Afferent hyperexcitability in neuropathic pain and the inconvenient truth about its degeneracy
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Neuropathic pain, which arises from damage to the nervous system, is a major unmet clinical challenge. Reversing the neuronal hyperexcitability induced by nerve damage is a logical treatment strategy but has proven frustratingly difficult. Here, we propose a novel explanation for that difficulty. Changes in several different ion channels are individually sufficient to cause hyperexcitability in primary somatosensory neurons. Despite offering multiple drug targets, this scenario is problematic: if multiple sufficient changes are triggered by nerve injury, then no single change is necessary for hyperexcitability. This so-called degeneracy compromises therapeutic interventions because drug effects on any one ion channel can be circumvented by changes occurring in other ion channels. Overcoming degeneracy demands a more integrative approach to drug discovery.

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Introduction
Neuropathic pain refers to pain caused by damage to or dysfunction of the nervous system [1]. It has several etiologies including trauma (e.g. nerve compression, spinal cord injury), disease (e.g. diabetes, multiple sclerosis) and toxicity (e.g. chemotherapeutic and antiretroviral agents). Unlike nociceptive pain, which is evoked by noxious stimuli, neuropathic pain is characterized by spontaneous pain and hypersensitivity to innocuous tactile and thermal stimuli. These sensory disturbances arise from changes, including neuronal hyperexcitability, that develop at multiple points along the neuraxis [2]. In many cases, excessive input from the periphery is necessary and sufficient to produce neuropathic pain [3–5], which suggests that neuropathic pain can be effectively treated by reversing primary afferent hyperexcitability. Significant research has been directed toward deciphering how afferent excitability is altered by nerve injury and which ion channels are involved, but these efforts have yet to yield clinically effective treatments [6,7]. This prompts the question of whether we are on the right track and have simply not yet reached the desired translational outcomes, or whether an unappreciated factor is impeding progress. We will argue in favor of the latter explanation.

The ion channels involved in pain processing by primary afferent neurons have been comprehensively reviewed elsewhere [4,8,9]. Here, we will focus on demonstrating that changes in diverse ion channels can produce similar patterns of cellular hyperexcitability and that many ion channel changes co-develop after nerve injury. Co-development of distinct molecular changes capable of producing equivalent cellular hyperexcitability is an example of degeneracy. Degeneracy means that an outcome does not have a unique basis [10**]. Degenerate processes — distinct processes that produce equivalent outcomes — confer robust phenotypes because disruption of any one process is compensated for by other processes [11,12**,13]. Robustness of a healthy phenotype is desirable but degeneracy may be co-opted under pathological conditions, stabilizing the pathological phenotype and thus rendering neuropathic pain refractory to treatment. The importance of degeneracy and robustness is gaining recognition in fields like cancer [14*] and infectious diseases [15], but has escaped the attention of pain researchers.

Primary afferent hyperexcitability and the ion channels responsible for it
Primary afferents are the first-order sensory neurons that relay sensory information from peripheral tissue to the central nervous system. Afferents are heterogeneous [16,17]. Nociceptive sensory information is normally conveyed by unmyelinated C fibers and thinly myelinated Aδ fibers [18] but, under pathological conditions, activation of thickly myelinated Aβ fibers can produce pain in the form of mechanical allodynia (i.e. hypersensitivity to light touch) [19]. Contrary to labeled lines in which each sensation (e.g. warmth, cool, touch, pain, etc.) is subserved by a different type of afferent, co-activation of differentially specialized afferents arguably dictates how somatosensory stimuli are perceived [20–22] but coding at the single cell level invariably relies on action potentials, or spikes. Excitability — defined here as the capacity to generate spikes — is therefore crucial for proper neural
Coding. Hyperexcitability refers to excessive spiking. Although consistent with the spontaneous pain and hypersensitivity characteristic of neuropathic pain, the development of hyperexcitability is perhaps counterintuitive insofar as the most likely consequence of nerve injury is loss, not gain, of function. Interestingly, neuropathic pain is associated with hyposensitivity (e.g., increased tactile detection thresholds) yet innocuous tactile and thermal stimuli are more likely to be mistakenly perceived as painful [23]. This mixture of sensory disturbances reflects the diversity of underlying changes [24,25].

Figure 1a summarizes the impact of hyperexcitability at different locations within a primary afferent neuron. Excitability is typically measured experimentally in the soma because its large size is conducive to patch clamp recordings. That said, somatic excitability does not necessarily reflect excitability elsewhere in the neuron [26], nor does excitability change uniformly throughout the neuron after injury. The excitability of peripheral axon terminals is especially important since that is where stimuli are detected and encoded, but it is difficult to measure the excitability of axon terminals and to disambiguate changes in excitability from changes in transduction of the stimulus, although innovative approaches have been developed to address these challenges [26,27]. Under pathological conditions, spikes can arise ectopically from the site of axonal damage or in the soma [28,29], which raises questions about how spikes originating from different sites interact [30]. The excitability of central axon branches affects spike propagation and synaptic

![Figure 1](image-url)
transmission, and it also dictates whether presynaptic inhibition can become paradoxically excitatory [31]. Even if only a single ion channel was affected by nerve injury (which is not the case; see below), its impact on excitability still depends on interactions with the other ion channels present, including those involved in sensory transduction (peripherally) or in synaptic transmission (centrally).

Qualitative changes in somatic excitability observed after nerve injury include a switch in spiking pattern, development of pronounced membrane potential oscillations, and spontaneous bursting (Figure 1b) [32]. According to computer simulations, this triad of excitability changes originate from a common underlying ‘switch’ in how ion channels interact during spike generation; furthermore, this switch can occur via a number of different ion channel changes [33*]. The latter observation is important in light of gene expression profiling, which has revealed that numerous genes, including several ion channels, are up or down regulated after nerve injury [34–40]. Traditional studies have tended to focus on one channel at a time, but surveying across those studies reveals the range of channels implicated in neuropathic pain. Sodium currents tend to increase whereas potassium currents tend to decrease, but hyperpolarization-activated cation current (Ih), leak currents, calcium currents, and calcium-activated currents of every stripe are all involved (for reviews, see [4,8*,9]). The scope of these changes should not come as a surprise if you consider, for example, that all three MAP kinases — extracellular signal-related kinase (ERK1/2), p38, and c-Jun N-terminal kinase (JNK) — are activated after nerve injury [41]. Activated ERK1/2 alone affects Na\textsubscript{v}1.7 [42], K\textsubscript{v}4.2 [43], Ca\textsubscript{v}2.2 [44], and calcium-activated potassium channels [45] in sensory afferents, and that list is sure to continue growing.

The causal link between ion channel changes and cellular hyperexcitability

Although nerve injury may induce hyperexcitability and alter the expression or function of a certain ion channel, the ion channel change may not cause (or contribute in any way to) the hyperexcitability. In this regard, many studies provide only correlational links between molecular changes and cellular hyperexcitability. To test if an ion channel is necessary for hyperexcitability, one must selectively block its function or reduce/eliminate its expression. Notably, an ion channel may be necessary for hyperexcitability without being altered by nerve injury; for instance, hyperexcitability due to decreased potassium conductance can be reversed by reducing sodium conductance, since relative densities rather than any one absolute density dictate how currents interact [33*,46]. One should ideally block the injury-induced change in the ion channel to determine if the change causes hyperexcitability, although doing so without affecting other channels is difficult if several channels are co-regulated (see above re. MAP kinases). But most importantly, the dependence of excitability on ion channel interactions means that the necessity of any given ion channel is contingent on other ion channels: An ion channel that is necessary for hyperexcitability in one context may be unnecessary in another context (i.e. neuronal type, subcellular location, or pathological stage).

Few studies have investigated which molecular changes are sufficient to cause hyperexcitability. Rho and Prescott [33*] and Ratté et al. [47*] used simulations and experiments, respectively, to demonstrate that an increase in Na\textsubscript{v}1.3-like current or reduction of K\textsubscript{v}1 current produce equivalent changes in excitability (Figure 2a). In theory, numerous molecular changes can produce the same outcome. The implications are critical: If multiple molecular changes are induced by nerve injury (see above), and more than one of those changes is sufficient to cause cellular hyperexcitability, then the basis for hyperexcitability is degenerate and no single change is necessary (Figure 2b). Therapies must target necessary changes to be effective. Degeneracy argues that multiple ion channels need to be simultaneously targeted to reverse hyperexcitability, or that therapies should target processes less degenerate than excitability.

Na\textsubscript{v}1.7 as a case study for choosing a drug target

Rare genetic disorders stemming from mutation of a single gene implicate particular ion channels in pain processing. For example, mutations causing a leftward (hyperpolarizing) shift in the voltage-dependent activation of Na\textsubscript{v}1.7 channels produce inherited erythromelalgia [48] and other mutations affecting inactivation of this same ion channel produce paroxysmal extreme pain disorder [49]. These phenotypes indicate that certain changes in Na\textsubscript{v}1.7 are sufficient to cause pain. Electrophysiological studies have confirmed that the mutated channels are sufficient to cause afferent hyperexcitability [50,51]. But interestingly, the same Na\textsubscript{v}1.7 mutation that makes unmyelinated sensory neurons hyperexcitable makes sympathetic ganglion neurons hypoexcitable because of the presence or absence, respectively, of Na\textsubscript{v}1.8 in each cell type [51]. These data suggest that blocking Na\textsubscript{v}1.8 would reverse Na\textsubscript{v}1.7-mediated hyperexcitability in neurons that express Na\textsubscript{v}1.8 without causing hypoexcitability in cells that do not express Na\textsubscript{v}1.8; by comparison, blocking Na\textsubscript{v}1.7 would risk causing hypoexcitability in cells that do not express Na\textsubscript{v}1.8. This example illustrates the importance of considering ion channel interactions.

Loss-of-function mutations in Na\textsubscript{v}1.7 [52] and other mutations that reduce Na\textsubscript{v}1.7 current at perithreshold voltages (by causing a rightward shift in activation or a leftward shift in inactivation) [53] result in congenital insensitivity to pain (CIP). According to mouse studies,
deleting NaV1.7 from nociceptive afferents blocks acute pain and the development of inflammatory pain [54]. Deleting NaV1.7 in adult mice [55] or knocking it down with antisense RNA [56] is also analgesic. Recently developed antagonists of NaV1.7 have shown promising results against nociceptive and inflammatory pain [57–59]; two of those studies also demonstrated efficacy against neuropathic pain [57,58]. These results argue that NaV1.7 is necessary for pain and, therefore, is an excellent drug target. However, not all forms of neuropathic pain are prevented by NaV1.7 deletion [55,60,61], and a case of neuropathic pain in a patient with NaV1.7-dependent CIP has been reported [62]. These observations hint at the contingency of NaV1.7’s necessity. With this in mind, we should learn from the failure of past clinical trials: The sodium channel antagonist 4030W92 initially reduced allodynia, but all benefits were lost by the end of the two-week treatment period [63]. We can only speculate that compensatory changes eventually offset the therapeutic benefits of sodium channel blockade, but it is notable that the aforementioned animal studies on NaV1.7 antagonists tested efficacy for no more than 3 days.

The necessity of NaV1.7 for neuropathic pain is key to whether it is a good drug target, but we must consider what is necessary for a degenerate phenotype. If injury-induced changes in other ion channels can produce hyperexcitability despite NaV1.7 blockade, then NaV1.7 will become unnecessary for hyperexcitability and the analgesic effects of its blockade will be lost. To be clear, acute blockade of NaV1.7 will invariably reduce excitability but the effect may be short lived if other ion channels continue to change. In fact, certain changes may occur in direct response to NaV1.7 blockade given evidence that blocking sodium channels in pyramidal neurons triggers compensatory changes, namely upregulation of sodium conductance and downregulation of certain potassium conductances [64,65]. As an aside, degeneracy is more likely to undermine the effects of very specific, single-target drugs; in this regard, the efficacy of multi-target drugs in treating complex diseases like epilepsy [66] is notable. Specificity is beneficial for avoiding off-target effects but it may be counterproductive when trying to prevent/reverse a degenerate phenotype like cellular hyperexcitability. A good compromise would be to combine specific, single-target drugs to give multi-target coverage.

The pathogenesis of hyperexcitability and its implications for degeneracy

Excitability is a complex phenotype that depends on nonlinear interactions between ion channels. Protein-protein interactions, whether direct or indirect, are quite limited compared with the large numbers of ion channels that can interact functionally by virtue of their mutual sensitivity to membrane potential. It is, therefore, not surprising that excitability is highly degenerate. This degeneracy allows excitability to be robustly regulated since the cell can readily adapt to disturbances in any one type of ion channel [12**]. But that begs the question of how excitability becomes pathologically disrupted despite degeneracy, and why hyperexcitability is so difficult.
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Pathogenesis of the hyperexcitable state. Under normal conditions (top panel), excitability is robustly maintained at a desired set point by feedback mechanisms regulating numerous ion channels. Hyperexcitability could develop because injury-induced changes in one or more ion channels overwhelm the feedback mechanisms, thereby displacing the system from its set point (scenario 1, middle panel). Under these conditions, the hyperexcitable state is unstable because feedback mechanisms continue working toward restoring normal excitability. Alternatively, injury may cause the set point to change (scenario 2, bottom panel). Under these conditions, maladaptive feedback stabilizes the hyperexcitable state. This is particularly problematic because maladaptive feedback can exploit degeneracy (i.e. misregulate many different ion channels) so as to robustly maintain the hyperexcitable state, thus making it refractory to treatment.

feedback works to help restore normal excitability, meaning feedback mechanisms and therapeutic interventions work toward the same goal, and simply removing the inciting pathology would allow the system to normalize. In contrast, scenario 2 predicts that intrinsic feedback strives to maintain the system in a hyperexcitable state, contrary to therapeutic interventions, which also means that the hyperexcitable state becomes self-perpetuating unless the set point can be normalized. Furthermore, the same degeneracy that normally ensures robust maintenance of normal excitability would, in the second scenario, robustly maintain hyperexcitability. If the second scenario is true, then treatment strategies must take degeneracy into account by simultaneously targeting multiple ion channels in order to protect against compensatory changes and/or by targeting upstream mechanisms, like MAP kinases, that coordinately misregulate multiple ion channels.

Conclusions and outlook
Diverse ion channels are implicated in pain processing. Many of those channels are altered under the pathological conditions leading to neuropathic pain. The changes occurring in each channel cannot be considered in isolation from the changes occurring in other channels, or in isolation from the unchanged ion channels present in each neuron. This runs counter to reductionist tendencies, but taking a more integrative approach is crucial if we seek to fully understand how neurons control their excitability, how that excitability becomes pathologically altered, and how we should intervene to restore normal excitability. Taking a more integrative approach has revealed that neurons can achieve the same excitability on the basis of many different ion channel combinations [12**,13]. Neurons can also become hyperexcitable on the basis of many different ion channel changes [33*,47**], which suggests that the same degeneracy that allows for robust regulation of excitability under normal conditions may be co-opted to help robustly maintain hyperexcitability under pathological conditions. If multiple changes that are individually sufficient to cause hyperexcitability are triggered after nerve injury, then no one of those changes is necessary for the hyperexcitability. Under those conditions, targeting a specific ion channel is not likely to produce lasting pain relief. Drug development strategies must look beyond the trees in order to see the forest, to recognize that molecules are constituents of larger, complex, adaptive systems.

Conflict of interest statement
Nothing declared.

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References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

* of special interest
** of outstanding interest

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