

## Hysteresis in voltage-gated channels

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### ABSTRACT

Ion channels constitute a superfamily of membrane proteins found in all living creatures. Their activity allows fast translocation of ions across the plasma membrane down the ion's transmembrane electrochemical gradient, resulting in a difference in electrical potential across the plasma membrane, known as the membrane potential. A group within this superfamily, namely voltage-gated channels, displays activity that is sensitive to the membrane potential. The activity of voltage-gated channels is controlled by the membrane potential, while the membrane potential is changed by these channels' activity. This interplay produces variations in the membrane potential that have evolved into electrical signals in many organisms. These signals are essential for numerous biological processes, including neuronal activity, insulin release, muscle contraction, fertilization and many others. In recent years, the activity of the voltage-gated channels has been observed not to follow a simple relationship with the membrane potential. Instead, it has been shown that the activity of voltage-gated channel displays *hysteresis*. In fact, a growing number of evidence have demonstrated that the voltage dependence of channel activity is dynamically modulated by activity itself. In spite of the great impact that this property can have on electrical signaling, hysteresis in voltage-gated channels is often overlooked. Addressing this issue, this review provides examples of voltage-gated ion channels displaying hysteretic behavior. Further, this review will discuss how *Dynamic Voltage Dependence* in voltage-gated channels can have a physiological role in electrical signaling. Furthermore, this review will elaborate on the current thoughts on the mechanism underlying hysteresis in voltage-gated channels.

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### Introduction

The discovery of “Animal Electricity” (*L'elettricità animale*) by Luigi Galvani in the 18<sup>th</sup> century is perhaps the earliest account of electrical activity in living beings. Today, the notion of bioelectrical phenomenon has been well established and it has been observed not only in animals, but in all life forms. Cellular electrical activity emerges from 2 basic properties of the plasma membrane: 1) *capacitance* (i.e.: the hydrophobic, non-conductive region of the membrane functions like a capacitor as it is placed between to conductive media), and 2) *resistance* (i.e.: specific membrane proteins allow the translocation of ions, making the plasma membrane to behave like a conductor). This latter property is commonly referred to as *conductance*, which is the inverse of the resistance.

The pioneering work of Kenneth Cole and Howard Curtis, in the 1930s, showed that there is a robust change in membrane conductance during action potentials in the squid giant axon.<sup>1</sup> Later, in 1952, a series of papers by Alan L. Hodgkin and Andrew F. Huxley demonstrated that voltage-dependent changes in the conductance of the plasma membrane for Na<sup>+</sup> and K<sup>+</sup> were responsible for action potentials in the squid giant axon.<sup>2-6</sup> Today, the molecular basis for these changes in conductance are well known, as *ion channels* have been identified as the proteins that form the main selective routes for ion conduction in many kinds of electrical events.<sup>7-9</sup> The activity of ion channels, combined with those from cations ATPase and transporters, generates a difference in electrical

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potential known as *membrane potential* which is not only a sign of life, but also a source of energy that drives electrical signaling and many other biological processes.

Ion channels allow the highest rate of translocation of ions across the plasma membrane than any other membrane protein.<sup>9</sup> For this reason, the activity of ion channels is critical for the maintenance and modulation of the membrane potential. In turn, the activity of a particular group of ion channels, known as *voltage-gated channels*, is controlled by the membrane potential. This interdependence between activity and voltage-dependence results in electrical signals in many living creatures.<sup>9</sup> In general, voltage-gated channels are responsible for cellular “fast” electrical signaling (e.g.: neuronal action potentials<sup>10</sup>).

The activity of voltage-gated channels is prone to hysteresis. Their response to changes in membrane potential depends on how the membrane potential is changing. An increasing number of reports suggests that hysteresis in voltage-gated channels’ activity can have important repercussions in cellular electrical signaling. Yet, this property is commonly overlooked. In addressing this issue, this review aims at providing an overview of the current understanding of hysteresis in voltage gated channels. In doing so, first, hysteresis will be defined in terms of voltage-gated channel activity. Second, this review will provide examples of ion channels displaying hysteretic behavior. Third, it will discuss the potential

physiological role of hysteresis. And fourth, this review will provide a perspective on the putative mechanism for hysteresis in voltage-gated channels and its thermodynamics.

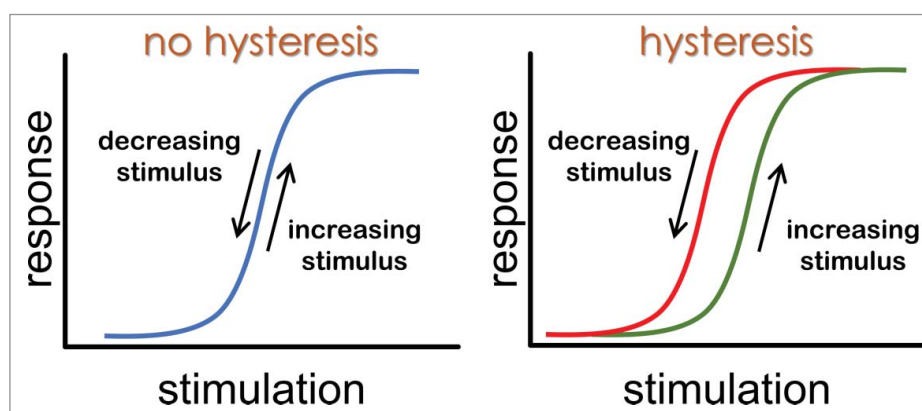
### What is hysteresis?

Hysteresis is a phenomenon observed in many physical and chemical systems in which the response of the system to stimulation depends on whether the intensity of the stimulus increases or decreases (Fig. 1, right). This notion often contrasts the common conception of any protein activity as a function of stimulation, which is typically expected to exhibit a “fixed” response-vs-stimulus relationship (Fig. 1, left).

A common consequence of hysteresis is the acquisition of “memory”; this emerges from the dependence of the system’s response to how the stimulus varies. As such, it could be said that voltage-gated channels can “remember their recent past,” altering their responsiveness (voltage-dependence) to changes in membrane potential. It can be argued, therefore, that *Dynamic Voltage Dependence* (DVD) in voltage-gated channels can have a strong impact on electrical signaling.<sup>11</sup>

### Hysteresis in electrical signaling: The case of the action potential

Action potentials are a fundamental unit of electrical communication in excitable tissues. They are also a good example of hysteresis in biological systems. During a prototypic action potential, the membrane



**Figure 1.** Comparison of the relationship between the activity and the magnitude of stimulation for a non-hysteretic and a hysteretic system. A) The response of a non-hysteretic system to stimulation is a “fixed” function of the stimulation, so no changes in the responses-stimulus relationship are observed except as the magnitude of a stimulus varies. B) In contrast, the activity of hysteretic systems changes as a function of the magnitude of the stimulus applied, but also depends on whether the magnitude of the stimulus is decreasing or increasing.

potential changes from a typical negative resting membrane potential (e.g.,  $\sim -60$  mV) toward 0 mV (depolarization) or to positive voltages (antipolarization). This change in membrane potential (the stimulus) drives the activation (the response) of both  $\text{Na}^+$ -selective voltage-gated ( $\text{Na}_V$ ) channels and  $\text{K}^+$ -selective voltage-gated ( $\text{K}_V$ ) channels; the action of  $\text{Na}_V$  channels further drives this process. Following activation,  $\text{Na}_V$  channels inactivate, while  $\text{K}_V$  channels remain active. This drives the membrane potential back to negative voltages (repolarization). As it can be seen, the 2 phases of an action potential follow different sequences of events (pathways). This is analogous to the situation depicted in Fig. 1 (right). As the stimulus increases, the responses involves currents through both  $\text{Na}_V$  and  $\text{K}_V$  channels. Then, when the stimulus decreases, only the  $\text{K}^+$  conductance is relevant, as  $\text{Na}_V$  channels are inactivated. In addition, the  $\text{Na}_V$  “remember” that they were activated, with inactivation acting as a “reminder.” Of course, they will soon “forget” the action potential because the membrane potential is set back to its initial negative (resting) potential, resetting the entire system.

In the case of an action potential, hysteresis emerges from combining the activity of 2 intrinsically independent proteins. This review, however, will focus instead on hysteresis in the activity of individual channels, or populations of channels to be precise.

### **Hysteresis in voltage-gated channels**

One of the earliest examples of hysteresis in voltage-gated channels was reported in 1982 from studies on *gating currents* in the squid giant axon.<sup>12</sup> Gating currents are transient, non-linear capacitive currents that derive from the movement of charges that are intrinsic of voltage-gated channels.<sup>13,14</sup> Charge movement drives conformational changes that controls activity, thus conferring voltage-sensitivity to voltage-gated channels.<sup>13,15</sup>

The relationship between the net mobilized gating charge ( $Q$ ) and the membrane potential ( $V$ ) that is associated with the activation of the voltage-dependent  $\text{Na}^+$  conductance in the squid giant axon shifts toward more negative voltages when the membrane is held at 0 mV, instead of  $-70$  mV.<sup>12</sup> These observations indicated that the voltage dependence of channels can vary with activity. It was suggested then that 2 processes were involved in the shift of the voltage

dependence: “one that develops within 50 ms and corresponds to the fast inactivation or immobilization of the gating charge, and a second process that develops within several seconds, which we will call slow inactivation of the gating charge.”<sup>12</sup>

Similar observations were made, a few years later, in studies on charge movement associated with excitation-contraction coupling in frog skeletal muscles. Like in the squid axon, charge *versus* membrane potential ( $Q$ - $V$ ) curves shifted over  $-80$  mV when the holding potential (H.P.) was set at 0 mV, instead of  $-100$  mV.<sup>16</sup> Later studies showed that gating currents from L-type  $\text{Ca}^{2+}$  voltage gated ( $\text{Ca}_V$ ) channels were the main contributor to charge movement in skeletal fiber<sup>17,18</sup> and that  $Q$ - $V$  curves from L-type  $\text{Ca}_V$  channels shifts over  $-60$  mV at depolarized H.P. (0 mV) with respect  $Q$ - $V$  curves at hyperpolarized H.P. ( $-110$  mV).<sup>19</sup>

$\text{K}_V$  channels are another example of voltage-gated channels with hysteretic behavior. During activation, the “human Ether-à-go-go-Related Gene” (hERG) channel ( $\text{K}_V11.1$ ) rapidly inactivates. Then, upon repolarization, hERG channels quickly reopen during deactivation,<sup>20-22</sup> with a deactivation voltage-dependence that is displaced over  $-60$  mV with respect to the activation voltage-dependence.<sup>23-28</sup> It was suggested that, like for  $\text{Na}_V$  channels, inactivation was likely responsible for the shift in voltage dependence.<sup>23,25</sup> This could be seen as a shift in voltage-dependence due to inactivation imposing a physical restriction to deactivation. Thus, because the membrane potential is a source of energy, such restriction could be overcome by applying a more negative potential.

To understand the previous idea, let us consider the following:  $\text{K}_V$  channels are formed by 6 trans-membrane segments, where the first 4 (S1-S4) segments constitute a voltage sensing domain (VSD) and the other 2 (S5-S6) segments constitute the pore forming domain. The fourth (S4) segment of the VSD typically bears positively charged residues functioning as the main voltage-sensing charges. The VSD produces non-linear capacitive currents known as *gating currents*<sup>13,15</sup> (or *sensing currents* in a more general sense) which are observed as these charges can be displaced when changing the membrane potential.<sup>29-34</sup> This movement is translated into conformational rearrangements that control the activity of the channel.<sup>13</sup> Back to the case of hERG, more recent studies suggest

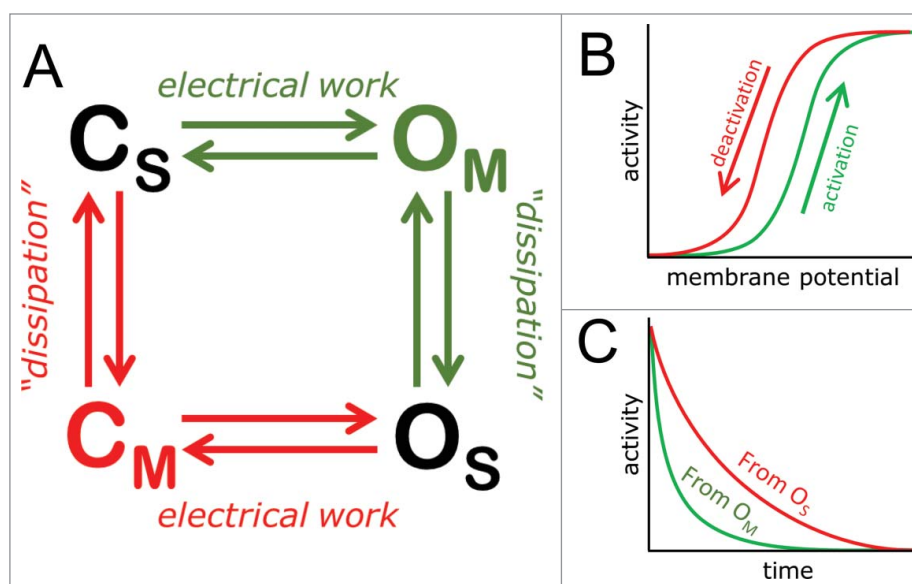
that alteration of the inactivation by pharmacological or genetic means do not abolish changes in gating currents' voltage dependence.<sup>26,28,35</sup> Therefore, inactivation may have a minor role in hysteresis of hERG. Instead, recent studies suggested that intrinsic changes in the voltage dependence of the voltage sensing machinery of hERG can account the shift in overall voltage dependence of the conductance.<sup>26,28</sup>

Another example of a voltage-gated channel displaying hysteresis is the Hyperpolarization-activated, cyclic-nucleotide-gated (HCN) ion channels. HCN channels are essential for the regulation of pace-making activity in the heart and brain. In contrast to their voltage-gated channel counterparts, HCN channels open their conductance as the membrane potentials becomes more negative, producing the so-called "funny current" or H-currents ( $I_H$ ).<sup>36-38</sup> Experimental and computer simulated data have shown that the stability of electrical rhythmic activity are critically dependent on the hysteretic activity of HCN.<sup>39-42</sup> In short, at depolarized potentials, the activation of HCN occurs at more negative potentials, so the channels tend to remain closed. In contrast, at more negative voltages, their voltage-dependence shifts to more positive values, facilitating the activation of these channels.<sup>39-43</sup> This shifting in mode of the channel's

operation is critical for their role in generation rhythmic patterns of electrical activity as it "tunes" channels to respond when "they are needed."

The term *mode shift* was coined from studies in HCN.<sup>39</sup> Mode shift is equivalent to the concept of *modal shift* developed from studies on the desensitization of Nicotinic Acetylcholine Receptors<sup>44</sup> and to *modal gating* developed to describe the activity of  $Ca_V$  channels<sup>45</sup> and from studies on the adaptation of Cardiac Ryanodine Receptors (RyR2) to describe its  $Ca^{2+}$ -dependent activation.<sup>46-49</sup> In this latter case, fast increase of  $Ca^{2+}$  (from  $\sim 50$  nM to 1-10  $\mu$ M) drives RyR2 into a high open probability ( $HP_O$ ) mode. Then, the RyR2 activity decays as a switch into a low open probability ( $LP_O$ ) mode takes place within a few seconds.<sup>49,50</sup> Although the decay in RyR2 activity resembles slow inactivation, further increases in  $Ca^{2+}$  concentration can further increase open probability.<sup>48,49</sup> Thus, the decay of the RyR2 activity was caused by a process distinct to inactivation which was dubbed "adaptation."<sup>49</sup>

Mode shift is a paradigm for hysteresis in which the channel's electrical sensitivity changes as a function of activity as follows: During depolarization, channels lingering within a set of stable deactivated (closed) states (Fig. 2 A,  $C_S$ ) are electrically driven to a



**Figure 2.** General scheme for hysteresis in the activity of voltage-gated channels. A) The basic scheme consists of 4 set of states: 2 sets of deactivated states ( $C_i$ ) and 2 sets of activated states ( $O_i$ ). These latter sets correspond to conformations in which the channel's activity shows open and closed events. The transitions linking C and O sets are voltage dependent, while transition between deactivated states and between activated states are non-voltage-dependent. The subscript "M" stand for Meta-stable set of states, the subscript "S" stands for stable set of states. B) Deactivation of channels can occur from either  $O_M$  or  $O_S$ . Because  $O_S$  is more stable than  $O_M$ , the voltage-dependence of deactivation can shift to more negative potentials as more energy is required to close the channels. C) Deactivation may become slower from  $O_S$  than from  $O_M$ , given this is a set of stable activated states.

metastable set of activated states (Fig. 2 A,  $O_M$ ). In  $O_M$ , open and closed events are observed. Holding the membrane potential at depolarized potentials keeps the channels activated, allowing for further conformational changes that brings channels into a set of stable activated states (Fig. 2 A,  $O_S$ ). This latter rearrangement occurs through a voltage-independent transition, as no further change in the membrane potential are needed for it to occur.

Completing the cycle, repolarization leads to channel deactivation through a voltage-dependent transition into a set of meta-stable deactivated state (Fig. 2 A,  $C_M$ ). Finally, the sustained negative polarization of the membrane grants time to channels to return to the initial set of stable states  $C_S$ . It is to be noted that the  $O_S$  correspond to the activated channels with the selectivity pore either conducting, as in non-inactivating channels (e.g.  $K_V7$  channels), or not conducting, as for  $K_V$  channels that display C-type inactivation (e.g.,; Shaker).

Stabilization of activated channels can result in a shift in voltage-dependence (Fig. 2 B). In the case of the *Drosophila*  $K_V1$  channel known as Shaker, Q-V curves are displaced toward negative potentials as channels are held at persistent depolarized membrane potentials. Noteworthy, reported changes in voltage-dependence of Shaker gating currents show substantial discrepancy among publications, ranging between  $-50$  mV and  $-10$  mV.<sup>51-54</sup> Such a discrepancy may be caused by the dramatic decrease in the deactivation rate observed in the Shaker gating currents after the membrane has been held depolarized.<sup>53,55,56</sup> A decrease the rate of deactivation implies that it is harder to leave  $O_S$ , which constitutes another evidence for post-activation stabilization of the channel's activated state (Fig. 2 A,C).

Both Shaker and the human  $K_V1.2$  channel display a remarkable decrease in their deactivation rates of both gating and ionic currents.<sup>53,55,56</sup> From these studies, it can be inferred that stabilization of the activated of a voltage-gated channel can decrease its deactivation rate with little or no effect on the voltage-dependence.

### Physiological role of hysteresis in $K_V$ channels

The physiological role of hysteresis remains unclear. However, it can be argued that “on demand” stabilization of the  $K_V$  channels' conductive conformation can

have a strong influence electrical signaling in cells. Also arguably, during the depolarization phase of action potentials, keeping  $K_V$  channels deactivated will result in a low  $K^+$  conductance, allowing  $Na_V$  (and  $Ca_V$ , depending on the cell type) to safely depolarize the membrane. On the other hand, shifting  $K_V$  channel's voltage-dependence for deactivation to more negative potential during the termination of an action potential would keep the  $K^+$  conductance active throughout repolarization, making this process more effective. Thus, DVD ensures that  $K_V$  channels are activated when most needed during the course of an action potential. Similarly, depolarization of the membrane during a cardiac pacemaking action potential could make the voltage dependence for opening of HCN channels shift to more negative potentials making this channels harder to be opened during repolarization.

In the case of cardiac action potentials, mode shifting the activation may limit the role of HCN to be more relevant to the diastolic depolarization.<sup>39,40</sup> Also in the heart, the shift in voltage dependence in hERG channels occurs within tens of milliseconds<sup>26,28</sup> as opposed to a time scale of second or minutes as reported with squid axon  $Na_V$  channels and skeletal muscle L-type  $Ca_V$  channels. This characteristic of the hERG activity indicates that channel deactivation follows a different pathway than activation. As deactivation has a voltage dependence that is shifted to more negative potential, the activity of hERG contributes with the repolarization of the membrane, even at potential at which it would not be normally activated.

In a similar fashion, the deactivation of  $K_V3.1$  channels is modulated by depolarization. Specifically, the deactivation rate of  $K_V3.1$  decreases within a few millisecond during activation.<sup>57</sup> This feature of its activity allows  $K_V3.1$  channels to quickly adapt to burst of neuronal activity, even during a single action potential, resulting in resurgent  $K^+$  currents that can guarantee the repolarization of the plasma membrane.<sup>57</sup> This implies that DVD in voltage-gated channels can have a great impact on electrical activity within millisecond-time scales.

It seems that the main physiological impact of hysteresis is on deactivation, rather than activation. This is the case of the heteromeric  $K_V7.2/K_V7.3$  channel. This channel constitutes the main molecular entity underlying M-currents in the central nervous system.<sup>58,59</sup> There, M-currents are critical in regulating

excitability.<sup>59-62</sup> In fact, mutations in these channels have been directly linked to several hyperexcitability-related neurological disorders.<sup>59,61,63-65</sup> During the past decade,  $K_V7$  agonists have become a target for research seeking to design new therapies for neurological disorders. It was shown that the  $K_V7$  agonist Retigabine, a clinically-used anticonvulsant, shifts the voltage dependence of activation of the heteromeric  $K_V7.2/K_V7.3$  channel to more negative potentials, facilitating activation.<sup>66-70</sup> More recently, the heteromeric  $K_V7.2/K_V7.3$  channel has a strong hysteretic behavior displaying, at least 2 modes,<sup>71</sup> analogous to what it has been discussed above. Retigabine preferentially targets the stable open mode of the heteromeric  $K_V7.2/K_V7.3$  channel, further stabilizing it.<sup>71</sup> This is consistent with the notion that the binding site for Retigabine is located in the pore domain<sup>66,69</sup> which can mediate the stabilization of the open conformation. Further, these observations indicated that distinct open modes of these channels can be pharmacologically discriminated, implying that these modes correspond to distinct protein conformations.<sup>71</sup> Furthermore, this constitutes the first example of a drug that enhances the hysteretic behavior of a channel, indicating that a particular mode of a voltage-gated channel's activity could be targeted to enhance drug specificity.

Mutations in the voltage sensor can alter the hysteretic behavior of the skeletal muscle  $Na_V$  channel ( $Na_V1.4$ ), causing pathologies such as normokalemic periodic paralysis (NormoPP).<sup>72</sup> Particularly, it was recently shown that a clinically-identified mutation in which the third arginine of the S4 segment in domain II of the channels is replaced with a glycine yields a non-selective pathway through the VSD with a conductance that increases as a function of activity.<sup>73</sup> Furthermore, the voltage-dependence of closing of this conductance is shifted to more negative potentials ( $< -100$  mV).<sup>73</sup>

A similar observation was reported from studies on Voltage-Sensitive Phosphatases (VSP). These proteins are constituted of 3 main regions: a VSD in the N-terminus, a Phosphatase Domain in the C-terminus, and a Phosphoinositide Binding Motif (PBM) linking both domains.<sup>31,32,74,75</sup> The VSD of these enzymes resembles those in voltage-gated channels both structurally<sup>76</sup> and functionally<sup>31,32</sup> similar. The VSD of VSPs confers voltage sensitivity to the activity of these enzymes.<sup>31,32,77-80</sup> The voltage sensor of the *Ciona intestinalis* (Ci-)VSP is a S4-based VSD formed by 4 trans-membrane

segments, with the S4 segment carrying the main voltage-sensing charges. Also, these VSDs produce non-linear capacitive currents known as *sensing currents*, which are equivalent to *gating currents*. Replacing the third arginine of the S4 segment of Ci-VSP with a histidine causes the trapping of the VSD in the so-called *relaxed* state of the sensor (see below for more details on VSD relaxation). In the relaxed state, the VSD is stabilized in a conformation that is reached through a voltage-independent transition that follows activation.<sup>81</sup>

Back to the case of  $Na_V1.4$ , it is noteworthy that the NormoPP  $Na_V$  mutation seemed innocuous when recording currents under voltage-clamp from single voltage-step protocols, as typically done in most of electrophysiological studies. However, a stronger effect of the mutation was observed when protocols involving repetitive or sustained activation were applied.<sup>73</sup> Under these experimental conditions, the hysteretic behavior of channels becomes relevant. These observations constitute an example of how changes in the hysteretic behavior of a voltage-gated channel can be altered by mutation that seems to have little or no effect on their activity. In other words, this example shows how mutations could enhance the hysteretic behavior of the VSD.

Another case that could involve hysteresis in  $Na_V$  channels is the mechanism of generation of  $Na^+$  resurgent currents in neurons<sup>82,83</sup> as resurgent currents seem to be related to modulation of slow inactivation.<sup>84</sup> In general, this type of observations highlights an emerging concern in the study of voltage-gated channels and their role in diseases, as it may be possible that variants of these proteins that are considered to display no pathological phenotypes, based on simple step-protocol based assays, may have dysfunctional hysteretic behavior that may disrupt their role in electrical signaling.

### Mechanisms of hysteresis

How hysteresis occurs in voltage-gated channels remains to be answered. However, hysteresis can be observed in both “isolated voltage sensor” and “isolated pore,” indicating that this property can emerge from the intrinsically hysteretic behavior of individual domains of the channel. The first example of hysteresis in “isolated voltage sensing domains” comes from studies on Voltage-Sensitive Phosphatases.<sup>31,32,34,85</sup>

Q-V curves from sensing currents Ci-VSP are displaced over  $-50$  mV when the H.P. is set to a high positive potential, instead of negative ones.<sup>34,53,76</sup> As mentioned above, this shift occurs through a process known as *VSD relaxation*.<sup>34</sup> Although the molecular mechanism of VSD relaxation remains unclear, it is thought to consist of a voltage-independent transition observed following the termination of sensing currents.<sup>34,75,76</sup> VSD relaxation seems not to be caused by the interaction of the VSD with the PBM or the phosphatase domain because voltage dependence shifts are also observed either when the phosphatase domain has been genetically deleted from the protein,<sup>34,53</sup> when the PBM was disabled by mutations,<sup>34,79</sup> or when the C-terminus has been replaced by fluorescent proteins as in the case of Genetically-Encoded Voltage Indicators (GEVI).<sup>33,86,87</sup> Thus, VSD relaxation is a hysteretic process that is intrinsic of VSDs.

The proton-selective voltage-gated channel,  $H_V1$ , is an example of a voltage-gated channels with a S4-based VSD protein that lacks an explicit pore domain.<sup>88,89</sup> It has been proposed that the activation of  $H_V1$  proceeds through several steps with different degrees of cooperativity,<sup>90-92</sup> where the last transition is likely voltage-independent step that is analogous to VSD relaxation.<sup>93</sup> As a consequence, activation and deactivation follows distinct pathways into a cycle, reminiscent of the definition of hysteresis.<sup>93</sup> This reinforces the idea that hysteresis can be observed in VSD protein with no explicit pore. Of course, the role of the intracellular domain of  $H_V1$  cannot be ruled out at this time.

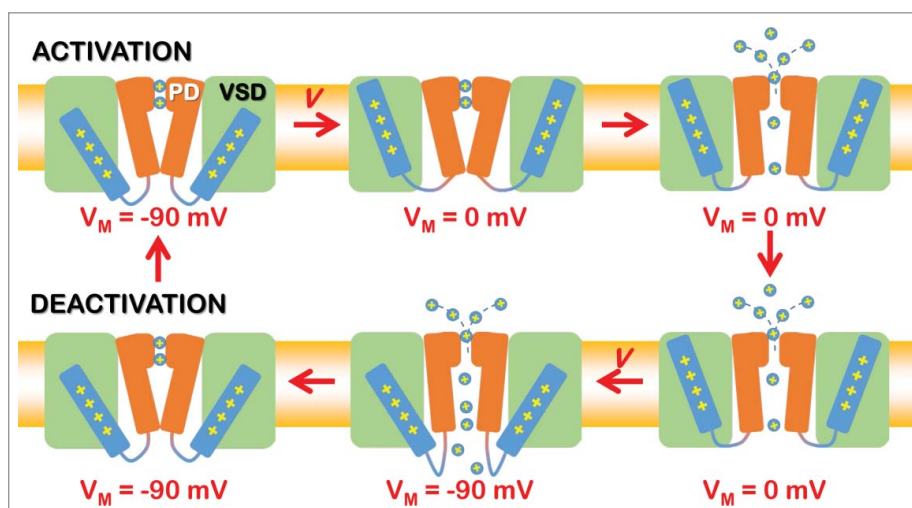
Having established that a VSD can display hysteresis, the attention now turns toward the pore domain. Can an “isolated” pore domain display hysteresis? KcsA is a  $K^+$  channel from the bacterium *Streptomyces lividans* with no explicit VSD.<sup>94,95</sup> Given its similarities to eukaryotic inwardly rectifying  $K^+$  channels and the pore domain of  $K_V$  channels, the structure and activity of KcsA has been intensely studied in the past 2 decades as a model protein for permeation and C-type or “slow” inactivation in ion channels.<sup>96</sup> The activity of KcsA is controlled by hydrogen ions; fast decrease of the pH in the intracellular side activates the channels. Upon activation, KcsA inactivates within a few seconds.<sup>97-100</sup> These observations can be interpreted as the channel sojourning between a  $HP_O$  and a  $LP_O$  mode, equivalent to the cardiac Ryanodine Receptor (see above),<sup>46,49</sup> except for the fact that KcsA inactivates. In spite of

these differences, both processes can be modeled as a cyclic scheme where the  $HP_O$  mode corresponds to a set of meta-stable states, while the  $LP_O$ /inactivated mode corresponds to a set of stable states, as recovery from inactivation is slower than deactivation and inactivation itself.<sup>100</sup> Therefore, based on the observations it can be concluded that an “isolated” pore can also display hysteresis in its activity.

Given that hysteresis can be observed in VSDs and pore domains, the next question is: What is the mechanism for hysteresis in a voltage-gated channels? The displacement of the Q-V has been attributed to C-type inactivation in channels, such as in the cases of Shaker<sup>51</sup> and the bacterial  $Na_V$  channel NaChBac.<sup>101</sup> In these instances, C-type inactivated channels are thought to be in a stable set of conformations, thus a deeper repolarization is required to bring the channels back to their deactivated/resting state. Based on this idea, it can be argued that shifting the voltage-dependence would require the pore and the VSD to be coupled. Along these lines, uncoupling the activation of Shaker activation by mutations in either the S4-S5 linker or the intracellular end of the S6 segment of has been shown to seemingly abolish VSD relaxation.<sup>54</sup> However, these considerations were made based on changes in the voltage-dependence of activity of these mutants. More recent studies, however, have shown that the main effect of relaxation on the activity of Shaker is to slow down deactivation, with a relative small effect on voltage-dependence for both gating and ionic currents.<sup>53,55,56</sup> This latter notion revealed the changes in voltage-dependence and kinetics are not necessarily straightforwardly correlated.

### **Thermodynamics of voltage-gated channel hysteresis**

To elaborate on the thermodynamics of voltage-gated channel hysteresis, let us consider a canonical tetrameric voltage-gated channel, consisting of 4 subunits in a radially symmetrical arrangement, with each subunit containing a VSD and a pore-forming region. Also, let us consider that voltage sensing is mainly carried out by charged residues in the S4 segment (S4 charges).<sup>13,15,102-110</sup> At negative potentials, a canonical voltage-gated channels is closed, as the VSDs are deactivated, with their S4 segment displaced toward the intracellular milieu, and the pore in a non-conductive conformation (Fig. 3, top left). Upon depolarization,



**Figure 3.** During activation, sensing charges in the voltage sensing domain (VSD) drive the conformational changes that are eventually transmitted to the pore domain, opening it. So, activation of the VSD precedes the activation of the pore, as expected. During deactivation, the electrically driven movements in the VSD also need to precede the closing of the channel. This indicates that the sequence of events involved in activation and deactivation follows different pathways.

S4 segments are driven toward the extracellular side of the plasma membrane (Fig. 3, top center). Subsequently, the VSDs lead the opening of the pore (Fig. 3, top right) through a transition that can be driven by a small fraction of the sensing charge.<sup>111</sup> Arguably, the opening of the pore *per se* may also be driven by transition that are not strictly voltage-dependent, as it has been suggested for H<sub>v</sub>1 channels.<sup>93</sup> Nevertheless, when the activation is sustained, channels tends to stabilize in their open conformation through transitions that are likely voltage independent.<sup>55-57,71</sup> On the other hand, during deactivation, the S4 segments are driven by the negative membrane potential back to their initial position and the closing of the pore follows (Fig. 3, bottom right and center). As noted above, the closing of channels is not achieved by simply reverting the sequence of events that led to their opening. Instead, the movement of the VSD, as the electrical transducer of the proteins, precedes this sequence of events (Fig. 3, bottom center and left). Although this notion seems to be trivial, it has important implications as it indicates that channels' activity may be intrinsically hysteretic.

In term of the thermodynamics of this process, during depolarization, the S4 segment is electrically driven to an activated position, as the electric field does work on the S4 charges. Thus, the VSD gains energy ( $E$ ), roughly  $E = Q \times \Delta V$ , where  $Q$  is the total gating (or sensing) charge and  $\Delta V$  is the difference between the H.P. and the voltage applied for

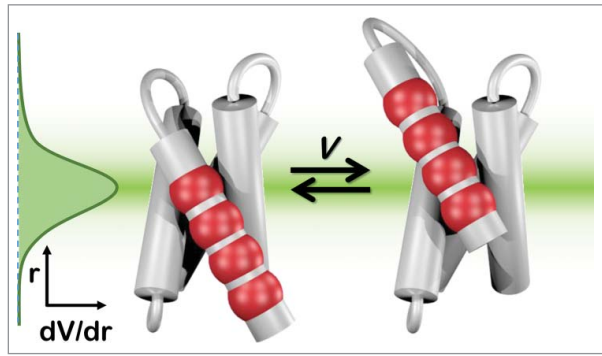
activation. A fraction of  $E$  is used to open pore. If the pore domains behaves as an elastic element of this system, then that fraction of energy used to open the pore is “stored,” so that no extra energy is required to bring the channel back to the initial state during deactivation. In contrast, if there is some energy lost during the interaction between the VSD and the pore, then a shift in voltage dependence would be observed as “extra” energy that will be needed for deactivation. This extra energy would be supplied by the electric field, thus the magnitude of  $\Delta V$  increases. Examples of this latter scenario can be observed in the case of changes in the voltage-dependence for charge movement of Shaker channels. Following a short depolarization of the membrane suffice to shift the voltage dependence of Shaker gating currents over  $-10$  mV.<sup>53</sup>

The energy gained by the VSD from electrical work ( $W_{ELECT}$ ) could be calculated using the expression:

$$W_{ELECT} = \int Q(r) E(r) dr \quad (1)$$

where  $Q(r)$  represents the charges of the VSD,  $E(r)$  is the electric field and  $r$  is position in space. Notice that  $Q$  and  $E$  are functions of the position of the charges. In general, crystal structures show that some of the S4 charges can be either embedded in the hydrophobic core of the VSD or exposed to water.<sup>76,112-115</sup> This is important because the electric field is focused on the core of the S4-based VSD,<sup>81,116-120</sup> implying that not





**Figure 4.** The electric field ( $dV/dr$ , in green) is focused on a narrow regions in the VSD core. Based on the current understanding of the structure of VSD proteins, it can be said that not all sensing charges (red balls) are located within the high intensity electric field volume (green). Therefore, the work done by the electrical field seems to takes place “one charge” at a time.

all charges (Fig. 4, red spheres) are sensing the electric field at all times (Fig. 4, green area).

Electric work is not a state function, so it is not independent of the path “taken” by channels toward a final state. In contrast, free energy (e.g. Gibbs free energy) is a state function, so it is independent of the route followed by the channel. As shown in the elegant work by Chowdhury and Chanda, the free energy for channel’s activation is given by

$$\Delta \bar{G}_{ACTIVATION} = Q_{MAX} V_M \quad (2)$$

where  $Q_{MAX}$  represents the total sensing charge involved in gating and  $V_M$  is the potential at which the VSD charges in the deactivated and activated states are equally populated.<sup>121</sup> In this case, the activation energy is calculated with no regard of the path followed by the VSD charges. In addition a built-in assumption in the Chowdhury-Chanda model is that the opening of the channels is strictly voltage-dependent, meaning that no voltage-independent transition precedes channel opening.

These two aforementioned views on the energetics of channel activation complement each other as they represent 2 different aspects of the channel’s dynamic. Equation (1) corresponds to the work done by the VSD to activate the channel; Equation (2) corresponds to the difference in energy between the deactivated and activated states. To further illustrate this, let us consider the work done when moving a bowling ball from the floor onto a high shelf. Because the ball is immersed in a gravitational field, the ball gains energy as it is lifted. The energy gained by the ball is

proportional to the height of the shelf with respect to the floor, regardless of how the ball got to the shelf. In contrast, the energy expended to place the ball on the shelf depended on the trajectory followed by the ball; the amount of work would be different if the ball was placed on the shelf following a straight trajectory or if it was lifted up to the ceiling and then placed on the shelf or the movement involves friction. In light of this idea, it can be concluded that

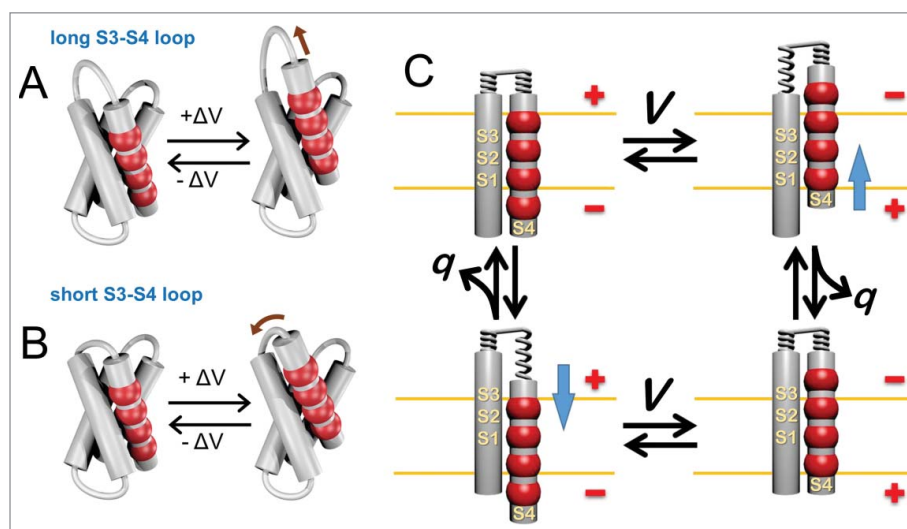
$$\Delta \bar{G}_{ACTIVATION} \leq W_{ELECT} \quad (3)$$

So the work done by the electric field on the S4 charges can be greater than the energy of VSD activation. This latter energy is compounded of 2 terms, one corresponding to the activation of the VSD ( $\Delta G_{VSD}$ ) and the second to the activation of the pore ( $\Delta G_{PORE}$ ). In addition, as the movement of charges takes places within a condensed system (proteins + water + lipids + ions), friction and non-voltage dependent transitions are expected to dissipate part of the energy gained from the electric work. Thus, it can be said that

$$W_{ELECT} \geq \Delta G_{VSD} + \Delta G_{PORE} + q_{DISSIP} \quad (4)$$

Where  $q_{DISSIP}$  accounts for the energy dissipated during this process. The inequality highlights that the VSD and surroundings are condensed systems, therefore a fraction of the work is inevitably lost to friction.

Because  $K_V$  channels can be opened and closed by changing the membrane potential, channel activation is often regarded to be “reversible” process. However, in terms of thermodynamics, reversibility refers to a process that causes no change to its surroundings. In other words, no changes in entropy are observed in a thermodynamically reversible process. A classic example is the isothermal expansion of an ideal gas in a piston. If the piston is released for a rapid expansion, the work done by the gas is minimized, while heat exchange is maximized, so there is a change in entropy. In contrast, releasing the piston in an infinite series of infinitesimal steps, each one in equilibrium, maximizes the work done by the gas with no change in entropy. In an equivalent situation, a hypothetical experiment using voltage-clamp, a thermodynamically reversible activation of *Shaker*,  $K_V1$ , or  $K_V4$  channels can be achieved by depolarizing the membrane using an infinitively long voltage ramp from a negative H.P., say from  $-90$  mV to  $0$  mV. At



**Figure 5.** Electromechanical model for VSD relaxation. The movement of the S4 segment could induce conformational changes in the S3 segments and the rest of the VSD depending on the length of the S3-S4 linker. Recent evidence suggest, not only that the length of the loop can change the kinetic and voltage dependence of activation in Shaker, but also makes VSD relaxation more prominent. A possible mechanism for this is that mechanical coupling between the S3 and the S4 segments is mediated by the loop linking these segments, so when this linker is longer (A) coupling decreases. In contrast, when the S3-S4 linker is shorter (B), mechanical coupling between this segments is enhanced. C) Based on this idea, a general mechanism for VSD relaxation could be based on electromechanical coupling between the S4 segment and the rest of the VSD through the S3-S4 linker and lateral interactions. These interactions are partially elastic, so that mechanical energy gained from the movement of the S4 segment can be “stored” in this components (springs). However, this energy will be eventually dissipated ( $q$ ) as the rest of the VSD rearranges to energetically satisfy the new position of the S4 segment.

the end of the ramp, the channels would have undergone C-type (slow) inactivation. Therefore, it can be concluded that the open channel corresponds to a metastable state, while the C-type inactivated state corresponds to the stable final state of this process. It is worth mentioning that based on this hypothetical experiment, one would expect that structural models of  $K_V$  channels that are available today represent the slow inactivated state which is the thermodynamically stable conformation – and, perhaps, also in the relaxed state of the VSD, since the structures are obtained at 0 mV.

The third term of Equation (4) refers to energy “dissipation” by the VSD. As aforementioned, the voltage-dependence of a VSD shifts to negative potentials following VSD relaxation. The mechanism for this process remains unclear. However, work by Priest and colleagues have shown that the S3-S4 loop is critically involved in VSD relaxation in *Shaker*.<sup>56</sup> Shortening this loop, which connects the S4 segment with the rest of the VSD, increases the effect of relaxation on the deactivation rate of gating currents in *Shaker*.<sup>56</sup> Intriguingly, the magnitude of the shift observed following VSD relaxation also seems to be correlated with the length of the S3-S4 loop among different

VSD proteins.<sup>56</sup> One interpretation of these observations could be that the S3-S4 loop constitutes a coupling element between charge movement and VSD relaxation. It has also been shown that the length of the S3-S4 linker affect the kinetics and voltage dependence of activation in *Shaker*.<sup>122-124</sup> Following these ideas, one may expect that, if a VSD has a long or unstructured (“flexible”) S3-S4 linker, the movement of the S4 segment could have little effect on the S3 segment and the rest of the VSD (Fig. 5 A). In contrast, if the S3-S4 loop is short or highly structured (“stiff”), then mechanical coupling between the S3 and S4 segment could be enhanced, causing the VSD to be rearranged to energetically satisfy the new position of the S4 segment after gating currents (Fig. 5 B).

The aforementioned notion leads to the formulation of a hypothetical mechanism of VSD relaxation which will be, hereafter, referred to as the “electromechanical hypothesis.” In an isolated VSD, sensing charges are located exclusively in the S4 segment. In this VSD, the segments S1 to S3 are mechanically coupled to S4 through the S3-S4 loop which constitutes an elastic component of the system (Fig. 5 C). When the membrane potential is negative, the S4 segment rests in its “down” position and with the rest of the

VSD is in a “low energy” conformation (Fig. 5 C, top left). Upon depolarization, the S4 segment moves to the “up” position, driven by the electrical work done by the electrical field on S4 charges (Fig. 5 C, top right). In this condition, the S3-S4 loop which is the elastic component is stretched “storing” or “saving” mechanical energy. If the membrane is immediately repolarized, the energy stored in the elastic component will contribute to the electrical work, so the changes in voltage-dependence of kinetics would be observed in charge movement. However, if the depolarization is prolonged, the elastic component relaxes, inducing a conformational change in the rest of the VSD (Fig. 5 C, bottom right). At this point, “saved” energy is “expended” or “dissipated” ( $q$ ) and the VSD enters in a new low energy state. From here, to return the S4 segment to its original position, the electric field will do work, driving the S4 segment to its “down” position and bringing the VSD to its resting conformation. This latter process involves extending again the elastic component (Fig. 5 C, bottom left). As a consequence, more work is required, resulting in a shift of the voltage-dependence, a decrease of the rate of deactivation, or both.

In light of these considerations, it becomes clear that, without accounting for friction, the term  $q_{\text{DISSIP}}$  in Equation (4) considers the energetic cost of VSD relaxation. Thus, it can be established that

$$q_{\text{DISSIP}} = \Delta \bar{G}_{\text{RELAXATION}} \quad (5)$$

where  $\Delta \bar{G}_{\text{RELAXATION}}$  can be calculated using Equation (2), where the  $V_M$  would be the membrane potential at which charges in the relaxed state and in the deactivated state are equally populated. Finally, the final form of Equation (4) is

$$W_{\text{ELECT}} \geq \Delta \bar{G}_{\text{ACTIVATION}} + \Delta \bar{G}_{\text{RELAXATION}} \quad (6)$$

and

$$\Delta \bar{G}_{\text{RELAXATION}} = Q_{\text{MAX}}(V_{\text{RELAX}} - V_M)$$

where  $V_{\text{RELAX}}$  is the equivalent  $V_M$  in Equation (2), but for the Q-V following VSD relaxation. Further refinement of these equations is required.

In summary, the goal of this article was, first, to review the current knowledge on hysteresis in voltage-gated channels and, second, to provide an initial

framework for understanding this phenomenon. Although action potentials can be generated without taking hysteresis into consideration (e.g., using the HH model<sup>2</sup>), it is clear that dynamics changes in the activity-voltage relationship in channels (here referred to as DVD) can have a significant effect in electrical signaling. On the other hand, one may hypothesize that mutations that are thought to be innocuous to channel activity, may affect hysteresis, influencing channel’s performance during prolonged activity, like during burst of action potentials or in pace-making.

### Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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