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Perspective

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A biophysical perspective on the resilience of neuronal excitability across timescales

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Abstract

Neuronal membrane excitability must be resilient to perturbations that can take place over timescales from milliseconds to months (or even years in long-lived animals). A great deal of attention has been paid to classes of homeostatic mechanisms that contribute to long-term maintenance of neuronal excitability through processes that alter a key structural parameter: the number of ion channel proteins present at the neuronal membrane. However, less attention has been paid to the self-regulating 'automatic' mechanisms that contribute to neuronal resilience by virtue of the kinetic properties of ion channels themselves. Here, we propose that these two sets of mechanisms are complementary instantiations of feedback control, together enabling resilience on a wide range of temporal scales. We further point to several methodological and conceptual challenges entailed in studying these processes — both of which involve enmeshed feedback control loops — and consider the consequences of these mechanisms of resilience.

Sections

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Introduction

"The living being is stable. It must be in order not to be destroyed, dissolved, or disintegrated by the colossal forces, often adverse, which surround it. By an apparent contradiction it maintains its stability only if it is excitable and capable of modifying itself according to external stimuli and adjusting its response to the stimulation. In a sense it is stable because it is modifiable — the slight instability is the necessary condition for the true stability of the organism."
Walter B. Cannon[1]

It is commonly understood that the behaviours of biological systems — including cells, networks, organs and organisms — are resilient to variations in the constituent elements that determine their function, absorbing a wide range of parameter changes, although maintaining overall functionality (Box 1). At the same time, biological systems must be plastic and capable of adjusting their behaviour when faced with new challenges[2,3]. The history of neuroscience is rich with attempts to illuminate the molecular and cellular mechanisms that enable neurons and circuits to cope with the apparent contradictions of these resilience–plasticity demands. These mechanisms operate on different timescales — from relatively rapid processes inherent in the kinetic properties of ion channel and receptor proteins to slower processes that almost certainly engage signal transduction, transcription and translation — and contribute to both health and disease[4–11].

Membrane excitability is a fundamental physiological phenomenon that drives the operation of the heart, endocrine, muscle and neural tissues, in which voltage-dependent changes in ionic conductances lead to action potentials. At least two opposing conductances are required to generate excitability, defined by their corresponding ionic gradients across the cell membrane: the depolarization is mainly caused by Na^+ or Ca^{2+} conductances, and the hyperpolarization is primarily driven by K^+ conductances. The flow of ions down their electrochemical gradients is modulated by voltage-dependent reaction rates that influence the probability that ion channel proteins will reside in the open (that is, conducting) state. The canonical conductance-based formalism proposed by Hodgkin and Huxley[12] in the 1950s and its various macroscopic and stochastic extensions[13–17] provided a theoretical framework that captures these biophysical mechanisms. At the core of the formalism is the Hodgkin–Huxley cycle[18], which translates membrane voltage to reaction rates that, in turn, change the probability that the membrane will be in different conducting states and thereby modulate ionic currents and change membrane voltage. However, the past 70 years of experimental analyses have revealed that some of the assumptions underlying these original models were oversimplifications. A spectrum of single-compartment models were therefore devised that facilitated integration of experimental data[15,19–30] and offered invaluable insights into the mathematical and physical nature of excitability[31].

The resilience of neuronal membrane excitability to extensive variations in conductances and kinetics was first observed and reported by Hodgkin and Huxley[12]. In those early measurements, as well as in many measurements and analyses conducted over the subsequent half a century, the time–voltage envelope of action potentials and their propagation along axons appeared similar, despite twofold or even threefold changes in the underlying densities of membrane proteins[26,32].

Experimental and theoretical analyses of neuronal resilience often borrow explicitly or implicitly from control theory concepts. In particular, there has been a focus on regulation by negative feedback[4,7,9], a well-developed theme in engineering[33]. It should be noted, however, that there are several fundamental differences between the challenges presented to an engineer with regard to regulation by feedback control and those facing physiologists who study how neuronal systems foster resilience in response to perturbations. For engineers, feedback control is a product that must be designed (engineered) and is implemented by a machinery that maintains a user-defined target output given a priori, well-defined ranges of inputs and disturbances. For physiologists, by contrast, feedback control is something that they must discover (via reverse engineering) and is a process that maintains (via mechanisms that are to be identified) the functional behaviour of developing, complex, nonlinear systems that continuously face unforeseen challenges. Thus, in the hands of the physiologist, the concept of feedback control is mostly used as a qualitative metaphor to generate hypotheses about regulation and maintenance of function, rather than a fully developed quantitative mechanistic explanation. Basic feedback control flow diagrams have proven useful in contextualizing research efforts to understand the resilience of membrane excitability[4,5,7,8,11,34]. However, as is the case with all metaphors, alongside its analytic power, the implementation of control theory in physiology entails subtleties, several of which are discussed subsequently in relation to the resilience of neuronal membrane excitability under widely variable conditions.

A basic feedback control process reads the output of a system, compares it to a desired target value and translates the outcome of that comparison to adjust relevant parameters. It involves sensors, actuators, comparators and other elements. In cell biology, there is an unspoken premise that these different blocks in the canonical engineered negative feedback loop (Fig. 1a) are implemented by different molecules. Some of the first homeostatic models of the regulation of intrinsic excitability[34,35] reflected this assumption. In these models, the protein molecules responsible for spike generation are separate from the proteins and signalling molecules (such as calcium ions) that sense the spiking activity and compare it to some 'target' behaviour (with information about this target reaching the system via yet another, separate molecular entity); deviations from the target performance give rise to changes in the numbers of ion channels in the membrane.

In this Perspective article, we start by describing the homeostatic regulation of channel densities in the membrane. We introduce the concept of homeostatic regulation from a computational, biophysical outlook, with the basic feedback control flow diagram in mind. The complex biochemical control of intracellular and intercellular signalling pathways that mediates this homeostatic regulation involves protein synthesis, post-translational modification, trafficking, endocytosis and degradation. (Although beyond the scope of the present essay, we refer the reader to further discussion of these processes in refs. 4,8,9,36,37.) Next, we describe a complementary biophysical mechanism for resilience, a mode of self-regulation of proteins of existing ion channels that emerge automatically from local feedback that relies on their slow kinetic properties. In the latter case, the system to be controlled — the mechanism of spike generation — is not separate from the control system, and no change in the numbers of channel proteins in neuronal membranes is required. We propose that resilience of neuronal excitability is achieved by using both modes of regulation: rapid, relatively simple self-regulating automatic processes that rely on the kinetics of proteins already present at the neuronal membrane and slower mechanisms that modulate the number of relevant membrane proteins. We suggest that cells take advantage of the wide repertoire of molecular machinery available to them to maintain function over timescales from milliseconds to years. We discuss, in this context, the

Box 1

Stability, robustness, resilience and adaptation

Stability

In this article, we refer to the stability of a system as its ability to return to the original, steady-state, balanced relations between the forces acting in it, after removal of a displacing disturbance. To illustrate this, we can think of a ball placed in a concave well. However, the concept of stability is less useful in thinking about biological systems, mainly because it is ahistorical (once back in place, the past of the system is irrelevant). The machinery underlying maintenance of biological functionality invariably relies on multiple processes in a high-dimensional parameter space, making it virtually impossible for a biological system to return to its original 'same' state. Therefore, we eschew the term stability when describing maintenance of biological structures and functions.

Robustness and resilience

Robustness and resilience are often used interchangeably. Here, we use robustness to describe intrinsic means enabling the system to withstand disruptions or input changes. For example, the pressure hull of a submarine is robust; it withstands the outside pressure while keeping normal atmospheric pressure inside. There are many examples of robust biological systems. For instance, the *lac* operon in *Escherichia coli* enables the cell to switch between glucose and lactose as sources of fuel, and the rod and cone cells in the retina enable vision in different ambient light intensities. We use resilience to describe the capacity of a system to recover from a wide range of parameter changes. Although the system is affected by these changes, it responds by adjusting its internal states to maintain overall function. Examples include the resilience of excitability in single neurons or neuronal networks to variations in ionic channel expression, stimulation frequency, changes in temperature, salinity and pH.

Adaptation

Adaptation carries many meanings. Sometimes, it denotes changes that promote system functionality. This concept is ubiquitous in neurophysiological texts, in which it is often used to refer to short-term changes in a neuron or process that occur in the continued presence of a stimulus.

concept of degeneracy — that neurons use multiple solutions to produce similar behaviour[11,38–43] — and highlight a possible physiological consequence of such degeneracy: the existence of cryptic or hidden effects that are seen only when systems are perturbed. Finally, we point to experimental challenges in the study of resilience and consider future directions in this field.

Regulation of channel number

Understanding the maintenance of excitability in the face of parametric variations is challenging, especially because extensive biophysical analyses have revealed that heart and neuronal cells may have as many as 10–15 different voltage-dependent conductances that contribute to their activity, beyond the basic two (Na^+ and K^+) described by Hodgkin and Huxley[44,45]. The genes that code for these different conductances can be differentially expressed in different tissues, in different neuronal types and in different parts of a given neuron. Furthermore, their expression levels can change over time[46–48]. The challenge of understanding the regulation of many channel types became immediately apparent when researchers tried to move from the relatively simple Hodgkin–Huxley model of axons to more complex models that incorporate additional membrane channels[49–51]. These so-called realistic models were invariably difficult to hand-tune, making it clear that biological neurons must implement mechanisms for tuning their own excitability. This, in turn, led to the development of homeostatic models that instantiated generic negative feedback control loops to tune the membrane densities of ion channel proteins[34,35,52].

One such generic feedback control scheme is presented in Fig. 1a: in this case, the scheme is instantiated for the case of maintaining a one-stimulus–one-spike response by changing the number of sodium and potassium channel proteins expressed in the membrane. Let us examine this scheme with a somewhat critical eye. The controlled system (the 'plant' in control theory terminology) is the mechanism of excitability that generates action potentials. In the life sciences, unlike engineering (in which plants are well defined and often the product of design), designation of a given physiological function as a system to be controlled involves the perspectives and assumptions of the observer. The input to the plant is an ionic current that, in biological neurons, usually results from synaptic activity. The output is a membrane voltage response that may fail to trigger an action potential (not-excitable), generate a single spike in response to the stimulus (excitable, a desired 'target'), or generate a long series of spikes (over-excitable). Here too, defining a target activity reflects the understanding of the observer (or intuition) about 'what really matters to the system', a non-trivial challenge[53,54]. Although the targets of some physiological processes, such as human body temperature, are well defined, defining the molecular and cellular bases of presumed neuronal and circuit targets has thus far been difficult. In all feedback control systems, some aspects of the output must be sensed. Determining the aspect to be sensed is not always straightforward; it requires identifying which of the possible 'reporters' of the output of the system (also known as observables) make biological sense. This might be the subject of control itself (the membrane voltage, for example) or a reflection of it. Furthermore, when identifying an observable, there are issues of specificity. For instance, using intracellular Ca^{2+} concentration as a proxy for electrical activity is common, but how specific is it, given the many processes for which intracellular calcium concentration allegedly serves as an observable? Observables are 'read' by biological proteins (sensors). Sensors may be specific receptor proteins, but this is not necessarily the case. If the observable is acidity, for example, any or every protein could act as a sensor, albeit with different sensitivities (all proteins are sensitive to pH, to a degree). Thus, although in most standard control diagrams, sensors are represented as a separate block from the plant, in real biological contexts, the sensor might be part of the plant; for instance, sodium channel proteins are part of the plant (according to the Hodgkin–Huxley model), but their functional state (open, closed or inactive) is sensitive to the output of the plant. Error signals are key to classic feedback control schemes, but in biological systems it is not always evident how the error signal is 'computed'. Given an error signal, a feedback control scheme translates the error

to a control signal, the actual 'tuning instructions' that are delivered to the plant. In the case of maintaining the Hodgkin–Huxley plant in a one-stimulus–one-spike mode, these tuning instructions change the density of Na^+ and K^+ channels in the membrane.

The aforementioned (partial) list of ambiguities involved in application of the generic feedback control loop to cell-biological settings suggests that it is one thing for an engineer to construct a feedback control process with electrical and mechanical elements that have defined and known properties. It is entirely different to figure out a naturally occurring biological control loop. In the latter case, we need to identify or guess the targets and observables, without knowing the mechanisms for computing error and control signals, in a network of coupled controllers with nonlinear and time-variant components. It is often not clear what kind of experimental designs are needed when facing such a daunting challenge. Nonetheless, the large body of experimental and computational work that has taken place in this field has been able to go some way towards resolving these ambiguities[5,8,9,37,55–65], supporting the idea that the control of neuronal excitability can be homeostatically regulated by the insertion into and removal from the membrane of channel proteins. Here, we highlight several milestones in the development of computational models that can self-assemble (that is, self-tune) to find a set of conductance densities that will result in a desired output pattern; these models have provided insight into intricacies involved in homeostatic mechanisms.

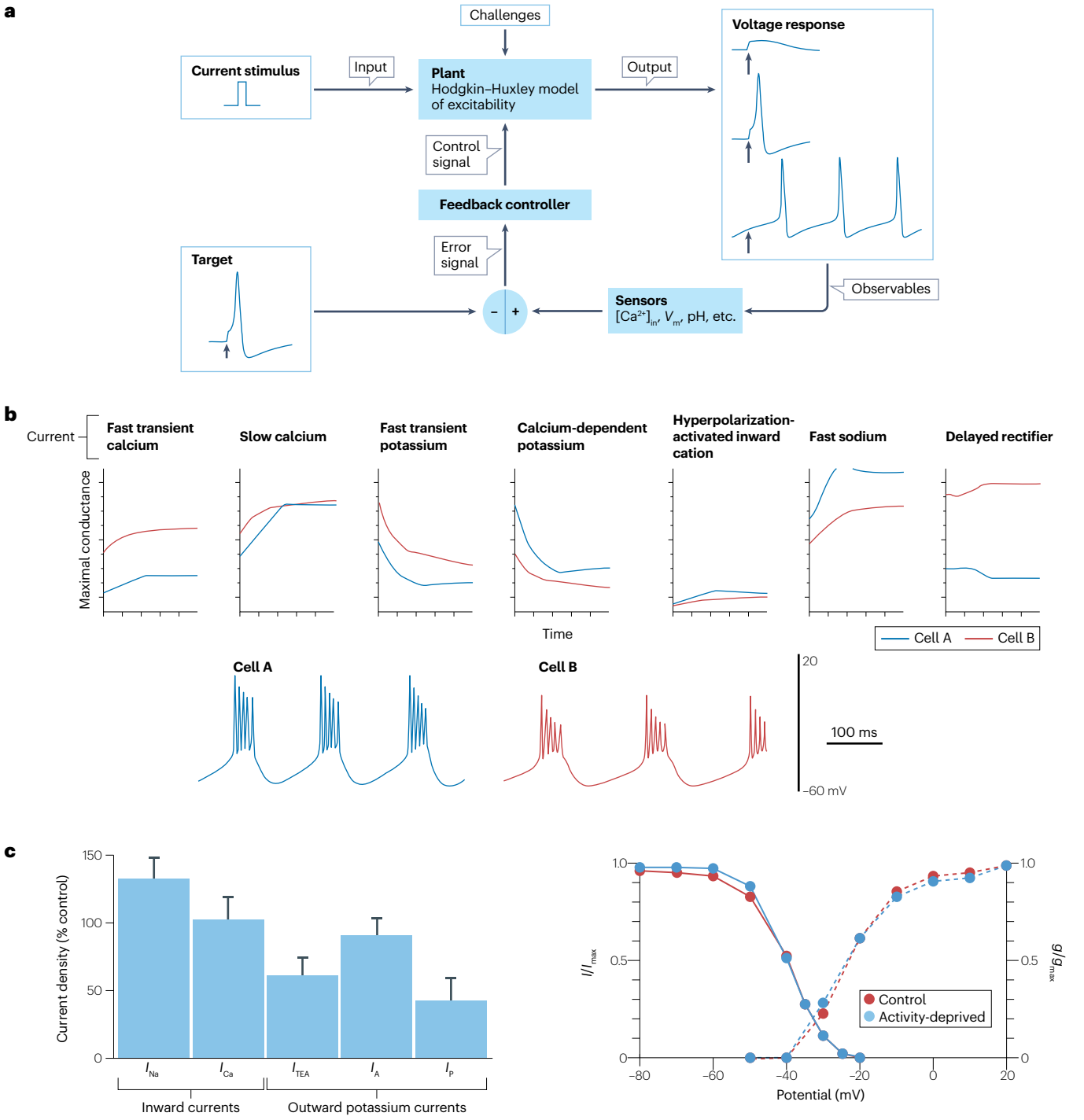
The earliest of these models consisted of a single negative feedback controller in which the mean intracellular Ca^{2+} concentration was used both as an integrated sensor of the activity of neuron and to control the insertion and removal of ion channels in the membrane. In response to external perturbations, this model adjusts its conductance densities to return the neuron to its target activity level[34,52] and thus provides a machinery that ensures resilience. Because this initial homeostatic model could not distinguish between different patterns of activity that resulted in the same mean intracellular Ca^{2+} concentrations, a second-generation model[35] was constructed with three sensors: these provided fast, slow and much-slower filters of the intracellular Ca^{2+} dynamics, introducing sensitivity to different temporal aspects of deviation from the target activity pattern. This model is akin to a proportional-integral controller, in which the deviation from a target value is derived from a combination of instantaneous 'error' and the accumulated, integrated errors over longer timescales. This model generates multiple solutions (different sets of conduction densities) with similar capacity to restore neuronal activity to target levels (it is degenerate), it self-assembles (Fig. 1b) and it recovers from numerous perturbations. However, the model in its original formulation had a flaw, as interactions between the three sensors eventually — after variable periods of relatively stable behaviour — resulted in exponentially increasing conductances. Recent work provides a remedy to this by bounding the conductances, reflecting the fact that the density of ionic channels is restricted by the possible numbers of proteins that can fit in biological membranes[66].

A third-generation, more biologically realistic, class of homeostatic model was developed in which each type of channel was assigned a specific turnover rate[67–69]. These models also self-assemble, generate multiple solutions and, importantly, generate correlations in the conductance densities of different ion channels that are similar to those measured experimentally[11,70–73]. This suggests that the correlations that are commonly seen in experimental data are a consequence of co-regulation of channel membrane expression and that these patterns of co-regulation ensure that a given cell type can display multiple, degenerate sets of channel densities within the membrane, but are restricted to a manifold that represents a small region in parameter space.

A recent theoretical and experimental analysis[74] addressed an aspect of membrane excitability that was not dealt with in the aforementioned homeostatic models: ionic channel pleiotropy — that is, the involvement of a given channel in two or more apparently unrelated cellular functions. The authors of this work concluded that, among the large set of degenerate solutions that satisfy the target value of one property (the 'single-output solution set'), only few satisfy the target value for other properties. Thus, resilience of excitability in pleiotropic systems entails a further challenge: identification of the few combinations of channel densities that would not interfere with cellular functions other than excitability. Moreover, they showed that, to successfully co-regulate n different properties, the number of adjustable conductances should be larger than n. These results — derived for the case of a space-less, single-compartment setting — further highlight the problems entailed in the control of closed loops that are enmeshed (that is, those in which the same individual components serve different, possibly mutually exclusive, roles).

The strength of a model is revealed by its ability to offer testable hypotheses. The models of homeostatic control described earlier initiated experiments designed to examine the mechanisms underlying the resilience of excitability at various levels of organization, from protein trafficking and neurotransmitter release to neuromodulation and neural circuits, in health and disease[4,5,8,9,36,37,58,73,75]. For example, a natural prediction of the homeostatic models of resilience of neuronal excitability described earlier is that long-term alterations of neuronal activity would result in changes of conductance density in the membrane. In a now classic paper[76], rat-cultured cortical pyramidal neurons were treated with tetrodotoxin (TTX), a potent Na^+ channel blocker, for 2 days to suppress their activity. TTX was then washed out and voltage-clamp was used to measure membrane conductances. This treatment did not change the kinetics of the Na^+ conductance but did change the current densities (Fig. 1c): the inward Na^+ current density was increased and the outward K^+ currents were decreased. Moreover, the results illustrated that it is not the Na^+ conductance per se that matters; rather it is the dynamic ratios of all the expressed conductances. This insight was taken further in a beautiful experimental study[77], in which the effects of acute (TTX-induced) partial reduction of Na^+ conductance, as well as long-term (genetically induced) complete deletion of Na^+ conductance, were measured in isolated mouse cerebellar Purkinje neurons. This study showed that the relative contributions of individual currents are variable among cells showing almost identical firing characteristics and that the neurons implement feedback mechanisms that make their spiking behaviour resilient to changes in Na^+ conductance, at both the short and the long timescales. In both cases, these mechanisms compensated for loss of Na^+ conductance by modulating the function (availability for action) of the channels as well as their actual, physical expression. These compensatory processes suggest the presence of a degenerate space of solutions, similar to those proposed in the models described earlier, and provide support for hypotheses (based on these models)[11,78,79] that there are multiple configurations in which the number of ion channels expressed by individual neurons with similar properties can vary considerably.

Several interesting features of neuronal resilience might result from the degeneracy suggested by these experiments. For instance, most biological neurons have multiple forms of Ca^{2+} and K^+ currents that vary and overlap in their voltage and time dependence.



The relative contribution of these currents to spike shape can change as a function of adaptation, membrane potential and temperature (see, for instance, the case of K^+ conductance[80–82]). Aligned with the report described earlier[77], this suggests that neurons use different mechanisms — having different timescales — to maintain function. Stated more generally, neurons and circuits may use several mechanisms that, in principle, could achieve similar outcomes in different contexts. Thus, neurons and circuits need not be hostage to a single process. They can maintain their function through activity-dependent mechanisms that include changes in membrane excitability, synaptic homeostatic plasticity and global modulatory network effects. Many excellent studies and reviews have been published that point to a multiplicity of homeostatic regulatory mechanisms, acting to provide short-term and long-term feedback at different levels. These include the homeostatic regulation of intrinsic excitability, synaptic scaling and presynaptic regulation of transmitter release[5,8,9,37,55–65].

Fig. 1 | Resilience of excitability by feedback regulation of channel protein expression. a, A generic feedback control scheme instantiated for the case of maintaining a one-stimulus–one-spike response by changing the number of sodium and potassium channel proteins expressed in the membrane. The system to be controlled (plant) is the mechanism of spike generation (in this case, the Hodgkin–Huxley model of excitability[12]). Its output is a voltage response that may be sensed by various means (for example, through changes in intracellular calcium (Ca^{2+}) levels, membrane voltage V_m or pH) and compared with a target activity. An error signal, reflecting the difference between the actual voltage response and the target activity, is translated by the feedback controller to a control signal (which drives an increase or decrease in channel protein membrane expression). Maintaining the target activity is challenged by parametric fluctuations, such as changes in channel kinetic parameters, the ratio between the numbers of different channel proteins, membrane capacitance and leak conductance during massive cell growth, and movement or contact of the cell with biological matrices that affect membrane surface tension. **b**, Implementation of a generic feedback control scheme in a model that self-assembles conductance densities, resulting in a desired output pattern[35]. This model generates multiple solutions with similar behaviour (it is degenerate): different initial conditions generate different combinations of conductances (top panel), resulting — at the end of the adjustment process — in two similar spiking behaviours in cell A and cell B (bottom panel). The model dynamically adjusts the maximal conductances of seven active currents (fast transient calcium (CaT), slow calcium (CaS), fast transient potassium, calcium-dependent potassium, hyperpolarization-activated inward cation (H), fast sodium conductance and delayed rectifier) over a period of 15 s, as shown in the top row of plots. CaT, CaS and H extend from 0 mS nF^{-1} to 2 mS nF^{-1}, whereas the range is 0–50 mS nF^{-1} for all other conductances. **c**, Such homeostatic regulation by control of membrane protein expression has been experimentally validated[76]. Increased inward Na^+ current density and decreased outward K^+ currents (I_{TEA}, I_A and I_P) were observed (left panel) in rat-cultured cortical neurons following tetrodotoxin-induced suppression of excitability for 2 days. The kinetics of the Na^+ conductance were not affected (right panel). Part **b** is adapted with permission from ref. 35, Society for Neuroscience. Part **c** is adapted from ref. 76, Springer Nature Limited.

Kinetic-based regulation

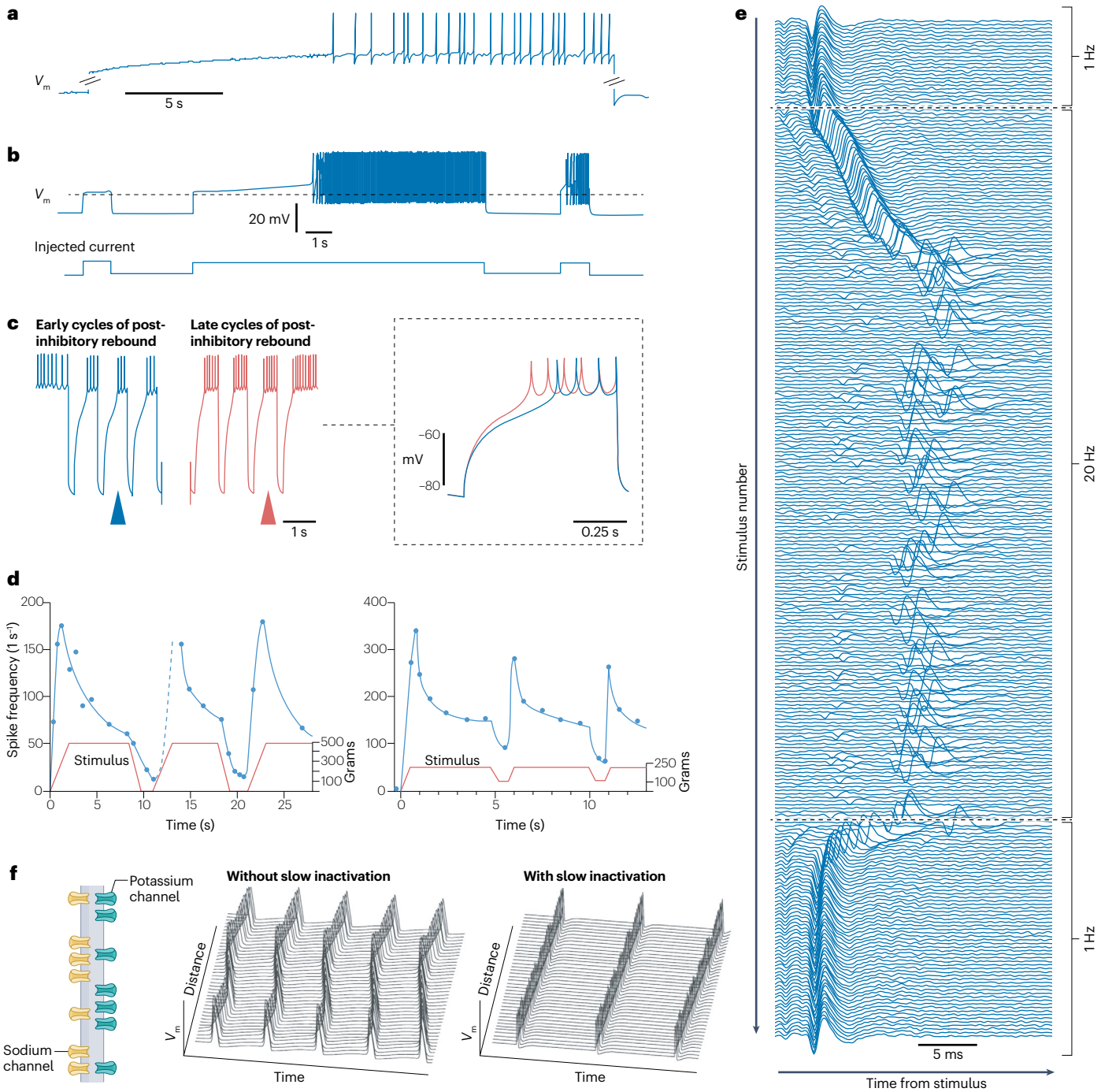
The aforementioned computational analyses use the homeostatic control framework to explain the regulation of structural parameters — the actual number of channel proteins in the membrane or their maximal conductances. Successful as they are, these models do not consider the rich and complex nature of ion channel proteins[6,83–89] and in particular do not take into account the existence of mixed voltage-dependent and voltage-independent reaction rates that span a wide temporal range that extends from sub-milliseconds to minutes and more (effectively equivalent to a continuum of scales)[89–94]. This complex feature of ion channel proteins opens a window to a complementary kind of feedback control — kinetic-based regulation — that supports resilience of spiking activity.

Activity-dependent reversible changes in spiking behaviour owing to the kinetic properties of channels and receptors occur over timescales that are orders of magnitude slower than the timescales over which an action potential is generated (Fig. 2a–c). This has been known ever since Adrian and Zotterman's classic series of papers in 1926 (refs. 95–97) (a 'must read' for every student of neurophysiology), which were the first to demonstrate spikes and their binary nature, to coin the term 'all-or-none' and to show the capacity of spiking activity to represent and adapt to features of sensory stimuli by means of temporal firing patterns, as well as by changes in the rate of spiking (Fig. 2d) . Kinetic-based regulation involves a battery of interacting processes in channel proteins and their modulation. Here, we describe one elementary kinetic-based mechanism underlying resilience that is intrinsic to the voltage-sensitive Na^+ channel.

Traditionally, the kinetics of voltage-sensitive Na^+ channels were assumed to be important only for the duration (on the millisecond scale) of a single spike. The common conception was that nothing in the present activity of Na^+ channels shows traces of their past activity beyond the timescale of a spike and the refractory period. But for many years, biophysicists who study the Na^+ channels that are critical for neuronal spike generation have shown that these channels do retain a deep 'memory' of past activation. This is expressed in a special kind of behaviour called slow inactivation, which is separate from the rapid inactivation (Hodgkin–Huxley's *h*-gate) that operates at the millisecond scale[84,88–90,93,94,98–100]. These observations clearly showed that slow inactivation spans a wide, practically inexhaustible, range of timescales; the longer the channels are activated, the slower the time constants for recovery from slow inactivation. There seems to be no temporal ceiling to this effect, which can be expressed as a power-law relationship between the duration of activation (T) and the time constant of recovery: $\tau = pT^D$, in which p is a constant kinetic set point and D, the scaling power, ranges from 0.5 to 0.8 (refs. 89,90,94). The importance of this power-law relationship may be appreciated when expressed in numbers: for instance, in the case of Nav1.2, the most abundantly expressed Na^+ channel isoform in the mammalian brain, the measured power-law relationship[89] translates to a 42-ms time constant for recovery from slow inactivation following a voltage clamp depolarization of 30 ms; it is 270 ms following a 300-ms depolarization, and 1.7 s following 3 s of depolarization. The phenomenon of slow inactivation is prominent when depolarizations are delivered in trains or bursts[90,94,101,102]. Because inactivated channels cannot contribute to the initiation of a spike, this translates to a refractory period that smoothly 'scales' with the duration and intensity of past depolarizations.

Although the exact mechanism of slow inactivation is still not fully understood[101,103], we know enough to picture it in broad terms. Negative membrane potential is essential for the Na^+ channel protein to be properly folded: that is, for it to have a 3D structure that responds to depolarization by opening and allowing ions to flow[101,103]. In other words, negative membrane potential organizes the proper, functional structure of the protein. By contrast, depolarization favours distortion of the selectivity filter of a channel and a marked breakdown of the fourfold symmetry around the central axis of the protein[101]. We hypothesize that this relationship between membrane potential and channel folding–unfolding dynamics has a powerful role as both the sensor and the control signal in a feedback control loop (Fig. 3). Imagine the following simplified feedback control process that makes excitability resilient to variations in maximal conductances. In this picture (Fig. 3b), the production and expression of channel proteins by the cell are independent of the number needed for spiking. Instead, the cell produces channels as its metabolic state enables (hence the origin of observed positive correlations in the expression of different channels). If this results in excess inward current, the neuron will be over-excited, independent of input. In this case, the persistent membrane depolarization will induce slow inactivation, effectively 'removing' Na^+ channels from the pool available to participate in spike generation, and the neuron will become less excitable. If the neuron becomes too quiet (not at all excitable, for example), Na^+ channels recover back from their slow inactivated non-available pool and make the neuron excitable again.

Thus, regulation of excitability need not be solely based on changes to the actual number of expressed proteins residing in the



membrane. As discussed in many experimental and modelling studies over the past decades, regulation of excitability may result from the kinetic properties of already expressed proteins and their interactions with each other[26,29,77,88,89,93,94,98,99,104–115]. This mechanism relies on biophysically known properties of channel proteins and covers a range of timescales from milliseconds to minutes and possibly hours. It partially relieves the cell of the challenge of tightly controlling the expression of different types of channel proteins. It does not entail storage of 'target behaviour', nor use of unique sensors, error signals and control signals. It enables local regulation of excitability in axons and dendrites with complicated morphologies and provides a wide range of potential excitability configurations for a cell to assume, while interacting with rich and dynamic inputs in multiple timescales.

There are several consequences of the aforementioned ideas about self-regulated automatic control of Na^+ channels by slow inactivation. One comes from analyses showing that the activity-dependent redistribution of channels between available (A) and non-available (slow inactive) states, $A \leftrightarrow (1 - A)$, may be grossly described by an expression

Fig. 2 | Examples of changes in spiking behaviour owing to kinetic-based regulation of channels and receptors. a, Prolonged depolarization of a rat hippocampal neuron results in an increase in excitation that is delayed owing to the presence of a slowly inactivating potassium (K^+) current[141]. **b**, A similar phenomenon is observed in a cultured stomatogastric ganglion neuron from the spiny lobster, *Panulirus interruptus*, where dynamic clamp is used to add a slowly inactivated and slowly recovering conductance mediated by Kv1.3 potassium channels. In this case, the 'memory' of rapid firing that is induced by a long depolarizing injected current persists for multiple seconds after the removal of the injected current, during which time the neuron does not fire[142]: in response to a subsequent depolarizing current, the latency to first spike is reduced. **c**, Post-inhibitory rebound (PIR) is a phenomenon in which increased excitability is observed after inhibition. The time course of PIR firing changes over many seconds in the lateral pyloric neuron of the stomatogastric ganglion of the crab *Cancer borealis*[143]. The figure shows examples of early (left) and late (right) cycles of PIR firing induced by a dynamic clamp protocol. Inset shows an overlay of the cycles indicated with the blue and red arrows, illustrating that the PIR depends on the history of inhibition. **d**, The canonical 100-year-old result of Adrian and Zotterman[97], showing that reversible changes in spike frequency following stimulus removal can occur over a timescale of many seconds in nerves of the plantar surface of hind foot of a cat. The reversible nature of the phenomenon, taken with the timescales involved, implies that such changes are not due to addition or removal of membrane channels or receptors. **e**, A reversible slow change in the spike delay is seen in an isolated cultured rat cortical neuron, stimulated with a sequence of short (400 µS) electrical pulses[105,107,116]. Shown are extracellular voltage response traces, each 20-mS long. The traces are shown in the order in which they were recorded from top to bottom and temporally aligned to the stimulation time. For visual clarity, only every other trace is plotted. In the example shown, the neuron is stimulated with a 1-Hz sequence for 1 min (top section of the figure, 60 traces). For this stimulation protocol, the response latency is stable and the response is reliable, implying constant excitability. The stimulation rate is then abruptly increased to 20 Hz for 2 min (middle section). After a transient period, in which latency is gradually increased (excitability decreases), the neuron reaches an intermittent steady state, in which it is barely excitable, spiking failures occur, and the response is irregular. When the stimulation rate is decreased back to 1 Hz (bottom section), the latency (excitability) recovers, and the stable steady-state response is restored[116]. The kinetic-based regulation in this case is attributed to slow inactivation of sodium channels[105,107,116]. **f**, A simulated axon with uneven distribution of channels shows discontinuities in its firing rate in the absence of slow inactivation of its Na^+ channels (left), but regular firing in the presence of slow inactivation (right)[115]. Part **a** is adapted from ref. 141, Springer Nature Limited. Part **b** is adapted with permission from ref. 142, APS. Part **c** is adapted with permission from ref. 143; copyright 2010 Society for Neuroscience. Part **d** is adapted with permission from ref. 97, Wiley. Part **e** is adapted with permission from ref. 116, Wiley. Part **f** is adapted from ref. 115, Zang, Y. et al., CC BY 4.0.

familiar from population dynamics[24,105,116]: $\dot{A} = -\gamma A + \delta A^D (1 - A)$. Here, the change in the fraction of available (not inactivated) channels is reduced by activity (γ) and increased by recovery that is proportional to the excitability status of the membrane (represented by δA); D is the scaling power of recovery from slow inactivation described earlier. This expression causes the system to spontaneously stabilize on the edge between excitable and not excitable upon intensive activation[105] (a second-order phase transition). Moreover, it has been predicted that the patterns of spiking activity about the phase transition will show features characteristic of self-organization at the critical point — that is, power-law relaxations, critical slowing down and adaptivity to a wide range of input statistics[116]. All these features were shown to exist in experimental analyses of individual cortical neurons in vitro[25,105–107,116,117] (Fig. 2e).

Figure 2f points to yet another consequence of the idea of automatic, local control by Na^+ channel slow inactivation, in which the plant and the controller are one entity. Surprisingly, despite evidence for non-homogeneous distributions of ionic channels along axons[46], propagation speed is remarkably stable; cortical axons conduct single action potentials with high temporal precision and reliability[118]. A recent numerical study shows that this reliability and precision of axonal spike propagation are at odds with what is expected given spatially variable conductances: in the absence of slow inactivation, most conductance distributions result in axonal spontaneous tonic activity. However, faithful axonal propagation is achieved with the introduction of Na^+ channel slow inactivation[115].

Another consequence emerges from a theoretical analysis aimed at parametrization of the Hodgkin–Huxley model of the squid giant axon action potential[26,32,111]. The full model is very sensitive to fluctuations that independently occur in its many parameters, but the outcome is in fact determined by simple combinations of these parameters along two physiological dimensions: structural and kinetic (denoted S and K, respectively). Slow inactivation serves as a powerful local homeostatic control mechanism that stabilizes excitability amid changes in both dimensions, S and K[26].

And finally, automatic local control by Na^+ channel availability has clinical consequences[119,120]. For instance, recovery from inactivation of Na^+ conductance is associated with the molecular basis of pain[121]. Particularly interesting are causal relations between impaired slow inactivation of the Na^+ channel owing to naturally occurring mutations and a resulting hyperexcitability of trigeminal neurons and persistent ocular pain[122].

Challenges

The study of neuronal and circuit resilience can be fraught with experimental difficulties. One of the most obvious of these concerns is studying processes that occur over extended timescales, requiring long recordings that can be challenging (especially if the dynamics are compromised by experimental deterioration). Another, deeper technical difficulty is inherent to the subject matter: both modes of regulation discussed here — rapid, self-regulating automatic processes that rely on the kinetics of already expressed proteins and slower mechanisms that depend on changes in the number of proteins (by means of synthesis and degradation) — involve interactions between structure and function. Structure–function relationships are much cited in physiology but are often interpreted as the way that a specific structure (mostly protein) enables its function. The cycle proposed here — in which channel protein state or expression (structure) gives rise to function that is, in turn, fed back to protein state or expression — is a genuine bidirectional relationship. This poses a challenge to physiologists who adhere to a long and successful scientific-engineering habit: defining independent and dependent variables. Conventionally, in the case of resilience of excitability, spiking activity would be the dependent variable, with conductance and kinetics being an independent variable. But when a biological process involves feedback, the idea of independent and dependent variables becomes ambiguous[123,124] because one cannot separate cause from effect. For instance, when the availability of Na^+ channels modifies, and is modified by, spiking activity, is channel availability the dependent or independent variable? As Dewey[125] wrote in his seminal 1896 paper on the reflex arc,

studying a piece of a circle "… gives us one disjointed part of a process as if it were the whole. It gives us literally an arc, instead of the circuit; and not giving us the circuit of which it is an arc, does not enable us to place, to center, the arc."

How then can closed-loop physiological feedback control be studied (or 'identified', in control theorists' jargon)? Hodgkin and Huxley paved the way for us. They 'sneaked into' the natural closed-loop dynamics of electrical membrane potential, linearizing the loop and converting the intractable to tractable[126], by building on the then revolutionary electrical engineering idea of a negative feedback amplifier (in which a fraction of the output is subtracted from the input[127]). Years later, system identification by feedback control was used to study the impacts of a given conductance on neuronal spiking behaviour by dynamic clamp (a method that uses computer-controlled feedback and essentially makes it possible to carry out simulations with biological cells and ionic channels)[128]. More recently, dynamic clamp was implemented to study the resilience of the Hodgkin–Huxley model in a bio-synthetic system, establishing a real-time closed-loop interaction between a genetically controlled population of excitability-relevant ion channels and a low-dimensional mathematical description of excitability[111]. A further step in the application of feedback control to identify circular processes was the introduction of a response clamp, in which a neuronal target behaviour (such as firing rate or stimulus–response delay) is stabilized by a negative feedback loop; this technique was implemented at different levels of organization, from single neurons through neural networks to human behaviour[124,129–132].

The most intriguing and far-reaching challenge entailed in the study of neuronal resilience is a consequence of the enmeshed networks of degenerate interdependent components of the feedback loops described earlier. It is related to pleiotropy[74], and its unseen, cryptic physiological toll. The finding that individual animals have different sets of neuronal and circuit parameters[5,70–72,133,134] led inevitably to the question of whether animals with different, degenerate solutions were differentially resilient to various perturbations. A sobering

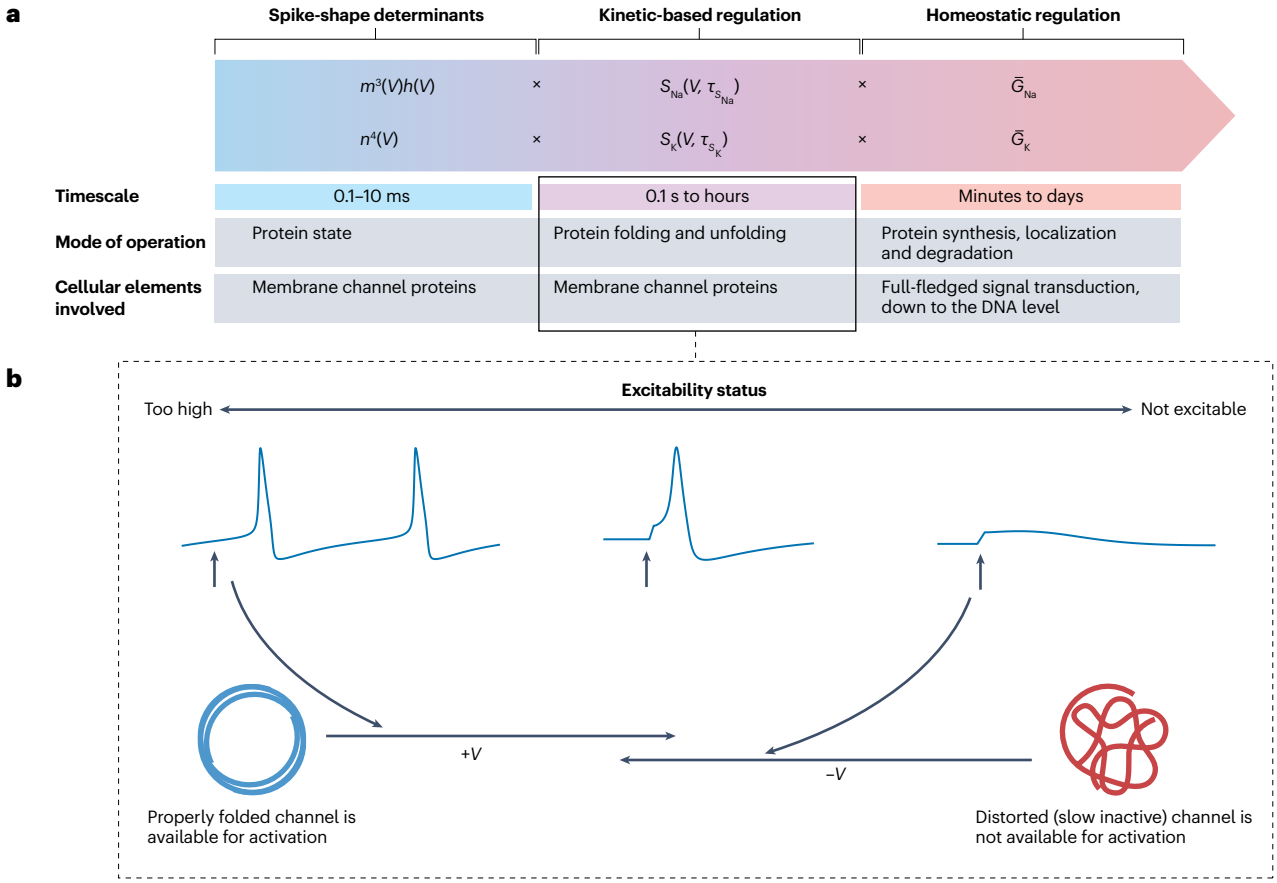


Fig. 3 | A hierarchy of closed loops, operating at different timescales. **a**, The schematic shows how a nested hierarchy of closed loops, operating at different timescales, can give rise to the emergence and regulation of spiking activity. Each row within the arrow at the top is an expression that relates to a different membrane current, showing maximal conductance ($\bar{G}$), slow inactivation gate $S(V, \tau)$ and rapid Hodgkin–Huxley's cycle opening and closing gates (m, n and h) for the current. The sum of these currents translates to a change ($\dot{V}$) in the membrane voltage (V) trajectory. The different components of each current equation operate on different timescales (depicted by colour gradient) and involve different cellular elements and modes of operation (depicted). We hypothesize that neurons can slide through these different mechanisms to maintain function. **b**, Kinetic-based regulation (middle column) involves a battery of different interacting processes in channel proteins and their modulation. One kinetic-based mechanism, a consequence of the slow inactivation of Na^+ channels, is depicted schematically: here, membrane depolarization ($+V$) induces slow inactivation of the channels owing to changes in channel protein configuration, effectively reducing the number of Na^+ channels available for participation in spike generation. Reduced spiking activity (and reduced membrane potential ($-V$)) favours recovery of the channels from slow inactivation, thereby making the membrane more excitable.

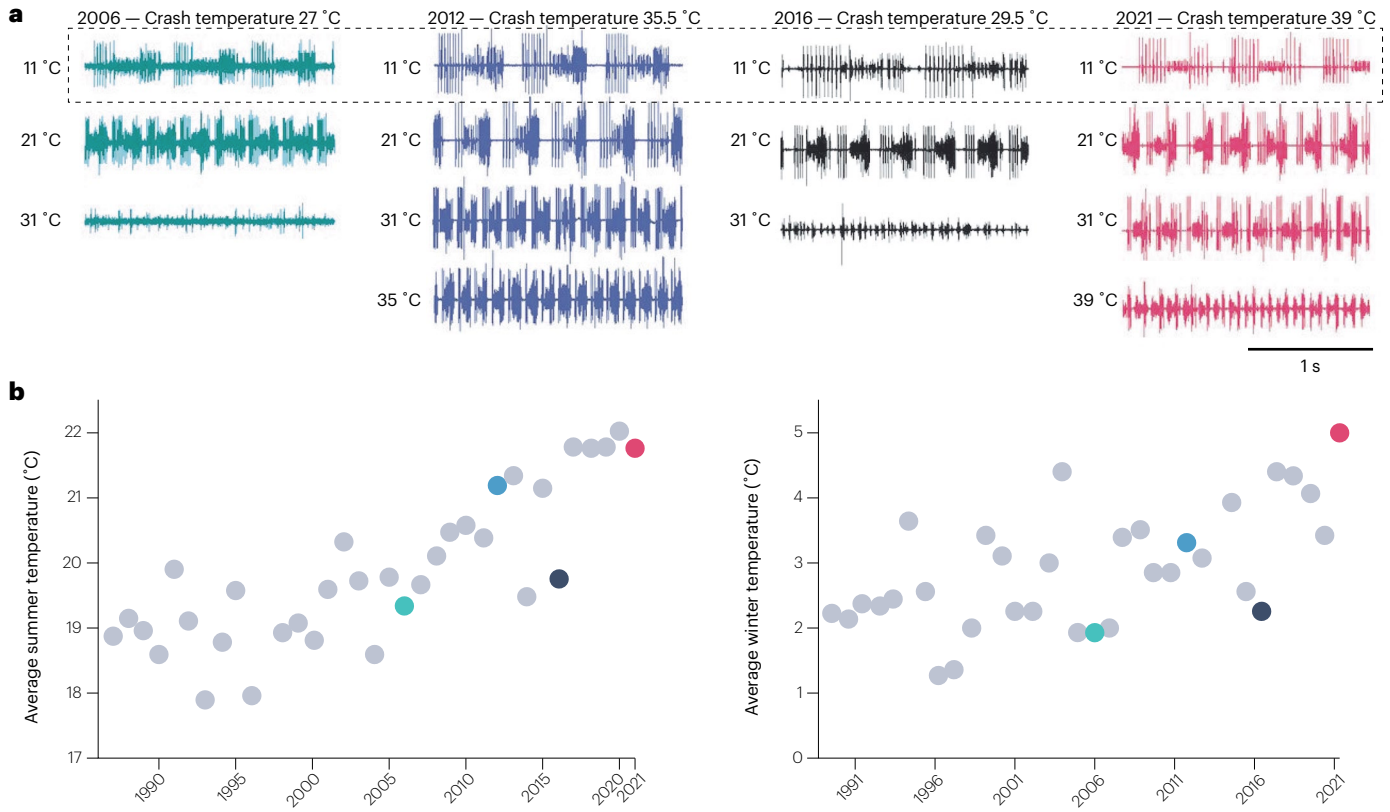


Fig. 4 | Stability of the pyloric circuit of crustacean stomatogastric ganglion at extreme temperatures correlates with changes in ocean temperature. **a**, Extracellular recordings from the crab lateral ventricular nerve at 11 °C (control), 21 °C and 31 °C taken from four experiments performed over 15 years[113]. In each case, activity increases with increasing temperature up to the point of a crash (loss of the characteristic triphasic rhythm seen at lower temperatures). For preparations that were robust at 31 °C, the last temperature step at which the preparation was still robustly triphasic is also shown. **b**, Average summer (June–August) and winter (December–February) sea temperatures 16NM east of Boston from 1985 to 2021. Winter temperature years correspond to the February date. Coloured dots represent the seasons from which we show example traces in part **a**. Figure adapted from ref. 140, Marder, E. & Rue, M. C. P., CC BY 4.0.

answer to that question comes from a series of studies conducted in the stomatogastric nervous system of the crab, *Cancer borealis*. This revealed that all animals showed robust pyloric and gastric rhythms at temperatures from 7 °C to about 25 °C, but at warmer temperatures, the rhythms were disrupted[135–139]. These 'crashes' varied across animals, both in terms of the temperature at which the disruption occurred and the characteristics of the disrupted rhythms. When crash temperatures were compared across years[140], there was a correlation between the surface water temperature of the Atlantic Ocean and the crash temperatures (Fig. 4). This indicates that long-term exposure to warmer water has shifted the temperatures over which the triphasic pyloric rhythm is maintained, presumably by engaging a series of molecular regulatory mechanisms. However, the most marked feature of these findings is that there was no indication in the control data collected when the preparations were studied at 11 °C that the nervous system had undergone a major change. That is, it requires a perturbation to reveal that these changes have occurred. These 'cryptic' or 'hidden' changes in response to climate may be similar to effects that occur in the brain under stressful situations, in which history-dependent changes may be invisible until the system is perturbed in a specific manner (as is seen, for example, in post-traumatic stress syndrome).

In summary, neurons and circuits are resilient over timescales from milliseconds to years. Here we propose that the underlying mechanisms responsible for resilience at these different timescales are interleaved, so that biological nervous systems can smoothly transition from one mechanism to another to maintain function against perturbations occurring on timescales ranging over many orders of magnitude. For instance, one might imagine resilience of excitability as being supported by a series of closed-loop mechanisms (Fig. 3a) that include slow structural (protein expression) changes that position the cell in a range of conductance densities within which changes in kinetic parameters fine-tune activity signatures over shorter timescales. The timescale over which structural changes take place is hours or more, whereas kinetic regulation operates on shorter physiological scales, from tens of milliseconds to hours. Voltage-gated conductances of the Hodgkin–Huxley cycle also instantiate a closed-loop mechanism that determines the shape of individual spikes at the millisecond scale. Such an overlapping continuum of timescales would suggest that we should extend our standard models of excitability and treat structural and kinetic dimensions as dynamic variables rather than parameters, beyond the timescale of a single action potential. Key to these notions is the concept that resilience — the capacity of a system to absorb a wide range of unforeseen parameter changes, while maintaining its

overall function — is not accompanied by a return to a previous state. Instead, as time moves forward, it inexorably leaves traces of the history of activation, which may be cryptic for long periods of time and can potentially result in unexpected circuit dynamics.

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