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Presynaptic NMDA receptors control nociceptive transmission at the spinal cord level in neuropathic pain

Meichun Deng^{1,2}, Shao-Rui Chen¹, and Hui-Lin Pan¹

¹Center for Neuroscience and Pain Research, Department of Anesthesiology and Perioperative Medicine, The University of Texas MD Anderson Cancer Center, Houston, TX 77030, USA;

²Department of Biochemistry and Molecular Biology, School of Life Sciences, Central South University, Changsha, Hunan 410013, China

Abstract

Chronic neuropathic pain is a debilitating condition that remains challenging to treat. Glutamate N-methyl-D-aspartate receptor (NMDAR) antagonists have been used to treat neuropathic pain, but the exact sites of their actions have been unclear until recently. Although conventionally postsynaptic, NMDARs are also expressed presynaptically, particularly at the central terminals of primary sensory neurons, in the spinal dorsal horn. However, presynaptic NMDARs in the spinal cord are normally quiescent and are not actively involved in physiological nociceptive transmission. In this review, we describe the emerging role of presynaptic NMDARs at the spinal cord level in chronic neuropathic pain and the implications of molecular mechanisms for more effective treatment. Recent studies indicate that presynaptic NMDAR activity at the spinal cord level is increased in several neuropathic pain conditions but not in chronic inflammatory pain. Increased presynaptic NMDAR activity can potentiate glutamate release from primary afferent terminals to spinal dorsal horn neurons, which is crucial for the synaptic plasticity associated with neuropathic pain caused by traumatic nerve injury and chemotherapy-induced peripheral neuropathy. Furthermore, $\alpha 2\delta$ -1, previously considered a calcium channel subunit, can directly interact with NMDARs through its C-terminus to increase presynaptic NMDAR activity by facilitating synaptic trafficking of $\alpha 2\delta$ -1–NMDAR complexes in neuropathic pain caused by chemotherapeutic agents and peripheral nerve injury. Targeting $\alpha 2\delta$ -1–bound NMDARs with gabapentinoids or $\alpha 2\delta$ -1 C-terminus peptides can attenuate nociceptive drive from primary sensory nerves to dorsal horn neurons in neuropathic pain.

Keywords

Calcineurin; K^+ - Cl^- cotransporter-2; synaptic plasticity; dorsal root ganglion; gabapentin; pregabalin

To whom correspondence should be addressed: Dr. Hui-Lin Pan, Department of Anesthesiology and Perioperative Medicine, Unit 110, The University of Texas MD Anderson Cancer Center, 1515 Holcombe Blvd., Houston, TX 77030. Tel: (713) 563-5838; Fax: (713) 794-4590; huilinpan@mdanderson.org.

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Introduction

Chronic neuropathic pain, often caused by peripheral nerve injury, diabetic neuropathy, postherpetic neuralgia, and chemotherapy, can result in prolonged suffering and remains an enormous unmet medical need. Neuropathic pain is often associated with spontaneous pain, hyperalgesia (increased pain sensation in response to a painful stimulus), and allodynia (pain from normally non-painful stimuli). The cellular and molecular mechanisms responsible for neuropathic pain have not been fully delineated, and effective treatments for this condition are still limited.

Glutamate is the dominant excitatory neurotransmitter and facilitates fast excitatory synaptic transmission in the central nervous system. The glutamate N-methyl-D-aspartate receptor (NMDAR) is involved in numerous physiological functions, such as learning and memory. NMDARs, especially those in the dorsal horn of the spinal cord, play pivotal roles in synaptic plasticity and have long been targeted for the treatment of neuropathic pain. It was shown about 28 years ago that intrathecal injection of NMDAR antagonists reduces nociceptive behaviors and pain hypersensitivity caused by nerve injury [1,2]. Because NMDARs were traditionally thought to be located postsynaptically in the central nervous system, NMDAR antagonists administered intrathecally were assumed to target postsynaptic NMDARs in the spinal cord [3].

NMDARs are now known to be also present at presynaptic nerve terminals and can powerfully shape synaptic transmission and neuronal plasticity in the amygdala [4], hypothalamus [5,6], striatum [7,8], and hippocampus [9]. Under physiological conditions, presynaptic NMDARs in the spinal dorsal horn are not functionally active in regulating neurotransmitter release. However, in opioid-induced hyperalgesia and chronic neuropathic pain conditions, presynaptic NMDARs in the spinal cord become tonically active and are stimulated by endogenous glutamate [10–14]. Here we review the distribution and function of presynaptic NMDARs in the spinal dorsal horn and their prominent role in regulating nociceptive transmission in chronic neuropathic pain.

NMDAR components and distribution in the spinal dorsal horn

NMDARs are tetrameric protein complexes comprising two obligatory GluN1 subunits with either two GluN2 subunits or a combination of one GluN2 and one GluN3 subunits. There are eight GluN1 subunits generated by alternative splicing from a single gene (*GRIN1*), four GluN2 subunits (GluN2A-2D, encoded by *GRIN2A-2D*), and two GluN3 subunits (GluN3A and GluN3B, encoded by *GRIN3A* and *GRIN3B*). The GluN1 subunits are required for the formation of functional NMDARs, whereas the GluN2 and GluN3 subunits modify channel properties [15,16]. GluN2 subunits are essential for glutamate-mediated NMDAR activation. The expression of different GluN2 subunits controls several NMDAR channel properties [16]. GluN2A-containing receptors show faster activation kinetics, higher open probability, and faster deactivation kinetics than GluN2B-containing receptors do [17,18]. On the other hand, GluN2C-NMDARs exhibit relatively low conductance, low open probability, and low sensitivity to Mg^{2+} block [19]. GluN2D is typically expressed early in the developmental stage and is characterized by a very slow decay time (about 4–5 s). The GluN3 subunits

function mainly as modulatory subunits that can reduce the vulnerability to Mg^{2+} block and attenuate the Ca^{2+} permeability of NMDARs [20]. GluN3A is primarily expressed early, whereas GluN3B levels increase over the development period [21].

Neurons in the dorsal root ganglion (DRG) are presynaptic because their central terminals synapse with second-order neurons in the spinal dorsal horn. Thus, many proteins are synthesized in DRG neurons and then transported to their central presynaptic terminals inside the spinal dorsal horn. GluN1 is expressed in large and small DRG neurons, including both peptidergic and nonpeptidergic neurons [22–24]. GluN2B and GluN2D are expressed at higher levels than GluN2A and GluN2C are in the DRG [25]. At the spinal cord level, NMDARs are abundant in the dorsal horn, with GluN1 and GluN2A subunits expressed throughout the gray matter, whereas GluN2B subunits are distributed mainly in laminae I–II [26]. The GluN1 immunoreactivity is present at the terminals of both unmyelinated and myelinated primary afferent nerves in the superficial dorsal horn [27,28]. In addition, NMDARs are present on GABAergic terminals in the superficial dorsal horn of the spinal cord [29].

Functional significance of presynaptic NMDAR in the spinal dorsal horn

NMDARs are permeable to Na^+ , K^+ , and Ca^{2+} . However, the channel permeability is normally blocked by extracellular Mg^{2+} , and strong neuronal depolarization is needed to release the Mg^{2+} block. For this reason, NMDARs are viewed as a coincidence sensor for coordinating presynaptic and postsynaptic neuronal activities [30]. Nonetheless, presynaptic NMDARs can promote spontaneous neurotransmitter release in the absence of neuronal depolarization [31], suggesting that these NMDARs can be activated by endogenous glutamate even without relief of the Mg^{2+} block [32]. In contrast, most postsynaptic NMDARs are blocked by Mg^{2+} at rest and therefore require neuronal depolarization in concurrence with glutamate binding to become functionally active [33]. Activation of presynaptic NMDARs may cause calcium influx to directly trigger vesicle exocytosis, cause the depolarization of the terminals to activate voltage-gated calcium channels, and indirectly trigger neurotransmitter release through downstream intracellular signaling cascades independent of ion flux [34,32,9].

Although NMDARs are present in primary sensory neurons and their central terminals in the spinal dorsal horn [27,35,36], they are normally in a quiescent state, as evidenced by the observation that blocking NMDARs with the specific NMDAR antagonist 2-amino-5-phosphonopentanoic acid (AP5) has no effect on spontaneous or evoked synaptic glutamate release to spinal lamina I or lamina II neurons in normal rats [13,10,11,37,14]. This finding is consistent with reports showing that blocking NMDARs at the spinal cord level does not alter acute nociception in normal conditions [1,37,11].

However, there are some inconsistent findings about the function of spinal presynaptic NMDARs in the normal state in studies using exogenous NMDAR agonists. For example, intrathecal administration of NMDA can evoke the release of substance P from the central terminals of a subset of primary sensory neurons in the rat dorsal horn [35]. On the other hand, bath application of NMDA inhibits synaptic glutamate release evoked from primary

afferent nerve stimulation [38]. Because the evoked glutamate release is likely mediated by voltage-gated Ca^{2+} channels, exogenous NMDA may induce calcium influx to activate calcineurin, which subsequently inhibits voltage-gated calcium channels [39,40]. Indeed, activation of presynaptic NMDARs can inhibit voltage-gated Ca^{2+} channels to attenuate evoked glutamate release at the calyx of Held synapse [41]. Intriguingly, it was reported that conditional knockout of GluN1 in DRG neurons in *Advillin*-Cre mice results in increased neuronal excitability and mechanical and thermal sensitivity [42]. This DRG neuronal hyperexcitability was shown to be due to the impairment of Ca^{2+} -activated slow conductance K^{+} channels in GluN1 conditional knockout mice [42]. Others have shown that conditional knockout of GluN1 in DRG neurons in *Peripherin*-Cre mice does not affect normal nociception [36]. The finding that removing NMDARs in DRG neurons paradoxically increases neuronal excitability and pain-like behaviors needs to be validated independently.

The first demonstration of increased activity of presynaptic NMDARs in the spinal cord is a study related to opioid analgesic tolerance, in which AP5 reduces the frequency of miniature excitatory postsynaptic currents (mEPSCs, a measure of quantal glutamate release from presynaptic terminals) and the amplitude of EPSCs of lamina I neurons evoked from the dorsal root (representing glutamate release from primary afferent nerves) in neonatal rat spinal cord slices [12]. Repeated opioid exposure in animal models and in patients can cause paradoxical hyperalgesia and analgesic tolerance, which are major obstacles to adequate pain management with opioids. Elevating endogenous glutamate levels using a glutamate transporter inhibitor produces an AP5-sensitive increase in the frequency of mEPSCs in opiate-naïve spinal cord slices [43], suggesting that the lack of presynaptic NMDAR activity may be due to an inadequate level of endogenous NMDAR agonists in normal conditions. Furthermore, long-term morphine treatment can substantially increase the amplitude of monosynaptic EPSCs evoked from primary afferent nerves and the frequency of spontaneous EPSCs and mEPSCs, and these changes were reduced principally by blocking of NMDARs [13,44]. Additionally, blocking NMDARs containing GluN2A or GluN2B significantly attenuates the frequency of spontaneous EPSCs and the amplitude of evoked EPSCs of lamina II neurons in morphine-treated rats [13]. In contrast to protein kinase C (PKC)- and mitogen-activated protein kinase-dependent activation of presynaptic NMDARs in the spinal cord, chronic morphine treatment diminishes postsynaptic NMDAR activity in the spinal dorsal horn [44,13]. This reduction is likely caused by the persistent increase in glutamate release from transient receptor potential vanilloid type 1-expressing primary afferents [13]. Strikingly, even bath application of an opioid agonist for 3 min can increase presynaptic NMDAR activity in spinal lamina I and lamina II neurons to elicit a long-lasting increase in glutamate release from nociceptive primary afferents [45]. These findings highlight the critical role of spinal presynaptic NMDARs in opioid-induced hyperalgesia and analgesic tolerance.

Increased presynaptic NMDAR activity potentiates spinal nociceptive transmission in neuropathic pain induced by peripheral nerve injury

Ketamine, a clinically used NMDAR antagonist, has been used for many years to treat neuropathic pain [46,47] and intrathecal injection of NMDAR antagonists, including MK-801 and AP5, effectively reduce neuropathic pain behaviors in animals subjected to peripheral nerve injury [1,48,49]. Thus, NMDARs at the spinal cord level play a major role in the development of central sensitization and neuropathic pain after nerve injury. The majority of neurons in spinal lamina II are glutamatergic excitatory interneurons involved in nociceptive transmission [50,51]. Consistent with the *in vivo* findings, bath application of AP5 rapidly and reversibly attenuates the increased frequency of mEPSCs in lamina II neurons in spinal cord slices from nerve-injured rats [52,10,14]. AP5 also normalizes the increased amplitude of monosynaptic EPSCs and the altered paired-pulse ratio (a measure of presynaptic effect) of evoked EPSCs in lamina II neurons in nerve-injured rats. These findings indicate that presynaptic NMDARs in the spinal dorsal horn are endogenously activated to potentiate glutamate release from nociceptive primary afferent terminals in neuropathic pain.

NMDAR activity is regulated predominantly by the phosphorylation status of NMDARs and/or their interacting proteins. NMDARs can be phosphorylated by many protein kinases, such as protein kinase A, PKC, casein kinase 2 (CK2), Ca^{2+} /calmodulin-dependent protein kinase II, and tyrosine kinases Src and Fyn [53,16,54,55]. Peripheral nerve injury in rats increases CK2 α and CK2 β protein levels in the spinal cord [56], and inhibition of CK2 or knockdown of CK2 β with specific siRNA at the spinal cord level can reverse nerve injury-induced pain hypersensitivity. These findings indicate that CK2 plays a major role in the potentiation of synaptic NMDAR activity of spinal dorsal horn neurons [56]. Furthermore, treatment with a PKC inhibitor, GF109203X, significantly reduces the amplitude of monosynaptic EPSCs of dorsal horn neurons in nerve-injured rats [14]. Various protein kinases, including CK2, PKC, and Src, are likely responsible for the increased presynaptic and postsynaptic NMDAR activity in the spinal dorsal horn in nerve injury-induced neuropathic pain [56,54,14].

Regulating presynaptic NMDAR activity by KCC2 and $\alpha 2\delta$ -1 in nerve injury-induced neuropathic pain

Impaired synaptic inhibition in the spinal dorsal horn due to downregulation of $\text{K}^+\text{-Cl}^-$ cotransporter-2 (KCC2, encoded by the *Slc12a5* gene) contributes substantially to the development of neuropathic pain [57]. Peripheral nerve injury impairs synaptic inhibition of spinal dorsal horn neurons through calpain-mediated KCC2 proteolysis resulting from the increased postsynaptic NMDAR activity [49]. Intrathecal *Slc12a5* gene transfer completely reverses mechanical hyperalgesia, tactile allodynia, and thermal hypersensitivity induced by nerve injury [10]. *Slc12a5* gene transfer at the spinal cord level via a lentiviral vector restores Cl^- homeostasis disrupted by nerve injury in both the spinal dorsal horn and DRG neurons. KCC2 normally is not expressed in DRG neurons. Intriguingly, ectopic expression of *Slc12a5* in the DRG and over-expression of *Slc12a5* in the spinal cord via a lentiviral

vector normalize both presynaptic and postsynaptic NMDAR activity increased by nerve injury [10], suggesting that disrupting Cl^- homeostasis at the spinal cord level affects the synaptic NMDAR activity in the spinal dorsal horn in nerve injury-induced neuropathic pain.

$\alpha 2\delta$ -1 (encoded by the *Cacna2d1* gene), commonly considered a calcium channel subunit, is expressed in the DRG, spinal cord, and discrete brain regions [58]. Recent studies indicate that $\alpha 2\delta$ -1 can form a protein complex with NMDARs and regulate NMDAR synaptic trafficking and activity in the brain [59,60,6,8]. Nerve injury profoundly increases the expression level of $\alpha 2\delta$ -1 in the DRG and spinal dorsal horn [61], and genetic ablation of $\alpha 2\delta$ -1 reduces pain hypersensitivity induced by nerve injury [62]. *In situ* hybridization analysis shows that $\alpha 2\delta$ -1 is mainly present in small- and medium-sized neurons in the normal DRG and that sciatic nerve injury predominantly increases the $\alpha 2\delta$ -1 expression in medium and large DRG neurons [63]. There are no studies showing the NMDAR subunit co-localization with $\alpha 2\delta$ -1 in the DRG. However, their co-localization is highly likely because of the widespread expression of NMDAR subunits and $\alpha 2\delta$ -1 in various types of DRG neurons.

Gabapentinoids, including gabapentin and pregabalin, have been used clinically to treat neuropathic pain conditions such as postherpetic neuralgia and fibromyalgia [64,65]. Although gabapentin and pregabalin bind to $\alpha 2\delta$ -1 [66], little has been known about how these drugs reduce neuropathic pain until recently. In a recent study, overexpression of *Cacna2d1* at the spinal cord level via a lentiviral vector causes a large increase in presynaptic and postsynaptic NMDAR activity in the spinal dorsal horn [52]. Conversely, siRNA knockdown of *Cacna2d1* or genetic knockout of *Cacna2d1* abolishes nerve injury-induced increases in presynaptic and postsynaptic NMDAR activity in the spinal dorsal horn (Figure 1). Further studies revealed that $\alpha 2\delta$ -1 can physically interact with NMDAR subunits *in vivo* and *in vitro* through its C-terminal domain, promoting the synaptic trafficking of NMDARs [52]. In addition, gabapentin treatment or disruption of the $\alpha 2\delta$ -1–NMDAR interaction with an $\alpha 2\delta$ -1 C-terminus peptide blocks nerve injury-induced potentiation of presynaptic and postsynaptic NMDAR activity in the spinal dorsal horn (Figure 2). Thus, $\alpha 2\delta$ -1 can increase presynaptic NMDAR activity in neuropathic pain by increasing the synaptic targeting and trafficking of NMDARs at primary afferent terminals and/or by reducing the Mg^{2+} block of GluN2A-containing NMDARs [52]. These findings reveal that $\alpha 2\delta$ -1-bound NMDARs are the relevant molecular target responsible for the therapeutic action of gabapentinoids in treating neuropathic pain.

Spinal presynaptic NMDARs mediate increased nociceptive input to dorsal horn neurons in chemotherapy-induced neuropathic pain

Chemotherapeutic agents, including paclitaxel and bortezomib, can induce painful peripheral neuropathy, a major dose-limiting adverse effect, in many cancer patients. Intrathecal injection of the NMDAR antagonist AP5 significantly reverses pain hypersensitivity induced by paclitaxel or bortezomib. Moreover, paclitaxel-induced pain hypersensitivity can be reduced by systemic treatment with memantine, a clinically used

NMDAR antagonist [11]. Although NMDARs are implicated in the development of chemotherapy-induced chronic pain, electrophysiological studies using spinal cord slices show that systemic treatment with paclitaxel or bortezomib does not affect postsynaptic NMDAR activity in spinal lamina II neurons [67,68].

Subsequent studies using spinal cord slice recordings indicate that treatment with paclitaxel or bortezomib in rats significantly increases the frequency of mEPSCs and the amplitude of monosynaptic EPSCs evoked from the dorsal root [11,68]. Bath application of AP5 quickly normalizes the frequency of mEPSCs and the amplitude of evoked EPSCs in dorsal horn neurons that had been increased by paclitaxel or bortezomib treatment [11,68]. Also, selective blocking of GluN2A-containing NMDARs decreases the frequency of mEPSCs and the amplitude of EPSCs evoked from the dorsal root in dorsal horn neurons from paclitaxel-treated rats [11], suggesting that chemotherapy primarily increases the activity of GluN2A-containing NMDARs at primary afferent terminals in the spinal cord.

Treatment with paclitaxel or bortezomib potentiates nociceptive input from primary afferent nerves via PKC-mediated tonic activation of presynaptic NMDARs as evidenced by the finding that inhibition of PKC with chelerythrine fully reverses the increased frequency of mEPSCs and amplitude of evoked EPSCs caused by paclitaxel and bortezomib [11,68]. Furthermore, paclitaxel-induced painful neuropathy is associated with increased activity of presynaptic mGluR5, which is an upstream signaling mechanism for activation of PKC and potentiation of presynaptic NMDAR activity in the spinal dorsal horn [69].

In a recent study, chronic treatment with paclitaxel in rats increases $\alpha 2\delta$ -1 expression levels in the DRG and spinal cord, $\alpha 2\delta$ -1–NMDAR interaction, and synaptic expression of $\alpha 2\delta$ -1–NMDAR complexes in the spinal dorsal horn [70]. Also, treatment with pregabalin or an $\alpha 2\delta$ -1 C-terminus peptide fully blocks the increased presynaptic NMDAR activity of spinal lamina II neurons in paclitaxel-treated rats but has no effect on mEPSCs or EPSCs monosynaptically evoked by dorsal root stimulation in vehicle-treated rats. In *Cacna2d1* knockout mice, chronic treatment with paclitaxel fails to significantly change the frequency of mEPSCs or the amplitude of EPSCs evoked by dorsal root stimulation. In addition, intrathecal injection of pregabalin or $\alpha 2\delta$ -1 C terminus–interfering peptide and *Cacna2d1* knockout markedly attenuate paclitaxel-induced pain hypersensitivity [70]. Taken together, these findings clearly indicate that chemotherapy-induced $\alpha 2\delta$ -1–NMDAR coupling is fully responsible for the increased presynaptic NMDAR activity that potentiates nociceptive drive to the spinal dorsal horn to maintain chronic pain. This study provides further evidence that the therapeutic target of pregabalin in neuropathic pain is $\alpha 2\delta$ -1–bound NMDARs that are expressed presynaptically in the spinal dorsal horn.

Presynaptic NMDARs are involved in potentiated spinal nociceptive transmission in calcineurin inhibitor-induced chronic pain

Calcineurin is a serine/threonine protein phosphatase, and its activation is dependent on Ca^{2+} /calmodulin. Calcineurin is highly expressed in the T cells of the immune system and in the nervous system, including neurons in the DRG and spinal cord [71,39,40]. Calcineurin inhibitors such as cyclosporin A and tacrolimus (FK506) are commonly used

immunosuppressants and used for the preservation of allograft function in patients. However, these drugs can cause severe, persistent pain, frequently referred to as calcineurin inhibitor-induced pain syndrome (CIPS) [72]. CIPS is characterized by severe pain and pain hypersensitivity that mostly affects the lower limbs, and the pain is often agonizing during standing and walking [73].

Calcineurin inhibition prolongs NMDAR channel opening [74]. Systemic administration of FK506 in rats leads to long-lasting nociceptive and mechanical hypersensitivity [37]. Blocking spinal NMDARs with AP5 can reverse the tactile allodynia and mechanical hyperalgesia caused by FK506 treatment. Also, systemic administration of the NMDAR antagonist memantine reduces pain hypersensitivity in a dose-dependent manner in FK506-treated rats [37]. Moreover, FK506 treatment increases the amplitude of NMDAR-mediated monosynaptic EPSCs of dorsal horn neurons evoked from primary afferent nerves and profoundly increases the frequency of mEPSCs in lamina II neurons. The increase in synaptic glutamate release can be readily normalized by AP5, suggesting that presynaptic NMDAR activity is augmented the calcineurin inhibitor, thereby potentiating glutamate release from primary afferent nerves in CIPS [37,75]. CK2 inhibition completely normalizes the amplitude of evoked NMDAR-EPSCs and the frequency of mEPSCs in dorsal horn neurons increased by FK506 treatment, suggesting that CK2 mediates the increased synaptic NMDAR activity in the spinal cord and the pain hypersensitivity associated with CIPS. In addition, intrathecal injection of CK2 inhibitors reverses tactile allodynia and mechanical hyperalgesia in FK506-treated rats [75]. Therefore, calcineurin and CK2 can reciprocally control NMDAR activity through phosphorylation, and inhibition of endogenous CK2 reverses calcineurin inhibitor-induced pain hypersensitivity by normalizing synaptic NMDAR activity in the spinal cord. The potential role of $\alpha 2\delta$ -1-bound NMDARs in CIPS-related synaptic NMDAR hyperactivity in the spinal dorsal horn is still being investigated.

Chronic inflammatory pain is not associated with increased presynaptic NMDAR activity in the spinal cord

Unlike neuropathic pain, there is currently no evidence about the involvement of spinal presynaptic NMDARs in inflammatory pain. A recent study examined changes in presynaptic and postsynaptic NMDAR activity of spinal dorsal horn neurons in rats 10–16 days after induction of tissue inflammation with complete Freund's adjuvant (CFA). Interestingly, the glutamatergic input, measured by the frequency of mEPSCs and spontaneous EPSCs, is markedly increased in lamina I, but not lamina II, neurons in CFA-treated rats [76]. However, bath application of AP5 has no effect on the CFA-induced increase in the frequency of spontaneous EPSCs in lamina I neurons. Furthermore, the amplitude of puff NMDA currents in lamina I neurons is similar in vehicle- and CFA-treated rats [76]. These findings suggest that chronic inflammatory pain is not associated with an increase in presynaptic or postsynaptic NMDAR activity in the spinal dorsal horn. Instead, presynaptic TRPA1 and TRPV1 channels play a major role in the augmented glutamatergic input to spinal lamina I neurons in chronic inflammatory pain [76]. It has been shown that CFA-induced pain hypersensitivity in the hindpaw is attenuated only for the first 2 days in mice in which spinal cord GluN1 is ablated [77]. Thus, although spinal NMDARs may play

a role in the initial induction of inflammatory pain, the NMDAR activity in the spinal dorsal horn is not required for the maintenance of chronic inflammatory pain.

Conclusions and Perspectives

Recent studies clearly indicate that presynaptic NMDARs in the spinal dorsal horn become tonically activated in neuropathic pain conditions. At the spinal cord level, presynaptic NMDARs are present mainly at the first sensory synapse, which is the interface between primary sensory nerves and spinal dorsal horn neurons, and can “gate” peripheral nociceptive input to the spinal cord in neuropathic pain. In neuropathic pain caused by traumatic nerve injury, chemotherapy, or calcineurin inhibitors, presynaptic NMDAR activity in the spinal cord is consistently increased. Consequently, presynaptic NMDARs may play a greater role than postsynaptic NMDARs in initiating and maintaining chronic neuropathic pain.

The increase in presynaptic NMDAR activity at the spinal cord level is associated primarily with $\alpha 2\delta$ -1-bound NMDARs in neuropathic pain (Figure 3) [52,70]. Thus, $\alpha 2\delta$ -1-bound NMDARs act mainly as a pathological form of NMDARs and represent an opportune target for treating neuropathic pain. Systemic administration of general NMDAR antagonists, such as ketamine, often produces various adverse effects in patients due to the interference of these antagonists with normal NMDAR function in the central nervous system. We have learned from recent studies that gabapentinoids reduce nociceptive input from primary sensory neurons to spinal dorsal horn neurons by inhibiting synaptic trafficking and activity of $\alpha 2\delta$ -1-bound NMDARs [52,70]. The $\alpha 2\delta$ -1Tat peptide that targets the C-terminus of $\alpha 2\delta$ -1 can disrupt the $\alpha 2\delta$ -1–NMDAR interaction and normalize the aberrant synaptic NMDAR activity in neuropathic pain. Thus, peptides and small molecules targeting the C-terminal domain of $\alpha 2\delta$ -1 may be developed clinically to treat chronic neuropathic pain.

Despite the progress being made, our knowledge about the role of presynaptic NMDARs in the development of neuropathic pain is still fragmentary. Many important questions remain: What signaling mechanisms prevent the tonic activation of spinal presynaptic NMDARs under normal conditions? Are any changes in presynaptic NMDARs in chronic pain associated with diabetic neuropathy and postherpetic neuralgia? Are separate protein kinases involved in regulating the function of presynaptic and postsynaptic NMDARs in neuropathic pain? What is the relationship between NMDAR phosphorylation/glycosylation and the protein-protein interaction between $\alpha 2\delta$ -1 and NMDARs in neuropathic pain? Do presynaptic NMDARs similarly regulate the glutamatergic input to excitatory and inhibitory dorsal horn neurons in neuropathic pain? Forthcoming studies will undoubtedly improve our understanding of the cellular and molecular mechanisms of chronic neuropathic pain and enable the design of effective therapies for this debilitating condition.

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The abbreviations used are:

AP5	2-amino-5-phosphonopentanoic acid
CFA	complete Freund's adjuvant
CIPS	calcineurin inhibitor-induced pain syndrome
DRG	dorsal root ganglion
EPSC	excitatory postsynaptic current
mEPSC	miniature excitatory postsynaptic current
NMDAR	N-methyl-D-aspartate receptor
PKC	protein kinase C

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Figure 1. $\alpha 2\delta$ -1 is essential for the increased presynaptic and postsynaptic NMDAR activity of spinal dorsal horn neurons after nerve injury.

(A) Original current traces and mean changes in NMDAR currents elicited by puff application of 100 μ M NMDA to spinal dorsal horn neurons in wild-type (WT, $n = 12$ neurons in each group) and *Cacna2d1* KO ($n = 11$ neurons in each group) mice 3 weeks after spared nerve injury (SNI) or sham surgery. Data are means $\pm$ s.e.m. * $P < 0.05$ (versus WT sham group). One-way ANOVA analysis followed by Tukey's post hoc test.

(B) Representative traces and mean changes in baseline values and the AP5 effect on the frequency and amplitude of mEPSCs of spinal dorsal horn neurons in wild-type (WT, $n = 11$

neurons in each group) and *Cacna2d1* KO (n = 12 neurons in each group) mice subjected to spared nerve injury (SNI) or sham surgery. Data are means $\pm$ s.e.m. *P < 0.05 (versus baseline). #P < 0.05 (versus baseline in the WT sham group). Oneway ANOVA analysis followed by Tukey's post hoc test.

(C,D) Representative traces (C) and mean changes (D) in baseline values and the AP5 effect on the amplitude of EPSCs of spinal dorsal horn neurons monosynaptically evoked by dorsal root stimulation in wild-type (WT) and *Cacna2d1* KO mice subjected to spared nerve injury (SNI, n = 11 neurons in each group) or sham surgery (n = 12 neurons in each group). Data are means $\pm$ s.e.m. *P < 0.05 (versus baseline). #P < 0.05 (versus baseline in the WT sham group). One-way ANOVA analysis followed by Tukey's post hoc test (Reproduced from [52]).

Figure 2. Gabapentin treatment normalizes presynaptic and postsynaptic NMDAR activity of spinal dorsal horn neurons after nerve injury.

(A) Original traces and mean effects of gabapentin (GBP, 100 μ M for 30 min) on currents elicited by puff application of 100 μ M NMDA or AMPA to spinal dorsal horn neurons in rats that had undergone sham surgery ($n = 12$ neurons in the vehicle group, $n = 13$ neurons in the gabapentin group) or spinal nerve ligation (SNL, $n = 11$ neurons in the vehicle group, $n = 12$ neurons in gabapentin group) 3 weeks after surgery. Data are means $\pm$ s.e.m. * $P < 0.05$ (versus sham rats treated with vehicle). One-way ANOVA analysis followed by Tukey's post hoc test.

(B,C) Representative traces, cumulative probabilities **(B)** and mean changes **(C)** of baseline values and the AP5 effect on the frequency and amplitude of mEPSCs of spinal dorsal neurons recorded from rats subjected to sham surgery (n = 10 neurons) or SNL (n = 12 neurons in the vehicle group, n = 11 neurons in the gabapentin group). Data are means $\pm$ s.e.m. *P < 0.05 (versus respective baseline). #P < 0.05 compared with the baseline in sham group. One-way ANOVA analysis followed by Tukey's post hoc test (Reproduced from [52]).

Figure 3. Schematic showing the potential role of $\alpha 2\delta -1$ in the regulation of synaptic NMDARs in the spinal dorsal horn in neuropathic pain.

Under normal conditions, most synaptic NMDARs are not associated with $\alpha 2\delta -1$ in the spinal dorsal horn. In the neuropathic pain condition, $\alpha 2\delta -1$ expression is increased and physically interacts with NMDARs, at presynaptic and postsynaptic sites, to promote synaptic trafficking of $\alpha 2\delta -1$ -NMDAR complexes. Gabapentinoids restore the normal activity of presynaptic and postsynaptic NMDARs by inhibiting synaptic trafficking of $\alpha 2\delta -1$ -bound NMDARs in the spinal dorsal horn, thereby reducing neuropathic pain.