living cells, the cell operates as an information-processing unit, receiving information from its environment, propagating that information through complex molecular networks, and using the information stored in its DNA and cell-molecular systems to mount the appropriate response (Kauffman et al. 2008, p. 28).

In the early twentieth century plant electrophysiological research focused on entire and complex behaviours, such as trap closure in the Venus flytrap *Dionaea*, or leaf movements in *Mimosa* (the sensitive plant). Much subsequent electrophysiological research was impelled by mechanistic materialist philosophies of science, with a mission to deconstruct complex behaviours into the simpler properties of components, beginning with cells, and continuing into further minute levels of ion channels and genes. At the same time prominent scientists drew attention to the problem of integration that such studies pose. For example, Peters (1969) postulated a network theorem of cell function, and argued for the existence of a cell cytoskeleton that we now take for granted.

There still remains the puzzle as to how the cell is integrated on a molecular basis, and adjusted to environmental stimuli and otherwise. Every change in the individual reactions of a cell is based upon some phase of chemistry or physical chemistry. Can we still believe, however, that the whole living cell is merely an extremely complex chemical equilibrium or have we still to look for some tenuous coordinating structure, fulfilling the role the nervous system does in the animal? (Peters 1969).

Peter's comments apply to other hierarchical levels of living systems, such as the whole plant. At the close of the twentieth century, increased computing power and the expansion of the World-Wide Web, coupled with the pursuit of genes and plant genomes, has enabled us to recognise the systems or network level of gene regulatory and metabolic networks, for example in *Arabidopsis* (reviewed, Yuan et al. 2008). If biology from the mid-nineteenth century and throughout the twentieth century had been dominated by the metaphor of the machine, as argued persuasively by Koestler (1978), the symbolism underlying twenty-first century biology has become that of the network or Web.

The proliferation of molecular data and rapid increase in computational power confirms that cells, organisms and ecosystems behave as networks of a special kind, the so-called small-world, or scale-free network (Strogatz 2001, 2003). These are not purely scale-free networks, but contain a hierarchy of modules (Ravasz et al. 2002) such as sets of genes, metabolons, cell groups or modules, tissues, organs interlinked by communication pathways and so on. Protein interaction networks, gene regulation networks, signal-transduction pathways, the large-scale topology of metabolism and cellular interactions, can all be described mathematically as modular small-world networks (reviewed, Albert 2005). In the 1990s cell biology began its ongoing shift from a molecular to a modular approach (reviewed by Hartwell et al. 1999) and this has revealed many surprising similarities between animals and plants. For example, defects in homologous modular gene networks common to *Arabidopsis* and humans leads in one instance to disrupted negative gravitropism in the plant, and neural crest defects resulting in craniofacial disease in humans (McGary et al. 2010).

Small-world networks seem to be inherent in the way living systems are organised. Not only protein to protein interactions, gene regulation, the topology of metabolism, but the human nervous system, gossip networks, networks of scientific collaboration and citation, Internet and peer group connectivity, and the structure of language behave as small-world networks (Strogatz 2001).

Small-world networks are collectives of nodes and the edges or paths that connect them as conduits for the flow of bio-information. On a cellular level, paths can be directed from one node to another, as in substrate to product, or non-directed, in which mutual interactions occur, such as the binding of one protein by another. Some nodes (hubs) are highly clustered, meaning that they connect to many or even all other nodes, whilst others are poorly connected. The power-law distribution of node connectivity makes these networks extremely robust and resilient with inbuilt redundancy. A key point is that the mathematical structure of small-world networks endows them with

enhanced signal propagation speed, computational power and synchronisability (Watts and Strogatz 1998).

The deconstruction of the behaviours of organisms and cells into the simpler properties of component molecules has culminated in the elucidation of genomes. So successful has this approach been that it has begun, like the ouroborus, to devour its own tail. The vast extent of networked epigenetic regulation has now been recognised, and the emerging concept of ‘a gene’ is now ‘a field of possibility’ (reviewed by Jorgensen 2011). Nucleic acid sequences are the foci of alternative chromatin states, which are responsive to developmental or environmental circumstances. Barbara McClintock’s concept of a genome that can be rapidly reorganised in response to specific or novel challenges (Keller 1983) has moved to the forefront.

In plants, gene expression is responsive to environmental stimuli transmitted via electrical signals between distant tissues. As a classic example, a flame wound activates proteinase inhibitor genes in distant tissues via an electrical signal (Wildon et al. 1992), called by them an action potential, but which was probably a variation or slow-wave potential (Davies 2004, 2006). Gene expression in plants is responsive to stimuli including light, osmotic conditions, and gravity, through the cellular calcium signalling network that Trewavas and Malho (1998) have called ‘the big network’.

The root–shoot polarity is fundamental to plant life. At the level of integrative signalling, the neural system of plants (reviewed by Barlow 2008) embraces the vascular tissues; phloem, now widely acknowledged as the living conduit through which fast-moving long-distance action potentials are transmitted, and xylem, through which the slow-wave or variation potentials stimulated by wounding may travel; as well as a postulated integrative centre or “root-brain” at the root apex transition zone (Baluska et al. 2004), whose cells emit synchronised electrical spikes (Masi et al. 2009). In terms of small-world network theory, this would be less a ‘command centre’ than a hub, a node which connects to most or all other nodes in the network.

The medium (network) is truly the message. Events perceived at the level of roots translate into adaptive action at the leaves. For example, water-stressed maize responds to onset of watering with root-initiated action potentials transmitted via the phloem to the leaves, where the rates of CO₂ and H₂O exchange subsequently increase (Fromm and Fei 1998). The guard cells regulating this gas exchange behave as networks. For example, conductance of stomata is ‘patchy’ in that coherent groups of stomata can independently adjust their conductance, and stomatal patches can oscillate in phase with distant patches on the same leaf, implying long-distance interaction (reviewed by Mott and Buckley 2000). Similarly, foraging by roots and leaves (now commonly referred to as ‘foraging strategy’) is plastic and adaptive and involves integration of local and systemic responses (reviewed by de Kroon et al. 2009). The plant is

...an interconnected network of modules, each with the ability to sense and respond to its environment (de Kroon et al. 2009).

Decisions about branching frequency are made locally and involve local signals within a module such as shoots or roots, but can be modified by signals emerging from other connected modules, resulting in

...integrated and adaptive response at the level of the whole plant to its whole environmental context (de Kroon et al. 2009, p. 705).

Although once controversial, it is now fully accepted that plants employ electrical signals in the integration of their responses to the world. Stimuli such as changes in light, temperature, water potential (or turgor pressure from a cell’s point of view), touch, wounding, sound (Telewski 2006) or volatile chemical signals, can induce electrical signals including receptor potentials, rapidly propagated action potentials and slow wave or variation potentials (reviewed Fromm and Lautner 2007).

In this age of networks, signal propagation, synchronisation and computation (in loose terms, a capacity for acquiring and assessing information, or decision making) have emerged as research priorities in integrative plant electrophysiology. These issues are central to our understanding of how, for example, roots may succeed in mining for mineral nutrients in the same place for hundreds or even thousands of years (Frommer 2010), and how, at the same time, leaves may position themselves for harvesting the light energy that is the portal for energy to enter the biosphere, whilst moderating the rate of photosynthesis according to water status of different regions of the root system (Fromm and Fei 1998).

Controversy over the use of terminologies usually not associated with plants (such as ‘intelligence’, ‘learning’, ‘memory’ or ‘nervous system’) is resolvable within the context of Living Systems Theory (Barlow 2008) where each level of biological organisation (e.g. cell, tissue, organ, organism and ecosystem) is supported by a set of critical subsystems, which repeat at each level, building a ‘self-similar organisational hierarchy’ (Barlow 2008). This concept endows the small-world network with the aspect that is critical to life- meaning. It removes the

loaded word ‘intelligence’ and considers instead information processing by living systems and their subsystems.

This returns us to the study of complex plant behaviours that began in the nineteenth and early twentieth centuries. Informed by the twenty first century concepts of the behaviour of networks, and with an impetus towards integration, we can employ the techniques of vast computational power, multiple electrode systems, faster data acquisition and analysis, and advanced imaging in an attempt to grasp the behavioural complexity of plants.

Histories are relational. As genes may be fields of possibility, existing in alternative states according to the circumstances surrounding them, histories unfold differently according to the focus of the compiler. The review that follows does not attempt to cover the rich and complex history of plant electrophysiology but takes as its focus the position of integrative plant electrophysiology. It includes a brief history of the research of Jagadis Chandra Bose, who had at the turn of the last century had so controversially argued that plants are integrated by the functional equivalent of a nervous system. In so doing the review refers to current and past advances in the electrophysiology of complex plant behaviours.

1.2 A Short History of ‘Animal and Vegetable Electricity’

1.2.1 *The Discovery of Animal Electricity: Galvani and Volta*

“I am attacked by two opposite sects- the scientists and the know-nothings”, wrote Luigi Galvani, “Both laugh at me, calling me the ‘frog’s dancing master’. Yet I know that I have discovered one of the greatest forces in nature” (cited by Verkhratsky et al. 2006).

Electrophysiology can be approached from two directions, the integrative and the reductive. Perhaps neither is complete without the other. The last in Galvani’s famous series of experiments with twitching frog’s legs can be called integrative, for he showed that muscles contracted when a frog’s leg was touched with the long sciatic nerves of the animal’s exposed spinal cord (Verkhratsky et al. 2006). In his 1791 publication, *De Viribus Electricitatis in Motu Musculari Commentarius*, Galvani regarded this ‘animal electricity’ or ‘electric fluid’ as the force integrating animal behaviour. His viewpoint, and perhaps his growing fame, led to a long-lasting and acrimonious scientific dispute with his compatriot, Volta.

In Galvani’s earlier series of experiments the frog leg muscles had contracted when an electrical circuit was made between nerve, two dissimilar metals in series, and another part of a frog’s body. Volta, sceptical and competitive, insisted that the electric current was reducible to the metallic interface in the circuit, and had nothing to do with ‘animal electricity’. Through sustained efforts to refute Galvani, Volta discovered bimetallic electrical conduction, and he invented the Voltaic battery in 1800 (Verkhratsky et al. 2006). After refusing to support Napoleon’s takeover of Bologna, Galvani suffered political persecution, lost his home,

his university position and his fortune. Volta, on the other hand, presented the battery to Napoleon, accepted a gold medal and became a successful politician.

Although criticised as a quasi-mystical vitalist by Volta and others, Galvani's explanation for 'animal electricity' (as the result of accumulated positive and negative charges on the inner and outer surfaces of nerve and muscle, where water-filled pores facilitated current flow; Piccolino 2006), anticipated Bernstein's later Membrane Theory of bioelectric potentials. The controversy over the existence of 'animal electricity' was resolved in 1779, shortly before Galvani's death, when von Humboldt proved the existence of both Galvani's 'animal electricity' and Volta's 'bimetallic electricity' (Becker and Marino 1982). Galvani and Volta were both partly right and partly wrong.

By the mid-nineteenth century, German mechanistic materialist philosophies had begun to influence the science of physiology, and hierarchically organised and bureaucratised research institutes began to emerge, in which scientists operated, in today's jargon, as technologists, managers and entrepreneurs (Veit-Brause 2002). The analytic–summative philosophy of science (Agutter et al. 2000) imbued the discipline of physiology with the idea that organisms are machines—Descartes' 'bete machine' (Drack et al. 2007). As a tenet of Descartes, philosophy, cells, tissues and organisms respond passively to the physical and chemical features of their environments as

...flotsam on a physico-chemical ocean... (Agutter et al. 2000).

The New Physicalist School, including Du-Bois-Reymond and his student Bernstein, focussed on the nerve action potential with the aim of dispelling 'Naturphilosophie' and what they perceived to be the fog of vitalism, seeking a purely physico-chemical explanation for 'animal electricity' (Veit-Brause 2002).

Nobili's 1858 invention of the galvanometer, an instrument that could detect the flow of electrical currents, enabled Du Bois-Reymond to show that stimulation of a nerve resulted in a propagated electrical disturbance (depolarisation) that provoked muscle contraction. Du-Bois-Reymond's student Julius Bernstein took this discovery further, inventing the 'rheotome' or 'current slicer', a galvanometer with a timer and sampling device with which, in 1868, he produced the first true recordings of nerve action potentials, measuring their velocity at 25–30 ms⁻¹ (Seyfarth 2006). In 1902, Bernstein formulated his Membrane Theory, incorporating Nernst's equations describing electrocatalytic theory, and Ostwald's concept of a semi-permeable lipid membrane. The Membrane Theory in essence described a cell as an electrolytic solution bounded by a selectively permeable membrane. This physico-chemical theory provided a satisfying mechanistic explanation for 'animal electricity', and it was widely accepted from the mid-1930s.

In 1873, a few years after the first measurement of animal action potentials, Sir John Burdon-Sanderson proved that trap closure in the Venus flytrap was brought about electrically, via action potentials that could travel at 20 cm s⁻¹. Later, Bose (1913) published detailed evidence that leaf movements in the sensitive plant *Mimosa* were induced by action potentials, propagated through the phloem, which he controversially argued was the plant equivalent of a nervous system.

This concept of nerve-like electrical signalling in *Mimosa* was unpopular at a time when many scientists sought to construct a purely physico-chemical theory of life (Agutter et al. 2000), exorcising vitalism, Romantic notions like Gustav Fechner's 'soul of plants' and interpretations of plant response that were reminiscent of animal behaviour.

The field of plant cellular electrophysiology was born within a theoretical scaffolding of membrane biophysics in the same era that saw acceptance of Bernstein's Membrane Theory. In 1930, Umrath succeeded in using intracellular microelectrodes to record action potentials from single giant *Nitella* cells, and continued improvements in electrometers, pen recorders and glass microelectrodes enabled the field of membrane biophysics to burgeon (reviewed by Hope and Walker 1975). The pace at which new techniques appeared and were applied accelerated (reviewed by Verkhatsky et al. 2006). Cole developed voltage-clamping in 1939; Hodgkin and Huxley applied it almost immediately, and produced the H-H membrane theory of excitation with indications of ion channel activity. Ling and Gerard developed a minimally invasive microelectrode technique in 1949, and Neher and Sakmann introduced patch-clamping of single ion channels in the 1980s. The techniques emerging from animal cell physiology were widely adapted and contemporaneously applied by plant cell electrophysiologists (reviewed by Hope and Walker 1975).

Whilst some researchers continued to focus on the electrophysiology of complex plant behaviours (reviewed, Sibaoka 1969, 1991; Pickard 1973; Davies 1987a; Wayne 1994), application of the patch-clamp technique had by the 1980s revealed the existence of plant ion channels underlying action potentials and other electrical signals (reviewed by Hedrich and Schroeder 1989), and a plethora of plant ion channels was identified. The molecular biology revolution pursued genes and plant genomes that specify such proteins, and the first complete genome sequence (*Arabidopsis*) was published in 2000.

1.2.2 The Discovery of Vegetable Electricity: Enter J.C. Bose

The discovery of 'vegetable electricity', in the sense of propagated electrical signals as integrative signals in all plants, took place in circumstances equally as contentious as the dispute between Galvani and Volta.

At the beginning of the twentieth century the Indian biophysicist Jagadis Chandra Bose (Fig. 1.1) was already well known and respected for his ingenuity and perspicacity in the field of microwave physics. In a prolific 5 years, from 1894 to 1899, Bose performed and published innovative research into the physics of electromagnetic waves. Between 1985 and 1900, Bose published ten papers in the *Proceedings of the Royal Society*, all of them communicated by Lord Rayleigh, and others in the "Philosophical Magazine" and "The Electrician" (Sengupta et al. 1998). This work was admired by distinguished physicists of the time, including Lord Rayleigh and J.J. Thompson. Many of Bose's inventions, including

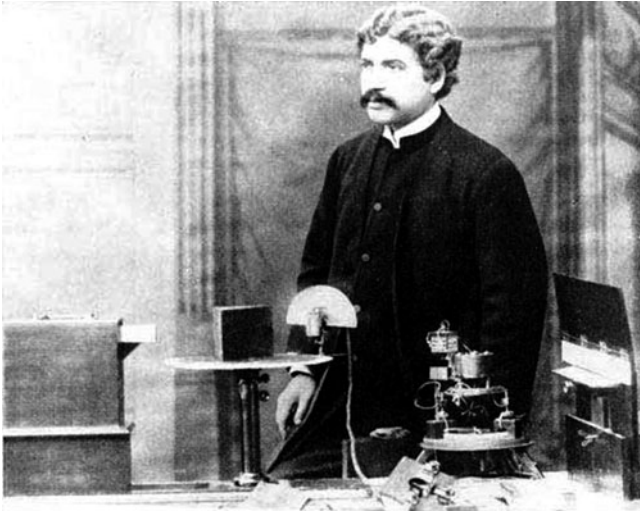


Fig. 1.1 J. C. Bose at the Royal Institution, London, with his radio equipment. The date is 1897, prior to his plant research

the world's first solid-state semi-conductor diode, are now devices taken for granted in contemporary microwave technology. Bose invented the 'coherer', a semiconducting diode device, which was adapted by Marconi for use in transatlantic wireless signalling.

Over a decade of research had established for Bose an enduring reputation as an inventor and physicist of extraordinary originality and perspicacity. Not only had he discovered millimetre waves, using ingenious devices of his own invention to generate them, but he laid bare most of their properties, and invented the 'eye' ('coherer') or receiver that would detect them (Engineer 2009). In the process, Bose published increasingly daring, original and inspired papers (Engineer 2009) in prestigious journals including *Proceedings of the Royal Society*, the "Philosophical Magazine" and "The Electrician" (Engineer 2009; Sengupta 2009; Sengupta et al. 1998; Bondyopadhyay 1998; Emerson 1997; Mitra 1997; Ramaseshan 1996).

This research, applauded in its time, continues to inspire today. Concepts from Bose's 1897 Royal Society paper were incorporated into the design of a 1.3 mm multibeam receiver, part of a 12 m telescope at the National Radio Astronomy Observatory in Tucson, Arizona (Emerson 1997) and Bose's one patented invention, the Detector for Electrical Disturbances, was the world's first solid-state semiconductor diode detector, a galena crystal detector sensitive to microwave/millimetre and optical waves (Engineer 2009; Bondyopadhyay 1998; Bose 1904).

Without semiconductors, today's networked world is truly unimaginable.

In around 1900, J.C. Bose began his plant electrophysiological research, becoming in the process one of the earliest biophysicists. He pursued this research

until his death in 1937 (Shepherd 1999, 2005). His hard-won reputation as a physicist of originality and insight was almost immediately overwritten, in Western botanical and electrophysiological circles, by a kind of notoriety, an image as mystic, maverick and outsider. Nonetheless, he established a nascent field of integrative plant biophysics.

Bose has been criticised for holding vitalist views (Nandy 1995), but he was actually critical of vitalism. Whilst the vitalists had asserted a dualism between living and non-living things, in which the former were animated by a non-material force, an entelechy or *vis viva* that some had equated with ‘animal electricity’, Bose argued against ‘vital forces’ on the basis that there existed no sharp demarcation between the realms of the living and non-living—these were parts of a continuum. Life did not emerge *de novo* from the physico-chemical realm, but rather its properties were pre-figured and already inherent in matter. In this, Bose was allied more closely with the later process philosophers, including Alfred North Whitehead.

Bose began by applying delicate instrumentation he had invented in his semiconductor research to deliver electrical stimuli and record electrical responses from various plant parts, including leafy stalks of horse chestnut, plane tree, celery, turnip, cauliflower and Eucharis lily, the storage roots of carrot and radish, the flower stalk of the Arum lily and the fruit of the eggplant (Bose 1902). He discovered that both living animal and plant tissues exhibited a diminution of sensitivity after continuous stimulation, recovery after rest, a ‘staircase’ or summation of electrical effects following mechanical stimulation, abolition of current flow after applying poisons and reduced sensitivity at low temperature. Strong and feeble mechanical stimuli produced bioelectrical responses of opposite polarity in the mechanically stimulated radish. The published record clearly shows what today would be called receptor potentials, small touch-induced transient depolarisations that precede an action potential. Furthermore, the form and polarity of these potentials depended on the condition and history of the plant and upon viability. Responsiveness disappeared when the tissue was killed with steam.

Having reported his results at numerous prestigious meetings and institutions, including the Friday Evening Discourse of the Royal Institution, May 1901, and at the Royal Society in June 1901, Bose wrote

... the wave of molecular disturbance in a living animal tissue under stimulus is accompanied by a wave of electrical disturbance; ... in certain types of tissue the stimulated region is relatively positive to the less disturbed, while in others it is the reverse; ... this characteristic of exhibiting electrical response under stimulus is not confined to animal, but extends also to vegetable tissues. In these the same electrical variations as in nerve and muscle were obtained ... (Bose 1902).

In the audience for the 1901 lecture at the Royal Society were the two prominent electrophysiologists, Sir John Burdon-Sanderson and Auguste Waller, each now poised to become a professional nemesis to Bose, who stated

...every plant, and even the organ of every plant, is excitable and responds to stimulus by electric response... (Bose 1913),

and postulated the existence of a ‘vegetable electricity’ as the counterpart of ‘animal electricity’.

It was in this lecture that Bose also drew analogies between the semiconducting characteristics of metals, and the changes in electric potentials he had measured following mechanical stimulation of plant tissues. Eager to prove his non-vitalist stance (that there exists no sharp demarcation between the living and the non-living), Bose drew analogies between changes in semiconducting properties of metals following ‘stimuli’ (such as fatiguing and poisoning) and the responses of living tissues to similar stimuli—as if matter (the metals), had, in some sense, proto life-like properties. This attempt to reconcile the difficulties inherent in explaining the meaningful nature of a plant’s response to stimulation in terms of physico-chemical theory (biology’s central problem, according to Bose’s contemporary J.S Haldane, the son of Burdon-Sanderson) was poorly received.

The professional conflicts that subsequently arose between Waller and Bose have been eloquently reconstructed and analysed in Dasgupta’s comprehensive article (Dasgupta 1998). Whilst Burdon-Sanderson, the first to have measured action potentials in the Venus flytrap, maintained that excitation was restricted to such strange and exceptional plants and strongly objected to the use of the physiological word ‘response’ in connection with metals, Waller, although hostile, made no comment. However, in November 1901 Waller published his own paper, ‘Electrical response of vegetable protoplasm to mechanical excitation’ in the *Journal of Physiology*, reporting, amongst other things, electropositive (depolarisation) responses to mechanical stimulus in parts of ordinary plants (e.g. the vine-shoot), exactly as Bose had done.

Both Waller and Bose then claimed priority for the discovery of ‘vegetable electricity’. Professors Vines and Howes of the Linnaean Society, who had read proofs of Bose’s ‘vegetable electricity’ manuscript, archived without publication by the Royal Society 5 months before Waller’s claim to priority, established a committee of inquiry of the Linnaean Society (Geddes 1920). The inquiry granted Bose priority, but the damage was done. The roots of a professional conflict were deeply established.

Burdon-Sanderson later scathingly reviewed and recommended rejection of a *Mimosa* paper submitted by Bose to the *Philosophical Transactions of the Royal Society of London*. Paul Simons, describing the incident, writes,

...The attitude of Burdon-Sanderson, the pioneer of plant electrophysiology, was altogether more baffling. He too refused to believe Bose’s results. Why he was so antagonistic amazes me. Was it professional jealousy because he himself had not investigated the *Mimosa*? Was it because Bose did not cite Burdon-Sanderson’s paper on the Venus Flytrap?

Simons (1992) writes that doubt was cast on Bose’s professional competence. Bose was controversial, he had said that there was no demarcation between life and non-life (had he actually said that metals are alive?), and furthermore the Victorian science establishment in England was not well-disposed towards mavericks. To Bose, the problem was more easily understood:

...I had unwittingly strayed into the domain of a new and unfamiliar caste system, and so offended its etiquette..., he wrote, '...an unconscious theological bias was also present.... To the theological bias was added the misgivings about the inherent bend of the Indian mind towards mysticism and unchecked imagination.... Thus no conditions could have been more desperately hopeless than those which confronted me for the next twelve years...' (Bose 1917).

1.3 Bose's Research: The Biophysics of Plant Behaviour and Response

I once did not know that these trees have a life like ours...they eat and grow...face poverty, sorrows and suffering. This poverty may...induce them to steal and rob...they also help each other, develop friendships, sacrifice their lives for their children... (JC. Bose cited in Nandy 1995, p. 46).

Seeking unifying principals underlying apparent disparities between animal and plant responses, Bose invented original and ingenious instruments that enabled him to simultaneously measure bioelectric potentials and to quantify very small movements in plants.

But since plants for the most part seem motionless and passive..... limited in their range of movement, special apparatus of extreme delicacy had to be invented, which should magnify the tremor of excitation and also measure the perception period of a plant to a thousandth part of a second. Ultra-microscopic movements were measured and recorded, the length measured being often smaller than a fraction of a single wave-length of light (Bose 1918).

From amongst the numerous plants with which Bose worked, this review concentrates on the suite of experiments employing touch-sensitive plants, including *Mimosa pudica*, plants that perform spontaneous movements, such as the Indian telegraph plant *Desmodium*, as well as some 'ordinary' plants (e.g. *Phoenix dactylifera*, the Praying Palm of Faridpur, or *Musa*, the banana palm) that were neither dramatically touch-sensitive nor spontaneously motile.

Bose viewed each of the experimental plants as an individual whose history determined the nature of its response to the environment. He noted that seedlings germinated from the same batch of seed and raised under three different environmental conditions responded differently to application of a poison—the first batch was killed, the second recovered and a third batch was stimulated. Therefore, Bose did not pool his experimental data or subject it to statistical analysis. Rather, he was interested in the individually variable behaviour of each plant. Whilst contemporary plant physiological experiments strive to control and make consistent the environmental conditions, Bose regarded constant environment change as being essential for plant behaviour to reveal itself;

...the continuance of normal functions depends on external stimulus...deprivation of stimulus reduces plants to an atonic condition in which all life-activities are brought to a standstill...rhythmic activities are maintained...by stimulus... (Bose 1923, p. 245).

The velocity of the transmitted electrical excitation in *Mimosa* depended on the tonic condition of the plant. A plant in optimum condition showed a rapid velocity of excitation, but excessive stimulation also resulted in rapid fatiguing of the response. A subtonic plant responded to stimulus with an excitation of slower velocity, but excessive stimulation actually enhanced the response. This dependence on the strength and duration of previous stimulations indicated a form of learning that had to be considered when interpreting experiments.

A plant carefully protected under glass from outside shocks looks sleek and flourishing, but its higher nervous function is then found to be atrophied. But when a succession of blows is rained on this effete and bloated specimen, the shocks themselves create nervous channels and arouse anew the deteriorated nature... (Bose 1917).

Thus, the velocity of electrical transmission was modified by

...individual vigour...temperature, and by the season. In summer, the velocity in thick petioles is 30 mm/s, in winter, as low as 5 mm/s..." (Bose 1926, p. 63). The age of organs was also influential; "...It is impossible to dissociate from the consideration of the age of a leaf its previous history as regards the stimulus of sunlight...the uppermost or youngest leaf of *Mimosa* [is] pre-optimum and less sensitive...the sensitiveness...[reaches a] maximum as we descend lower...continuing to descend...excitability [is] progressively decreased... (Bose 1913, p. 267).

The standardised conditions of many plant physiological experiments, with constant light period, constant temperature, uniform watering, may not only produce the effete and bloated specimens Bose deplored, but the application of statistical analyses to such experiments will conceal the subtleties of individual plant behaviours that were a focus of Bose's research.

Many of Bose's experiments were published in books, probably because the research papers he submitted to prominent journals were archived for years, without publication. Of hundreds of intricate experiments using original and ingenious apparatus, reported in books, research papers and essays, I confine myself to a brief overview of those studies where Bose coupled specific plant behaviours with electrical and hydraulic signals. Four books are considered here; "Researches into the Irritability of Plants" (1913), "Life Movements in Plants" (1918), "The Ascent of Sap" (1923) and "The Nervous Mechanisms of Plants" (1926).

Bose (1913, 1918, 1923, and 1926) aimed to compare and contrast three kinds of responses. These were:

1. *Contractility* (plant movements, following a stimulus). For example, the *Mimosa* or "touch-me-not" plant folds its leaflets and dips the entire leaf as a response to being touched.

2. *Rhythmicity* (plant movements taking place automatically, analogous to a heartbeat: Bose 1913, p. 202). The Indian Telegraph Plant *Desmodium* (Bon Charal or “forest churl”) has a trifoliate leaf, whose two small lateral leaflets make mysterious spontaneous gyrations of regular periods. Bose found that *Biophytum* was capable of both contractile and rhythmic responses. Stimulus-induced and spontaneous movements both took place in the same plant, depending on the strength of the stimulus and the individual’s history (Bose 1913, p. 289). All plants showed rhythmic or pulsatile growth.
3. *Conductivity* (transmission of electrical excitation associated with plant movements).

Bose also investigated ordinary plants that made no obvious dramatic movements. These included *Chrysanthemum*, trees such as *Ficus*, *Nauclea*, the mango, monocotyledons including the banana (*Musa*), palms, and fruits and other organs, including the tomato, turnip, carrot and potato.

Bose invented unique instruments for simultaneously measuring bioelectric potentials and for quantifying very small movements in plants (Figs. 1.2a–d, 1.3a–e). Many of these instruments are still in working order, and housed in the Museum of the Bose Institute in Kolkata, India. With its frictionless jewelled bearings, and lightweight aluminium lever connected to the leaf, the delicate Resonant Recorder (Bose 1913) used a vertical lever to ‘write’ leaf movements (plant response) on a smoked glass plate that moved at a regular rate using a clockwork mechanism. The problem of friction of the writer against the smoked glass plate was solved by having the writer vibrate or resonate, making intermittent contacts with the plate (Bose 1926, p. 55). Leaf movements were recorded with precision (at intervals of 1/100th of a second)—and “the record is thus its own chronogram” (Bose 1913, p. 22).

Other extraordinary delicate instruments included the High Magnification Crescograph, which magnified increments of growth up to ten thousand times. A plant holder was connected via a series of gear wheels driven by a falling mass and controlled by a fan governor, and descended at various rates, compensating for the growth of the plant. When the rate of growth was exactly balanced with the movement of the plant holder, the record showed a horizontal line. An increase or decrease in the growth rate was reflected in a rise or fall in the record. Bose wrote that the Crescograph could detect and plot a change in growth rate as small as one in 27,000.

The Electric Probe, an early microelectrode, consisted of a fine platinum wire enclosed in a glass capillary, except at the tip. In circuit with a galvanometer, the probe could be advanced into plant tissue at fine intervals of 0.1 mm.

In addition to these instruments for measuring and plotting plant movements and changes in electrical polarity, Bose introduced techniques for electrical stimulation of different intensity. An induction coil, using a slide (potentiometer) to generate feeble (0.5–8 μA) or strong (100 μA) currents could be appended to any of the instruments so that movements or changes in electric potential could be monitored whilst stimulation from electric current was applied. Bose reported that

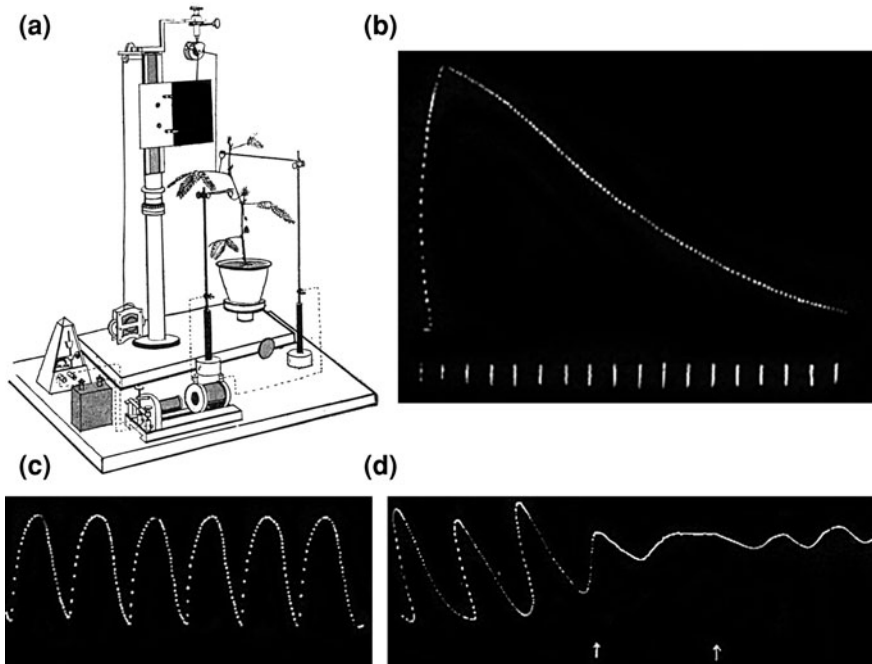


Fig. 1.2 Some of Bose's equipment and some measurements he made with it. **a** The resonant recorder (reproduced from Fig. 4, Bose 1913). This device had "frictionless" jewelled bearings, a fine lightweight *horizontal* lever connected to the pulvinus or leaf, and a *vertical* lever for writing the response on a smoked glass plate, which moved at a uniform rate using a clockwork mechanism. In this configuration, the duration of an "induction shock" applied to *Mimoso* was determined by a metronome, which completed the electric circuit. The illustration shows a *Mimoso* plant ready for measurement of leaf movements. **b** The record shows the leaf-dropping response in *Mimoso* measured with the resonant recorder (reproduced from Fig. 14, Bose 1913). Dots are at 1/10 s intervals during the "contractile" or leaf-dropping phase and at 10 s intervals during recovery. *Vertical marks*, 1 min intervals. **c**. The rhythmic gyrations of the leaflets of the telegraph plant *Desmodium* (reproduced from Fig. 145, Bose 1913). Individual dots are 2 s apart. This leaf was measured in summer and the whole period is about a minute, although in winter this increased to 4–5 min. **d**. Arrest of spontaneous movements in *Desmodium* by a cut applied at the first arrow. The pulsatile movement was revived by an electric shock at the second arrow. An electrical stimulus could substitute for a mechanical one. (Reproduced from Fig. 145, Bose 1913)

plants were in some cases more sensitive to electrical currents than animals. *Biophytum* responded to a feeble stimulating current of about 0.5 μA (Bose 1913, p. 27), which was too feeble for his own tongue to detect (Bose 1923). The

...sensitiveness of *Mimoso* to electrical stimulation is high and may exceed that of a human subject. (Bose 1913, p. 51).

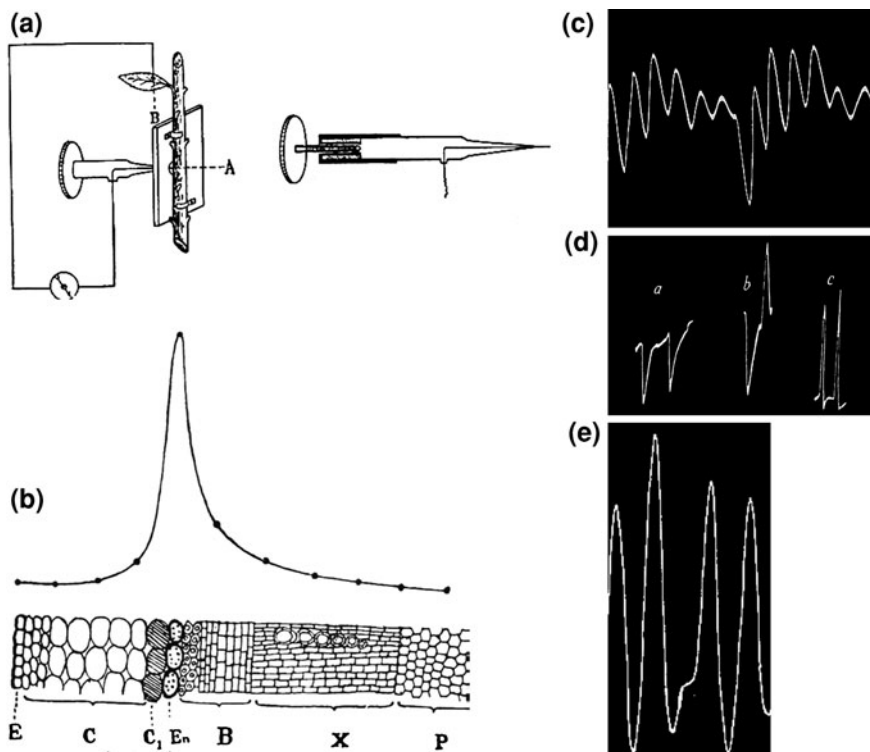


Fig. 1.3 **a.** The Electric Probe (reproduced from Fig. 75, Bose 1923). The tip of the Probe was in circuit with either a sensitive or Einthoven galvanometer, and the device could be driven, by small (0.1 mm) increments into the tissue by turning the screw. Bose achieved remarkable precision of measurement—a deflection of 1 mm PD between electrodes was equivalent to a 1 mV deflection of the galvanometer. In some cases, he measured potentials as small as 0.1 mV. The tip of the probe enters at A, and a reference contact is made with a distant or dead leaf. The micrometric screw enables the probe to be gradually introduced. **b.** A section of a *Brassica* petiole showing the relative cellular activity in terms of electromechanical pulsations, measured with the electric probe. The pulsations occur mainly in the inner cortical layer abutting the endodermis. Reproduced from Fig. 77, Bose 1923. **c.** Regular electromechanical pulsations in the cortical cells of *Musa*, the banana. Bose used an Einthoven galvanometer to measure the amplitude of these pulsations in *Nauclea* as *0.4 mV, and lasting *13.5 s. Reproduced from Fig. 71, Bose 1913. **d.** Three kinds of electrical response to electrical stimulus in *Musa*, the banana. The *first* (a) shows hyperpolarisation following indirect stimulus (feeble stimulus applied at a distance from the responding point), and this was coupled with the leaf erectile response. The *second* (b) shows biphasic response with stronger stimulus—a hyperpolarisation followed by a depolarisation. The *third* (c) shows the depolarisation response with direct stimulus, applied close to the responding point, and accompanied by turgor decrease. Reproduced from Fig. 80, Bose 1918. **e.** Periodic groupings of the electrical oscillations in the pulvinus of *Desmodium* (reproduced from Fig. 69, Bose 1923), which accompanied the mechanical oscillations of leaflet position

His numerous experimental set-ups enabled Bose to perform complex experiments, which would be challenging to execute today. He could simultaneously measure plant movements and electric potentials, measure very small electrical oscillations, apply mechanical stimuli and electrical stimuli, vary hydrostatic pressures, apply chemical inhibitors or poisons (e.g. KCN, HCl, NH₄, H₂S, NO₂, SO₂, anaesthetics such as chloroform and ether), suddenly modify temperature, vary light conditions and measure tiny growth increments over very short time intervals.

1.3.1 *Plant Nervous Systems: The Mimosa and Desmodium Work*

1.3.1.1 Intimate Coupling of Hydraulic and Electrical Signalling

Bose interpreted his results as constituting evidence that plants possess the equivalent of a well-defined nervous system. All plants co-ordinate their movements and integrate their responses to the environment via propagated electrical signals. All plants have an electromechanical pulse, and are capable of intelligent behaviour, memory and learning. Plants have receptors for stimuli, conductors (plant nerves), which electrically code and propagate the stimulus, and effectors, or terminal motor organs. The

...physiological mechanism of the plant is identical with that of the animal... (Bose 1926, p. 9).

...All plants and their organs are excitable, the state of excitation being manifested by an electric response of galvanometric negativity [relative depolarisation] (Bose 1926, p. 95).

It can only be in virtue of a system of nerves that the plant constitutes a single organised whole, each of whose parts is affected by every influence that falls on any other (Bose 1913, p. 121).

Bose's contemporaries Pfeffer and Haberlandt had reasoned that the collapse of leaves following touch stimulus in *Mimosa* was not a true excitation, but rather a sort of 'hydraulic lift', 'a disturbance of hydrostatic equilibrium within the transmitting elements', which were situated within the leptome, or phloem (reviewed, Haberlandt 1928). Haberlandt reasoned that the shock-induced movement of a leaflet exerted pressure on these transmitting cells, and succeeding pressure waves then acted as shock or touch stimuli on other leaflets. Scalding, he reported, was without effect and so this was not a true, protoplasmic excitation.

Bose (1914, 1926) reported to the contrary. In *Mimosa*, the excitatory response could be induced by touch, sudden temperature change, by initiation or cessation of a constant current and by induction shock. Crucially, the mechanical (touch) stimulus could be substituted for by an electrical one, the onset or cessation of an electric current. The excitation was bipolar, moving both with and against the

direction of the transpiration stream, unlike the non-discriminative hydro-mechanical model. The action potentials travelled at rates of 20–29 mm s⁻¹, at similar rates to Bernstein's measurements of the nerve impulse. Bose concluded that electrical signals (including action potentials) controlled the leaf movements.

Transmission of excitation in the plant is a process fundamentally similar to that which takes place in the animal, in the one case as in the other, a propagation of protoplasmic charge (Bose 1913).

Bose determined that the type of response depended on the strength of the stimulus. A non-electrical stimulus (light) applied to the upper half of the leaf produced either of two responses—an increase of turgor (and leaf lifting) if the light stimulus was moderate or short-lived, and an abrupt leaf-dropping response (loss of turgor on the lower half) if the light stimulus was strong.

The former response (leaf lifting) was associated with increased turgor pressure, expansion of cells, and “galvanometric positivity” [relative hyperpolarisation]. The latter (leaf-folding) involved a true excitation, a propagated wave of “galvanometric negativity” [relative depolarisation], cell contraction and abrupt loss of turgor pressure. The range of electrical responses showed a hyperpolarisation with mild stimulus, a biphasic response—both depolarisation and hyperpolarisation—with moderate stimulus, and a depolarisation with a strong stimulus, as in *Musa* (Fig. 1.3d). Bose determined that there are two forms of travelling signal, the first hydraulic and associated with leaf-lifting and turgor increase, the second a true propagated excitation, associated with turgor decrease and collapse of leaves. The first involved a

“...quick absorption of water which causes a hydrostatic impulse; this travelling with great rapidity delivers a mechanical blow at the distant responding point...” to cause the second, true propagated excitation, “... the excitatory response of contraction and galvanometric negativity [depolarisation]...” (Bose 1923, p. 205).

The motor organ in both *Desmodium* and *Mimosa* is the pulvinus, a joint-like thickening at the base of a petiole, which supports the leaf. Increase or decrease of turgor pressure in pulvinar cells causes the leaf to collapse or rise. One of Bose's remarkable discoveries was that only the electrical or excitatory response decreased turgor pressure in the *Mimosa* pulvinus sufficiently to collapse the leaves. The hydraulic and electrical systems of a plant cell were intimately coupled, Bose reasoned, and the plant nervous system was complex, with both sensory and motor components. A mild unilateral stimulus was conducted only on the stimulated side. However, if repeated, or if the stimulus was increased to a critical intensity, the slow sensory or hydraulic impulse associated with it was converted into a fast motor impulse, a true excitation, in the pulvinus. The ascending hydraulic impulse was converted into a descending, true excitation after crossing over at the apex of the stem (Bose 1926, p. 42, p. 204).