# Persistent nociceptor hyperactivity as a painful evolutionary adaptation

Edgar T. Walters **Correspondence:** edgar.t.walters@uth.tmc.edu
Department of Integrative Biology and Pharmacology, McGovern Medical School, The University of Texas Health Science Center at Houston, Houston, TX, 77030 USA.
Robyn J. Crook
Department of Biology, San Francisco State University, San Francisco, CA 94132, USA
G. Gregory Neely
Charles Perkins Centre and School of Life and Environmental Sciences, The University of Sydney, NSW 2006, Australia
Theodore J. Price
Department of Neuroscience and Center for Advanced Pain Studies, University of Texas at Dallas, Richardson, TX 75080, USA
Ewan St John Smith
Department of Pharmacology, University of Cambridge, Tennis Court Road, Cambridge CB2 1PD, UK

**Keywords**
Darwinian fitness, human, invertebrate, neuropathic pain, primary afferent neuron, spontaneous activity

## Abstract

Chronic pain caused by injury or disease of the nervous system (neuropathic pain) has been linked to persistent electrical hyperactivity of the sensory neurons (nociceptors) specialized to detect damaging stimuli and/or inflammation. This pain and hyperactivity are considered maladaptive because both can persist long after injured tissues have healed and inflammation has resolved. While the assumption of maladaptiveness is appropriate in many diseases, accumulating evidence from diverse species challenges the assumption that neuropathic pain and persistent nociceptor hyperactivity are always maladaptive. We review studies indicating that persistent nociceptor hyperactivity has undergone evolutionary selection in widespread, albeit selected, animal groups as a physiological response to increase survival long after bodily injury, using both highly conserved and divergent underlying mechanisms.

## Neuropathic pain and nociceptor hyperactivity persisting chronically are nearly always assumed to be maladaptive

Evolutionary medicine recognizes that some disease symptoms commonly assumed to be maladaptive, such as diarrhea and fever, are in fact evolutionary adaptations promoting survival and reproductive success – notwithstanding the distress they cause [1]. One malady almost universally considered maladaptive is chronic **neuropathic pain** (see **Glossary**) [2,3], which contrasts with acute pain that has clear protective functions. In humans and rodents, many examples of neuropathic pain have been linked to persistent electrical **hyperactivity** in primary **nociceptors** (Figure 1, Box 1) [reviewed by 4,5,2]. Because nociceptor activation evokes conscious pain in humans [6,7], and because nociceptor activity is sufficient and sometimes necessary for behaviors in rodents that indicate pain [8–11], continuing hyperactivity in nociceptors is recognized as a major source of persistent mammalian pain. Importantly, nociceptor hyperactivity is often generated "spontaneously" at "ectopic" sites near axonal injury or in nociceptor cell bodies rather than in the peripheral terminals where normal activation occurs (Figure 1), and both it and the associated pain sometimes persist after apparent healing of an initiating injury. In principle, persistent hyperactivity may have many different causes, both extrinsic (Figure 2A) and intrinsic (Figure 2B) to the nociceptor. Extrinsic (often inflammatory) and intrinsic (cell-autonomous) alterations overlap and work cooperatively. For example, transient exposure to serotonin may trigger an intrinsic hyperactive state that lasts minutes or hours after the signal is removed, but prolonged or repeated exposure to serotonin or other inflammatory signals may sustain hyperactivity for long periods or induce a much more persistent, memory-like hyperactive state [12–15]. Here we review evidence from mammals and invertebrates indicating that persistent nociceptor hyperactivity can be evolutionarily adaptive.

## Primary nociceptors and evolution

Because nociceptors detect injury, and injury affecting survival and reproduction can occur in any animal, cells with this function should be widespread. Indeed, nociceptors have been identified in diverse animals, including roundworms, fruit flies, leeches, snails, squid, fish, birds, rodents, cats, pigs, monkeys, and humans [16,17]. Nociceptors thus provide an opportunity for broad comparative studies into evolutionary selection pressures for nociceptive plasticity and the roles of shared and divergent physiological and molecular mechanisms. Adding to the evolutionary interest of nociceptors, adaptive responses to injury or the threat of injury may have antedated the earliest animals, shaping core mechanisms later used for nociception and neural function [18–20]. Nociceptors probably evolved early and perhaps multiple times, and thus they may be especially useful for defining ancient and more recently evolved mechanisms for adaptive injury responses.

## Taxa in which persistent nociceptor hyperactivity may not be functionally important

Insight into the adaptive roles of a physiological trait can be gained by comparing the animal groups and ecological conditions in which the trait is prominent with those in which it appears to be weak or absent. We begin with nociceptors exhibiting limited evidence of persistent hyperactivity after bodily injury.

### *Nematodes*

*Caenorhabditis elegans* has polymodal nociceptors that repeatedly respond to noxious mechanical or heat stimuli and receive neuromodulatory input to prevent habituation to noxious stimuli [21]. However, *C. elegans* neurons primarily signal by graded depolarizing responses rather than **action potentials** (APs, see Figure 1) [22], and neither persistent depolarization nor AP

generation has not been reported in nematode nociceptors after injury. The short lifespan of *C. elegans* (about 20 days), tiny body size, and rarity of survivable injuries from predators within their soil habitat [23] might explain a lack of persistent nociceptor alterations in these animals.

***Insects***

Larvae of the fruit fly *Drosophila melanogaster* show behavioral **sensitization** after tissue injury, including a lowering of threshold for heat- or mechanically induced defensive rolling following UV irradiation of the epidermis [24,25]. Behavioral sensitization was linked to various molecular changes [26], and accompanied by an increase in the range of temperatures that evoked continuing generation of APs in heat-sensitive nociceptors [27]. "Spontaneous" pain produced by continuing activity in heat-sensitive nociceptors resulting from a lowering of temperature threshold after injury has been implicated in rodents [28] (see also Figure 2B). However, for heat allodynia in *Drosophila*, UV-induced **hypersensitivity** of defensive behavior was recently found to depend more on alterations in central neurons than nociceptors [29], suggesting that heat-dependent nociceptor hyperactivity may contribute little to the behavioral alterations. Results from adult *Drosophila* also show that alterations in the central nervous system (CNS) can mediate injury-induced hypersensitivity of a defensive behavior without obvious contributions from injured primary nociceptors [30]. Amputation of one leg lowered the threshold for jumping escape to heat stimulation for at least 3 weeks. Initial nociceptor activation was necessary for inducing permanent central alterations (excitotoxic loss of GABAergic inhibitory neurons) and sensitization to heat test stimuli. However, no nociceptor alterations were reported other than the death of all nociceptors in the amputated limb. The use of amputation in this study highlights an unusual feature of *Drosophila* nociceptors that may be important for the evolution of injury-induced persistent nociceptor hyperactivity. Unlike in leeches, *Aplysia*, squid, and vertebrates, where nociceptor cell bodies occur within centrally located ganglia, *Drosophila* nociceptor cell bodies are located just

beneath the epidermis where they can easily be killed by even superficial tissue injuries. This anatomical feature in insects should prevent effective nociceptor sensitization at a site of injury, which is consistent with the lack of enhanced sensory activity recorded from nerves innervating a previously injured region in larvae of the moth *Manduca sexta* [31].

***Mole-rats***

Most investigations of persistent nociceptor hyperactivity have used laboratory rats, *Rattus norvegicus*, and mice, *Mus musculus*, which show robust, complex alterations of both behavior and nociceptor function after injury. Quite different nociceptive reactions have been found in naked mole-rats (*Heterocephalus glaber*), which show distinctive traits related to their subterranean existence [32]. Two differences have been found between nociceptors of naked mole-rats and laboratory rodents that are consistent with limited injury-induced nociceptor hyperactivity in mole-rats.

First, mole-rats have far fewer cutaneous nociceptors. In most mammals, the skin is innervated by ~4 times as many unmyelinated C-fibers as myelinated A-fibers, and most cutaneous C-fibers are nociceptors [33]. In adult naked mole-rats, the ratio of cutaneous C- to A-fibers is ~1:1 [34,35]. Fewer nociceptors in a subterranean animal might represent an evolutionary response to reduce the energy costs of maintaining defensive neurons that are less useful in an environment where predation is reduced, as has been suggested for a central defensive neuron, the Mauthner cell, in a cave fish species [36,37].

Second, nociceptors in naked mole-rats exhibit adaptations that limit their ability to drive pain under inflammatory conditions. Presumed nociceptors in mammals are often identified by sensitivity to capsaicin, the pain-evoking ingredient of chili peppers, and by expression of the

capsaicin receptor, transient receptor potential vanilloid 1 (TRPV1). In other mammals, TRPV1+ nociceptors are important for driving inflammatory pain. Unexpectedly, in naked mole-rats the synapses of TRPV1+ sensory neurons are distributed randomly throughout the dorsal spinal cord [34], rather than being concentrated in the superficial dorsal horn of the spinal cord that is critical for relaying nociception in other mammals. Another adaptation is related to acid sensitivity. Tissue injury and inflammation are accompanied by tissue acidosis, and most mammals utilize a plethora of acid-sensitive receptors and ion channels to activate nociceptors and drive inflammatory pain [38]. However, naked mole-rats live in large colonies in poorly ventilated burrows, and thus experience high $CO_2$ levels. $CO_2$ can produce carbonic acid, releasing protons that activate acid receptors of naked mole-rats and other rodents. Surprisingly, acid fails to evoke APs or consequent pain behavior in naked mole-rats. This is because a mutation in voltage-gated $Na^+$ channel $Na_V1.7$ enables protons to block the channel and prevent AP generation under acidic conditions [39]. A third adaptation is reduced function of the naked mole-rat tropomyosin receptor kinase A (TrkA) for nerve growth factor (NGF), which is released in mammals during injury and inflammation. This TrkA hypofunctionality results in an absence of NGF-induced hyperalgesia in naked mole-rats and might also contribute developmentally to the reduced nociceptor number. These adaptations in the naked mole-rat and additional adaptations in related mole-rats [40] should reduce the likelihood of persistent nociceptor hyperactivity being induced by injury and inflammation (although this prediction has yet to be tested directly). Loss of nociceptor hyperactivity might have been promoted during evolution by the relative safety from predation afforded by subterranean environments.

## Taxa in which persistent nociceptor hyperactivity is likely to be functionally important

All the species (including humans) in which persistent nociceptor hyperactivity has been observed after nerve or tissue injury have probably been subject to high levels of predation in their recent evolutionary history, and this hyperactivity is generated both near a site of injury and sometimes also in nociceptor cell bodies that lie in central ganglia distant from the body surface (Figure 1).

### *Gastropod molluscs*

The marine snail, *Aplysia californica*, lacks a shell and is one of the larger invertebrates, reaching weights of ~1 kg in its 2-year lifespan. Identified *Aplysia* nociceptors are activated preferentially by pinching or crushing stimuli and are unresponsive to chemical or thermal stimuli. Hyperactivity lasting days to months after experimental injury (nerve crush or wounding that transects major nerve branches) is expressed as reduced AP threshold and **afterdischarge** (Figure 1C) generated in nociceptor peripheral terminals [41], the injured axon [42], and the cell body [43]. The function of nociceptor cell body hyperactivity in *Aplysia* is, via afterdischarge, to amplify the number of APs reaching central synaptic terminals, thereby enhancing defensive behavioral responses [44]. Many invertebrates, including *Aplysia* and leeches, lack image-forming eyes and depend upon chemical and mechanical senses to detect predatory threats. In *Aplysia*, nociceptive sensitization after injury not only increases nociceptor responses to noxious stimuli, but also reduces nociceptor mechanical threshold into the innocuous range [45], which may promote escape at the first contact with soft, slowly moving carnivorous gastropods that prey upon *Aplysia* [46].

### *Cephalopod molluscs*

Nociceptors that respond to intense mechanical stimuli and fail to respond to heat have also been identified in the squid *Doryteuthis pealeii*. Activity recorded in fin-innervating nerves showed that nociceptors are activated by forces that can damage the fin, and, following noxious activation, the

nociceptor mechanical activation threshold decreased dramatically (~50 fold) [47]. Two findings in this study were unexpected. First, injury produced either experimentally to the fin in vivo or more naturally to various body regions (caused by attacks from other squid) produced **spontaneous activity** (Figure 1D) lasting at least 24 hours after injury. This remains the only reported invertebrate example of injury-induced spontaneous activity in primary afferent neurons. Second, after injury to one side of the animal, both nociceptor sensitization to mechanical stimulation and spontaneous activity were found in nociceptors innervating the contralateral fin. This generalized hyperactivity contrasts with hyperactivity in mammalian nociceptors that is usually restricted to neurons near an injury site.

***Mammals***

Most studies of persistent nociceptor hyperactivity have utilized rat or mouse neuropathic pain models [reviewed by 4,5,2]. Hyperactivity can manifest in nociceptors that normally are electrically silent as increased generation of APs evoked by extrinsic signals or generated by intrinsic alterations (including spontaneous activity) (Figures 1C, 1D, 2A, 2B, Box 1). Cell body hyperactivity is typically assumed to be an uncommon and less important parallel to hyperactivity generated in peripheral terminals. The more severe an injury is, however, the more likely peripheral nerve branches are to be crushed or cut, disconnecting the affected nociceptors from their normal sources of excitation. Peripherally disconnected nociceptors may still contribute ongoing information about injury because sensory receptors and ion channels accumulate at the tips of cut axons, which along with other alterations, can impart sensitivity, hyperexcitability, and spontaneous activity to axons at the injury site [48–50]. Ongoing pain in humans from peripheral injury or neuropathy [51] can be eliminated rapidly by blocking peripheral nerve conduction with a voltage-gated sodium channel ($Na_V$) inhibitor, lidocaine, which also can partially ameliorate pain from amputation [52]. In addition, persistent spontaneous activity may be generated in the cell body

[53,54]. For example, lidocaine delivered directly to human dorsal root ganglia (DRG) innervating an amputated limb at concentrations that block local AP generation without blocking conduction through the ganglia rapidly and reversibly relieved phantom limb pain [55] (Figure 3), providing strong evidence for the clinical importance of spontaneous activity generated in sensory neuron cell bodies. Spontaneous activity also occurs in cultured DRG neurons from cancer patients with neuropathic pain who have undergone thoracic vertebrectomy surgery that removes DRGs [56]. An experimental advantage of cell-body generation of hyperactivity in mammalian (and gastropod) nociceptors is that the mechanisms can be investigated more directly than is possible in less accessible compartments of the nociceptor.

## Evidence that pain-like behavior associated with nociceptor hyperactivity is adaptive in cephalopod molluscs

As mentioned, injury to different parts of the body surface of squid induces long-lasting nociceptor spontaneous activity, which was suggested to drive a state of generalized hypervigilance [47,57]. The adaptiveness of this state was tested by examining the effects on survival of squid in staged interactions with fish that are their natural predators (Figure 3) [58]. A relatively minor injury, distal amputation of one of a squid's eight arms, produced immediate defensive behavior and sensitization of responses to tactile and visual stimuli, but no lasting effects on motor function [57]. When exposed to predatory sea bass (*Centropristis striata*), squid with minor amputation injury were more likely to be attacked than uninjured squid, but they also began their escape behavior sooner than uninjured squid [58]. Briefly blocking nociceptor activation/sensitization during amputation by transient delivery of an anesthetic dose of $MgCl_2$ resulted in injured squid later failing to show early responses to approaching sea bass and being less likely to escape. This result demonstrates how nociceptive sensitization enhances evolutionary fitness by increasing

survival during predatory encounters and, combined with earlier electrophysiological observations, it suggests that persistent nociceptor spontaneous activity can drive a hypervigilant state that protects animals from attack during periods of increased vulnerability. Analogous results were found in mice experiencing chronic pain in a spared nerve injury model (Figure 3). When neuropathic mice had to choose between long and short routes to a food reward, they were more likely than healthy mice to avoid the short route if it exposed them to the smell of fox urine, suggesting that continuing awareness of their injury influenced their behavioral choices during elevated predatory risk [59]. Persistent injury-induced hypervigilance may be expressed as anxiety [60], which is a common comorbidity of chronic pain in humans and rodents [61].

Are the hypervigilant states driven by nociceptor hyperactivity in cephalopods associated with motivational properties of affective pain in mammals? This question has not yet been addressed experimentally in squid, but a study with octopus [62] using another noxious stimulus, acetic acid, shows that nociceptive states with pain-like aversive and cognitive features occur in a cephalopod. Octopuses remembered a chamber in which an arm was injected with acetic acid and subsequently avoided it (conditioned place aversion). When acetic acid injection was followed by lidocaine injection into the same site immediately prior to placement in a different chamber, octopuses later chose to spend time in the chamber associated with lidocaine's block of ongoing nociceptive activity (conditioned place preference). Thus, the octopus's voluntary behavior revealed a pain-like link between flexible cognitive processing (including complex associative learning, memory, and decision making) and a potent aversive state induced by noxious stimulation. Although recordings could not be made from primary afferent neurons in the octopus arm, downstream electrical activity recorded in a central pathway from the injected arm to the brain continued for at least 30 minutes after acetic acid injection. The immediate block of this activity by lidocaine injected into the acetic acid injection site is consistent with ongoing nociceptor

activity generated at an injury site driving pain-like motivational and cognitive processes that enable place aversion and place preference in octopuses. These findings [62] parallel observations in rodents where spontaneous nociceptor activity is associated with a tonic aversive state that can be revealed in place preference or aversion experiments.

## Anatomical features and electrophysiological specializations of mammalian nociceptors foster persistent hyperactivity after injury

Spontaneous activity generated in mammalian nociceptor cell bodies and continuing long after peripheral injury might function, like the spontaneous activity in squid [47,58], to drive painful hypervigilance after injuries severe enough to increase an individual's vulnerability to attack by predators or conspecifics [63]. In mammals, primary sensory neuron cell bodies are likely to survive serious peripheral injury because they are protected, not only by their central location far from their receptive fields (Figure 1A), but also by the bone and dura covering sensory ganglia. The cell bodies also are uniquely exposed to injury signals (Figure 2) that can induce or persistently drive hyperactivity. These signals may be conducted electrically or conveyed by axonal transport from the nociceptors' peripheral processes, or come from molecules both in the cerebrospinal fluid and in the blood (because of the absence of an effective vascular permeability barrier in the ganglia), from resident or infiltrating immune cells, and potentially from molecules received by sensory neuron presynaptic terminals within the CNS from postsynaptic neurons and glia [63,64]. While these features have many explanations, plausible functions are to enable nociceptors to survive peripheral injury, integrate information about injury severity in tissues that may have been disconnected from the CNS, and continue to inform the CNS about persistent peripheral dysfunction.

One criterion proposed for a biological process to be an adaptation is that it employs complex, complementary mechanisms for a plausible function [65]. Assuming persistent hyperactivity of mammalian nociceptors has a protective function after injuries severe enough to induce neuropathic pain, this criterion is met for hyperactivity generated in the cell body. In terms of membrane potential, there are only three possible alterations that can result in AP generation in the absence of excitation from fast synaptic potentials (which mammalian nociceptors do not receive) or sensory receptor potentials (Figure 1B, which are unlikely to be generated in the cell body): depolarization of **resting membrane potential** (RMP) to reach AP threshold, hyperpolarization of AP threshold to reach RMP, and enhancement of depolarizing spontaneous fluctuations of RMP (DSFs, Figure 1D) to bridge the gap between RMP and AP threshold (Figure 2B). Recordings from rodent nociceptors in a variety of different persistent pain models showed that all three properties are altered to promote spontaneous activity [66,67] [68] [69]. Spontaneous activity and all three contributing electrophysiological alterations also were found in probable nociceptors isolated from human cancer patients suffering from neuropathic pain caused by compression of spinal nerve roots [56,70]. Importantly, these coordinated effects occurred almost exclusively in nociceptors taken from ganglia innervating dermatomes reported by the patients to have ongoing pain.

RMP, action potential threshold, and DSFs in nociceptors each involve numerous ion channels, and the interactions between membrane potential, channel activity, and associated intracellular signaling are highly complex. Although spontaneous activity in mammalian pain models might be a purely pathological side effect of various insults to the body and nervous system, the intricate, functionally cohesive electrophysiological alterations that underpin spontaneous activity suggests that many damaging conditions can recruit a persistent, specialized state that was selected during evolution to ensure that nociceptor hyperactivity continues after injury severe enough to produce

physical impairment detectable by predators and competitors. Continuing pain would promote protective behavior (including hypervigilance, sometimes manifested in mammals as anxiety [61]) during increased vulnerability and risk of attack, which could be chronic for severe injuries such as amputation (Figure 3) [63].

## Shared signaling mechanisms support nociceptor hyperactivity in a gastropod mollusc and in rodents

From an evolutionary perspective, persistent nociceptor hyperactivity is interesting because it is found in two distantly related animal phyla (chordates and molluscs), suggesting common functions and potentially ancient mechanisms. However, even within placental mammals, nociceptor physiology shows clear divergences, raising questions about whether fundamental hyperactivity mechanisms are shared across the enormous phylogenetic distances between invertebrates and mammals .

Nociceptors in rodents and *Aplysia* share fundamental mechanisms important for synaptic plasticity [71] and hyperactivity. In *Aplysia,* long-lasting hyperactivity in dissociated nociceptors induced by serotonin was found to require local protein synthesis [72]. Persistent axonal hyperactivity induced by nerve injury or nerve depolarization using elevated extracellular $K^+$ to mimic the depolarization produced by axotomy [42], or serotonin application to a nerve [12]*,* also required protein synthesis, which was controlled by the mechanistic target of rapamycin (mTOR) pathway. Detailed studies in mice showed that activity-dependent mRNA translation regulated by mitogen-activated protein kinase (MAPK)-interacting kinase (MNK) phosphorylation of eukaryotic translation initiation factor 4E (eIF4E) is necessary for nociceptor hyperexcitability after peripheral nerve injury or inflammatory signals [73,74]. *Aplysia* has MNK1 and eIF4E proteins [75], with

conservation of the eIF4E serine residue that is phosphorylated by MNK1 and required in mice for nociceptor hyperactivity induced by inflammatory mediators [73]. A selective MNK inhibitor potently inhibited the axonal hyperexcitability induced by depolarization of an *Aplysia* nerve, as did general inhibitors of tyrosine receptor kinases (known upstream activators of MNKs) and of extracellular receptor kinases (ERKs, which activate MNKs) [75].

A cell signal that plays prominent but complex roles in persistent hyperexcitability in *Aplysia* nociceptors is cyclic adenosine monophosphate (cAMP) [76–78]. Cyclic AMP has long been known to increase mammalian nociceptor excitability [79] and cAMP signaling is essential for maintaining rat nociceptor hyperactivity months after spinal cord injury, via protein kinase A [80], exchange protein activated by cAMP (EPAC) [68], and indirect positive feedback through depolarization and consequent C-Raf and ERK signaling, plus a reduction of adenylyl cyclase's sensitivity to inhibition by opioids [81]. Thus, MNK-dependent protein synthesis, ERK signaling, which is upstream of MNK [82], and cAMP signaling all are major contributors to nociceptor hyperactivity in species whose last common ancestor lived ~600 million years ago.

## Continuing evolution of nociceptor phenotypes in mammals may explain unique aspects of human neuropathic pain

The last common ancestor of placental mammals, including rodents and primates, lived ~65 million years ago [83], far more recently than when molluscs and chordates diverged [71]. During this period several notable differences emerged between human and rodent nociceptors that may contribute to the prominence of neuropathic pain in our species. Rodent nociceptors are typically divided into two classes, peptidergic and non-peptidergic. Peptidergic nociceptors express TrkA into adulthood and contain neuropeptides such as calcitonin gene-related peptide (CGRP); they

typically innervate deeper tissues and connect to neurons in lamina I of the spinal dorsal horn. Non-peptidergic nociceptors innervate the skin, do not express TrkA in adulthood, and connect to neurons in lamina II of the dorsal horn. The two classes appear specialized for thermal and mechanical pain, respectively [84], although genetic tools are revealing finer functional subdivisions within each class. Unexpectedly, in adult humans, all nociceptors express TrkA and most express CGRP, suggesting that human nociceptors are generally peptidergic [85,86]. All human nociceptors also express TRPV1, which can be sensitized by diverse inflammatory mediators such that their temperature activation threshold sometimes falls into the range of core body temperature (Figure 2B). TRPV1 is only expressed by ~half of rodent nociceptors [87]. Thus, human nociceptors appear more polymodal than rodent nociceptors, and possibly than other primate nociceptors [86,88–90]. This would make them sensitive to a broader range of inflammatory mediators, which can persist long after nerve injury [56], thereby increasing extrinsically driven nociceptor hyperactivity and inducing intrinsic hyperactivity (Figure 2).

Human nociceptors seem unusually excitable compared with other animals. DRG neurons cultured after recovery from organ donors exhibit a greater density of tetrodotoxin (TTX)-sensitive and TTX-resistant $Na_V$ current than rodent nociceptors [91]. RNA sequencing and functional experiments suggest that Nav1.7 (SCN9A) expression is higher in human than rodent nociceptors [92]. Accordingly, a greater ratio of TTX-sensitive to TTX-resistant current has been observed in human nociceptors, and the TTX-sensitive current is activated at more hyperpolarized potentials [91]. Furthermore, a lack of use-dependent inactivation of TTX-resistant currents in human nociceptors [91] should promote spontaneous activity, as observed in cultured DRG neurons from neuropathic pain patients [56,70]. Electrophysiological differences are not limited to $Na_V$ currents. Different densities and kinetics of voltage-gated calcium ($Ca_v$) currents suggest that activity-

dependent increases in intracellular $Ca^{2+}$ and consequent cell signaling effects may be smaller in human than rodent nociceptors [93]. If human evolution led to a greater propensity towards chronic pain [94], perhaps via continuing nociceptor hyperactivity, then smaller $Ca^{2+}$ responses could help protect against toxicity from excessive $Ca^{2+}$ accumulation during continuing hyperactivity.

## Concluding remarks and future perspectives

In humans and rodents, persistent nociceptor hyperactivity is a major source of chronic neuropathic pain. Finding that persistent nociceptor hyperactivity both in mammals and molluscs is induced by injuries sufficiently severe to damage nerves indicates it might have a common function in diverse animal species. Evidence from squid and mice suggests that persistently enhanced vulnerability resulting from injury-induced physical impairment can be compensated for by behavioral alterations, including hypervigilance, driven by prolonged nociceptor hyperactivity. The lack of clear evidence thus far for injury-induced persistent nociceptor hyperactivity in naked mole-rats, *Drosophila*, and *C. elegans* suggests that a protective hypervigilance function has not been evolutionarily significant in some lineages (e.g., because of limited predation or short lifespans), and/or that alternative mechanisms (e.g., hyperexcitability in central neurons) were preferentially selected. In rodent and human nociceptors, synergistic electrophysiological alterations underpinning spontaneous activity are consistent with hyperactivity functioning biologically to drive persistent hypervigilance. Future perspectives emphasize further exploration of functional and mechanistic differences between species having and lacking persistent nociceptor hyperactivity (see Outstanding Questions). Also needed is expanded investigation across diverse taxa into common mechanisms (such as those shared by *Aplysia* and mice) as well as divergent mechanisms driving persistent hyperactivity. Finally, unexpected molecular differences between nociceptors in humans and other mammals encourage further study into the

possibility that very recent evolution of human nociceptors increased their susceptibility to persistent hyperactivity, and thus that humans potentially are more prone than other animals to chronic pain.

**Box 1. Hyperactivity and hyperexcitability are not the same.**

The terms hyperexcitability and hyperactivity are rarely distinguished, but hyperactivity is more important physiologically and probably evolutionarily. In studies of neuronal plasticity involving alterations of AP function, the most common experimental indicator of an altered electrophysiological state is modified electrical excitability – either hyperexcitability or hypoexcitability. Hyperexcitability is demonstrated when electrical stimulation more readily triggers an AP. Experimental injection of depolarizing current through a recording pipette, either in a series of increasingly depolarizing current steps or in response to a smooth depolarizing ramp of current, is often used to reveal that an AP is generated by stimulation with less injected current (sometimes referred to as a decrease in rheobase) or is triggered at a more negative membrane potential. Under natural conditions, hyperexcitability would make it more likely that a physiological stimulus such as a sensory generator potential (Figure 1) or synaptic potential would reach AP threshold and produce activity.

For most neurons in the animal kingdom, the frequency and pattern of APs (i.e., electrical activity) are the critical physiological endpoints of excitability. These both determine a neuron's immediate effect on its postsynaptic targets and produce activity-dependent synaptic plasticity to modify the effects of its subsequent activity. Hyperexcitability usually promotes hyperactivity, but *many* other intrinsic and extrinsic states of a neuron and its inputs will determine whether a neuron is hyperactive and what its level and pattern of activity are. As shown with examples in Figure 2, numerous drivers of hyperactivity in mammalian nociceptors may contribute after bodily injury, including intrinsic hyperexcitability, intrinsic hypersensitivity to extrinsic excitatory chemical signals, increased exposure to extrinsic excitatory signals, hyposensitivity to inhibitory signals, and decreased exposure to extrinsic inhibitory signals.

Evolutionary selection could change any or all of the intrinsic and extrinsic drivers of persistent nociceptor hyperactivity to make some species more or less likely to experience persistent pain-related states driven by continuing activity of nociceptors. The accessibility of nociceptors in diverse animal taxa enables direct inquiry into potentialy adaptive functions of persistent nociceptor hyperactivity, as well as into similarities and differences in the underlying mechanisms.

## Glossary

**Afterdischarge:** APs generated in response to a preceding AP or burst of APs, prolonging the electrical activity.

**Depolarizing spontaneous fluctuation (DSF):** brief, intrinsically generated depolarizing fluctuations of RMP that in nociceptors under hyperexcitable conditions may intermittently reach AP threshold.

**Hyperactivity:** state of increased discharge of APs resulting from drivers intrinsic or extrinsic to a neuron.

**Hyperexcitability:** electrophysiological state in which the likelihood of generating at least one AP by depolarizing stimulation is increased.

**Hypersensitivity:** state of increased responsiveness to a given sensory stimulus or chemical signal. In contrast, increased responsiveness to an electrical signal is termed hyperexcitability.

**Neuropathic pain**: pain produced by injury or disease of any part of the nervous system.

**Nociceptor:** a primary sensory neuron that is selectively activated by stimuli causing actual or impending tissue injury, or by signals reliably associated with tissue injury (e.g., inflammatory signals).

**Resting membrane potential (RMP):** electrical potential difference across the plasma membrane in the absence of APs, sensory potentials, or synaptic potentials. If DSFs occur, they are considered transient components of RMP.

**Sensitization:** a state in which a neuron, neuronal pathway, or animal exhibits increased responsiveness to sensory stimuli or chemical signals (including electrically driven chemical signals). The term often is used more broadly to indicate any increase in responsiveness.

**Spontaneous activity:** (formally) ongoing generation of action potentials by a cell without concurrent extrinsic sources of depolarization [66]. Because it is usually impractical to identify

background excitatory or sensitizing contributions to ongoing activity, ongoing activity generated in neurons in the absence of sensory generator potentials or synaptic potentials is commonly referred to as spontaneous activity.

## Acknowledgements

ETW was supported by NIH grants NS091759 and RO1NS111521. RJC was supported by an Allen Distinguished Investigator Award, a Paul G. Allen Frontiers Group advised grant of the Paul G. Allen Family Foundation, and NSF IOS 204-733. GGN was supported by Australian National Health and Medical Research Council grants APP1026310, APP1028887, and APP1046090. TJP was supported by NIH grants NS111929 and NS102161. EStJS was supported by a grant from the Medical Research Council MR/W002426/1.

## References


1 Williams, G.C. and Nesse, R.M. (1991) The dawn of Darwinian medicine. Q Rev Biol 66, 1–22

2 Finnerup, N.B. et al. (2021) Neuropathic Pain: From Mechanisms to Treatment. Physiol Rev 101, 259–301

3 Sommer, C. et al. (2018) Inflammation in the pathophysiology of neuropathic pain. Pain 159, 595–602

4 Roza, C. and Bernal, L. (2022) Electrophysiological characterization of ectopic spontaneous discharge in axotomized and intact fibers upon nerve transection: a role in spontaneous pain. Pflugers Arch 474, 387–396

5 Walters, E.T. (2021) Nociceptors and chronic pain. In *Oxford Research Encyclopedia of Neuroscience* (Murray, S. Oxford University Press

6 Ackerley, R. and Watkins, R.H. (2018) Microneurography as a tool to study the function of individual C-fiber afferents in humans: responses from nociceptors, thermoreceptors, and mechanoreceptors. J Neurophysiol 120, 2834–2846

7 Kleggetveit, I.P. et al. (2012) High spontaneous activity of C-nociceptors in painful polyneuropathy. Pain 153, 2040–2047

8 Cowie, A.M. et al. (2018) Optogenetic Inhibition of CGRPα Sensory Neurons Reveals Their Distinct Roles in Neuropathic and Incisional Pain. J Neurosci 38, 5807–5825

9 Beaudry, H. et al. (2017) Distinct behavioral responses evoked by selective optogenetic stimulation of the major TRPV1+ and MrgD+ subsets of C-fibers. Pain 158, 2329–2339

10 Daou, I. et al. (2016) Optogenetic Silencing of Nav1.8-Positive Afferents Alleviates Inflammatory and Neuropathic Pain. eNeuro 3,

11 Yang, Q. et al. (2014) Persistent pain after spinal cord injury is maintained by primary afferent activity. J Neurosci 34, 10765–10769

12 Weragoda, R.M. and Walters, E.T. (2007) Serotonin induces memory-like, rapamycin-sensitive hyperexcitability in sensory axons of Aplysia that contributes to injury responses. J Neurophysiol 98, 1231–1239

13 Reichling, D.B. and Levine, J.D. (2009) Critical role of nociceptor plasticity in chronic pain. Trends Neurosci 32, 611–618

14 Liu, R.Y. et al. (2011) The requirement for enhanced CREB1 expression in consolidation of long-term synaptic facilitation and long-term excitability in sensory neurons of Aplysia. J Neurosci 31, 6871–6879

15 Lopez, E.R. et al. (2021) Serotonin enhances depolarizing spontaneous fluctuations, excitability, and ongoing activity in isolated rat DRG neurons via 5-$HT_4$ receptors and cAMP-dependent mechanisms. Neuropharmacology 184, 108408

16 Smith, E.S. and Lewin, G.R. (2009) Nociceptors: a phylogenetic view. J Comp Physiol A Neuroethol Sens Neural Behav Physiol 195, 1089–1106

17 Walters, E.T. (2020) Evolutionary aspects of nociception and pain. In *The Senses: A Comprehensive Reference, Vol 5, Pain* (Fritzsch, B. et al. eds.), pp. 463–480, Elsevier, Academic Press

18 Monk, T. and Paulin, M.G. (2014) Predation and the origin of neurones. Brain Behav Evol 84, 246–261

19 Brunet, T. and Arendt, D. (2016) From damage response to action potentials: early evolution of neural and contractile modules in stem eukaryotes. Philos Trans R Soc Lond B Biol Sci 371, 20150043

20 Moroz, L.L. et al. (2021) Neural versus alternative integrative systems: molecular insights into origins of neurotransmitters. Philos Trans R Soc Lond B Biol Sci 376, 20190762

21 Marques, F. et al. (2021) Signaling via the FLP-14/FRPR-19 neuropeptide pathway sustains nociceptive response to repeated noxious stimuli in C. elegans. PLoS Genet 17, e1009880

22 Jiang, J. et al. (2022) C. elegans enteric motor neurons fire synchronized action potentials underlying the defecation motor program. Nat Commun 13, 2783

23 Quach, K.T. and Chalasani, S.H. (2022) Flexible reprogramming of Pristionchus pacificus motivation for attacking Caenorhabditis elegans in predator-prey competition. Curr Biol 32, 1675–1688.e7

24 Babcock, D.T. et al. (2009) Cytokine signaling mediates UV-induced nociceptive sensitization in Drosophila larvae. Curr Biol 19, 799–806

25 Jang, W. et al. (2018) Duox mediates ultraviolet injury-induced nociceptive sensitization in Drosophila larvae. Mol Brain 11, 16

26 Follansbee, T.L. et al. (2017) Drosophila nociceptive sensitization requires BMP signaling via the canonical SMAD pathway. J Neurosci 37, 8524–8533

27 Im, S.H. et al. (2015) Tachykinin acts upstream of autocrine Hedgehog signaling during nociceptive sensitization in Drosophila. Elife 4, e10735

28 Banik, R.K. and Brennan, T.J. (2009) Trpv1 mediates spontaneous firing and heat sensitization of cutaneous primary afferents after plantar incision. Pain 141, 41–51

29 Gu, P. et al. (2022) Nociception and hypersensitivity involve distinct neurons and molecular transducers in Drosophila. Proc Natl Acad Sci U S A 119, e2113645119

30 Khuong, T.M. et al. (2019) Nerve injury drives a heightened state of vigilance and neuropathic sensitization in Drosophila. Sci Adv 5, eaaw4099

31 Tabuena, D.R. et al. (2017) Central neural alterations predominate in an insect model of nociceptive sensitization. J Comp Neurol 525, 1176–1191

32 Buffenstein, R. et al. (2022) The naked truth: a comprehensive clarification and classification of current ‘myths’ in naked mole-rat biology. Biol Rev Camb Philos Soc 97, 115–140

33 Schmalbruch, H. (1986) Fiber composition of the rat sciatic nerve. Anat Rec 215, 71–81

34 Park, T.J. et al. (2008) Selective inflammatory pain insensitivity in the African naked mole-rat (Heterocephalus glaber). PLoS Biol 6, e13

35 Omerbašić, D. et al. (2016) Hypofunctional TrkA Accounts for the Absence of Pain Sensitization in the African Naked Mole-Rat. Cell Rep 17, 748–758

36 Gallman, K. et al. (2020) Differences in behavior between surface and cave Astyanax mexicanus may be mediated by changes in catecholamine signaling. J Comp Neurol 528, 2639–2653

37 Tanvir, Z. et al. (2021) Evolutionary and homeostatic changes in morphology of visual dendrites of Mauthner cells in Astyanax blind cavefish. J Comp Neurol 529, 1779–1786

38 Pattison, L.A. et al. (2019) Evolution of acid nociception: ion channels and receptors for detecting acid. Philos Trans R Soc Lond B Biol Sci 374, 20190291

39 Smith, E.S. et al. (2011) The molecular basis of acid insensitivity in the African naked mole-rat. Science 334, 1557–1560

40 Eigenbrod, O. et al. (2019) Rapid molecular evolution of pain insensitivity in multiple African rodents. Science 364, 852–859

41 Dulin, M.F. et al. (1995) Recovery of function, peripheral sensitization and sensory neurone activation by novel pathways following axonal injury in Aplysia californica. J Exp Biol 198, 2055–2066

42 Weragoda, R.M. et al. (2004) Memory-like alterations in Aplysia axons after nerve injury or localized depolarization. J Neurosci 24, 10393–10401

43 Walters, E.T. et al. (1991) Similar neuronal alterations induced by axonal injury and learning in Aplysia. Science 253, 797–799

44 Gasull, X. et al. (2005) Evidence that long-term hyperexcitability of the sensory neuron soma induced by nerve injury in Aplysia is adaptive. J Neurophysiol 94, 2218–2230

45 Billy, A.J. and Walters, E.T. (1989) Long-term expansion and sensitization of mechanosensory receptive fields in Aplysia support an activity-dependent model of whole-cell sensory plasticity. J Neurosci 9, 1254–1262

46 Pepino, C. et al. (2022) Sensitized by a sea slug: Site-specific short-term and general long-term sensitization in Aplysia following Navanax attack. Neurobiol Learn Mem 187, 107542

47 Crook, R.J. et al. (2013) Squid have nociceptors that display widespread long-term sensitization and spontaneous activity after bodily injury. J Neurosci 33, 10021–10026

48 Devor, M. et al. (1992) Eicosanoids, but not tachykinins, excite C-fiber endings in rat sciatic nerve-end neuromas. Neuroreport 3, 21–24

49 Bernal, L. et al. (2016) Spontaneous activity in C-fibres after partial damage to the saphenous nerve in mice: Effects of retigabine. Eur J Pain 20, 1335–1345

50 Yatziv, S.L. and Devor, M. (2019) Suppression of neuropathic pain by selective silencing of dorsal root ganglion ectopia using nonblocking concentrations of lidocaine. Pain 160, 2105–2114

51 Haroutounian, S. et al. (2014) Primary afferent input critical for maintaining spontaneous pain in peripheral neuropathy. Pain 155, 1272–1279

52 Buch, N.S. et al. (2019) The role of afferent input in postamputation pain: a randomized, double-blind, placebo-controlled crossover study. Pain

53 Wall, P.D. and Devor, M. (1983) Sensory afferent impulses originate from dorsal root ganglia as well as from the periphery in normal and nerve injured rats. Pain 17, 321–339

54 Bedi, S.S. et al. (2010) Chronic spontaneous activity generated in the somata of primary nociceptors is associated with pain-related behavior after spinal cord injury. J Neurosci 30, 14870–14882

55 Vaso, A. et al. (2014) Peripheral nervous system origin of phantom limb pain. Pain 155, 1384–1391

56 North, R.Y. et al. (2019) Electrophysiological and transcriptomic correlates of neuropathic pain in human dorsal root ganglion neurons. Brain 142, 1215–1226

57 Crook, R.J. et al. (2011) Peripheral injury induces long-term sensitization of defensive responses to visual and tactile stimuli in the squid Loligo pealeii, Lesueur 1821. J Exp Biol 214, 3173–3185

58 Crook, R.J. et al. (2014) Nociceptive sensitization reduces predation risk. Curr Biol 24, 1121–1125

59 Lister, K.C. et al. (2020) Chronic pain produces hypervigilance to predator odor in mice. Curr Biol 30, R866–R867

60 Walters, E.T. (1994) Injury-related behavior and neuronal plasticity: an evolutionary perspective on sensitization, hyperalgesia, and analgesia. Int Rev Neurobiol 36, 325–427

61 Kremer, M. et al. (2021) How to study anxiety and depression in rodent models of chronic pain. Eur J Neurosci 53, 236–270

62 Crook, R.J. (2021) Behavioral and neurophysiological evidence suggests affective pain experience in octopus. iScience 24, 102229

63 Walters, E.T. (2019) Adaptive mechanisms driving maladaptive pain: how chronic ongoing activity in primary nociceptors can enhance evolutionary fitness after severe injury. Philos Trans R Soc Lond B Biol Sci 374, 20190277

64 Walters, E.T. (2012) Nociceptors as chronic drivers of pain and hyperreflexia after spinal cord injury: an adaptive-maladaptive hyperfunctional state hypothesis. Front Physiol 3, 309

65 Andrews, P.W. et al. (2002) Adaptationism--how to carry out an exaptationist program. Behav Brain Sci 25, 489–504; discussion 504

66 Odem, M.A. et al. (2018) Isolated nociceptors reveal multiple specializations for generating irregular ongoing activity associated with ongoing pain. Pain 159, 2347–2362

67 Bavencoffe, A.G. et al. (2022) Macrophage Migration Inhibitory Factor (MIF) Makes Complex Contributions to Pain-Related Hyperactivity of Nociceptors after Spinal Cord Injury. J Neurosci JN–RM

68 Berkey, S.C. et al. (2020) EPAC1 and EPAC2 promote nociceptor hyperactivity associated with chronic pain after spinal cord injury. Neurobiol Pain 7, 100040

69 Laumet, G. et al. (2020) Interleukin-10 resolves pain hypersensitivity induced by cisplatin by reversing sensory neuron hyperexcitability. Pain 161, 2344–2352

70 North, R.Y. et al. (2022) Electrophysiological alterations driving pain-associated spontaneous activity in human sensory neuron somata parallel alterations described in spontaneously active rodent nociceptors. J Pain S1526–5900(22)00047

71 Orvis, J. et al. (2022) The evolution of synaptic and cognitive capacity: Insights from the nervous system transcriptome of Aplysia. Proc Natl Acad Sci U S A 119, e2122301119

72 Dale, N. et al. (1987) Serotonin produces long-term changes in the excitability of Aplysia sensory neurons in culture that depend on new protein synthesis. J Neurosci 7, 2232–2238

73 Moy, J.K. et al. (2017) The MNK-eIF4E Signaling Axis Contributes to Injury-Induced Nociceptive Plasticity and the Development of Chronic Pain. J Neurosci 37, 7481–7499

74 Jeevakumar, V. et al. (2020) IL-6 induced upregulation of T-type $Ca^{2+}$ currents and sensitization of DRG nociceptors is attenuated by MNK inhibition. J Neurophysiol 124, 274–283

75 Mihail, S.M. et al. (2019) MNK-eIF4E signalling is a highly conserved mechanism for sensory neuron axonal plasticity: evidence from Aplysia californica. Philos Trans R Soc Lond B Biol Sci 374, 20190289

76 Scholz, K.P. and Byrne, J.H. (1988) Intracellular injection of cAMP induces a long-term reduction of neuronal K+ currents. Science 240, 1664–1666

77 Liao, X. et al. (1999) Activation of protein kinase A contributes to the expression but not the induction of long-term hyperexcitability caused by axotomy of Aplysia sensory neurons. J Neurosci 19, 1247–1256

78 Zhang, Y. et al. (2021) Quantitative description of the interactions among kinase cascades underlying long-term plasticity of Aplysia sensory neurons. Sci Rep 11, 14931

79 Gold, M.S. et al. (1998) Modulation of TTX-R INa by PKC and PKA and their role in PGE2-induced sensitization of rat sensory neurons in vitro. J Neurosci 18, 10345–10355

80 Bavencoffe, A. et al. (2016) Persistent Electrical Activity in Primary Nociceptors after Spinal Cord Injury Is Maintained by Scaffolded Adenylyl Cyclase and Protein Kinase A and Is Associated with Altered Adenylyl Cyclase Regulation. J Neurosci 36, 1660–1668

81 Garza Carbajal, A. et al. (2020) Depolarization-Dependent C-Raf Signaling Promotes Hyperexcitability and Reduces Opioid Sensitivity of Isolated Nociceptors after Spinal Cord Injury. J Neurosci 40, 6522–6535

82 Yousuf, M.S. et al. (2021) Pharmacological Manipulation of Translation as a Therapeutic Target for Chronic Pain. Pharmacol Rev 73, 59–88

83 O'Leary, M.A. et al. (2013) The placental mammal ancestor and the post-K-Pg radiation of placentals. Science 339, 662–667

84 Cavanaugh, D.J. et al. (2009) Distinct subsets of unmyelinated primary sensory fibers mediate behavioral responses to noxious thermal and mechanical stimuli. Proc Natl Acad Sci U S A 106, 9075–9080

85 Shiers, S.I. et al. (2021) Convergence of peptidergic and non-peptidergic protein markers in the human dorsal root ganglion and spinal dorsal horn. J Comp Neurol 529, 2771–2788

86 Tavares-Ferreira, D. et al. (2022) Spatial transcriptomics of dorsal root ganglia identifies molecular signatures of human nociceptors. Sci Transl Med 14, eabj8186

87 Julius, D. (2013) TRP channels and pain. Annu Rev Cell Dev Biol 29, 355–384

88 Middleton, S.J. et al. (2021) Studying human nociceptors: from fundamentals to clinic. Brain 144, 1312–1335

89 Nguyen, M.Q. et al. (2021) Single-nucleus transcriptomic analysis of human dorsal root ganglion neurons. Elife 10, e71752

90 Yang, L. et al. (2022) Human and mouse trigeminal ganglia cell atlas implicates multiple cell types in migraine. Neuron 110, 1806–1821.e8

91 Zhang, X. et al. (2017) Voltage-gated $Na^+$ currents in human dorsal root ganglion neurons. Elife 6, e23235

92 Wangzhou, A. et al. (2020) Pharmacological target-focused transcriptomic analysis of native vs cultured human and mouse dorsal root ganglia. Pain 161, 1497–1517

93 Hartung, J.E. et al. (2022) Voltage-gated calcium currents in human dorsal root ganglion neurons. Pain 163, e774–e785

94 Williams, A.C.C. (2019) Persistence of pain in humans and other mammals. Philos Trans R Soc Lond B Biol Sci 374, 20190276

95 Donnelly, C.R. et al. (2020) How Do Sensory Neurons Sense Danger Signals. Trends Neurosci 43, 822–838

96 Clatworthy, A.L. et al. (1994) Induction of a cellular defense reaction is accompanied by an increase in sensory neuron excitability in Aplysia. J Neurosci 14, 3263–3270

97 Adamo, S.A. and McMillan, L.E. (2019) Listening to your gut: immune challenge to the gut sensitizes body wall nociception in the caterpillar Manduca sexta. Philos Trans R Soc Lond B Biol Sci 374, 20190278

98 Smith, A.K. et al. (2013) Mechanical sensitization of cutaneous sensory fibers in the spared nerve injury mouse model. Mol Pain 9, 61

99 Tappe-Theodor, A. et al. (2012) Gα(q/11) signaling tonically modulates nociceptor function and contributes to activity-dependent sensitization. Pain 153, 184–196

## Figure Legends

**Figure 1. Nociceptor hyperactivity.** (A) Schematic of a primary nociceptor in vertebrates, gastropod molluscs, and leeches. The cell body is centrally located and distant from peripheral terminals where survivable injury and inflammation are most likely. In vertebrates, the cell body is in a ganglion near the central nervous system (CNS). In molluscs and leeches, nociceptor cell bodies are in ganglia within the CNS. (B) Normal nociceptive activity initiated by noxious stimulation of peripheral terminals, which produces a depolarizing sensory generator potential that reaches threshold for action potentials (APs) that are conducted to the CNS. (C) Illustration of sensitization of nociceptive activity evoked by the same noxious stimulus as in panel B, with the evoked hyperactivity following injury caused by a larger sensory generator potential, increased terminal excitability, and afterdischarge triggered by the evoked APs. Experimentally, injection of pulses or ramps of depolarizing current (usually into the cell body) is often used to reveal hyperexcitability (see Box 1). (D) Spontaneous activity after injury, defined as ongoing activity generated (sometimes by depolarizing spontaneous fluctuations, DSFs) in the absence of concurrent extrinsic stimulation of a neuron [66]. However, "spontaneous activity" commonly describes any activity occurring without evident ongoing noxious stimulation, including potentially common cases where intrinsic (cell autonomous) hyperexcitability and unobserved inflammatory signals combine to drive ongoing hyperactivity. Spontaneous activity and/or afterdischarge have been found in peripheral terminals, injured axons, and cell bodies of mammals and molluscs. In mammals, nociceptor hyperactivity can induce prolonged hyperresponsiveness of central nociceptive and pain pathways (termed central sensitization).

**Figure 2. Sources of nociceptor hyperactivity following injury.** (A) In mammals, numerous extrinsic chemical signals have been found to excite and/or sensitize nociceptors, promoting

physiologically significant hyperactivity and pain [95]. Hyperactivity can also be promoted by reducing ongoing inhibition from other chemical signals (disinhibition). Few injury- and inflammation-related extrinsic chemical signals that affect nociceptor activity have been identified in other animal taxa, but they are likely to exist [96,97]. (B) In principle, nociceptor hyperactivity can be promoted by intrinsic hyperexcitability via any or all of the listed electrophysiological alterations [66,70]. Hyperactivity may also be promoted by increasing a nociceptor's intrinsic sensitivity to excitatory or sensitizing sensory stimuli or chemical signals, and by decreasing intrinsic sensitivity to inhibitory chemical signals. Abbreviations: AP, action potential; ATP, adenosine triphosphate, DAMPs, damage-associated molecular patterns; DSF, depolarizing spontaneous fluctuation; GABA, gamma-aminobutyric acid; GDNF, glial-derived neurotrophic factor; GPCR, G protein-coupled receptor; IL-1$\beta$, interleukin-1$\beta$; IL-10, interleukin 10; LPS, lipopolysaccharide; MIF, macrophage migration inhibitory factor; NGF, nerve growth factor; PAMPs, pathogen-associated molecular patterns; RMP, resting membrane potential; TNF$\alpha$, tumor necrosis factor $\alpha$; TRP, transient receptor potential; VGPC, voltage-gated potassium channel; VGSC, voltage-gated sodium channel; VIP, vasoactive intestinal peptide.

**Figure 3. Evidence that persistent nociceptor hyperactivity can be adaptive.** (A) Squid survival after injury. Peripheral tissue injury (minor amputation) causes long-lasting nociceptor hyperactivity expressed over much of the body surface [47] (red). In staged encounters with fish predators, nearly half of injured squid survived, despite being selectively targeted, as they benefitted from escalated escape behavior [58]. More uninjured squid survived, while waiting longer to initiate escape behavior. Far fewer squid survived that were injured without nociceptive sensitization and persistent nociceptor hyperactivity (because transient block of nociceptor activity during amputation eliminated persistent hyperactivity), indicating the survival benefit of persistent nociceptor hyperactivity after injury. Persistent hyperactivity also increased protective

reflex sensitivity (hyperreflexia) [57]. (B) Avoidance of a predator cue by mice with neuropathic pain. Mice with a spared nerve injury model exhibit nociceptor hyperactivity (red) in the hindpaw [98] and DRG [99]. When exposed to fox urine, injured mice chose a route that took them farther from the predator odor [59]. This suggests an injury state involving persistent nociceptor hyperactivity that causes hypervigilance and increased risk aversion. (C) Speculative benefit of chronic nociceptor hyperactivity in ancestral humans at high risk for predation after amputation injury. The top two rows parallel the arguments illustrated in the experiments in panel A. The bottom row shows that transient blockade of DRGs in amputees blocked ongoing pain [55], consistent with spontaneous activity in DRGs persistently driving painful hypervigilance that might be protective for an individual in the vicinity of highly threatening predators.

## Outstanding Questions

- What types of animals exhibit persistent nociceptor hyperactivity, and do species that display relatively little inflammatory or neuropathic pain, such as naked mole-rats, lack persistent nociceptor hyperactivity after injuries that damage nerves?

- To what extent are biophysical and molecular mechanisms (intrinsic and extrinsic) that drive persistent nociceptor hyperactivity conserved across vertebrate and invertebrate species, and between mammalian species?

- Do conserved cell signaling mechanisms underlying persistent hyperactivity in diverse species also contribute to synaptic potentiation and growth (sprouting) of peripheral and central nociceptor branches that also can enhance the effects of nociceptor activity and resulting pain?

- To what extent are properties of human nociceptors that differ from rodent nociceptors shared with primates having differing degrees of phylogenetic separation from humans?

- Are the high excitability of human nociceptors and unusually extensive polymodality evident in their transcriptomes linked to exceptional susceptibility of humans to persistent nociceptor hyperactivity and consequent pain?

- Many clinical conditions in humans can cause neuropathy, altered sensory function, and chronic pain (e.g., diabetes, various kinds of chemotherapy, radiculopathy, postherpetic neuralgia). Do some or all such conditions involve the accidental recruitment of a persistent nociceptor hyperactivity state that evolved originally as an adaptation to severe peripheral injury, or do purely pathological effects of neuropathy on nociceptors (such as metabolic impairment) account entirely for most cases of persistent hyperactivity encountered clinically?

## Highlights

Injury to the nervous system can generate chronic pain driven by persistent hyperactivity of nociceptors, sensory neurons specialized to detect damaging stimuli.

Recent evidence argues against the near-universal assumption that nociceptor hyperactivity and resulting chronic pain are always maladaptive.

Widespread conservation of mechanisms of nociceptor function and hyperactivity are well documented, as well as distinct adaptations to divergent nociceptive needs.

Persistent nociceptor hyperactivity could have evolved to drive painful hypervigilance during persistent physical impairment, enhancing survival by decreasing subsequent risk of predatory or aggressive attack.

Differences between rodent and human nociceptors suggest that human nociceptors are unusually excitable and sensitive to diverse noxious stimuli, perhaps associated with exceptional susceptibility of humans to persistent pain.

Figure 1 PDF

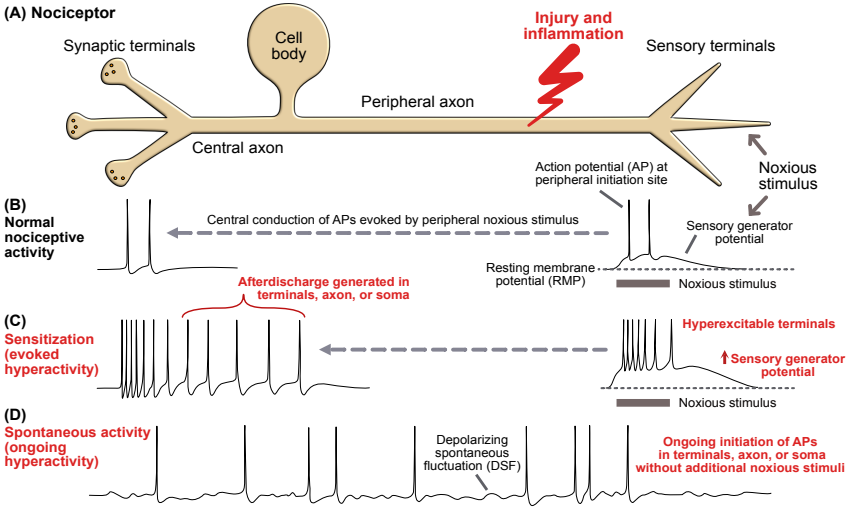

(A) Nociceptor
Synaptic terminals
Cell body
Injury and inflammation
Sensory terminals
Peripheral axon
Central axon
Noxious stimulus
Action potential (AP) at peripheral initiation site
(B) Normal nociceptive activity
Central conduction of APs evoked by peripheral noxious stimulus
Sensory generator potential
Resting membrane potential (RMP)
Noxious stimulus
Afterdischarge generated in terminals, axon, or soma
(C) Sensitization (evoked hyperactivity)
Hyperexcitable terminals
Sensory generator potential
Noxious stimulus
(D) Spontaneous activity (ongoing hyperactivity)
Depolarizing spontaneous fluctuation (DSF)
Ongoing initiation of APs in terminals, axon, or soma without additional noxious stimuli

Figure 2 PDF

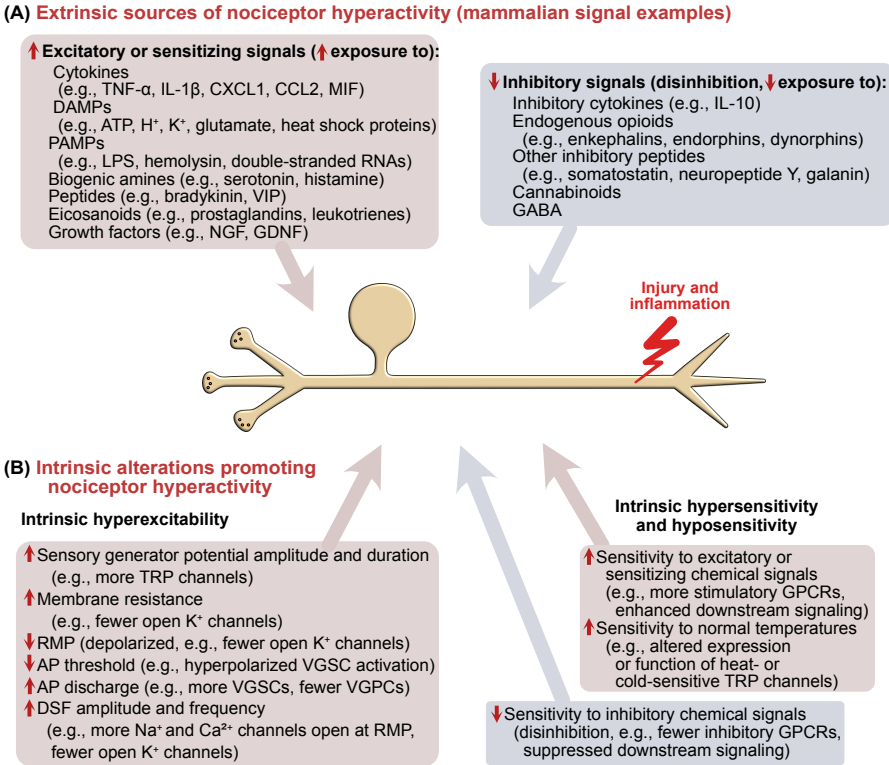
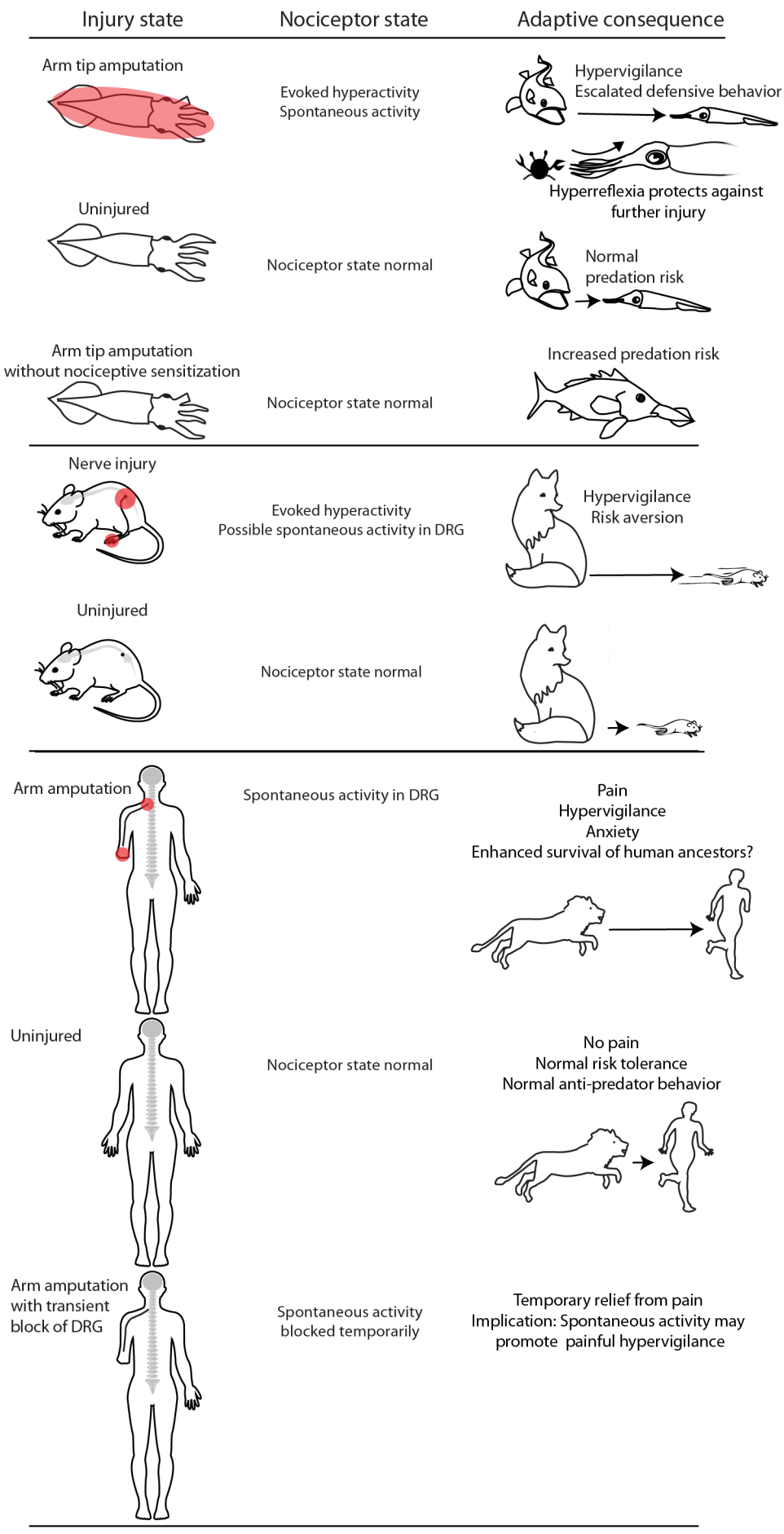

(A) Extrinsic sources of nociceptor hyperactivity (mammalian signal examples)
↑Excitatory or sensitizing signals (↑exposure to):
Cytokines
(e.g., TNF-α, IL-1β, CXCL1, CCL2, MIF)
DAMPs
(e.g., ATP, H+, K+, glutamate, heat shock proteins)
PAMPs
(e.g., LPS, hemolysin, double-stranded RNAs)
Biogenic amines (e.g., serotonin, histamine)
Peptides (e.g., bradykinin, VIP)
Eicosanoids (e.g., prostaglandins, leukotrienes)
Growth factors (e.g., NGF, GDNF)
↓Inhibitory signals (disinhibition, ↓exposure to):
Inhibitory cytokines (e.g., IL-10)
Endogenous opioids
(e.g., enkephalins, endorphins, dynorphins)
Other inhibitory peptides
(e.g., somatostatin, neuropeptide Y, galanin)
Cannabinoids
GABA
Injury and inflammation
(B) Intrinsic alterations promoting nociceptor hyperactivity
Intrinsic hyperexcitability
↑Sensory generator potential amplitude and duration
(e.g., more TRP channels)
↑Membrane resistance
(e.g., fewer open K+ channels)
↓RMP (depolarized, e.g., fewer open K+ channels)
↓AP threshold (e.g., hyperpolarized VGSC activation)
↑AP discharge (e.g., more VGSCs, fewer VGPCs)
↑DSF amplitude and frequency
(e.g., more Na+ and Ca2+ channels open at RMP,
fewer open K+ channels)
Intrinsic hypersensitivity and hyposensitivity
↑Sensitivity to excitatory or sensitizing chemical signals
(e.g., more stimulatory GPCRs, enhanced downstream signaling)
↑Sensitivity to normal temperatures
(e.g., altered expression or function of heat- or cold-sensitive TRP channels)
↓Sensitivity to inhibitory chemical signals
(disinhibition, e.g., fewer inhibitory GPCRs, suppressed downstream signaling)

Figure 3 PDF


Injury state
Nociceptor state
Adaptive consequence
Arm tip amputation
Evoked hyperactivity
Spontaneous activity
Hypervigilance
Escalated defensive behavior
Hyperreflexia protects against further injury
Uninjured
Nociceptor state normal
Normal predation risk
Arm tip amputation without nociceptive sensitization
Nociceptor state normal
Increased predation risk
Nerve injury
Evoked hyperactivity
Possible spontaneous activity in DRG
Hypervigilance
Risk aversion
Uninjured
Nociceptor state normal
Arm amputation
Spontaneous activity in DRG
Pain
Hypervigilance
Anxiety
Enhanced survival of human ancestors?
Uninjured
Nociceptor state normal
No pain
Normal risk tolerance
Normal anti-predator behavior
Arm amputation with transient block of DRG
Spontaneous activity blocked temporarily
Temporary relief from pain
Implication: Spontaneous activity may promote painful hypervigilance