Cellular Neuroplasticity Mechanisms Mediating Pain Persistence

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Transmission of noxious-stimulus-evoked inputs in the spinal and trigeminal systems is mediated primarily through excitatory glutamatergic synapses using alpha amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid (AMPA), kainate and N-methyl-D-aspartate (NMDA) subtypes of glutamate receptors. Glutamatergic synapses exhibit multiple forms of short-lasting and long-lasting synaptic plasticity. Persistent enhancement of nociceptive transmission, known as "central sensitization," is a form of lasting plasticity that is similar mechanistically to long-term potentiation of glutamatergic transmission in other regions of the central nervous system. This potentiation of AMPA/kainate transmission is dependent upon the activity of NMDA receptors, which become enhanced following noxious peripheral stimulation as a result of several convergent mechanisms. Central sensitization is thus an expression of increased synaptic gain at glutamatergic synapses in central nociceptive-transmission neurons and thereby contributes importantly to pain hypersensitivity. In addition, recent evidence has revealed a new player in the mechanisms underlying pain hypersensitivity following nerve injury—microglia. Understanding of the roles of microglia may lead to new strategies for the diagnosis and management of neuropathic pain. J OROFAC PAIN 2004;18:318–324

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A key insight in neuroscience over the past 2 decades is that synaptic connections between neurons are in a near-continual state of change. These modifications are highly dependent upon the electrical activity and the state of multiple intracellular biochemical signaling networks in the pre- and postsynaptic neurons. During development, the biasing of these intracellular signaling networks by temporally appropriate molecular signals is responsible for diverse processes such as axonal pathfinding and the formation, establishment, and consolidation of synaptic contacts. In the developed nervous system, the continual interplay of these intracellular signaling network processes serves to produce synaptic modifications (ie, plasticity) that underlie physiological processes such as learning and memory. The same molecular signaling cascades that produce these normal forms of plasticity may, if aberrant, lead to pathologic excitatory processes including epilepsy, neurodegeneration, and chronic pain. This review discusses the role of the commonalities in plasticity of excitatory synaptic transmission in the context of the function of the spinal dorsal horn and trigeminal brainstem subnucleus caudalis that may contribute to the pathogenesis of persistent pain. The focus of this discussion is on mechanisms of plasticity that do not involve changes in gene

Fig 1 Activation of nociceptive transmission neurons by glutamatergic fast EPSPs. The diagram illustrates the monosynaptic excitatory connection of nociceptive primary afferent neurons with nociceptive-transmission neurons in the spinal dorsal horn or the trigeminal subnucleus caudalis. The nociceptive neurons also receive inhibitory inputs from local glycine/GABA neurons that are excited by inputs from non-nociceptive primary afferents. Adapted from Woolf and Salter.[5] Abbreviations in this and subsequent figures: KAI = kainate glutamate receptor; VGCC = voltage-gated calcium channel; NK1 = neurokinin 1 receptor; NSC = nonselective cation channel; GLY = glycine; mGluR = metabotropic glutamate receptor. Other abbreviations as in text.
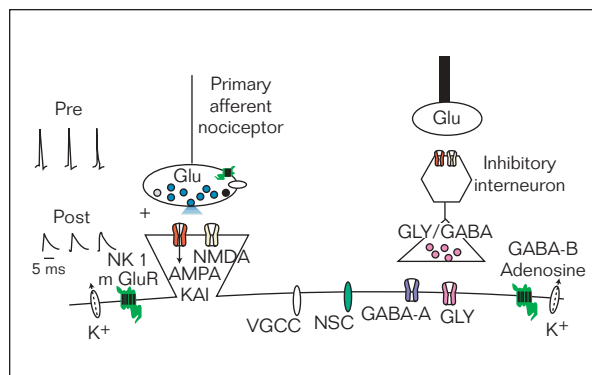
expressions. Nociceptive plasticity mechanisms mediated by alterations in gene expression are discussed in detail elsewhere.[1]

Glutamatergic Fast Synaptic Transmission at Central Primary Afferent Synapses

Like the vast majority of excitatory synapses in the central nervous system (CNS), fast excitatory neurotransmission at most central terminals of primary afferent neurons is caused by released glutamate, which activates ionotropic glutamate receptors (GluRs) that are strategically localized in the postsynaptic neurons.[2] The excitatory postsynaptic potentials (EPSPs) resulting from single presynaptic action potentials are caused primarily by activation of the alpha amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid (AMPA) and kainate subtypes of GluR.[3,4] The N-methyl-D-aspartate (NMDA) subtype of GluR, which is also localized at excitatory synapses, contributes little to the responses to single presynaptic action potentials because these receptors are tonically suppressed by extracellular magnesium (Mg^{2+}), which blocks NMDA channels. This type of fast excitatory synaptic transmission occurs even at synapses of "slow" primary afferents, which are predominantly nociceptive (Fig 1). A mild noxious stimulus to the periphery causes low-frequency discharges in nociceptors that elicit EPSPs in spinal dorsal horn and subnucleus caudalis neurons that signal the onset, duration, and intensity of the stimulus.[5]

Comparing "Windup" with "Central Sensitization"

More intense or sustained noxious peripheral stimulation induces primary afferent nociceptors to discharge at higher frequencies and results in release of peptide neuromodulators, such as Substance P and calcitonin gene-related peptide, together with glutamate from central terminals of nociceptive neurons, which leads to slow synaptic potentials lasting tens of seconds,[6] as illustrated in Fig 2. These slow EPSPs provide substantial opportunities for temporal summation of fast EPSPs,[7] and the cumulative depolarization is boosted by the recruitment of NMDA receptor current upon relief of the Mg^{2+} suppression of the current flow through the channels. The sustained depolarization may also recruit voltage-gated calcium (Ca^{2+}) currents, causing a further boost in the level of intracellular Ca^{2+} and triggering plateau potentials mediated by calcium-activated nonselective cation channels. The net effect of these multiple intracellular signaling cascades in second-order nociceptive neurons is a progressive increase in the action potential discharge elicited by subsequent close-spaced stimuli, a phenomenon known as "windup."[5]

Nociceptive spinal dorsal horn and subnucleus caudalis neurons show an additional and mechanistically separable form of enhanced responsiveness to nociceptive inputs which is often referred to as "central sensitization."[8] Central sensitization is considered a major mechanism underlying inflammatory and neuropathic pain.[9–11] Central sensitization, like windup, is initiated by peripheral nociceptor input but not by low-threshold peripheral inputs. In contrast to windup, sensitization of central nociceptive neurons outlasts, sometimes by many hours, the duration of the nociceptor inputs that initiate it. These inputs cause the engagement of multiple intracellular signaling cascades that were dormant during activation, leading to an orchestrated modification of neuronal behavior consisting of enhanced excitatory postsynaptic responses and depressed inhibition. The engagement of these signaling cascades functionally increases the gain of the nociceptive neurons, resulting in amplified responses not only to noxious inputs but also to innocuous inputs. Because most
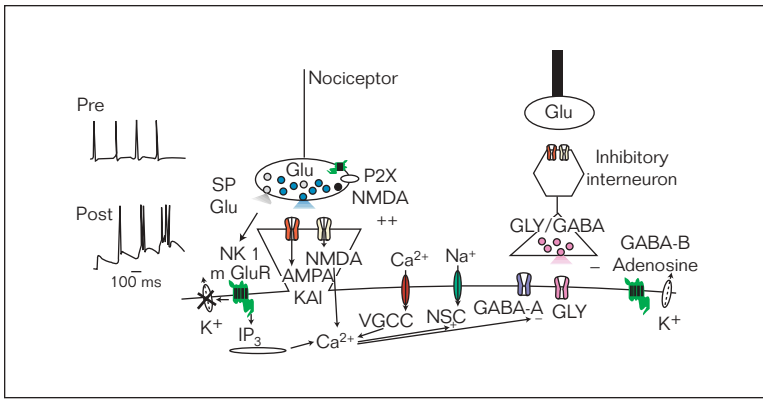
Fig 2 High-frequency discharge of primary afferent neurons causes enhanced glutamatergic responses through slow EPSPs, plateau potentials, and windup. The diagram illustrates the temporal and spatial summation of excitatory synaptic responses of postsynaptic dorsal horn and trigeminal nociceptive-transmission neurons. The critical excitatory neurochemical mediators are glutamate and substance P. SP = substance P; P2X = purinoceptor 2X; IP_3 = inositol triphosphate. Adapted from Woolf and Salter.[5]

nociceptive-transmission neurons have a large excitatory subliminal fringe, the increased gain also results in the unmasking of subthreshold inputs, causing the neurons to become sensitized to stimuli in the periphery.[1] Thus, not only are the responses of individual neurons amplified, but the number of nociceptive-transmitting neurons activated by a given peripheral input is increased. The presumed clinical implication of this enhanced transmission is the development of pain hypersensitivity.[5,10,11]

Central sensitization is initiated and sustained over the short term (ie, seconds to hours) primarily through posttranslational alterations in the function of the complement of gene products already expressed by nociceptive-transmission neurons. However, the signaling cascades activated by nociceptor inputs also produce changes that maintain the increase in gain in nociceptive pathways over a longer period of time through altering expression of a repertoire of genes, which changes the phenotype of central transmission neurons[5] and may even disrupt or kill inhibitory neurons.[12]

Persistent Enhancement of Excitatory Synaptic Transmission

A conceptually simple way to sensitize central nociceptive-transmission neurons is to increase the synaptic efficacy at the excitatory primary afferent synapses onto these neurons. In numerous studies, primary afferent-evoked responses of nociceptive spinal dorsal horn and subnucleus caudalis neurons have been shown to be enhanced by a wide variety of conditioning stimuli.[10,13,14] Whether these represent enhanced efficacy at primary-afferent-to-second-order synapses is unclear, because often the neurons studied receive long-latency monosynaptic responses that overlap temporally with polysynaptic responses, or the responses are evoked by stimuli producing asynchronous discharges of primary

afferents, and the requisite timing information is lost. Nevertheless, from the most rigorous studies of monosynaptic responses, which have by necessity been carried out on superficial dorsal horn neurons, it is clear that brief-duration, high-frequency primary afferent stimulation may induce potentiation of AMPA receptor- mediated responses at synapses onto second-order neurons.[15,16] The potentiation is prevented by pharmacologic blockade of NMDA receptors and persists for as long as experimentally observable, up to many hours.

Lasting enhancement of excitatory synaptic responses at primary-afferent-to-second-order synapses in nociceptive pathways shares a common signaling cascade with the so-called NMDA receptor-dependent form of long-term potentiation (LTP) of excitatory synaptic transmission that is observed in many regions of the CNS,[17,18] including the trigeminal system.[10,13] The mechanisms of NMDA receptor-dependent LTP that have been examined in the most detail are Schaffer collateral synapses onto CA1 neurons in the hippocampus, where a core-signaling cascade for initiating LTP has been proposed.[19–22] This requires calcium influx through NMDA receptors during the tetanic stimulation, which is accomplished by temporal summation of EPSPs that diminishes the Mg^{2+} blockade of the channel. Enhancement of NMDA channel function by the tyrosine kinase Src is also necessary, and a coincident rise in postsynaptic sodium concentration may additionally contribute to boosting NMDA receptor activity.[22] The resultant influx of calcium sets off a cascade leading to activation of calcium/calmodulin-dependent kinase II (CAMKII) and phosphorylation of the AMPA receptor subunit protein GluR1, which causes AMPA channels to move to a high conductance state.[23] Phosphorylation of AMPA receptors may also cause increased cell-surface expression of AMPA receptors and allows conversion of "silent synapses,"[21] those lacking AMPA receptors, into active ones.

Fig 3 A model for molecular mechanisms producing central sensitization through facilitation of AMPA/kainate receptor function and/or cell-surface expression in nociceptive-transmission neurons. CAK = cell adhesion kinase. *Activated.
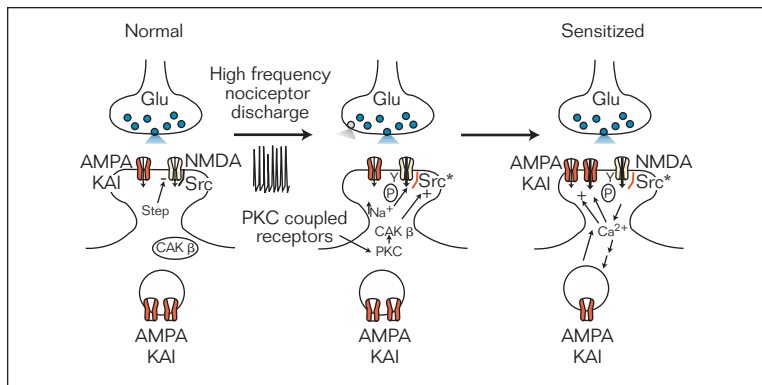
The general form of this core-signaling cascade—NMDA receptor activation leading to postsynaptic enhancement of AMPA receptor function or cell-surface expression—is likely applicable in spinal and trigeminal nociceptive transmission neurons,[10,13] as illustrated in Fig 3. In particular, there is evidence for silent synapses in dorsal horn neurons and for the conversion of these to active synapses, a process requiring PDZ domain interactions of AMPA receptors.[24,25] As in many regions of the CNS, silent synapses in the dorsal horn are most prominent at early developmental stages; there may be few if any silent synapses in the dorsal horn in the adult. Thus, in the adult dorsal horn and trigeminal brainstem nuclei, LTP may be expressed primarily by enhanced single-channel conductance of AMPA channels or enhanced cell-surface expression of AMPA receptors, although these mechanisms remain to be shown directly in the spinal dorsal horn and in the trigeminal system.

The applicability of the entire signaling cascade described in CA1 is likely limited to a subpopulation of neurons in the dorsal horn of the spinal cord and perhaps also to the trigeminal system.[10,14] The administration of exogenous CAMKII has been shown to enhance AMPA responses of dorsal horn neurons, but within the spinal dorsal horn, expression of endogenous CAMKII is restricted to a subgroup of neurons.[26,27] A protein kinase that is a candidate to substitute for CAMKII is protein kinase C (PKC); it causes potentiation of synaptic transmission in dorsal horn neurons and elsewhere. PKC has been implicated in initiating LTP in CA1 neurons, but recent evidence indicates that it is likely upstream of the protein tyrosine kinase Src and that its effects are mediated via the protein tyrosine kinase CAKβ/Pyk2.[28] In the dorsal horn, PKC could play a dual role, phosphorylating AMPA receptors and stimulating CAKβ/Pyk2-Src signaling, or alternatively, phosphorylation of AMPA receptors may be produced by an as yet unidentified serine/threonine kinase.

In the adult CNS, it appears CAKβ/Pyk2-Src signaling to NMDA receptors is normally at a low level and is offset by activity of the phosphotyrosine phosphatase known as striatal enriched phosphatase (STEP).[29] In the spinal cord, STEP has been shown to be a component of the NMDA receptor protein complex and to downregulate NMDA receptor channel activity.[29] Thus, enhancement of EPSPs in nociceptive neurons may require suppression of STEP activity as well as facilitation of CAKβ/Pyk2-Src signaling. A growing number of regulators of Src signaling to NMDA receptors have been identified,[22] and additional molecular modulators of central sensitization that utilize this signaling pathway are expected to be found in the near future.

The mitogen-activated protein kinase (MAPK, also known as ERK) pathway is another kinase signaling cascade that appears to be required for induction of LTP in CA1 neurons. MAPK is activated upon phosphorylation by mitogen-activated kinase (MEK) and inhibitors of MEK block induction of LTP.[19,30] Importantly, the early phase of LTP may be prevented by MEK inhibition too early to be accounted for by changes in gene expression known to be induced by MAPK. In the superficial spinal dorsal horn, MAPK phosphorylation increases following nociceptive stimulation.[31] Spinal inhibition of MEK suppresses the second phase of the formalin test[31] and enhances responses following peripheral inflammation.[32] Thus, it has been hypothesized that the MAPK pathway is necessary for amplification in spinal nociceptive pathways.[18]

An additional mechanism for lasting enhancement of excitatory transmission is through activation of AMPA receptors lacking the edited form of the GluR2 subunit. Such GluR2-less AMPA receptors are permeable to Ca^{2+}. This provides the potential to bypass a requirement for NMDA receptors to initiate synaptic plasticity that depends upon raising postsynaptic Ca^{2+}. Neurons expressing Ca^{2+}-permeable AMPA receptors are preferentially localized in the superficial dorsal horn. Lasting enhancement of

synaptic transmission mediated by GluR2-less AMPA receptors by a postsynaptic mechanism has been demonstrated at spinal dorsal horn synapses.[33] Such enhancement may contribute to certain types of amplification of responsiveness of central nociceptive neurons.

As well as postsynaptic mechanisms such as those described, a presynaptic increase in the release of glutamate might also result in sustained increase in the gain of nociceptive transmission. This could be produced by direct facilitation of transmitter release or by suppression of tonic presynaptic inhibition. Release of transmitter could be enhanced by stimulating receptors on primary afferent terminals, including the P2X$_3$ subtype of purinergic receptors and NMDA autoreceptors.[34] However, the effects of such receptor stimulation may be relatively short-lived. In contrast, sustained enhancement of the release of glutamate may be produced by the neurotrophin brain-derived neurotrophic factor (BDNF), which contributes to inflammatory nociceptive hypersensitivity.[35]

Lasting Suppression of Inhibition

Potentially of equal importance as sustained enhancement of excitatory transmission in spinal and trigeminal nociceptive pathways is persistent suppression of inhibitory mechanisms. One mechanism for reducing inhibition is to decrease the excitatory drive onto the inhibitory neurons. For example, long-term depression (LTD) of transmission at primary afferent synapses onto inhibitory dorsal horn neurons is elicited by activation of Aδ primary afferents.[15] The depression, which requires NMDA receptor activation and a subsequent rise in postsynaptic Ca^{2+}, is mechanistically similar to LTD in other regions, such as the hippocampus or cerebellum. The molecular basis for LTD in both hippocampus and cerebellum has emerged as clathrin-mediated endocytosis of synaptically localized AMPA receptors.[36] Thus, it is likely that LTD at primary afferent synapses onto inhibitory dorsal horn neurons is due to internalization of cell-surface AMPA receptors.

An additional mechanism for suppressing glycine/γ-aminobutyric acid (GABA) transmission in central nociceptive neurons is down-regulation of postsynaptic glycine and GABA receptors. There is recent information that glycine α3 receptors may be down-regulated by PGE$_2$, a mechanism contributing to inflammatory pain hypersensitivity.[37]

Role of Microglia in Nerve-Injury Pain Plasticity

For most of the past 100 years, the predominant theme in research in neurobiology, and specifically in pain, has been to understand the role of neurons. However, neurons are outnumbered approximately 10:1 by glial cells in the CNS. The view that these cells serve primarily "housekeeping" roles in the nervous system has changed radically in the last half decade, particularly in regard to pain resulting from peripheral nerve injury. A large and rapidly growing body of findings in the spinal dorsal horn indicates that hyperalgesia, allodynia, and ongoing pain involve the active participation of glia. Products released by activated glia, including cytokines, have been implicated by several groups in contributing directly to the pathology of nerve-injury pain.[38–40] Thus, understanding the functions of glia is an important key to understanding pain hypersensitivity following nerve injury.

Three types of glia—astrocytes, oligodendrocytes, and microglia—are present in the CNS, and microglia have emerged as a central character in nerve-injury pain. Microglia are macrophages, ie, cells of hematopoietic rather than of neural crest lineage, that are present throughout the CNS and make up 5% to 10% of the total amount of glia. Under normal circumstances, microglia are at rest, in a surveillance mode where they act as "sensors" to various stimuli that threaten physiological homeostasis. Once activated, microglia show a stereotypic program of changes in morphology, gene expression, function, and number.[41]

Activated microglia change their morphology from a resting, ramified shape into an active, amoeboid shape. They up-regulate expression of numerous cell-surface proteins, including the complement receptor 3, which is recognized by the antibody OX42. An increase in labeling for OX42, together with a characteristic change in morphology to the ameboid phenotype, are widely used as the key diagnostic markers characterizing microglia as activated. In the activated state, microglia are capable of phagocytosing pathologically damaged cells. In addition, activated microglia release chemical mediators that can act on neurons to alter their function. Various cytokines have activating effects and others, inhibitory effects. Thus, microglial activation is reversible and tightly regulated.

The role of microglial activation in neuropathic pain has become an intense area of study over the past several years.[42] Activation of microglia in the spinal dorsal horn is concomitant with the development of pain in a wide variety of nerve injury

models: spinal nerve ligation, chronic constriction injury, and dorsal rhizotomy. Many reports have shown a correlation between activation of microglia and signs of pain hypersensitivity, but only recently were microglia shown to have a causal role in these pain behaviors following nerve injury.[43,44] These studies have implicated p38 MAPK and $P2X_4$ receptors, respectively, in pathological pain behaviors resulting from spinal nerve ligation.

The $P2X_4$ receptor is a subtype of the P2X family of ligand-gated ion channels activated by adenosine triphosphate (ATP). It was found that mechanical allodynia following spinal nerve ligation is acutely reversed by intrathecal administration of a $P2X_4$ antagonist.[44] Contrary to expectations, $P2X_4$ receptors are found neither in neurons nor astrocytes, but in microglia. Expression of $P2X_4$ receptors, which is low in the naive spinal cord, progressively increases in the days following nerve injury, paralleling the development of mechanical allodynia. Moreover, inhibiting the rise in $P2X_4$ level by anti-sense RNA administered intrathecally prevents the development of allodynia. These results indicate that microglial $P2X_4$ receptors in the spinal cord are necessary for mechanical allodynia after spinal nerve injury. Moreover, in otherwise naive animals, mechanical allodynia develops progressively in the 3 to 5 hours following the intrathecal administration of microglia in which $P2X_4$ receptors have been stimulated in vitro. In contrast, unstimulated microglia do not cause allodynia, nor does the administration of ATP-only controls. The findings imply that stimulation of $P2X_4$ receptors in microglia is both necessary and sufficient for producing mechanical allodynia following nerve injury.

It was also found that inhibiting MAPK pharmacologically by means of an inhibitor administered intrathecally can reverse mechanical allodynia following spinal nerve ligation.[43] Infusion of the p38 inhibitor beginning prior to the nerve injury prevented allodynia from developing. The nerve lesion leads to persistent activation of p38 MAPK, as assessed by phospho-p38 MAPK labeling, which is entirely restricted to microglia.

Taken together, the findings from these 2 studies[43,44] lead to a model in which the activation of microglia, $P2X_4$ receptors, and p38 MAPK are central to allodynia and neuropathic pain following nerve injury. Because $P2X_4$ receptors are nonspecific cation channels that are permeable to Ca^{2+}, a likely scenario is that ATP stimulation of these receptors leads to Ca^{2+} influx, which indirectly activates p38 MAPK and possibly also other downstream signaling proteins, resulting in release of a factor or factors that enhance(s) transmission through the spinal nociceptive network. This enhanced transmission might be because of facilitation of glutamatergic synaptic transmission or through reversal of GABA/glycinergic inhibition.[45]

While such studies have yet to be made in the trigeminal system, an overall picture is emerging in which peripheral spinal nerve damage stimulates changes in the spinal cord that cause microglia to activate. The trigger by which a peripheral nerve injury initiates activation of spinal microglia has not been established. It is clear, however, that microglial activation only occurs following neuropathic injury and is not observed following peripheral inflammation. These newly activated microglia change the properties of the spinal neurons in their vicinity to bring the onset and the maintenance of nerve-injury pain. The differential microglial activation may explain why drugs that are effective as analgesics for inflammatory pain in humans are typically ineffective for neuropathic pain. Thus, understanding of the key role of microglia may lead to new strategies for the diagnosis and management of neuropathic pain, strategies not previously anticipated by a view of pain plasticity centered on neurons.

Concluding Remarks

Neurons in spinal and trigeminal nociceptive pathways show multiple forms of persistent enhancement of synaptic transmission. These forms of synaptic plasticity are mechanistically similar to persistent enhancement of excitatory synaptic transmission found in other regions of the CNS. Enhancement of excitatory responses in dorsal horn and subnucleus caudalis nociceptive neurons is 1 of the key active processes that occur centrally and peripherally, leading to an increased gain of nociceptive transmission and to pain hypersensitivity.

Acknowledgments

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