Luigi Galvani and animal electricity: two centuries after the foundation of electrophysiology

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Luigi Galvani and his famous experiments on frogs carried out in the second half of the 18th century belong more to legend than to the history of science. Galvani not only laid the foundations of a new science, electrophysiology, but also opened the way for the invention of the electric battery, and thus for the development of the physical investigations of electricity. However, in spite of the widespread celebration of his work, Galvani's scientific endeavours have been largely misrepresented in the history of science. The scholar of Bologna has a stereotyped image as an 'occasional' scientist, who started his studies by chance, largely ignored the scientific theories of his time and wandered aimlessly in mental elaborations until the physicist of Pavia, Alessandro Volta, entered the field, correctly interpreted Galvani's results and eventually developed the electric battery. With the present understanding of electrical phenomena in excitable membranes, it is now time to reconsider the real matter raised by Galvani's discoveries and by his hypothesis of an intrinsic 'animal electricity', and to make a clearer evaluation of a revolutionary phase of scientific progress.

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THE STORY IS WELL KNOWN: a frog preparation, consisting of the lower body half with exposed nerves and a metal wire inserted across the vertebral canal, starts contracting vigorously when one of Galvani's collaborators touches its crural nerves with a lancet, and a spark rises from a distant electric machine[1] (Fig. 1). To understand the mechanism of this surprising effect, Galvani first assessed the most appropriate conditions for obtaining contractions with artificial electricity, by using various devices capable of producing or accumulating electricity (electric machines, Leyden jars, Franklin's magic squares). Next, to investigate the effects of natural atmospheric electricity, one stormy evening he connected the frog nerve to a long metallic wire pointing toward the sky, obtaining strong contractions in conjunction with thunder and lightning. Afterwards, to ascertain if quiet atmospheric electricity could also elicit contractions, on a clear day he hung prepared frogs on the iron railing of his house balcony and waited. Since nothing happened for a long time, Galvani manipulated the frogs and, to his surprise, obtained lively contractions by pushing the metallic hooks inserted into the spinal cord towards the iron bars of the railing. The contractions, however, bore no relation to atmospheric events, and could also be obtained indoors, by substituting the balcony railing with an iron plate. It was enough to connect leg nerves and muscles through metallic conductors, thus realizing a circuit 'similar to that which develops in a Leyden jar' (Ref. 1) by connecting the internal and external plate. Contractions failed if an insulating body was used for connection, or if the metallic circuit ('arc') was interrupted by non-conductive material. From such observations, Galvani concluded that intrinsic electricity is present in the animal, and that external conductors induce contractions by allowing for the flow of this internal electricity. This 'animal electricity' is mainly accumulated in muscle: every muscle fibre would correspond to a tiny Leyden jar with nerve fibres penetrating into its interior and making possible a flow of electric fluid toward the exterior, similar to that occurring through the conductor of the Leyden jar.

Volta and the power of metals

After reading Galvani's main work, the famous *Commentarius* published in 1791 (Ref. 1), Volta expressed his admiration for the great discovery of animal electricity[2]. However, with the progress of his own work on this subject, his attitude changed, particularly when he obtained frog contractions by connecting, through a bimetallic arc, two points of the same nerve without any contact with muscle[3]. Contractions thus seemed not to require a flow of current from the inside to the outside of muscle, a finding which clearly contradicted Galvani's conception of muscle fibres as reservoirs of electricity. Afterwards, Volta, becoming aware of the extreme responsiveness of frogs to external electric influences, conceived the possibility that the contractions, ascribed by Galvani to animal electricity, could derive instead from a small amount of external electricity inadvertently produced by experimental manipulations. Having noticed the particular efficacy of arcs made by different metals (Ref. 4, p. 39), Volta assumed that electricity could derive from the contact between dissimilar metals, and that frogs react to this electricity as they react to other forms of external electricity. To verify this assumption he explored the effects of bimetallic arcs on his tongue, and correctly interpreted the acid taste

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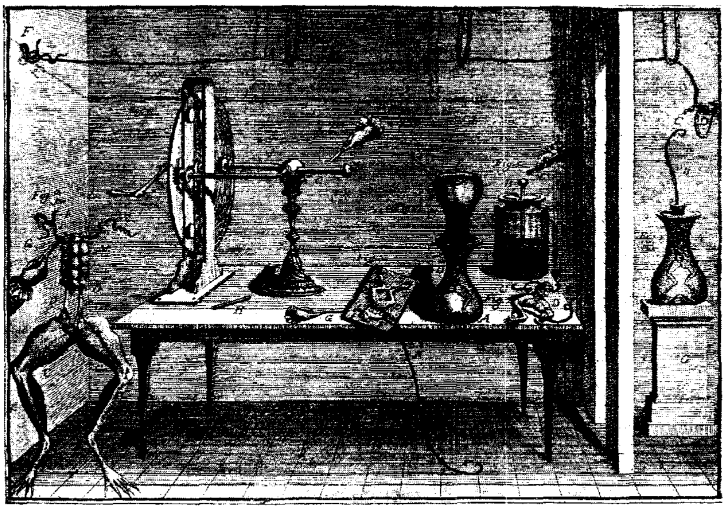

Fig. 1. ***Plate I of the* Commentarius.** *The prepared frog and the electric machine on the left allude to the spark experiment.*

perceived as caused by stimulation of gustatory nerves[5]. These findings provided additional support for the view that muscles need not be included in the circuit of current flow in order to activate nervous conduction. Volta proposed that dissimilar metals may act as 'motors' of electricity, producing and maintaining artificial disequilibrium, and, afterwards, he elaborated his 'contact theory', ranking metals by their tendency to generate electricity when put into reciprocal contact.

Galvani–Volta controversy and the critical experiments

Against Volta's objections, in 1794 Galvani remarked that contractions could be elicited by using a monometallic arc, and by connecting nerve and muscle through a cut piece of tissue, and even simply

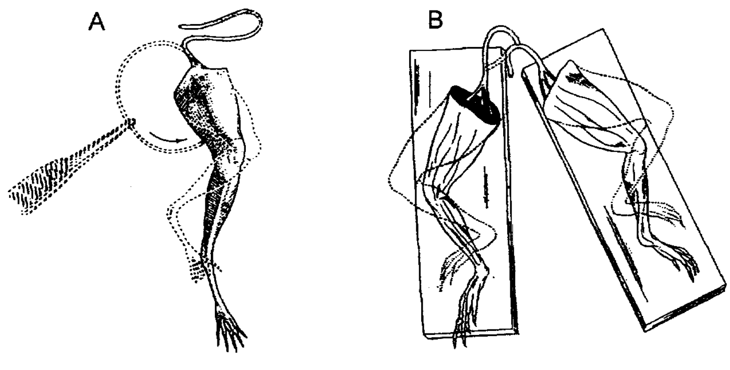

Fig. 2. ***Galvani experiments of contractions without metals.*** **(A)** *The 1794 experiment: when the surface of sections of the nerve touches the muscle the leg contracts.* **(B)** *The 1797 experiment: when the surface of a section of the right sciatic nerve touches the intact surface of the left sciatic nerve, both legs contract. Galvani's interpretation of these experiments was correct as to the main issue raised (the existence of animal electricity), but not for the mechanism of excitation proposed. The contact did not simply allow for passive electrical conduction, but resulted in a local stimulation caused by the potential difference existing between injured and intact tissue surface. The necessity of contact between injured and intact tissues for the experimental manifestation of animal electricity emerged only with Carlo Matteucci's studies (1838–1844) and, moreover, only following Bernstein's membrane hypothesis (1902) was it clear that this is because of the low resistance path to the intracellular medium created by lesion.* Figure derived from Ref. 24.

by putting the tip of the sectioned crural nerve in direct contact with the muscle surface[6] (Fig. 2A). Volta refused, however, to see in these experiments the definitive proof of the existence of animal electricity. After arguing that a mechanical (or chemical) irritation could underlie nerve stimulation, he proposed that, besides dissimilar metals, humid bodies of different species or composition can also generate an electrical force at contact[7].

To this new objection, Galvani replied in 1797 by producing contractions without any heterogeneous contact[8] in an experiment considered 'the most capital experiment of electrophysiology', the foundation of this new science[9] (see Fig. 2B). However, even this experiment, which implies an exclusive contact between nervous tissues, did not succeed in earning final acceptance for the animal electricity theory, and it passed by, practically unnoticed by the scientific community.

At the moment that Galvani made his 'decisive' experiment, Volta was providing similarly 'decisive' evidence to support the power of metal in generating artificial electricity. In 1796 he could measure with physical instruments the tiny quantity of electricity generated upon contact between silver and zinc[10], and, afterwards, he succeeded in generating a substantial quantity of electrical force by interposing, between alternating couples of dissimilar metals, disks of paper 'moistened with water or better with salt solution'. This was the invention of the electrical battery, communicated to the Royal Society of London on 20 March, 1800 (Ref. 11). The immense success of this invention, and the increased scientific authority it gave to Volta, explains why animal electricity studies were confined, henceforth, to Galvani's few followers, and were, afterwards, practically abandoned for about three decades. Galvani had died on 4 December 1798, in poor conditions, after being stripped of his professorship because of his refusal to accept the principles of the new political system.

In his initial communication, Volta referred to the electrical battery as an 'artificial electrical organ', the physical equivalent of the electrical organ of the fish. The fish organ, with its stack-like arrangement of modular elements, was the mental image that guided him in assembling his battery and in interposing humid disks between metallic elements. However, Volta concluded his communication by noting that electricity was produced by the fish organ according to the same physical principles of the battery, that is by alternation of different conductors acting as motors of electricity, and thus it could not be considered genuinely animal. He refuted Galvani's hypothesis of the involvement of animal electricity in frog contraction with stronger vigour. Either the metal-derived electricity acted as a pure stimulus activating some internal, non-electric force; or the external electric force moved an electric fluid inside the organism which had nothing especially animal, and was in no way related to the living state. According to this last view, which finally prevailed, the electricity of animal body was simply the 'common' type of electricity, present in animal tissues as in any humid conductor.

Galvani's prophetic insights

After two centuries of electrophysiological studies, we know that Volta was wrong in almost all his conclusions about animal electricity. Genuine animal electricity is present in all living beings, and is involved in fundamental processes such as muscle contraction and nerve conduction. As Galvani supposed, animal electricity exists in a state of 'disequilibrium', and it is ready to move in response to internal stimuli or following external influences. According to Galvani '...in the animal there is a particular machine capable of generating such disequilibrium, and it will be convenient to refer to this form of electricity as to animal electricity to denote, not a type of electricity whatsoever, but a particular one referred to a particular machine' (Ref. 12). Now we know that this machine, which once appeared 'totally occult to the most acute sight', corresponds to the cell membrane with its complex organization of ionic pumps and ion channels. These create dissimilar Na^+ and K^+ concentrations, and convert concentration gradients into an electric potential difference between the intracellular and extracellular medium. Well before the advent of cell theory, Galvani assumed that electricity in its duplex forms, that is, positive and negative, is accumulated in individual muscle fibres because, as he said, any fibre probably bears two opposite surfaces, internal and external, corresponding to the internal and external plate of the Leyden jar[1]. Galvani somewhat anticipated by more than one century Bernstein's membrane hypothesis of bioelectric potentials. Moreover, to account for the electrical flow from the interior to the exterior of nerves involved in the experimental contractions of his frogs, Galvani conceived structures similar to ionic channels. By referring to the metallic conductor, which, in his Leyden jar model, represented the equivalent of 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 that concerns the conductor. Then let one moist with water or with some other conductive fluid all the plastering, having care that the fluid penetrate in the above mentioned holes, and come 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' (Ref. 12).

The electric nature of nervous impulse

The difficulties underlying the controversy between Galvani and Volta largely depended on the peculiar nature of the involvement of electricity in muscle and nerve physiology, difficult to account for within the narrow limits of 18th-century physical science. As we now know, although signalling in nerve and muscle is a genuine electric process, it differs profoundly from the simple 'passive' conduction along electric cables. The involvement of electricity in this process is twofold: the organism is endowed with an electrical disequilibrium providing the energy for charge movement; however, charges do not move unless the intrinsic electric force is released by a distinct electric influence, which is not itself the effective cause of charge flow. Historically, the complex nature of the electric signalling mechanism in nerve emerged clearly when Hodgkin, Huxley and Katz depolarized the membrane of the squid giant axon with their voltage clamp apparatus and recorded an inward going current in the initial phase of the response[13]. This current was totally inexplicable as a pure charge movement under the influence of the applied electric field, because a positive going change of internal potential should have induced an outward going current. The current was in fact due to a movement of ions (mainly sodium ions) under the effect of a pre-existing energy gradient originating from the cell metabolic activity (and thus fully 'animal'), and it was activated by the extrinsic stimulus because it produced a specific change of membrane permeability capable of releasing the energy of the gradient.

Volta excluded the involvement of intrinsic electrical forces in the metal-induced contractions based on his contention that, if metals generated an electrical force, there was no need to assume another, internal, force to account for electrical flow in nerve. Although apparently inspired to the correct scientific reasoning, Volta's conclusion was misleading in the circumstances of frog experiments, because metallic electricity was indeed only 'gating' the process responsible for the release of the internal electric force, and not itself causing the main current flow in nerve and muscle.

The reasons for the complexity of the mechanisms involved in nerve signalling are to be found in the enormous physical difficulties encountered by evolution in developing effective electric signalling in small diameter fibres of considerable length using poorly conductive materials. More recently, these difficulties have been vividly depicted by Hodgkin, who showed that a long and thin nerve fibre may have a resistance comparable to that of an ordinary electric cable extending several times the distance between the earth and Saturn[14]. To overcome the attenuation inherent in passive electric conduction, the electric nerve signal needs to be regenerated during its propagation, and an electric energy must be present locally along the entire extension of the nerve fibre ready to be discharged. Nerve signal propagation resembles the progress of ignition along a gun-powder fuse, as well shown by Lucas and Adrian at the beginning of this century[15].

The difference between ordinary electric conduction and 'active' propagation along nerve fibres has represented the greatest conceptual difficulty in understanding the mechanism of electric signalling in excitable cells. This difficulty was already implied in a famous objection raised in Galvani's time by Felice Fontana and others: tightly ligating a nerve abolished nervous conduction, thus preventing movements or sensations, but did not abolish electric current flow along the nerve[16]. Moreover, the speed of conduction in frog motor nerve, measured in 1850 by von Helmholtz (less than 30 metres per second), seemed too slow for electric propagation. Although, in 1868, Bernstein showed that nerve electric response propagated with a similar low speed, for a long time it seemed probable that electric events were only epiphenomena in nervous signalling, and a chemical hypothesis long challenged the electric theory.

Galvani, the spark and the doctrine of irritability

How did Galvani arrive at the discovery of animal electricity and his far-reaching hypothesis? The answer may come from careful consideration of the spark experiment. In spite of the apparent casualness of the observation, Galvani (Fig. 3) had already been interested in the study of the physiological effects of electricity, since his frogs were clearly purposely prepared for investigating electrical phenomena in living organisms. According to Galvani[17] 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, to avoid possible complications arising from the influence of 'will' and 'soul', reduced preparations from recently killed animals should be used in preference to intact living animals.

Fig. 3A. *Contemporary portrait of Luigi Galvani.*

Galvani's excitement at seeing the frog contracting in the spark experiment has been interpreted as evidence that he 'ignored the correct theory of electrical influence' (Ref. 4), proposed some years before to explain the 'return stroke' phenomenon[18]: a current may pass across a charged body when another one, situated at some distance, is suddenly discharged. It has been said, moreover, that were Galvani sufficiently acquainted with the physics of electricity, he would not have been surprised by the phenomenon[19]. This type of criticism overlooks an important aspect of Galvani's observations in the study of the phenomenon. Contractions were more effectively excited by a distant spark, than when the frog was directly connected to the charged machine[17], although this last situation was clearly more favourable to the transfer of 'electrical fluid' to the frog nerve. Thus, there was some particular property in the way electrical influence acted on the frog making the spark particularly effective in eliciting contraction. To calculate how external electricity could induce frog contractions, Galvani frequently used words evoking a sudden impulsive action, such as *impulso* (impulse), *impeto* (impetus) and *urto* (push)[17]. Moreover, he noticed that increasing the electric discharge strength beyond a certain level did not result in stronger contractions, while progressively reducing it might suddenly result in a complete disappearance of the contractions, and, furthermore, he remarked that, if contractions disappeared after repeated stimulations, they could be reobtained if the preparation was left unstimulated for a while[17,20].

All these findings roused Galvani's suspicion that contractions did not result directly from the extrinsic electrical influence, but were caused by some internal force, proper to the animal, set in motion by the external electrical agent. Indeed, von Haller's doctrine of 'irritability' was well known to Galvani[21]: it was a fundamental conceptual elaboration of the 18th-century physiology, which stipulated that the way an organism reacts to external influences is an expression of its internal organization, and independent of the specific nature of external influence. In the framework of this doctrine, which largely circulated in Italy and had a particularly strong echo in Bologna, external electricity appeared to Galvani to be a stimulator, an 'exciting cause' of the contraction rather than the direct 'efficient' cause of the phenomenon. Galvani's suspicion was supported by other experiments in which he established how small was the least quantity of electricity capable of stimulating the contraction. At one stage, Galvani summarized his views 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' (Ref. 20). In pursuing his studies further, Galvani eventually came to the conclusion that this mobile principle is itself electrical, a specific form of electrical force generated as a consequence of life processes. The conceptual transition to the new hypothesis probably occurred with the first experiments with metals, because these experiments evidently involved an agent possessing all the characteristics of electric fluid, and since, moreover, nothing in the apparatus used seemed capable of generating or accumulating external electricity. The importance of these experiments in Galvani's elaboration of the animal electricity hypothesis explains why he could not afterwards accept Volta's view that electricity might derive from metal contacts, although, well before the physicist of Pavia, he became aware of the particular efficacy of dissimilar metals in inducing contractions[17,20]. There were, however, important difficulties in assuming the existence of an intrinsic electrical force inside the organism, because of the conductive properties of animal tissues, which would likely dissipate any electric disequilibrium. The physical device capable of maintaining an electrical imbalance for a long time, the Leyden jar, implied an insulating material separating two conductors. Although in Galvani's time there was no clear evidence for the cell structure of animal tissues, nevertheless it seemed plausible for Galvani to assign some special characteristic (that is, an insulating property) to the ill-defined structure enclosing the substance of muscle fibres. The next step was to find out how a functional communication could be established between the interior and the exterior of muscle fibre in spite of the physical separation between the two compartments. The evidence that nerves penetrate deeply and diffusely into the muscle tissue led Galvani to assign this role to nerve fibres, which he supposed entered every muscle fibre. The electric mechanisms underlying muscle contraction and nervous conduction were thus tightly related, although the role of passive conductors of muscle electricity assigned to nerves is clearly a weak point of Galvani's elaboration. On that point a further difficulty arose when Volta elicited contractions by connecting two points of a nerve without any muscle contact. Galvani tried to undermine the relevance of this observation by assuming the existence of an 'occult arc' through which the current could reach muscle from the nerve points stimulated[20].

As already discussed, in the discovery of animal electricity, an important interpretative notion, was the concept of 'irritability'. Irritability may sound ancient now, and reminiscent of time-worn vitalistic creeds; however, it was an insightful notion that penetrated into one of the most characteristic aspects of functional organization of living organisms. The reaction of the organism to external (or internal) influence largely depends on its internal organization and is not a simple 'physical' result of the influence acting on it. Implicit in the notion of irritability is the idea that the organism is prepared to react in some specific way and that the energy for the reaction is accumulated in its inside, and is not directly related to the external energy. The discrepancy between the stimulus and response energy was vividly depicted by Fontana in the 'spark-gunpowder' analogy, which was probably known to Galvani when he 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' (Ref. 21).

Now we can appreciate this discrepancy between external stimulus and physiological response in quantitative terms, by noting, for instance, that the energy of the single photon response in a retinal rod is about one hundred thousand times larger than that of the photon itself. The energy of the response evidently depends on an internal energy, generated by metabolic processes and thus 'vital'. We now know in detail the complex chain of events which, following photon absorption, release the energy accumulated across the photoreceptor membrane leading to the potential change which initiates vision[23]. The global process set in motion by photon absorption is the specific form by which the photoreceptor expresses what we could call its 'irritability'.

Fig. 3B. ***Contemporary portrait of Lucia Galeazzi, the wife of Galvani.*** *Lucia Galeazzi assisted Galvani in his experiments, some of which were carried out in the house of her father, Domenico Gusmano Galeazzi, a Professor at the University of Bologna and member of the scientific élite.* Photographs by F. Petrucci, courtesy of the Ferretti family.

It is perhaps not straying too far from the truth to say that the notion of irritability has anticipated subsequent developments in abiological sciences, too, and, in particular, the development of automatisms in modern machines; and also, in some way, the structural logic block-design and some computational paradigms in computer science and engineering. The idea that different components of a complex system may interact through relations based on the exchange of control commands (and thus information) rather than of energies precedes important conceptual developments in modern science.

Although physical sciences were of great importance in promoting the general progress of science, particularly in the 19th century, scientific thinking nevertheless, extends well beyond the limits of physical schemes. The history of science shows that some fundamental notions on the organization and functioning of living bodies did not derive from the application of the laws of physics to biology. The theory of evolution, the laws of genetics and immunology, the principles of enzymatic action, the notions of homeostasis and regulation are eminently biological conceptions, primarily derived from the study of living organisms, even though all the biological processes involved therein ultimately obey physical laws. Natural and biological sciences, and particularly physiology, have provided important conceptual schemes useful for the general progress of science. For instance the concept of feedback control emerged first in biology and was later usefully incorporated into physical and technological sciences.

The impact of a biological scheme on physical discoveries is illustrated by the invention of the electric battery. In constructing this epoch-making device, Volta created an apparatus which, in the functional arrangement of its components, was clearly more related to complex machines of 'organic' type, derived from the realm of biology (the electric fish organ), rather than to the physical devices of his time. On his side, Galvani, through the notion of irritability, could see deep in the organization of the animal machine and thus succeeded, as he said, in '...somewhat touching with hands and extracting from nerves the electricity concealed in them and, in some way, in putting it under everyone's eyes'. If, as Dostoevsky says, all stories of 19th-century Russian literature derived from the cloak of Gogol's, then perhaps we could say that much of the electrophysiology of these last two centuries sprang from the frogs of Galvani.

Selected references

1 **Galvani, L.** (1791) *Bon. Sci. Art. Inst. Acad. Comm.* 7, 363–418 (English Transl. by M. Glover Foley, 1953, Burndy)
2 **Volta, A.** (1792) in *Le opere di Alessandro Volta-Edizione Nazionale* (Vol. I), pp. 16–25, Hoepli (1918)
3 **Volta, A.** (1792) in *Le opere di Alessandro Volta-Edizione Nazionale* (Vol. I), pp. 43–75

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4 **Volta, A.** (1792) in *Le opere di Alessandro Volta-Edizione Nazionale* (Vol. I), pp. 36–40
5 **Volta, A.** (1792) in *Le opere di Alessandro Volta-Edizione Nazionale* (Vol. I), pp. 151–159
6 **Galvani, L.** (1794) *Supplemento al trattato dell'arco conduttore*, S. Tommaso d'Aquino
7 **Volta, A.** (1795) in *Le opere di Alessandro Volta-Edizione Nazionale* (Vol. I), pp. 290–301
8 **Galvani, L.** (1797) *Memorie sulla elettricità animale a L. Spallanzani*, Sassi
9 **du Bois-Reymond, E.** (1849–1884) *Untersuchungen über thierische Elektricität*, Reimer
10 **Volta, A.** (1796) in *Le opere di Alessandro Volta-Edizione Nazionale* (Vol. I), pp. 418–431
11 **Volta, A.** (1800) *Philos. Trans. R. Soc. London* 90, 403–431
12 **Galvani, L.** (1794) *Dell'uso e dell'attività dell'arco conduttore*, S. Tommaso d'Aquino
13 **Hodgkin, A.L., Huxley, A.F. and Katz, B.** (1952) *J. Physiol.* 116, 424–448
14 **Hodgkin, A.L.** (1964) *The Conduction of the Nervous Impulse*, Liverpool University Press
15 **Lucas, K.** (1917) *The Conduction of the Nervous Impulse*, Longman Green
16 **Fontana, F.** (1792) *Giornale Fisico-Medico* 4, 116–118
17 **Galvani, L.** (1786) in *Memorie ed esperimenti inediti*, Cappelli (1937)
18 **Stanhope, C.** (1779) *Principles of Electricity, Containing Divers New Theorems and Experiments*, Elmsy
19 **Arago, J-F-D.** (1854) in *Oeuvre complètes* (Vol. I), pp. 187–240, Gide et Baudry
20 **Galvani, L.** (1841) *Opere edite ed inedite* (Gherardi, S., ed.), Dall'Olmo
21 **von Haller, A.** (1755) *Dissertation sur les parties sensibles et irritables des animaux*, Tissot
22 **Fontana, F.** (1760) *Mémoires sur les parties sensibles et irritables du corps animal* (Vol. 3) (von Haller, A., ed.), pp. 157–243, D'Arnay
23 **McNaughton, P.A.** (1990) *Physiol. Rev.* 70, 847–884
24 **Sirol, M.** (1939) *Galvani et le galvanisme*, Vigot Frères

LETTERS TO THE EDITOR

More on calcium currents

This letter is a rebuttal of comments from Garcia and Carbone[1], ostensibly in response to a review by Dolphin[2], casting aspersions on the veracity of data published in a series of six papers authored by myself[3–8]. The argument raised in the letter[1], that prepulse facilitation in bovine chromaffin cells is due solely to relief of a tonic G-protein inhibition of non-L type Ca^{2+} currents rather than a phosphorylation-dependent activation of an L-type channel, does not apply to the cells used in my studies. Prepulse facilitation in my experiments was blocked entirely by nisoldipine and was due to the activation of a 27 pS Ca^{2+} channel as exhaustively documented by single-channel recordings[5]. As previously reported[9] the inclusion of GDPβS (which should block any tonically active G proteins) or GTPγS (which should fully activate G proteins) in the patch pipette has no effect on the recruitment of facilitation L-channels by prepulses or cAMP in bovine calf chromaffin cells. This experiment directly demonstrates that facilitation cannot be due to the removal of a tonic G-protein inhibition of this channel. This result sharply contrasts with other systems where GDPβS blocks prepulse facilitation as it inactivates the G-proteins that are inhibiting Ca^{2+} channels and where GTPγS increases prepulse facilitation when this is mediated by G-proteins as it fully activates heterotrimeric G proteins that are responsible for the effect[2]. This distinction was quite clearly drawn in Dolphin's review[2].

The probable explanation for the discrepancy between the results on bovine chromaffin cells published by Carbone, Garcia and co-workers[1] and ours is a very simple one: the expression of the facilitation L-channel is strongly dependent on the age of the animals from which chromaffin cells are prepared. We have recently performed a direct comparison of cells from calves and adult cows showing that the facilitation L-channel is present in the former and virtually absent in the latter[10]. All the studies in my published work[3–8] are from cells derived from bovine calves of ages 10–12 weeks while many other studies, including those conducted in the laboratory of Garcia, are performed on tissue obtained from adult cows of indeterminate age, but generally at least 2–3 years old. Others have reported that the level of the facilitation L-current is very low in cells taken from adult animals[11]. As it is impossible to obtain significant current from the facilitation L-channel in cells from adult animals it is very likely that any effects of prepulses on Ca^{2+} currents are mediated by channels other than this one. Indeed, the explanation for the results of Doupnik and Pun[12], also presumably on cells from adult animals, is almost certainly voltage-dependent relief of a tonic inhibition of some non-L-type Ca^{2+} channel brought about by substances released from the cells, as suggested by Garcia and Carbone[1]. However, it should be emphasized that all the experiments reported in Refs 3–8 were done under conditions where rapid flow rates were maintained in the bath; in this case the effects of any substances released from chromaffin cells during secretion would be negligible due to their rapid removal from the bath, a question raised by Dolphin in her reply to Garcia and Carbone[13]. It is not that surprising that cells derived from animals of different ages should have a different complement of channels: developmental regulation of Ca^{2+} channels has been shown in a number of systems (see, for example, Ref. 14). Moreover, other differences between chromaffin cells derived from young and old bovines have previously been described[15].

Prepulse facilitation of L-type Ca^{2+} channels is being recognized as a very widespread phenomenon with new examples appearing on a regular basis. Recently, a Ca^{2+} current with very similar properties to that described by us in calf chromaffin cells was described in rat chromaffin cells[16]. As detailed by Dolphin in her reply[13] to the letter of Garcia and Carbone, the example of facilitation in skeletal muscle is particularly pertinent as the cloned α1c L-type Ca^{2+} channel subunit has been shown to undergo voltage-dependent facilitation in a heterologous system. Whether channels that behave in such a manner will turn out to be related at the molecular level remains to be determined. The burgeoning literature in this field persuasively argues that voltage-dependent but G-protein independent facilitation of L-type channels is a reality, not an artefact as suggested by Garcia and Carbone[1].

Our hypothesis that the facilitation L-channel is critical to the massive catecholamine release seen in the 'fight-or-flight' response needs testing at the level of whole perfused glands and this has not yet been attempted. It is significant, however, that such studies that have been done on cholinergically-stimulated intact glands (for example, adult bovine and cat) reveal a dominant role for L-type Ca^{2+} channels in secretion[17,18]. It seems reasonable, therefore, to expect events that regulate channel activity to be focussed on such channels rather than on other channel types that contribute little to secretion. Indeed, one study showed that preincubation of perfused bovine adrenal glands under depolarizing conditions resulted in a facilitation of catecholamine release that was