Common patterns of plasticity contributing to nociceptive sensitization in mammals and Aplysia

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In contrast to innocuous stimuli, which only have transient effects when applied to the body surface, noxious stimuli generate persistent changes in the nervous system. This nociceptive memory manifests itself most prominently as a post-injury sensitization where, after tissue damage, the avoidance reaction and pain that result from subsequent stimuli are exaggerated and prolonged and can be initiated by low intensity stimuli. Similarities between nociceptive sensitization in mammals (including humans) and the mollusc Aplysia californica *suggest that fundamental mechanisms contributing to injury-induced behavioral modifications might be widespread in the animal kingdom.*

All organisms encounter threats to their physical integrity in the environment and defensive responses to noxious stimuli are found in all major animal phyla[1]. Although defensive adaptations are quite diverse in the animal kingdom, two basic needs are shared by virtually all animals – the need to escape from a source of injury and the need to protect recuperating parts from further disturbance. The latter need is met by the generation of a state of sensitization. The term 'sensitization' has been used independently by physiologists and psychologists to describe an increase in sensitivity and/or magnitude of a response. Here we only consider the sensitization induced by injury or by physiological signals that normally signify injury. We call this form of sensitization 'nociceptive sensitization' because it involves tissue-damaging noxious stimuli that activate nociceptors; this distinguishes it from the sensitization produced by innocuous events such as appetitive or novel stimuli.

The most common manifestation of nociceptive sensitization is a hypersensitivity of the site of an injury and of surrounding areas. If an animal has been injured it is to the animal's advantage that defensive responses to subsequent stimuli near the site of injury are quicker and are generated at a lower threshold than normal. Recent investigations in a number of different mammals, including humans, and in the mollusc *Aplysia californica* have shown that nociceptive sensitization involves peripheral alterations at the site of the injury and central alterations within the neuronal circuits representing the injured region.

In humans the readiness to protect an injury is expressed as hyperalgesia (the lowering of pain thresholds and increased pain by normally painful stimuli) and allodynia (the production of pain by innocuous stimuli). This nociceptive sensitization spreads to areas beyond the site of damage, increasing the sensitivity of uninjured tissue; this phenomenon is known as secondary hyperalgesia. Two general mechanisms contribute to post-injury hypersensitivity states in mammals: peripheral sensitization and central sensitization (Fig. 1A).

Peripheral sensitization in mammals

On the basis of their responsiveness to low and high intensity stimuli, primary afferents can be characterized as having a low or high threshold. Because they respond only to noxious stimuli, primary afferents with a high threshold are known as nociceptors. Peripheral sensitization involves a reduction in the threshold and an increase in the gain of the transduction processes of the primary afferent nociceptors. Studies on single afferent fibers have demonstrated alterations in the sensitivity of thermoreceptive nociceptors in the immediate area of injury[2] but not in the surrounding uninjured area[3]. Mechanical hypersensitivity is a prominent feature at the site of injury and in remote areas[4,5]. An example of peripheral mechanical sensitization is seen in a subset of articular afferents with very high thresholds that begin to respond to innocuous movements of the joint after experimental arthritis[6]. However, changes in the thresholds of mechanoreceptive nociceptors are rare or absent in the zone of secondary mechanical hyperalgesia[7].

Several sources of chemical signals contribute to peripheral sensitization. Histamine, serotonin (5-HT), hydrogen ions (H^+), potassium ions (K^+) and neuropeptides (including bradykinin and the neurokinins) are released from injured afferents, damaged tissue and inflammatory cells[8] and generate a sensitizing 'soup', in which the chemicals appear to act synergistically to alter the sensitivity of afferent terminals[9]. The mechanisms responsible are not known but might involve effects mediated by second messengers on ion channels or receptors. In addition, the axon terminals of sympathetic postganglionic neurons release amines, purines and eicosanoids that also act on nociceptor terminals, altering their responsiveness[10].

Central sensitization in mammals

The lack of a change in the sensitivity of afferents in areas of secondary hyperalgesia[8] implies that modifications in central neurons must be involved in generating the sensory disturbances in these regions. 'Central sensitization' is the term that is used to describe alterations in the responsiveness of spinal cord neurons to normal inputs after a conditioning noxious stimulus or peripheral tissue damage. Direct evidence for central sensitization first appeared in a study of the flexion withdrawal reflex in rats[11]. Injury of peripheral tissue was shown to reduce the intensity of stimuli required to initiate a flexion reflex and to expand the cutaneous receptive field of flexor motorneurons. Once these changes were established, a local anesthetic block of the injury site did not return the facilitated reflex to its baseline level, suggesting that the afferent signal associated with the injury could induce a state of prolonged facilitation in the spinal cord[11,12]. This effect could be mimicked by brief electrical stimulation of peripheral nerves (1 Hz for 20 s), provided C afferents were recruited[13].

The conditioning stimuli that induce a prolonged facilitation of the flexion reflex also produce marked alterations in the receptive fields of dorsal horn neurons, such as a reduction in threshold, expansion of receptive fields and the recruitment of novel

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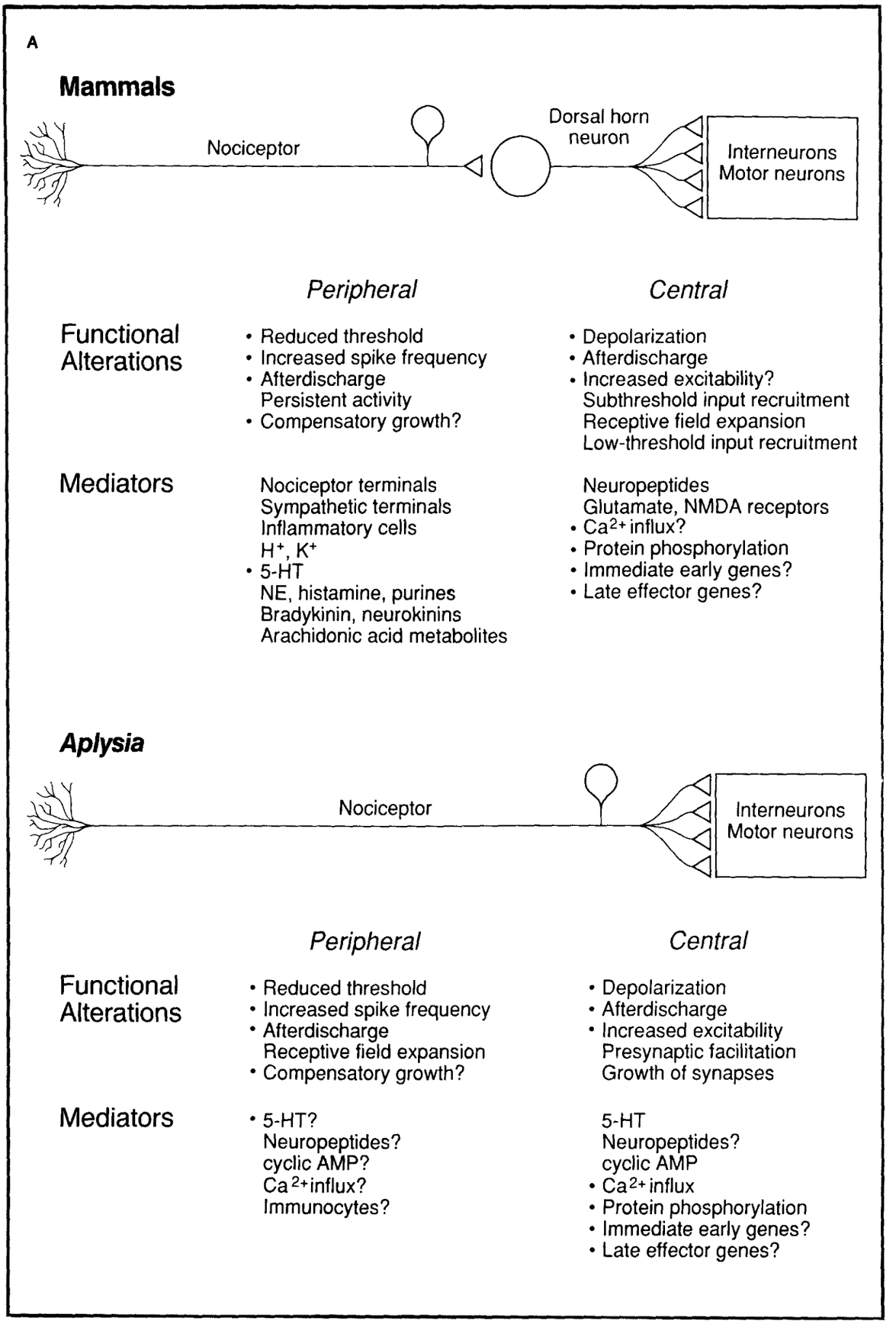

Fig. 1. *Functional alterations and mediators implicated in nociceptive sensitization in* **(A)** *mammals and* **(B)** Aplysia. *All entries have received some experimental support; interesting possibilities that have not yet been examined are not listed. Question marks indicate alterations or mediators for which the evidence is indirect. Dots indicate features that have been observed in both mammals and* Aplysia. *Compensatory growth of nociceptor axons in mammals might only occur when injury is severe enough to cause denervation.* (Compiled from data reviewed in text.)

inputs[14]. Similar changes have been observed in the dorsal horn of cats and monkeys[15,16]. In humans the cutaneous application of the irritant capsaicin generates a large area of secondary mechanical hyperalgesia[17]. Inputs produced by intraneural stimulation of low-threshold A beta afferents with receptive fields in such an area of secondary hyperalgesia then begin to elicit pain, whereas before the application of capsaicin these afferents produce only innocuous sensations[18]. These sensory changes and many of the clinical features of acute pain are therefore likely to be the consequence of an injury-induced sensitization of central neurons.

The capacity of dorsal horn neurons to enlarge their receptive fields after noxious stimuli seems to be due to these receptive fields having an excitatory input that is mostly subthreshold. Intracellular recordings from dorsal horn neurons show that their receptive fields can be divided into a high-probability firing zone, where a stimulus elicits a discharge of action potentials, and a low-probability firing fringe, where a stimulus elicits a distinct subthreshold response but a small, variable or absent action potential discharge[19]. By virtue of these subthreshold inputs, the receptive fields of dorsal horn neurons can change if either synaptic efficacy and/or membrane excitability increases[20].

The mechanism responsible for the triggering of central sensitization in dorsal horn neurons is not established yet. However, one clue lies in the differences in the synaptic activation that can be shown in *in vitro* preparations to be produced by those afferents that can induce central sensitization (small diameter, high-threshold afferents) and those that cannot (large diameter, low-threshold A beta afferents). A beta afferents generate 'fast' excitatory postsynaptic potentials (EPSPs) of short duration, while A delta and C afferents produce slow EPSPs[21–23]. These slow EPSPs last up to 20 s after a single stimulus and, by summating, enable a cumulatively incrementing depolarization to occur on repeated stimulation[23]; this increases the number of spikes elicited per stimulus and is the phenomenon of 'windup'[24].

The slow potentials and their summation are substantially diminished by the NMDA antagonist D-amino-5-phosphonovaleric acid (D-APV)[23], as is windup[25]. An implication of this is that nociceptive afferent inputs can uniquely generate a progressively incrementing response that, because of the removal of the voltage-dependent magnesium ion (Mg^{2+}) blockade of the NMDA receptor–ion channel complex[26], can be further amplified in a non-linear way. The transmitters responsible for the slow potentials need to be established, but the neurokinins are likely candidates[21].

The NMDA ion channel permits calcium ions (Ca^{2+}) to enter the cell[27]; this could alter the phosphorylation states of various proteins[28]. Therefore, the potential exists for translating a brief cumulative depolarization into a prolonged alteration in excitability that could, because of the subthreshold response repertoire of dorsal horn neurons, produce dramatic changes in sensory processing in the spinal cord – such as 'nociceptive-specific' dorsal horn neurons becoming responsive to non-nociceptive inputs[20]. That the NMDA receptor has a key role in these changes is confirmed by the demonstration in the rat that the NMDA receptor antagonist MK-801 (dizocilpine) prevents the induction of central sensitization *in vivo* and can abolish it once sensitization has been established[29].

A feature of nociceptive sensitization is its persistence long after the initiating stimulus has terminated[12,13]. A nociceptive memory has been established, which, by contributing to an animal's exaggerated response to a potentially harmful situation, is adaptive. However, it should be considered whether this sensitization is always advantageous. Indeed the problem of chronic pain could be the maintenance of a state of nociceptive sensitization where such sensitization no longer has an obvious protective role.

Nociceptive sensitization in *Aplysia*

Although behavioral and cellular alterations following noxious stimulation have been studied intensively in *Aplysia* for about two decades, it is only recently that parallels between these alterations and those in the nociceptive systems of mammals have been recognized. Such parallels were not appreciated until it was discovered that identified mechanosensory neurons in *Aplysia* function as nociceptors. While these sensory neurons have a wide dynamic range, responding with one or a few action potentials to moderate intensity stimuli, they respond preferentially to noxious mechanical stimuli, firing prolonged high-frequency bursts in response to tissue-damaging stimuli[30].

This finding led to a series of behavioral and cellular studies, the results of which showed that the sensitizing effects of noxious stimulation in *Aplysia*, as in mammals, are site specific – being greatest in the region of noxious stimulation. Behavioral studies showed that a brief sequence of either ten severe pinches or electric shocks to the skin caused site-specific sensitization of the siphon- and tail-withdrawal responses. This sensitization was demonstrated by test stimuli applied near the site of trauma[31]. This long-term sensitization, lasting a week or more, was not seen when test stimuli were applied away from the site of trauma. However, if noxious stimulation sequences are repeated several times over a period of hours or days, sensitization of withdrawal responses can spread to test sites distant from the site of trauma[32,33].

Mechanisms of nociceptive sensitization in *Aplysia*

Although nociceptive sensitization in *Aplysia* involves alterations in sensory, motor and interneurons[34], analysis has largely focused on nociceptive mechanosensory neurons of wide dynamic range that innervate the siphon and tail. These neurons have peripheral receptive processes in the skin that are connected by long axons to somata and presynaptic terminals in the CNS (abdominal or pleural ganglia). Each sensory neuron appears to combine some of the integrative functions that, in mammals, are distributed across primary sensory neurons and interneurons of wide dynamic range in the dorsal horn of the spinal cord[30]. Nociceptive sensitization involves alterations in both the peripheral and central regions of the *Aplysia* sensory

neurons (Fig. 1B). The peripheral regions of sensory neurons innervating a traumatized region show a decrease in mechanosensory threshold and an increase in the number of sensory action potentials evoked by a cutaneous test stimulus[35–37]. This peripheral sensitization is mimicked by infusing 5-HT or molluscan small cardioactive peptide B (SCP_B) into the body wall[38,39]. Central regions of the sensory neurons show both general and site-specific alterations after noxious stimulation. A strong tail shock, 45 s in duration, causes a general facilitation of inactive sensory neurons that lasts about 30 min and is expressed at the synapses of sensory neurons with receptive fields outside the shocked region[35]. Specific facilitation (five times the magnitude of general facilitation and lasting at least one day) occurs only in neurons whose receptive fields are activated by the shock. This activity-dependent memory of the site of injury is functionally similar to primary hyperalgesia in mammals[35], while the general sensitizing effect appears to be analogous to secondary hyperalgesia in mammals. Since the localized induction of a cellular immune reaction in *Aplysia* is accompanied by an enhancement of the excitability of nearby sensory neurons, it seems possible that immunocytes (perhaps similar to mammalian inflammatory cells) might play a role in nociceptive sensitization in molluscs[40].

Alterations in sensory neurons activated during trauma show quantitative, but not qualitative, differences from alterations in sensory neurons that remain silent. This suggests that trauma releases chemical modulators that influence all the mechanosensory neurons ('heterosynaptic facilitation', see Ref. 41), and that these extrinsic influences are amplified in sensory neurons that are firing action potentials at the time of modulation. This activity-dependent extrinsic modulation (ADEM) was first proposed to explain classical conditioning[42,43], but a more common function of ADEM in nociceptive neurons is probably to encode memory of a site of injury[31,36]. An important modulator released by noxious stimulation in *Aplysia* might be 5-HT[44], which enhances sensory synaptic transmission and excitability in both the CNS[38,41,45] and periphery[39,46]. The central effects are mediated at least in part by cyclic AMP, which causes a depression of one or more K^+ conductances, and an increase in Ca^{2+} transients and neurotransmitter mobilization in presynaptic terminals of the sensory neurons[47]. Amplification of extrinsic modulatory effects by spike activity involves Ca^{2+} entry into the cell; this enhances adenylate cyclase activity, increasing the rate of cyclic AMP synthesis[48].

Behavioral sensitization in *Aplysia*, as in mammals, can last for weeks[31–33]. A week after the tail is injured or shocked, the mechanosensory thresholds of tail sensory neurons are lowered and the receptive fields are enlarged[36]. Long-term changes also occur in the central regions of sensory neurons: sensory neurons innervating a traumatized area make stronger synaptic connections within the CNS to follower neurons than do sensory neurons innervating other areas[35]. This is paralleled by an increase in excitability of the centrally located sensory neuron soma[35,49]. If strong shock is repeated over hours or days, similar changes occur in sensory neurons that have unstimulated receptive fields[33]. Repeated strong shock also leads to morphological changes in these cells, doubling the number of presynaptic varicosities and active zones within the CNS[50,51]. Indirect evidence suggests that peripheral growth is involved in the long-term increase in the size of the receptive field near an injury[36]. A potentially similar injury-induced expansion of mechanonociceptor receptive fields in mammals is suggested by observations of activity-dependent growth of nociceptors into denervated skin of the rat[52].

Are primitive mechanisms of plasticity involved in nociceptive sensitization?

In mammals and *Aplysia*, nociceptive sensitization is associated with profound changes in the processing of sensory inputs. Common features include enhanced sensitivity of peripheral sensory elements, enlargement of receptive fields, long-term modifications of central neurons, and activity-dependent plasticity (Fig. 1A,B). Because the ancestors of molluscs and mammals diverged very early in the history of the animal kingdom, it is likely that some of these similarities are due to convergent evolution of analogous processes after divergence of the groups. These similarities might reflect common solutions to a ubiquitous problem, that is, the dangers that follow sublethal injury. Such processes might appear similar at a functional level, but actually involve different molecular mechanisms. For example, nociceptive circuits representing an injured region show a similar hyper-responsiveness in *Aplysia* and mammals. However, one molecular mechanism, a cumulative depolarization by the voltage-dependent unblocking of the NMDA receptor–ion channel complex, appears to contribute to hyper-responsiveness in mammals but probably does not occur in *Aplysia*.

On the other hand, some of the similarities in nociceptive sensitization between mammals and *Aplysia* might reflect mechanisms descended from a primitive common ancestor of these evolutionarily divergent groups. At present, not enough is known about the molecular mechanisms of nociceptive sensitization in either group to conclude that functional similarities involve homologous mechanisms. However, the arguments supporting a very early origin of mechanisms of nociceptive plasticity[53], and the involvement of common cellular regulatory processes in various forms of plasticity in both groups, suggest that homologies will be found in mammalian and molluscan mechanisms of nociceptive sensitization. In this regard it will be interesting to compare the roles of various protein kinases. For example, given the central role of cyclic AMP in depressing K^+ conductances and triggering morphological changes in *Aplysia* nociceptors[41,54], it is noteworthy that cyclic AMP can also depress K^+ conductances and enhance regenerative growth in vertebrate sensory neurons[55–57]. Similarly, it is intriguing that sensitizing stimulation leads to the transient expression of 'immediate–early' genes in rat dorsal horn neurons[58] and *Aplysia* neurons[59].

Because of the intense efforts underway to identify stimulation-associated proteins in *Aplysia*[59] and mammals[60], it seems likely that interphyletic comparisons of sensitization-associated proteins that display various degrees of homology will soon become possible. Identification of common cellular processes

that contribute to sensitization in mammals and molluscs might provide clues about the early evolution of mechanisms that have widespread importance for memory and pain.

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