REVIEWARTICLE

CANADIAN ASSOCIATION OF NEUROSCIENCE REVIEW: Cellular and Synaptic Insights into Physiological and Pathological Pain

EJLB-CIHR Michael Smith Chair in Neurosciences and Mental Health Lecture

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ABSTRACT: Neurons and synapses in the central nervous system are plastic, undergoing long-term changes throughout life. Studies of molecular and cellular mechanisms of such changes not only provide important insight into how we learn and store new knowledge in our brains, but they also reveal the mechanisms of pathological changes that occur following injury. The author proposes that during induction, neuronal mechanisms underlying physiological functions, such as learning and memory, may share some common signaling molecules with abnormal or injury-related changes in the brain. Distinct synaptic and neuronal network mechanisms are involved in pathological pain as compared to cognitive learning and memory. Nociceptive information is transmitted and regulated at different levels of the brain, from the spinal cord to the forebrain. Furthermore, N-methyl-D-aspartate receptor-dependent and calcium-calmodulin activated adenylyl cyclases (AC1 and AC8) in the anterior cingulate cortex play important roles in the induction and expression of persistent inflammatory and neuropathic pain. Neuronal activity in the anterior cingulate cortex can also influence nociceptive transmission in the dorsal horn of the spinal cord by activating the endogenous facilitatory system. Our results provide important synaptic and molecular insights into physiological responses to injury.

RÉSUMÉ: Considérations sur le rôle des cellules et des synapses dans la douleur physiologique et pathologique. Les neurones et les synapses du système nerveux central sont plastiques et subissent des changements à long terme pendant toute la vie. Des études sur les mécanismes moléculaires et cellulaires impliqués dans ces changements fournissent des informations importantes sur les mécanismes d'apprentissage et de storage de nouvelles connaissances ainsi que sur les mécanismes impliqués dans les changements pathologiques suite à une lésion. L'auteur propose que pendant l'induction, des molécules qui servent à la signalisation et qui sont impliquées dans les mécanismes neuronaux sous-jacents aux fonctions physiologiques comme l'apprentissage et la mémoire, sont également impliquées dans les changements observés suite à une lésion cérébrale. Les mécanismes au niveau des réseaux synaptiques et neuronaux sont différents dans la douleur pathologique et dans l'apprentissage cognitif et la mémoire. L'information nociceptive est transmise et régulée à différents niveaux du cerveau, de la moelle épinière au prosencéphale. De plus, l'adényl-cyclase dépendante du récepteur N-méthyl-D-aspartate et l'adényl-cyclase activée par le couple calcium/calmodulin (AC1 et AC8) dans le cortex cingulaire antérieur jouent des rôles importants dans l'induction et l'expression de la douleur persistante d'étiologie inflammatoire et névropathique. L'activité neuronale dans le cortex cingulaire antérieur peut aussi influencer la transmission nociceptive dans la corne postérieure de la moelle épinière en activant le système facilitateur endogène. Nos résultats contribuent à la compréhension des mécanismes synaptiques et moléculaires impliqués dans la réponse physiologique à une lésion.

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Pain is the unpleasant experience or sensation induced by a noxious stimulus. The nociceptive information enters the brain through the spinal-brain projecting system and it continues to project widely to different brain areas. Most of all, painful inputs enter the forebrain areas, including the anterior cingulate cortex (ACC) and insular cortex, and trigger an unpleasant sensation or experience. Painful inputs that project to the somatosensory

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cortex determine the location and quality of painful stimuli. The hippocampus, a structure known to be important in spatial memory, is also activated by painful stimuli and may contribute to the formation of pain-related spatial memory. Neuronal inputs to the amygdala and its related structures play an important role in the formation of fear memory and pain-emotional responses. Furthermore, nociceptive inputs activate endogenous analgesic systems including neurons in the periaqueductal gray (PAG) and brainstem rostral ventromedial medulla (RVM). Activation of the endogenous analgesic system excites the descending inhibitory systems and modulates sensory transmissions at the level of the spinal cord, as well as supraspinal structures. By activating descending inhibitory systems, painful information entering the central nervous system is significantly reduced. Thus, acute or physiological pain is bearable and it does not become chronic or pathological pain.

Previous studies of plastic changes related to pathological pain are mainly focused on the dorsal root ganglion (DRG) and spinal cord dorsal horn. However, recent studies demonstrate that central plasticity happens after injury within the ACC. There are three major reasons why the study of central cortical plasticity is important in pathological pain. First, pain or pain-related unpleasantness is encoded in the forebrain areas such as the ACC. Second, higher brain structures play important roles in mental dysfunction related to chronic pain and the long-term use of pain medicine. Finally, central activity itself may produce pain sensations and plays an important role in spontaneous pain or central pain.

Pain has been divided into two groups: physiological pain and pathological pain. Physiological pain is a very important physiological function for survival. Depending on their previous pain experience, animals and humans gain knowledge of potentially dangerous stimuli from their environment and this pain-related unpleasantness helps to form a memory of long-term avoidance for protection. Although animals have the capability to enhance their sensitivity, as well as monitor their responses to subsequent noxious stimuli, an animal's ability to distinguish pain from other sensory stimuli is intact, or at least not permanently altered. Pathological pain only occurs after injury (e.g., tissue or nerve injury) and it is not a result of the repetitive application of physiological pain.[1] After injury, long-term changes are likely to occur peripherally and centrally. Consequently, the site of injury and injury-related areas undergo long-term plastic changes, and pain sensation is significantly enhanced (hyperalgesia) or non-noxious stimuli cause pain (allodynia). It should be pointed out that allodynia is one of the major problems in pathological pain. Because allodynia is induced by non-noxious stimuli, it is most likely that central plastic changes play important roles.

Pathological pain is likely the result of long-term plastic changes along the somatosensory pathway from the periphery to the cortex. Due to the long-term plastic changes in the central regions, pain specificity is lost in the somatosensory pathway, at least from those areas where allodynia was reported. Thus, drugs developed based on physiological pain mechanisms may not be useful for treating pathological pain. Understanding pathological pain requires an understanding of the plastic changes in the somatosensory pathways, mainly the central nervous system. In this article, the current understandings of the basic synaptic mechanisms in pain transmission, regulation and plasticity are reviewed. Focus is on

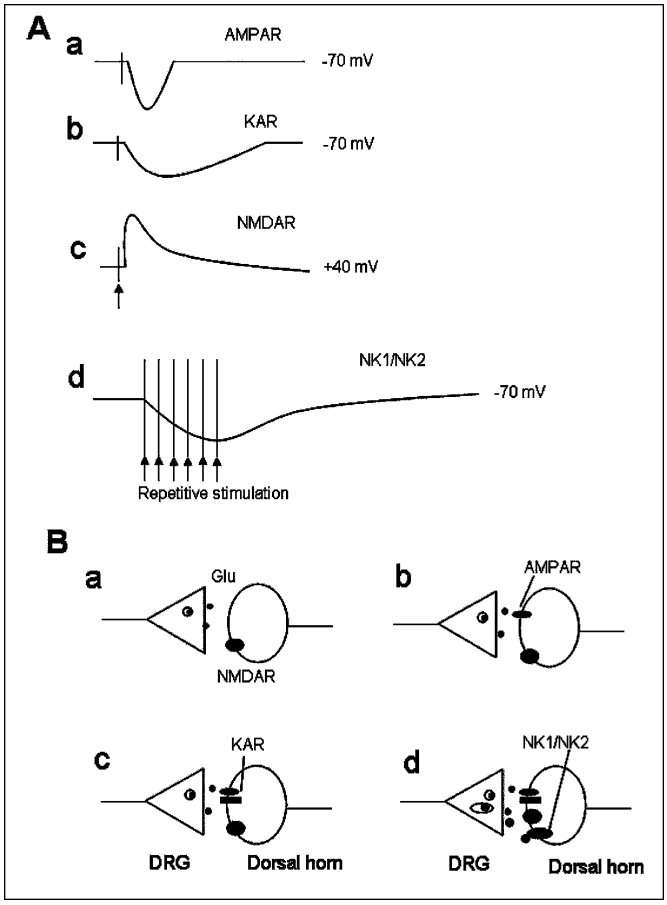

Figure 1: *Spinal sensory transmissions are mediated by glutamatergic and neuropeptidergic receptors*
(A) Synaptic currents recorded at resting membrane potentials are mostly mediated by AMPA receptors (a). Some synaptic currents at dorsal horn neurons receiving high-threshold inputs are mediated by KA receptors (b). In young and adult dorsal horn neurons, some sensory synapses are 'silent'and contain only functional NMDA receptors (c). These pure NMDAsynapses are revealed when cells are held at +40 mV, as well as at more positive potentials. In adult dorsal horn neurons, some pure NMDA receptor synapses are even detected at the resting membrane potential. When a train of stimulation is applied, neuropeptide-mediated responses are recruited. Both postsynaptic NK1 and NK2 receptors contribute to SP- and NKA-mediated excitatory postsynaptic currents (d). (B) Models for glutamate-containing and glutamate- and neuropeptide-mixed sensory synapses in the spinal cord dorsal horn. At least four different synapses are found: (a) Synapses receiving low-threshold sensory inputs contain only postsynaptic NMDA receptors; (b) Synapses receiving low-threshold sensory inputs contain both AMPA and NMDA receptors; (c) Synapses receiving both low- and high-threshold sensory inputs contain postsynaptic AMPA, KA, and NMDA receptors; (d) Synapses receiving low- and high-threshold sensory inputs contain AMPA, KA, and NMDA receptors as well as peptidergic NK1 and NK2 receptors.

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recent findings from the ACC and a model for neuronal network mechanisms for pathological pain is proposed.

Spinal dorsal horn: the beginning of a painful journey

AMPA and kainate receptor-mediated responses

Neurons in the spinal cord dorsal horn and related areas receive sensory inputs, including noxious information, and convey them to supraspinal structures.[2] Studies using pharmacological and behavioral approaches show that glutamate and neuropeptides, including substance P (SP), are excitatory transmitters for mediating pain. Electrophysiological investigations of sensory synaptic responses between primary afferent fibers and dorsal horn neurons provide evidence that glutamate is the principle fast excitatory transmitter. While α-amino-3-hydroxy-5-methyl-4-isoxazole propionate (AMPA) receptors mediate a large component of postsynaptic currents[3] (Figure 1Aa), kainate (KA) receptors preferentially contribute to synaptic responses induced by higher (noxious) stimulation intensities[4,5] (Figure 1Ab). Consistent with this, antagonism of KAand AMPA receptors yields a greater analgesic effect in adult animals than AMPA receptor antagonism alone.[5] These findings suggest that sensory modalities may be encoded, in part, by different postsynaptic neurotransmitter receptors.

Pure N-methyl-D-aspartate (NMDA) receptor-mediated responses

Silent glutamatergic synapses have been documented in the spinal cord dorsal horn.[6-8] In silent synapses, no effective AMPA/KA receptors are available to detect the release of glutamate from the presynaptic terminals. Consequently, these synapses do not conduct any synaptic transmission at the resting membrane potential. It is important to point out that silent synapses should not be confused with potential 'silent synaptic transmission'. The definition of 'silent synapse' is the condition when the postsynaptic cell is experimentally clamped at –70 mV. As defined by silent synapses, there are abundant NMDA receptors located in these 'silent' synapses (Figure 1Ac). In an unclamped cell, these NMDA receptors may conduct sensory synaptic transmissions, such as 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 the spinal sensory connection, between the primary afferent fibers and the dorsal horn neurons. These silent synapses provide a key synaptic mechanism for explaining the recruitment of ineffective synapses as measured by neuronal spikes after injury (see Wall[9]).

Possible developmental factors have been raised as concerns in the study of silent synapses because most of such experiments are performed in spinal cord slices from young animals (e.g., postnatal days 2-4). Recordings from adult neurons show that fewer silent synapses, or none at all, are found.[10] To examine the synaptic regulation by serotonin (5-HT), we performed intracellular recordings in the spinal cord slices of the adult mouse. We found that in the sensory synapses of the adult mouse, some of the synaptic responses (26.3% of a total of 38 experiments) between the primary afferent fibers and the dorsal horn neurons were almost completely mediated by NMDA receptors.[11] Dorsal root stimulation did not elicit any detectable AMPA/KA receptor-mediated responses in these synapses.

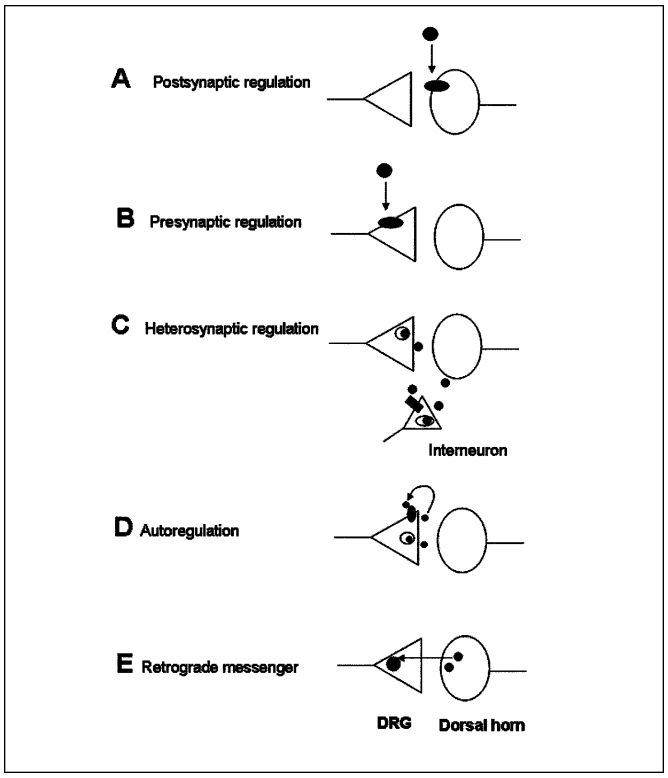

Figure 2: *Spinal sensory synaptic transmission is regulated by different mechanisms*
Models for spinal sensory transmission and modulation by postsynaptic regulation (A); presynaptic regulation (B); heterosynaptic regulation (C); autoregulation (D); and retrograde messengers (E). in case of heterosynaptic regulation, glutamate released from the central terminals of the primary afferent fibers may regulate spinal local inhibitory transmission through the activation of presynaptic KA receptors.

Neuropeptide-mediated responses

In addition to glutamate, several neuropeptides, including SP, are thought to act as sensory transmitters. For many years, there was a lack of electrophysiological evidence supporting SP-mediated monosynaptic responses because SP-mediated responses have a very slow onset. Recent studies using whole-cell patch-clamp recordings reveal relatively faster SP and neurokinin A-mediated excitatory post-synaptic currents (EPSCs) in synapses between primary afferent fibers and spinal dorsal horn neurons[12] (Figure 1Ad). Together with glutamate-mediated synaptic responses, these neuropeptide-mediated EPSCs may cause dorsal horn neurons to fire action potentials at a higher frequency for a longer period of time. The combination of glutamate- and neuropeptide-mediated EPSCs allows nociceptive information to be conveyed from the periphery to the central nervous system (Figure 1B).

Regulation of spinal sensory transmission

Although the dorsal horn of the spinal cord is often regarded as a simple relay for sensory transmission, recent studies reveal

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that synaptic transmission in the dorsal horn of the spinal cord undergoes complicated, biphasic, and activity-dependent regulation. By doing so, sensory inputs from the periphery are appropriately encoded and conveyed into the brain. Figure 2 shows models for some of major forms of synaptic regulation in the spinal cord dorsal horn.

Postsynaptic regulation: DRG-dorsal horn synapses

Neurotransmitters or neuromodulators bind to receptors located postsynaptically on spinal dorsal horn neurons. Activation of these postsynaptic receptors leads to changes in AMPA/KA receptor-mediated synaptic responses. They include acetylcholine, serotonin, opioids, norepinephrine, and oxytocin[7,13,14] (Figure 2A).

Presynaptic regulation: DRG-dorsal horn synapses

Sensory transmitters or neuromodulators bind to their target receptors on the central terminals of DRG cells in the spinal cord dorsal horn. Activation of these presynaptic receptors will lead to changes in the release of sensory transmitters in response to peripheral sensory stimulation. Many neurotransmitters and peptides, such as adenosine triphosphate, serotonin, and opioids, have been reported to produce presynaptic regulatory effects in the DRG-dorsal horn synapses[4,15-17] (Figure 2B).

Heterosynaptic regulation: DRG-spinal inhibitory neurons

In the spinal cord dorsal horn, glutamate-containing sensory fiber terminals come into close proximity with γ amino butyric acid (GABA)- and glycine-containing boutons of local interneurons at synaptic glomeruli.[18,19] In a recent study, we found evidence that glutamate released from primary afferent sensory fibers regulates spinal inhibitory transmission by activating KA receptors on the local inhibitory neurons. This data suggests that heterosynaptic regulation of transmitter release by presynaptic ligand-gated ionic channels may be reciprocal between sensory fibers and dorsal horn interneurons. Since synaptically-released glutamate suppresses evoked inhibitory transmission, inhibitory tone may be reduced, possibly facilitating the relay of sensory information to higher brain centers[20,21] (Figure 2C).

Autoregulation

In addition, neurotransmitters act on their target receptors that are also expressed on presynaptic terminals. This can either be an excitatory glutamate or inhibitory GABA synapse. In the glutamate synapse, glutamate may act on the presynaptic KA receptors expressed on the central terminals of primary afferent fibers, thereby regulating the release of glutamate.[21,22] Similar autoregulation of GABA release is also reported in the spinal cord[1,22] (Figure 2D).

Retrograde messengers

In central synapses, activation of postsynaptic receptors often leads to the production of diffusible messengers, such as nitric oxide and carbon monoxide.[23,24] In the spinal cord dorsal horn, enzymes that produce retrograde messengers are found in the dorsal horn neurons. It is very likely that diffusible retrograde messengers affect the presynaptic release of glutamate and/or neuropeptides (Figure 2E).

Long-term plasticity of spinal sensory synapses

Long-term potentiation

Studies of long-term potentiation (LTP) in spinal dorsal horn neurons draw much attention because it is believed that the potentiation of sensory responses after injury may explain chronic pain.[25-27] Consistently, it has been demonstrated that the spike responses of dorsal horn neurons to peripheral stimulation are enhanced after injury (see Willis[26]), however it remains to be investigated if enhanced spike responses are simply due to enhanced synaptic transmission between DRG cells and dorsal horn neurons. Unlike synapses in areas such as the hippocampus, synaptic potentiation in the spinal dorsal horn neurons is not induced by strong tetanic stimulation.[28] Recent studies further show that LTP only occurs in some of the spinal projecting cells.[29] In those spinal cord dorsal horn neurons that did not express SP receptors, potentiation was not observed. Furthermore, activation of neurokinin 1 (NK1) receptors or NMDA receptors is required for LTP. However, in other areas of the brain, there is no requirement of SP receptor activation for the induction of NMDA receptor dependent LTP.[29] It will be important in the future to investigate why LTPcannot be induced at neurons that do not express SP receptors.

Long-term facilitation

Spinal dorsal neurons receive innervation from descending 5-HT systems originating in the brainstem.[30,31] Application of 5-HT or 5-HT receptor agonist induced long-term facilitation of synaptic responses.[32,33] One mechanism for facilitation is the recruitment of silent synapses by interacting glutamate AMPA receptors with proteins containing postsynaptic density-95/Discs large/zona occludens-1 (PDZ) domains. Both glutamate receptor (GluR) subtype 2 and 3 are widely expressed in sensory neurons in the superficial dorsal horn of the spinal cord.[32,34,35] Glutamate receptor-interacting protein (GRIP), a protein with seven PDZ domains that binds specifically to the C-terminus of GluR2/3, is expressed in spinal dorsal horn neurons.[32,36] In many dorsal horn neurons, GluR2/3 and GRIP coexist.[32] Long-term over-expression of the C-terminus of GluR2 in hippocampal neurons reduces the number of synaptic AMPA receptor clusters,[36] 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, the author made a synthetic peptide corresponding to the last 10 amino acids of GluR2 ("GluR2-SVKI": NVYGIESVKI) that disrupts binding of GluR2 to GRIP.[32] As expected, the 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.[32] Experiments with different control peptides consistently indicate that the interaction between the c-terminus of GluR2/3 and GRIP/ABP (also known as GRIP1 and GRIP2)[37] is important for 5-HT-induced facilitation. Furthermore, synaptic facilitation induced by the Ca^{2+}-phospholipid-dependent protein kinase (PKC) activator phorbol 12,13-dibutyrate (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.[32]

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The cyclic adenosine monophosphate (cAMP) signaling 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, has been reported to raise cAMP levels. 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 ACs 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.[11] 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 cAMPand 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 (Figure 3).

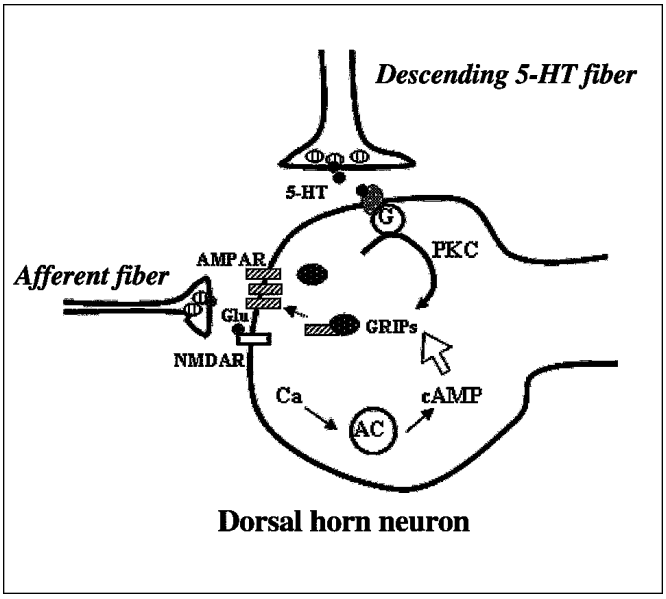

Figure 3: *Recruitment of postsynaptic AMPA receptors contribute to serotonin-induced facilitation*
Neurons in the RVM project to the spinal dorsal horn and modulate sensory synaptic transmission in the spinal cord. Serotonin is most likely the transmitter that mediates this facilitatory effect. The facilitation induced by serotonin requires the activation of specific subtypes of serotonin receptors and coactivation of cAMP signaling pathways to induce facilitation in adult spinal dorsal horn neurons. Serotonin activates postsynaptic PKC through G-protein receptors. PKC activation and subsequent AMPA receptor and GRIP interactions cause the recruitment of AMPA receptors to the synapse. Due to the enhanced synaptic efficacy between primary afferent fibers and dorsal horn neurons, spike (action potential) responses to the stimulation of afferent fibers were enhanced, as were the behavioral nociceptive responses (e.g., decrease in response latencies).

Long-term disinhibition

In addition to homo- and hetero-synaptic potentiation, a recent study reported that long-term disinhibition may contribute to persistent pain caused by nerve injury.[38] Injury triggers a trans-synaptic reduction in the expression of the potassium-chloride exporter KCC2 and as a consequence, spinal dorsal horn sensory excitability significantly increases. It is likely that such changes occur at a late phase of chronic pain and may play an important role in enhanced spinal excitability after injury.

Anterior cingulate cortex: making it painful

Animal and human studies consistently suggest that forebrain neurons play an important role in nociception and pain perception. In animal studies, lesions of the medial frontal cortex, including the ACC, significantly increased acute nociceptive responses, while formalin injection induced aversive memory behaviors.[39,40] In patients with frontal lobotomies or cingulotomies, the unpleasantness of pain is abolished (see Zhuo[41] for review). Electrophysiological recordings from the ACC neurons revealed that neurons within the ACC respond to noxious stimuli, including nociceptive specific neurons.[42,43] Neuroimaging studies further confirm these observations and show that the ACC, together with other cortical structures, are activated by acute noxious stimuli.[44-47] Thus, an understanding of synaptic mechanisms within the ACC will greatly help us gain insight into plastic changes related to central pain in the brain.

It is important to point out here that, in addition to pain, the ACC has been proposed as the neurobiological substrate for executive control of cognitive and motor processes. Human imaging studies demonstrate that the ACC region is activated by different factors including: motivational drive, reward, gain or loss, conflict-monitoring or error prediction, and attention or anticipation. The neuronal mechanisms for these different functions within the ACC remain unknown because of the limitations of human studies. These nonselective roles of the ACC further support the critical role of the ACC in chronic pain-related mental disorders. The contribution of the ACC in humans is unlikely limited to pain, but it may also include pain-related depression, drug addiction, suicide, and loss of interest.

Long-term plasticity in the ACC

Long-term potentiation

Glutamate is the major fast excitatory transmitter in the ACC.[48] Different types of glutamate receptors, including AMPA, KA, NMDAand metabotropic glutamate receptors, are found in the ACC. Fast synaptic responses induced by local stimulation or stimulation of the thalamocortical projection pathways are mediated by AMPA/KA receptors since bath application of 6-cyano-7-nitroquinoxaline-2,3-dione completely blocks fast synaptic responses. In addition to fast synaptic responses in adult ACC slices at physiological temperatures, NMDA receptor-mediated slow synaptic responses were also recorded from the

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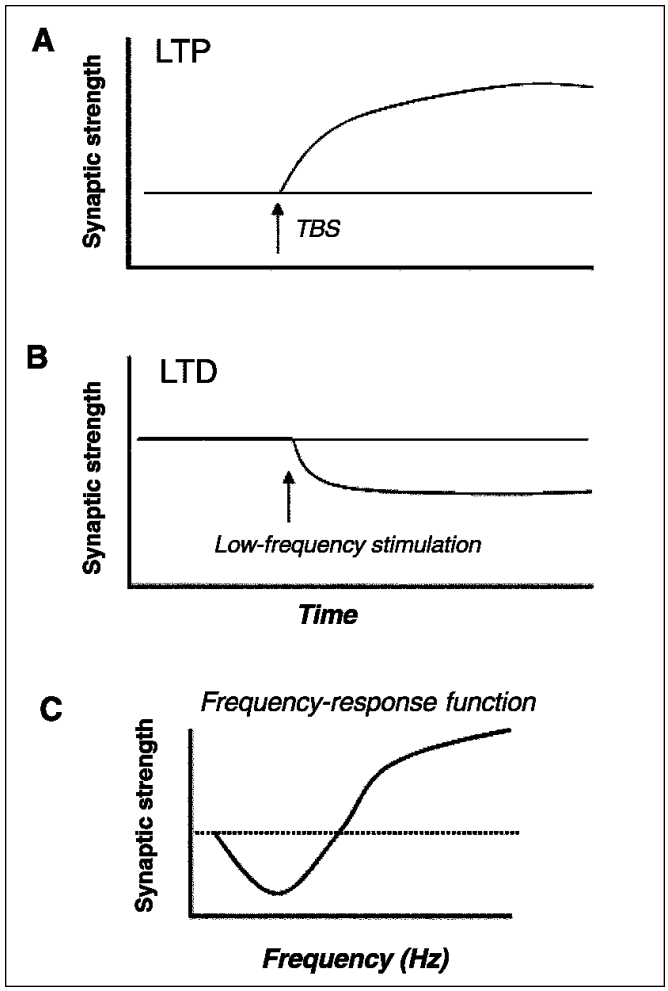

Figure 4: *Frequency-dependent synaptic plasticity in the ACC Biphasic synaptic plasticity in the ACC. Both LTP (A) and LTD (B) are observed in the ACC. In particular, injury or theta-burst stimulation (TBS) causes a long-lasting enhancement of synaptic responses. Biphasic synaptic plasticity is frequency-dependent (C) Low-frequency stimulation induces LTD, whereas theta-burst stimulation (TBS) induces LTP.*

ACC,[49] suggesting that NMDA receptors are tonically active in this region.

Glutamatergic synapses in the ACC can undergo long-lasting potentiation in response to theta-burst stimulation, a paradigm more closely related to the activity of ACC neurons. The potentiation lasted for at least 40 to 120 min[50] (Figure 4A). The cAMP signaling pathways, which are important for LTP in central synapses such as the hippocampus, are required for the induction of the LTPof the ACC. Preliminary studies using gene knockout mice and pharmacological activators/inhibitors found that calcium-stimulated AC1 and AC8 contribute to the induction of LTP in the ACC. In addition, CaM-dependent protein kinase IV (CaMKIV), another protein kinase responding to calcium-calmodulin (calcium-CaM), is also required for the induction of LTP.[50]

Long-term depression

Long-term depression (LTD) has been thought to be a reversed form of plasticity from LTP. In the ACC slices of adult rats and mice, LTD can be induced by repetitive stimulation for a long time period (15 min).[48] Prolonged, low frequency stimulation (1 Hz for 15 min) produced long-lasting depression of synaptic responses. Depression is input-specific, and unstimulated pathways remain unchanged (Figure 4B). There are several properties of LTD in the ACC that differ from those in the hippocampus. First, repetitive stimulation at 5 Hz (3 min) induces LTD in the ACC but not in the hippocampal slices. Second, unlike hippocampal LTD that requires the activation of NMDA receptors, induction of LTD requires the activation of metabotropic glutamate receptors and L-type voltage-gated calcium channels. Finally, LTD in adult ACC slices is easily detected,[48] while LTD in adult hippocampal slices is difficult to detect.

Alterations of ACC plasticityafter injury

Long-term enhancement of synaptic responses in the ACC after injury

One important question related to the ACC plasticity is whether injury causes prolonged or long-term changes during synaptic transmission in the ACC of whole animals. To test this question, we first measured synaptic responses to peripheral electrical shocks. We placed a recording electrode in the ACC of anesthetized rats.[51] At high intensities of stimulation, sufficient to activate A δ and C fibers in the periphery, evoked field excitatory postsynaptic potentials (EPSPs) were found in the ACC. The field EPSPs recorded from the ACC were obviously polysynaptic in nature, likely involving primary afferent fibers, spinothalamic tracts, and thalamocortical tracts (the estimated latency for the onset of the field EPSPs was 12.0 ± 0.1 ms). To detect central plastic changes, we amputated the contralateral hindpaw to which stimulation was delivered. Interestingly, after amputation of a central digit of the hindpaw, we observed a rapid enhancement of sensory responses to peripheral electrical shocks delivered to the normal hindpaw. The potentiation was long-lasting; evoked responses remained enhanced for at least 120 minutes.[51] To address the issue of synaptic changes occurring locally in the ACC, we measured field EPSPs to focal ACC electrical stimulation. Consistently, we observed a long-lasting potentiation of field EPSPs after amputation that lasted for at least 90 min.[51] The amount of potentiation is not significantly different from field recordings evoked by hindpaw stimulation. We hypothesize that LTP within the ACC is likely due to abnormal activity during and after amputation. One important question is whether potentiated sensory responses require persistent activity from the injured hindpaw. To test this, we locally injected a local anesthetic, known as QX-314, into the hindpaw (5%, 50 μl) at 120 min after amputation. We found that the QX-314 injection did not significantly affect synaptic potentiation induced by amputation.[51]

Loss of long-term depression

In support of plastic changes in the ACC after injury, activity-dependent immediate early genes, such as c-fos, Egr1, adenosine 3',5'-monophosphate response element binding protein (CREB),

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are activated in the ACC neurons after tissue inflammation or amputation.[48,52] Furthermore, these plastic changes persist for a long period of time, from hours to days. Studies using AC1 and AC8 double-knockout or NR2B over-expressed mice show that the NMDAreceptors, AC1 and AC8, contribute to the activation of immediate early genes by injury.[52,53] In parallel with these dramatic gene expression changes, synaptic plasticity recorded *in vitro* from ACC slices is also altered. In the ACC slices of animals with amputation, the same repetitive stimulation produced less or no LTD. The loss of LTD is regionally selective, and no change was found in other cortical areas.[48] One possible physiological mechanism for LTD in the ACC is to serve as an autoregulatory mechanism. The LTD, induced during low-frequency repetitive stimulation, maintains an appropriate neuronal activity within the ACC by reducing synaptic transmission. In amputated or injured animals, the loss of synaptic tone autoregulation leads to over-excitation in the ACC neurons and contributes to the enhancement of pain or unpleasantness related to the injury.

Genetic evidence link the ACC neurons with chronic pain

In order to investigate molecular and cellular mechanisms for pain-related plasticity in the ACC, we decided to use genetic approaches together with integrative neuroscientific techniques. First, we wanted to test if persistent pain is enhanced by genetically enhanced NMDA receptor functions, a key mechanism for triggering central plasticity in the brain.[41] Functional NMDA receptors contain heteromeric combinations of the NR1 subunit plus one or more of NR2A-D. While NR1 subunits are widely distributed in the brain, NR2 subunits are dispersed regionally. In humans and rodents, NR2A and NR2B subunits predominate in the forebrain structures. Each of the NR2A and NR2B subunits confer distinct properties to the NMDA receptors; heteromers containing NR1 plus NR2B mediate a current that decays three to four times more slowly than receptors composed of NR1 plus NR2A. Unlike other ionotropic channels, NMDAreceptors are five to ten times more permeable to calcium, a critical intracellular signaling molecule, than to sodium or potassium. These 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 over-expression, the normal developmental change in NMDA receptor kinetics (i.e., the gradual shortening of the EPSC duration of the NMDAchannel) was reversed.[54] This 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 ACC and insular cortex, NR2B expression was significantly increased, and NMDA receptor-mediated responses were enhanced.[52] The NMDA receptor-mediated responses in the spinal cord, however, were not affected. Both NR2B transgenic mice and wild-type mice were indistinguishable during tests of acute nociception, however NR2B transgenic mice exhibited enhanced behavioral responses after peripheral injection of formalin. Late phase nociceptive responses, not early phase responses, were enhanced. Furthermore, mechanical allodynia measured in the complete Freund's adjuvant model were significantly enhanced in NR2B transgenic mice. These findings are the first to provide genetic evidence of forebrain NMDA receptors playing a critical role in chronic pain.

Next, we wanted to know if inhibition of NMDA receptor-dependent and calcium-stimulated signaling pathways in the ACC reduce chronic pain, while keeping acute pain sensation intact (this is critical for animal or human self-protection). The two major CaM-stimulated adenyl cyclases (ACs) in the brain, AC1 and AC8, couple NMDA receptor activation to cAMP signaling pathways. In the ACC, strong and homogeneous patterns of AC1 and AC8 expression were observed in all cell layers.[53] Behavioral studies found that wild-type, AC1, AC8, or AC1 and AC8 double-knockout mice were indistinguishable in tests of acute pain, such as in the tail-flick test, hot-plate test, and the mechanical withdrawal response test. However, behavioral responses to peripheral injection of two inflammatory stimuli, formalin and complete Freund's adjuvant, were reduced in AC1 or AC8 single-knockout mice. Deletion of both AC1 and AC8 in AC1 and AC8 double-knockout mice produced a greater reduction in persistent pain.[53] More importantly, microinjection of the AC activator, forskolin, rescued defects in chronic pain in the AC1 and AC8 double knockout mice. Pharmacological interventions of NMDA receptors, as well as cAMP signaling pathways within the ACC, also produced inhibitory effects during persistent pain in wild-type (normal) animals, thereby 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.[53] A recent study showed that persistent pain induced by tissue inflammation or nerve injury was significantly reduced in PDZ-93 knockout mice, in part due to the lower level of NR2B expression at the spinal and cortical levels of knockout mice.[55]

Model for the molecular mechanism of injury-related potentiation in the ACC

We believe that the molecular and cellular mechanisms of central plasticity in the ACC are beginning to be revealed from pharmacological and genetic studies. Figure 5 is a proposed model based on current studies. Neural activity triggered by injuries releases the excitatory neurotransmitter glutamate in the ACC synapse. The activation of glutamate NMDA receptors leads to an increase in postsynaptic calcium in dendritic spines. Calcium is an important intracellular signal for triggering a series of biochemical events that contribute to LTP expression. Calcium binds to CaM, leading to the activation of calcium-stimulated signaling pathways.[56] Among them, calcium-CaM stimulated ACs, including AC1 and AC8 and calcium-CaM-dependent protein kinases, PKC and CaMKII. The calcium-CaM-dependent protein kinases phosphorylate glutamate AMPA receptors, thereby increasing their sensitivity to glutamate. Activation of CaMKIV, a kinase predominantly expressed in the nuclei, triggers CaMKIV-dependent CREB. In addition, activation of AC1 and AC8 leads to the activation of PKA, as well as CREB. In turn, CREB, as well as other immediate early genes, activate targets that are thought to lead to structural changes (Figure 5).

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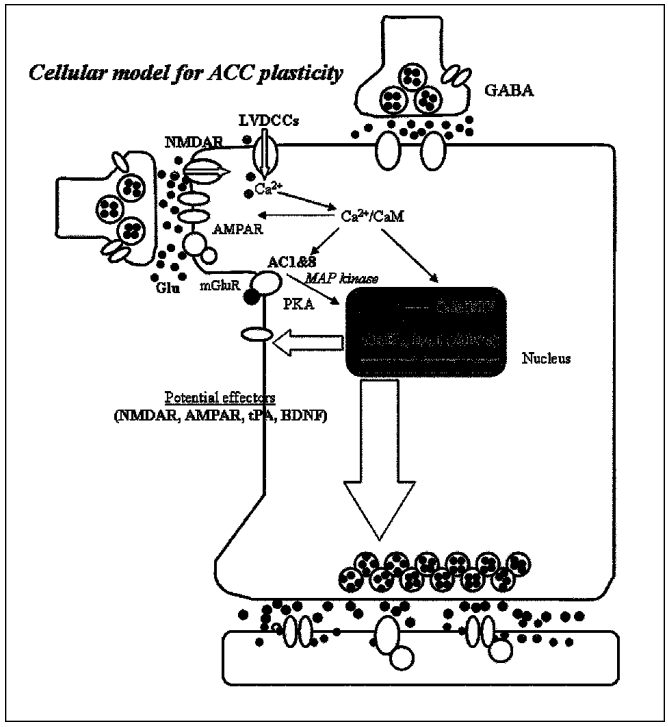

Figure 5: *Model of signaling pathways in the ACC for plastic changes related to injuries*
The neural activity triggered by injuries releases the excitatory neurotransmitter glutamate (Glu: filled circles) in the ACC synapses. Activation of glutamate NMDA receptors leads to an increase in postsynaptic calcium in dendritic spines. Calcium serves as an important intracellular signal for triggering a series of biochemical events that contribute to the expression of LTP. Calcium binds to CaM and leads to the activation of calcium-stimulated ACs, including AC1 and AC8 and calcium-CaM-dependent protein kinases (PKC, CaMKII and CaMKIV). The calcium-CaM-dependent protein kinases phosphorylate glutamate AMPA receptors, increasing their sensitivity to glutamate. The activation of CaMKIV, a kinase predominantly expressed in the nuclei, will trigger CaMKIV-dependent CREB. In addition, activation of AC1 and AC8 lead to the activation of PKA, as well as CREB. CREB as well as other immediate early genes (e.g., Egr1), in turn, activate targets that are thought to lead to more profound structural changes.

ENDOGENOUS ANALGESIC SYSTEM AND FACILITATORY SYSTEM: TOP-DOWN MODULATION

Endogenous analgesic/anti-nociceptive systems

Spinal nociceptive transmission is modulated by an endogenous antinociceptive or analgesic system, which consists of the PAG and the RVM in the midbrain[30,57-59] (Figure 6). The RVM serves as an important relay for descending influences from the PAG to the spinal cord. Activation of neurons in the RVM inhibits spinal nociceptive transmission and behavioral nociceptive reflexes. The inhibitory effect is mediated directly by descending pathways projecting bilaterally in the dorsolateral funiculi, as well as indirectly by descending activations of local spinal inhibitory neurons.[60-63] In the spinal cord, muscarinic, noradrenergic, and serotonergic receptors are important for the descending inhibition of behavioral nociceptive reflexes. Electrophysiological studies using intracellular or whole-cell patch-clamp recordings of dorsal horn neurons allow an investigation into the cellular mechanisms for the antinociceptive or analgesic effects. In anesthetized whole animals, electrical stimulation applied to sites within the nucleus raphe magnus or PAG produced inhibitory postsynaptic potentials in the dorsal horn neurons, including the ascending projection spinothalamic tract cells. A more detailed pharmacological analysis came from those studies using *in vitro* brain or spinal cord slice preparations. In the trigeminal nuclei, all of the three major transmitters (acetylcholine, serotonin, norepinephrine) are reported to inhibit glutamatergic transmission.[64,65] In the lumbar spinal cord, activation of the postsynaptic muscarinic receptors inhibits excitatory sensory transmission. Unlike carbachol, an agonist that is attenuated by postsynaptic G-protein inhibition, agonists of serotonin and α2-adrenergic receptors produce inhibitory modulatory effects through presynaptic and postsynaptic receptors, since post-synaptic G-protein blockade allowed partial attenuation.[12] These findings are consistent with anatomical evidence that both presynaptic and postsynaptic receptors are found in these sensory synapses. Future studies are clearly needed to explore the inhibitory molecular mechanisms of postsynaptic glutamate-mediated responses.

Endogenous facilitatory systems

In addition to descending inhibition, descending excitatory or facilitatory influences from the brainstem or forebrain have been characterized[60-63,66,67] (Figure 6B). Biphasic modulation of spinal

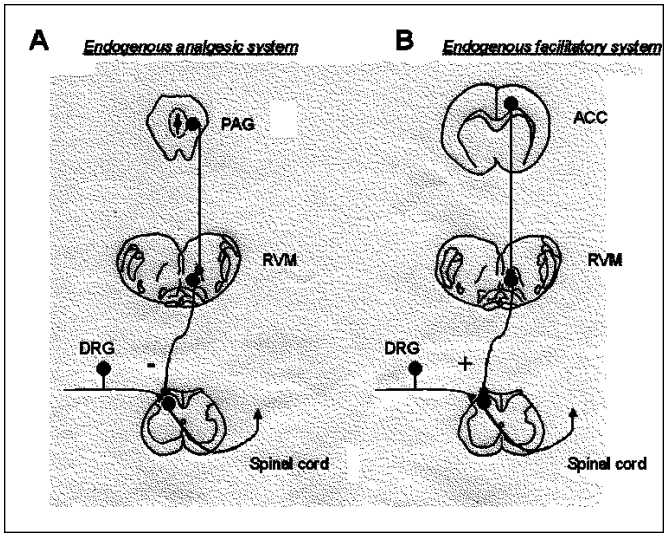

Figure 6: *Top-down descending endogenous analgesic and facilitatory systems.*
Spinal sensory transmission is under biphasic modulation by endogenous analgesic/antinociceptive systems (A) and facilitatory systems (B). These endogenous biphasic modulatory systems provide fine regulatory mechanisms to allow the brain control of the amount of sensory inputs entering the central nervous system.

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nociceptive transmission from the RVM, perhaps reflecting the different types of neurons identified in this area, offer fine regulation of spinal sensory thresholds and responses. While descending inhibition is primarily involved in regulating suprathreshold responses to noxious stimuli, descending facilitation reduces the neuronal threshold to nociceptive stimulation.[60-63,66] Descending facilitation has a general impact on spinal sensory transmission and it induces sensory inputs from cutaneous and visceral organs.[68,69] Descending facilitation can be activated under physiological conditions, and one physiological function of descending facilitation is to enhance an animal's ability to detect potential danger signals in the environment. Indeed, neurons in the RVM not only respond to noxious stimuli, but they also show 'learning'-type changes during repetitive noxious stimuli. More importantly, RVM neurons undergo plastic changes during and after tissue injury and inflammation. Descending facilitation is likely activated after injury, contributing to secondary hyperalgesia.[13,70] The blockade of descending facilitation, by a lesion of the RVM or spinal blockade of serotonin receptors, is antinociceptive.[71-73] The descending facilitatory system therefore serves as a double-edged blade in the central nervous system. On the one hand, it allows neurons in different parts of the brain to communicate with each other and enhance sensitivity to potentially dangerous signals; on the other hand, prolonged facilitation of spinal nociceptive transmission after injury speeds up central plastic changes related to chronic pain.

Conclusions and future directions

In order to gain more knowledge about nociception and pain, two extreme attitudes toward pain-related research should be avoided. First, it is important to keep in mind that many animal models can provide knowledge about the injury mechanisms, although they may not be related to pain nor possess a brain to feel or report pain. Simply pursuing or funding clinically-relevant studies or human studies may only produce superficial data without molecular insights into the mechanisms. Second, it is important for biologists to keep in mind that pain is a function of the whole central nervous system; it is not a function of the spinal cord, dorsal horn or peripheral DRG cells. Furthermore, memory-like mechanisms from memory-related regions, such as the hippocampus, may not explain plasticity in the other brain areas related to pathological pain. It is necessary and reasonable to request integrative neurobiological, neurophysiological and neuropharmacological approaches to be used for studying physiological and pathological pain-related mechanisms. The author is confident that new generations of pain therapies will be discovered which benefit human beings in the near future.

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