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HISTORY OF NEUROSCIENCE

Animal electricity and the birth of electrophysiology: The legacy of Luigi Galvani

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ABSTRACT: Preceded by a companion paper on Galvani's life, this article is written on the occasion of the bicentenary of the death of Luigi Galvani. From his studies on the effects of electricity on frogs, the scientist of Bologna derived the hypothesis that animal tissues are endowed with an intrinsic electricity that is involved in fundamental physiological processes such as nerve conduction and muscle contraction. Galvani's work swept away from life sciences mysterious fluids and elusive entities like "animal spirits" and led to the foundation of a new science, electrophysiology. Two centuries of research work have demonstrated how insightful was Galvani's conception of animal electricity. Nevertheless, the scholar of Bologna is still largely misrepresented in the history of science, because the importance of his researches seems to be limited to the fact that they opened the paths to the studies of the physicist Alessandro Volta, which culminated in 1800 with the invention of the electric battery. Volta strongly opposed Galvani's theories on animal electricity. The matter of the scientific controversy between Galvani and Volta is examined here in the light of two centuries of electrophysiological studies leading to the modern understanding of electrical excitability in nerve and muscle. By surveying the work of scientists such as Nobili, Matteucci, du Bois-Reymond, von Helmholtz, Bernstein, Hermann, Lucas, Adrian, Hodgkin, Huxley, and Katz, the real matter of the debate raised by Galvani's discoveries is here reconsidered. In addition, a revolutionary phase of the 18th century science that opened the way for the development of modern neurosciences is reevaluated. © 1998 Elsevier Science Inc.

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INTRODUCTION

And still we could never suppose that fortune were to be so friend to us, such as to allow us to be perhaps the first in handling, as it were, the electricity concealed in nerves, in extracting it from nerves, and, in some way, in putting it under everyone's eyes [32].

With these words in 1791 Luigi Galvani (Fig. 1, and see [20]), an eminent Professor of the University of Bologna and member of the *Accademia delle Scienze* expressed in a somewhat hesitating, but still proud way the gratification of the discoverer, conscious of the importance of his scientific achievement: he had been the first to provide evidence for the electrical nature of the mysterious fluid (long referred to as "animal spirits") involved in nerve conduction and muscle contraction. The publication in 1791 of Galvani's main work (the famous *De Viribus Electricitatis in Motu Musculari Commentarius* [31]), summarizing and discussing more than 10 years of research on the effect of electricity on animal preparations (mostly frogs), had an impact on the scientific audience comparable to the social and political impact of the French revolution in those same years [26]. Wherever frogs were available, not only scientists, but also laymen tried to reproduce Galvani's experiments in order to induce contractions in the leg muscles of recently dead animals. The effects of electricity were also studied in other species, and in several occasions even in human beings. The experiments made in 1803 in London by Galvani's nephew, Giovanni Aldini (1762–1834), who applied electricity to the head of an executed criminal, had a particularly strong echo [8]. The possibility, suggested by these experiments, that electricity could be used to "revive" dead corpses inspired Mary Shelley's famous character *Frankenstein*.

In the scientific community, however, after the initial excitement and enthusiasm, some doubts emerged concerning Galvani's main hypothesis (elaborated in the *Commentarius*) that the electricity involved in the contraction of frog muscles had an animal origin. The controversy that developed over Galvani's interpretation had, as its protagonist, Alessandro Volta, a young and brilliant physicist of the University of Pavia, who became interested in Galvani's studies in 1792, after reading the *Commentarius* [107]. Opposing Galvani's hypothesis, Volta put forward the view that frogs, although capable of reacting, like sensitive electroscopes, to external electricity, were themselves devoid of intrinsic electricity. According to Volta, electricity originated from the metals normally used for connecting the frog nerve and muscle. The controversy between Galvani and Volta is considered one of the most important

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FIG. 1. (Top) Contemporary portraits of Galvani and of his wife Lucia Galeazzi (courtesy of family Ferretti, Ferrara); (Bottom) Galvani's autograph of the experimental log of the spark experiment (left) with the passage alluding to his wife collaboration in this experiment (shown on the right at higher magnification): ". . . if someone, the wife or somebody else, approached the finger to the conductor [of the electric machine] and a spark was being extracted, if at the same time I rubbed the crural nerves or the spinal cord by means of an anatomical knife with a bony handle, or simply approached it to the said spinal cord or nerves, contractions did appear even though no conductor was on the glass where the frog was. They appeared in the said way if the person who discharged the conductor . . ." (by courtesy of the *Accademia delle Scienze* of Bologna).

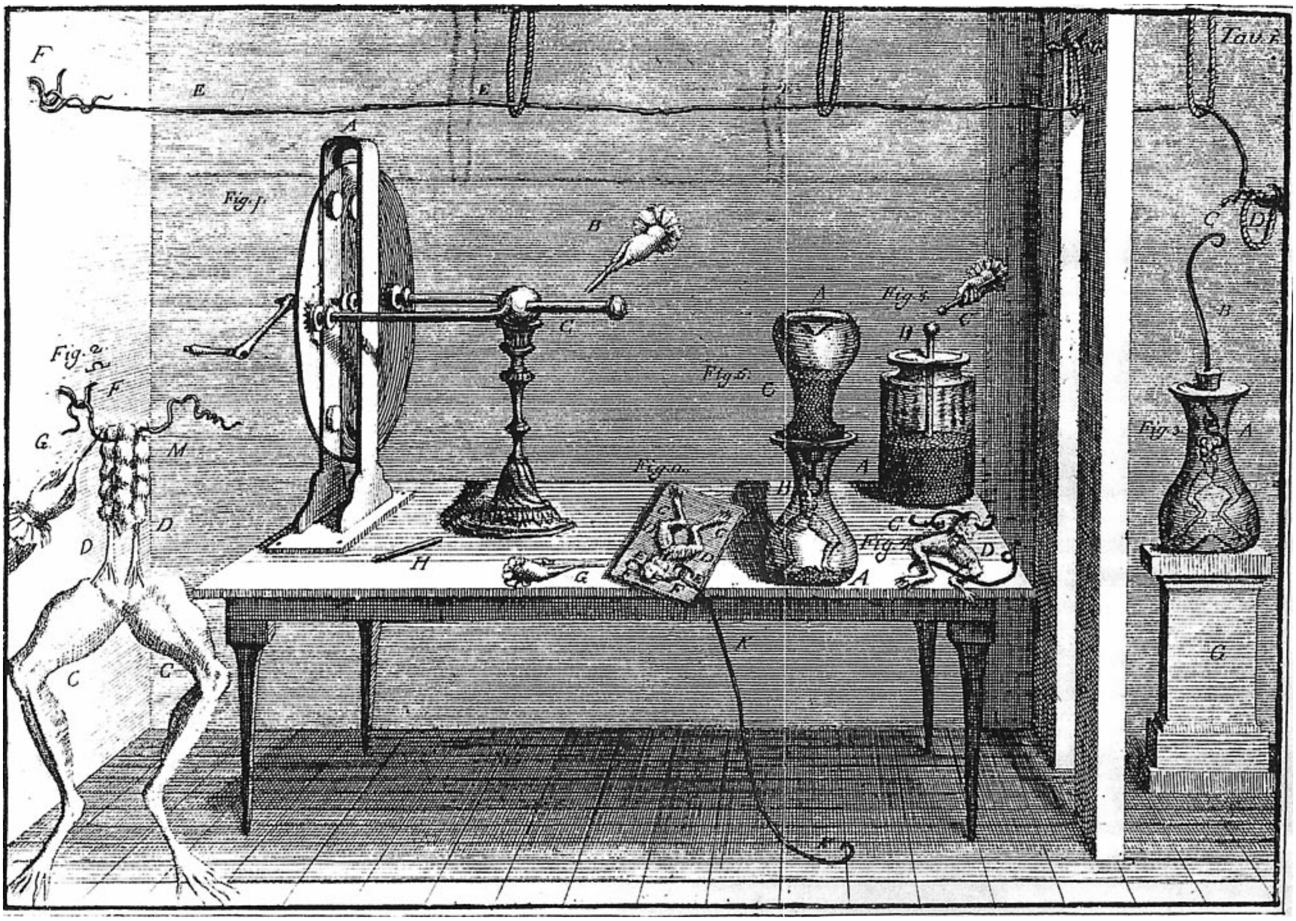

FIG. 2. Plate I of the *Commentarius* (1791 edition). The prepared frog and the electric machine on the left allude to the spark experiment.

scientific discussions in the history of science, because it led both great competitors to carry out fundamental experiments to support their respective hypotheses. Volta's experiments led to the invention of the electrical battery, the famous Voltaic pile, which opened a path to the tremendous subsequent development of the physical investigations of electricity, electrochemistry, electromagnetism, and related phenomena. Galvani's studies, on the other hand, laid down the foundations of electrophysiology, a science that in more recent times has had a development comparable to that of the physical studies of electricity in the first half of the 19th century. The controversy between Galvani and Volta is also interesting from another standpoint because it makes it clear how the apparent success of a scientific demonstration depends not only on its intrinsic "truthfulness," but also on other factors, even of an extra-scientific character. Furthermore, viewed retrospectively, it is noteworthy how a fundamental achievement in the history of science has been incorrectly represented by a tradition largely dominated by a partisan spirit that has ignored much of the progress of electrophysiology. With the present progress in our understanding of electrical phenomena in excitable membranes, particularly after the Hodgkin–Huxley fundamental studies on squid giant axon and, more recently, after the development of patch-clamp technology, we now understand better the difficulties encountered by the supporters of "animal electricity" in the infancy of electrophysiology two centuries ago.

THE SPARK EXPERIMENT

In opening his *Commentarius*, Galvani recounts how he became interested in the study of the effects of electricity on animals. He had made a frog preparation (consisting of the inferior limbs with the crural nerves exposed in their course from the spinal cord to the limbs, and a metal wire inserted across the vertebral canal). The frog was at some distance from a charged electrical machine (Fig. 2). When one of the collaborators, probably Galvani's wife Lucia Galeazzi (see Fig. 1 and [34] p. 254) touched delicately with a lancet the internal crural nerves, all leg muscles started contracting vigorously ([33], p. 63). Apparently the phenomenon occurred at the moment in which a spark arose from the electric machine. Galvani says that he was so impressed by the novelty of the finding that he decided immediately to verify and explain the phenomenon. A careful consideration of the importance that Galvani attached to this type of observation can shed light on the development of the idea of intrinsic animal electricity in Galvani's mind, and, at the same time, it can make justice of the erroneous evaluation frequently adopted of Galvani's attitude in interpreting the phenomenon.

First it is clear that Galvani had been interested in the study of the effect of electricity on the organism before this observation, because the frog, which happened to contract in that occasion, was clearly purposely prepared for the investigation of electrical phenomena in living organisms. In the introduction to an unpublished essay written in 1782 ([34] p. 4) Galvani explicitly declares that the study of an electrical influence on nerve function should be limited to the investigation of muscle movement "which makes itself sensible to the observer eye," and should not consider the sensation "which is totally occult for the observer." Moreover, according to Galvani, in studying movements one should avoid the possible complications arising from the influence of will and of "soul," and therefore, "reduced" preparations obtained from recently killed animals should be preferred to intact living animals.

Galvani's excitement in seeing the frog contracting in the spark experiment has been interpreted as evidence that he "ignored the correct theory of electrical influence" ([107] p. 175), because the mechanism leading to the contractions could be easily explained on the basis of the laws of the electrical atmospheres as particular case of the "return stroke" phenomenon (or counter blast) described some years before by Charles Stanhope (Lord Mahon; [103]). A current may pass across a charged body when another one, situated at some distance, is suddenly discharged. In modern times we would say that this occurs because of a capacitive influence between two separated conductors when the potential in one of them changes abruptly. It is also possible that the electrical influence in this type of experiment could be ascribed, at least in some conditions, to the electromagnetic radiations originated by the sudden discharge of the electrical machine (see for instance [104]). It has been said that were Galvani sufficiently acquainted with the physics of electricity, he would have not been surprised by the phenomenon, and perhaps he would not have started his investigations [9]. This type of criticism overlooks an important aspect of Galvani's observations during the study of the phenomenon. Galvani noticed that the muscle contractions were more easily excited, and were more vigorous, when the spark was elicited from the electrical machine separated from the frog, than when the frog was directly connected to the charged machine through the metallic wire inserted in the spinal cord (see for instance [34], pp. 9, 268). This last situation was clearly more favorable to the transfer of the "electrical fluid" to the frog nerve, compared to the electrical influence arising when a spark originated from a distant electrical machine, and still it was less effective in producing contractions. Therefore, there was some peculiar property in the way the electrical influence acted on the frog preparation which made the electricity associated with the spark particularly effective as stimulus for the contractions.

In the description of this experiment (generally referred to as Galvani's first experiment), particularly in the unpublished essays and memoirs written before the *Commentarius*, in order to figure out how external electricity can induce frog contractions, Galvani frequently utilizes words evoking a sudden impulsive action, a shock, such as *impulso* (impulse) *impeto* (impetus), *urto* (push, impact, shock; see for instance [34] pp. 6,11,21,169,289,322). Moreover, although he realizes that some relationship exists between the intensity of the electrical discharge and the occurrence and the strength of muscle contractions, Galvani notices that this relationship works only within a certain range. Increasing the discharge strength beyond a certain maximum does not result in stronger contractions, while progressively reducing it may suddenly result in a complete disappearance of the contractions (see [34] pp. 30, 99, 256). Finally, on several occasions Galvani remarks that if in a given preparation the contractions had disappeared after repeated applications of the electrical fluid, they could be reobtained if the animal was left unstimulated for a while or received some particular treatment (see for instance [34] pp. 14, 265, [33] p. 160).

It is reasonable to suppose that in the mind of an acute observer well aware of the scientific ideas of the time, as Galvani surely was, all these findings were likely to rouse the suspicion that muscle contractions did not directly result from the action of the extrinsic electrical force, but were instead due to some internal force, proper to the animal, which was set in motion by the external electrical agent. Galvani was well aware of the doctrine of "irritability," one of the most important conceptual elaborations of 18th century physiology. This doctrine was developed by Albrecht von Haller [41] after the notion (and the word) introduced by Francis Glisson one century earlier [36]. Implicit in the notion of irritability was the idea that the way an organism reacted to an external influence was an expression of its internal functional organization, and was in some way independent of the specific nature of external influence that acted as an "irritation."[1] According to Galvani, in the framework of the doctrine of irritability, external electricity was acting as an excitatory stimulus (as a trigger we would say now) for the contraction, and not as the direct "efficient" cause of the observed phenomenon.[2] Galvani's suspicion that external electricity was setting into motion some specific internal force responsible for the contraction was supported by the results of other experiments in which he established how small was the minimal quantity of electrical force of an effective stimulus. Contraction could be obtained by using a Leyden jar almost completely discharged so as to be undetectable with the most sensitive electroscope (see for instance [34] pp. 19, 21, 243, 295). How could it be that such a tiny electrical force would produce muscle contraction if it were not setting in motion some internal force?

At a certain stage of his work, Galvani summarizes the view emerging in his mind on the contraction induced by the spark with these words:

"The electrical atmosphere hit, and pushed, and vibrated by the spark is that, which brought to the nerve, and similarly pushing, and commoting some extremely mobile principle existing in nerves excites the action of the nerveo-muscular force." ([32] p.

[1] According to Haller, "irritability" was a specific property of the muscle and consisted in the tendency of muscle to contract in response to any external (or internal) stimulus (or "irritation"). Although influenced by Haller's conceptions Galvani had a different view on the role played by nerves in the mechanism of the contraction induced by electric stimuli (see for instance [34], pp. 23, 257). It is worth reminding here that the scientific milieu in which Galvani started his work was well aware and profoundly interested to Haller's notion of irritability. One of the most important contributors to the doctrine of irritability was Leopoldo Caldani, Professor at the Bologna University from 1755 to 1760. Among other things, Caldani employed electricity as a tool to induce contractions in animal preparations [21], and it is likely that the young Galvani attended some of his experimental demonstrations on irritability. In 1772, Galvani himself presented to the Academy a memoir on the "*Irritabilità Halleriana*." Although this memoir has been lost, the original Galvani's manuscripts deposited at the *Accademia delle Scienze* of Bologna indicate that since 1770 Galvani had started experiments on the animal irritability using mechanical and chemical stimuli (i.e., the basic stimuli used by Haller in most of his works) (see [35], pp. 11–16).

[2] With reference to the muscular contractions induced by external manipulations or physiological actions, the distinction between an "efficient cause" (*causa efficiens*) and an "excitatory cause" (*causa excitans* or *irritans*) is particularly well developed in the works of Felice Fontana [27–29]. Conceptually this distinction represents a major advancement with respect to the physic of the time based on mechanical interactions of a continuous type, implying the exchange of forces (or energies), which act as the immediate, sufficient, cause of the effects produced. In some way, the concept of "exciting cause" anticipates the idea that different parts of a natural or artificial organism (i.e., a complex system) may interact through relationships based on the exchange of control commands rather than of energies, a notion that anticipates conceptual developments of modern science (see later).

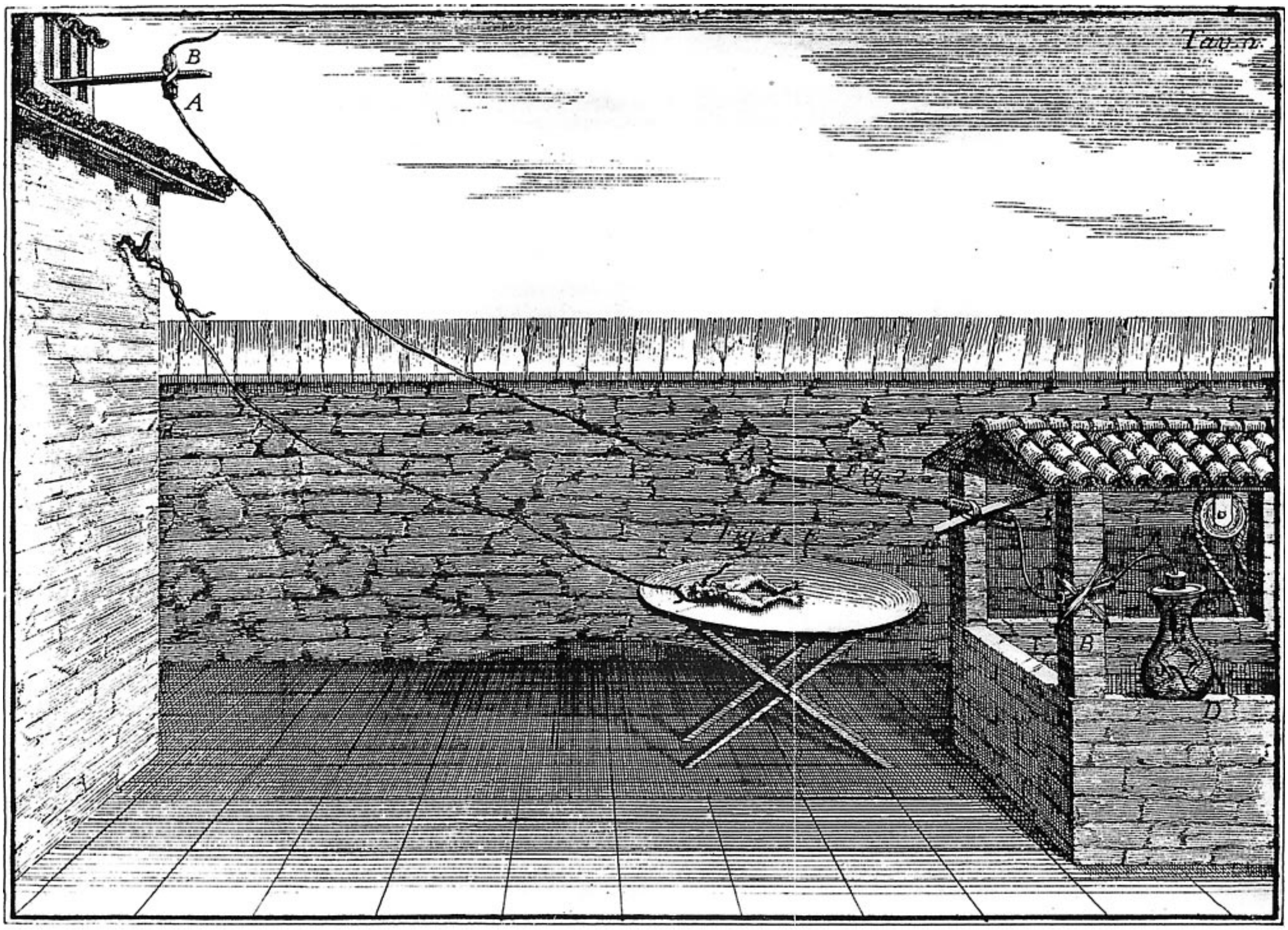

FIG. 3. Plate II of the *Commentarius* (1791 edition): The experiment with the stormy atmospheric electricity.

18). In further pursuing his studies, Galvani will eventually come to the conclusion that this extremely mobile principle is itself electrical, a specific form of electrical force generated in the organism as a consequence of the life process.

ATMOSPHERIC AND ANIMAL ELECTRICITY

After assessing the most appropriate conditions for obtaining contractions with various devices capable of producing electricity or of accumulating it (e.g., electrical machines, Leyden jars, Franklin magic squares, electrophorus), Galvani decided to investigate the effects of natural atmospheric electricity. Prior to Galvani, the most important practical achievement of electrical science in the 18th century had been indeed the demonstration, given by Benjamin Franklin in 1750, that thunder and lightning were produced by the discharge of a natural electricity present in the atmosphere [30]. Galvani, therefore, set up the study of the effect of natural electricity in a stormy evening. He connected the frog nerve to a long metallic wire pointing toward the sky, in the highest place of his house and ". . . in correspondence of four thunders, contractions not small occurred in all muscles of the limbs, and, as a consequence, not small hops and movements of the limbs. These occurred just at the moment of the lightnings; they occurred well before the thunders when they were produced as a consequence of these ones." (Fig. 3).

The next step was to investigate whether, besides the violent electricity of the storm, the natural electricity present in the atmosphere of a calm day could succeed in evoking contractions. He prepared the frogs "in the usual way" and hung them up on the iron railing of the balcony of his house in a clear and calm day (September 20, 1786) and waited. But nothing happened for a long time. Finally "tired of the vain waiting" he came near the railing and started manipulating the frogs. To his great surprise, the contractions appeared when he pushed and pressed, toward the iron bars of the railing, the metallic hooks inserted into the frog spinal cord.[3] The contractions bore no relation with the atmospheric events, however,

[3] The same experiment is described in the 1791 *Commentarius* and in a unpublished memoir, also in Latin, written in 1786. As pointed out long ago by Silvestro Gherardi [35], there is an important difference between the two descriptions: in the 1786 memoir, the hook inserted in the spinal cord is apparently made of iron (*ferreo uncino*) and, therefore, of the same metal of the balcony railings (*feriata* according a word created by Galvani from the Latin word *ferrum*, iron). In the later writing the hooks appear to be made of bronze (*uncis aereis*) and, therefore, of a metal different from that of the railings (*ferreis cancellis*). It is probable that in the time elapsed between the two writings, Galvani became aware of the stronger power of dissimilar metals in evoking contractions and, therefore, changed the description of this experiment as to such critical detail.

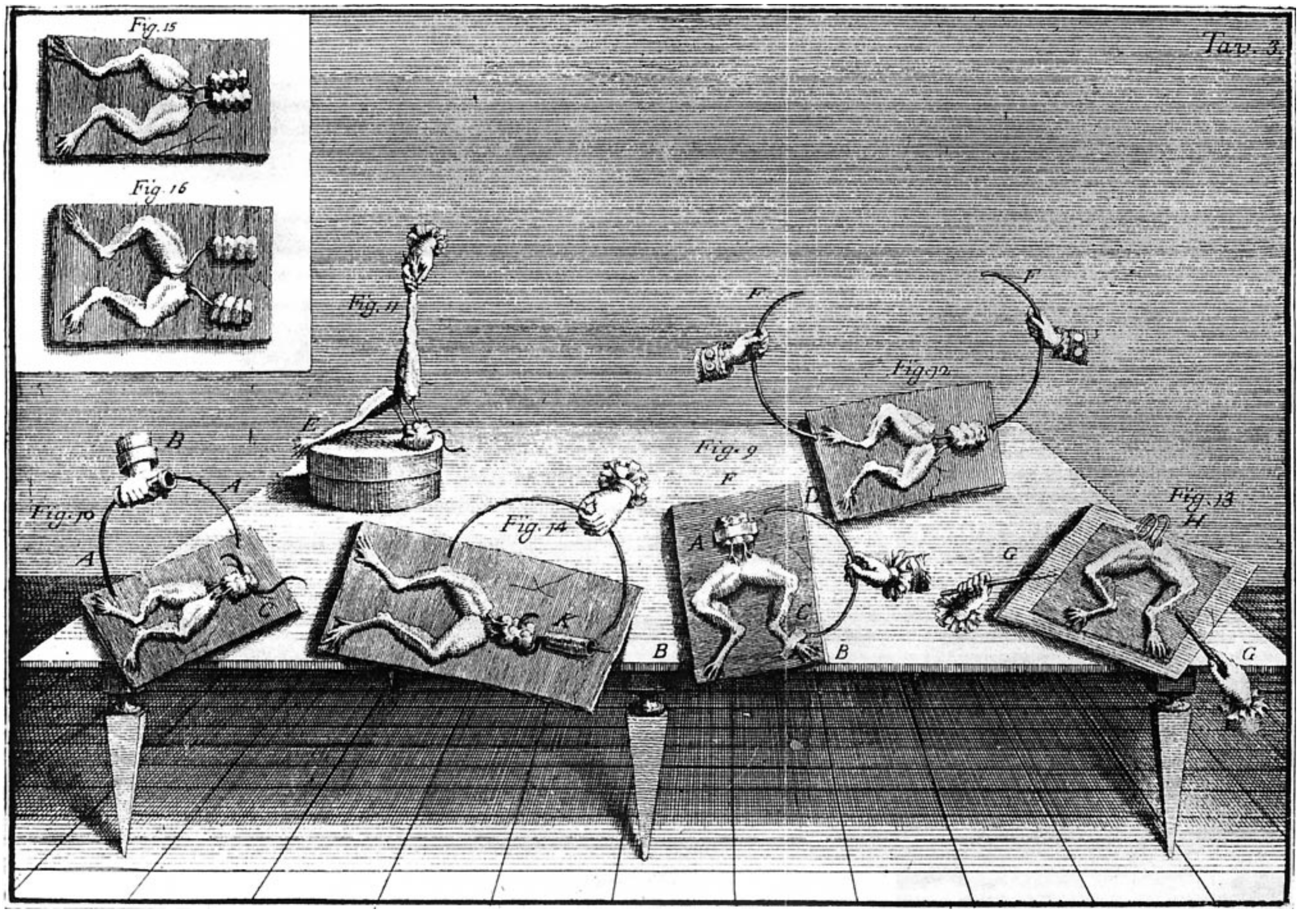

FIG. 4. Plate III of the *Commentarius* (1791 edition): the experiments with metallic arcs.

and they could also be obtained if the experiment was repeated indoors, "in a closed room," by substituting the balcony railing with an iron surface. Therefore, they seemed not to involve atmospheric electricity.

Galvani soon started a long series of experiments (Fig. 4), and realized that, in order to get contractions, it sufficed to connect through a metallic conductor the nervous structures (crural nerves or spinal cord) and the leg muscles, therefore, creating a circuit "similar to that which develops in a Leyden jar" ([33] p. 80) when the internal and external plates are connected. Contractions did not appear if an insulating body was used for the connection, or if the metallic arc was interrupted in some way by the interposition of a nonconductive material. The different materials used for the arc displayed different efficacies in inducing the contractions, which corresponded to their efficacies as electric conductors (gold and silver being very powerful, lead or iron less so, and glass ineffective). Liquid bodies could also be used, but only if they were electrically conductive (e.g., contractions appeared using water but not using oil).

On the basis of these findings (and of the results of a multiplicity of studies in which experimental conditions were varied in an amazing number of ways), Galvani came to the conclusion that some form of intrinsic electricity was present in the animal, and that connective nerve and muscle together, by means of conductive materials, induced contractions by allowing for the flow of this internal electricity. Galvani will refer to such intrinsic electricity as "animal electricity" (*animalis electricitas*) using a terminology first introduced by Pierre Berthollon some years before [16]. Therefore, at some moment of his intellectual elaboration, Galvani became fully convinced that the "extremely mobile principle" necessary to explain the contractions in the spark experiment is in fact of an electrical nature. The "mental image" that took possession of his mind since that moment was that of the Leyden jar, the first accumulator of electrical energy, the first capacitor ([33] p. 101). In Galvani's view, similar to the physical device, the animal is indeed capable of storing the electrical fluid and to maintain it in a state of "disequilibrium," ready to be set in motion by means of the conductive arc (or following other external or internal influences). In the case of the physical device, the motion of the electric fluid is capable of producing a series of effects (e.g., sparks, luminescence, "electrical wind," heating). In an analogous way, the motion of the animal electricity from the internal to external surface of the muscle, through the nerve fibers, would normally produce muscle contraction.[4]

In Galvani's mind, the force of the Leyden jar image was, however, not only "functional" and "mechanistic," but also visual.

[4] In the view of Galvani, intrinsic animal electricity accumulated in muscle was the "efficient" cause of the whole muscle contraction process. Now we know that a genuine form of electricity is involved only in the excitation of the membrane of muscle (and nervous) cells, whereas the energy used by the

In particular, a frog leg, with the crural nerves isolated after their emergence from the thigh muscles evoked the image of the physical instrument, the jar itself with its internal and external plates corresponding to the muscle mass, and the metallic wire, connected to the internal plate and protruding through the jar orifice, corresponding to the nerve (see [33] p. 206). This visual suggestion was probably one of the reasons why Galvani supposed that electricity was mainly accumulated in muscles, and that nerves had only a conductive function. Another possible reason, also related to the analogy with physical capacitors, came from the consideration that a relatively large device is required to accumulate large amounts of electrical fluid. Compared to muscle mass, nerves are excessively tiny tissues, and Galvani was reluctant to suppose that they could accumulate electricity to a significant degree. The view that nerves are devoid of an intrinsic electricity and behave as simple conductors of an electrical force accumulated in muscles is clearly a misconception. However, it is not without some admiration that we discover in the *Commentarius* the insightful conception that electricity in its duplex forms (i.e., positive and negative) is likely to be accumulated in each single muscle fiber because any fiber probably bears two opposite surfaces, one internal and the other external, which would correspond to the internal and external plate of the Leyden jar: "It is even more difficult that the existence of a duplex electricity in every muscular fibre itself could be denied if one thinks not difficult, nor far from truth, to admit that the fibre itself has two surfaces, opposite one to the other; and this from consideration of the cavity that not a few admit in it, or because of the diversity of substances, which we said the fibre is composed of, diversity which necessarily implies the presence of various small cavities, and thus of surfaces" ([33] p. 102).

GALVANI-VOLTA CONTROVERSY AND THE POWER OF METALS

After reading the *Commentarius* in 1792, Alessandro Volta repeated and confirmed Galvani's observations and expressed his admiration for the great discovery of animal electricity ([107] pp. 17–25). However, with the progress of his own work on this subject, which would grow in the following 8 years with an extraordinary crescendo, Volta progressively changed his attitude. At the beginning, the criticism did not concern the main hypothesis of Galvani (i.e., the existence of animal electricity itself) and was directed to some relatively secondary aspects of Galvani's theory, as for instance the interpretation of the experiment of the spark-induced contractions ([107] pp. 37, 41), and the exact localization of the negative and positive electricity between the inside and the outside of the muscle.

As to Galvani's view of the frog as a Leyden jar (with the nerve behaving as a simple conductor of the electricity accumulated in the muscle), an important difficulty emerged from noting that contractions could be obtained by connecting, through a bimetallic arc, two points of the same nerve without any contact with the muscle ([107] p. 59). It was, therefore, not necessary to suppose that muscle contractions required a current flow through the nerve from the inside to the outside of the muscle, as Galvani assumed, and this finding was, therefore, in contrast with Galvani's conception of the muscle as the reservoir of electricity (according to the Leyden jar model). Moreover, since the liquid humor surrounding the external nerve surface established an electrical communication between the points contacted by the bimetallic arc, how could one suppose the existence of a natural electrical disequilibrium between these points? A Leyden jar would have been rapidly discharged if an electrical communication existed between its internal and external plates and, on the other hand, a true (i.e., physical) Leyden jar could not be discharged by connecting two points of the same plate.

It is interesting to note that Galvani had already recognized in his experiments the possibility of inducing contractions by putting into contact two points of the same nerve with a metallic arc (see, for instance, [34] p. 35). However, he did not consider this observation as particularly conflicting with his views, and was able to reaccommodate it within his hypothesis, by assuming the existence of an "occult arc" through which the current could reach the muscle from the two stimulated points of the nerve (see [33] pp. 186–187, 192).

Subsequent experiments by Volta cast additional doubts on the origin of the electrical disequilibrium involved in the excitation of frog contractions. By repeating Galvani's experiments, the physicist of Pavia was surprised to find out how small was the amount of electricity capable of eliciting frog contractions. Having realized that the frog was more sensitive than his own more sophisticated physical instruments, Volta started to view the frog as an extremely sensitive biological electroscope. This attitude implied a radical viewpoint change, because the animal preparation was henceforth considered more for its capacity to reveal external electricity than as a source of internal electricity (an "animal electrometer," see [107] pp. 55, 147). According to Volta, moreover, if an extremely small external electricity could induce muscle contractions, it was not unlikely that many phenomena ascribed to an internal, animal, electricity derived instead from a small amount of external electricity produced inadvertently by experimental manipulations. In particular, it was necessary to exclude the intervention of external electricity in the famous experiments with the metallic arc from which Galvani derived the main evidence in support of his hypothesis of animal electricity. Volta was becoming progressively aware that an arc made by two different metals was more effective in inducing contractions than a monometallic arc ([107] p. 39). Again, a similar observation had also emerged in Galvani's experiments (see, for instance, [34] pp. 40, 124; [32] pp. 37–39, 328–329) but it had not been considered as an evidence against animal electricity. On the contrary, because according to Galvani, conductors formed by different metals were also more effective in stimulating the discharge of the electric fish (a process in which the involvement of an intrinsic electricity seemed unquestionable, see [33] p. 463 or [34] p. 40), the stronger efficacy of bimetallic arcs could be considered as an evidence consistent with animal electricity. As noted above, the various procedures capable of inducing contractions were considered by Galvani as different ways to set in motion an internal electrical agent, supposed to be in some state of disequilibrium in the animal tissue. The observation that bimetallic arcs were more effective than monometallic ones appeared to Galvani as "really wonderful" ([34] p. 40), and did not upset his confidence in animal electricity theory. Volta, on the contrary, started considering the possibility that the electricity was external in origin and derived from the difference of the metals used for the connection ([107] p. 117). The frog reacted to

contraction machinery is the chemical energy accumulated in molecules containing high-energy phosphate bonds as final products of metabolic processes. It is interesting to note here that in an unpublished memoir presented at the Academy of Science of Bologna around 1783 ([33] pp. 451–458), Galvani tried to establish a correlation between the processes involved in animal respiration, ordinary combustion, and in the appearance of an "electric flake" (*electricum penicillum*) from the pointed conductor of a charged Leyden jar. We think that in suggesting this correlation Galvani aimed at proposing an unitary view of apparently diverse "energetic" processes.

this metal-derived electricity as it reacted to other forms of external artificial electricity.

From the moment the idea emerged in Volta's mind that electricity could depend on metals, the progress of experiments and elaborations made by the great scholar of Pavia must be considered under two different perspectives, logically distinct, although largely interdependent. The first concerns the development of the physical studies of electricity, and will be extraordinary successful, culminating in 1800 with the invention of the battery, an epoch-making event from which Volta derived an imperishable fame, and also great honors at his times. The second perspective concerns the progress of studies on animal electricity, eventually leading to the birth of modern electrophysiology. Here the influence of Volta was in some aspects detrimental to the advancement of scientific knowledge and prevented many of the contemporaries from realizing how insightful were the views of Galvani on this field. It must be recognized, however, that the development of electrophysiology in the 19th century depended critically on both the technical achievements and theoretic elaborations made possible by the great progress of the physical studies of electricity that followed the invention of the voltaic battery.

THE CRUCIAL EXPERIMENTS

One of the reasons why Volta stumbled on the particular efficacy of bimetallic arcs in inducing muscle contractions came from his tendency to vary the type of experimental preparations, and to make frequent use of relatively intact animals in which nerves and muscles had not been "prepared" according to Galvani's indications (i.e., with the skin and connective tissues removed, nerves carefully unsheathed, as Galvani, who was a skillful anatomist and surgeon, routinely did, see [20]). The frogs "prepared in the "usual" (i.e., Galvani's) way were particularly sensitive to any external electrical influence, because of the smaller current dispersion in the perinervous and perimuscular tissues. This tended to obscure the different efficacy of the various stimuli. Intact or semiintact animals, on the contrary, were less excitable, and did not produce any reaction when a monometallic arc was applied to the skin (or to the connective tissues overlying nerves or muscles), while they contracted vigorously in response to the application of a bimetallic arc.

In his search for less conventional and more intact preparations (and also in order to assess whether human tissues reacted to metal contacts similarly to those of other living beings), Volta decided to explore the effects of a bimetallic arc on the tongue, a place where muscle tissue appeared to be almost directly exposed to external influence ([107] pp. 154, 157). No contraction was produced by using a lamina of tin (or of lead or zinc) in contact with a lamina of silver (or gold), but a clear acid taste was instead perceived. After an initial deception, Volta correctly interpreted this effect as due to stimulation of nerve fibers coming from the gustatory papillae. Next he tried a similar experiment on the eye and he found that the bimetallic contact induced there a sensation of light (see [107] p. 146). These findings and further experiments led Volta to anticipate Johannes Müller's doctrine of "specific nervous energies" that stipulates that the physiological effects of nerve stimulation essentially depend on the type of nerve stimulated and not on the type of stimulus used ([107] p. 138). Moreover, these data supported Volta's hypothesis that it was not necessary to include muscles in the circuit of current flow in order to excite nervous function. In the course of his studies, and in particular in the tongue experiment, Volta observed that the sensation evoked by the bimetallic arc lasted as long as the metals were maintained in contact with the tissues ([107] p. 73). This observation seemed to provide further evidence against the view that the particular efficacy of bimetallic arcs was simply the expression of a special conductive property that could facilitate the flow of an electrical fluid, present in the organism in a condition of natural disequilibrium, as in a capacitor. With prolonged application of metals, the disequilibrium should eventually disappear and the physiological effects of current passage terminate. It appeared more correct for Volta to suppose that dissimilar metals were themselves capable of producing and maintaining an artificial disequilibrium or, in other words, that they acted as "motors" of electricity (similar to electric machines) rather than simply as conductors ([107] p. 146).

E' la differenza de' metalli che fa ("It is the difference of metals which does it"; [107] p. 71).

With this somewhat vague, but powerful and allusive sentence, Volta expressed his view while he was searching for a coherent explanation of this entirely new experimental observation. Later he will elaborate his theory of metal contacts, which stipulates that an electromotive force arises from the contact between different metals, and he will rank metals on the basis of their tendency to generate positive or negative electricity when put into reciprocal contact ([107] p. 134).

The new hypothesis, however, encountered important objections from Galvani and from other supporters of animal electricity. Against Volta's new interpretation, Galvani started remarking on the importance of the finding that contractions could also be elicited by using a single metal for connecting nerve and muscle ("a monometallic arc;" to the old observations of Galvani, new, carefully-controlled experiments were added by Giovanni Aldini, see [34] pp. 196–197 and [7]), and that they could be produced even by connecting nerve and muscle by means of a cut piece of muscle (or of some other tissue, or even simply of moist paper, [33], pp. 202–203).

The theory of animal electricity received further support from an experiment made by Galvani in 1794 ([33] pp. 211–212), and from a similar experiment done by the Pisan scientist Eusebio Valli [106]. A contraction was obtained in a "prepared" frog by gently putting the tip of the sectioned crural nerve in contact with the surface of leg muscle, without the interposition of any extraneous substance[5] (Fig. 5A). This experiment seemed, at first, conclusive and unquestionable. Nevertheless, Volta refused to see in it the definitive proof of the existence of real "animal" electricity. Initially he argued that in these circumstances a mechanical (or chemical) irritation could underlie nerve stimulation ([107] p. 280). Later, with a bold change of his theoretical formulations, he proposed that not only dissimilar metals, but also different conductors of any type, and in particular humid bodies of different species or composition, when put into contact could generate an electrical force ([107] p. 291).

In those days, Volta's new attitude clearly appeared as an *ad hoc* reaction, somewhat like a veritable and surprising "fencing move," made to blunt the force of the new experimental arguments of the supporters of animal electricity, but apparently devoid of any experimental basis. If accepted, it seemed to leave little room for Galvani, at least within the original view of animal electricity

[5] Interestingly, Galvani noticed that the experiment failed if the muscle surface, where the tip of the cut nerve dropped, was injured. The explanation of this failure will be at hand solely after Matteucci's work (see later), when it will become clear that a difference of potential appears only between the intact and the injured surface of animal tissues. If the two tissues coming in contact are both injured, the difference is smaller or absent, and, therefore, no excitation will ensue from the contact.

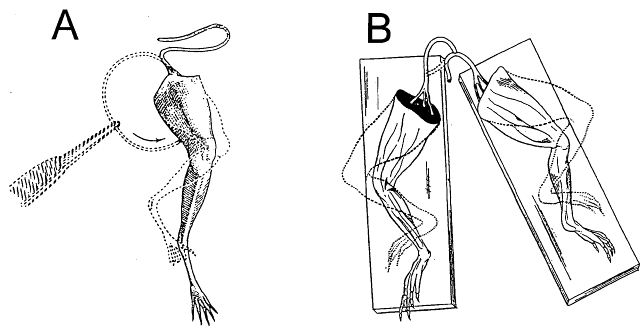

FIG. 5. Galvani experiments of the contraction without metals. (A) The 1794 experiment: when the surface of section of the nerve touches the muscle the leg contracts; (B) the 1797 experiment: when the surface of section of the right sciatic nerve touches the intact surface of the left sciatic nerve, both legs contract. (modified from [99]).

visualized in the Leyden jar image. It appeared indeed impossible to force the movement of the intrinsic electrical fluid from the inside of the muscle to the outside by the way of the nerve, if nerve and muscle were not to be connected together (in order to avoid the contact between dissimilar bodies). Galvani, however, convinced of the importance of providing new facts in scientific controversies, sought an experimental design capable of facing even the new objections of his competitor, and succeeded in doing this in an experiment published in 1797, an experiment that has been considered as "the most capital experiment of electrophysiology" [26], the real foundation of this new science.

Galvani separated and prepared the two legs of a frog with their respective sciatic nerves sectioned near their emergence from the vertebral canal, and placed them somewhat apart. With a glass rod, he then moved the nerve corresponding to one of the legs so that it came to touch in two different points the other nerve, bent to form a small arc. If care was taken during the manipulation, so that one of the parts of the first nerve used to establish the contact was its cut mouth, then the contraction appeared in the first leg and, frequently, also in the second one ([33] pp. 322–323; Fig. 5B).

Without any doubt this experiment appeared, in principle, capable to face any possible criticism from Volta's side, because it did not involve any contact between dissimilar bodies. The tissues coming into contact were indeed those of two corresponding nerves of the same frog. One could expect that this experiment would convince the scholar of Pavia, as well as Galvani's other opposers, because of its intrinsic "truthfulness," because of the obvious self-evidence of the demonstrated fact. But, even in science, nothing is so self-evident to obtain general acceptance in a simple and undisputed way. In the circumstances of the controversy on animal electricity, the "crucial experiment" did not succeed in convincing Volta and his followers, and it passed by, practically unnoticed by the scientific community.

At the moment in which Galvani made his "decisive" experiment in support of animal electricity, Volta, on his side, was providing a similarly "decisive" evidence to support the power of metals in generating artificial electricity. Volta was well aware of the necessity of obtaining an objective physical measure of "metallic electricity" without having recourse to the animal preparation, in order to avoid any possible criticism from Galvani's side. However, the initial experiments he made by using the most sensitive electrometers and electroscopes were unsuccessful. Volta supposed that this was due to the extremely small quantity of electrical force generated in the contact between two metals. He tried several experimental arrangements, and finally succeeded in achieving his goal. The tiny electricity generated upon the contact between a silver and zinc lamina was made capable to cause the movement of the indicators of his condenser electroscope, which consisted of a sensitive electroscope associated with a capacitor. The trick was to first transfer metal electricity to one dish of the capacitor, which was, thereafter, separated from the other dish. The separation, by causing a reduction of the electrical capacity of the condenser, led to an increase of the electrical potential (to be later called voltage), therefore, making the potential detectable by the electroscope. A similar principle underlay other experimental procedures used by Volta to reveal the tiny quantity of electricity produced by a bimetallic contact ([107] pp. 420, 435–436).

From this moment, Volta directed all his experimental effort to generate, from the arrangement of metal plates, a substantial quantity of electricity, comparable to that produced by electrical machines. In order to multiply the electrical force generated by a single bimetallic contact, he decided to arrange, in a stack-like fashion, several disks of two dissimilar metals in an alternating fashion ([108] pp. 224–225). In the beginning, however, he did not achieve any positive result by this method. Eventually Volta interposed, between the alternating couples of dissimilar metal pieces, disks of paper "moistened with water or better with a salt solution," and the long-sought dream was finally achieved. This was the invention of the electrical battery. The invention was communicated in a letter addressed on 20 March 1800 to the secretary of the Royal Society of London, of which Volta was fellow since 1791 ([107] pp. 565–587).

The immense success of this invention, and the increased scientific authority it gave to Volta, explain why the studies on animal electricity were confined, henceforth, to a small group of Galvani's followers and were, afterwards, practically abandoned for about three decades. Galvani had died on 4 December 1798, two centuries ago, in poor conditions after having been deprived of his professorship by the Napoleonic authorities who ruled in the North of Italy at the time. This was due to Galvani's refusal to accept the principles of the new political system, principles that Galvani considered in contrast with his religious faith. Napoleon, on the other hand, sanctioned in a particularly splendid way the success of Volta by attending personally the demonstration that the scholar of Pavia gave of his invention in Paris in 1801.

The electrical battery, which belongs to the history of physics rather than of biology. However, in this context, it is interesting to note that this device, lately named Voltaic "pilière" or "pile" in France, was referred to by Volta, in his initial communication to the Royal Society, as an "artificial electrical organ," the physical equivalent of the electrical organ of Torpedo and the electric eel ([107] p. 566). The electrical organ of fish, with its stack-like arrangement of modular elements, was indeed the mental image that guided Volta in assembling, as he did, the elements of his battery. It is also likely that, at least in part, Volta derived from the fish organ the idea of interposing human disks between the metallic elements, which resulted to be the veritable *atout* in the effort to build up the new electrical machine. Although recognizing that salt solutions (or other types of solutions) were more effective than simple water for moistening the paper disks interposed between the metallic elements, Volta considered the humid disks only as a peculiar type of conductor. He was completely unaware that his battery was in fact transforming the chemical energy of these solutions into electric energy, and that the presence of solutes of a particular species was, therefore, a fundamental constituent of his device. Convinced of the pure metallic origin of electricity in the battery, Volta assumed that, in principle, his device could promote an inexhaustible circulation of the electric fluid, a kind of *motus perpetuus* ([107] p. 576).

The 18th century, therefore, ended with the apparent triumph by Volta's conceptions, and, as already mentioned, the hypothesis of animal electricity was abandoned for many years. However, although successful with the invention of the electrical battery, Volta was wrong in many of his conclusions on animal electricity. In 1782, long before starting his studies on the effects of electricity on animals, the physicist of Pavia had lucidly indicated that the expression "animal electricity" should be used to indicate exclusively a form of electricity "which would be essentially linked to life, which would depend on some of the functions of animal economy" ([107] p. 8). At that time he considered this expression appropriate to denote only the kind of electricity discovered in Torpedo and in the electric eel. In his second memoir on animal electricity, written 10 years later, Volta wrote that Galvani had first succeeded in showing that a similar form of electricity was present in essentially any animal, and that it represented the nervous fluid whose identity had been long sought by scientists ([107] pp. 24–25). With the progress of his studies, Volta reached the point of considering Galvani's hypothesis of the presence of an intrinsic electricity in any animal to be without any foundation. Eventually he reached the conclusion that even the electricity of the electric fish could not be defined as a genuine "animal electricity." Although recognizing his initial inspiration from the natural organ of the electric fish in the invention of the electric battery, Volta concluded his famous communication to the Royal Society by noting that the production of electricity by the Torpedo and electric eel was indeed based on the same physical principles of the battery (i.e., the alternation of different conductors acting as motors of electricity at their contact). Therefore, in some way, according to Volta, the natural organ of the fish was a copy of the artificial electrical organ ([107] p. 582).

Of course, the basic principles of physics apply also to living organisms. However, although some operational aspects of the electrical organ can be reminiscent of the functioning of some forms of electrical batteries (concentration batteries), the strong similarity between the battery formed by different metallic layers and the fish electrical organ, strongly advocated by Volta, was clearly a misconception. For instance, in order to explain why the animal battery of the electric fish did not get discharged by a current flux through the humid tissues of the electric organ, Volta was obliged to admit that the animal battery was not ready to work in normal conditions, but it became so after a contact was established between the alternating layers of dissimilar materials, just at the moment of the discharge ([107], pp. 573–574). Volta strongly opposed Galvani's conception that in the Torpedo organ the electricity was constantly in a state of disequilibrium and ready to be discharged. Moreover, he did not believe in the presence in animal tissues of insulating components capable of maintaining this state of disequilibrium.

Contrary to Volta's views, the electricity of electric fishes, as well as the electricity discovered by Galvani in the muscles and nerves of a variety of animal species, was fully "animal" according to the criteria initially established by Volta himself in 1782, because it clearly depended "on the functions of animal economy" and was "essentially linked to life" (see later).

AN OUTLINE OF THE EVOLUTION OF ELECTROPHYSIOLOGY AFTER GALVANI

A rapid sketch of the evolution of the study of electrical phenomena in animals after Galvani and Volta will be now given with two aims. First to outline the fundamental moments of the history of science whereby it has been possible to reach the present understanding of the mechanisms responsible for the generation and conduction of the nervous signal. Second, to appreciate and better understand the problems and difficulties encountered by Galvani and Volta in their controversy on animal electricity two centuries ago.

Nobili and Matteucci

While physical studies of electricity saw an extraordinary progress after Volta, no substantially new achievement was obtained in the study of animal electricity for about three decades after Galvani. The influence of the apparent triumph of Volta's theories in this field is well illustrated by the interpretation that Leopoldo Nobili (1784–1835), a physicist of Reggio Emilia who worked in Florence, gave in 1828 of the experiment in which he first measured with a physical device animal electricity in the frog [95]. With his "astatic" device Nobili had substantially improved the sensitivity of the electromagnetic galvanometers of his times, by reducing the influence of the earth magnetism on the magnetic needle. He prepared a frog in Galvani's way, and put the skinned leg with intact muscles in a receptacle of salted water, while the lumbar nerves with the cut muscle surface were immersed in a second similar receptacle. When the two receptacles were put into contact through moist cotton, a contraction was obtained, consistent with Galvani's description. If the moist cotton was removed, and the two receptacles were put into contact through the platinum extremities of the astatic galvanometer, a current flow was observed. Nobili called this current *corrente di rana* or *corrente propria* ("frog current" or "intrinsic current"). Paradoxically, however, Nobili underscored the importance of the studied phenomenon. He attributed the measured current to a thermoelectrical effect due to the unequal cooling of nerve and muscle produced by evaporation, rather than to a genuine life mechanism. How poor was the credit of the animal electricity theory after Volta!

The first real progress in electrophysiology after Galvani was due to the work of Carlo Matteucci, a physicist born in Forlì in 1811 and appointed professor of physics in Pisa in 1840. Matteucci repeated Nobili's experiment and interpreted it correctly in 1838. Among the evidence suggesting that the measured current was truly biological in origin was the observation that it disappeared when the muscle was in a tetanic state [84,87].

In his experiments Matteucci also showed that a current could be measured from the cut muscular tissue alone, if care was taken to place one of the galvanometer electrodes on the intact muscular surface, and the other on the sectioned surface (this being always of negative polarity compared to the intact surface) [82]. The necessity of connecting the two different types of surface in order to record the "muscle current" was clearly recognized by Matteucci. He succeeded in providing an unequivocal demonstration of the biological origin of the muscle current with the ingenious preparation of the pile of sectioned frog thighs arranged in series, in such a way that the intact surface of one thigh was in contact with the sectioned surfaces of the next one [83] (Fig. 6). The deflection of the galvanometer needle increased in proportion with the number of the elements of the "biological pile," therefore, excluding the possibility that the measured current was due to the contact of the muscular tissue with the metal of the electrodes.

The experiments of Matteucci, therefore, provided an objective, instrumental evidence of the existence of animal electricity, the electricity supposed by Galvani on the basis of his pure biological experiments.[6] The existence of a "muscle current," moreover, confirmed Galvani's supposition that an electrical disequilibrium exists in muscle tissue, and that in some way the muscle is a biological equivalent of the Leyden jar. The current measured by Matteucci between the intact and the sectioned surface of the

[6] It seems worth to recall here the words that Volta wrote in 1798 in an anonymous letter addressed to Galvani's nephew Giovanni Aldini (and published

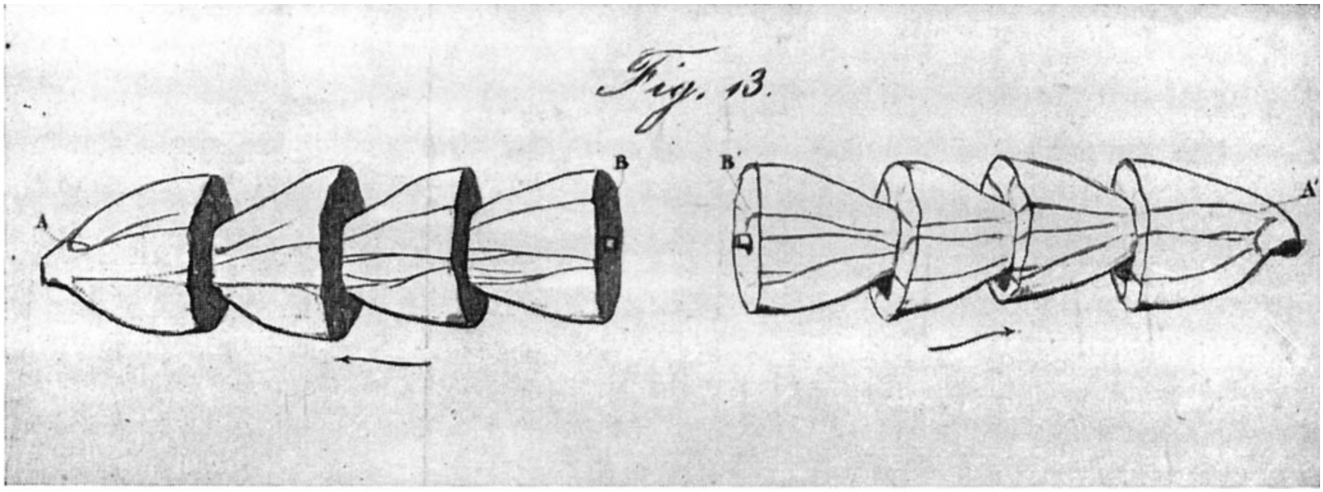

FIG. 6. «Pile» of half-thighs of frogs used by Matteucci to show that the current measured from muscles was not generated by the contact between the metal of the galvanometer electrodes and the animal tissue. The intensity of measured current increased in proportion of the number of the half-thighs composing the pile, whereas the number of metal-to-tissue contacts did remain constant. (From [83]).

muscle was indeed an expression of a difference of potential between the internal and the external compartments of the muscle fibers, because the lesion of the plasma membrane creates a path of smaller resistance to the interior of muscle fibers (although this was only recognized many years later with the development of Bernstein's membrane theory) [13].

The observation that the biological current measured by Matteucci disappears during muscle tetanus later prompted the studies of Emile du Bois-Reymond and his collaborators in this field [26]. Another of Matteucci's discoveries seminal for the development of modern electrophysiology was that of the "induced-twitch" phenomenon first described in 1842. Two frogs are prepared in Galvani's way. The leg nerves of a frog are laid down on the intact muscle surface of another frog. When a contraction is elicited in this second frog by some stimulus (electrical or mechanical), a contraction also appears in the first frog [85]. This induced-contraction was shown by Matteucci not to depend on any mechanical artifact, but, at least initially, it was considered as the expression of an electrical influence of a contracting muscle of one frog on the nerve of the other frog ([83], p. 134).

The German School: du Bois-Reymond and Von Helmholtz

After Matteucci, the study of the electrical phenomena involved in nerve and muscle excitation was taken over by Emile du Bois-Reymond, who initially confirmed Matteucci's findings [26]. Moreover, du Bois-Reymond succeeded in measuring with a galvanometer the electrical events associated with nerve excitation. The electrical phenomenon accompanying the excitation of muscle and nerve will be referred to by du Bois-Reymond as to the "*negative Schwankung*" ("the negative variation"). Its discovery represents the first instrumental recording of what were to be called "action current" and "action potential." The term "negative" described two related aspects of these electrical responses. "Negative" meaning "diminution" was used to indicate that the difference of potential between an intact and an injured surface decreased (or vanished) when the excitation flowed along the intact tissue. It also meant that, during excitation, the outer membrane surface of the nerve and muscle became negative with respect to a distant inactive region.

The other fundamental event in the 19th century was the measurement carried out in 1850 of the speed of propagation of the nervous signal. This appeared to be not simply a technical achievement, because the mere possibility of obtaining it seemed to contradict the expectations of the science of the time. The great physiologist of Berlin, Johannes Müller, was convinced that the "nervous principle" should be somewhat akin to light ("an imponderable fluid or a mechanical undulation") and it should, therefore, propagate along nerve fibers at an extremely high speed. Consequently, he stated in 1844 that any attempt to measure the time necessary for the propagation of nervous signal along a nerve trunk of limited size was doomed to failure ["Probably we will never have the means to measure the speed of the nervous action, since we lack, in order to establish comparisons, these immense distances whereby we can calculate the speed of light, which, under this respect, has some relation with it (i.e., with the nervous action)"] ([88] p. 581).

However, a few years after Müller's statement, one of the most brilliant of Müller's disciples, Hermann von Helmholtz, taking advantage of a simple nerve–muscular preparation and of rather simple experimental devices, succeeded in measuring the time required for the nerve signal to propagate a short distance. In 1850 von Helmholtz used a method derived from the "ballistic" procedures employed in artillery for determining the speed of cannon balls or bullets. He measured the duration of the interval elapsing from the application of an electric shock to the nerve until the mechanical response of the muscle. To this purpose he used an apparatus in which the muscle contraction directly interrupted the flow of a current initiated by the application of the stimulus to a given point of the nerve. He then stimulated a point of the nerve closer to the muscle and noticed that the measured interval in-

in a scientific journal of *Come*, [107] p. 555): "Galvani on the contrary neither has demonstrated in any way the hypothetical animal electricity in his experiments (on the other hand very beautiful, and surprising), i.e. that electricity, which he pretends to be moved by some vital force or organic function; nor he has succeeded in making it manifest to the electrometer; nor I think he will ever succeed."

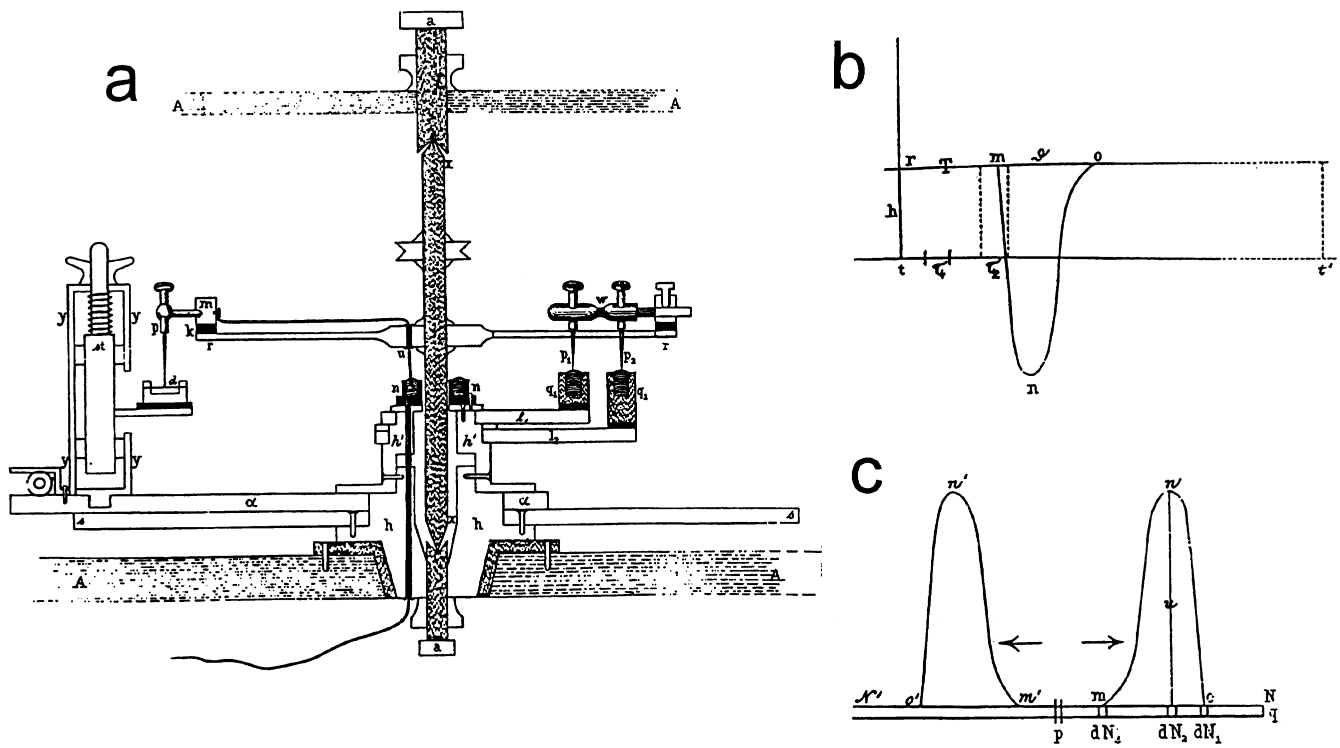

FIG. 7. The recording of the electric activity of nerve and the measurement of its conduction speed by Bernstein. (a) The "differential rheotome" used for delivering electric stimuli to the nerve and for opening, at the same time, the "sampling window" of the recording system at different intervals after the stimulus; (b) the first published recording of the time course of the action current ("negative variation") in the nerve. At the peak of the electrical response represented here by a downward deflection, the current overshoots by a large amount the baseline level that corresponds to the resting current level; (c) plots of the magnitude of the action current versus the distance along the nerve, at a given time (From [13] and [14]).

creased. Von Helmholtz correctly ascribed the difference to the time necessary for the propagation of the nervous impulse between the two stimulated points, and calculated a conduction speed of only a few tens of m instead of several hundreds of km per s [43,44]. In 1851 von Helmholtz used a smoked drum to record graphically muscle contraction and to calculate the speed of propagation of nerve signal and he obtained a measure for conduction speed that closely corresponded to that obtained with the previous method [45]. These results were graphically illustrated in an article published in 1852 [46].

The experiments of von Helmholtz were a real milestone in the history of science, and their importance goes far beyond the limits of the specific studied problem. For the first time an event bearing on the most elevated and elusive functions of animal organization, a manifestation of nervous phenomena akin to immaterial entities, such as psyche or soul, was measured with precision by means of a physical instrument. This was indeed a great step forward for science out from conceptions dominated by vitalistic doctrines with their multitude of spirits and animal forces.

Julius Bernstein and the First Measurement of the Time Course of the Electrical Nerve Impulse

Although von Helmholtz was prompted to carry out his experiment by considerations on the electrical model of nerve conduction proposed by du Bois-Reymond (see later), his results appeared to cast doubts on the electrical nature of nerve fluid because the measured speed was by far smaller than that of propagation of light and of electrical field. To reestablish a confidence in the identity between nerve activity and electrical phenomena it became, therefore, crucial to show that the "negative variation" propagated along the nerve at a speed corresponding to that of the nerve signal. However, du Bois-Reymond initially failed to measure the propagation speed of his negative variation because of the slow response characteristics of the electrometers of those days.

The problem was taken over by Julius Bernstein, a student of both von Helmholtz and du Bois-Reymond, who succeeded in 1868 in this difficult technical achievement by taking profit of his "differential rheotome." By means of a mechanical device, this ingenious instrument allowed for an electrical recording based on a "timing, sampling and holding" procedure [13]. The electrical event appearing at a given point of a nerve, within a definite time interval after the stimulation of a distant point, was recorded by performing a time summation of the effects of stimulations repeated at high frequency. The entire time course of the electrical process could be determined by varying the interval between the stimulus and the acquisition time window. The conduction speed could be measured by changing the stimulated point and noting the change in the delay of the appearance of the electrical event. It turned out that the speed of propagation of the negative variation and of the nerve signal corresponded to each other closely, and this was strong evidence for the identity of the two events.

Besides demonstrating the similarity of conduction speed between nerve and electrical signals, with his experiment Bernstein provided the first faithful recording of the time course of nerve excitation (Fig. 7a–c), and showed that it was a transient phenomenon lasting about 1 ms. In some of his experiments he noticed that the amplitude of the current measured during the excitation phase exceeded the level of the resting current measured between an intact and an injured segment of the nerve. This indicated that the excitatory event could be more than a simple "destruction" of the resting electrical state of the nerve. Bernstein's observation on the possible "overshoot" by the nerve signal of the injury current was afterwards largely forgotten [39]. It reappeared in the main path of the science only in 1939 with the studies of Hodgkin and Huxley (see later).

Nerve Conduction and Electrical Conduction Along a Cable

From the experiments of von Helmholtz and Bernstein, it became clear, however, that although the nerve signal was always accompanied by an electrical event, the propagation of this event did not follow the simple laws of the pure passive propagation of electrical currents, and that a more complex and specific mechanism was involved. The idea that the propagation of the nerve signal differed from that of an ordinary electrical current along a conductor had been already recognized by several scientists including von Haller [42], and it explains some of the reluctance to accept Galvani's views after the publication of the *Commentarius*. According to Felice Fontana, the first scientist to provide evidence that nerves were constituted by distinct microscopic fibers [11], the nerve fluid could not be one and the same thing as the electrical fluid. This could be shown by observing the effects produced by ligating a nerve with a thread: the ligature abolished the passage of the nerve signal, therefore preventing movements or sensations (as already shown centuries before by Galen), but it did not abolish the passage of an electrical current [12]. A similar view was also maintained by Alexander Monro [86].

The strength of this argument was probably one of the main reasons why Johannes Müller initially refused to accept the identity of the nervous and electrical fluid. Not only a ligated nerve, but also a crushed nerve, or a nerve resulting from suturing two sectioned stumps, could differentiate between the passage of the electrical fluid (which persisted) and the passage of the nerve signal that was blocked (see [88] pp. 541–542).[7]

In the epoch of du Bois-Reymond, objections to the idea that the nerve signal was of an electrical nature were also raised by Carl Ludwig who considered the longitudinal resistance of nerve fibers to be too high to permit an effective propagation of an electrical signal (in the form of a passive electrical conduction along a cable, [79]). In modern days the force of this argument was expressed in a particularly vivid way by Hodgkin, who calculated the longitudinal resistance of a long and thin nerve fiber and showed that it was comparable to that of an ordinary electrical cable extending several times the distance between the earth and the planet Saturn [56].

In order to explain electrical phenomena associated with the propagation of excitation in nerve and muscle, du Bois-Reymond had assumed that an electromotive power existed in the two tissues due to the presence of minute particles ("electrical molecules") bearing a positive charge in their middle (equatorial) zone and two negative charges at their polar regions (one each). In his conception, the arrangement and orientation of these electrical molecules in the excitable tissues somewhat corresponded to the way magnetized particles are assembled together in a large magnet. Normally (i.e., at rest) the particles are arranged longitudinally in an ordered fashion. This corresponds to an electrical condition that could be revealed if the fibers were sectioned transversally, therefore producing the "muscle" or "nerve current" that would flow between an intact surface and the injured region. An electrical stimulus would result in a disturbance of this ordered arrangement, producing an "electrotonic condition" that could eventually lead to the appearance of the "negative variation" [22,26]. The model of du Bois-Reymond with its ordered arrangements of charged elements anticipated the idea of an electrical "polarization" of excitable membranes. Moreover, it suggested that the propagation of the nervous signal did not consist of a flow of electricity in a simple form. As mentioned previously, from considerations on this model von Helmholtz was inspired to try the measurement of the speed of propagation of nervous signal, in spite of Müller's skepticism of the possibility that this could be done with success. According to von Helmholtz, the movement of the charged molecules of du Bois-Reymond's model required a definite time and this should result in a relatively slow speed of propagation of the electrical disturbance along the nerve fiber.

Von Hermann and the Local Circuit Theory

The next important advancements in the electrophysiology of nerve and muscle excitability were due to the work of two other German scientists. One of them, Ludimar Hermann, a student of du Bois-Reymond, pointed the attention to the fundamental fact that the signal propagating along a nerve fiber consisted of a negativity of the external surface of the nerve, and that at the same time the fiber was stimulated when its external surface was made more negative [48,49]. This suggested the possibility that, in physiological conditions, the external surface negativity produced by the arrival of the negative variation in a region of nerve could, in turn, serve to stimulate a region ahead, therefore closing a loop capable of propagating the nerve signal. It appeared that the propagation of the signal could be due to a local excitation of the resting fiber due to a flow of current from the active region. To account for the propagation of local currents along the nerve fiber, Hermann proposed his well-known local circuit theory (as it will be later denoted) [48,49], by reference to the theory of electrical current flow formulated some years before by Lord Kelvin [70]. This theory was based on the idea that the nerve fiber consisted of a conductive core separated from an external fluid phase by a relatively insulating coating, and that any electrical disturbance originated in a point of the nerve could influence near regions through local current loops involving the internal core, the insulating sheet, and the external fluid.

Bernstein and the Membrane Theory of Bioelectrical Potential

Another fundamental contribution to the electrophysiology of nervous and muscle excitation was due to the work of another student of du Bois-Reymond, the same Julius Bernstein who had first succeeded in measuring the speed of propagation of the "negative variation." Bernstein applied to biology the theory of electrical potentials arising from diffusion of ions developed by Nernst [93,94]. He put forward the hypothesis that in the resting

[7] A possible response to the "ligature" objection is contained in the third memoir that Galvani addressed in 1797 to Lazzaro Spallanzani: "Let us finally suppose that the nerves exert such (conductive) task with their intimate part and medullary substance; and therefore that, if this part is divided and interrupted by means of ligature, or otherwise in general the circulation of the electricity is suspended, although in the nerves ligated, or otherwise vitiated, the membranes remain there, and there will still be the humidity which could draw electricity out of the muscle." ([33], p. 346). In proposing that nerves conducted electricity with their internal core, Galvani assumed that the interior of the nerve was insulated from the external surface by means of an "oily matter" (see for instance [33], p. 241).

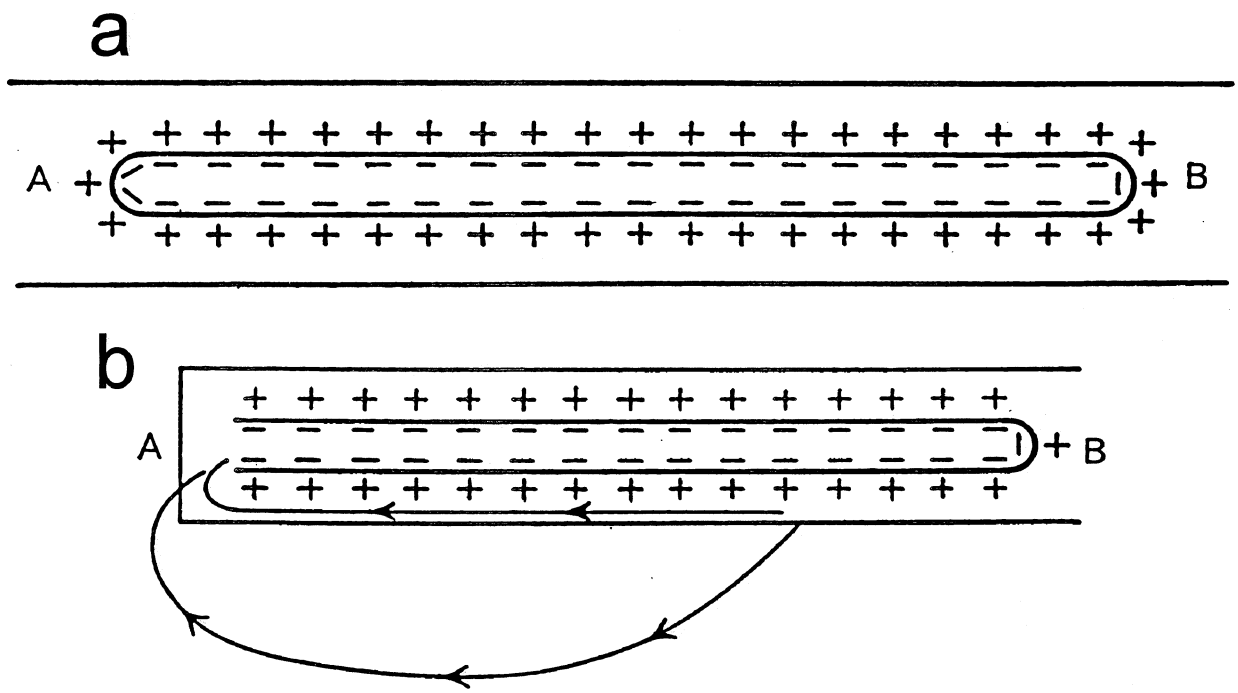

FIG. 8. Scheme of Bernstein's theory of membrane polarization. (a) A muscle fiber in the resting state, with an excess of negative charges inside the membrane and of positive charge outside; (b) a lesion has removed the local barrier to ion passage provided by the membrane, and, therefore, A becomes negative with respect to B, allowing for a current flow from B to A. According to Bernstein [15], a similar phenomenon would occur during excitation A representing then the active region of the membrane (From [15]).

state, excitable cells generated a diffusion potential across their membrane as a consequence of the selective permeability of the membrane for potassium ions and of the higher concentration of these ions in the intracellular compartment compared to the extracellular medium. According to Bernstein, the potential arose because the outward movement of the positive-charged potassium ions could not be compensated by movements of other ions, because of the exclusive permeability of the membrane for potassium. This tended to leave an excess of negative charges in the interior of the cell and to create an excess of positive charges outside (Fig. 8a and b).

To account for the excitatory events in nerve and muscle, Bernstein assumed that the excitation consisted in a disappearance of the polarization present in the resting state (i.e., in a "depolarization") consequent to a sudden and generalized increase of the ion permeabilities of the membrane. Bernstein's theory will be referred to as membrane theory of excitation, and one of its implications, confirmed by later studies (see later) was that membrane electrical resistance decreased during excitation [15].

With Bernstein the focus on electrical phenomena of excitable cells was, therefore, based clearly on the properties of the cell membrane. This still undefined surface of separation between the intracellular and extracellular media started to acquire a physico–chemical connotation. Apparently one of the reasons that led Bernstein to develop his membrane theory of nerve excitation was the consideration of the similarity between the negativity of the current measured from an injured nerve and the negativity of the action current. Because injury was likely to produce an "exposure" of the nerve interior to the recording electrode, it could be assumed that a similar phenomenon occurred during the "negative oscillation" and therefore that nerve (and muscle) excitation consisted in a reversible "rupture" of the membrane separating the intracellular and extracellular media.

The English School: Lucas and Adrian

After Bernstein the progress of knowledge on the mechanisms of electrical excitability was mainly due to the work of English physiologists. A fundamental acquisition concerned the "all-or-nothing" character of electrical excitation in nerve and muscle. This character, whose origin can be traced to the work of Felice Fontana [28,29,68], had already emerged from studies carried out on heart muscle in 1871 by the American physiologist H. P. Bowditch working in Carl Ludwig's laboratory in Leipzig [17]. Bowditch noticed that if an electrical stimulus applied directly to the isolated apex of frog heart was capable of inducing a contractile response (i.e., if its intensity was larger than a minimum value), the strength of contraction remained constant when the intensity was further increased. On the other hand, stimuli of intensity smaller than the minimum effective value did not result in any detectable contraction. For several years this "all-or-nothing" property seemed, however, to belong exclusively to the heart muscle, because the strength of contraction elicited in a striated muscle, stimulated either directly or through its motor nerve, increased apparently in a relatively smooth way by increasing the stimulus intensity. In 1902, Francis Gotch put forward the hypothesis that this pattern was basically due to an increase of the number of excited fibers, rather than an increase of the response amplitude in any excited fiber, which he supposed to be a constant quantity [37]. Following Gotch, Keith Lucas, working in Cambridge, succeeded in showing that other electrically excitable tissues indeed follow the "all-or-nothing" law. In 1905 he reduced, by careful dissection, the number of active muscle fibers in the dorso-cutaneous muscle of the frog to 20 or less, and excited the muscle directly with an electrical stimulus. By increasing the intensity of the stimulus he found that the strength of the resulting contraction

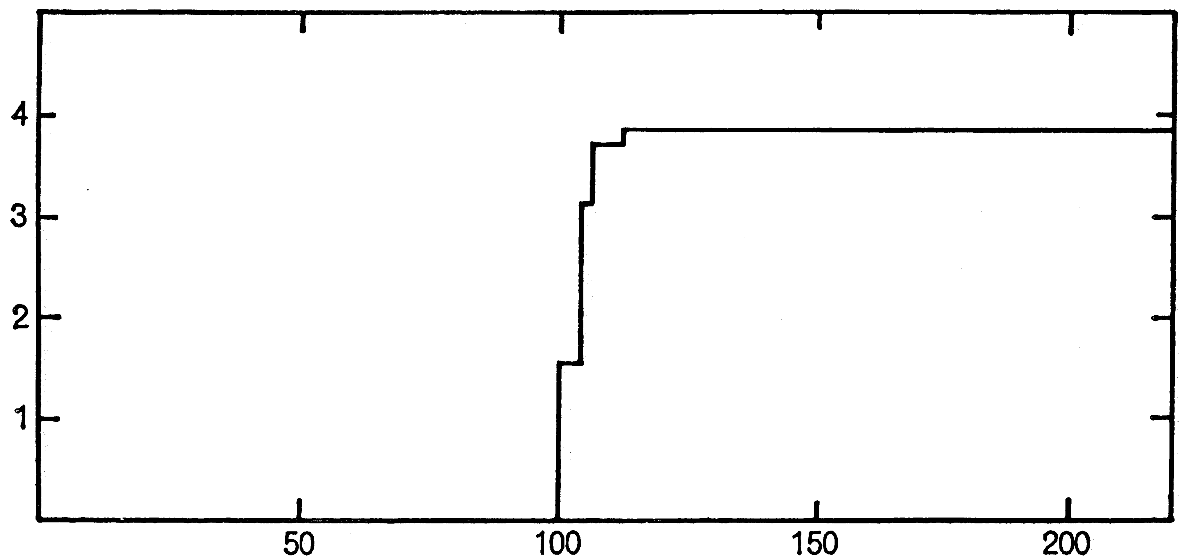

FIG. 9. The 1909 experiment of Lucas and the all-or-nothing law. The abscissa represents the strength of the electrical stimulus applied to the motor nerve and the ordinate the mechanical response of the muscle (height of the contraction of the *cutaneous dorsi* muscle of the frog). Notice the step-like transitions in the muscle response as the intensity of the stimulus is increased within a critical range (From [76]).

increased in discrete steps, whose number was in general less than the number of active muscle fibers [74].

In 1909 Lucas made a comparable experiment using a similar preparation but elicited muscle contraction through a stimulus applied to a motor nerve [76]. Again the contractile response increased in discrete steps whose number was normally smaller, in this case, than the number of the active nerve fibers (Fig. 9).

A direct demonstration of the all-or-nothing character of the propagated electrical signal in nerve emerged from the first recording of the electrical activity of single nervous fibers, an epoch-making discovery obtained by Lucas' student, Adrian in 1926. Adrian, in his first study, using the capillary electrometer and a vacuum tube amplifier, succeeded in revealing the presence of individual electric oscillations in the discharge of various sensory fibers of both frog and cat in response to physiological stimulation [4] (Fig. 10A–C). In a second study, carried out in collaboration with Zottermann [6], in order to better isolate the electrical activity of a single fiber, Adrian made recourse to the nerve supplying the sterno-cutaneous muscle of the frog on the assumption that this muscle might contain just one muscle spindle. If the muscle was cut carefully in order to minimize the number of active sensory units, and a low mechanical load applied, the response recorded from the nerve consisted in a rather regular train of impulse-like deflections of constant amplitude and duration, which were shown to represent the activity of a single sensory fiber. Increasing the intensity of the stimulus led to an increase of the discharge frequency without any change of amplitude and duration of every individual impulse. These features were later confirmed in a variety of nervous (and muscular) fibers leading to the understanding that an impulse-code modulation is the ordinary means used by many nerve fibers (and by other excitable cells) to code information.

Another property of the nervous signal, somewhat related to the all-or-nothing feature, was revealed by the results of other experiments carried out by Lucas and Adrian. In these experiments small segments of nerve were more or less completely inactivated by exposure to alcoholic vapors or to other procedures interfering with the conduction process [1,2,5,77,78]. In a frog nerve-muscular preparation, a long exposure time was necessary to produce the conduction block if the treatment was applied to a short nerve segment. A much shorter time was effective in blocking the nerve if a long segment was exposed. However, if the treatment was applied to two similar short segments, separated by a relatively large untreated portion, the time of application needed to produce the block corresponded closely to that necessary in the application to a short single segment (Fig. 11a and b). Clearly, in the untreated portion the signal was regenerated, regaining its original amplitude, if the block did not exceed a certain value. The words Lucas wrote with reference to Adrian's experiments penetrate a fundamental aspect of the "energetics" of nerve signal propagation, and are worth quoting at length:

"A disturbance, such as the nervous impulse, which progresses in space must derive the energy of its progression from some source; and we can divide such changes as we know into two classes according to the source from which the energy is derived. One class will consist of those changes which are dependent on the energy supplied to them at their start. An example of this kind is a sound wave or any strain in an elastic medium which depends for its progression on the energy of the blow by which it was initiated. A sound wave will lose its initial energy if the medium in which it progresses is imperfectly elastic, because the medium will be heated in its deformation. Suppose a sound wave travelling through air and then encountering a tract of treacle. In this passage through the treacle it will lose its energy more rapidly than in the passage through the air, but on emerging into the air again it will have suffered permanent loss, and will not recover the energy which it had before it entered the treacle. A second class of progressive disturbance is one which depends for its progression on the energy supplied locally by the disturbance itself. An example of this type is the firing of a train of gunpowder, where the liberation of energy by the chemical change of firing at one point raises the temperature sufficiently to cause the same change at the next point. Suppose that the gunpowder is damp in part of the train; in this part the heat liberated will be partly used in evaporating

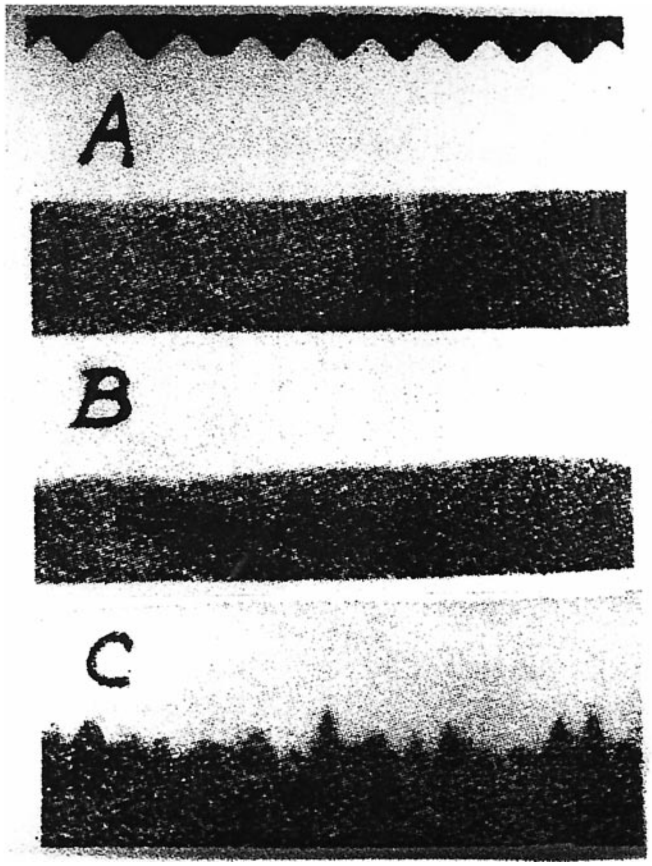

FIG. 10. The first published recording of unitary electrical activity in sensory nerve fibers elicited by physiological stimuli. The electrical responses were derived with a capillary electrometer from the sciatic nerve of frog, and a mechanical stimulus applied to the gastrocnemius in order to activate muscle sensory receptors therein. The trace above the recordings is a time calibration provided by a tuning fork oscillating at the frequency of 200 Hz. (A) Control, nerve killed near the muscle, no response; (B) muscle relaxes, no weight, no response; (C) wt. of 100 g applied for 10 s: clearly detectable, upward pointing deflections appear in the recording trace (From [4], modified).

water, and the temperature rise will be less, so that the progress of the chemical change may even be interrupted; but if the firing does just succeed in passing the damp part, the progress of the change in the dry part beyond will be just the same as though the whole train had been dry [78]."

From the studies of Adrian and Lucas, it became increasingly evident that the amplitude and time course of the propagated signal in nerve and muscle depend in an essential manner on the local conditions of the nerve region in which it is recorded (or otherwise tested), and not on the intensity of the stimulus, nor on the conditions of the nerve region from which it originates (provided that the stimulus is of sufficient intensity and the propagation is not completely blocked in between).

In the work of Lucas, and in the initial studies of Adrian, the nervous signal is generally referred to as "propagated disturbance" to distinguish it from the local effects induced by electrical stimulation near the site of stimulus application, effects that could be revealed especially by using subthreshold stimuli. "Propagated disturbance" was also a noncommittal term employed to avoid possible objections against the notion that the nerve signal was essentially an electrical phenomenon. As a matter of fact, although it was generally recognized that nerve excitation was constantly associated with an electrical event, it was still unsettled whether electricity was the fundamental process of nerve signaling, or simply one of its many possible manifestations (such as heat production, chemical modifications), a sort of epiphenomenon of an underlying, more essential, process.

It may seem surprising that the electrical nature of nerve signal was still matter of debate more than one century after Galvani, and long after Matteucci and du Bois-Reymond. As a matter of fact, theories of nerve conduction giving a predominant importance to chemical mechanisms have, for a long time, challenged hypotheses based on the preeminence of electrical phenomena. For instance in 1946, Nachmansohn proposed that acetylcholine could serve as a "messenger" for the conduction of the nerve impulse [89] and this hypothesis has persisted in the paths of science as late as 1975 [90].

The unequivocal evidence that the nervous signal is fundamentally an electrical event, as Galvani had supposed at the end of the 18th century, had to await for the most modern epoch of electrophysiology (i.e., the period of studies of nerve physiology carried out by Hodgkin and his collaborators in Cambridge), which led to the final elucidation of the mechanism underlying the generation and propagation of the nerve signal. However, the seeds for this modern development can be traced back to the work of Lucas and Adrian.

Before considering some aspects of this work in detail, we should recognize that the difficulties in accepting the view that the nerve signal is an electrical event were not without logical grounds. The conduction of the nervous signal seemed to be too slow for an event based on the propagation of a typical electrical signal along a metallic wire. Moreover, starting from the initial observations of von Helmholtz [44,47], it appeared that temperature exerted a profound effect on the rate of propagation of nervous impulses [101,102], and evidence had been accumulating pointing to a strong temperature dependence of other kinetic parameters of the propagated nervous disturbance [3,10,18,38,75]. Because a high temperature coefficient was generally considered to be an indication of the involvement of chemical reactions, these findings led to envisioning the nervous impulse fundamentally as a chemical event [72], although the straight-forward applicability of this reasoning to the case of the phenomena of cell excitation and conduction was questioned by some authors [3,72].

From the studies of Adrian, Lucas, and their predecessors it appeared increasingly evident that the electrical stimuli of intensity insufficient to generate the "propagated disturbance" (or subthreshold in modern terminology) were nevertheless capable of increasing the electrical excitability of a narrow segment of nerve around the point of application, and for a limited time duration [1,2,5,77,78]. A stimulus that was subthreshold if applied alone, could lead, through this mechanism, to the appearance of a fully developed nerve signal if applied in combination with another similar stimulus. Besides, this explained why a train of electrical stimuli could be more effective in stimulating a nerve when compared to a single electrical pulse [5]. These findings suggested that "local responses" did not follow the all-or-nothing law as propagated responses did. The different character of the two phenomena was also evident in that the arrival of a propagated response was followed by a temporary reduction of local excitability (a phenomenon already recognized in the heart muscle by Felice Fontana [29,68], and denoted as the "refractory phase" by Marey in 1876 [80]). This reduced excitability contrasted with the increase in electrical excitability which, as we have seen, appeared to be associated with weak stimuli. On the other hand, notwithstanding the obvious differences between local and propagated

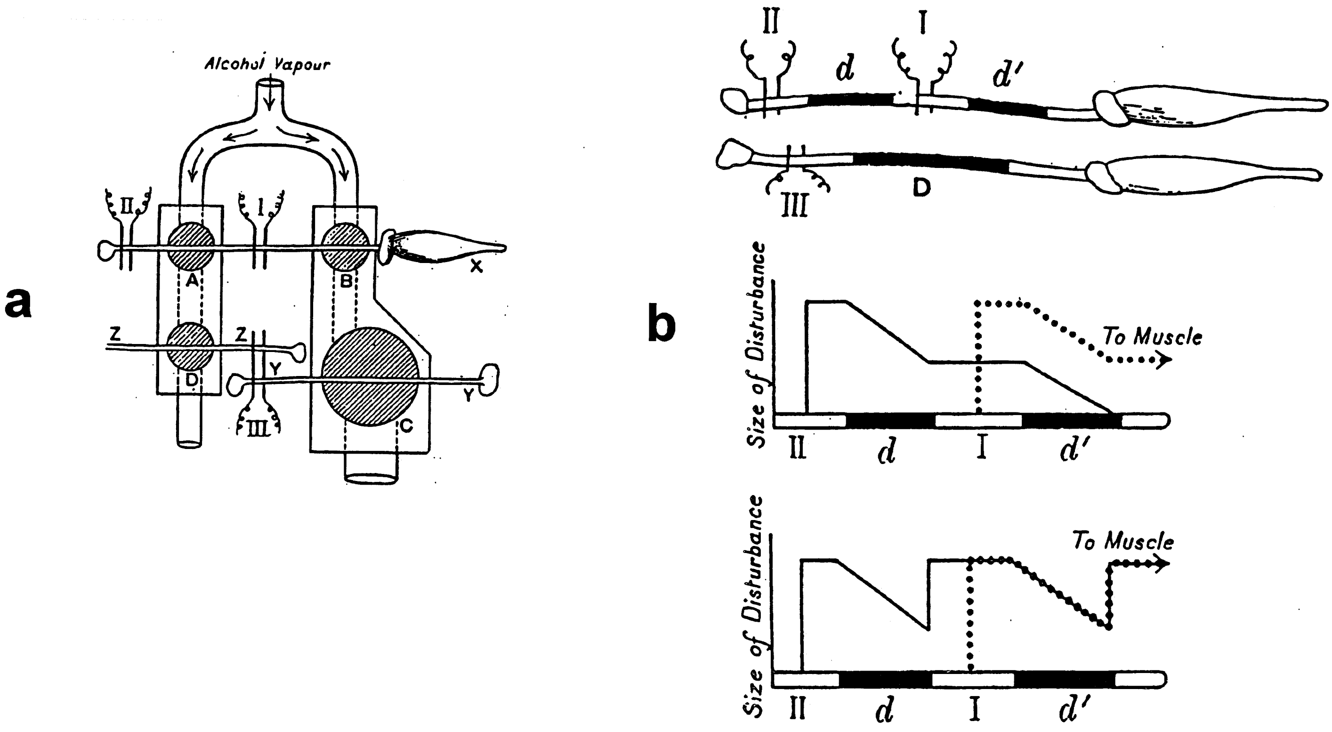

FIG. 11. Adrian's experiment of partial block of nerve conduction by local application of alcohol vapors. (a) The experimental apparatus used for testing at the same time the effects of alcohol on three different nerve-muscle preparations of the frog (denoted as X, Y, and Z, respectively). A, B, C and D indicate the experimental chambers where portions of the nerves were exposed to alcohol, and I, II and III indicate three stimulating electrode pairs. (b) Hypothetical experimental outcomes based on the two alternative possibilities of a "propagated disturbance," which does, or does not, recover its original amplitude while travelling along the untreated zone separating the two narcotised portions. In the first case (corresponding to the actual experimental result) the conduction should fail at the same time (calculated from the beginning of alcohol application) for a stimulus at position I and II. It should persist longer than for an application to a larger portion (as indicated in D) (From [1]).

responses, there was an evident functional relationship between the two phenomena. First, the local response could develop into a full propagated response if the stimulus was increased beyond a threshold value. Moreover, if a nerve was blocked by some treatment applied to a short segment of its course, an increase of the excitability could be detected, upon the arrival of the propagated disturbance, in the region beyond the block. This indicated that some event, similar to that induced by an artificial local stimulation, accompanied the propagated signal, a finding well in line with the local circuit theory of Hermann. In normal conditions, the leading wave of the "propagated disturbance" would be able to increase the excitability of the nerve region ahead, to such a degree that a new propagated disturbance would be generated there.

The Modern Era: Alan Hodgkin and the Cambridge School

The most modern phase of the studies on the electrical phenomena in excitable cells began in Cambridge around 1934. As with Adrian, who had published his fundamental study of nerve block in the nerve–muscular preparation of the frog when he was an undergraduate student at the Trinity College, another undergraduate student of the same College, Alan Hodgkin, began studying the effects of nerve block in the frog. As with his predecessor, Hodgkin was to publish the results of his first study in the *Journal of Physiology* at the age of 23 years (in 1937, exactly two centuries after Galvani's birth) [51,52]. Hodgkin induced a local block by cooling a short segment of the sciatic nerve, and investigated the effect induced in the blocked region by the electrical stimulation of the proximal region of the nerve, using muscle contraction to monitor of nerve excitation (Fig. 12a and b). The initial purpose of this experiment was to ascertain whether the arrival of a nerve impulse near the blocked region brought about a local decrease of the membrane resistance [58,59], as implied by Bernstein's membrane theory of excitation.

Initially Hodgkin positioned the two test electrodes at the two ends of the blocked region, and noticed that a smaller current was necessary to induce the muscle contraction when the current was applied in temporal coincidence with the arrival of the nerve excitation induced by the conditioning stimulus. He soon recognized, however, that this effect was not due to the sought-for membrane resistance change, because it could also be observed when both test electrodes were positioned beyond the blocked point, downhill with respect to the flow of nerve message. Hodgkin correctly interpreted the phenomenon as an expression of a local increase of excitability, a phenomenon consistent with the local circuit theory of Hermann, and already observed by Lucas and by Adrian some 20 years before.

To provide a more direct indication in support of the local circuit theory, Hodgkin succeeded in demonstrating that the increased excitability observed beyond the blocked region was indeed accompanied by a measurable local electrical response, having the characteristics of a current spreading passively along the nerve fiber with a geometrical law similar to that underlying the spread of the excitability increase. Besides supporting the local circuit theory of Hermann, these findings helped to strengthen the

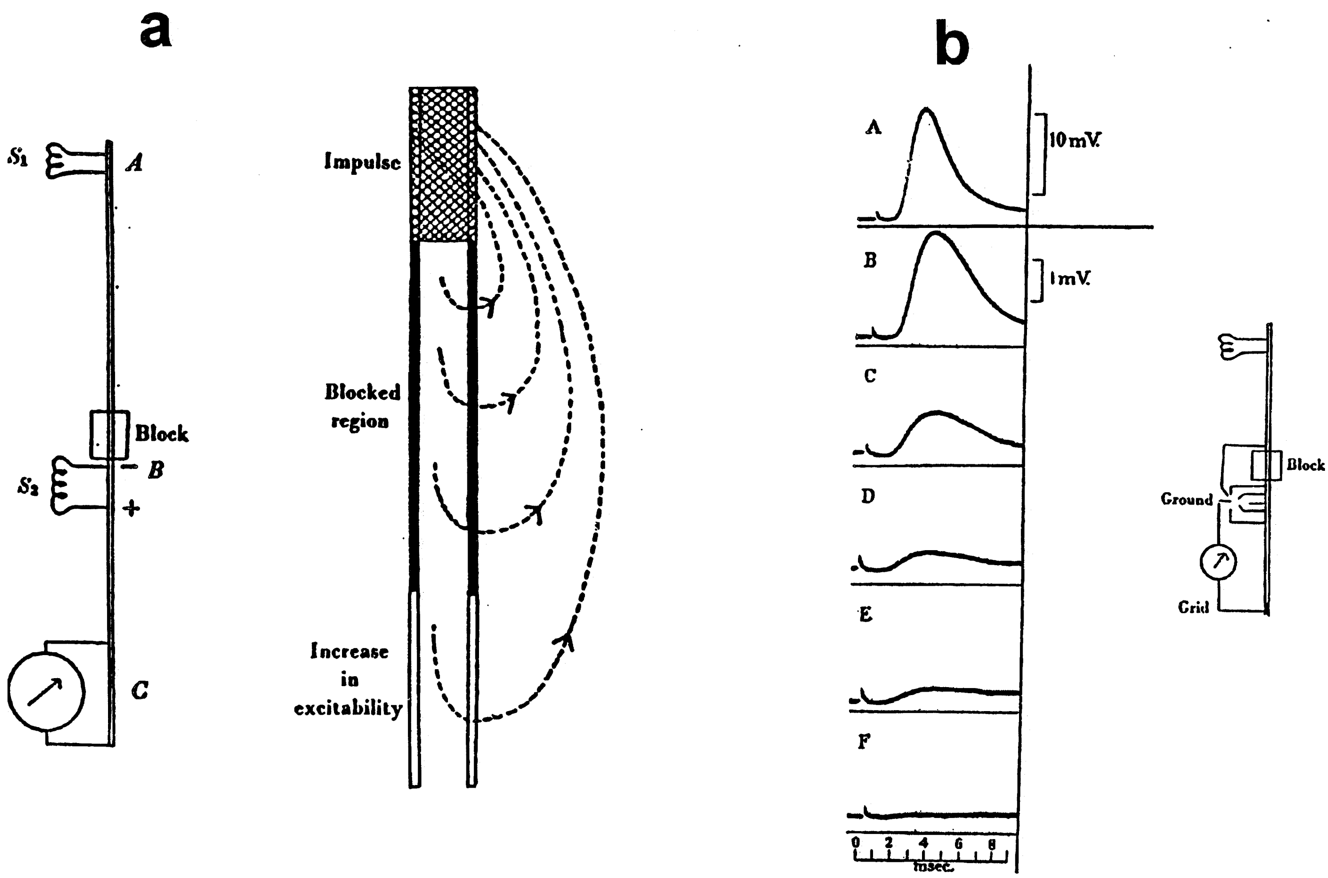

FIG. 12. The initial experiments of Hodgkin. (a) Increase of excitability; (b) appearance of subthreshold depolarizations in the region distal to the site of a block induced by local cooling. In (a) the increase of excitability is observed only if the test shock (S_2) is applied after the control shock (S_1) with a delay corresponding to the conduction time between the two sites of stimulation. (b) The upper record, A, represents the action potential recorded proximally to the block, while the lower recordings B–F are the potentials recorded at progressively longer distances beyond the blocked site. Note the progressive decrease of potential with the distance from the block, and the calibration difference between A and B–F. (From [51]).

case for a close relationship between signal propagation and electrical events in the nerve. This relationship was further supported by the results of a subsequent study aimed at investigating the changes of conduction speed induced in both crab and squid nerves by modifications of the conductivity of the extracellular medium. It was found that the speed decreased when the conductivity was decreased (by immersing the nerve in oil or reducing the volume of conductive fluid around it) and, on the contrary, it increased when the conductivity was increased by making recourse to metallic conductors [54].

In the crab nerve, moreover, Hodgkin succeeded in showing that a local electrical response, comparable to that appearing after a partial conduction block, could be elicited near the site of application of a subthreshold electrical stimulus. An electrode was used to stimulate a nerve with short electrical pulses of different intensities, and the resulting electrical responses were recorded in the immediate vicinity with another electrode [53]. The responses brought about by anodal stimuli (i.e., stimuli that produced a positive potential on the external nerve surface, and were, therefore, of opposite polarity to those capable of eliciting a nerve impulse) appeared to be largely the result of a passive spread of current along the nerve fibers. With cathodal (i.e., external negative) stimuli the responses were mirror images of those obtained with anodal stimulation only as far as small intensities were concerned. Large stimuli evoked responses characterized by a prominent tail. These responses did rather abruptly develop into full impulsive events, when the stimulus exceeded a “threshold” value. Again this finding confirmed the expectations of Lucas and Adrian, based on the observations of local excitability changes elicited by weak electrical stimuli in frog nerves. It remained to be investigated what functional relationship existed between the subthreshold responses evoked by cathodal stimuli and the full-blown nerve response, and how the localized potential changes, which accompanied the leading edge of this response, intervened in the conduction process. This required major advances in the experimental preparation and technique, advancements that were on the way after the “rediscovery” by J. Z. Young of the existence in the squid of a “giant axon” which, by its size, appeared ideal for electrophysiological recordings [111]. Using extracellular electrodes, Cole and Curtis provided clear evidence that the action potential in this axon was accompanied by a decrease in membrane resistance [24] (Fig. 13).

Both Cole and Curtis, and Hodgkin and Huxley, succeeded afterwards in inserting an electrode inside the axon and in measuring directly the transmembrane potential in the resting state and during excitation [25,60]. The existence of the resting membrane polarization postulated by Bernstein was confirmed by these studies (the interior of the cell being about 50 mV negative with

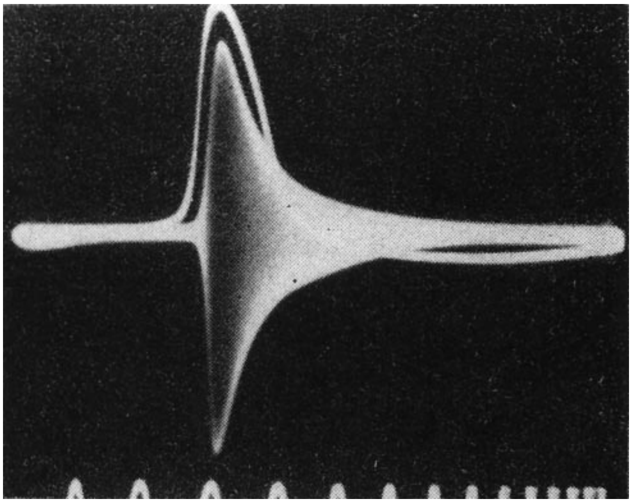

FIG. 13. The classical demonstration of the increase of conductance in the squid axon during the discharge of the action potential. Upper line: action potential; white-dark band: measure of the membrane impedance obtained with the Wheatstone bridge method by applying a high frequency (20 KHz) sinusoidal signal to two electrodes placed on the opposite site of a giant axon. From a measure of the impedance changes obtained at various frequencies (and proportional to the width of the band) the change of conductance was estimated to be approximately 40 times at the peak of the action potential relative to rest. Time marks: 1 ms apart. (From [24]).

respect to the exterior [60] (Fig. 14). Unexpectedly, however, it appeared that during excitation the potential overshot the zero level by several tens of millivolts, a finding that was clearly in contrast with Bernstein's membrane theory (no substantial difference of potential should exist across the membrane if this became permeable to all ion species), but well in line with the initial observation, made by Bernstein himself (and in less ancient times by Shaefer [98]) that the amplitude of the action current could sometimes exceed that of the injury current. The interpretation of these results was postponed by the Cambridge group because of the events of war.

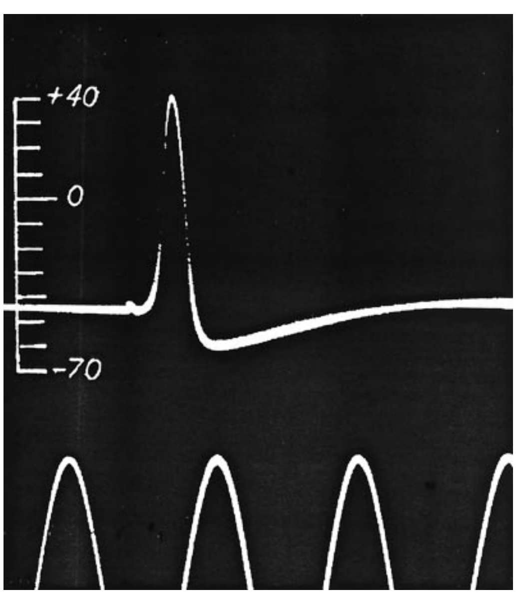

FIG. 14. The first published intracellular recording of the action potential in the squid axon. Time course of the difference between the internal and external potential, in the resting state and during the discharge of an action potential. Time mark, 500 Hz. Notice the large positive overshoot of the membrane potential during the action potential, which contrasted with the expectation of the Bernstein's theory (From [60]).

In 1949, Hodgkin and Katz succeeded in showing that the action potential of the squid nerve decreased in amplitude by decreasing the extracellular concentration of sodium ions, and suggested that this was the consequence of the involvement of a selective increase of membrane permeability for sodium ions in the discharge of the action potential [66]. Because sodium ions are largely in excess in the extracellular medium compared to the axon interior, the resulting electrochemical potential would be positive (axon interior vs. extracellular compartment), and therefore, membrane potential would change polarity during excitation as a consequence of the sodium permeability increase. In other words, the membrane potential, which in the resting state depended on the permeability for potassium ions, would be largely dominated during the excitation by the permeability for sodium ions, and the passage from the one to the other of the two conditions would be the consequence of a specific change of membrane permeability induced by the electrical stimulus. The notion that the membrane became selectively permeable to sodium at the peak of the action potential was an important revision of the Bernstein's theory, which assumed an unselective passage of all ions during the excitation, a theory which, as already mentioned, implied the almost complete disappearance of any difference of membrane potential whatsoever during nerve excitation.

In order to verify whether the new theory, based on a transition between two permeability states of the membrane, could account for the basic properties of nerve excitation and conduction, it was necessary to study in an accurate and quantitative way the changes of membrane currents and permeability accompanying the appearance of the action potential. However, a study of this kind was difficult with conventional techniques, because the "regenerative" and "explosive" properties of the action potential discharge did not permit a study of the relationship between membrane current and membrane voltage during the excitation process. To obtain accurate information on the electrical events responsible for the action potential it was necessary to circumvent the explosive character of this event, by forcing the membrane potential to assume, and to maintain, certain levels in spite of its tendency to evolve spontaneously when the threshold value was exceeded.

The Voltage Clamp, the Squid Axon, and the Hodgkin-Huxley Model

The sought for technique that made possible the study of membrane events underlying the generation of action potentials was the voltage-clamp devised in 1949 by Cole [23] and by Marmont [81]. It was applied in a masterly way to the squid axon by Hodgkin and Huxley in collaboration with Katz, in a series of studies published in 1952, which represent a fundamental milestone in the investigations on animal electricity [61–65]. In the epoch of Lucas and Adrian, the explosive character of the propagated response had been somewhat subdued by using procedures based on partial block of small segments of the nerve, but these procedures could give little information about what would happen once the threshold was exceeded.

In voltage clamp, the "block" of the electrical events underlying the explosive character of the action potential is not based on the application of narcotic agents or temperature changes, but relies on the use of a feedback circuit capable of providing the transmembrane current necessary for moving and clamping the potential at any desired value.

Using the voltage-clamp technique, Hodgkin and Huxley, working in collaboration with Katz, clarified the mechanisms underlying the generation of the action potential in nerve fibers. They provided the final, unequivocal evidence that the nerve conduction is fundamentally an electrical event and, as Hodgkin formulated it, that "the action potential is not just an electrical sign of the impulse, but the causal agent in the propagation" [57]. As a matter of fact, after the studies of Hodgkin and Huxley the nervous impulse elicited by an electrical stimulus could no longer be considered as a purely passive physical consequence of the stimulus. On the contrary, it will appear to be an active process, which depended on a particular form of electrical energy, accumulated between the interior and the exterior of the nerve fiber, as a consequence of physiological processes clearly "belonging to the domain of life," a true "animal electricity" with characteristics corresponding to the fundamental intuition of Galvani. Moreover, nervous signaling appeared to be a genuine electrical phenomenon also because it involved a change in the electrical potential of the membrane as a necessary, "causal" link for the release of the intrinsic electrical energy and for the consequent development of signaling event.

This resulted in a clear and simple way from the comparison between membrane currents elicited by voltage pulses of opposite polarities (Fig. 15a–d). The currents elicited by hyperpolarizing pulses (i.e., by pulses which increased the negativity of the cell interior relative to the extracellular medium) were of small amplitude and could be accounted for largely by simple movements of charges under the effect of the applied, extrinsic potential. These currents were inward-going (i.e., positive charges entered the membrane, as expected) because the cell interior was made more negative by the applied stimulus. Their size and their time course corresponded to those calculated on the basis of the pure physical characteristics of the membrane (i.e., resistance and capacitance).

Something different occurred with depolarizing voltage pulses (i.e., with pulses that made the cell interior less negative or more positive) with respect to the exterior, particularly when these stimuli were of an amplitude that would have been suprathreshold in normal conditions (i.e., in the absence of voltage clamp). The initial phase of the membrane response was dominated by a large inward current, a current totally inexplicable as a pure charge movement under the influence of the changed electrical field. Because a depolarizing stimulus moves the internal membrane potential in the positive direction, the current directly produced by this stimulus should be outward-going (i.e., an excess of positive charges should leave the cell interior under the effect of the changed field). The fact that the main experimental current was actually inward-going, under these conditions, suggested that the charge movement was not directly caused by the change in electrical field across the membrane induced by the applied stimulus. It was the consequence of changes in membrane properties induced by the depolarizing stimulus, which resulted in a movement of ions under the effect of a preexisting energy gradient, a gradient originating from the metabolic activity of the cell. The membrane modification consisted of an increase of the permeability to sodium ions that allowed for the entrance of these ions under their electrochemical gradient through a process that will be later denoted as "gating."

The increase of permeability, induced by depolarizing stimuli, was assumed by Hodgkin and Huxley to result from an electrical influence on charged particles present in the membrane and regulating the membrane capability to allow for a selective passage of sodium ions, via a mechanism that will be denoted as "activation." The voltage-dependence of the ion permeability changes involved in the discharge of the nervous impulse links electricity in a fundamental way to this event, and makes unlikely any hypothesis that considers the electrical phenomenon only as one of the many possible functional expressions of nerve excitation.

A fundamental consequence of the gating properties of the membrane permeability for sodium is that the entry of sodium ions, brought about through the influence of depolarization on the membrane, results in a net increase of the positive charge influx, and, therefore, in a further depolarization that in turn would cause an additional increase of sodium permeability. This positive feedback cycle controlling the entry of sodium ions activated by membrane depolarization [55], later denoted as Hodgkin cycle, is at the heart of the mechanism of nervous signal generation, and accounts for the twofold electrical nature of this signal: a signal generated by the release of an intrinsic electrical energy which, however, requires an electrical influence to be released (Fig. 16). The threshold depolarization required to initiate the explosive cycle is just the *"impeto," "impulso," "urto"* invoked by Galvani as a fundamental aspect of the mechanism capable of setting in motion the intrinsic animal electricity.

The model of the nerve signal generation proposed by Hodgkin and Huxley, on the basis of the activation characteristics and kinetic properties of the membrane currents identified in their studies, accounts for most of the properties of the excitation and conduction process along the nerve fiber.

In this model the equilibrium potentials of the various membrane currents correspond to the values of the electrical membrane potential at which the tendency of the ions to move, as a consequence of their transmembrane concentration gradients, is counteracted by the electrical field. These equilibrium potentials for the ion mechanisms of the membrane are modeled by three electrical batteries. Being distributed along the axon fiber, these batteries provide the local energy for the propagation of the nervous impulse, an energy that corresponds to that of the gunpowder in the vivid analogy proposed by Lucas (as noted previously).

The electrical behavior of the axon membrane during the generation and conduction of action potential is accurately described by the Hodgkin–Huxley model in all its fundamental aspects. Among other parameters, the model accounts for the all-or-nothing character of nervous impulses with the presence of a threshold and of a refractory period. It describes in a satisfactory way the time course of both the local and propagated electrical signals and of the accompanying conductance changes. Moreover, according to the model, the strong temperature dependence of the processes of excitation and conduction can be explained as a result of the strong influence that temperature exerts on the gating mechanisms controlling the ion currents involved in action potential generation [65,69].

Ion channels, Patch-Clamp, and Galvani

Most of the fundamental predictions of the Hodgkin–Huxley model were confirmed by subsequent studies. It was shown that the early inward and the delayed outward current, carried by sodium and potassium ions, respectively, depends on two specific and independent membrane mechanisms, because each of them can be blocked by specific pharmacological agents (the sodium current by the puffer fish poison tetrodotoxin [91], and the delayed potassium current by tetraethylammonium, [105]). Moreover, it will become clear that, as assumed by Hodgkin and Huxley, the ion currents involved in electrical excitation of nerve and muscle membranes are due to fluxes of ions along their electrochemical gradients, and that active metabolic phenomena (to be called "ion pumps") do not intervene directly in producing the currents [67,97,100,110]. Metabolic processes intervene indirectly in electrical membrane phe-

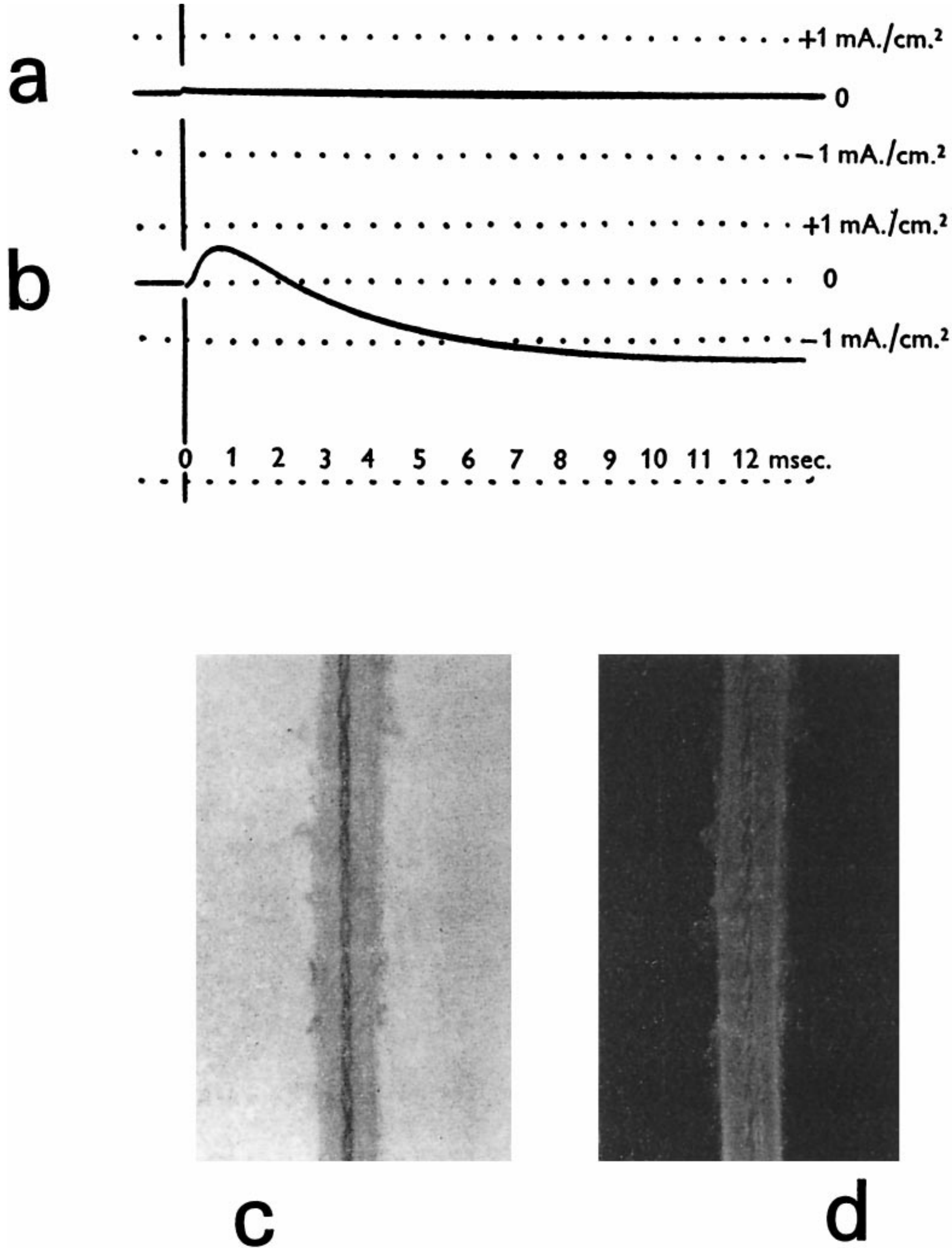

FIG. 15. Voltage clamp currents elicited in squid axon by a hyperpolarizing (a), and a depolarizing (b) voltage step. Contrary to the nowadays conventions, in both tracings an upward deflection indicates a current entering into the cell, while a downward deflection indicates a current flow from the inside to the outside of the cell. In the upper panel the potential is displaced from the resting value of −65–−130 mV and this results in a very small inward flow of current (almost undetectable at the used amplification). In the lower panel the voltage is displaced from −65–0 mV resulting in a large biphasic current with an early, inward-directed component carried by sodium ions that enter the cell, and in a late, outside-directed component corresponding to the outflow of potassium ions; (c, d) two photomicrographs of the squid axon penetrated with intracellular metallic electrodes for voltage-clamp experiments, obtained with transmitted light or dark field illumination, respectively. (From [65]).

nomena, because normally they serve to establish and maintain the ion gradients, from which the electrical potential at both rest and during excitation originate.

The questions left largely unresolved by the Hodgkin–Huxley studies concerned the mechanism of ion permeation through the membrane. The hypothesis oscillated between a diffusion process through a pore-type device, and a permeation mechanism involving a carrier molecule.

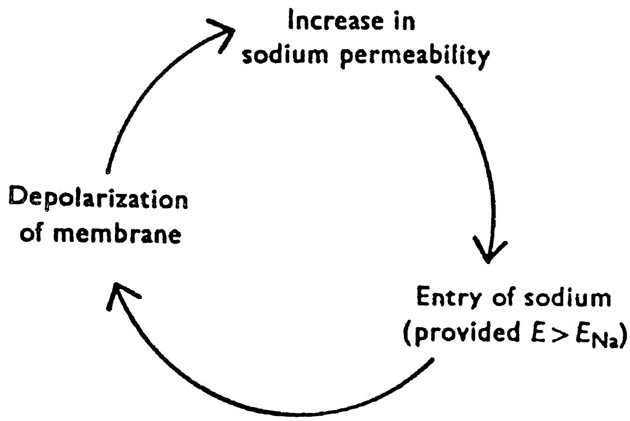

FIG. 16. The Hodgkin cycle of the sodium current in the discharge of the action potential: membrane depolarization increases the permeability to sodium ions with a consequent increase of the flow of sodium ions entering the cell. In turn, this results in a further depolarization of the membrane and, therefore, in an additional increase of sodium permeability. The process will be of a "regenerative" or "explosive" character and will tend to bring the membrane potential to a level capable of contrasting the tendency of sodium ions to enter the cell, because of their larger extracellular concentration (From [55]).

The studies carried out after Hodgkin and Huxley have clarified the mechanism of ion permeation involved in membrane electrical excitability with a molecular resolution, and this is probably the most important achievement of contemporary membrane electrophysiology. Besides conceptual advancements, the success of these studies was largely made possible by the invention, in 1976, by Erwin Neher and Bert Sakmann of a patch-clamp technique for recording the elementary electrical events of the membrane, the currents passing through a single molecule of the permeation device present in the membrane structure [92,109].

Patch-clamp studies have provided definite evidence that membrane currents involved in the electrical behavior of the cells are carried by ions passing through protein structures embedded in the membrane. These structures, indicated as ion channels, provide hydrophilic paths whereby ions can permeate in spite of the hydrofobic properties of the lipid constituents of the membrane [50].

This digression on the studies of "animal electricity" after Galvani ends here, and I return to the main theme of this article, with the hope that the considerations on the historical aspects of electrophysiology will help us to better understand the various aspects of the scientific discussion on animal electricity at the epoch of Galvani. Before leaving the subject of ion channels, however, it is perhaps apropriate to mention here that in order to account for the possible passage of electricity from the interior to the exterior of nerves necessary for explaining the experimental contractions in his frogs, Galvani came very near to conceiving the possible existence of structures similar to ion channels. By referring to the metallic conductor, which, in his Leyden jar model, represents the equivalent of the nerve, he wrote:
". . . let one plaster then this conductor with some insulating substance, as wax . . . let one make small holes in some part of the plastering which concerns the conductor. Then let one moist with water or with some other conductive fluid all the plastering, having care that the fluid penetrates in the above-mentioned holes, and comes in contact with the conductor itself. Certainly, in this case, there is communication through this fluid between the internal and the external surface of the jar." ([33] pp. 246–247).

GALVANI AND VOLTA AND ANIMAL ELECTRICITY

The electrophysiological studies carried out in these last two centuries have fully confirmed Galvani's insightful hypothesis of the existence of an "animal electricity . . . a kind of electricity essentially linked with life itself, and inherent in some animal functions," as Volta himself defined it in a famous letter sent in 1782 to Mme. Le Noir de Nanteuil ([107] pp. 8–12). At that time, Volta took for granted that such form of electricity was present only in electric fishes. We know now that this electricity is present practically in all animal beings.

The animal electricity supposed by Galvani and proved by subsequent studies is "essentially linked to life" because it depends on an organization typical of living beings, an organization based on the existence of membranes that contain structures capable of separating ions and of establishing ion gradients by using metabolic energy (the ion pumps). They are capable, moreover, of converting these ion gradients into electric potential differences.[8] As Galvani supposed, animal electricity exists in normal conditions in a state of "unbalance" or "disequilibrium," and it is ready to be set in motion by internal physiological processes or following the influence of external agents. This electricity is involved in fundamental physiological processes such as muscle and nerve excitation, and it corresponds to the elusive "animal spirits" of the medical science of the classic period. In some way animal electricity is accumulated in animal tissues as in electrical capacitors, and the Leyden jar, which represented in Galvani's epoch the prototypical capacitor, appeared, therefore, to be an appropriate model for the electrical condition of animal tissues.

In many respects, therefore, Galvani's ideas have been confirmed by studies made after him, and it seems appropriate to consider Galvani the real founder of electrophysiology, as the new science of animal electricity was called by Carlo Matteucci. In retrospect, it is also appropriate to consider decisive, in support of animal electricity, some of Galvani's experiments and in particular those experiments in which he produced muscle contraction without recourse to metals (*la contrazione senza metallo*), the 1794 experiment in which the contraction was obtained through a direct contact between nerve and muscle, and, finally, the experiment of 1797 in which the contraction was produced in the absence of any contact between heterogeneous tissues.

For reasons previously explained in this article, Galvani considered muscle tissue as the main accumulator of animal electricity in the organism, and he assumed that nerves behave as simple passive conductors, allowing for the reequilibration of the elec-

[8] It is interesting to recall here the words that Galvani wrote in 1794, with reference to his hypothesis of a disequilibrium of electric fluid in animal tissues: "Such a disequilibrium in the animal either must be there naturally or should result from artificium. If it is there naturally, we should admit that in the animal there is a particular machine capable of generating such a disequilibrium, and it will be convenient to refer to this form of electricity as to an animal electricity in order to denote, not an electricity whatsoever, but a particular one referred to a particular machine: . . . But what will it be this animal machine? We cannot establish it with certitude; it remains totally occult to the most acute sight; we can do nothing else than figure out its properties, and, from these, somewhat envision its nature." ([33] pp. 201, 205). The membrane, with its ion pumps that creates dissimilar concentrations of Na^+ and K^+, and with ion selective channels capable of converting concentration differences into an electrical potential, is just the hypothetical machine supposed by Galvani more than two centuries ago.

tricity present in the muscle in a state of unbalance. The role assigned to nerves was undoubtedly a weak point in Galvani's theory, which was aimed at by Volta, when the physicist of Pavia realized in his experiments that muscle contraction could be induced with a bimetallic arc connecting two different points of the same nerve in the absence of any contact with muscle tissue.

The problem roused in that regard by the objections of Volta presented to Galvani's theory was a major one, involving one of the most serious difficulties that precluded the acceptance of the doctrine of animal electricity by many important scientists of the 18th century. If electricity existed in the organism, and if it was involved in the functioning of nerve and muscles, how could it be in a state of unbalance in animal tissues that were mainly composed by liquid bodies, known for being "deferent" (i.e., good conductors of electricity?[9] In physics, the capacitor was the only device capable of maintaining electricity in a state of unbalance for a long time, and this explains why Galvani made recourse to the Leyden jar analogy in his theory. However, a capacitor implied an insulating material separating two conductors, and it was therefore necessary to assume an organization of animal tissues compatible with the presence of a possible insulator separating two conductive compartments. Although at the epoch of Galvani there was no clear evidence for the cell structure of animal tissues, and the existence of a membrane separating an internal and external compartment was only a vague possibility, it seemed plausible to Galvani to assign some special characteristic (i.e., an insulating property) to the ill-defined structure enclosing the muscle fibers. The next logical step in the development of Galvani's hypothesis was to find a possible way whereby a functional communication could be established between the interior and the exterior of the muscle fiber in spite of the hypothetical insulating material separating the two compartments. The evidence that nerves penetrate deeply and diffusely in muscle tissue allowed Galvani to assign this role to the nerve fibers that he supposed to penetrate inside every muscle fiber.

Galvani could not accept, therefore, the main point raised by Volta with his experiment of the effectiveness of a bimetallic arc connecting two points of a nerve. He tried to envision explanations that could undermine its relevance (such as the previously mentioned "occult arc"), because it was impossible for him to figure how an electrical disequilibrium could exist between two similar zones of the nerve that were not separated by any insulating material. It was previously mentioned that Galvani became well aware of the possibility that external electricity could set in motion internal electricity. It may, therefore, appear hard to conceive why he did not accept the suggestion of Volta that metals were acting as a source of external electricity, similar to the case of the spark from an electric machine and of the lightning from atmospheric electricity. In the author's opinion this depends on the fact that the first experiments with metals ("the experiment on the balcony") marked the fundamental transition in the conceptual elaboration of Galvani toward the doctrine of "animal electricity." Undoubtedly Galvani had started his experiments with the aim of verifying the neuroelectrical hypothesis of muscle contraction proposed in 1733 by Stephen Hales in England, and in Bologna strongly advocated by Tommaso Laghi [20,40,73]. However, up to the experiments with metals, Galvani thought that his results could only demonstrate that electricity was acting as an external agent capable of stimulating an unknown internal force. Following the experiment with metals, he became fully convinced that the internal force was electrical (what other kind of fluid could permeate a metal without any apparent difficulty?). For Galvani, to renounce to the idea that in these experiments metals were acting basically as conductors of an internal electrical fluid was, therefore, a possibility totally unreconcilable with his mental elaboration on the hypothesis of animal electricity.

Many differences undoubtedly existed between Galvani and Volta from several points of view, ranging from their scientific formation to their human attitudes. On one side Galvani, the anatomist, an old style biological scientist, the *savant* of the *ancien régime*, with a tendency to present the results of his investigation to a small circle of colleagues in Bologna, rather than to write scientific articles or memoirs to be spread around Europe, preferring to use Latin or Italian for his scientific papers many of which were left unpublished, reluctant to accept the social and political changes of his time. On the other side Volta, the brilliant modern physicist, in close relationship with the most important academies and scientific institutions of Europe, frequently travelling outside his country, publishing in French, English, and German the results of his studies, apparently much more at his leisure in a world undergoing revolutionary transformations.

The differences between Galvani and Volta have, however, been exaggerated by a tradition more inclined to create a legend around the two great scholars than to investigate the real matter of their scientific debate. To attribute to these differences the difficulties that the two competitors had in accepting the point of view of the adversary and in reaching a common view, would be to underestimate the importance of their scientific controversy. The real problem was that it appeared hard to conceive, two centuries ago, that nerve conduction was electrical, not only because it implied a flow of an internal electricity existing in a state of unbalance (Galvani's view), but also because this electricity was set in motion only by an electrical stimulus. In normal conditions this stimulus was derived from a variation of the same internal electricity, according to a self-exciting cycle, while in the conditions of Galvani's or Volta's experiments it was provided by external electrical influences. There was no model in the physics of those days for an electrical fluid that had in itself the tendency and the energy for moving, but which required, nonetheless, a stimulating electrical action to start its actual motion. Science had to wait a long time before seeing completely elucidated, with the discovery of the Hodgkin cycle, the deep reasons for the conflict between Galvani and Volta on the mechanism of nerve and muscle excitability.

Galvani's interpretation of his experiments carried out in 1794 and 1797 can be considered correct as to the main raised issue (the existence of animal electricity), but not for the proposed mechanism of excitation. In none of the two circumstances was the electrical excitation due to a simple conduction mechanism along artificial or natural arcs. In all cases, the excitatory stimulus was the difference of potential generated by the contact between an injured and an intact tissue surface. This stimulus depolarized locally the nerve (or muscle) membrane and, therefore, set in motion the internal electricity accumulated across it in a condition of unbalance. In Galvani's writings we can find some indication that he was aware of the particular effectiveness of injured tissue

[9] Although convinced that electricity was "the most powerful stimulant" of muscle contraction, Haller nevertheless refused the identification of the nervous and electrical fluid on the basis of the impossibility to conceive how electricity could move along restricted paths in organisms composed of electrically conductive tissues. "All animal matter" he said "is from that kind of bodies that assume electric nature through communication, & all animal parts are equally apt to receive it. Now, assume that either sciatic nerve or muscle is full of electric matter, and incited to motion; certainly that matter will diffuse anywhere, being not restricted by any limit, to the adipose tissue around, to nearby muscles, until an equilibrium will be born." ([42] p. 255)

in evoking contractions. For instance, in the 1797 experiment he recognizes that the contraction occurs if the contact is established through the "small mouth" of one of the two nerves (i.e., through its cut section) ([33] p. 323). However, only with Matteucci would it become clear that intrinsic animal electricity manifests itself experimentally only by connecting an injured and an intact tissue surface.

If Galvani could not easily accept Volta's views on the role of metals in producing the electricity in the bimetallic-arc experiment, Volta, on the other hand, could not accept Galvani's hypothesis of an intrinsic animal electricity and at the same time maintain his confidence in the electromotive force of bimetallic contacts. For Volta this meant accepting the idea that the form of electricity he had discovered (the metallic one) was acting as a stimulating cause capable of setting in motion the other electricity (i.e., the intrinsic, animal one). As already mentioned, there was not a simple physical model that could support a similar possibility (i.e., an electrical force that manifested itself only under the action of another similar electrical force). In that regard, Volta's attitude oscillated between two mutually exclusive possibilities. External electricity could be acting as a pure stimulus (similar to a chemical or to a mechanical excitor) activating some internal nonelectric force, and nothing was, therefore, intrinsically electrical in the physiological processes leading to muscle contraction from nerve excitation. Alternatively, the external electrical force was causing the motion, inside the organism, of an electrical fluid that had nothing of especially animal and was in no way especially related to the living state. According to this last view, which finally prevailed in Volta's elaboration, animal tissues were simple conductive bodies, similar to other humid conductors, and particularly to saline solutions. If the metals were electromotive, why should one assume the presence in the organism, of another possible cause of motion of the electrical fluid? In Volta's opinion, if an electricity existed in the animal body, this was simply the "common" type of electricity, present in animal tissues as in any conductive body. Besides other reasons, there was, therefore, an argument derived from the economy of scientific thinking that prevented Volta from accepting Galvani's conception of intrinsic animal electricity: if a sufficient cause for the motion of electricity in animal tissues was at hand (i.e., the electromotive power of metals), why should one invoke for the same effect, another additional cause (i.e., the intrinsic unbalance of electric fluid)? The problem here is that sometimes the paths leading to scientific discoveries, particularly to discoveries capable of causing a true revolution in the progress of science, do not follow necessarily the way indicated by the formalism of scientific method elaborated by scientists, or, more often, by philosophers of science.

GALVANIC FLUID AND THE VOLTAIC BATTERY

The cause of the apparently stubborn attitude of Volta in refusing to accept the idea of an animal electricity is to be found in this kind of logical difficulties.

The discussion concerning the involvement, in nerve and muscle excitation, of a specific animal form of electricity, or of common electricity, had other historically interesting implications, besides those pertaining closely to the Galvani–Volta controversy, which are interesting to consider here.

Before the introduction of the electrical battery, the electricity commonly used in the various forms of experiments carried out in 18th century was basically that provided by electrical machines, and from these brought to, and accumulated in, capacitor devices (i.e., Leyden jars or Franklin's magic squares). Generally this electricity was characterized by extremely high voltages and by small amounts of total charge. It manifested itself quite evidently with a series of visible, or otherwise perceptible effects, such as sparks, electric stars or "aigrettes," electrical wind or electrical odor, etc. In those days these effects were considered as fundamental qualifications for the presence of the electrical fluid. For instance, in 1836, many years after Galvani, the presence of sparks as evidence of animal electricity was still considered so important that a fervid discussion occurred at the *Académie des Sciences* of Paris on a question of priority, raised against Carlo Matteucci by Santi Linari, concerning the possibility of obtaining sparks from Torpedo [87].

However, except in electric fishes, in no other circumstances did it appear possible to obtain such sparks, or other manifest electrical signs, to support the presence of an electrical force in animal tissues as was assumed by Galvani. This seemed a serious difficulty. Galvani hesitated between two different conceptual attitudes. The absence of overt electrical signs could be the consequence of the extreme exiguity of the electrical force involved in nerve and muscle excitation. Or it could be hypothesized that the electrical phenomena of organisms depended on a peculiar form of electrical fluid that resembled common electricity for some of its properties (e.g., the rapidity of propagation, the capacity of flowing along metal bodies and other "conductive" materials), but it differed from common electricity because of the absence of sparks and of other evident electrical signs. This second possibility should have appeared to Galvani as a convenient response to the objections of Volta (see for instance [33], pp. 232–233, 304–306). The special electrical fluid proper to animal tissues were to be commonly denoted as "Galvanic fluid," where "Galvanic" was used more to signify the unusual, and somewhat ambiguous character of this fluid than to give credit and honor to Galvani for its discovery. However, Volta did not content himself by seeing that his adversary moved a step back in his theory of animal electricity, by recognizing the special character of the electricity present in living organisms. At some stage of his elaboration, Volta definitely excluded the involvement of any form of special electricity in muscle and nerve functions. As already noted, when Volta became fully convinced of the electromotive power of metals, he attacked Galvani with new arguments. It was an electrical fluid that underlay muscle and nerve excitation, but not a "Galvanic" type of fluid. It was simply the common electrical fluid present in all conductive bodies.

Ironically, the history of science took some revenge against Volta in this respect. After the invention of the electrical battery, it soon became evident that the electrical force generated by the new "wonderful" device differed from the typical force of the electrical machines and of Leyden jars, and was more similar to the supposed animal electricity of Galvani. As a matter of fact, the electrical force of the battery did not easily produce sparks or other classical electrical signs. Nevertheless, it was powerful in producing other effects that also seemed related to electricity. For instance it was very effective in producing chemical and thermal reactions. We know now that this is due to the fact that in contrast to the electrical machines, the electricity of normal batteries is characterized by a small voltage and a large amount of charge. The apparently ambiguous character of the electricity of the battery explains why the term "Galvanic" was used also to indicate the calm but powerful form of electricity developed by the battery, which contrasted with the tumultuous, but relatively ineffective, force of electric machines. It is somewhat paradoxical that the name of Galvani was to become somewhat associated, in this way, to the main invention of his great competitor.

CONCLUDING REMARKS: BIOLOGICAL CONCEPTS AND PHYSICAL LAWS

It has been proposed that among the reasons for the difficulty of the two great adversaries to recognize what was valid in the hypothesis of the antagonist was also the impossibility of reconciling in the 18th century science a biological and a physical approach to an experiment intrinsically "double-faced." The interest of Galvani was to discover a biological mechanism, while Volta was studying a particular aspect of the "physics" of electricity. Like ambiguous images, the "prepared" frogs presented two different and mutually exclusive aspects, and Galvani or Volta were able to perceive only one of them [96]. As already mentioned, in communicating the invention of the battery to the Royal Society, Volta eventually speculated that if electricity exists in the electric fish, this is a common form of electricity generated according to the physical principles underlying his newly invented "artificial electric organ" (i.e., the battery). As a matter of fact, in the view of the savant of Pavia, the electric fish was copying, in the organization of its electrical bodies, the arrangement of his battery ([107] p. 582). Apart from the excessive boldness of this conclusion, one could be tempted to recognize in the words of Volta the modernity of an attitude that asserted the general applicability to living organisms of the principles of physics, in an epoch still dominated by vitalistic conceptions. As a matter of fact, the great progress of biology in the following century corresponded to the definitive acquisition of the validity and applicability of physical laws in biological processes, according to a program that was particularly pursued by the great scientists of the German biophysical school (e.g., du Bois-Reymond, von Helmholtz, Ludwig, Brücke).

But some caution is necessary here. The history of science shows that some fundamental notions on the organization and functioning of living bodies did not derive from the application to biology of the laws of the physics. The theory of evolution, the laws of genetics and immunology, the principles of enzymatic action, the notions of homeostasis and regulation, and the concept of feedback control are eminently biological conceptions, primarily derived from the study of living organisms even though all the biological process involved therein must ultimately obey the laws of physics.

As already discussed in this article, in the case of Galvani, an important interpretative notion, seminal to the discovery of animal electricity was the concept of "irritability." The word "irritability" may sound ancient now, and somewhat reminiscent of a time-worn vitalistic creed. However, irritability was an insightful notion that penetrated into one of the most characteristic aspect of functional organization of living organisms. The reaction of the organism to an external (or internal) influence largely depends on its internal organization and is not a simple "physical" result of the influence acting on it. As already mentioned, implicit in the notion of irritability was the idea that the "force" (or the energy, according to a modern word), necessary for the expression of the specific reaction is accumulated in the organism, and is not directly related to the force (or energy) of the external influence. At the time of Felice Fontana the discrepancy between the energy of the stimulus and that of the response was clearly vividly depicted by the famous analogy "spark-gunpowder," an analogy that was probably present in Galvani's mind when the scientist of Bologna interpreted the mechanism of muscle contraction in his "spark" experiment:

"The contractile energy of the entire muscle can surpass that of the stimulus. It is thus that a tiny spark ignites a great mass of gunpowder, the energy of which is prodigious. The spark could hardly move a pebble, while the air imprisoned in an infinity of grains of powder in developing its elastic powers, upsets boulders. The spark is not the cause of its enormous effort, which greatly exceeds it in force, it is only the exciting cause, which liberates in the powder the energy of an agent which is enclosed in it.

The needle that pricks the heart does what the spark does . . . (Fontana, [27,68,71])."

Now we can better appreciate this discrepancy between external stimulus and physiological response in quantitative terms, by noting for instance that the energy of the response induced in a retinal rod by a single photon is about one hundred thousand times larger than the energy of the photon itself. The energy of the response evidently depends on an internal energy (generated by metabolic processes and, therefore, "vital"), which is released following the absorption of a photon. We know now in detail the complex chain of events that lead from photon absorption to the generation of the photoresponse, and how, through these events, the energy accumulated across the photoreceptor membrane leads to a movement of charges and to a change of potential that initiates the vision process. Phototransduction depends on a complex structural and functional organization of the photoreceptor cell, which makes possible the integration of many different events in a highly elaborated process [66]. This process, set in motion by photon absorption, is the specific form whereby the photoreceptor expresses what we could call its irritability.

It is perhaps not straying too far from the truth to say that the notion of irritability has anticipated subsequent developments in nonbiological sciences too, and, in particular, it precedes conceptually the development of automatisms in modern machines, and also, in some way, of "digital" logic in information science and technology.

An overview of history of science, particularly in 19th century, clearly shows the prominent importance of physical sciences in the progress of scientific knowledge and in the evolution of scientific thinking. However, scientific knowledge extends beyond the limits of physics, at least of the physics in the ordinary, reductionist meaning we normally assume today (as a matter of fact "physics" derives from the Greek "physis," nature, and at the time of Galvani and Volta it was still used to denote natural sciences in a general sense, and, in this respect, it was akin to "physiology," which means literally "science of nature"). Natural and biological sciences, and particularly physiology, have provided important conceptual schemes useful for the general progress of scientific knowledge. This is for instance the case for the concept of feedback control, which emerged first in biology and was later usefully incorporated into the realm of physical and technological sciences.

The impact of a biological scheme on a physical discovery is illustrated by the discovery of the electrical battery. Whatever was the mental elaboration guiding Volta to the invention of battery, it is certain that in constructing this epoch making device, Volta's attitude was in fact that of creating an apparatus which, in the morphological and functional arrangement of its components, was clearly much more related to complex machines of "organic" type, derived from the realm of biology (the electric organ of the fish), than to the simple physical devices of his time ([108] pp. 61–63).

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REFERENCES

1. Adrian, E. D. On the conduction of subnormal disturbances in normal nerve. J. Physiol. (Lond.) 45:389–412; 1912.

2. Adrian, E. D. The all-or-none principle in nerve. J. Physiol. (Lond.) 47:460–474; 1914.
3. Adrian, E. D. The temperature coefficient of the refractory period in nerve. J. Physiol. (Lond.) 48:453–464; 1914.
4. Adrian, E. D. The impulses produced by sensory nerve endings. Part I. J. Physiol. (Lond.) 61:49–72; 1926.
5. Adrian, E. D.; Lucas, K. On the summation of the propagated disturbance in nerve and muscle. J. Physiol. (Lond.) 44:68–124; 1912.
6. Adrian, E. D.; Zotterman, Y. Impulses from a single sensory endorgan. J. Physiol. (Lond.) 61:151–184; 1926.
7. Aldini, G. De animali electricitate dissertationes duae. Bonomiae: Typographia Instituti Scientiarum; 1794.
8. Aldini, G. Essai théorique et expérimentale sur le galvanisme (2 vol.). Paris: Fournier et Fils; 1804.
9. Arago, J.-F.-D. Alexandre Volta, in "Oeuvre complètes" (Vol. I). Paris: Gide et Baudry; 1854.
10. Bazett, H. C. Observations on the refractory period of the sartorius of the frog. J. Physiol. (Lond.) 36:414–430;1907.
11. Bentivoglio, M. 1896–1996: The centennial of the axon. Brain Res. Bull. 41:319–326; 1996.
12. Bernabeo, R. Carlo Matteucci (1811–1868): Profilo della vita e dell'opera (con 10 documenti inediti). Quaderni di Storia della Scienza e della Medicina. 11:1–41; 1972.
13. Bernstein, J. Ueber den zeitlichen Verlauf der negativen Schwankung des Nervenstroms. Pflüger's Arch. f. d. ges. Physiol. 1:173–207; 1868.
14. Bernstein, J. Untersuchungen über den erregungsvorgang in nerven and muskelsystheme. Heidelberg: Carl Winter; 1871.
15. Bernstein, J. Elektrobiologie. Braunschweig: Vieweg und Sohn; 1912.
16. Bertholon, P. De l'électricité du corps humain dans l'état de santé et de maladie. Paris: Chez Didot; 1780.
17. Bowditch, H. P. Über die eigenthümlichkeiten der reizbarkeit welche die Muskelfasern der Herzens zeigen. Ber. Sachs. Ges. (Akad.) Wiss., 23:625–689; 1871. 1871:
18. Boykott, A. E. Note on the muscular response to two stimuli of the sciatic nerve (frog). J. Physiol. (Lond.) 24:144–154; 1899.
19. Bresadola, M. La biblioteca di Luigi Galvani. Annali di Storia delle Universitá Italiane 1:167–197; 1997.
20. Bresadola, M. Medicine and science in the life of Luigi Galvani (1737–1798). Brain Res. Bull. [In this Issue] 46:367–380; 1998.
21. Caldani, L. M. A. Sull'insensività, ed irritabilità di alcune parti degli animali. In: Fabri, G. B., ed. Sulla insensitività ed irritabilità halleriana, vol. 1. Bologna: Corciolani ed Eredi Colli; 1757:269–336.
22. Clarke, E.; O'Malley, C. D. Nerve function. The human brain and spinal cord. Berkeley and Los Angeles: University of California Press; 1968:139–259.
23. Cole, K. S. Dynamic electrical characteristics of the squid axon membrane. Arch. Sci. Physiol. 3:253–258; 1949.
24. Cole, K. S.; Curtis, H. J. Electric impedance of the squid giant axon during activity. J. Gen. Physiol. 22:649–670; 1939.
25. Curtis, H. J.; Cole, K. S. Membrane action potentials from the squid giant axon. J. Cell Comp. Physiol. 15:147–157; 1940.
26. du Bois-Reymond, E. Untersuchungen über thierische elektricität, 2 voll. Berlin: Reimer, 1848–1884.
27. Fontana, F. Dissertation épistolaire adressée au R. P. Urbain Tosetti. In: Haller, A., ed. Mémoires sur les parties sensibles et irritables du corps animal. Vol. III, Lausanne: S. D'Arnay; 3:157–243;1760.
28. Fontana, F. De irritabilitatis legibus nunc primum sancitis et de spiritum animalium in movendis musculis inefficacia. Lucca: I. Riccomini; 1767.
29. Fontana, F. Richerche filosofiche sopra la fisica animale. Firenze: Cambia-gi; 1775.
30. Franklin, B. Benjamin Franklin's experiments. Cambridge: Harvard University Press; 1941.
31. Galvani, L. De viribus electricitatis in motu musculari commentarius. Bon. Sci. Art. Inst. Acad. Comm. 7:363–418; 1791.
32. Galvani, L. De viribus electricitatis in motu musculari. Commentarius (1791). In: Gherardi, S., ed. Opere edite ed inedite del Professore Luigi Galvani. Bologna: Dall'Olmo; 1841:59–125.
33. Galvani, L. Opere edite ed inedite del Professore Luigi Galvani raccolte e pubblicate dall'Accademia delle Science dell'Istituto di Bologna. Bologna: Dall'Olmo; 1841.
34. Galvani, L. Memorie ed esperimenti inediti. Bologna: Cappelli; 1937.
35. Gherardi, S. Rapporto sui manoscritti del celebre professore Luigi Galvani. In: Gherardi, S., ed. Opere edite ed inedite del Professore Luigi Galvani. Bologna: Dall'Olmo; 1841:3–106.
36. Glisson, F. Tractatus de ventriculo et intestinis cui praemittitur alius de naturae eiusque tribus facultatibus. London: Henry Broome; 1677.
37. Gotch, F. The submaximal electrical response of nerve to a single stimulus. J. Physiol. (Lond.) 28:395–416; 1902.
38. Gotch, F. The delay of the electrical response of a nerve to a second stmulus. J. Physiol. (Lond.) 40:250–258; 1910.
39. Grundfest, H. Julius Bernstein, Ludimar Hermann and the discovery of the overshoot of the spike. Arch. Ital. Biol. 103:483–40; 1965.
40. Hales, S. Statical essays; containing haemostaticks, vol. 2. London: W. Innys et al.; 1733.
41. Haller, A. Mémoires sur les parties sensibles et irritables du corps animal (I-IV tomes) 1756–1760. Lausanne: Sigismond d'Arnay; 1756–1760.
42. Haller, A. Elementa physiologiae corporis humani—Tomus Quartus—Editio prima veneta. Venetiis: Aloysium Milocco; 1768.
43. Helmholtz, H. Über die Fortpflanzungsgeshwindikeit der Nervenreizung. Arch. Anat. Physiol. Wiss. Med. 71–73; 1850.
44. Helmholtz, H. Note sur la vitesse de propagation de l'agent nerveux dans les nerfs rachidiens. C. R. Acad. Sci. (Paris) 30:204–206; 1850.
45. Helmholtz, H. Deuxième note sur la vitesse de propagation de l'agent nerveux. C. R. Acad. Sci. (Paris) 32:262–265; 1851.
46. Helmholtz, H. Messungen über fortpflanzungsgeschwindigkeit der reizung in den nerven–zweite reihe. Arch. Anat. Physiol. Wiss. Med. 199–216; 1852.
47. Helmholtz, H. On the later views of the conduction of the electricity and magnetism. Annual report of the Smithsonian Institution for 1873. Washington: 1874;247–253.
48. Hermann, L. Ueber eine wirkung galvanischer ströme auf muskeln und nerven. Archiv. f. ges. Physiol. 6:312–350; 1872.
49. Hermann, L. Weitere Untersuchungen uber den electrotonus, insbesondere über die erstreckung desselben au die intramusculären nervendenden. Archiv. f. ges. Physiol. 7:301–364; 1873.
50. Hille, B. Ionic channels of the excitable membranes. Sunderland: Sinauer Associates; 1992.
51. Hodgkin, A. L. Evidence of electrical transmission in nerve. Part I. J. Physiol. (Lond.) 90:183–210; 1937.
52. Hodgkin, A. L. Evidence for electric transmission in nerve. Part II. J. Physiol. (Lond.) 90:211–232; 1937.
53. Hodgkin, A. L. A local electric response in a crustacean nerve. Proc. R. Soc. Lond. B 126:87–121; 1938.
54. Hodgkin, A. L. The relation between conduction velocity and the electrical resistance outside a nerve fibre. J. Physiol. (Lond.) 94:560–570; 1939.
55. Hodgkin, A. L. The ion basis of electrical activity in nerve and muscle. Biol. Rev. 26:339–409; 1951.
56. Hodgkin, A. L. The conduction of the nervous impulse. Liverpool: Liverpool University Press; 1964.
57. Hodgkin, A. L. The ion basis of nervous conduction, The Nobel Lecture for 1963. Nobel Lectures, Physiology and Medicine, 1963–1970. Amsterdam: Elsevier; 1972:32–48.
58. Hodgkin, A. L. Chance and design in electrophysiology: An informal account of certain experiments on nerve carried out between 1934 and 1952. J. Physiol. (Lond.) 263:1–21; 1976.
59. Hodgkin, A. L. Chance and design. Cambridge: Cambridge University Press; 1992.
60. Hodgkin, A. L.; Huxley, A. F. Action potentials recorded from inside a nerve fibre. Nature 144:710–711; 1939.
61. Hodgkin, A. L.; Huxley, A. F. Currents carried by sodium and potassium ions through the membrane of the giant axon *Loligo*. J. Physiol. (Lond.) 116:449–472; 1952.
62. Hodgkin, A. L.; Huxley, A. F. The components of membrane conductance in the giant axon of *Loligo*. J. Physiol. (Lond.) 116:473–496; 1952.
63. Hodgkin, A. L.; Huxley, A. F. The dual effect of membrane potential on sodium conductance in the giant axon of *Loligo*. J. Physiol. (Lond.) 116:497–506; 1952.

64. Hodgkin, A. L.; Huxley, A. F. A quantitative description of membrane current and its application to conduction and excitation in nerve. J. Physiol. (Lond.) 117:500–544; 1952.
65. Hodgkin, A. L.; Huxley, A. F.; Katz, B. Measurement of current-voltage relations in the membrane of the giant axon of *Loligo*. J. Physiol. (Lond.) 116:424–448; 1952.
66. Hodgkin, A. L.; Katz, B. The effect of sodium ions on the electrical activity of the giant axon of the squid. J. Physiol. (Lond.) 108:37–77; 1949.
67. Hodgkin, A. L.; Keynes, R. D. Active transport of cations in giant axons from *Sepia* and *Loligo*. J. Physiol. (Lond.) 128:28–60; 1955.
68. Hoff, H. E. The hystory of the refractory period. A neglected contribution of Felice Fontana. Yale J. Biol. Med. 14:635–672; 1942.
69. Huxley, A. F. Ions movements during nerve activity. Ann. N. Y. Acad. Sci. 81:221–246; 1959.
70. Kelvin, W. T. On the theory of electric telegraph. Proc. R. Soc. (Lond.) 7:382–389; 1855.
71. Knoefel, P. K. Felice Fontana, life and works. Animal Physics. Trento: Società di Studi Trentini di Scienze Storiche II; 1984:191–209.
72. Knowlton, F. P.; Starling, E. H. The influence of variations in temperature and blood-pressure on the performance of the isolated mammalian heart. J. Physiol. (Lond.) 44:206–219; 1912.
73. Laghi, T. De sensitivitate atque irritabilitate halleriana. Sermo alter. In: Fabri, G. B., ed. Sulla insensitività ed irritabilità halleriana, vol. 2. Bologna: Corciolani ed Eredi Colli; 1757:326–345.
74. Lucas, K. On the gradation of the activity in a skeletal muscle fibre. J. Physiol. (Lond.) 33:125–137; 1905.
75. Lucas, K. The temperature coefficient of the rate of conduction in nerve. J. Physiol. (Lond.) 37:112–129; 1908.
76. Lucas, K. The "all or none" contraction of the amphibian skeletal muscle fibre. J. Physiol. (Lond.) 38:113–133; 1909.
77. Lucas, K. The effect of alcohol on the excitation, conduction and recovery processes in nerve. J. Physiol. (Lond.) 42:470–505; 1911.
78. Lucas, K. The conduction of nervous impulse. London: Longmans Green and Co.; 1917.
79. Ludwig, C. Über die krafte des nervenprimitevenrohres. Wien. Med. Wochscr. 729:129;1861.
80. Marey, E. J. Des excitations électriques du coeur. Travaux du Laboratoire de M. Marey II:63–86; 1876.
81. Marmont, G. Studies on the axon membrane. I. A new method. J. Cell Comp. Physiol. 34:351–382; 1949.
82. Matteucci, C. Deuxième mémoire sur le courant électrique propre de la grénouille et sur celui des animaux à sang chaud. Ann. Chim. Phys. 6 (ser. 3):301–339; 1842.
83. Matteucci, C. Traité des phénomènes électro-physiologiques des animaux suivi d'études anatomiques sur le systhème nerveux et sur l'organe électrique de la torpille par Paul Savi. Paris: Fortin, Masson et C.ie; 1844.
84. Matteucci, C. Sur le courant électrique ou propre de la grénouille. Bibl. Univ. Genève 15:157–168; 1938.
85. Matteucci, C. Expériences rapportées dans un paquet cacheté déposé par M. Dumas, au non de M. Matteucci, et dont l'auteur, présent a la séance, désire aujourd'hui l'ouverture. C. R. Acad. Sci. (Paris) 15: 797–798; 1942.
86. Monro, A. Experiments on the nervous system with opium and metalline substances made chiefly with the view of determining the nature and effects of animal electricity. Edinburgh: Adam Neill and Co; 1793.
87. Moruzzi, G. The electrophysiological work of Carlo Matteucci (translated by M. Piccolino, P. Witkovsky and A. Fiorentini; original paper published in Italian in "Physis" 4:101–140; 1964). Brain Res. Bull. 40:69–91; 1996.
88. Müller, J. Manuel de physiologie. Paris: Baillière; 1845.
89. Nachmansohn, D. Chemical mechanism of nerve activity. Ann. N. Y. Acad. Sci. 47:395–428;1946.
90. Nachmansohn, D.; Neumann, E. Chemical and molecular basis of nerve activity. New York: Academic Press; 1975.
91. Narahashi, T.; Moore, J. W.; Scott, W. R. Tetrodotoxin blockage of sodium conductance increase in lobster giant axons. J. Gen. Physiol. 47:965–974; 1964.
92. Neher, E.; Sakmann, B. Single-channel currents recorded from membranes of denervated frog muscle fibres. Nature 260:799–802; 1976.
93. Nernst, W. Zur kinetik der in lösung befindlichen körper: Theorie der diffusion. Z. Chem. Phys. 613–637; 1888.
94. Nernst, W. Zur Theorie des elektrischen reizes. Pflügers Arch. f. d. ges. Physiol. 122:275–314; 1908.
95. Nobili, L. Comparaison entre les deux galvanometres les plus sensibles, la grenouille et le moltiplicateur a deux aiguilles, suivie de quelques resultats noveaux. Ann. Chim. Phys. 38:225–245; 1828.
96. Pera, M. The ambiguous frog. The Galvani-Volta controversy on animal electricity (translated by J. Mandelboun; original paper published in Italian. Torino: Einaudi; 1986)
97. Schatzmann, A. J. Herzglykoside als Hemmstoffe für den aktive kalium and natriumtransport durch die erythrocytenmembran. Helv. Physiol. Acta 11:101–122; 1953.
98. Shaefer, H. Untersuchungen über den muskelaktionstrom. Pflüger's Arch. f. d. ges. Physiol. 237:329–355; 1936.
99. Sirol, M. Galvani et le galvanisme. Paris: Vigot Frères; 1939.
100. Skou, J. C. The influence of some cations on an adenosine triphosphatase from peripheral nerves. Biochim. Biophys. Acta 23:394–401; 1957.
101. Snyder, C. D. A comparative study of the temperature coefficients of the velocities of various physiological actions. Am. J. Physiol. 22: 309–334; 1908.
102. Snyder, C. D. The temperature coefficient of the velocity of nerve conduction (second communication.). Am. J. Physiol. 22:179–201; 1908.
103. Stanhope, C. L. Principles of electricity. London: P. Elmsy; 1779.
104. Süsskind, C. Observations of electromagnetic wave radiations before Hertz. Isis 55:32–42; 1964.
105. Tasaki, I.; Hagiwara, S. Demonstration of two stable potential states in squid axon under tetraethyiammonium chloride. J. Gen. Physiol. 40:859–885; 1957.
106 Valli, E. Lettera XI sull'elettricità animale. Mantova: G. Braglia; 1794.
107. Volta, A. Le opere di Alessandro Volta (edizione nazionale), I vol. Milano; Hoepli; 1918.
108. Volta, A. Le opere di Alessandro Volta (edizione nazionale), II vol. Milano: Hoepli; 1918.
109. Walsh, J. On the electric property of torpedo: In a letter to Benjamin Franklin. Philos. Trans. R. Soc. (London) 63:478–489; 1773.
110. Whittham, R. Potassium movement and ATP in human red cells. J. Physiol. (Lond.) 140:479–497; 1958.
111. Young, J. Z. Structure of nerve fibres and synapses in some invertebrates. Cold Spring Harbour Symp. Quant. Biol. 4:1–6; 1936.