Homeostasis, failure of homeostasis and degenerate ion channel regulation

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Most neurons express a wide variety of ion channels with diverse properties, providing a rich toolbox for tuning neural function. Coexpressed channel types are often degenerate: they share overlapping roles in shaping electrophysiological properties. This can allow one set of channels to compensate the role of others, thus making nervous systems robust to perturbations such as channel deletions and mutations, expression noise or environmental disturbances. In tandem, activity-dependent homeostatic mechanisms can actively regulate channel expression to counteract perturbations by sensing changes in physiological activity. However, recent work shows that in spite of degeneracy and homeostatic regulation, the compensatory outcome of a perturbation can be unpredictable. Sometimes a single mutation in an ion channel gene can be catastrophic, while in other contexts a similar loss of function might be compensated. Compensation sometimes fails even when there may be many potential ways to compensate using available channels. Theoretical models show how homeostatic mechanisms that regulate degenerate conductances can fail and even cause pathologies through aberrant compensation.

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1. Introduction

In all nervous systems there are many kinds of ion channels and channel receptors with diverse biophysical properties. Mammalian genomes contain hundreds of distinct channel genes and even the nervous systems of supposedly simple invertebrates contain many tens [1,2••,3,4••,5]. Allowing for alternative splicing, subunit combinations and post-translational modification [6–8] increases the effective palette of channel types to the thousands. Why are there so many degrees of freedom available for shaping neural activity?

One possibility is that neuronal properties require such intricate tuning that a large set of knobs and dials is a prerequisite for a functioning nervous system. However, this does not require each individual current to be tuned to a precise value. All living systems need to be robust to biological variation and the insults that life throws at them. A weight of evidence shows that nervous system properties can be robust to significant variation in channel expression [2••,4••,9•,10–16]. Genetic variants that change the gating properties of ion channels, or result in complete loss of expression of a channel type sometimes lead to subtle phenotypes in an otherwise functional system [2••,10,15,17–20]. A wide array of ion channels is therefore useful not only for fine-tuning physiological properties, but for compensating perturbations.

On the other hand, it is obvious that nervous systems have a finite capacity for compensation. Biophysical properties and expression characteristics of ion channels can potently alter neural function [8,21,22], and often mean the difference between life and death. Debilitating diseases such as epilepsy [23–27] can be traced to alterations in specific channel subunits [22] and disorders of excitability such as chronic pain can be attributed to subtle interactions between multiple membrane currents [28••,29]. Of course, there are situations where a current may be absolutely essential, or where cumulative insults reduce the compensatory capacity of the available set of currents in an obvious way. However, experiments indicate that there are also situations where we see no evidence of compensation even though the complement of available channels could, in principle, be regulated to provide it [21,28••,30,31].

Together, these observations show that ion channel perturbations can have disparate and unpredictable outcomes. Sometimes a change in membrane current might result in a subtle phenotype, or be almost completely compensated; other times a similar change might disrupt the properties of a neuron or circuit drastically. Variability to perturbations can also occur in organisms with the same genetic background. Physiologists continually face these conundrums when designing and interpreting experiments to unravel the roles of ionic currents [32] and they pose a fundamental obstacle to developing tools for basic science and medicine [23,25,29,32,33].

This review will explore ways that multiple ion channel types allow neurons to tune physiological properties while compensating for perturbations and biological variability. There are two kinds of compensation I will focus on. The first arises due to *degeneracy*, which means that distinct channel types overlap in their biophysical properties and can thus contribute collectively to specific physiological phenotypes. Deletion or alteration of a degenerate current may allow other currents present in the membrane to immediately fill its role. Changes in overall physiological properties can therefore be buffered, and in some cases this buffering effect may be sufficient to preserve the function of a neuron or circuit in the face of substantial variability and external insults [2**,4**,29,34,35].

The second form of compensation — usually called *homeostatic plasticity* — is an active form of compensation whereby currents are regulated and reconfigured by feedback mechanisms in neurons [36,37]. These feedback mechanisms operate on internal biochemical signals that are coupled to physiological activity. Deviations in these signals from some kind of set point result in up or down-regulation of channel expression to recover the set point. Homeostatic regulation mechanisms are ubiquitous across species and preparations, and they are believed to be essential for the development and ongoing maintenance of healthy nervous system function [16,35,37–44]. Because they involve integration of activity signals over long timescales and rebalancing of channel expression, homeostatic plasticity mechanisms are necessarily slower than degenerate compensation, allowing both mechanisms to be disambiguated in certain cases. However, both mechanisms coexist, so the net outcome of an ion channel perturbation can be a complex mixture of interactions between the two.

Recent theory and experimental work [9*,11,12,14,16,40,42,43,45–51] reveals that homeostatic coregulation of multiple currents permits tight regulation of specific physiological properties like firing rates and subthreshold integration in the face of significant channel expression variability and perturbations. However, counterintuitive things can happen when degenerate sets of conductances are coregulated by homeostatic feedback mechanisms [11,20,28**,32,43,50,52]. Homeostatic mechanisms that tune specific physiological properties and compensate for some perturbations can paradoxically turn other, apparently benign perturbations into pathological chronic states [50].

Nervous system disorders are often attributed to failure of compensatory mechanisms [53]. I will discuss examples of degenerate systems that fail to compensate for a particular perturbation, even though degeneracy might seem to offer a compensatory path. I will then outline a peculiar and overlooked kind of homeostatic failure that can arise when homeostatic plasticity operates on degenerate sets of conductances.

The ideas discussed here are far from complete. It is safe to say that we are a long way from having a full understanding of homeostatic compensation in the nervous system. Nonetheless, the counterintuitive behaviour of degenerate ion channel regulation can be captured and rigorously understood even in simple dynamical models, which aid the interpretation of sometimes confusing experimental results. This highlights the importance of combining quantitative, systems-level experiments with mathematical modelling.

## 2. Ion channel degeneracy, flexible tuning and compensation

A single channel type rarely has a monopoly on a specific physiological process. Strong overlap exists in the biophysical properties of different channels and in how channel types shape neural activity. This overlap is often termed *degeneracy* [28**,29,46**,49,54*]. For example, the activation potentials and time-constants of many currents have overlapping windows where multiple currents make very similar contributions to membrane properties. This raises the question of why a biological system would spend time and energy expressing more types of conductances than appear necessary for physiological function.

Figure 1a shows very recent channel expression and physiology data from cortical neurons, obtained using new techniques that provide measurements of firing properties and gene expression in single identified neuron types [4**]. Although the palette of available channel types is very rich, the biophysical properties of the neurons fall into a relatively narrow class of excitable behaviours. Why do neurons express such a rich array of channel types? Would fewer types be sufficient?

Potential answers to these questions can be found in recent modelling and experimental studies. Drion *et al.* [46**] analysed a simple conductance based model of a spiking neuron with two slow currents, a calcium current and an A-type potassium current (Figure 1b). These currents have a degenerate effect on firing properties: both currents can modulate the transition from type-I to type-II spiking; in other words, they can determine a nonzero minimum firing rate for the neuron, as shown in the FI curves in Figure 1b. Both currents can also determine the current threshold. However, neither current can determine both properties simultaneously. Therefore, a neuron expressing both currents has more freedom to control and modulate firing properties. This is one example of how ion channel degeneracy permits flexibility in physiological function.

Recent experimental work shows analogous interactions between membrane currents and excitability in substantia nigra neurons. In a series of elegant experiments Kimm and colleagues [54*] directly isolated the effects of BK and Kv2 channels on firing rate. Although both

Figure 1
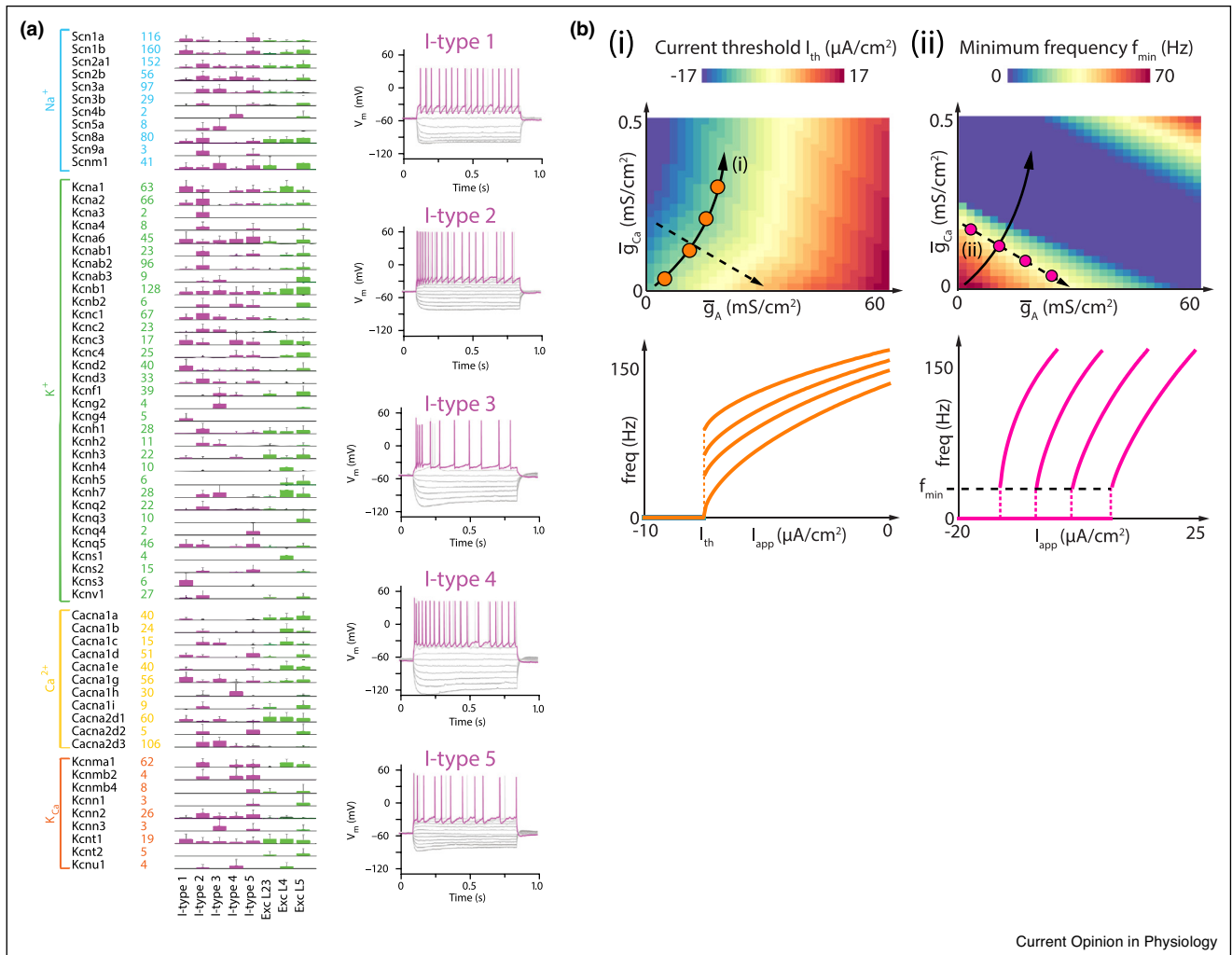
Neurons express many kinds of ion channels that often have degenerate functions. **(a)** Single-neuron mRNA expression data for identified cortical neurons shows the wide variety and significant variability (error bars) of channels expressed in neurons with relatively simple physiological properties (traces, right). Figure reproduced from [4••]. **(b)** A simple conductance-based model with four voltage-dependent conductances shows how current threshold (the minimum amount of steady current, I_{app}, required to elicit spiking, left panels) and minimum firing frequency (the minimum frequency at which repetitive firing can be sustained, right panels) have a degenerate dependence on a calcium current (g_{Ca}) and A-type potassium current (g_A). This degeneracy allows both properties to be tuned by regulating expression density of each channel. Regulation of both channel densities simultaneously allows independent tuning of both properties. Figure reproduced from [46••].

types of current contributed to repolarisation of the membrane, they had opposing effects burst firing frequency. Interestingly, inhibition of one current led to additional recruitment of the other, illustrating how co-regulation of these currents can permit partial compensation as well as tuning of important membrane properties.

Other recent modelling work has shown how degeneracy allows neural circuits to buffer channel expression variability and maintain physiological function. Dendritic integration properties of CA1 hippocampal neurons [13] and synaptic plasticity dynamics [52] can be almost perfectly invariant to substantial differences in channel

expression, provided there are enough channel types. These studies illustrate the generality of earlier, seminal studies in models of invertebrate central pattern generators, which established that there are multiple, potentially disparate ways that channel expression can produce rhythmic motor patterns in a neural circuit [55–58].

3. Degeneracy complicates the outcome of channel perturbations

Degeneracy therefore allows a certain amount of compensation between channel types at the same time as allowing important electrophysiological properties to be tuned. If one kind of potassium channel is blocked,

altered or deleted, the remaining ensemble of channels might partially substitute for the change in dynamics. Degeneracy is present at the single neuron and circuit level. Just as there are multiple channel types that have overlapping contributions to membrane properties, synaptic pathways often provide multiple configurations that allow a neural circuit to perform its functions.

The downside of degeneracy is that it can make experimental manipulations difficult to interpret. Establishing the role of a particular ion channel is problematic when other channels can compensate. Similarly, perturbations to the nervous system involving loss of channels or alterations to channel function can have variable and counterintuitive outcomes.

A landmark study [2••] of healthy humans and those with idiopathic epilepsy found hundreds of channel variants that correlated with symptoms. However, the study also documented a very large set of genetic variants affecting symptomatic individuals that were also present in healthy controls. The asymptomatic control group showed a large number of deleterious mutations in coding regions of channel genes, sometimes with multiple such mutations in the same healthy individual. In a study of this kind it is impossible to determine the precise mechanism of compensation that presumably explains the lack of symptoms in asymptomatic cases. Despite this, the authors provided a partial answer by showing that multiple deletions or 'hits' in a simple conductance-based model generically give rise to complex outcomes, as can be seen from the membrane potential traces in Figure 2a. Moreover, the reduction of multiple currents sometimes led to milder phenotypes than reduction of a single current. This hints at degeneracy having a role in the varied outcomes in epilepsy.

A simple conceptual picture of degenerate channel interactions can explain why compound channel perturbations can sometimes result in milder outcomes than a single perturbation. Figure 2b shows a situation similar to Figure 1b, where two channel types, g_1 and g_2, interact to produce a functional phenotype (indicated by the shaded region). Reducing the expression of one channel (as might occur in a mutation) destroys function. However, a further perturbation that reduces the expression of the other channel restores function.

Recent experimental studies of the electrophysiological basis of neuropathic pain showed that hyperexcitability can be induced and reversed by degenerate interactions between multiple membrane currents. Ratte et al. [28••] studied excitability of nerves under healthy, control conditions, where steady current injection elicits a single spike (Figure 2c, top panel). Individually applied changes in sodium and potassium conductances leave excitability intact, but when both changes are combined they interact

to produce hyperexcitability (Figure 2c, bottom panel). This makes it difficult to assign roles to individual channel types in producing hyperexcitability. At the same time, hyperexcitability induced by nerve damage (Figure 2d) can be reversed by targeting individual currents. The authors of the study argue that degenerate channel interactions of this kind explain the variable and often disappointing outcomes of generic pharmacological interventions for treating neuropathic pain [29].

4. Adding activity-dependent channel regulation to the picture

The findings discussed so far show that neurons coexpress degenerate conductances such that their densities lie inside a functional space. The shape of this space and its relation to individual conductances can explain some compensatory effects as well as sensitivity to perturbations, as shown in Figure 2. However, this does not explain how neurons establish and maintain channel expression inside functional spaces, nor does it address the question of how slower activity-dependent mechanisms contribute to compensation.

As neural circuits grow and develop throughout life, they are subjected to ongoing plasticity processes, environmental perturbations and biological noise, all of which can change neural activity and circuit function. Activity-dependent channel regulation mechanisms have been identified as a key means by which the nervous systems compensate for such perturbations and allow neurons to self-tune their properties [37]. It is important to distinguish this form of compensation, referred to as homeostatic plasticity [37,39,41], from the immediate compensation that can arise due to degeneracy. Homeostatic plasticity mechanisms employ activity sensors that control channel expression using feedback: when activity deviates from a set-point, channel expression is up or down regulated to return the system to the set-point [34,37,39–41,50,59]. In this way, homeostatic channel regulation allows neurons to configure their channel densities inside a functional space, and in some cases return to a functional configuration following a perturbation.

Experiments in a variety of species and systems indicate that expression of many (but not all) neuronal ion channels and receptors are controlled by so-called master regulator pathways. These master regulators are signalling cascades that respond homeostatically to physiological variables that depend on neural activity, such as calcium influx [60]. Examples of putative master regulators include transcription factors [61] and calcium sensitive enzymes [42]. Activity dependent coregulation makes sense from the point of view of degeneracy: up and down-regulating several conductances together can preserve their interactions, helping to keep them inside a functional space under certain conditions.

Figure 2
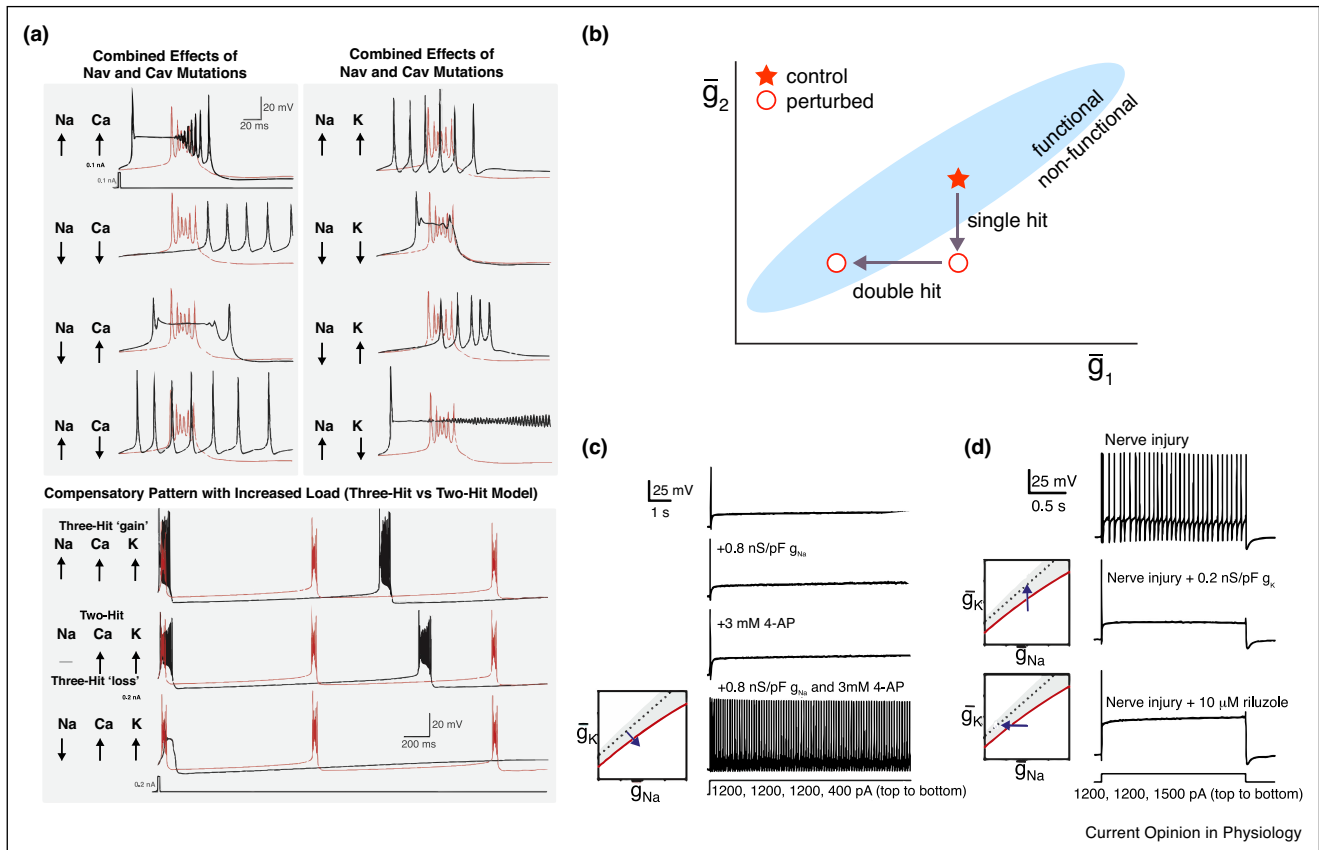
Degenerate channel interactions pose problems for understanding the effects of channel deletions, mutations and pharmacological perturbations. **(a)** Variable outcomes from a model of changes in gene dosage due to idiopathic epilepsies (reproduced from [2••]). The voltage trace of a normal, control pyramidal cell model are shown in red, with various combinations of ion channel density perturbations. Top panels show effects of pairwise ('two hit') manipulations of sodium (Na) calcium (Ca) and potassium (K) current densities. Bottom panel shows mixed outcomes from triple ('three-hit') manipulations. **(b)** Conceptual diagram showing two conductances that interact in a degenerate way to produce functional behaviour (shaded region). Reduction in the density of one conductance ('single hit') can result in a non-functional cell, while reduction in both ('double hit') preserves function. **(c)** Peripheral nerve recordings showing the effect of pharmacological and dynamic clamp-induced changes in sodium (g_{Na}) and potassium (g_K) conductances. A normal, healthy nerve responds to current injection with a single action potential (top trace). Combined increases in g_{Na} and decrease in g_K result in a transition to hyperexcitability (bottom trace), while either manipulation in isolation leaves normal function intact. The inset shows the combinations of channel densities that produce normal excitability (grey shaded region) and the boundary where hyperexcitability occurs (red curve). **(d)** Hyperexcitability induced by nerve injury exhibits degeneracy: manipulation of either g_{Na} or g_K by the appropriate amount restores normal function. (Figures c, d reproduced from [28••]).

This is shown conceptually in Figure 3 in a very simple thought experiment. Suppose the expression levels of two ion channel types, g_1 and g_2, are under the control of an activity-dependent regulation mechanism in a neuron (Figure 3a). Because the two conductances are co-regulated, the mechanism will increase or decrease their densities together, imposing a direction of coregulation in conductance space (Figure 3b). For simplicity, we assume the strength of regulation is equal between the channels, so the direction of regulation lies along the diagonal.

Both conductances contribute to average activity (Figure 3c), which is sensed by an intracellular mechanism that feeds back to control conductance expression

on a slow timescale, as depicted by the feedback arrow in Figure 3a. When activity is above a set point, the conductances are down-regulated, and vice versa when activity is too low. Because activity depends on a combination of both channel densities, the activity set point forms a contour in conductance space, which could be complex in shape [49]. Finally, both conductances also contribute in a degenerate way to neural properties, with the functional space of densities shown as a shaded region in Figure 3d.

How will this hypothetical neuron behave when we perturb it? Consider a control condition where the conductances are in the functional space and the sensor is at its homeostatic set-point (star in Figure 3e). Acute reduction of both conductances by the same amount will

Figure 3
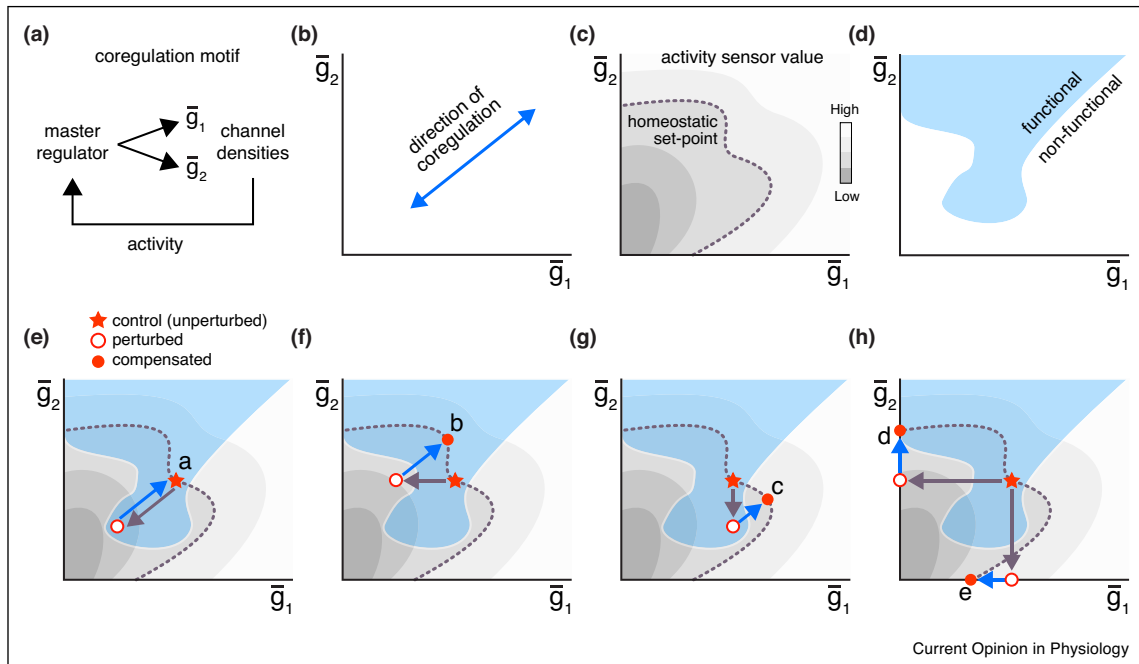
Counterintuitive compensatory outcomes are possible from a simple activity-dependent coregulation mechanism acting on degenerate conductances. **(a)** Activity dependent regulation controls the expression of two ion channel types, g_1 and g_2. **(b)** Both conductances are up and downregulated together, imposing a direction of coregulation in conductance space. **(c)** Average intracellular activity values depend on the expression of both conductances, such that the homeostatic set-point (dotted curve) traces out a curve in conductance space. **(d)** Specific combinations of both conductances produce functional behaviour (blue shaded area); the shape of the functional space can be complex [2••,13,49,55,57]. Outcomes of acute perturbations to channel densities from a control value (filled star) can be homeostatic or pathological. **(e)** Reduction of both channel types to 50% of their control values reduces the activity sensor value while leaving the cell functional. The change in activity causes coregulation that opposes the perturbation, restoring conductance densities to their original values (point a). **(f)** Reduction of g_1 to 50% of its control value renders the cell non-functional. The compensatory response restores function, but with different expression levels to control values (point b). **(g)** Reduction of g_2 to 50% initially leaves the cell functional, but the compensatory response destroys function as the coregulation mechanism restores activity to the set-point (point c). **(h)** Complete deletion of each conductance has varied outcomes. Deletion of g_1 confines the system to regulate g_2 alone; restoration of the set-point in this case restores function by upregulating g_2 (point d). Deletion of g_2 results in a nonreciprocal effect: g_1 is regulated in the opposite direction and in this case downregulated to a value (point e) that fails to restore function even though the activity sensor is at its set-point.

decrease average activity (Figure 3e), resulting in the system gradually moving back along its direction of regulation until it reaches the homeostatic set-point (point **a** in the figure). In this case the conductances return to control values over time.

Perturbing g_1 to 50% of its control value results in loss of function (Figure 3f). Function is later recovered as the system returns along its direction of compensation, but to different conductance values to control (Figure 3F, point **b**). On the other hand, the same perturbation to g_2 initially leaves the neuron functional. However, function is lost as the regulation mechanism returns to the sensor set-point (Figure 3g, point **c**). This is an example of aberrant compensation, a phenomenon where a mechanism that behaves homeostatically in one context can cause pathologies in other contexts. Aberrant compensation can readily occur when multiple conductances are coregulated by activity-dependent feedback [50].

Deletion of either conductance has divergent outcomes (Figure 3h). Because a conductance is missing, the regulation mechanism is confined to move along the axis of the remaining conductance. When g_1 is deleted, function is initially lost, then restored by activity dependent upregulation of g_2 (Figure 3e, point **d**). When g_2 is deleted, function is lost and a nonreciprocal downregulation of g_1 occurs, which in this example fails to restore function (Figure 3e, point **e**).

The outcomes of the thought experiment in Figure 3 are not merely hypothetical. Very straightforward models of activity-dependent ion channel regulation reproduce all of the main outcomes of homeostatic and aberrant compensation [50]. This is shown in the results reproduced in Figure 4, which summarise the behaviour of a simulation of an activity-dependent transcriptional feedback loop operating on multiple conductances in a model of an invertebrate pacemaker neuron. For different channel

Figure 4
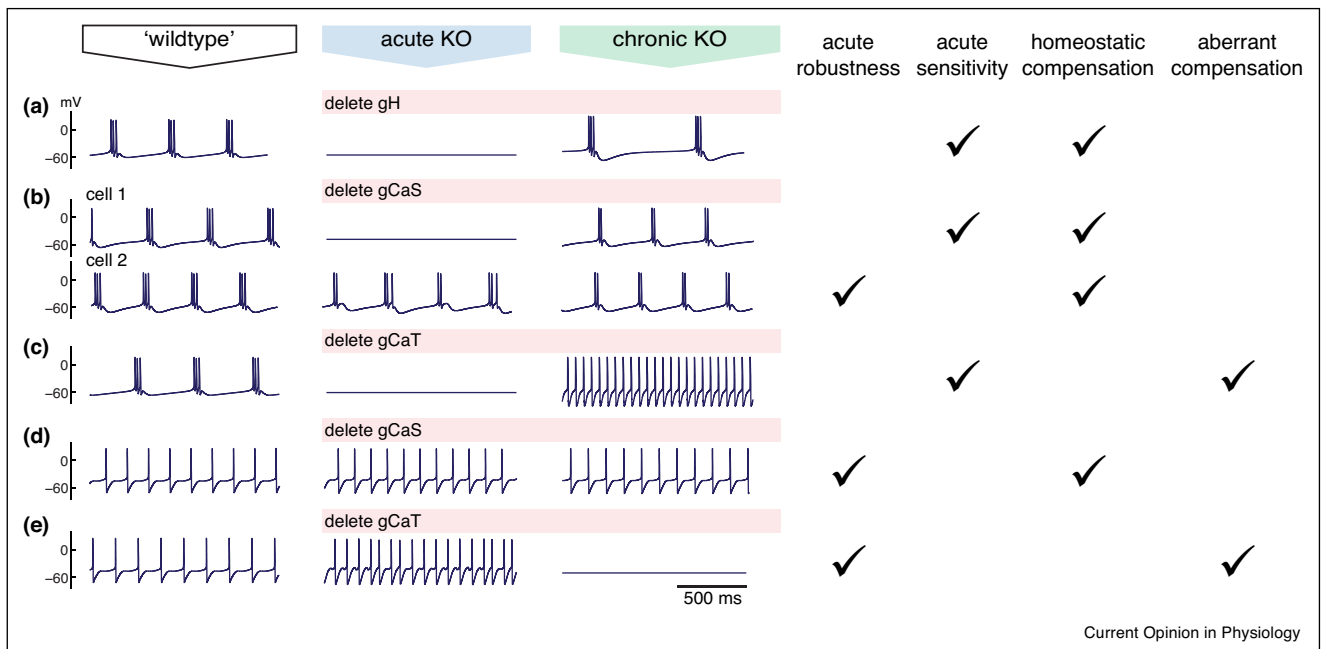
Activity-dependent feedback regulation of a degenerate set of conductances can produce homeostatic compensation or aberrant compensation, depending on context. A conductance-based model with multiple conductance regulated by long-term average intracellular calcium influx [50]. Each row shows firing behaviour of the model subjected to acute and long-term channel deletions, with deleted channel identities indicated above membrane potential traces. **(a)** A bursting pacemaker neuron is sensitive to deletion of an I_H channel (g_H). Over time the regulation mechanism almost fully compensates the loss of the channel. **(b)** Two different cells selected from a variable population controlled by the same regulation model show divergent effects when a calcium channel (g_{CaS}) is deleted acutely. On longer timescales the regulation mechanism homeostatically compensates for channel loss. **(c)** Deletion of a different calcium channel (g_{CaT}) destroys pacemaking acutely, as in (a and b), however, in this case the regulation mechanism produces an aberrant compensatory response, resulting in tonic firing. **(d)** A tonic pacemaker is acutely and chronically robust to deletion of one calcium conductance (g_{CaS}), however, in **(e)** the same neuron type is robust to deletion of a different calcium conductance (g_{CaT}) but the regulation mechanism causes pathological loss of function on a longer timescale. Figure reproduced from [50].

perturbations, the model automatically produces homeostatic compensation (e.g. Figure 4a, b, d) or aberrant compensatory effects (Figure 4c, e). These occur generically, without needing to tune the model, suggesting that both kinds of effect are generic features of feedback mechanisms operating on degenerate components. Similarly, counterintuitive effects such as nonreciprocal coregulation are observed experimentally [43]. It is therefore reasonable to expect these kinds of outcomes to occur more generally and they may explain some nervous system pathologies.

We must take care in interpreting simple models of kind shown in Figures 3 and 4. The key assumption is that *some* degenerate set of channels (not necessarily *all channels*) is under control of a common regulatory pathway [42,43]. This pathway could be transcriptional, but it need not be — it could be a postranscriptional or postranslational mechanism acting on multiple targets, as some experimental studies have found evidence for [18]. Secondly, it is obviously crude to define a hard boundary between 'functional' and 'non-functional' in all cases, as physiological properties can have a graded dependence on

channel expression. Nonetheless, there will often be critical points where physiological properties deviate to a level that destroys circuit function. Finally, regulation mechanisms may be more sophisticated than a simple homeostatic feedback loop. However, there are limits to the number of physiological variables a cell can sense and severe limits on how well these signals can control the expression of intracellular components [62,63].

Thus, although homeostatic plasticity and ion channel degeneracy can together promote robustness to variability and perturbations, we should expect situations where these compensatory mechanisms do not always work, or worse still, exacerbate an otherwise benign insult. This may also explain why nervous systems are sometimes unable to compensate for perturbations even when the raw ingredients are present and intact.

5. Failure of homeostatic regulation in degenerate systems
A growing number of studies have identified loss-of-function or gain-of-function variants in channel subunits that reliably lead to epileptic seizures [64]. One well-

studied model of a severe epilepsy, Dravet syndrome, results from a loss-of function mutation in a specific sodium channel family, NaV 1.1. Interestingly, there is evidence for compensatory upregulation of another sodium channel gene, NaV 1.3 in animal models [22,27]. In spite of this obvious homeostatic response, the resulting reorganisation of channel expression does not rescue the absence of NaV 1.1. In this example, it seems as though the affected neurons sense a change in excitability, but fail to organise the remaining currents to compensate in the appropriate way. It is important to note that pathologies such as epilepsy have complex etiologies and discrepant effects of specific genetic perturbations can sometimes be attributable to genetic background [25]. Furthermore, it is possible that some disease-associated changes to channel genes cannot feasibly be compensated for by the remaining channelome [10,25,65].

Certain kinds of severe ataxia result from mutations in potassium channels expressed in the cerebellum. A recent study showed that the loss of function of Kv1.1 led to an aberrant increase in cerebellar GABA release [21], which tended to dampen excitability of postsynaptic Purkinje neurons. There are multiple ways the affected circuit could compensate, for example, by a compensatory balancing of excitatory synaptic currents or perhaps via a change in the intrinsic excitability of the target cells, as has been reported elsewhere in the cerebellum [38]. However, in this example, no compensation was detected at the cellular level, coinciding with very obvious impairments at the behavioural level.

The crab Stomatogastric ganglion (STG) is an extensively studied neural circuit that generates stereotyped rhythmic motor patterns in response to modulatory input from other parts of the nervous system. Several studies revealed that the STG and its constituent cells are capable of autonomously generating a rhythm in the prolonged absence of modulatory input, indicating the presence of necessary, modulator-independent currents [44,66,67]. However, more recent experiments in the crab Stomatogastric ganglion show that on very long timescales, removal of modulatory input that maintains rhythmic network activity is *not* compensated by endogenous currents in the network [30]. This failure of homeostasis occurs even though the same circuit is known to compensate for loss of modulatory input and maintain normal activity on shorter timescales [66,67].

Owing to the experimental challenges in making precise perturbations and monitoring potentially widespread effects on channel expression, it is difficult to enumerate cases where homeostatic compensation could occur, but for some reason fails. As with any biological mechanism, there are many potential failure modes and it is difficult to imagine any biological system evolving to cope with all kinds or perturbations. However, the examples cited here

are at least consistent with compensation failing to occur in situations where there may be a potential compensatory path that the nervous system could follow, and when there is no reason to expect the activity-dependent homeostatic mechanisms themselves to be impaired.

Regulation mechanisms are tuned to produce and maintain specific physiological properties in some, but not all contexts. As long as there is degeneracy (and, as a consequence, flexibility) in channel expression, there is also the potential for aberrant compensation. This is because multiple channel configurations can satisfy a feedback sensor without necessarily generating functional behaviour.

Probing examples of compensatory failure in more detail can therefore be enormously useful in understanding the regulatory logic of ion channels in neurons. Conversely, a quantitative, dynamical understanding of homeostatic feedback regulation and ion channel degeneracy can help us understand and potentially remedy some kinds of channel dysregulation.

Advances in experimental techniques [4••,12,68] that allow single-cell resolution measurements of channel expression and physiology allow us interrogate living systems in similar mechanistic detail to the way we can interrogate models. This will enable us to make sense of the widespread and counterintuitive effects of channel regulation in neurons. In tandem, insights from models will enable us to design and interpret new experiments.

Conflict of interest
I have no conflicts of interest to declare.

Acknowledgements

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- • of special interest
- •• of outstanding interest

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