Glutamate receptors and persistent pain: targeting forebrain NR2B subunits

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Glutamate is the fast excitatory transmitter in mammalian brains. It binds to two major classes of glutamate receptors: ionotropic and metabotropic receptors. Ionotropic receptors contain three subtype receptors, including N-methyl-D-aspartate (NMDA) receptors. Activation of NMDA receptors is important for initiating long-lasting changes in synapses. In the forebrain structures that are known to contribute to the formation and storage of information, NMDA receptors have an important role in persistent inflammatory pain by reinforcing glutamate sensory transmission. Mice with enhanced forebrain NMDA receptor function demonstrate selective enhancement of persistent pain and allodynia. Drugs targeting NMDA NR2B subunits in the forebrain could serve as a new class of medicine for controlling persistent pain in humans.

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▼ Tissue injury often leads to pain that lasts for an extended period of time after the injury (persistent pain). Hyperalgesia and allodynia are associated with persistent pain. In hyperalgesia, the response to noxious stimuli and the intensity of pain are increased. Allodynia is a pathological state in which the nociceptive threshold is decreased and a normally non-noxious stimulus can induce pain. Both peripheral and central sensitization contributes to persistent pain (i.e. the sensitivity to subsequent stimuli is enhanced). Peripheral sensitization reflects increased sensitivity of primary afferent nociceptors, and includes lowered thresholds and an increased responsiveness of the skin. Furthermore, both during and after injury, synaptic transmission in the central nervous system (CNS) undergoes long-lasting changes. Some of these central changes are permanent, altering the brain's perception of future sensory stimuli [1,2]. Despite recent progress in dissecting the pathophysiological mechanisms of persistent pain, cellular and molecular mechanisms for the induction and maintenance of chronic pain remain unclear. An understanding of molecular and cellular mechanisms of pain transmission and modulation is essential to the development of clinical strategies aimed at alleviating chronic pain. This review presents a new genetic approach, using mouse NR2B overexpression for investigating mechanisms of persistent pain.

Spinal dorsal horn: a primary site for controlling persistent pain

Glutamate and substance P as sensory transmitters

Neurons in the spinal cord dorsal horn and related areas receive sensory inputs, including noxious stimuli, and convey them to supraspinal structures [2]. The identification of molecules that are selectively involved in pain transmission is the major research focus and holds hope for the treatment of pain, including persistent pain. Studies using pharmacological and behavioral approaches showed that glutamate and neuropeptides including substance P (SP) are probable transmitters for pain [3–5]. Electrophysiological investigation of sensory synaptic responses between primary afferent fibers and dorsal horn neurons provide evidence that glutamate is the principal fast excitatory transmitter, and synaptic responses are mediated by postsynaptic glutamate receptors [6–9]. Although α-amino-3-hydroxy-5-methyl-4-isoxazole propionate (AMPA) receptors mediate the largest component of postsynaptic currents, kainate receptors preferentially contribute to synaptic responses induced by higher (noxious) stimulation intensities [9]. Consistent with this, antagonism of both kainate and AMPA receptors yields

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greater analgesic effects in adult animals than AMPA receptor antagonism alone [9]. These findings suggest that sensory modality could be coded in part by different postsynaptic neurotransmitter receptors.

Not all sensory synapses are functional or effective in normal conditions [7,10,11]. In young animals, silent glutamatergic synapses, or synapses containing only NMDA receptor-mediated responses, were found between sensory afferent fibers and dorsal horn neurons. Conversion of such silent synapses to a functional, or activated, state contributes to enhancement of synaptic responses by serotonin, an important transmitter of descending projecting pathways [7,12]. Furthermore, pure NMDA receptors were also reported in spinal dorsal horn of adult animals [13]. These synapses are functional because of possible distal dendrite locations or insensitivity to magnesium blockade.

In addition to glutamate, several neuropeptides, including SP, are also thought to act as sensory transmitters. For many years, there has been a lack of electrophysiological evidence that SP can mediate monosynaptic responses, because SP-mediated responses had a very slow onset [14]. Recent studies using whole-cell patch-clamp recordings reveal rather fast SP and neurokinin A-mediated synaptic currents in synapses between primary afferent fibers and dorsal horn neurons [15]. Together with glutamate-mediated synaptic responses, these neuropeptide-mediated excitatory postsynaptic currents (EPSCs) might cause dorsal horn neurons to fire action potentials at a high frequency for a long period of time. The combination of glutamate- and neuropeptide-mediated EPSCs allows nociceptive information to be conveyed from the periphery to the CNS.

NMDA receptors as a synaptic enhancer

What makes glutamatergic synapses mostly unique is that they can undergo long-lasting plastic changes, lasting from hours to days. Although AMPA and kainate receptors mainly contribute to sensory transmission, NMDA receptors can mediate long-term plastic changes [16–19]. Excitatory synapses contain an electron-dense thickening in the postsynaptic membrane, known as the postsynaptic density (PSD), containing receptors and their interacting proteins, including membrane-associated guanylyl kinase (MAGUK) proteins. In mammals, the MAGUK family includes PSD-95, synapse-associated protein (SAP)97, PSD-93 and SAP102. They contain several protein–protein interaction domains, including PDZ domains, an SH3 domain and a guanylyl kinase domain. PSD-95, SAP102 and PSD-93 interact with the NMDA receptor subunits NR2A and NR2B and play a role in the clustering and synaptic targeting of NMDA receptors [20,21].

One major feature of NMDA receptors is their voltage-dependence. At resting membrane potentials, NMDA receptors are inactive because of pore blockade by extracellular Mg^{2+}, even in the presence of glutamate. Thus, to activate NMDA receptors at synapses, two events need to happen simultaneously. First, glutamate needs to be released and then bind to NMDA receptors; second, the postsynaptic membrane needs to be depolarized so that the extracellular Mg^{2+} blockage can be removed. NMDA receptor-mediated calcium influx from the extracellular space into the postsynaptic cells then activates a series of signaling molecules within postsynaptic cells, including protein kinases, protein phosphatases, immediate early genes (i.e. genes that can be activated rapidly, or third messengers), as well as enzymes producing diffusible retrograde messengers [17,22,23]. This unique property of NMDA receptors makes them the best candidates for mediators of memory formation in the brain. In learning-related central nuclei, such as the hippocampus and neocortex, synaptic potentiation or long-term potentiation, is NMDA-receptor-dependent. Inhibition of NMDA receptor function pharmacologically or genetically causes memory defects, and enhancing NMDA receptors leads to the superior performance of animals in various memory tests [24]. NMDA receptors are known to contribute to physiological learning and memory; they also get activated when abnormal activity is induced. It is well known that NMDA receptors are activated in the context of excessive neuronal activity during noxious insults or injuries [25]. NMDA receptors trigger plastic changes along sensory pathways, neuronal death in certain brain regions, as well as emotion-related memory [26].

Gate theory: a heterosynaptic form of inhibition

In the early 1960s, Melzack and Wall proposed the gate control theory, based on evidence collected from neurophysiological and anatomical approaches [27]. This form of heterosynaptic inhibition between different types of afferent sensory fibers provides a gating mechanism for regulation of spinal nociceptive transmission. Recent studies using brain slice preparations enable physiologists to investigate this mechanism in detail. It is known that glutamate serves as the major fast excitatory transmitter between sensory afferent fibers and dorsal horn neurons, including both non-nociceptive and nociceptive fibers [6,9]. Within the spinal cord dorsal horn, γ-aminobutyric acid (GABA) and glycine serve as major inhibitory transmitters [28]. Activation of non-nociceptive Aβ fibers form direct excitatory connections with spinal projecting cells, as well as inhibitory interneurons. These inhibitory interneurons, containing both GABA and glycine as their transmitters, form secondary connections with dorsal horn

cells, including projection cells (Fig. 1). Recent studies further reveal that glutamate that is released from the central terminals of afferent fibers can act on the presynaptic terminals of inhibitory neurons. Antagonists that block both postsynaptic and presynaptic kainate receptors on dorsal horn interneurons would be expected to have analgesic properties. Furthermore, sensory fibers themselves also express presynaptic kainite receptors, and activation of these receptors with exogenous agonists suppressed sensory transmission [28,29]. Because the kainate receptors on peripheral sensory neurons could be pharmacologically separated from those expressed on spinal neurons [28,29], manipulation of spinal kainate receptors with selective agonists and antagonists could represent a viable therapeutic strategy for the treatment of pain.

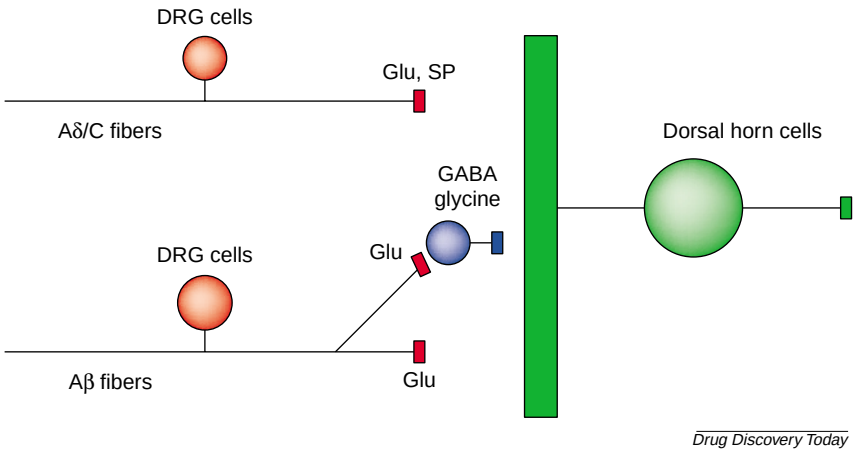

Figure 1. Heterosynaptic inhibition in the spinal dorsal horn sensory synapses. Spinal dorsal horn neurons receive sensory inputs from nociceptive Aδ/C fibers and non-nociceptive Aβ fibers. Glutamate (Glu) is the principal fast excitatory transmitter at synapses between afferent fibers and dorsal horn neurons, regardless of the type of afferent fibers. In addition to Glu, substance P (SP) and neurokinin A are also released from nociceptive afferent fibers. Large diameter, non-nociceptive Aβ fibers can also activate spinal inhibitory neurons. These inhibitory neurons then form inhibitory synapses with dorsal horn neurons as well. Activation of non-nociceptive afferent fibers could thus inhibit spinal nociceptive transmission by activating local spinal inhibitory influences. A recent study further suggests that Glu release from primary afferent fibers can also act on presynaptic kainate receptors on inhibitory terminals and modulate spinal inhibitory transmission [28].

Biphasic descending modulation

In addition to the neuronal interactions between different types of sensory afferent fibers, spinal sensory transmission, including pain transmission, receives strong descending modulation from the brain [30–34]. Spinal sensory transmission receives numerous descending innervation from central nuclei directly or indirectly, including the anterior cingulate cortex (ACC), amygdala, periaqueductal gray (PAG) and brainstem. Among them, a major descending pathway consists of the PAG-rostroventral medulla (RVM) and spinal cord connections. Many other central nuclei interact with this so-called endogenous analgesia system and produce antinociceptive or analgesic effects. As the last step of relay nuclei, neurons in several nuclei in the brainstem have important roles in descending inhibition of spinal sensory transmission. In addition to descending inhibition, descending excitatory or facilitatory influences from the brainstem or forebrains have also been characterized [35–39] (see Fig. 2 and Box 1). Biphasic modulation of spinal nociceptive transmission from the RVM, perhaps reflecting the different types of neurons identified

Table 1. Descending facilitation and inhibition of spinal nociceptive transmission

	Descending inhibition	Descending facilitation
Central origins	NGC, NGCα, NRM	NGC, NGCα, NRM, ACC
Spinal pathways	Dosolateral funiculi (DLF)	Ventrolateral and ventral funiculi (VLF/VF)
Descending projection	Bilateral	Bilateral
Stimulation intensity	High (50–100 μA)	Low (5–25 μA)
Spinal transmitters	Ach, NE, 5-HT	5-HT
Synaptic modulation	Pre- and post-synaptic modifications	Postsynaptic 'silent' glutamatergic synapses
Stimulation-response function	Peak response	Threshold
Latency for dorsal horn responses	~80 msec	~232 msec
Latency for cardiovascular responses	Mixed, 5 sec	Mixed, 5 sec

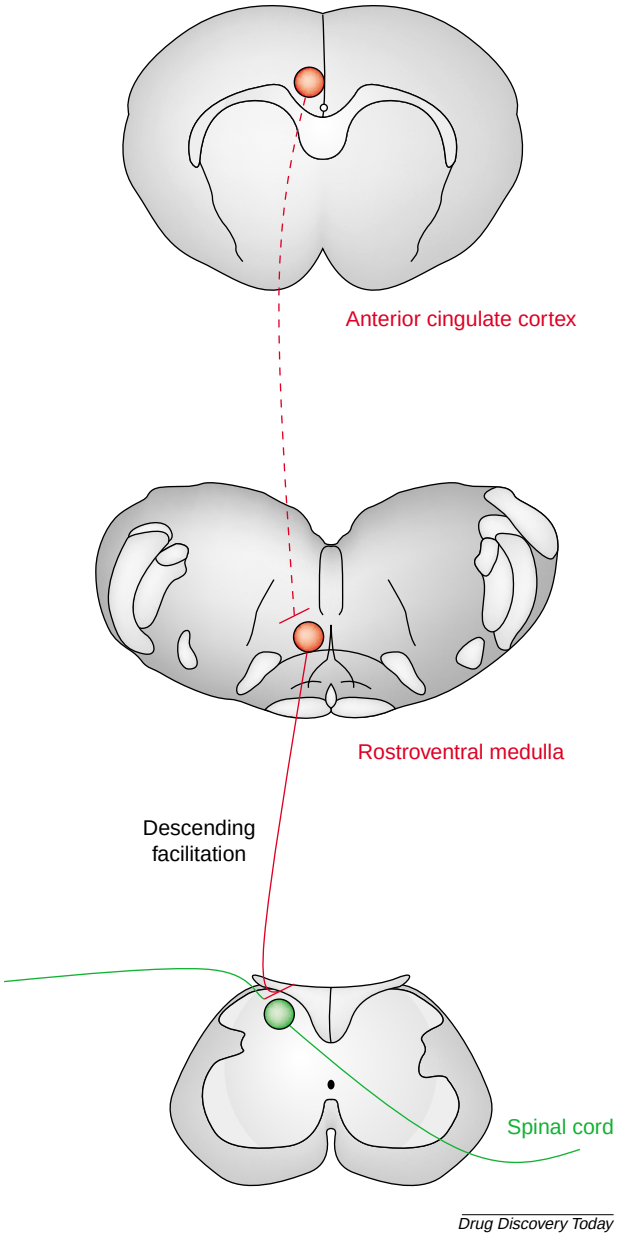

Figure 2. Model for descending facilitatory modulation from supraspinal structures on spinal nociceptive transmission. Neurons in the rostroventral medulla (RVM) project to the spinal dorsal horn (DH) and modulate sensory synaptic transmission in the spinal cord. Serotonin is the most likely transmitter for mediating this facilitatory effect. The facilitation induced by serotonin likely requires activation of specific subtypes of serotonin receptors and coactivation of cAMP signaling pathways to induce facilitation in adult spinal DH neurons [13]. Because of enhanced synaptic efficacy between primary afferent fibers and DH neurons, spike (action potential) responses to stimulation of afferent fibers were enhanced, as were behavioral nociceptive responses (e.g. decrease in response latencies). Stimulation of neurons in the anterior cingulate cortex (ACC) also activated descending facilitation, and activity within the RVM is required for mediating descending facilitation from the ACC to the spinal DH [35].

in this area, offers fine regulation of spinal sensory thresholds and responses. Although descending inhibition is primarily involved in regulating suprathreshold responses to noxious stimuli, descending facilitation reduces the neuronal threshold to nociceptive stimulation (Table 1).

Pain medicine targeted at spinal cord sensory transmission

Because neurons in the dorsal horn directly or indirectly convey nociceptive information from periphery to the brain, drugs that interfere or inhibit such transmission are potentially analgesic. Based on sites of action, there are at least four major classes of drugs: (1) drugs that directly inhibit sensory transmitter-mediated transmission, (2) drugs that block central plasticity, (3) drugs that mimick descending inhibitory modulation, and (4) drugs that affect spinal heterosynaptic inhibition of excitatory transmission [40] (Table 2). Animal models of acute pain and persistent pain are of great help in evaluating potential analgesic properties of different chemicals. For acute pain, commonly used testing models include the tail-flick (TF) reflex, the hot-plate (HP) test, mechanical withdrawal and the cold-plate (CP) test. For persistent inflammatory pain, the formalin test and the complete Freund's adjuvant (CFA) model were used. For neuropathic pain and amputation, injury or total dissection of nerves has been reported [40].

For the first class of compounds, antagonists for different subtypes of glutamate receptors have been reported to be analgesic. These include AMPA receptor antagonists, kainate receptor antagonists, as well as mixed AMPA and kainate receptor antagonists. The efficacy of these drugs in controlling pain might be enhanced during persistent pain. Increases in postsynaptic AMPA and possibly kainate receptor-mediated responses have been suggested in persistent pain conditions. In terms of allodynia, it is quite unlikely that transmitters released selectively during high-intensity stimulation will have important roles here. Instead, glutamate, which is released upon gentle, non-noxious stimulation, has a crucial role [2].

NMDA receptor antagonists were reported to be analgesic through the blockade of NMDA receptor-mediated plasticity. Because of the potentially long-lasting changes in synapses after an injury, NMDA receptors become more excitable or tonically active. This explains why NMDA receptor antagonists are effective in the treatment of chronic pain. Because glutamate is a common transmitter in many regions of the brain, it is likely that drugs targeted at these receptors will have some side effects on other physiological functions, including the ability to learn and memory.

For the third group of drugs, spinal pain transmission receives descending inhibitory modulation from supraspinal

Box 1. Descending facilitatory system: a novel modulatory system

It is well documented that spinal nociceptive transmission receives descending inhibitory modulation from supraspinal structures. Recent studies indicate that there is also descending facilitatory modulation of supraspinal structures, including the rostroventral medulla (RVM) and anterior cingulate cortex (ACC) [a]. In the ACC, electrical and/or chemical stimulation induce only facilitation of spinal nociceptive reflexes. In the RVM, electrical or chemical stimulation of neurons in several nuclei, such as the nuclei reticularis gigantocellularis (NGC), gigantocellularis pars alpha (NGCα) and raphe magnus (NRM), facilitated the responses of spinal dorsal horn neurons to peripheral stimuli as well as spinal nociceptive tail-flick reflex [b,c]. In the spinal cord, serotonin receptors are involved in facilitation of synaptic responses as well as behavioral reflexes. One synaptic mechanism for serotonin-mediated facilitation is the recruitment of functional α-amino-3-hydroxy-5-methyl-4-isoxazole propionate (AMPA) receptors at pure NMDA receptor-containing synapses [d]. Postsynaptic G protein-coupled activation of protein kinase C (PKC) is important for serotonin-produced facilitation. Furthermore, the interaction between AMPA receptors and the PDZ protein glutamate receptor-interacting protein (GRIP) likely contribute to the recruitment of functional AMPA responses [e]. Descending facilitation can be activated under physiological conditions, and one possible physiological significance of descending facilitation is to enhance animals' ability to detect potential dangerous signals in the environment. Indeed, neurons in the RVM not only respond to noxious stimuli, but also show 'learning'-type changes during repetitive noxious stimuli [f]. More importantly, RVM neurons can undergo plastic changes during and after tissue injury and inflammation. Descending facilitation is likely activated after the injury, contributing to secondary hyperalgesia [g,h]. Blocking descending facilitation, by lesion of the RVM or spinal blockade of serotonin receptors, is antinociceptive. The descending facilitatory system therefore serves as a double-edged sword in the central nervous system: it allows neurons in different parts of the brain to communicate with each other and enhance sensitivity to potentially dangerous signals, but also prolonged facilitation of spinal nociceptive transmission after injury speeds up central plastic changes related to chronic pain.

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Table 2. Pain medicines currently used and their synaptic mechanisms

Classes	Clinical drugs	Type of drug	Major synaptic mechanism	Clinical pain conditions
Type I	SNX111	N-type calcium channel blocker	Inhibiting transmitter release	Cancer pain
	LY293558	AMPA/kainate receptor antagonist	Inhibiting postsynaptic excitatory currents	Hyperalgesia and/or allodynia
Type II	Ketamine, CPP	NMDA receptor antagonist	Blocking glutamatergic synaptic plasticity and transmission	Neuropathic pain, post-operative pain
Type III	Clonidine	α2 Adrenoceptor agonist	Inhibiting sensory synapses	Cancer pain, neuropathic pain, postoperative pain
	Neostigmine	Cholinesterase inhibitor	Inhibiting sensory synapses	Cancer pain, postoperative pain
	Morphine and other similar drugs	μ-Opioid receptor agonist	Inhibiting sensory synapses	Cancer pain, postoperative pain
	Celebrex	Cyclooxygenase subtype 2 inhibitor	Inhibiting sensory synapses	Cancer pain, postoperative pain
Type IV	GABApentin	Has several mechanisms of action	Enhancing inhibitory transmission	Neuropathic pain

structures. This descending inhibition is believed to contribute to stimulus-produced analgesia and opiate analgesia [30,41,42]. Within the dorsal horn, multiple transmitter receptors mediate inhibitory modulation. Drugs that act on enhancing activity of these receptors could be analgesic. These include: (1) cholinergic muscarinic receptor agonists, (2) subtype-selective serotonin receptor agonists, (3) adrenergic receptor agonists, and (4) opioid receptor agonists. Finally, drugs enhancing inhibitory synaptic transmission in the spinal dorsal horn or supraspinal structures could also be potentially important for controlling pain.

Forebrain areas: potential new targets for controlling persistent pain

Forebrains in pain perception

Forebrain neurons are important for pain-related perception. Recent studies from both human and animal tests consistently suggest that the anterior cingulate cortex (ACC) and its related areas are important for processing pain perception. ACC neurons respond to nociceptive stimuli in animals and humans [43–47]. Human studies indicate that activity within the ACC could relate to the unpleasantness or discomfort of somatosensory stimuli [44].

Glutamatergic synapses and plasticity

Just as in other areas in the brains, glutamate is the major fast excitatory transmitter in the ACC and other pain-related cortical areas [48–50]. Different types of glutamate receptors, including AMPA, kainate, NMDA and metabotropic receptors are distributed in the ACC, insular cortex and somatosensory cortex. Fast synaptic responses induced by local stimulation or stimulation of thalamocortical projection pathways are mediated by AMPA or kainate receptors. Glutamatergic synapses in forebrain areas can undergo long-lasting plastic changes, such as long-term depression (LTD). LTD can also be observed in the ACC of adult animals. Prolonged, low-frequency stimulation (1 Hz for 15 min) produced long-lasting depression of synaptic responses. Depression is input-specific, and unstimulated pathways remained unchanged. There are several properties of LTD in the ACC that differ from the hippocampus; for example, 5 Hz stimulation (3 min) induced LTD in the ACC but not in hippocampal slices. Unlike hippocampal LTD, which required activation of NMDA receptors, LTD induction required activation of metabotropic glutamate receptors and L-type voltage-gated calcium channels. Therefore, sensory synapses in pain-related forebrain areas are plastic and can undergo LTD.

Modifications of plasticity in the ACC by the injury

What makes ACC more interesting is that neuronal activity show 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 neurons after tissue inflammation or amputation [48]. Furthermore, these plastic changes persist for a long period of time. For example, in the case of amputation, the pattern of expression remained altered after one week. In parallel with these dramatic changes in gene expression, synaptic plasticity in both *in vitro* slices and *in vivo* in animals is also altered. In ACC slices of animals with amputation [48], the same repetitive stimulation produced less or no LTD. In lightly anesthetized animals, amputation caused long-lasting enhancement of sensory synaptic responses in the ACC to either peripheral stimulation or local stimulation [51]. There are two possible implications of these studies. First, LTD in the ACC during low-frequency repetitive stimulation could serve as an autoregulatory inhibition to maintain neuronal activity. In amputated animals, the loss of autoregulation of synaptic tone might lead to overexcitation in the ACC neurons. Second, synaptic potentiation induced by theta burst stimulation can also enhance synaptic transmission in the ACC. Considering the important roles of ACC in pain, enhanced neuronal excitability can directly contribute to the discomfort or pain or indirectly affect subsequent sensory entry at the level of spinal cord [52].

NR2B overexpression

Functional NMDA receptors contain heteromeric combinations of the NR1 subunit plus one or more of NR2A-D [53]. Although 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. NR2C and NR2D are expressed in selected areas of the brain, such as cerebellum. NR2A and NR2B subunits confer distinct properties to 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, NMDA receptors are 5–10 times more permeable to calcium, a crucial 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 [24,54–57]. NR2B subunit expression, driven by the α-calcium–calmodulin-dependent protein kinase II (α-CaMKII), 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 [58]. However, NMDA receptor-mediated responses in the spinal cord were not affected. NR2B transgenic and wild-type mice were indistinguishable in tests of acute nociception, and NR2B transgenic mice exhibited enhanced behavioral responses after peripheral injection of formalin. Late-phase nociceptive responses but not early responses were enhanced. Furthermore, mechanical allodynia measured in the complete Freund's adjuvant model were significantly enhanced in NR2B transgenic mice. These findings provide the first genetic evidence that forebrain NMDA receptors have a crucial role in chronic pain.

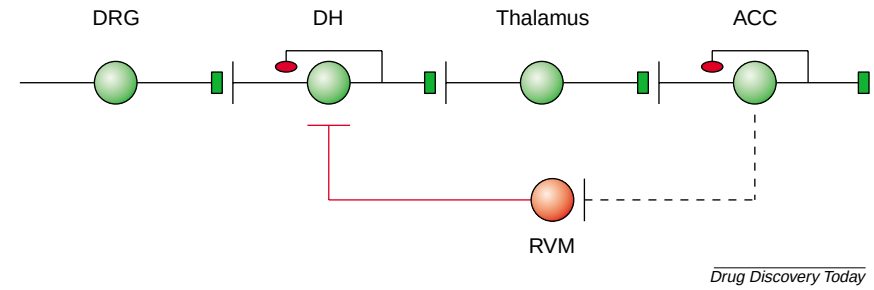

Figure 3. Positive feedback control as a key central mechanism for persistent pain or central pain. Sensory inputs inducing painful sensations enter the brain through three major synaptic relays, including the dorsal horn (DH), thalamus and anterior cingulate cortex (ACC). At each synaptic relay, glutamate is the major fast excitatory transmitter. Although α-amino-3-hydroxy-5-methyl-4-isoxazole propionate (AMPA) and kainate receptors mediate most of the synaptic response at resting conditions, NMDA receptors serve as a coincidence detector to enhance synaptic responses in an activity-dependent manner. Long-lasting potentiation is likely to occur at each sensory synapse. In addition, DH 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 or excitatory 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.

Positive feedback control: a key enhancer for persistent pain

It could be argued that positive feedback control serves as the key pathological mechanism for chronic pain. Such positive enhancement occurs not only at single synapses, but also between multiple neuronal synapses at different parts of the brain (Fig. 3). Several mechanisms could contribute to synaptic enhancement: (1) postsynaptic regulation of glutamate receptors, including phosphorylation and dephosphorylation, (2) recruitment of functional glutamate receptors (such as in spinal dorsal horn neurons, recruitment of postsynaptic functional AMPA receptors), (3) presynaptic enhancement of glutamate release, and (4) structural changes in synapses. At network levels, heterosynaptic facilitation or disinhibition can also lead to enhancement. It is well documented that dorsal horn neurons receive descending facilitatory modulation from the brainstem neurons [36–39]. Recent study further suggests that the ACC can also facilitate spinal responses [35].

This positive feedback control will lead central neurons to a much enhanced and overexcited status; a weak input will lead to significantly greater neuronal action potentials. Such a mechanism most likely contributes to several chronic pain-related states, such as allodynia and central pain.

NR2B receptors as a potential target for controlling persistent pain

Because of the important role of NR2B in positive regulation of neuronal excitability in the forebrain, it is conceivable that NR2B antagonists might be analgesic. Recent animal studies by different investigators using different NR2B receptor antagonists found that NR2B antagonists are analgesic in animals with persistent inflammatory pain or neuropathic pain. Taniguchi *et al.* (1997) reported that CP-101606, an NR2B selective NMDA receptor antagonist, inhibited inflammation-related mechanical allodynia [59]. In addition, capsaicin-induced nociceptive behavioral responses were also inhibited. Boyce *et al.* (1999) confirmed the analgesic effect of CP-101606 in inflammation-related hyperalgesia, and further demonstrated that two other selective NR2B receptor antagonists, ifenprodil and Ro 25-6981, also produced antinociceptive effects in both inflammatory pain and neuropathic pain [60]. Because the drugs were injected systemically, it is difficult to determine the site of action. In normal animals, higher levels of NR2B are found in forebrain areas, suggesting that forebrains are most likely the site for drug action. Indeed, Chizh *et al.* (2001) reported that ifenprodil did not produce any significant inhibitory action in the spinal cord, supporting the hypothesis that forebrain structures are the most likely targets for NR2B receptor antagonists [61]. Our recent data with forebrain-targeted NR2B overexpressing mice support this conclusion, although the possible role of spinal NR2B or other NMDA receptors cannot be completely excluded. One possible side effect of NR2B receptor antagonists is inhibiting cognitive functions of the brain. However, recent studies from animals indicate that NR2B inhibitors could

affect new memory formation selectively without significant effects on existing memory [62].

Future strategies for discovering new treatments for persistent pain

Understanding molecular and cellular mechanisms for central changes in various pain-related states offers hope for the treatment of chronic pain. Although many animal models are currently available for different types of chronic pain, more animal models are still needed for other types of chronic pain, such as back pain, migraine and phantom pain. In addition to the previously described four major classes of drugs, future research also needs to focus on the following potential new chemicals:

- Drugs that block descending facilitatory influences; including antagonists for certain serotonin receptor subtypes;
- Drugs that block the induction and/or expression of synaptic potentiation in different sensory related areas. For example, NMDA NR2B receptor antagonists and other selective NMDA receptor antagonists; and
- Drugs targeted at intracellular signaling pathways for chronic pain; drugs targeted at intracellular signaling pathways involved in synaptic potentiation might also be effective.

Among these potential new drug targets for chronic pain, we believe that NR2B NMDA receptors serve as a most promising target. Long-lasting pain is most likely because of central plasticity triggered by NMDA receptor-related signaling pathways. Mice with NR2B overexpression provide a powerful tool for us to understand the central mechanism for persistent pain, as well as a useful tool for searching chemicals that could selectively inhibit persistent pain.

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