

Endogenous Facilitation: From Molecular Mechanisms to Persistent Pain

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Abstract: It is well documented that sensory transmission, including pain, is subject to endogenous inhibitory modulatory influences at dorsal horn of the spinal cord. Recent results, from behavioral to molecular studies, demonstrate that endogenous modulatory systems are bi-phasic, including inhibitory as well as new facilitatory systems. In this review, we propose the existence of endogenous facilitatory systems in the brain, and review evidence supporting the hypothesis. We believe that understanding molecular and cellular mechanisms of endogenous facilitatory systems hold the hope for better future treatment of patients with chronic pain.

INTRODUCTION

Brain activity is able to affect sensory transmission through descending modulatory systems. For many years, it has been believed that endogenous modulatory systems are mainly inhibitory, or 'analgesic'. Recent studies, however, reveal that descending modulation of spinal sensory transmission is biphasic, including inhibitory and facilitatory influences. Descending influences from supraspinal, central nuclei directly or indirectly modulate spinal sensory transmission and include the anterior cingulate cortex (ACC), amygdala, periaqueductal gray (PAG), and rostroventral medial medulla (RVM), which may function as the last relay between brain centers and the spinal cord. Biphasic modulation of spinal nociceptive transmission from the RVM, perhaps reflecting the different types of neurons identified in this area, offers fine regulation of spinal sensory thresholds and responses. Integrative approaches have been used to investigate the mechanisms for descending facilitation, including electrophysiological, pharmacological, behavioral, and biochemical studies. In this review, we will summarize data using whole animal preparation, *in vitro* spinal and brain slices, and genetically manipulated mice to support the hypothesis that the positive feedback mechanism within the synapses or between different brain regions is a key mechanism for persistent pain caused by injury (Fig. 1).

ENDOGENOUS FACILITATORY SYSTEMS

The investigation of descending facilitatory systems has been carried out systematically in the brainstem RVM. At the level of the whole animal, electrophysiological, pharmacological, and behavioral experiments have been performed to characterize facilitation of responses of spinal sensory neurons to peripheral noxious stimuli as well as behavioral responses to noxious stimuli (Zhuo and Gebhart, 1990a,b; 1991; 1992; 1997; 2002a,b; Zhuo *et al.*, 2003). Facilitation affects spinal nociceptive transmission from somatocutaneous areas as well as from visceral organs.

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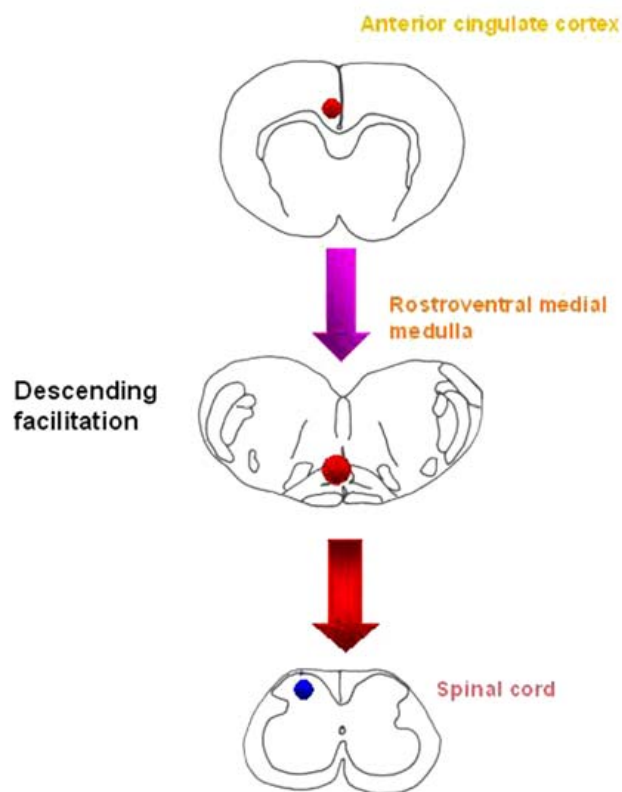


Fig. (1). Model for descending facilitatory modulation from the anterior cingulate cortex (ACC) on spinal nociceptive transmission. Neurons in the ACC and rostroventral medial medulla (RVM) project directly or indirectly to the spinal dorsal horn and modulate sensory synaptic transmission in the spinal cord. Serotonin is the most likely transmitter for mediating this facilitatory effect.

Furthermore, facilitation is a common form of modulation of sensory transmission, affecting both noxious and non-noxious inputs (Zhuo and Gebhart, 2002b). These unique features indeed raise the possibility that descending facilitation may serve as a key central mechanism to contribute to injury-related central pain or allodynia.

Biphasic Modulation

A key feature of descending facilitation is that it is intensity-dependent. Whether facilitation or inhibition is observed, in part, depends on the intensity of stimulation applied. According to effects on spinal sensory neuronal responses, we characterize sites within the brainstem into three different groups: biphasic, inhibitory and facilitatory sites (Fig. 2a, b). At biphasic sites of stimulation, it is typical that electrical stimulation facilitates spinal nociceptive transmission at lesser intensities (5-25 μ A) and inhibits responses of the same neurons at greater intensities (50-100 μ A). At inhibitory sites, electrical stimulation only reduces

and inhibits responses of spinal sensory neurons. At facilitatory sites, we found that electrical stimulation only caused increases in responses of spinal sensory neurons. To determine whether facilitatory or inhibitory effects were simply due to different groups of spinal dorsal horn neurons recorded, we also investigate the effect of electrical stimulation at one intensity, but different sites in the RVM on the same spinal neuron. We found that the responses of the same spinal sensory neurons can be either inhibited or facilitated by electrical stimulation applied to different sites in the brainstem. Thus, spinal units receive both facilitatory and inhibitory influences descending from the brainstem.

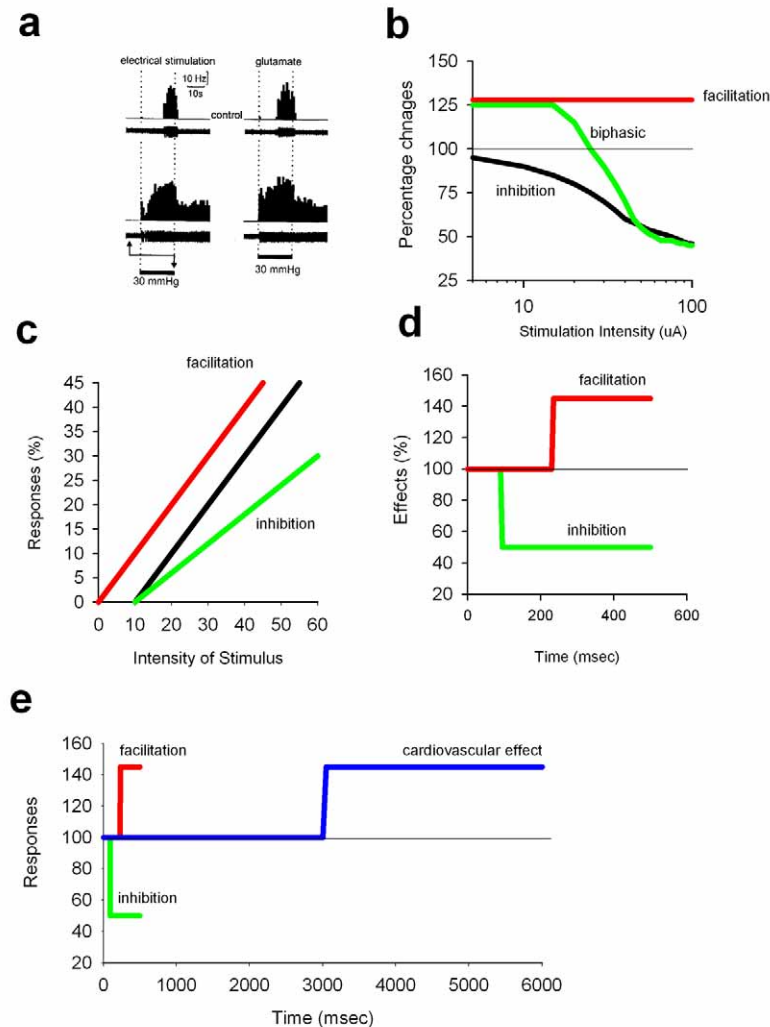


Fig. (2). Descending facilitation from the RVM.

(a) Example of descending facilitation of spinal visceral transmission produced by activation of the nucleus raphe magnus (NRM). Peristimulus time histograms (1-s binwidth) and corresponding ocillographic records in the absence (*top histograms*) and presence (*bottom histograms*) of electrical stimulation (25 μ A) and glutamate (5 nmoles) given in the same site in NRM. The intensity and duration of colorectal distension is illustrated below; the period of electrical stimulation (25 s) is indicated by the arrows.

(b) Model of intensity-dependent biphasic modulation of spinal nociceptive transmission.

(c) Model of different effects of descending facilitatory vs inhibitory modulation on the SRFs of spinal dorsal horn neurons.

(d) Model of the latency for descending facilitatory vs inhibitory effects from the RVM.

(e) Model of electrical stimulation in the RVM on the latencies of cardiovascular vs neuronal effects.

There is no clear anatomical separation between these different effects produced by stimulation in the brainstem. Biphasic effects are often produced at sites of stimulation adjacent to sites from which only inhibition is produced by similar intensities of stimulation. Further, inhibitory effects are produced at biphasic sites of stimulation adjacent to other biphasic sites from which facilitatory effects are produced. It is unlikely that effects are simply due to activation of fibers passing through the RVM because microinjection of glutamate or selective receptor agonists into the RVM also produces similar biphasic effects.

Stimulation-Response Function

For studying the effects of facilitation on stimulation-response functions (SRFs), responses of spinal neurons to graded, noxious, cutaneous or visceral stimuli were studied (Zhuo and Gebhart, 1992; 1997; 2002b; Zhuo *et al.*, 2002). In most cases, in particular within the range of noxious intensities of cutaneous or visceral stimulation, descending inhibition significantly reduces the slopes of SRFs. In contrast, descending facilitation facilitates responses and causes a parallel shift of the SRF to the left without changing its slope (see Fig. 2c). These different outcomes on the encoding properties of spinal neurons suggest that descending facilitation is likely to be mechanistically different from descending inhibition.

Latency

The latency to stimulation-produced facilitation and inhibition is determined by employing a cumulative sum technique and bin-by-bin analysis of unit responses and further supports that the mechanisms and pathways leading to inhibition and facilitation are different (Zhuo and Gebhart, 1992; 1997; 2002b; Zhuo *et al.*, 2002). Brainstem stimulation is given during a relatively stable rate of neuronal response to noxious stimulation. The first 500 ms period of recording is averaged to generate a reference baseline, and the cumulative sum of unit activity 500 ms before and 1500 ms after the onset of stimulation is plotted. The latency to effect is defined as the time from the onset of stimulation to the point when the cumulative sum of the histogram departs steadily from the reference baseline. The mean latency of stimulation-produced inhibition from the RVM is about 90 ms whereas the apparent mean latency to facilitation by electrical stimulation is greater than 200 ms (Fig. 2d). This suggests that descending facilitatory influences likely involve sites rostral to the RVM (e.g. ACC, see below).

Unmasking Effects of Descending Facilitation

Facilitatory influences are apparent only at lesser intensities of stimulation (or lesser concentrations of glutamate). In early studies, the presence of descending facilitation from the brainstem may be missed because brain stimulation intensity-dependent functions were not performed. Descending inhibitory and facilitatory influences are likely to be simultaneously activated, and prepotent inhibitory effects masked facilitatory effects. This notion has been confirmed in experiments investigating spinal pathways for descending modulation. Bilateral transections of the

dorsolateral funiculi (DLFs) in the thoracic spinal cord not only abolished descending inhibition produced by electrical or chemical stimulation in the RVM, but also unmasked descending facilitatory influences on spinal sensory neurons at the same high intensities of stimulation that only produced inhibition before the DLF transections (Zhuo and Gebhart, 1991; 1992). These findings suggest that descending inhibitory and facilitatory influences can be simultaneously engaged by activation of sites in the RVM and that removal of the route conveying the inhibitory influences uncovers descending facilitatory effects on spinal sensory neurons.

Neuronal Versus Cardiovascular Effects of Stimulation

It is well documented that brainstem sites are important for the regulation of cardiovascular functions. In biphasic sites of stimulation in the RVM, electrical stimulation (10 μ A) that facilitated responses of spinal neurons to noxious stimuli did not affect mean arterial blood pressure. At intensities of electrical stimulation that significantly inhibited responses of spinal neurons, mean arterial pressure was decreased, increased, or unaffected (Zhuo and Gebhart, 1992). The latency for electrical stimulation-produced increases or decreases in blood pressure was estimated by employing the cumulative sum technique described above. The mean apparent latency to increase blood pressure was about 4.0s. The estimated latency to decrease arterial blood pressure was about 3.0 (see Fig. 2e). The latency for cardiovascular changes is about 100 times slower than changes in spinal neuronal responses, indicating that modulation of sensory transmission in the spinal cord precedes effects on the cardiovascular system, suggesting independence of these descending systems, at least from the RVM.

Supraspinal Inputs to the RVM

Recent studies from both humans and non-human animals consistently suggest that the ACC and its related areas are important for processing pain perception. Lesions of the medial frontal cortex including the ACC significantly increased acute nociceptive responses as well as injury-related aversive memory behaviors (Lee *et al.*, 1999; Johansen *et al.*, 2000). In patients with frontal lobotomies or cingulotomies, the unpleasantness of pain is abolished (see Zhuo, 2002 for review). Electrophysiological recordings both from animals and humans demonstrate that neurons within the ACC respond to noxious stimuli and include neurons that respond only to noxious input (Sikes and Vogt, 1992; Hutchison *et al.*, 1999). Neuroimaging studies further confirm these observations and show that the ACC, together with other cortical structures, is activated by acute noxious stimuli (Rainville *et al.*, 1997; Talbot *et al.*, 1991; Casey, 1999). It has been proposed that the ACC may activate the endogenous pain modulatory system due to its projecting connections to the PAG in the midbrain. To investigate the roles of the ACC in descending modulation, we applied electrical stimulation or chemical microinjection into the ACC and found that activation of ACC neurons only produces facilitation of spinal nociceptive reflexes (Calejesan *et al.*, 2000). Furthermore, activation of glutamate mGluRs in the ACC also produced similar facilitatory effects. The descending facilitatory modulation from the

ACC is likely conveyed through the RVM because blockade of activity in the RVM or AMPA receptor antagonists given into the RVM attenuated or abolished the facilitation.

SPINAL MOLECULAR MECHANISMS

Molecular mechanisms for descending facilitation in the spinal cord are carried out using different approaches, including *in vivo* and *in vitro* experiments. For *in vivo* experiments, intrathecal application of neurotransmitter receptor antagonists is used to identify spinal transmitters/receptors that mediate the facilitatory effects (Zhuo and Gebhart, 1991). For *in vitro* experiments, the effects of a prominent spinal neurotransmitter, serotonin (5-HT), on AMPA receptor-mediated excitatory postsynaptic currents in spinal neurons have been investigated (Li and Zhuo, 1998; Li *et al.*, 1999; Wang and Zhuo, 2002). Genetic studies are also performed to examine roles of two major calcium-stimulated adenylyl cyclases subtype 1 (AC1) and subtype 8 (AC8) in spinal facilitation (Wang and Zhuo, 2002).

Stimulation-Produced Facilitation and Drug Pretreatment

5-HT-containing neurons in the RVM send descending projection fibers to targets in the spinal cord, including the superficial dorsal horn. Activation of these descending pathways can facilitate or inhibit spinal nociceptive transmission. Behavioral and pharmacological experiments have been performed to determine the spinal receptors that mediate descending modulation from the RVM. The advantage of intrathecal administration of drugs is that it allows rapid, reversible and localized blockade of neurotransmitter receptors (e.g., in the lumbosacral spinal cord). Intrathecal (i.th.) pretreatment with the non-selective 5-HT receptor antagonist methysergide completely reversed the facilitatory effect of electrical stimulation in the RVM (Zhuo and Gebhart, 1992; 1997). Interestingly, the baseline nociceptive reflex response latency as well as the mean arterial blood pressure was not affected by i.th. pretreatment with the same dose of methysergide. Neither the opioid receptor antagonist naloxone nor the cholinergic nicotinic receptor antagonist mecamylamine affected the facilitatory effect of electrical stimulation in the RVM. Intrathecal pretreatment with the cholinergic muscarinic receptor antagonist atropine or the adrenergic receptor antagonist phentolamine also failed to influence the facilitation of nociceptive reflexes produced by stimulation in the RVM.

Facilitation of Spinal Synaptic Transmission by 5-HT

It is important to show that these modulatory effects are due to changes in spinal sensory synaptic transmission, and not due to modulation of pre-motor spinal interneurons or spinal local inhibitory synapses. Consistent with the biphasic modulatory effects of 5-HT on spinal nociceptive transmission and behavioral reflexes, we found that 5-HT produced biphasic modulation of excitatory synaptic responses in spinal cord slices. 5-HT at high doses produces inhibition of AMPA/kainate receptor mediated excitatory postsynaptic currents (EPSCs), while a low dose of 5-HT or a selective 5-HT₂ receptor agonist induces facilitation of fast

EPSCs in the lumbar spinal cord (Li and Zhuo, 1998; Fig. 3a). 5-HT at low doses could facilitate fast EPSCs in the

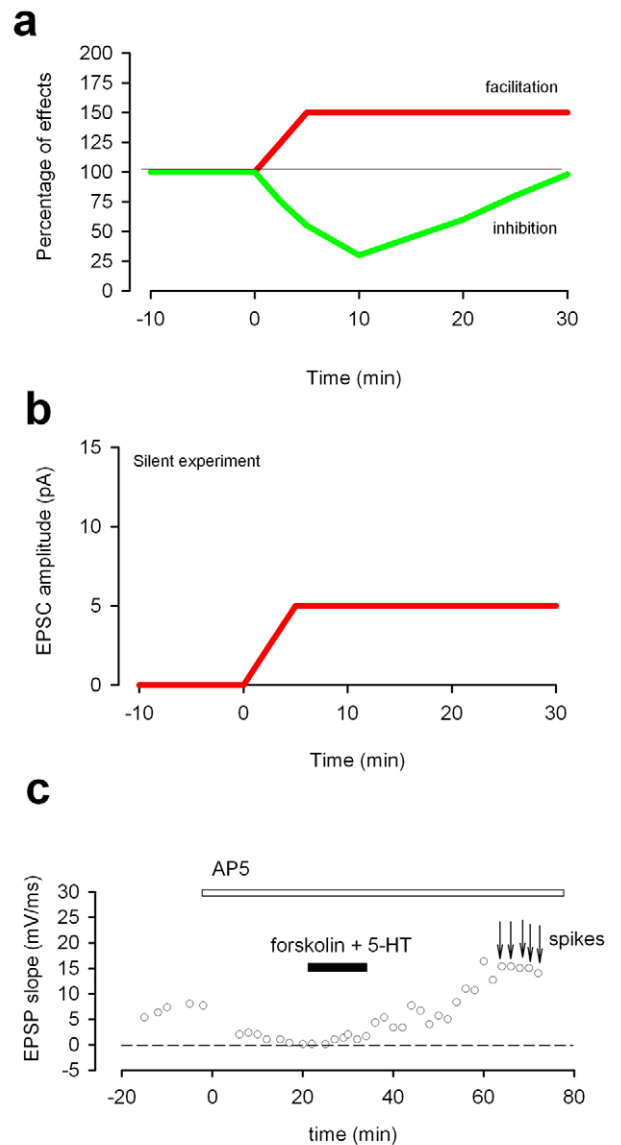


Fig. (3). Serotonin (5-HT) produces facilitation and recruitment of silent synapses in spinal dorsal horn neurons.

(a) Descending facilitation of spinal sensory synaptic transmission is long-lasting, while descending inhibition induced by different neurotransmitters are transient;

(b) One key mechanism for long-term facilitation of 5-HT is the recruitment of silent glutamatergic synapses in young spinal cord slices. Activation of 5-HT receptors changes previously silent synapses into functionally glutamatergic synapses by the insertion of AMPA receptors or the activation of previously un-functional AMPA receptors. (c) In adult spinal sensory synapses, some of sensory responses induced by stimulation of primary afferent fibers are mediated by pure NMDA receptors. Co-activation of 5-HT receptors and adenylyl cyclases (ACs) lead to the recruitment of AMPA receptor mediated responses in the continuous presence of NMDA receptor blocker AP-5. The enhancement is long-lasting, and in some cases, action potentials are induced by AMPA receptors.

5-HT may affect spinal sensory transmission by acting on presynaptic or postsynaptic receptors (Hori *et al.*, 1996; Lopez-Garcia and King, 1996; Millan, 1999). Postsynaptic application of G protein inhibitors, introduced through the recording pipette, abolishes the effect of 5-HT to facilitate synaptic transmission, suggesting that postsynaptic 5-HT receptors are critical for the effect (Li and Zhuo, 1998). In support of this notion, we found postsynaptic Ca^{2+} -dependent processes to be required for 5-HT-induced facilitation. In experiments with chelating postsynaptic Ca^{2+} with BAPTA in the pipette solution, the facilitatory effect of 5-HT was abolished, indicating that an increase in postsynaptic Ca^{2+} is required. Additional evidence against a mechanism of 5-HT-induced synaptic facilitation involving modulation of presynaptic glutamate release comes from the observation that while 5-HT application clearly caused AMPA receptor mediated EPSCs, NMDA receptor mediated EPSCs were significantly decreased by 5-HT in the same neurons (see Li *et al.*, 1999; Fig. 5). This result suggests that postsynaptic enhancement of AMPA receptor mediated currents by 5-HT are selective.

Silent Glutamate Synapses and 5-HT Produced Facilitation

Recently, using the whole-cell patch-clamp recording technique in brain slices, silent glutamatergic synapses have been documented in various regions of the CNS, including the hippocampus (Isaac *et al.*, 1995; Liao *et al.*, 1995; Durand *et al.*, 1996), neocortex (Issac *et al.*, 1997; Rumpel *et al.*, 1998) and spinal cord dorsal horn (Li and Zhuo, 1998; Bardoni *et al.*, 1998; Zhuo, 2000) and ventral horn motor neurons (Li and Zhuo, unpublished data). In silent synapses, no effective AMPA/kainate receptors are available to detect the release of glutamate from presynaptic terminals. Consequently, these synapses do not conduct any synaptic transmission at the resting membrane potential. We have established the existence of silent synapses between sensory fibers and dorsal horn neurons in the lumbar spinal cord (Li and Zhuo, 1998). We recorded from sensory neurons in the superficial dorsal horn of spinal cord slices using the whole-cell patch-clamp technique and tested for the possible existence of silent glutamatergic synapses. Fast EPSCs were induced when cells were held at -70 mV. To detect silent synapses, the intensity of stimulation was decreased so that no fast EPSC was detected at -70 mV. The holding potential was then changed to +40 mV to detect possible NMDA receptor mediated EPSCs. In about 59% of dorsal horn neurons tested, synaptic responses were found at +40 mV but not at -70 mV. Synaptic responses at +40 mV were abolished by the selective NMDA receptor antagonist AP-5 (50 μM). This sensitivity to AP-5 and voltage-dependence of channel activation indicates that synaptic responses measured at +40 mV are NMDA receptor mediated. The intensity of stimulation used in these experiments is low, and it is likely that only low-threshold afferent fibers are activated.

It is important to point out that silent synapses should not be confused with potential 'silent synaptic transmission'. The definition of 'silent synapses' is related to the condition when the postsynaptic cell is clamped at -70 mV. As defined by silent synapses, there are abundant NMDA receptors located in these 'silent' synapses. In an unclamped cell, these

NMDA receptors may contribute to sensory synaptic transmission, for example, in the case of high intensity sensory fiber activity induced by tissue injury. These results consistently suggest that different types of glutamatergic synapses exist in spinal sensory connections between primary afferent fibers and dorsal horn neurons.

Coactivation of Calcium-Stimulated Adenylyl Cyclases in Adult Sensory Neurons

Possible developmental factors have been raised as concerns in the study of silent synapses because most such experiments are performed in spinal cord slices from young animals (e.g., postnatal days 2-4 old). Recordings from adult neurons have been reported, and less or no silent synapses have been found (Baba *et al.*, 2000). To study synaptic regulation by 5-HT, we performed intracellular recordings in adult mouse spinal cord slices. We found that in sensory synapses of adult mouse, some synaptic responses (26.3 % of a total of 38 experiments) between primary afferent fibers and dorsal horn neurons were almost completely mediated by NMDA receptors (Wang and Zhuo, 2002). Dorsal root stimulation did not elicit any detectable AMPA/kainate receptor-mediated responses in these synapses. Unlike young spinal cord, 5-HT alone does not produce any long-lasting synaptic enhancement in adult spinal dorsal horn neurons.

cAMP signal pathways have been implicated in the function of spinal dorsal horn neurons. Activation of several receptors for sensory transmitters such as glutamate and calcitonin gene related peptide (CGRP) has been reported to raise cAMP levels. In slices or isolated cells from young animals, a cAMP analog enhanced glutamate receptor mediated synaptic responses (Cerne *et al.*, 1992; 1993) or had no effect on AMPA/kainate receptor mediated synaptic responses (Hori *et al.*, 1996). In a recent study, application of forskolin did not significantly affect synaptic responses induced by dorsal root stimulation in slices of adult mice. However, co-application of 5-HT and forskolin produced long-lasting facilitation of synaptic responses. Possible contributors to the increase in the cAMP levels are calcium-sensitive adenylyl cyclases (AC). We found that the facilitatory effect induced by 5-HT and forskolin was completely blocked in mice lacking AC1 or AC8, indicating that calcium-sensitive adenylyl cyclases are important. Our results demonstrate that in adult sensory synapses, cAMP signaling pathways determine whether activation of 5-HT receptors causes facilitatory or inhibitory effects on synaptic responses. Unlike synapses from young animals, 5-HT alone did not induce reliable and long-lasting facilitation of synaptic responses (see Li and Zhuo, 1998). Instead, 5-HT at the same low dose induced no effects, short-lasting increases or inhibition in neurons from adult animals. However, co-application of the same dose of 5-HT with forskolin produced significant long-lasting enhancement of synaptic responses (Fig. 3c). This finding provides a possible explanation for the regulation of two different signaling pathways under physiological or pathological conditions. Postsynaptic increases in cAMP levels by sensory transmitters may favor 5-HT-induced facilitation. The interaction between cAMP and 5-HT may provide an associative heterosynaptic form of central plasticity in the

spinal dorsal horn to allow sensory inputs from the periphery to act synergistically with central modulatory influences descending from the brainstem RVM. We think it is unlikely that cAMP acts additionally to 5-HT signal pathways. First, 5-HT at a higher dose produces opposite effects—that is, inhibition of synaptic responses—and forskolin alone did not produce any facilitation. Second, in neurons from young animals, it has been reported that PKC is required for the effects of 5-HT (Li *et al.*, 1999).

Protein-Protein Interactions and 5-HT Induced Facilitation

One possible mechanism for the recruitment of silent synapses is the interaction of glutamate AMPA receptors with proteins containing postsynaptic density-95/Discs large/zona occludens-1 (PDZ) domains. GluR2 and -3 are widely expressed in sensory neurons in the superficial dorsal horn of the spinal cord (Tachibana *et al.*, 1994; Popratiloff *et al.*, 1996; Li *et al.*, 1999). Glutamate receptor-interacting protein (GRIP), a protein with 7 PDZ domains that binds specifically to the C-terminus of GluR2/3, is also expressed in spinal dorsal horn neurons (Dong *et al.*, 1997; Li *et al.*, 1999). In many dorsal horn neurons, GluR2/3 and GRIP coexist (Li *et al.*, 1999). Long-term overexpression of the C-terminus of GluR2 in hippocampal neurons reduced the number of synaptic AMPA receptor clusters (Dong *et al.*, 1997), suggesting that an interaction between GluR2/3 and PDZ proteins is involved in the postsynaptic targeting of AMPA receptors. To examine the functional significance of GluR2/3-PDZ interactions in sensory synaptic transmission, we made a synthetic peptide corresponding to the last 10 amino acids of GluR2 (“GluR2-SVKI”: NVYGI^{ES}VKI), which disrupts binding of GluR2 to GRIP (Li *et al.*, 1999). As expected, GluR2-SVKI peptide blocked the facilitatory effect of 5-HT. The effect of GluR2-SVKI on synaptic facilitation is rather selective because baseline EPSCs and currents evoked by glutamate application did not change over time in these neurons (Li *et al.*, 1999). Experiments with different control peptides consistently indicate that the interaction between the c-terminus of GluR2/3 and GRIP/ABP (or called GRIP1 and GRIP2) (Dong *et al.*, 1999) is important for 5-HT-induced facilitation. Furthermore, synaptic facilitation induced by PDBu is also blocked by GluR2-SVKI, suggesting that synaptic facilitation mediated by PKC activation is similar to that produced by 5-HT in its dependence on GluR2/3 C-terminal interactions (Li *et al.*, 1999). A working model for the 5-HT-induced recruitment of silent synapses and spinal long-term potentiation (LTP) is shown in (Fig. 4).

DESCENDING PROJECTIONS AND CHRONIC PAIN

Neurons in the brain that contribute to descending modulatory systems are clearly important to the normal processing of nociceptive input. Peripheral noxious stimuli activate not only neurons in the spinal cord, but also neurons in the RVM and ACC. Such activation is brief, but when peripheral tissues are injured, long-lasting changes in neuronal activity can occur in these brain areas. Here, we first propose a positive feedback model, then provide evidence to support its contribution to chronic pain.

Positive Feedback Model

A *positive feedback control* is proposed to serve as the key pathophysiological mechanism for chronic pain (Fig. 5). Such positive enhancement occurs not only at single synapses, but also between multiple neuronal synapses in different parts of the brain. Several mechanisms may contribute to synaptic enhancement: (1) postsynaptic regulation of glutamate receptors, including phosphorylation and dephosphorylation of the receptor; (2) recruitment of functional glutamate receptors (for example, in spinal dorsal horn neurons, recruitment of postsynaptic functional AMPA receptors); (3) presynaptic enhancement of glutamate release; and (4) structural changes in synapses. At the level of neuronal networks, heterosynaptic facilitation or disinhibition can lead to enhancement as well. It is well documented that dorsal horn neurons receive descending facilitatory modulation from brainstem neurons. The consequence of this *positive feedback control* will lead central neurons to an enhanced and overexcited status. Accordingly, a weak input can lead to significantly greater consequences than would normally obtain from that intensity input (e.g., significantly more neuron action potentials). The enhanced excitability of central nervous system neurons has been termed “central sensitization” and most likely contributes to chronic pain states characterized by allodynia and includes central pain. Studies of mechanisms of central sensitization have primarily focused on the spinal cord and have generally excluded consideration of central nervous system contributions such as positive feedback from supraspinal sites (see Urban and Gebhart, 1999; Porreca *et al.*, 2002 for recent commentary).

ACC

What makes the ACC interesting as a contributor to positive feedback control mechanisms is that ACC neuronal activity shows plastic changes after tissue injury or amputation. Activity-dependent immediate early genes, such as *c-fos*, *Egr1* and adenosine 3',5'-monophosphate response element binding protein (CREB), are activated in cingulate and insular cortex neurons after tissue inflammation or digit amputation (Wei *et al.*, 1999; 2001). Furthermore, these plastic changes persist for a long period of time. For example, in the case of digit amputation, the pattern of expression remained altered after one week. In parallel with these dramatic changes in gene expression, synaptic plasticity both in *in vitro* and *in vivo* is also altered. In slices of the ACC taken from animals after amputation, repetitive stimulation produced less or no LTD (Wei *et al.*, 1999). That is, only potentiative, but not depressant, effects on synaptic events were found, in contrast to normal animals where both LTP and LTD can be documented. Similarly, amputation caused long-lasting enhancement of sensory synaptic responses in the ACC *in vivo* and this effect was evident either for peripheral stimulation or local stimulation (Wei and Zhuo, 2001). There are two possible implications of these studies. First, LTD in the ACC during low-frequency repetitive stimulation may serve as an auto-regulatory inhibition to maintain neuronal activity. After amputation, the loss of auto-regulation of synaptic tone may lead to overexcitation in ACC neurons. Second, synaptic potentiation induced by theta burst stimulation can also

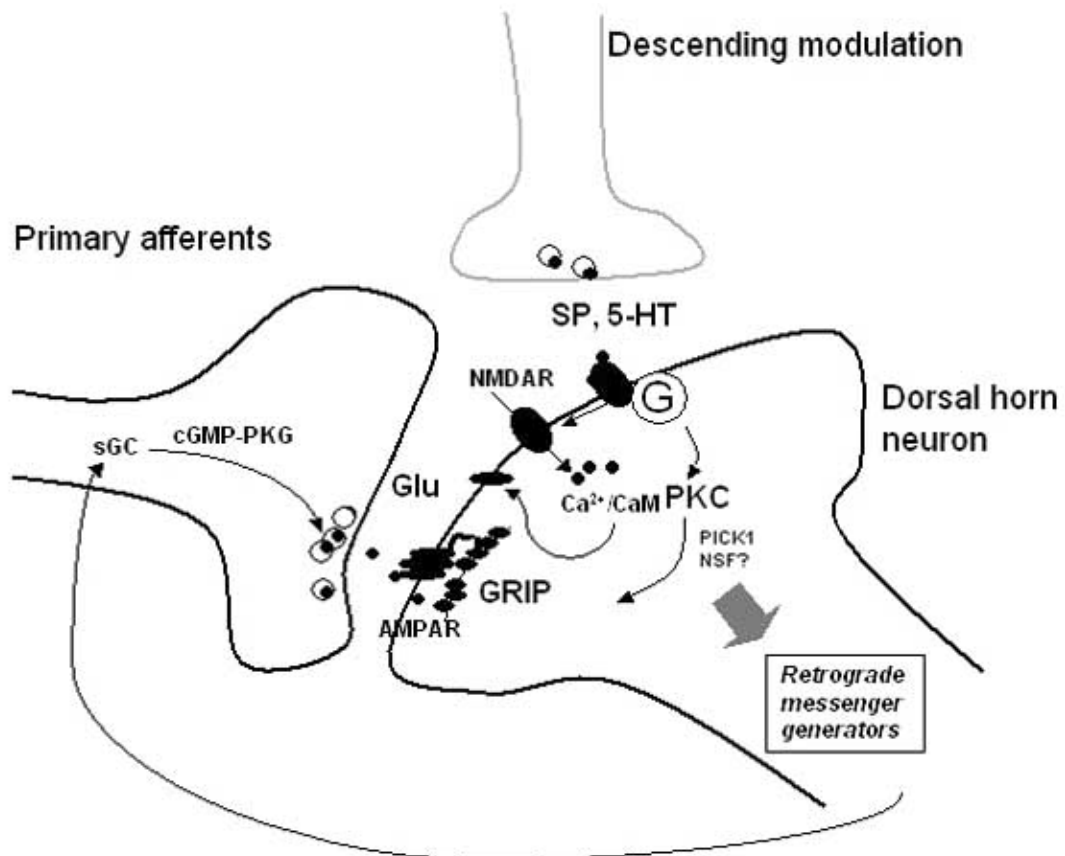


Fig. (4). Long-term facilitation in spinal dorsal horn neurons.

Peripheral tissue injury activates nociceptors and causes the release of glutamate (filled circles) as well as substance P, CGRP and other transmitters (not shown) from the central terminals in the spinal dorsal horn. Activation of glutamate NMDA receptors leads to an increase in postsynaptic Ca^{2+} in dendritic spines. Ca^{2+} serves as an important intracellular signal for triggering a series of biochemical events that contribute to the expression of spinal LTP. Ca^{2+} binds to CaM and leads to activation of various calcium-dependent enzymes and release of potential diffusible retrograde messengers, such as nitric oxide, carbon monoxide, arachidonic acid, platelet-activating factor and neurotrophins. Retrograde messengers will enhance transmitter release from presynaptic terminals by regulating: presynaptic ion channels and/or synaptic vesicle release and/or recycling pathways. Activation of protein kinases and protein phosphatases regulate phosphorylation and dephosphorylation of different target proteins to enhance postsynaptic excitability. Several G protein coupled receptors can also contribute to the regulation of spinal synaptic transmission.

enhance synaptic transmission in the ACC. Considering the important role of the ACC in pain, enhanced neuronal excitability can directly contribute to altered pain states.

In order to investigate molecular and cellular mechanisms of pain-related plasticity in the ACC, we used genetic approaches together with integrative neuroscience techniques to investigate synaptic mechanisms in the ACC. First, we tested whether persistent pain may be enhanced when NMDA receptor function, a key mechanism for triggering central plasticity in the brain (Zhuo, 2002), is genetically increased. Functional NMDA receptors contain heteromeric combinations of the NR1 subunit plus one or more of NR2A-D subunits. While NR1 shows a widespread distribution in the brains, NR2 subunits exhibit regional distribution. In humans and rodents, NR2A and NR2B

subunits predominate in forebrain structures. NR2A and NR2B subunits confer distinct properties to NMDA receptors; heteromers containing NR1 plus NR2B mediate an inward current that decays three to four times more slowly than receptors composed of NR1 plus NR2A. Unlike other ionotropic channels, NMDA receptors are 5-10 times more permeable to calcium, a critical intracellular signaling molecule, than to Na^{+} or K^{+} . NMDA receptor mediated currents are long lasting compared with the rapidly desensitizing kinetics of AMPA and kainate receptor channels. In transgenic mice with forebrain-targeted NR2B overexpression, the normal developmental change in NMDA receptor kinetics was reversed (Tang *et al.*, 1999). NR2B subunit expression was observed extensively throughout the cerebral cortex, striatum, amygdala, and hippocampus, but not in the thalamus, brainstem, or cerebellum. In both the

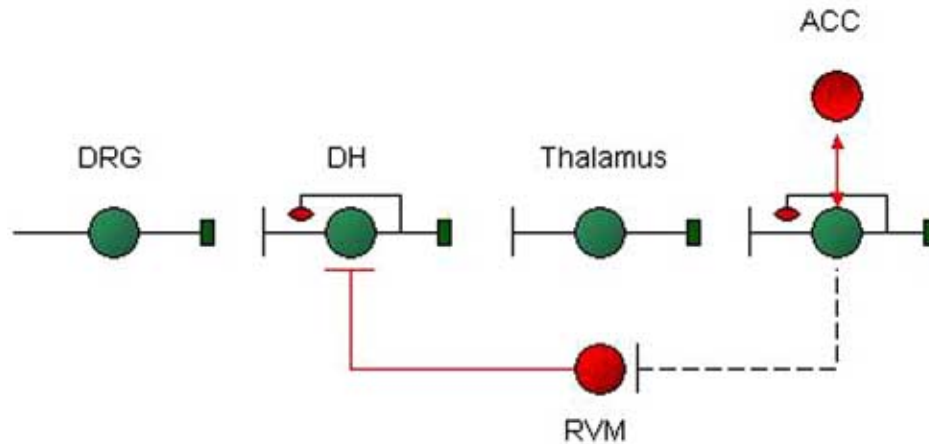


Fig. (5). Positive feedback control as a key central mechanism for persistent pain or central pain.

Sensory inputs enter the brain through three major synaptic relays, including the dorsal horn (DH), thalamus and anterior cingulate cortex (ACC). At each synaptic relay, glutamate is the major fast excitatory transmitter. Long-lasting potentiation is likely to occur at each sensory synapse. In addition, dorsal horn sensory synapses receive heterosynaptic facilitatory modulation from supraspinal structures, including the forebrain and brainstem. The RVM in the brainstem is likely to serve as a final relay for this descending facilitatory modulation. Both homosynaptic and heterosynaptic enhancement will lead central sensory neurons to an enhanced excitatory status, so that a gentle trigger or stimulation can cause massive firing of action potentials and thus cause pain. In the case of central pain, spontaneous activity of neurons in the network itself can also lead to action potential firing and pain.

ACC and insular cortex, NR2B expression was significantly increased, and NMDA receptor mediated responses were enhanced (Wei *et al.*, 2001). NMDA receptor mediated responses in the spinal cord, however, were not affected. NR2B transgenic and wild-type mice were indistinguishable in tests of acute nociception, but NR2B transgenic mice exhibited enhanced behavioral responses after peripheral injection of formalin. Late phase nociceptive responses to formalin but not the early responses were enhanced. Furthermore, mechanical allodynia assessed in the complete Freund's adjuvant (CFA) model was significantly enhanced in NR2B transgenic mice. These findings provided the first genetic evidence that forebrain NMDA receptors play a critical role in chronic pain.

We also wanted to know whether inhibition of NMDA receptor dependent, calcium-stimulated signaling pathways in the ACC may help to reduce chronic pain while keeping acute pain sensation intact (that is critical for animal or human self-protection). AC1 and AC8, the two major CaM-stimulated ACs in the brain, couple NMDA receptor activation to cAMP signaling pathways. In the ACC, strong and homogeneous patterns of AC1 and AC8 expressions were observed in all cell layers (Wei *et al.*, 2002). Behavioral studies found that wild type, AC1, AC8 or AC1plus AC8 double knockout mice were indistinguishable in tests of acute pain, including the tail-flick test, hot-plate test and the withdrawal responses to mechanical stimulation. However, behavioral responses to peripheral injection of two inflammatory stimuli, formalin and CFA, were reduced in AC1 and AC8 single knockout mice. Deletion of both AC1 and AC8 in double knockout mice produced greater

reduction in persistent pain (Wei *et al.*, 2002). More importantly, microinjection of an AC activator, forskolin, rescued defects in chronic pain in AC1 and AC8 double knockout mice. It has been consistently shown that pharmacological interventions targeting NMDA receptors or cAMP signaling pathways in the ACC also produce inhibitory effects on persistent pain in normal or wild-type animals, supporting the role of the ACC in persistent pain. Microinjection of NMDA receptor antagonists or cAMP-dependent protein kinase (PKA) inhibitors reduced or blocked mechanical allodynia related to inflammation (Wei *et al.*, 2002).

RVM

RVM neurons have been classified by Fields and colleagues into three physiological classes (Fields *et al.*, 1999). Among them, the lack of response of cells termed "neutral" to acute noxious stimulation and to morphine suggested that this cell class might not play an important role in acute nociception or opioid-dependent antinociception. Our recent results, however, suggest that NEUTRAL cells may participate in the response to prolonged nociceptive stimulation or inflammation (Robinson *et al.*, 2002).

Considerable evidence reveals a significant contribution of supraspinal influences to development and maintenance of hyperalgesia (see Urban and Gebhart, 1999; Porreca *et al.*, 2002 for recent reviews). For example, spinal cord transection, which removes descending modulatory influences on the spinal cord, prevents development of secondary, but not primary mechanical and/or thermal

hyperalgesia after topical mustard oil application, carrageenan inflammation or nerve-root ligation. In all of these models, hypersensitivity to stimuli applied to uninjured tissue adjacent to or at some distance from the site of tissue injury either does not develop or is not maintained. Similarly, inactivation of the RVM attenuates hyperalgesia and central sensitization in several models of persistent pain. As in the spinal cord, inhibition of medullary NMDA receptors or generation of one of its downstream mediators, NO, attenuates both somatic and visceral hyperalgesia. In support, topical mustard oil application or colonic inflammation increases expression of NO synthase in the RVM. These data suggest a significant role for the RVM in mediating the sensitization of spinal neurons and development of secondary hyperalgesia. Results to date suggest that peripheral injury and persistent input engage spinobulbospinal mechanisms that may be important contributors to some chronic pain states. Thus, like the ACC, the RVM is an important contributor, and may be the final common pathway, in positive feedback control of spinal nociceptive processing.

FUTURE RESEARCH DIRECTIONS RELATED TO ENDOGENOUS DESCENDING FACILITATION SYSTEM

Understanding molecular and cellular mechanisms for central changes in various pain-related states holds hope for improved understanding and thus treatment of chronic pain. From the basic science point of view, it is important to understand molecular and cellular mechanisms for long-term plastic changes in the ACC, RVM and spinal dorsal horn after peripheral tissue insult. At the level of neuronal circuits or networks, it is necessary to understand how ascending projection fibers and descending projection fibers affect local neuronal activity and contribute to neuronal plasticity. Such information will provide clues for testing new drug targets – for example, blocking descending facilitatory influences at different levels of the central nervous system (e.g., the ACC and RVM). It is clear that improved understanding of endogenous facilitatory systems provide not only knowledge about basic physiological mechanisms related to sensory transmission, modulation and neuron plasticity, but also knowledge that can lead to improved management of persistent and chronic pain states in patients.

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ABBREVIATIONS

Glu	=	Glutamate
mGluRs	=	Metabotropic glutamate receptors
AMPARs	=	-Amino-3-hydroxy-5-methyl-4-isoxalepropionate receptors
NMDARs	=	N-methyl-D-aspartate receptors
SP	=	Substance P

5-HT	=	Serotonin
GRIP	=	Glutamate receptor interacting protein
sGC	=	Souble guanylyl cyclase
PKG	=	cGMP-dependent protein kinase
PICK1	=	Protein interacting with C kinase

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