HHS Public Access

Author manuscript

Brain Behav Evol. Author manuscript; available in PMC 2024 November 30.

Published in final edited form as:

Brain Behav Evol. 2023 ; 98(6): 314–330. doi:10.1159/000535552.

Exaptation and Evolutionary Adaptation in Nociceptor Mechanisms Driving Persistent Pain

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Abstract

Background: Several evolutionary explanations have been proposed for why chronic pain is a major clinical problem. One is that some mechanisms important for driving chronic pain, while maladaptive for modern humans, were adaptive because they enhanced survival. Evidence is reviewed for persistent nociceptor hyperactivity (PNH), known to promote chronic pain in rodents and humans, being an evolutionarily adaptive response to significant bodily injury, and primitive molecular mechanisms related to cellular injury and stress being exapted (co-opted or repurposed) to drive PNH and consequent pain.

Summary: PNH in a snail (*Aplysia californica*), squid (*Doryteuthis pealeii*), fruit fly (*Drosophila melanogaster*), mice, rats, and humans has been documented as long-lasting enhancement of action potential discharge evoked by peripheral stimuli, and in some of these species as persistent extrinsically driven ongoing activity and/or intrinsic spontaneous activity (OA and SA, respectively). In mammals, OA and SA are often initiated within the protected nociceptor soma long after an inducing injury. Generation of OA or SA in nociceptor somata may be very rare in invertebrates, but prolonged afterdischarge in nociceptor somata readily occurs in sensitized *Aplysia*. Evidence for the adaptiveness of injury-induced PNH has come from observations of decreased survival of injured squid exposed to predators when PNH is blocked, from plausible survival benefits of chronic sensitization after severe injuries such as amputation, and from the functional coherence and intricacy of mammalian PNH mechanisms. Major contributions of cAMP-PKA signaling (with associated calcium signaling) to the maintenance of PNH both in mammals and molluscs suggests that this ancient stress signaling system was exapted early during the evolution of nociceptors to drive hyperactivity following bodily injury. Vertebrates have retained core cAMP-PKA signaling modules for PNH, while adding new extracellular modulators (e.g., opioids) and cAMP-regulated ion channels (e.g., TRPV1 and Nav1.8 channels).

Key Messages: Evidence from multiple phyla indicates that PNH is a physiological adaptation that decreases the risk of attacks on injured animals. Core cAMP-PKA signaling modules make

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Author Contributions

Edgar T. Walters planned the review and wrote the manuscript.

Conflict of Interest Statement

The author has no conflicts of interest to declare.

major contributions to the maintenance of PNH in molluscs and mammals. This conserved signaling has been linked to ancient cellular responses to stress, which may have been exapted in early nociceptors to drive protective hyperactivity that can persist while bodily functions recover after significant injury.

Keywords

Aplysia ; Cyclic AMP signaling; Excitability; Mammals; Spontaneous activity

Introduction

Why do one in five adults (one in three above the age of 65) [1] suffer from chronic pain? This is a question of clear clinical importance and substantial social significance, given the societal burden and economic costs of uncontrolled pain plus the links between chronic pain and opioid abuse [2, 3]. However, it is also an interesting question from the perspective of evolutionary biology. Evolutionary considerations have led to several suggested answers to the question of why chronic pain is so common in people. These include assertions that 1) chronic pain today is promoted by a mismatch between humans' more protected modern environments and sedentary lifestyles compared to those of their ancestors [4], 2) excessively sensitive and prolonged responses to stimuli that threaten bodily integrity are less costly than insufficient responses to noxious stimuli, resulting evolutionarily in a bias towards persistent and frequent pain (smoke detector principle) [5], 3) pain and other chronic ailments increase with age as injuries and illnesses accumulate, and these are not subject to countervailing evolutionary selection pressures past the child-bearing phase of life [6], and 4) some mechanisms that drive chronic pain were selected during evolution because they enhanced survival and thus reproductive success.

These reasons for why chronic pain is so common in modern humans are not mutually exclusive, all four are likely true. Here I review evidence for the fourth reason (chronic pain is promoted by evolutionarily adaptive traits), focusing on one physiological alteration, persistent nociceptor hyperactivity (PNH). I extend previous reviews supporting the evolutionary adaptiveness of PNH after significant bodily injury [7, 8] and consider evidence that cAMP signaling, which has primordial links to stress responses, may have been exapted in nociceptors to drive biologically adaptive hyperactivity that promotes clinically maladaptive chronic pain.

Persistent Nociceptor Hyperactivity as an Adaptive Response to Consequential Injury

Chronic pain depends upon numerous interacting mechanisms in many parts of the human body and nervous system, including the spinal cord and brain [9–12]. While central alterations are clearly important for chronic pain [13–15], my focus is on a peripheral mechanism, PNH – a sustained enhancement of action potential [AP] generation in nociceptors (Fig. 1), which can activate central sensitization mechanisms and may often be essential for maintaining central alterations [16, 17]. Nociceptor alterations in addition to hyperactivity are important for driving central pain mechanisms, including potentiation

of a nociceptor's synapses and sprouting of its peripheral and central terminals [18, 19]. However, these additional mechanisms in nociceptors are beyond the scope of this review. PNH is quite interesting from an evolutionary perspective because the availability of detailed information about specific molecular contributions to the same well-defined cellular alteration (hyperactivity) in an identified cell type (nociceptor) with the same type-defining functions (nociception and nociceptive sensitization) across distantly related lineages enables plausible conjectures about the exaptation of ancient molecular mechanisms for functions that are of great importance to humans.

Nociceptors are defined as primary sensory neurons that are specialized to detect signals of injury and inflammation. They have been identified not only in multiple classes of chordates (mammals, birds, reptiles, fish) but also in annelids, molluscs, nematodes, and arthropods [20–26]. As reviewed below, nociceptor hyperactivity persisting for days, weeks, or months after noxious events has been found in invertebrates, mammals, and humans. PNH has been observed as increases in evoked discharge and in the development of spontaneous discharge in peripheral terminals, injured axons, and cell bodies (somata) of nociceptors.

PNH Manifested as Sensitization and Ongoing Activity in Nociceptor Peripheral Terminals

Sensitization of the peripheral branches of nociceptors associated with hyperalgesia (enhanced pain evoked by normally painful stimuli) was the first type of injury-induced PNH discovered, with the threshold for eliciting APs by heat stimuli decreasing and the number of APs generated by subthreshold thermal stimuli increasing in recordings from anesthetized cats [27, 28], monkeys [29] and rodents [reviewed by 30], and from humans [31]. Increased AP discharge during heating and possibly cooling following damaging ultraviolet irradiation that sensitizes defensive behavior has been shown in polymodal nociceptors (class IV) of *Drosophila* larvae [32, 33]. It is not known if this hyperactivity originates in peripheral branches of the nociceptors or in the soma, which is located close beneath the skin of a larva in the center of the peripheral arbor. Peripheral sensitization of electrical activity by noxious stimulation or injury, expressed as increased AP discharge to suprathreshold mechanical stimuli has been described in molluscs (Fig. 1a) [34–36], humans [31], and rodents [e.g., 37, 38–40]. Interestingly, acute sensitization of AP discharge after noxious stimulation appears to be rare or absent in fish nociceptors [41]. While sensitization of molluscan and mammalian nociceptors can be long lasting, persisting for 1–2 months after nerve injury in *Aplysia* (a substantial fraction of its 1-year lifespan) [34, 42, 43] and for months, years, or longer in rodents and humans [e.g., 44, 45], PNH often resolves after tissue healing and nerve regeneration [40, 43]. The eventual recovery of normal excitability that can occur after repair of injuries severe enough to damage innervating nerves is consistent with the inference that long-lasting PNH and behavioral sensitization function to protect organisms during prolonged healing following serious injury [20, 46].

The primary nociceptors that have been examined electrophysiologically in various species, including leeches, snails, fish, mice, rats, guinea pigs, cats, monkeys, and humans, are usually electrically silent; APs are generated when evoked by noxious stimuli delivered to their receptive fields but otherwise APs are absent or infrequent (Fig. 1a) [25]. Polymodal nociceptors in vertebrates [e.g., 27], the leech [47, 48], and *Drosophila* [32, 49] sometimes

show low-frequency spontaneous discharge under basal recording conditions, which in some polymodal nociceptors may be caused by stimulation of thermosensitive ion channels. During nociceptive sensitization, another type of PNH – afterdischarge – can occur, in which stimuli that normally evoke a few APs evoke higher-frequency, longer-lasting discharge. For example, sensitized *Aplysia* nociceptors that usually respond to a moderate intensity tap stimulus with a few APs will respond with many APs generated in the periphery and sometimes initiated as well in the soma; responding as if to an intense pinch (Fig. 1a) [43, 50, 51].

In some species, significant injury and/or inflammation can result in yet another type of PNH: persistent generation of continuing discharge in the peripheral terminals and axons (especially at neuromas) of some nociceptors in the absence of additional mechanical or thermal stimulation. I use the term ongoing activity (OA) to describe any continuing discharge that does not depend upon brief, transient depolarization from extrinsic inputs; e.g., from conventional sensory generator potentials or excitatory postsynaptic potentials. Many investigators have used the term spontaneous activity (SA) for any form of OA, but it is useful mechanistically to define SA as a subtype of OA that is generated by intrinsic alterations [52] resulting from a change in cellular state that outlasts immediate effects of triggering events such as axonal membrane rupture or transient exposure to sensitizing neuromodulators. Persistent, low-frequency, irregular OA (possibly involving intrinsic SA) in presumed nociceptors occurs in rodents after peripheral nerve injury [53–56] or spinal cord injury (SCI) [39], and it also occurs in squid after bodily injury caused by attacks from other squid [36]. Enhanced OA in class IV nociceptors in *Drosophila* larvae was observed across a wide range of temperatures 24 hours after epidermal damage resulting from ultraviolet irradiation [32]. In rodent nociceptors, persistent OA can be driven by long-lasting peripheral inflammation [55, 57, 58]. Furthermore, peripheral generation of persistent OA in C-fibers (composed primarily of nociceptors) is prominent in various human neuropathic conditions [45, 59, 60]. Blocking OA in peripheral nerves of human patients effectively interrupts spontaneous neuropathic pain [16] and, more surprisingly, can also block poststroke pain [61].

PNH Manifested as Ongoing Activity and Afterdischarge in Nociceptor Somata

After nerve injury, PNH may involve APs generated ‘ectopically’ in the nociceptor cell body (soma) as well as more peripherally. In *Aplysia* nociceptors, somally generated afterdischarge can be evoked immediately and potentiated persistently by noxious peripheral stimulation (Fig. 1a) or by nerve injury, with the afterdischarge adding substantially to the number of APs reaching the central nervous system (CNS) and excite motor neurons mediating defensive behavior [43, 62, 63]. Afterdischarge is produced by a depolarizing afterpotential generated in or near the nociceptor soma, which requires Ca^{2+} influx [43, 51]. Unlike unmyelinated C-fiber nociceptors, which have very small receptive fields (e.g., ~1 mm diameter in cats) [27], *Aplysia* nociceptors’ receptive fields are quite large relative to the size of the body (diameters often >2 cm in individuals weighing ~250 g) [34, 64], similar to receptive field sizes of Aδ nociceptors in cats [65]. Consequently, *Aplysia* nociceptors and Aδ nociceptors are much less likely to be disconnected completely from their receptive fields during injury. It may be that in small C-fiber nociceptors of mammals, the most

prominent form of somal hyperactivity is OA rather than afterdischarge because a C-fiber nociceptor innervating severely injured tissue is likely to lose its entire receptive field and with it the usual source of afferent AP discharge that triggers afterdischarge in the central soma.

While PNH manifested as OA and afterdischarge generated in the receptive fields and neuromas of mammalian nociceptors has long been evident [reviewed by 66], only recently has OA initiated in mammalian nociceptor somata been recognized as a major form of PNH and driver of persistent pain. Most *in situ* and *in vivo* studies of somally generated OA have been on sensory neurons other than nociceptors; specifically, large, low-threshold, A-fiber mechanoreceptors (LTMRs) [e.g., 67, 68–72, 55, 73]. In these studies, little or no somally initiated OA was found in C-fiber nociceptors under neuropathic conditions. However, technical limitations of the three methods used to record somally generated OA *in situ* or *in vivo* have precluded accurate detection of single APs in the thin axons and small somata of C-fiber nociceptors. First, teased fiber recording from nerves is an indirect monitor of APs initiated in distant somata, and even when conditions are optimal, only APs in the largest C-fibers may be detectable. Second, sharp electrode recording is more informative but suffers from a widely overlooked limitation – even very sharp microelectrodes decrease input resistance dramatically (often >10-fold). Although this inadvertent shunting may have relatively little effect on the generation of APs (because enormous voltage-gated Na^+ currents provide a large safety factor for producing overshooting APs – see Figure 1a) or on resting membrane potential (RMP, because of powerful homeostatic mechanisms), the decreased input resistance during sharp electrode recording will make normally small spontaneous depolarizations proportionally smaller and much less likely to reach AP threshold. Third, Ca^{2+} imaging is necessarily a coarse and indirect monitor of APs and, while it continually improves, its limited sensitivity and temporal resolution has hampered detection of single APs. For example, ongoing clustered OA initiated in DRG neuron somata in vivo was recently reported to be correlated with spontaneous, paroxysmal pain in Pirt-Cre/GCaMP6s mice [74]. However, each imaging frame used to measure Ca^{2+} transients produced by APs was 238 ms, far longer than the 1–5 ms duration of single APs. All these technical limitations mean the incidence and importance of ectopic somal OA in C-fiber nociceptors may often be underestimated.

On the other hand, strong evidence for OA (including intrinsic SA, Fig. 1b) generated within nociceptor somata has come from dissociated DRG neurons recorded with the whole-cell, gigaseal patch technique. Although this method also has limitations, including the dialysis of soluble intracellular constituents that may be important for the phenomena under investigation, it provides unmatched fidelity for measurement of detailed electrophysiological properties, such as small spontaneous fluctuations of RMP. This method revealed SA continuously generated in mammalian nociceptor somata isolated after various neuropathic conditions, including chronic constriction injury (CCI) of the sciatic nerve in rats [75], spinal cord injury (SCI) in rats [44] (Fig. 1b) and mice [76], paclitaxel treatment in rats [77], cisplatin treatment in mice [78], and compression of spinal nerves by tumors in humans [79]. These findings show that diverse neuropathic conditions induce hyperexcitability that (quite remarkably) survives the cellular trauma and stresses caused by subsequent DRG excision, dissociation, and neuronal culturing [80]. Countering the

logical possibility that somally generated SA might only be an artifact of dissociation are the findings that 1) neuropathy-induced somal SA was preserved when recording from a DRG neuron *in vivo* and then after it was isolated from the DRG [69], 2) the irregular pattern and low discharge frequency of ongoing discharge (perhaps extrinsically driven OA plus intrinsic SA) after SCI when recorded *in vivo* from dorsal root filaments (in or near C-fiber and Aδ somata within a DRG) show remarkable parallels to intrinsic SA generated in dissociated nociceptor somata after SCI [44], and 3) SA in dissociated nociceptor somata after SCI continues to be regulated by potent cell signaling pathways (including cAMP and ERK pathways; see below) under whole-cell recording conditions [81, 82]. Thus, somally initiated SA appears to be a robust nociceptor specialization that can operate similarly *in vivo* and in dissociated rodent and human DRG neurons.

Multiple ion channels contribute to the generation of somal SA in dissociated rodent nociceptors, including voltage-gated Na^+ and Ca^{2+} channels and TRP channels [83]. One of these, Nav1.8 (which is expressed almost entirely in primary somatosensory neurons, mainly nociceptors), was shown to be required for SA in sensory axons after peripheral nerve transection in mice [84]. After SCI in rats, both SA in dissociated nociceptors and correlated measures of spontaneous pain, heat hyperalgesia, and mechanical allodynia were eliminated by antisense oligodeoxynucleotide knockdown of Nav1.8 [85].

While it seems likely that spontaneous discharge recorded in dissociated neurons represents, at least in part, intrinsic SA driven by a persisting hyperexcitable state in DRG neurons rather than continuing stimulation by extrinsic factors, these cultures contain heterogeneous cell types that may continually release excitatory factors. Indeed, in the presence but not the absence of conditioned medium from DRG neuron cultures prepared from SCI rats, neurons cultured from naïve control rats exhibited OA when artificially depolarized to −45 mV for 30 seconds [86]. This SCI-dependent extrinsic excitatory factor present in the culture medium drove OA at −45 mV but was not sufficient to drive OA at RMP, suggesting that the extrinsic excitants work together with persistent intrinsic hyperexcitability after SCI to drive OA at RMP. Interestingly, the OA produced by conditioned medium was prevented by an inhibitor of a widely expressed proinflammatory cytokine, macrophage migration inhibitory factor (MIF) [86], known to chronically increase in humans after SCI [87]. An important open question concerns the degree to which PNI driving ongoing pain in any condition represents persistent intrinsic alterations of nociceptors versus persistently altered exposure of the nociceptors to extrinsic modulators such as cytokines, and how the relative contributions of each may change over time.

Clinically Maladaptive but Evolutionarily Adaptive PNH Is Linked to Pain and Anxiety

Pain and associated PNH that persist after healing are assumed almost universally to be purely pathological, maladaptive consequences of injury or disease [11, 88, 89]. In the modern world, chronic pain seems to bring no benefit to those afflicted, and it not only diminishes quality of life, it also is linked to many health risks, including substance abuse and suicide [1]. Nevertheless, several arguments suggest that, while maladaptive from a modern clinical perspective, the PNH that often drives persistent pain has been subject to positive evolutionary selection. These arguments have been detailed elsewhere [7, 8, 90]

and are briefly summarized here. The first argument was an extension of arguments for the adaptive value of acute nociceptive sensitization, namely that nociceptor hyperactivity protects a wounded animal, and especially the injured site, until healing has occurred, and it helps to compensate for lost sensory function in tissue denervated by injury [20, 46, 91]. This argument was based on the obvious need to protect injured sites from additional stress during movements of an animal, on the long periods needed for recovery from severe sublethal injury, and on numerous field observations showing that injury increases the risk of attack from predators that often selectively target wounded prey [e.g., 92]. Direct evidence for the adaptiveness of nociceptor hyperactivity came from squid in which bodily injury had been shown to induce widespread, long-lasting nociceptor sensitization and OA [36]. Staged encounters with predatory fish revealed that the fish selectively target previously injured squid, which showed heightened vigilance to the predators (initiating earlier escape responses) but were still killed more frequently than uninjured squid [93]. However, squid in which the injury site was transiently anesthetized (previously shown to prevent nociceptor sensitization and OA [36]) during the experimental injury later showed significantly higher mortality than squid that had not received local anesthesia at the time of injury. This indicated that injury-induced PNH can increase survival under conditions of high predation risk by driving continuing hypervigilance [93]. Analogous results [94] were found in mice using a spared nerve injury pain model that can induce somal OA in DRG neurons [74]. When injured mice had to choose between long and short routes to a food reward, they avoided the short route when it exposed them to the smell of fox urine, suggesting that ongoing awareness of chronic injury driven by nociceptor OA altered their behavior to reduce predatory risk [94]. Such injury-induced hypervigilance appears functionally similar to generalized anxiety in humans [20]. Anxiety is a common comorbidity of chronic pain in humans and rodents [95] and it seems likely that PNH may promote ongoing generalized anxiety as well as ongoing pain.

The second argument extends the first by noting that predators often look for signs of physical impairment, including indicators that may not resolve after healing (e.g., limping after amputation) and that result in life-long behavioral alterations detectable by predators and aggressive conspecifics [7, 90]. Amputees in various species have been reported to survive and reproduce long after injury. Importantly, in human patients, ongoing phantom limb pain and non-painful phantom sensations (both of which can be permanent) are continuously driven by sensory neuron OA initiated and generated within DRGs [96]. This finding supports the idea that PNH (and possibly OA in some low-threshold mechanosensory neurons) promotes hypervigilance that might be prompted by ongoing pain and which, during our evolutionary history, enhanced the survival of severely and sometimes permanently injured individuals having detectable physical impairments that increase their risk of attack by predators and members of their own species [7, 8]. Most species are not social (most invertebrates don’t provide care for their young), and they are unlikely to benefit from communication of pain states to others (humans and domesticated animals being notable exceptions). In asocial and minimally social species, including many mammals, ongoing pain does not appear to be manifested in obvious behavioral cues (e.g., ongoing vocalization, clear grimacing) that could invite attack [97, 98].

The third argument is for the adaptiveness of OA in the mammalian nociceptor soma [7, 8, 52, 90, 99], and is based on arguments for a complex 'functional design' [100, 101] of primary nociceptors in mammals that enables potentially beneficial somal generation of OA after bodily injury. First, mammalian nociceptor somata can be continuing sources of OA after severe injury because they are likely to survive, residing in sensory ganglia that are centrally located and protected by bone and dura. Second, nociceptor somata are in a unique position to integrate numerous local and systemic signals of bodily injury when 'deciding' whether to generate OA because they are exposed to humoral signals both in the blood (sensory ganglia lack an effective vascular permeability barrier) and in cerebrospinal fluid, in addition to injury- and inflammation-related electrical and molecular signals conveyed from their peripheral and central terminals by action potentials and axonal transport. Third, OA itself shows functional specialization by virtue of multiple, redundant mechanisms [52, 83, 99]. In particular, changes in RMP, input resistance, AP threshold, and the frequency of large depolarizing spontaneous fluctuations (DSFs) of membrane potential drive OA in diverse persistent pain conditions in rodents and humans (Fig. 1b) [44, 52, 76, 78, 86, 99, 102]. These changes involve complementary contributions from numerous ion channels [83, 85, 103, 104] and multiple cell signaling pathways [81, 82, 86]. Although PNH might be induced in modern humans by various pathological conditions that had nothing to do with its selection during evolution, such as chemotherapy or SCI (SCI is almost always fatal without modern medical support), these arguments suggest that PNH is a physiological specialization that was selected in part for its ability to increase survival after injury or disease that persistently increased the risk of being attacked.

Exaptation and Mechanisms of Persistent Nociceptor Hyperactivity

The existence of PNH in multiple species across several phyla, coupled with increasing knowledge about underlying mechanisms, enables a consideration of primitive cellular and molecular plasticity mechanisms that may have been exapted in the evolution of PNH. The term 'exaptation' was coined by Stephen Jay Gould and Elizabeth Vrba, who defined it as a trait (previously evolutionarily neutral or having a different function) that was co-opted for a new function [105]. They termed the preexisting trait an 'aptation', which they considered less loaded teleologically than the older but equivalent word 'preadaptation,' which is used by some evolutionary biologists [e.g., 106, 107, 108]. The concept of exaptation – repurposing preexisting traits for new functions - was recognized by Charles Darwin [109] and many other evolutionary biologists as a fundamental evolutionary process [107], and the idea of exaptation followed by progressive adaptation (e.g., scales being exapted into insulating feathers and then further adapted for flight) has become a core principle of evolution that has been applied at anatomical, physiological, and molecular levels of organization [e.g., 106, 110].

Possible exaptation of early adaptive cellular responses to injury for plasticity in nociceptors and other neurons

Among neurons, primary somatosensory neurons, including nociceptors, are unusual in interfacing directly with one of the strongest evolutionary selection pressures – bodily injury. Because damage to cells and tissues has always had a direct impact on survival,

it seems safe to assume that adaptations to reduce damage and promote recovery from injury were among the earliest to appear during evolution. Furthermore, the survival benefits of rapid detection of injury probably drove the early evolution of molecular sensors of noxious events [111] and, after the appearance of animals, the evolution of nociceptive neurons specialized to detect incipient injury and trigger adaptive responses. Activation of primeval nociceptors may have directly excited muscle or ciliary cells for withdrawal and escape (and possibly stimulated innate immune cells to combat microbes that enter open wounds [112]), and, as more complex behaviors and circuits evolved, nociceptors excited interneurons controlling defensive behaviors and their integration with other activities [20]. Two general arguments have been made for primitive cellular reactions to injury being exapted for neuronal mechanisms that later became important for pain.

One argument was spurred indirectly by evidence for independent origins of neurons and nervous systems in different lineages [113]. Moroz argued that neurons could have evolved independently in different animals because injury-induced repair, regeneration of asymmetric morphological processes, and secretion of cellular metabolites and peptides were readily exapted for new functions – such as directed chemical signaling -- that now define neurons [114]. Rupturing a cell releases intracellular molecules, including glutamate, ATP, and peptides, which are used as secreted signals in diverse contemporary eukaryotes. These organisms include some unicellular species, animals that lack neurons (placozoa and sponges), and by most if not all animals with nervous systems [108, 115–117]. Even plants use glutamate as a wound signal [118]. While biological demands in addition to responding adaptively to injury were certainly important for the evolution of neuronal signaling [119, 120], various observations support the hypothesis that some injury-related molecules and receptors were exapted for chemical signaling specializations during the early evolution of neurons and nervous systems in different animal lineages [108].

A second argument was suggested by a discovery in nociceptive sensory neurons of *Aplysia*. Persistent hyperexcitability and synaptic potentiation induced rapidly by activity-dependent learning mechanisms were found to be indistinguishable from delayed alterations induced by crushing a nerve containing the axons of the tested sensory neurons – under conditions that prevented AP generation and acute release of neuromodulators [63]. The fact that the same hyperfunctional neuronal state could be induced in primary nociceptors either by molecular injury signals conveyed by axonal transport after axotomy [121–123] or by activity-dependent signals important for learning in *Aplysia* and mammals [124, 125] suggested a link between mechanisms underlying adaptive neuronal responses to injury and mechanisms underlying learning and memory. Given the probable universality of injury as a mediator of natural selection and the ubiquity of predation as a major source of injury during most of animal evolution [20, 126], a further suggestion was that adaptive responses to cellular injury present in the earliest animals [111] and perhaps in their single-celled ancestors [127] were exapted in some of the earliest sensory neurons to detect bodily injury and to produce hyperfunctional alterations after tissue injury, with the adaptive alterations likely to include regeneration and sprouting into denervated tissue, hyperexcitability, and increased excitatory synaptic transmission to cells with defensive functions [91]. Mechanisms of nociceptor hyperexcitability at peripheral injury sites might have then been exapted in larger animals to more central nociceptor somata that were more likely to survive peripheral injury. Even

in very simple nervous systems, hyperfunctional alterations in sensory neurons might serve both to compensate for loss of function in a damaged region and to sensitize protective behavior in a dangerous environment. Subsequently, injury-dependent plasticity mechanisms might have been exapted for additional functions in other neuronal types as nervous systems and behavior became more complex. Notably, ancient injury-related mechanisms might have been exapted for learning and memory [18, 20, 91, 128].

Possible exaptation of ancient injury-linked cell signaling pathways for PNH

Despite the enormous range of form and function across the eukaryotic kingdoms, core cellular constituents and processes are highly conserved, and the evolution of new cellular functions in animals largely depended on the exaptation and re-exaptation of proteins and other molecules that already existed when this kingdom first emerged [110, 129]. However, stimulus transduction and cell signaling pathways have diversified greatly in animals (metazoans), raising the possibility that signaling pathways critical for adaptive responses to injury diverged significantly across different metazoan lineages. On the other hand, strong continuing selection pressures, such as injury, can maintain ancient molecular adaptations [129]. Two of the signaling systems that appeared early in evolution, long before the emergence of animals, are Ca^{2+} signaling and cAMP signaling. These interacting signaling pathways are associated with many functions in a wide range of organisms, including responses to cellular injury, such as wound repair and regeneration, as well as responses to other kinds of organismic stress [130–136]. Both signaling pathways are also closely linked to synaptic plasticity [135, 137–139]. Ca^{2+}-dependent processes for cellular repair probably appeared at the dawn of eucaryotic evolution 1.5–2 billion years ago, when early eukaryotic cells lost the protective, rigid cell walls of prokaryotes, and these early repair processes have been highly conserved in metazoans [111, 140]. One ancient repair process was Ca^{2+}-induced vesicle fusion that seals ruptured plasma membrane in response to Ca^{2+} entry through membrane breaches and sometimes also through voltage-gated Ca^{2+} channels (activated by the depolarization resulting from membrane damage), a process that may have been exapted in early animals to trigger chemical synaptic transmission [111, 127].

Given the plausibility of primitive cellular injury and stress responses being exapted for use as mechanisms of learning and memory, it is interesting that early discoveries in *Aplysia* sensory neurons implicated cooperative activation of Ca^{2+} signaling and cAMP signaling as important for synaptic plasticity, leading to the first proposed cell signaling mechanism of associative learning [141, 142]. In *Aplysia*, the coincidence of activity-dependent Ca^{2+} signaling and stimulation of a G protein-coupled receptor (GPCR) by serotonin (5-HT) produced cooperative activation of an adenylyl cyclase (AC) homologous with AC1 in mammals to amplify cAMP synthesis and produce short- and long-term memory-like effects [143]. The same coincident activation of AC1 by Ca^{2+} signaling and neuromodulators to promote learning and memory occurs in *Drosophila* brain [144, 145] and the mammalian brain [124]. Integrated activation of these and associated cell signaling pathways (notably ERK signaling, well known for its roles in cellular differentiation, growth, and survival in response to growth factors) is now recognized as fundamental to the induction and consolidation of many forms of learning and memory in mammals [137–139].

cAMP signaling in nociceptors may represent a fundamental exaptation for PNH

It is often overlooked that the *Aplysia* sensory neurons used for studies of associative learning [141, 142, 146–148] function as primary nociceptors, with direct exposure to peripheral trauma [35, 128, 149]. Studies of plasticity in these nociceptive neurons have usually been modeled on nonassociative or associative aversive learning paradigms. In all cases an aversive stimulus that activates nociceptors (often electric shock to skin or nerve) or the presumed major extracellular modulator released by noxious stimulation, serotonin (5-HT), has been used. This makes these *Aplysia* studies at least partly equivalent to nociceptive sensitization and hyperalgesia paradigms in mammalian pain studies. In addition to revealing critical roles for PKA, ERK, and other protein kinases in potentiatiing nociceptor synapses [125, 150, 151], *Aplysia* studies showed that PKA, ERK, tyrosine kinase, MNK, and PKG signaling contribute to nociceptor hyperexcitability [151–158]. All these protein kinases have been associated with pain in mammals, but I will focus on PKA and cAMP signaling (Fig. 2) because of how much is known about cAMP-PKA contributions to nociceptive plasticity in mammals and invertebrates.

Drosophila melanogaster has been used for many seminal investigations of nociception and nociceptive sensitization. Although multiple alterations in *Drosophila* class IV polymodal nociceptors and other neurons have been reported during nociceptive sensitization [26, 32, 159–162], very little attention has been paid to cAMP signaling in these neurons. However, photoactivation of a membrane-anchored AC selectively expressed in class IV nociceptors in *Drosophila* larvae can elicit defensive behavior [163]. This finding plus observations of enhanced AP discharge in polymodal nociceptors during heating after damaging ultraviolet irradiation [32] and of cAMP-PKA stimulation driving hyperactivity in other *Drosophila* neurons [164] suggest that cAMP might promote PNH in insect nociceptors after tissue injury, even though central sensitization mechanisms may be more important than peripheral sensitization mechanisms for nociceptive behavioral alterations in insects [8, 165–168].

In *Aplysia* nociceptors (Fig. 2a), ongoing PKA activity contributes to the maintenance of somal hyperexcitability after nerve injury [154]. However, more attention has been paid in *Aplysia* to cAMP signaling for the induction of nociceptor alterations [125, 153]. Ca^{2+}-calmodulin (CaM) directly activates AC1. Ca^{2+} enters through voltage-gated Ca^{2+} channels during bursts of APs, and a coincidence of bursts with excitatory neuromodulatory input (notably, from 5-HT) enhances cAMP synthesis [169, 170] by AC1 [143] and produces long-lasting depolarization of RMP [171, 172], which increases excitability by bringing membrane potential closer to AP threshold (see Fig. 1a). *Aplysia* nociceptors also express AC5 and/or highly similar AC6 as well as AC1 [143], but possible roles of AC5/6 in *Aplysia* PNH have yet to be explored. These ACs activate PKA in *Aplysia*, which is tethered to a scaffolding A-kinase anchoring protein (AKAP) by a regulatory subunit (RII) of PKA (Fig. 2A) that is a close ortholog of mammalian RII [173] (Fig. 2).

In mammalian nociceptors, more is known about AC5/6 (see below) than AC1, and mRNA for AC5 and AC6 is more highly expressed than mRNA for AC1 both in rat DRGs [81] and in most rodent and primate nociceptors [174, 175]. However, indirect evidence suggests a contribution of nociceptor AC1 to sensitization in mice [176]. This could be explained by contributions from the relatively small population of myelinated Aδ nociceptors, which

express more AC1 than do nociceptors in unmyelinated C-fiber subpopulations in mammals [174, 175], and which display striking similarities to *Aplysia* nociceptors in rapid axonal conduction relative to other nociceptor types in the same species, large receptive fields, sensitivity to a wide range of intensities of mechanical stimuli, and sensitization of mechanosensory responses by noxious mechanical stimulation [35, 65, 177].

In rodents (Fig. 2b), at least three downstream signaling effectors of cAMP are reported to enhance nociceptor function in pain models: 1) PKA in mechanical and heat hypersensitivity in inflammatory and neuropathic models [72, 81, 178–181], 2) EPAC in inflammatory and SCI models [76, 182–184], and 3) HCN channels in inflammatory models [185, 186]. While many excitatory modulators are likely to stimulate cAMP-dependent hyperactivity in various mammalian nociceptors, it is interesting that at least one of these, 5-HT, is the same as in *Aplysia* [187–189]. The most direct links between cAMP signaling and PNH have been shown in rodent dissociated nociceptor somata months after SCI (Fig. 2b). In rats and mice, ongoing EPAC activity was required for SCI-induced SA, depolarization of RMP, reduction of AP threshold, and enhanced DSFs [76]. In rat nociceptors, SCI-induced SA and depolarization of RMP required continuing activation of PKA by adenylyl cyclases (AC5 and/or highly similar AC6) scaffolded to A-kinase anchoring protein 150 (AKAP150) in rodents or its ortholog AKAP79 in humans [81]. As in *Aplysia* nociceptors, the RII regulatory subunit binds PKA to an AKAP in rodent primary somatosensory neurons [190, 191], in this case to AKAP150. Ion channels in rodent sensory neurons known to be scaffolded with and/or phosphorylated by PKA include TRPV1, Nav1.8, Cav1.2, and KCNQ channels [192–194].

Unexpectedly, SCI decreased the sensitivity of AC to inhibitory Gi in isolated DRG membranes [81], suggesting that an additional hyperactive effect in vivo might result from induced resistance of AC5 and/or AC6 in nociceptors to endogenous and clinically applied opioids (Fig. 2b). Indeed, SCI reduced the in vitro inhibitory effects of mu-opioid agonists on PKA activated by forskolin treatment [82]. One of the hyperexcitable alterations after SCI is sustained depolarization of RMP [44, 52]. Experimental depolarization was found to activate C-Raf (upstream to ERK signaling), and C-Raf activation decreased the inhibition of AC by the G protein Gi [82] (Fig. 2b). Blocking MEK (downstream from C-Raf and upstream of ERK) blocked SA, DSF enhancement, and sustained depolarization of RMP. Thus, both cAMP signaling and ERK signaling are likely to depolarize nociceptor RMP after SCI, and cAMP signaling via PKA and EPAC probably contributes to the C-Raf-MEK-ERK effects by helping to depolarize RMP, while C-Raf activation by depolarization in the presence of endogenous or exogenous opioids should enhance cAMP signaling by reducing ongoing opioid inhibition of AC. These positive feedback loops and others involving translational and transcriptional regulation by these pathways (not shown in Fig. 2) are likely to help maintain PNH. Associated negative feedback loops, including inhibition of AC5/6 by Ca^{2+} (Fig. 2b) and AKAP-scaffolded phosphodiesterases and phosphatases (not shown) are probably important in nociceptors for protecting against inappropriate or excessive hyperactivity and possible excitotoxicity [86, 195, 196].

Because the last common ancestor of molluscs and chordates lived ~600 million years ago, it is striking that the same isoforms of AC (AC1 and AC5/6) and PKA regulatory

subunit RII are expressed in nociceptors that exhibit prominent cAMP-dependent PNH both in *Aplysia* and rodents. AKAP-scaffolded cAMP signaling arose near the onset of multicellular eukaryotic evolution [197] and it would have been available to modulate early nociceptors as they evolved to detect, integrate, and respond to information about injury to an organism. It is also notable that PNH depends upon ACs that are directly modulated by Ca^{2+}, perhaps reflecting primordial properties of Ca^{2+} both as a signal of cellular injury and as a potential cellular toxin, which might have shaped fundamental nociceptive functions of AC1 and AC5/6, respectively. Thus, AKAP-scaffolded cAMP signaling may have been exapted from even more ancient injury- and stress-related functions of this signaling to drive PNH in ancient animals, prior to the divergence of molluscs and chordates (or exapted independently in each lineage later in evolution for common hyperexcitability functions). In contrast, the identities of the ion channels involved in PNH differ markedly across phyla. For example, PKA-regulated Nav1.8 and TRPV1 channels are important for SA in rodent nociceptors [83, 85, 102], but ion channels have diverged substantially over the course of evolution [198, 199]. Indeed, Nav1.8 and TRPV1 are unique to chordates and thus cannot contribute to PNH in *Aplysia* or *Drosophila*. Other channels may have more ancient, conserved roles. For example, PKA can also contribute to PNH by reducing background activity in K^+ leak channels in rodents and *Aplysia* (TREK-1 and TREK-1-like channels, respectively) under sensitizing conditions [200–204]. Another late evolutionary development is inhibitory regulation of cAMP signaling by opioids and opioid receptors, which only occur in the vertebrate lineage, although other G_i-coupled GPCRs found on mammalian nociceptors, such as somatostatin receptors, are found in multiple phyla [205, 206]. Thus, while core cAMP signaling mechanisms linked to PNH have been highly conserved, downstream ion channels and upstream GPCRs in nociceptors may exhibit greater evolutionary diversification.

Conclusions

An evolutionary perspective can provide insights into why chronic pain is so prevalent and difficult to treat. One of several explanations for the pervasiveness of chronic pain in modern life is that some mechanisms in primary nociceptors that predispose humans to chronic pain were selected during evolution because they promoted the survival of seriously injured individuals, and these mechanisms might also be activated pathologically in other neuropathic conditions. One physiological mechanism, PNH, has been associated with enhanced anti-predator responses after injury in squid and mice, indicating its adaptive value. The few species in which PNH has been investigated mechanistically (primarily *Aplysia*, rats, mice, and humans) have revealed many similarities and some differences between *Aplysia* and mammals. Evidence for essential contributions of scaffolded cAMP-PKA signaling (with associated Ca^{2+} signaling) to the maintenance of PNH in rodents and *Aplysia* suggest that ancient cell signaling modules linked to primordial organismic injury responses were exapted during the early evolution of nociceptors to drive persistent hyperactivity and adaptive behavior following significant bodily injury. Much remains to be learned about the pain-maintaining roles of these pathways and other mechanisms underlying PNH, but current knowledge about PNH mechanisms already has therapeutic implications. One is that the cellular signaling pathways found to be important for PNH thus

far (cAMP, Ca^{2+}, and ERK signaling) are important for many organismic functions and thus may be poor candidates for targeting chronic pain without unwanted side effects. In addition, redundancy has been found in the complex physiological and molecular mechanisms of PNH in mammals, which may impede effective therapeutic targeting. The complexity and functional coherence of these PNH mechanisms in mammals are consistent with shaping and maintenance by strong, continuing evolutionary pressures. On the other hand, recent evolution may offer new molecular targets to treat pain in vertebrates. For example, PNH in mammals depends upon the activity of three ion channels that are mainly expressed in nociceptors (Nav1.8, Nav1.9, and, with somewhat less specificity, Nav1.7) [207] and these channels first appeared in tetrapod vertebrates [208]. Specific inhibition of one or more of these recently evolved channels might suppress PNH with few side effects. For example, experimental targeting of Nav1.8 (whose activity is enhanced by cAMP-PKA signaling) alleviates persistent pain behavior in several rodent neuropathic pain models at least in part by blocking nociceptor SA [84, 85, 209]. Recent results from phase 2 clinical trials for a highly selective Nav1.8 blocker [210] encourage a strategy of treating chronic pain by inhibiting ion channels that are important for PNH and are preferentially expressed in nociceptors.

Acknowledgements

The author is grateful to Dr. Carmen Dessauer for her leadership of the mammalian cell signaling studies discussed in this review and for her valuable comments on the manuscript, and to Drs. Alexis Bavencoffe, Anibal Garza Carbajal, Jinbin Tian, and Michael Zhu for their many contributions to the research and ideas presented here.

Funding Sources

Scholarship by the author during the preparation of this article and some of the research reviewed was supported by NIH grants NS091759 to Carmen W. Dessauer and Edgar T. Walters, and NS111521 to Edgar T. Walters and Michael X. Zhu, and by the Fondren Chair in Cellular Signaling.

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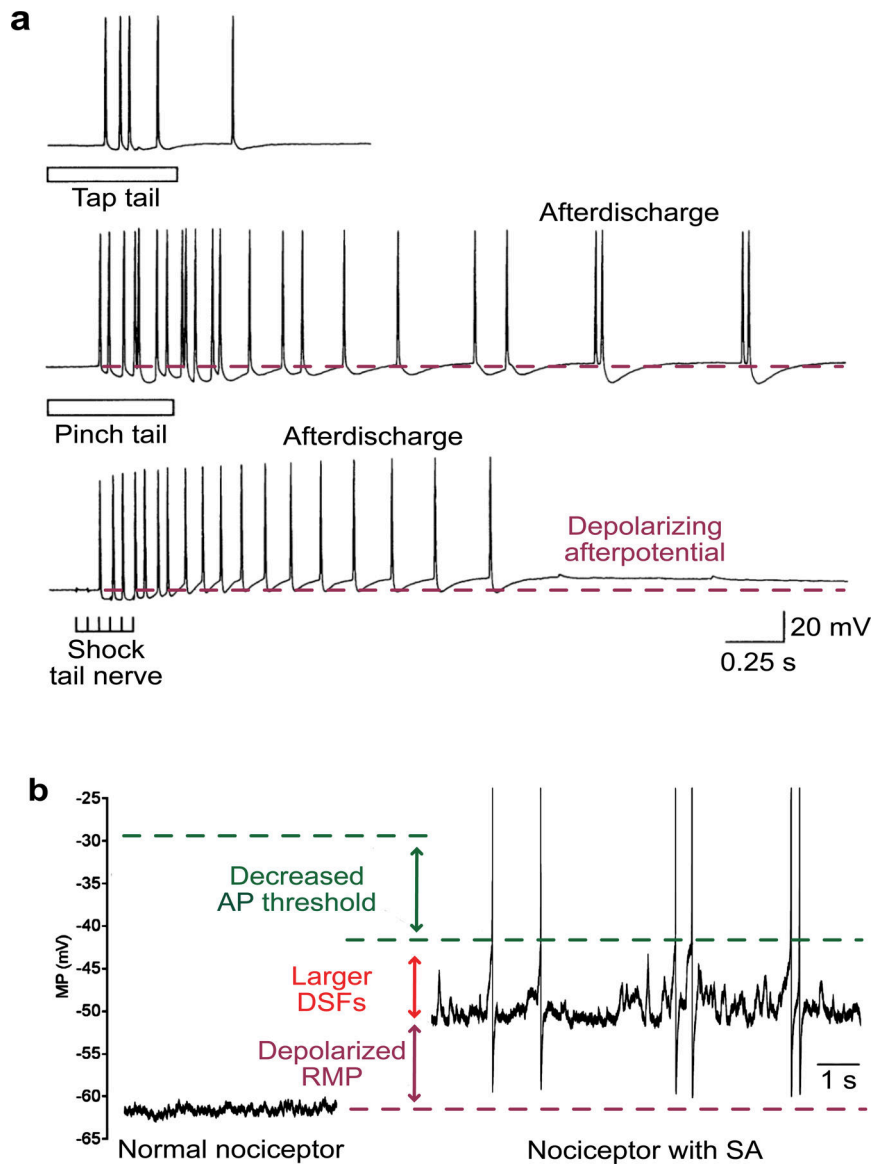

Fig. 1.

Two types of somal hyperactivity in nociceptors. **a** Afterdischarge generated by three kinds of peripheral stimulation delivered sequentially to the same *Aplysia* nociceptor in a semi-intact preparation with the tail attached to central ganglia containing the nociceptor somata: top panel, 0.5-s tap (75 g/mm^2) to the neuron's receptive field on the animal's tail; middle panel, ~0.5-s pinch to the same receptive field; bottom panel, 0.25-s train of 2-ms shocks (20 Hz) to the nerve containing the nociceptor's axon. The dashed purple line indicates the resting membrane potential (RMP) for comparison to the depolarizing afterpotential that can bring the soma membrane to action potential (AP) threshold. Afterdischarge when this potential is small, as shown here immediately after tail pinch, is generated peripheral to the soma, probably within the distant receptive field. The larger depolarizing afterpotential evoked in the soma by subsequent tail nerve shock triggers afterdischarge as a long-lasting consequence of the prior pinch. Modified from [51]. **b** Examples of RMP and one form of

ongoing activity (OA) in nociceptors dissociated from dorsal root ganglia excised from a previously uninjured rat (left) and from a rat 2 months after spinal cord injury. Compared to the isolated control nociceptor shown on the left, the nociceptor from the previously injured rat exhibited OA at RMP (likely intrinsically generated spontaneous activity, SA), depolarization of RMP, enhanced depolarizing spontaneous fluctuations (DSFs) of membrane potential (MP), and decreased voltage threshold for AP initiation. The dashed purple line indicates RMP in the control nociceptor, and the dashed green line indicates AP threshold for each nociceptor. Modified from [52].

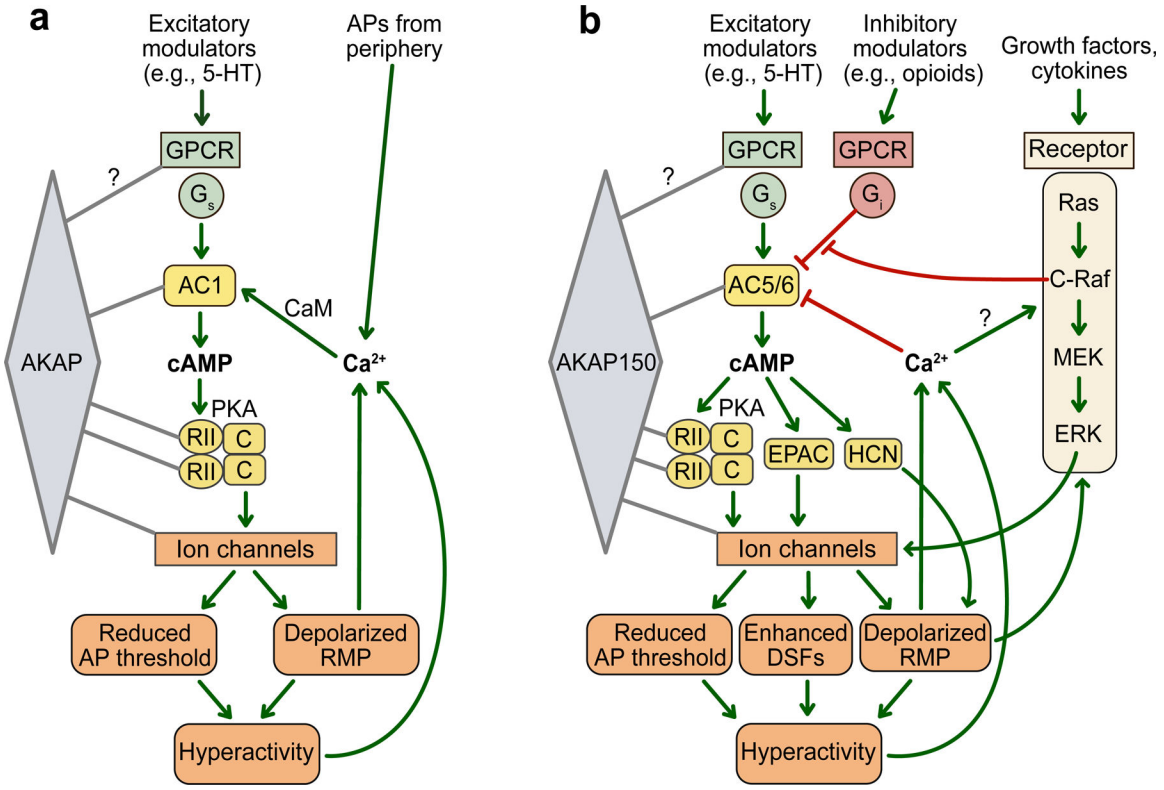

Fig. 2.

Core cAMP signaling modules described in *Aplysia* and rodent nociceptors that might have been exapted during early animal evolution to promote hyperactivity and persistent pain after bodily injury. **a** Scaffolded AC1-centered signaling implicated in the induction and possible maintenance of PNH in *Aplysia*. Some evidence suggests that AC1 signaling might also contribute to PNH in mouse Aδ nociceptors and possibly in *Drosophila* nociceptors (see text). The yellow elements are close orthologs in *Aplysia* and rodent nociceptors. It is not yet known whether other cAMP signaling elements (e.g., EPAC or HCN channels) function in *Aplysia* nociceptors. Most cAMP research on these neurons has focused on 5-HT stimulation, and the ion channels phosphorylated by PKA have not been identified, except for a TREK-1-like leak K^+ channel inhibited by PKA. **b** AC5/6-associated signaling that contributes to the maintenance of PNH in rodent nociceptors. AC5/6 is also prominently expressed in *Aplysia* nociceptors, but their potential contribution to PNH in these neurons has not been explored. In rodents, numerous excitatory and inhibitory modulators and GPCRs can stimulate or inhibit AC5/6 in primary sensory neurons. AC5/6 is inhibited by Ca^{2+} and by opioids and other modulators coupled to G_i, and the G_i-mediated inhibition can be reduced by depolarization of RMP, at least in part through C-raf in the ERK pathway. Among the ion channels regulated by scaffolded PKA in these neurons are TRPV1, Nav1.8, Cav1.2, and KCNQ channels (not shown). Neither other scaffolded proteins such as phosphodiesterases and phosphatases nor pathways regulating DNA transcription and mRNA translation downstream from AC5/6 or ERK are shown. Activating effects are indicated by green arrows, inhibitory effects by red bars. 5-HT, serotonin; AC5, adenylyl cyclase 5; AC5/6, adenylyl cyclase 5 and/or AC6; AKAP150, A-kinase anchoring

protein 150; AP, action potential; DSF, depolarizing spontaneous fluctuation; C, catalytic subunit of PKA; CaM, calmodulin; C-raf, Raf-1 proto-oncogene; cAMP, cyclic adenosine monophosphate; EPAC, exchange protein directly activated by cAMP; ERK, extracellular signal-related kinase; GPCR, G-protein-coupled receptor; G_i, inhibitory G-protein $G_{\alpha i}$; G_s, stimulatory G-protein $G_{\alpha s}$; HCN, hyperpolarization-activated, cyclic nucleotide-gated channel; MEK, mitogen-activated protein kinase kinase; PKA, protein kinase A; RII; regulatory subunit II of PKA; Ras, “rat sarcoma virus” GTPase.