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Topical Review

Slow Changes in the Availability of Voltage-gated Ion Channels: Effects on the Dynamics of Excitable Membranes

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Introduction

Excitability stands at the basis of many physiological systems (e.g., neural systems, cardiac cells, hormone-releasing cell clusters). In most cases, the action potential (the excitation event itself) operates on a relatively fast time scale, whereas the system is modulated at time scales that are many orders of magnitude slower. The search for mechanisms to bridge this time gap usually leads to the addition of complex intracellular biochemical modulation pathways and intercellular communication. Recent experiments and theoretical considerations suggest that intrinsic activity-dependent gating mechanisms of voltage-gated ion channels (the molecules of excitability), and in particular slow changes in the availability of the channels for activation, might contribute significantly to long lasting modulations in excitable systems. These modulations are independent of intra- and intercellular mechanisms. The present topical review is aimed at summarizing these experimental findings and theoretical considerations.

Ion channels of Excitable Membranes are Complex Molecular Machines

In the 1940s and 1950s, Cole, Goldman, Hodgkin, Huxley, Katz, and other pioneers of membrane physiology, conducted a series of studies aimed at uncovering the relations between the ionic environments, membrane conductance, fluxes and voltage changes in excitable membranes (for an interesting overview of these 'heroic' times *see* Hodgkin, 1992). Their efforts led to the elegant and symmetrical mathematical model of Hodgkin and Huxley (1952) which accounts for conduction and excitation in quantitative terms. The impact of the Hodgkin-Huxley model was so strong that forty-five years after its conception, it still serves as a canon for most membrane physiologists who study excitability. The Hodgkin-Huxley mathematical description assumes that the 'particles' that allow for ionic conduction (*n*, *m*, and *h* gates) are voltage-sensitive, independent of each other, and operate within a relatively narrow range of time scales. These assumptions were appropriate for what Hodgkin and Huxley aimed at in 1952, that is to ''. . . cover only the short-term responses of the membrane . . .'' (p. 541). Biophysical knowledge that accumulated during the past 40 years, particularly since the invention of the single channel recording technique (Sakmann & Neher, 1983), made it possible to correlate the abstract Hodgkin-Huxley 'particles' with structures and functions in ion channel proteins. Detailed kinetic analyses of ion channel gating mechanisms reveal that the key assumptions of Hodgkin and Huxley are not appropriate when longer term responses of the membrane are sought. Instead, gating of ion channels of excitable membranes is a process which includes:

- coupling of transitions between functionally different conducting states;

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- a mix of voltage-dependent and voltage-independent steps; and,
- a wide range of reaction time scales.

It is instructive to think of the gating of voltage-gated ion channels of excitable membranes within the framework of the following simplified three-states scheme,

$$C \underset{\beta(v)}{\overset{\alpha(v)}{\Leftrightarrow}} O \underset{\delta}{\overset{\gamma}{\Leftrightarrow}} I$$

where ion channels can reside in one of three states: closed (nonconducting) states (C), open (conducting) states (O), and inactivated (unavailable) states (I). The $C \Leftrightarrow O$ transitions are usually within the time scale of a single action potential. These transitions are voltage-dependent and conform with the general idea of the Hodgkin and Huxley model (Hille, 1991; and references therein). In contrast, the time scale of the $O \Leftrightarrow I$ transitions is widely distributed (depending on channel type) and can reach many seconds (e.g., Ehrenstein & Gilbert, 1966; Adelman & Palti, 1969; Chandler & Meves, 1970; Schauf, Peneck, & Davis, 1976; Brismar, 1977; Rudy, 1978; Aldrich, 1981; Almers, Stanfield & Stühmer, 1983; Simoncini & Stühmer, 1987; Stühmer et al., 1987; Iverson & Rudi, 1990; Choi, Aldrich & Yellen, 1991; Hoshi, Zagotta & Aldrich, 1991; Ruben, Starkus & Rayner, 1992; Marom & Levitan, 1994; Fleidervish, Friedman & Gutnick, 1996; Cummins & Sigworth, 1996; Bertoli, Moran & Conti, 1996). These reactions are largely voltage-independent. Besides the marked time scale separation between the $C \Leftrightarrow O$ and the $C \Leftrightarrow I$ reactions, it is important to realize that the reactions are coupled: the process of inactivation is state-dependent, i.e., it depends on a prior residence of the channel in the open set of states. The three-states scheme is highly simplified, and each set of states has a complex internal structure. Nevertheless, this simple scheme captures the main features of the gating of most voltage-gated ion channels: These channels are, by definition, activated by voltage, and then proceed to an inactive unavailable state at a wide range of time scales and voltage dependencies. In the following sections, the simplified three-states scheme will serve as a tool to convey the main message of this review: 'real' ion channels in excitable membranes are very clever, much more than anticipated by the pioneers of membrane physiology. In particular, we will be dealing with the remarkable potential of ion channels to temporally integrate past electrical activities of the cell over a wide range of time scales.

Temporal Integration of Episodic Activity Requires State-dependent Cumulative Inactivation

From the general three-states kinetic scheme above, it is expected that a long enough depolarizing pulse will cause a decrement in the availability of ion channels. ''Long enough'' being measured in terms of $(\alpha(v) + \beta(v))^{-1}$, which is the characteristic time of the $C \Leftrightarrow O$ relaxation; the depolarizing voltage should be such that $\alpha(v)/(\alpha(v) + \beta(v))$, the steady-state probability of the channels to be found in the open state, approaches unity. Under these conditions the process of inactivation reaches a steady state following a time course determined by γ and δ. The resulting decreased availability due to inactivation affects the inertia of the cell to fire action potentials via modulation of the threshold potential, resting membrane potential or both. The biological significance of such a process is obvious in excitable systems that go through periods of long lasting depolarization. However, for the more general case, where depolarizing input occurs in episodes of various (mostly short) duration and frequencies, inactivation must *accumulate* and persist between repetitive action potentials in order to become biologically relevant. This general case is discussed below.

The degree of *accumulation* of channels in the unavailable inactive state, in response to a short episodic input, depends on several considerations: (i) The probability of a channel to be found open during a short episodic depolarizing input; (ii) the probability that an open channel will inactivate after the end of the episodic input; (iii) the probability that an inactive channel will recover fully to the closed state before the arrival of the next episodic input; and finally, (iv) the effects of accumulation of channels in the inactive state on the probability of other channels to become inactive in response to subsequent input episodes. We will now deal with these considerations one at a time. Since the $C \Rightarrow O$ reaction rate, $\alpha(v)$, is usually fast, one may assume that the probability of a channel to open during an episodic depolarizing input is given by the steady-state term $\alpha(v)/(\alpha(v) + \beta(v))$. In other words, an input episode which lasts several milliseconds will redistribute the available channels (those that are not inactivated) between the open and closed states according to the membrane potential. What fraction of these open channels will 'fall' into the inactive state *after* the end of the depolarizing episode, when the membrane returns to its hyperpolarized resting potential? Once the short episodic input that opens the channels subsides, the open channels undergo a partitioning process. The channels can return to the closed state, which renders them available for participation in the subsequent electrical activity, *or* fall into the inactive state, which renders them unavailable to actively participate in a subsequent electrical activity. This partitioning process depends critically upon the ratio between the rate of deactivation, $\beta(v)$, and the rate of inactivation, γ. Indeed it is the fraction $\gamma/(\beta(v) + \gamma)$ around the resting membrane potential, that determines how many channels are lost due to a single action po-

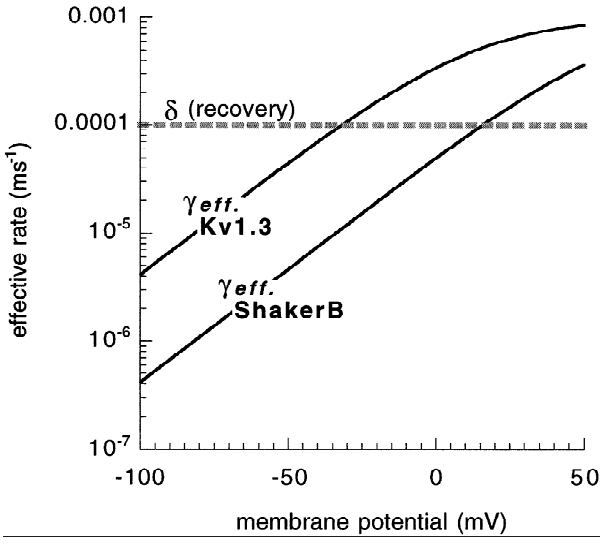

Fig. 1. The ShakerB and Kv1.3 channels have similar rates of entry to (γ) and recovery from (δ) slow inactivation. The coupling is taken into account by multiplying γ and the probability that the open channel is not deactivated. A difference emerges between the channels: the effective rate of inactivation of the Kv1.3 channel is higher compared to that of ShakerB, particularly in the range of negative membrane potentials. This allows for accumulation of inactivation between episodes of activity to readily occur in Kv1.3, less so in ShakerB.

tential. Note that it is imperative that γ not be strongly voltage-dependent. If γ was strongly voltage-dependent, in the Hodgkin-Huxley sense (where hyperpolarization causes channels to *recover* from inactivation), inactivation could not have taken place after the end of the short action potential, and it would have been difficult to see how slow inactivation can be more then a laboratory curiosity. This idea is demonstrated in Fig. 1 where the effective rate of inactivation (γ_{eff}), which takes into account the 'competition' between $\beta(v)$ and γ over an open channel, is plotted as a function of membrane potential. The figure shows the effective rate of inactivation for two types of voltage-gated ion channels. The channels, ShakerB and Kv1.3, have similar rates of entry into, γ, and recovery from, δ, slow inactivation (Lee & Deutch, 1990; DeCoursey, 1990; Hoshi et al., 1991; Lopez-Barneo et al., 1993; Marom et al., 1993; Marom & Levitan, 1994; Kupper et al., 1995). Despite these similar $O \Leftrightarrow I$ rates, the two channels differ significantly in their probabilities to inactivate during the post episodic membrane potential due to a marked difference in their deactivation rate, $\beta(v)$. In other words, slow inactivation of the ShakerB channel is less effective as an integrator of episodic activity, in comparison to the Kv1.3 channel; this difference between the channels is caused by the relatively slow *effective* rate of inactivation of ShakerB at membrane potentials that are below 0 mV. Note that at a membrane potential of about −40 mV, the rate of recovery from inactivation, δ, of the Kv1.3 channel, is very close to the effective rate of inactivation. This suggests that after each action potential with a post-spike depolarization of the kind that one often sees in excitatory (but not inhibitory) cortical neurons (McCormick et al., 1985; Baughman et al., 1991), a significant amount of Kv1.3-like ion channels will become unavailable. This is not what one expects to see in neurons with ShakerB-like channels. To summarize, the dependence of the deactivation rate, $\beta(v)$, on membrane potential is a very important determinant of the ability of a channel to integrate episodic activity.

The third point to consider about accumulation of inactivation in a physiological context has to do with the probability that an inactive channel will recover to the closed state *before* the arrival of the next episodic input. This probability depends on the ratio between the rate of input arrival and the rate of recovery from inactivation; therefore, the value of δ determines the characteristic input frequency that is required in order to accumulate channels in the unavailable pool. If the input frequency is within the range of δ or higher, accumulation will occur. And finally, one should consider the effects of accumulation of channels in the inactive state on the probability of other channels to become inactive in response to subsequent input episodes: Excitability is an opponent process which involves a delicate counterbalance between exciting and restoring forces. A loss of exciting force channels (e.g., sodium conducting channels) from the available pool during activity will cause an adaptation (or depression) of activity, and will therefore act as a negative feedback loop, decreasing the chances of more channels to inactivate. In contrast, accumulation of potassium conducting channels (restoring force) in the unavailable inactivated state will cause a sensitization (or potentiation) of the excitable system, which in turn will increase the probability of further inactivation. Therefore, accumulation of potassium channels in the inactive state acts as a positive feedback signal.

Many studies provide compelling evidence for the importance of slow cumulative changes, in the availability of ion channels, as a mechanism underlying the phenomenology of temporal integration of electrical activity in excitable membranes. Several of these studies are reviewed below.

Temporal Integration of Electrical Activity by Slowly Inactivating Conductance

Slowly inactivating potassium currents were suggested to allow for temporal integration over many seconds in several types of cells, including sympathetic (Wang & McKinnon, 1995), thalamic (McCormick, 1991), neostriatal (Nisenbaum et al., 1994), and cortical neurons (Schwindt et al., 1988; Foehring & Surmeir, 1993). An

elegant exploration of this possibility can be found in a study done by Storm (1988) on hippocampal neurons. The hippocampus is believed to be involved in memory storage, therefore mechanisms of temporal integration and activity dependent regulation of excitability in hippocampal neurons are particularly interesting. It has been known for quite a while that hippocampal pyramidal neurons can be driven to spike in response to a subthreshold depolarizing input provided that this input lasts long enough. This delayed excitation phenomenon was reported also in other types of mammalian and molluscan neurons (e.g., Byrne, 1980; Getting, 1983; Yarom & Llinas, 1987). Storm (1988) clearly showed that the delayed excitation in the rate CA1 hippocampal neurons is due to a slowly inactivating potassium current (named 'ID'). Moreover, Storm showed that the effect of this current on excitability can accumulate, allowing the cell to integrate separate episodes of depolarization on a time scale of seconds. In fact, it is now known that hippocampal neurons express significant amounts of Kv1.4 (Beckh & Pongs, 1990), a channel type with a marked long lasting inactivation (Pardo et al., 1992) due to a relatively slow deactivation rate; more then 20 seconds are needed to allow complete recovery from state-dependent inactivation produced by a brief stimulus (Bertoli, Moran & Conti, 1996). It was suggested, based on these and related data, that slow kinetics of potassium channels can produce hippocampal cellular memory that is independent of changes in synaptic efficacy (Turrigiano, Marder & Abbott, 1996). Interestingly, such a memory mechanism was mentioned by Bliss and Lømo (1972) in their original report on LTP; they demonstrated a nonsynaptic memory effect that lasts even longer than Storm's. In their seminal manuscript, they introduced two independent mechanisms that are responsible for long-lasting potentiation in response to a repetitive stimulation of the hippocampal formation: (a) an increase in the efficacy of synaptic transmission; (b) an increase in the excitability of the granule cell population which can last for many hours, independent of synaptic mechanisms. The first, synaptic process, caught the imagination of neurobiologists and occupies thousands of printed pages throughout the scientific literature. The second phenomenon (coined ''spike potentiation'' by Bliss and Lømo) was practically ignored by neurobiologists. (Perhaps this lack of response of neurobiologists can be understood when the Hebbian tradition is considered: Single neurons are simple millisecond time scale integration machines, therefore long lasting effects should be sought for at the level of connectivity (Hebb, 1949)). As shown here, sensitization based solely on the gating of potassium selective ion channels of excitable membranes, can be a powerful mechanism for long lasting modulation.

Slow to recover cumulative inactivation of potassium channels makes the system more excitable as a function of past activity. In contrast, slow cumulative inactivation of sodium channels would act to restore an overactive cell, a process called adaptation. However, as Cannon (1996*a*) put it ''. . . for many years, slow inactivation of sodium channel was viewed as a laboratory curiosity with uncertain physiological significance. . . .'' In recent years it is becoming clear that sodium channels cannot be regarded as simple 'on' switches of the action potential. They too take part in what was considered for years as the role of their more colorful partners, the potassium channels—that is to modulate longer term patterns of firing. Two recent examples for the involvement of sodium channels in shaping the input-output response of excitable systems, on a time scale of seconds, are brought here: slow cumulative adaptation of cortical neurons, and the involvement of slow sodium inactivation in hyperkalemic periodic paralysis. Fleidervish, Friedman and Gutnick (1996) explored the effect of slow sodium conductance cumulative inactivation on the adaptation of mouse and guinea-pig neocortical neurons in slices. They found that repetitive intracellular stimulation at intervals of up to 5 seconds caused slow cumulative adaptation of spike firing which was associated with a use-dependent removal of sodium channels from the available pool by a process of inactivation. Their kinetic analysis allowed them to suggest that in the mammalian cortex each action potential leaves in its wake a prolonged period of a slightly decreased pool of available sodium channels. This aftermath lasts several *seconds* thus allowing cells to integrate previous activities on a time scale of short-term memory. Fleidervish, Friedman and Gutnick (1996) suggested that slow sodium inactivation plays a role also in the phenomenon of frequency dependence of back propagation in dendritic trees (Spruston et al., 1995). Similar accommodation effects were recently suggested by Elliot (1997) to play a role in the sculpturing of typical spontaneous bursts of sensory neurons.

Cummins and Sigworth (1996) provided evidence for the involvement of impaired slow changes in the availability of sodium channels in hyperkalemic periodic paralysis (HPP). HPP patients experience episodic weakness which lasts for hours. During this period the muscles are depolarized and unable to produce action potentials. The phenomenon is attributed to a defective rapid inactivation machinery (Cannon, 1996*b;* and references therein). Ruff (1994) submitted that although a defective rapid inactivation can explain the development of a *short* episodic weakness, it is insufficient to explain *long* periods of depolarized weak muscles. Ruff (1994) argued that there must be, in addition to the defective rapid inactivation, a defect in the machinery of slow inactivation. Cummins and Sigworth (1996) tested Ruff's inference in cloned muscle sodium channels containing the disease mutation. They showed that this mu-

tant recovers very quickly from prolonged depolarization. This rapid recovery from slow inactivation results in ~25% of sodium channels being available for activation even after a depolarization which lasts as long as 20 minutes. For comparison, only 3% of the wild-type channel are available under the same conditions. As pointed out by Cannon (1996*a*) this study of Cummins and Sigworth (1996) provides a clear example that an impairment of slow inactivation may predispose a cell to prolonged periods of depolarization and failure of impulse generation. Cannon speculates that the presence of slow inactivation defect determines which of the many known mutations that interfere with fast inactivation of the skeletal muscle lead to prolonged episodes of weakness.

In the studies described above, the involvement of slowly inactivating conductance in temporal integration is inferred from indirect electrophysiological data. To categorically demonstrate that a slowly inactivating conductance confers nonsynaptic, long-lasting, activity-dependent changes of excitable membrane input-output function, one needs to have a control over the slowly inactivating conductance while measuring the dynamics of excitability at the relevant time scales. Studies of this kind are described below.

Cellular Short-term Memory in Experimental Model Systems

Lester and his colleagues (Hsu et al., 1993) expressed a combination of sodium and potassium voltage-gated ion channels of excitable membranes in Chinese hamster ovary cells using a vaccinia virus vector system. Doing so, they generated synthetic excitable cells with predefined mixtures of excitable ion channels of known kinetics. In these 'synthetic neurons,' slow and incomplete inactivation of voltage-gated ion channels regulates the input-output function in a way that is predicted from the microscopic kinetics of the channels. Since they expressed the ShakerB potassium channels (a relatively poor temporal integrator of episodic input, *see* Fig. 1) Lester and his colleagues had to induce inactivation by applying long lasting depolarizing input. They demonstrated that with appropriate sodium to potassium current ratios, maintained stimulation eventually led to a change in the voltage response of the membrane. In a somewhat similar approach, Marom, Toib and Braun (1995) took advantage of the *Xenopus* expression system in order to produce miniature point excitable systems in detached patches of membranes from oocytes that were injected with mRNA coding for sodium and potassium channels. In this case, Kv1.3 channels were used, allowing to reveal the cumulative nature of episodic integration (Marom & Abbott, 1994). These point systems exhibit dynamics on time scales that are several orders of magnitude longer then a single spike.

Perhaps the most compelling evidence for the involvement of slow gating processes in activity dependent modulation of excitability, at time scales of many seconds, comes from a recent study by Turrigiano, Marder and Abbott (1996). They used the dynamic clamp method (Sharp et al., 1993) in order to incorporate, in a controlled manner, a computer generated Kv1.3 potassium conductance into a cultured stomatogastric ganglion neuron. These electronically compact neurons move between two modes of firing (tonic or bursting) according to the kinetics and magnitudes of their driving conductances (Turrigiano, Lemasson & Marder, 1995). As discussed above, the Kv1.3 conductance exhibits state-dependent cumulative inactivation due to a relatively high $\gamma/(\beta(v) + \gamma)$ ratio (Marom & Levitan, 1994). In simulation studies in which this channel was added to a single compartment Hodgkin-Huxley cell, the presence of Kv1.3 conductance produced temporal integration and memory effect that lasted seconds (Marom & Abbott, 1994) to 'lifetime' (Marom, 1994). In their dynamic-clamp study, Turrigiano et al. (1996) prove, experimentally, that the slow kinetics of a cumulative inactivating potassium conductance can produce a form of cellular short-term memory that is independent of any changes in synaptic efficacy. Recently, a similar effect was demonstrated when Kv1.3 was expressed transgenically in *Xenopus* signal neurons (D. Dagan, *unpublished results*).

Effects of Slow Gating Processes on Resting Membrane Potential and its Fluctuations

So far we have discussed changes in the threshold, due to slow changes in the availability of voltage-gated sodium and potassium channels, as a function of past electrical activity. It is important to note that excitability is also a function of the dynamics of the resting membrane potential. In recent years there has been a growing interest in the fluctuations of resting membrane potential in the context of neural coding (e.g., Softkey & Koch, 1993; Shadlen & Newsome, 1994; Mainen & Sejnowski, 1996). One key question in this context is—how much of the 'noisy' nature of membrane potential in neurons is contributed by intrinsic stochastic mechanisms of ion channels. This is not a simple question because of the following causal circularity: ion channels need to be available in order to affect the membrane potential, but at the same time the membrane potential determines how many ion channels are available. When the density of the channels and their complex kinetics, including state-dependent inactivation, are considered, the path to the answer is even more tortuous. Under natural conditions the value of resting potential and its fluctuations are affected by many types of voltage-gated channels. Some insight into the principles that govern the interaction of voltage-gated channels and resting membrane potential

can be gained by examining the case of potassium channels. It is accepted that voltage-gated potassium channels participate in the stabilization of resting membrane potential in cells of many tissues. For example, the resting membrane potential in airway smooth muscle cells (Fleischmann, Washabau & Kotlikoff, 1993), dorsal root ganglion cells (Wang, Van den Berg & Ypey, 1994), and human retinal pigment epithelial cells (Hughes, Takahia & Segawa, 1995), was shown to be controlled by voltage-gated potassium channels. However, as shown below, this view that attributes a stabilizing role to voltage-gated potassium channels might be misleading when state-dependent slow inactivation is considered. Recently, Braun, Marom and colleagues (Salman, Soen & Braun, 1996; Marom et al., 1996) expressed slowly inactivating ShakerB mutant channels that lack fast inactivation in oocyte membranes, and systematically studied the behavior of the membrane potential of detached patch membranes as a function of channel density. They concluded that the mean value and the fluctuation of the membrane potential interact with the channel density and kinetics in the following manner: At high channel densities, the mean value and fluctuations of the resting membrane potential are sensitive to the activation-deactivation kinetics (the $C \Leftrightarrow O$ reaction) but are not sensitive to the inactivation kinetics; in contrast, at low channel densities, the mean value and fluctuations of resting membrane potential are very sensitive to the rates of entry and recovery from inactivation (the $O \Leftrightarrow I$ reaction). As will become clear in a minute, how much is 'high' and how much is 'low' depends on the kinetics of inactivation of the channel: In the case of Shaker B, for example, a channel density of slightly more than $1/\mu m^2$ is considered high (Marom et al., 1996; Salman et al., 1996). At high channel densities, if an excessive number of potassium channels become open, the membrane 'slips' downwards to more hyperpolarized potentials, forcing the potassium channels to become closed, and allow the leak conductance to 'pull' the membrane potential back to more depolarized levels. If, on the other hand, the membrane potential is perturbed to depolarized levels, more voltage-gated potassium channels become open, bringing the potential down again. It is easy to see that when the density of channels is high, the membrane potential value around which the membrane stabilizes is dictated by the voltage-dependent rates of the $C \Leftrightarrow O$ reaction. One is tempted to speculate that the resting potential of many types of excitable cells is at ~−60 mV because the $C \Leftrightarrow O$ kinetics of many potassium channels creates a characteristic knee at ~−60 mV of their open probability *vs.* voltage curve (e.g., Hodgkin & Huxley, 1952; Hoshi & Aldrich, 1988; Stühmer et al., 1989; Kirsch et al., 1991; Perozo et al., 1992; Marom et al., 1996). What happens at lower channel densities? Here, each channel counts, and the stochasticity of the channels becomes evident in the form of membrane potential fluctuations; even a small number of channels that become inactive change the balance between the potassium and the leak currents, in favor of the later. Consequently, the membrane potential becomes more depolarized, forcing more channels to inactivate and depolarize the membrane even further. This avalanche phenomenon might play an important role in the generation of 'noisy' membrane potential. This is particularly relevant in cells that express channels with a high effective inactivation rate, in cellular regions with a small density of voltage-gated channels, or after a period of intense activity.

Computer Modeling of Voltage-independent State-dependent Inactivation While Adhering to the Hodgkin-Huxley Formalism

Inactivation of many ion channels conform to the simplified three-states description presented above. This state-dependent inactivation can not be described by the standard Hodgkin and Huxley formalism, which assumes independence of reactions. Several researchers overcome this problematic issue by substituting the three-states scheme, in which a voltage-independent inactivation is coupled to a voltage-dependent activation, with two separate voltage-dependent reactions. In other words, they 'transform' the coupled voltage-independent inactivation to an autonomous voltage-dependent inactivation. However, this transformation does not achieve, even qualitatively, a correct macroscopic description in the case of cumulative inactivation. Marom and Abbott (1994) extended the Hodgkin-Huxley formalism for modeling macroscopic behavior to account for state-dependent inactivation. They expressed the coupling between opening and inactivation by introducing the probability of being open as a parameter of the rate of inactivation. Note that the effective rate of inactivation in a three-states coupled scheme should be equal to the fraction of open channels (n according to the Hodgkin-Huxley terminology) times the rate of inactivation (γ). Therefore, in the simple case of a three-states scheme dh/dt becomes equal to $\delta \cdot (1-h) - \gamma \cdot n \cdot h$. Marom and Abbott (1994) used this method to provide an accurate description of cumulative inactivation of potassium channels, as well as to gain new insight into other state-dependent voltage-independent inactivation processes in sodium channels. In single compartment model neurons, the inclusion of state-dependent cumulative inactivation produced a novel short-term memory effect and firing delays similar to those seen in hippocampal neurons, as well as a long-term memory effect (Marom, 1994) that is independent of changes in synaptic efficacy.

Multiplicity of Inactivation States: Scaling of Rates?

One of the most intriguing observations in excitable membrane studies during the past fifty years is that elec-

trophysiologists do longer experiments and report ever increasing time constants for inactivation. Hodgkin and Huxley were content with the millisecond sodium inactivation and no potassium inactivation. Since the 60s, many reports of slower inactivation of sodium channels appeared, mostly at the tens of millisecond time scale, and potassium channel inactivation entered the arena. Most of these slower processes (excluding the A-type 'fast' potassium inactivation (Connor & Stevens, 1971)) did not make a significant impression on neurobiologists who are interested in encoding of information by neurons, since these slower changes of conductance were usually attributed to voltage-sensitive gates (rather than to coupled, voltage-insensitive cumulative inactivation): It is easy to see that most excitable membranes do not depolarize for long enough in order to affect and be affected by slow voltage-sensitive gating mechanisms. This fact did not stand in the way of membrane biophysicists to discover even longer time scales of inactivation: To date, the inactivation of the sodium channel, for example, is known to proceed on time scales ranging from milliseconds to many minutes (e.g., Stühmer et al., 1987; Ruben et al., 1992; Cummins & Sigworth, 1996). The emerging picture is that the internal structure of state *I* of the three-states kinetic scheme actually includes a set of many states so that one can imagine a cascade of the form

$$C \overset{f(v)}{\Longleftrightarrow} O \Leftrightarrow I_1 \Leftrightarrow I_2 \Leftrightarrow I_3 \Leftrightarrow \ldots \Leftrightarrow I_{n-1} \Leftrightarrow I_n.$$

One wonders if there is some deep physiological meaning for this multiplicity of inactivation states. An interesting treatment of a similar chain of states was explored theoretically by Millhauser, Salpeter and Oswald (1988) who showed that when so many states are coupled to each other there is a scaling relation between the duration of activity (or in our terms duration of depolarizing pulse or duration of pulse series) and the effective rate of *recovery* from inactivation (Fig. 2). Upon membrane activation the *I* states absorb channels so that as the duration of the activity becomes longer, the distribution of the channels shifts farther and farther to the right. Upon hyperpolarization, *C* is an absorbing state, and the effective recovery rate becomes dependent on the distribution of channels between the different *I* states. These relations between activity and recovery rates imply that rates (derivatives), and not simply quantities (populations) can serve as integrators of past electrical activity, an intriguing possibility that needs more exploration.

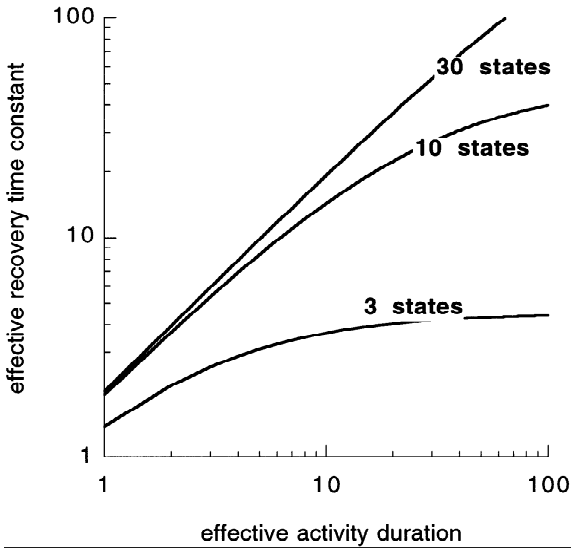

Fig. 2. A demonstration of how the number of inactivation states in a serial cascade affects the effective rate of recovery from inactivation as a function of activity duration. Note that this simulation assumes a pure serial cascade organization with identical rates along the cascade. If these constraints are relaxed, it takes less states to obtain the same results. The computer simulation is based on Millhauser et al. (1988); time units are arbitrary.

A Concluding Remark

Modulation of excitability at the level of a single cell is the outcome of two classes of mechanisms: biochemical and physicochemical. Biochemical mechanisms involve enzymatic reactions ranging from gene expression regulation down to ion channel phosphorylation. The characteristic time scale of biochemical processes is long in comparison to the time envelope of a single action potential. These reactions are very complex and not unique to excitable systems. Physicochemical mechanisms for modulation of excitability are intrinsic to the voltage-gated ion channel proteins that underlie excitability. Fifty years ago, when physiology of excitable membranes was at its prime, knowledge of physicochemical mechanisms was confined to the time scale of a single action potential. The separation between the time scales of physicochemical and biochemical processes led to the following inference: While intrinsic gating processes in voltage-gated ion channels 'shape' the envelope of the action potential and refractory period, biochemical processes are responsible for longer time scale activity-dependent effects at the single cell level. As demonstrated in this review, it is evident that the repertoire of physicochemical kinetic mechanisms of ion channels is sufficiently rich to support modulation of input-output function of excitable cells over a very wide range of time scales. This means that the mechanism of excitability cannot be treated as an inert process that needs intracellular biochemical assistance in order to adapt. Processes such as potentiation and adaptation (depression) can be shown to occur already at the level of the machinery of excitability. Time will tell how the ever-increasing com-

plexity which seems to be level independent comes to form the patterns that we see in extended excitable systems.

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