


# Enhanced function of NR2C/2D-containing NMDA receptor in the nucleus accumbens contributes to peripheral nerve injury-induced neuropathic pain and depression in mice

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## Abstract

N-methyl-d-aspartate receptors (NMDARs) dysfunction in the nucleus accumbens (NAc) participates in regulating many neurological and psychiatric disorders such as drug addiction, chronic pain, and depression. NMDARs are heterotetrameric complexes generally composed of two NR1 and two NR2 subunits (NR2A, NR2B, NR2C and NR2D). Much attention has been focused on the role of NR2A and NR2B-containing NMDARs in a variety of neurological disorders; however, the function of NR2C/2D subunits at NAc in chronic pain remains unknown. In this study, spinal nerve ligation (SNL) induced a persistent sensory abnormality and depressive-like behavior. The whole-cell patch clamp recording on medium spiny neurons (MSNs) in the NAc showed that the amplitude of NMDAR-mediated excitatory postsynaptic currents (EPSCs) was significantly increased when membrane potential held at  $-40$  to  $0$  mV in mice after 14 days of SNL operation. In addition, selective inhibition of NR2C/2D-containing NMDARs with PPDA caused a larger decrease on peak amplitude of NMDAR-EPSCs in SNL than that in sham-operated mice. Applying of selective potentiator of NR2C/2D, CIQ, markedly enhanced the evoked NMDAR-EPSCs in SNL-operated mice, but no change in sham-operated mice. Finally, intra-NAc injection of PPDA significantly attenuated SNL-induced mechanical allodynia and depressive-like behavior. These results for the first time showed that the functional change of NR2C/2D subunits-containing NMDARs in the NAc might contribute to the sensory and affective components in neuropathic pain.

## Keywords

Nucleus accumbens, NMDA receptor, NR2C, NR2D, neuropathic pain, depression

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## Introduction

Peripheral nerve injury-induced neuropathic pain is a major clinical challenge. The nucleus accumbens (NAc) within the ventral striatum participates in many behaviors including reward, motivation, as well as sensory processing, and its dysfunction has been implicated in neuropathic pain and related affective behaviors.<sup>1–3</sup> Recent studies showed that the glutamatergic synaptic inputs to the NAc medium spiny neurons (MSNs) are reduced in inflammatory and neuropathic pain.<sup>4–6</sup> The N-methyl-d-aspartate receptors (NMDARs) are one of major glutamate

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receptors in the spinal cord and brain and associated with numerous physiological functions. Intrathecal injection of NMDAR non-selective antagonists reduces nociceptive behaviors and pain sensitivity caused by nerve injury.<sup>7,8</sup> NMDARs are heterotetrameric complexes composed of NR1 subunit, NR2 subunits (NR2A, NR2B, NR2C and NR2D), and NR3 subunits (NR3A and NR3B), giving rise to NMDARs with distinct pharmacological and physiological properties.<sup>9,10</sup> Indeed, the distinct functional properties of NMDARs primarily dependent on the subunits combinations, and in particular on NR2 subunits.<sup>11</sup> Accumulative evidence has indicated that the changes in the expression and function of NR2B or NR2A subunits in the spinal cord,<sup>12,13</sup> anterior cingulate cortex,<sup>14,15</sup> periaqueductal grey,<sup>16</sup> hippocampus,<sup>17</sup> and insular cortex<sup>17</sup> play a crucial role in chronic pain. More recent investigations further indicated that enhancement of NR2B in NAc MSNs contributes to the pain hypersensitivity and related negative emotions.<sup>5,18</sup> However, evidence for the function of NR2C/2D subunits-containing NMDARs at the NAc in neuropathic pain modulation is still lacking.

Different from NR2B and NR2A subunits-containing NMDARs, the NR2C or NR2D containing-NMDARs have less voltage-dependency properties, lower conductance, and higher affinity for glutamate.<sup>10,19</sup> NR2C and NR2D subunits are mainly expressed in the adult forebrain, cerebellar granule cells, thalamus, and pontine,<sup>20,21</sup> and functionally contribute to various neurological diseases.<sup>22-24</sup> A recent investigation demonstrated that repeated cocaine experience increases NR2C/2D-containing NMDARs function and unmasked NMDAR-dependent long-term depression at synapse of thalamo-NAc MSNs.<sup>25</sup> Here, we provide evidence showing that the functional up-regulation of NR2C/2D subunits-containing NMDARs in the NAc was involved in regulating mechanical hyperalgesia and related emotional behavior in a neuropathic pain animal model.

## Materials and Methods

### *Animals and surgery*

Adult ICR mice (male, 8 weeks old) were purchased from Experimental Animal Center of Nantong University. The animals were maintained on a 12:12 light-dark cycle at a room temperature of  $22 \pm 3^\circ\text{C}$  with free access to food and water. All animal procedures performed in this study were reviewed and approved by the Animal Care and Use Committee of Nantong University and were conducted in accordance with the guidelines of the International Association for the Study of Pain. To produce a spinal nerve ligation (SNL), animals were anesthetized with isoflurane and the L6 transverse process was removed to expose the L4 and L5 spinal nerves. The L5 spinal nerve was then isolated and tightly ligated with 6-0 silk thread.<sup>26</sup> For sham operations, the L5 spinal nerve was exposed but not ligated.

### *Behavioral testing*

Animals were habituated to the testing environment daily for at least 2 days before baseline testing. All the behavioral experiments were done by individuals that were blinded to the treatment mice.

**Von Frey test.** The animals were put in boxes on an elevated metal mesh floor and allowed 30 min for habituation before measure. As described in our previous study,<sup>18</sup> the plantar surface of the hindpaw was stimulated with a series of von Frey hairs with logarithmically incrementing stiffness (0.02–2.56 g, Stoelting, IL), presented perpendicular to the plantar surface (2–3 s for each hair). The 50% paw withdrawal threshold was determined using Dixon<sup>27</sup>'s up-down method.

**Hargreaves test.** The animals were put in a plastic box placed on a glass plate, and the plantar surface was exposed to a beam of radiant heat through a transparent glass surface (IITC model 390 Analgesia Meter, Life Science, CA). The baseline latencies were adjusted to 12–15 s with a maximum of 20 s as a cut-off to prevent potential injury. The latencies were averaged over three trials and separated by a 5-min interval.<sup>28</sup>

**Forced swim test (FST).** FST was performed according to the previous research.<sup>29</sup> Briefly, each animal was handled and placed for 15 min into a glass beaker with water at  $25^\circ\text{C}$  filled to 25 cm height, one day before the measure. For the behavior test, the animal was placed into the glass beaker again under the same condition for 6 min and recorded the swimming behavior. The total duration of immobility (non-swimming) within the last 4 min was calculated.

### *Brain slices preparation*

NAc slices preparation and whole cell patch-clamp recordings were conducted as described previously.<sup>30</sup> In brief, adult (8–12 weeks) male ICR mice were deeply anesthetized with isoflurane and decapitated. The brains were quickly removed from the skull and immersed in ice-cold artificial cerebrospinal fluid (aCSF). Sucrose-rich aCSF containing (in mM): 235 sucrose, 25  $\text{NaHCO}_3$ , 2.5 KCl, 1.25  $\text{NaH}_2\text{PO}_4$ , 1  $\text{CaCl}_2$ , 2.5  $\text{MgCl}_2$ , and 10 glucose, bubbled with 95%  $\text{O}_2$  plus 5%  $\text{CO}_2$ . Sagittal brain slices (300  $\mu\text{m}$ ) containing the NAc shell was cut by using a vibratome (Series 1000; Leica, Germany). The slices were stored with oxygenated (95%  $\text{O}_2$  plus 5%  $\text{CO}_2$ ) normal aCSF containing (in mM): 125 NaCl, 3 KCl, 1.25  $\text{NaH}_2\text{PO}_4$ , 2.4  $\text{CaCl}_2$ , 1.2  $\text{MgCl}_2$ , 26  $\text{NaHCO}_3$ , and 10 glucose for 30 min at  $34^\circ\text{C}$  and subsequently incubated at room temperature at least 1 h prior experiment recording.

### *Electrophysiological recording in NAc shell slices*

MSNs in the NAc shell were visualized using a BX51WI infrared-differential interference contrast (IR-DIC) microscope (Olympus, Japan) and with an IR CCD camera (IR-

1000, USA). Whole-cell recordings of excitatory postsynaptic currents (EPSCs) on neurons in NAc shell were made using a patch clamp amplifier (Multiclamp 700B; Axon Instruments, CA). Data acquisition and analysis were performed using a digitizer (DigiData 1400A; Axon Instruments, CA) and the analysis software Clampfit 10.4 (Molecular Devices, Sunnyvale, CA), respectively. MSNs within the NAc shell were identified by their small morphology (somatic diameters, < 20  $\mu\text{m}$ ) and hyperpolarized resting membrane potential ( $-75$  to  $-85$  mV). The resistance of patch pipettes was 4–8 M $\Omega$  when filled with an intracellular solution containing (in mM): 120 cesium methanesulfonate, 2 NaCl, 20 HEPES, 0.4 EGTA, 5 tetraethylammonium-Cl, 2.5 Na<sub>2</sub>ATP, and 0.3 GTP-Tris, 2.5 mM QX314, pH 7.2–7.4 (adjusted by CsOH). Afferents were stimulated (by Master 8; AMPI Technologies) at 0.1 Hz (pulse of 100 $\mu\text{s}$  duration) by a concentric bipolar electrode (FHC, Bowdoinham, ME) placed at the border between the prefrontal cortex and NAc.<sup>31</sup> All neurons in this study had a graded evoked current response (NMDAR-EPSC) to the increasing stimulation intensity (ranging from 25 to 150  $\mu\text{A}$ ), and an intensity giving 50–60% of the maximum evoked synaptic response was adopted to evoke test currents. The GABA<sub>A</sub> receptor antagonist picrotoxin (PTX, 100  $\mu\text{M}$ ) and AMPAR antagonist (CNQX, 20  $\mu\text{M}$ ) was present in the aCSF throughout the experiment. For each cell at each stimulus intensity tested, 10–15 consecutive EPSCs were recorded and the peak amplitudes averaged. MSNs were held at a range of membrane potentials from  $-70$  mV to 0 mV. A stimulation electrode was positioned at the border of prefrontal cortex and NAc, which primarily activates glutamatergic PFC fibers projecting to the NAc.<sup>31</sup> Series resistance (<30 M $\Omega$ ) and input resistance were monitored on-line with a small depolarization voltage (100 ms, +10 mV) followed with each stimulus, and data were not counted if the resistance changed more than 25%. All Data were filtered at 2 kHz and digitized at 10 kHz.

### Brain cannula implantation and drug injection

The animals were anesthetized with pentobarbital sodium (40–50 mg/kg, i.p.). Guided cannulas was implanted above the bilateral NAc (AP:  $-1.53$  mm; lateral:  $\pm 0.8$  mm; depth:  $-4.0$  mm from skull). An injection needle (32 G, Hamilton Company, USA) was inserted through the guide cannula, and drug solution or vehicle (0.5  $\mu\text{L}$ ) was slowly injected over 5 min using a Hamilton syringe. Locations of the cannula placement were confirmed at the time of tissue harvest.

### Data Analysis

All data were expressed as mean  $\pm$  SEM. The behavioral data were analyzed by repeated measures (RM) two-way ANOVA followed by Bonferroni test. Electrophysiological data variance was analyzed using one-way ANOVA or two-tailed

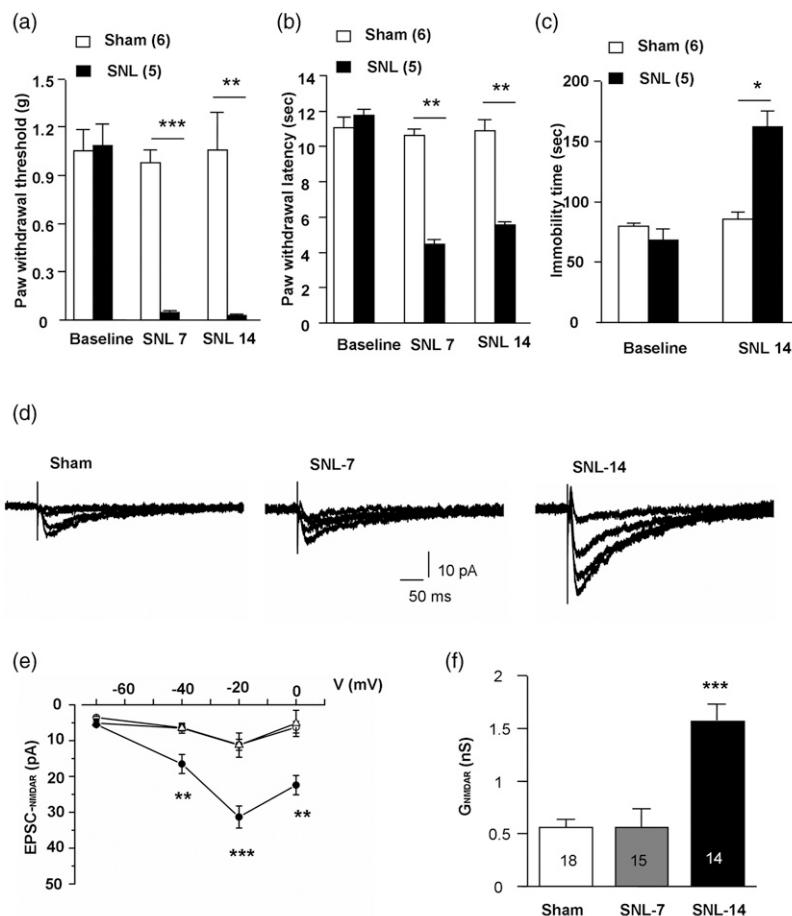
Student's *t*-test. The criterion for statistical significance was  $p < 0.05$ .

## Results

*SNL-induced neuropathic pain modulates kinetic properties of NMDAR-EPSCs.* We used the SNL model to construct the time course of the development of chronic pain and depressive behavior.<sup>18</sup> We examined the mechanical allodynia behavior at day 7 after the SNL procedure, and this abnormal sensory behavior persisted for at least 14 days following SNL (Figure 1(a),  $p < 0.001$ , two-way ANOVA RM test). In contrast, the sham group did not show any sensory abnormality (Figure 1(a)). In addition, the paw withdrawal latency was significantly decreased 7 and 14 days after the SNL operation (Figure 1(b),  $p < 0.01$ , two-way ANOVA RM test). We then measured the forced swimming behavior, which indicates depressive performance in mice.<sup>29</sup> The immobility time of SNL-treated mice was markedly increased than sham group mice (Figure 1(c),  $p < 0.05$ , two-way ANOVA RM test). These data indicate that spinal nerve injury induces a persistent sensory abnormality and depressive-like behavior in mice.

To examine the impact of SNL on kinetic properties of NMDAR-EPSCs, we analyzed the current-voltage relationship of evoked EPSC in the NAc shell MSNs from sham and SNL-operated mice. NMDAR-EPSCs were obtained at synapses of MSNs which held at a range of potentials from  $-70$  mV to 0 mV (Figure 1(d)). The peak amplitude of NMDAR-EPSCs were significant larger in SNL-14 group than that in sham- and SNL-7 groups (Figure 1(e),  $p < 0.001$ , two-way ANOVA RM). Moreover, The NMDAR channel conductance was further markedly increased in SNL-14, but not changed in SNL-7 group (Figure 1(f),  $p < 0.001$ , one-way ANOVA test).

*NR2C/2D-containing NMDAR-EPSCs increased in NAc shell MSNs.* NR2C/2D containing NMDARs have large inward current response at lower membrane potentials due to its weak Mg<sup>2+</sup> block.<sup>21</sup> To examine whether the NR2C/2D containing NMDARs contribute to the enhancement of synaptic NMDAR-EPSCs after SNL, we tested the effect of PPDA<sup>32</sup> (a most potent and selective NR2C/2D-perferring antagonist) on peak amplitude of EPSCs at synapse of NAc MSNs. We found that the PPDA caused a larger decrease in peak amplitude of NMDAR-EPSCs in SNL mice than that in sham-operated mice (Figure 2(a) and (b),  $p < 0.05$ , Student's *t*-test). To further confirm the contribution of NR2C/2D-containing NMDARs in SNL, we applied a selective potentiator of NR2C/2D, CIQ (20  $\mu\text{M}$ ) in brain slices.<sup>33,34</sup> CIQ significantly enhanced the evoked NMDAR-EPSCs in SNL-operated mice, but no change in sham-operated mice (Figure 2(c) and (d),  $p < 0.01$ , Student's *t*-test), supporting that SNL enhanced the function of NR2C/2D-containing NMDARs.



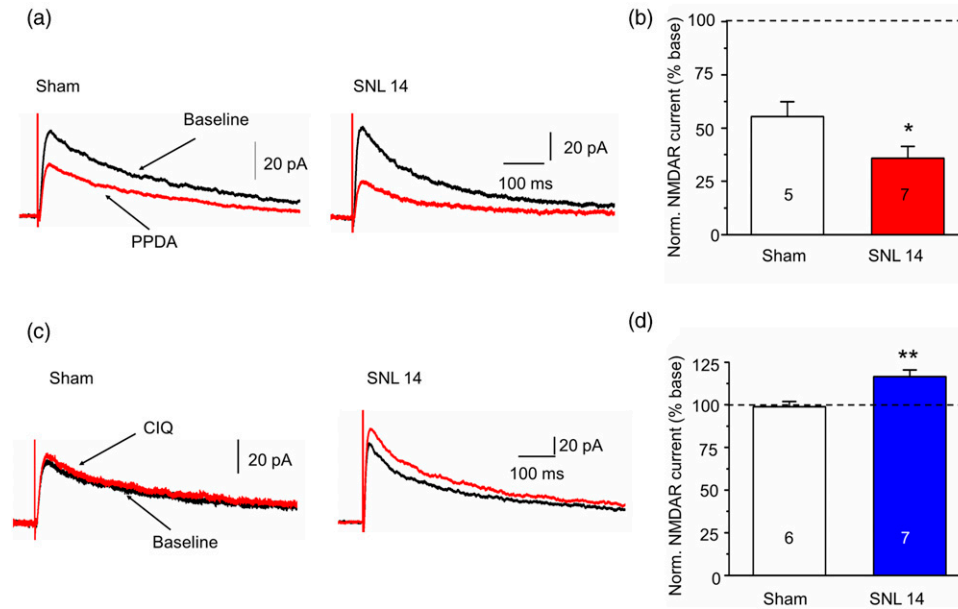
**Figure 1.** Spinal nerve injury modulates the kinetic properties of N-methyl-d-aspartate receptor excitatory postsynaptic currents in the nucleus accumbens shell medium spiny neurons. **A**, spinal nerve ligation-operated mice developed persistent mechanical allodynia after surgery (ANOVA with Bonferroni post-test,  $n = 5-6$ ,  $***p < 0.001$ ,  $**p < 0.01$ ). **B**, spinal nerve ligation-treated mice showed shorter paw withdrawal latency than sham-treated mice (ANOVA with Bonferroni post-test,  $n = 5-6$ ,  $**p < 0.01$ ). **C**, spinal nerve ligation increased immobility time of forced swimming behavior on postoperative day 14 ( $n = 5-6$ , ANOVA with Bonferroni post-test,  $*p < 0.05$ ). **D**, Samples of evoked N-methyl-d-aspartate receptor excitatory postsynaptic currents were recorded at synapse of nucleus accumbens shell medium spiny neurons when the membrane potentials were holding from  $-70$  to  $0$  mV from sham, after 7 days and 14 days of spinal nerve ligation-operated (spinal nerve ligation-7 and -14) mice. **E**, The peak amplitude of N-methyl-d-aspartate receptor excitatory postsynaptic currents was plotted against the holding potentials in sham, spinal nerve ligation-7 and spinal nerve ligation-14 animals. **F**, Summary data show the N-methyl-d-aspartate receptor conductance in sham, spinal nerve ligation-7 and SNL14 mice of nucleus accumbens shell medium spiny neurons, when the membrane potential held at  $-20$  mV ( $n = 14-18$ ,  $***p < 0.001$ , one-way ANOVA followed by Bonferroni post-test).

*Application of PPDA into the NAc relieves SNL-induced neuropathic pain and depression.* To examine whether the effects of PPDA were specific to SNL-induced neuropathic pain, we injected PPDA into the NAc. As shown in Figure 3, NAc injection of PPDA significantly attenuated paw withdrawal threshold 3 h after administration in SNL-operated mice (Figure 3(a),  $p < 0.05$ , PPDA 0.1 ng/ul vs. sham;  $p < 0.01$ , PPDA 0.5 ng/ul vs. sham, two-way ANOVA RM test). However, PPDA administration did not change paw withdrawal latency to radiant heat (Figure 3(b)). Furthermore, the immobility time of SNL-treated mice were significantly prevented by PPDA treatment compared to that of the saline-treated group (Figure 3(c),  $p < 0.01$ , PPDA 0.1 ng/ul vs. sham;  $p < 0.001$ , PPDA 0.5 ng/ul vs. sham, two-way ANOVA

RM test). These results suggested that specific blunting NR2C/2D-containing NMDARs at the NAc potentially relieves neuropathic pain and depressive-like behavior.

## Discussion

In this study, we identified physiological properties of NMDAR-EPSCs at synapses of the NAc shell MSNs after peripheral nerve injury. Our results revealed a functional up-regulation of NR2C/2D-containing NMDAR in NAc shell MSNs following SNL at 14 days post-operation. We further demonstrated that pharmacological blocking this type of NMDAR subunits at the NAc relieved SNL-induced mechanical allodynia and related depressive-like behavior. As

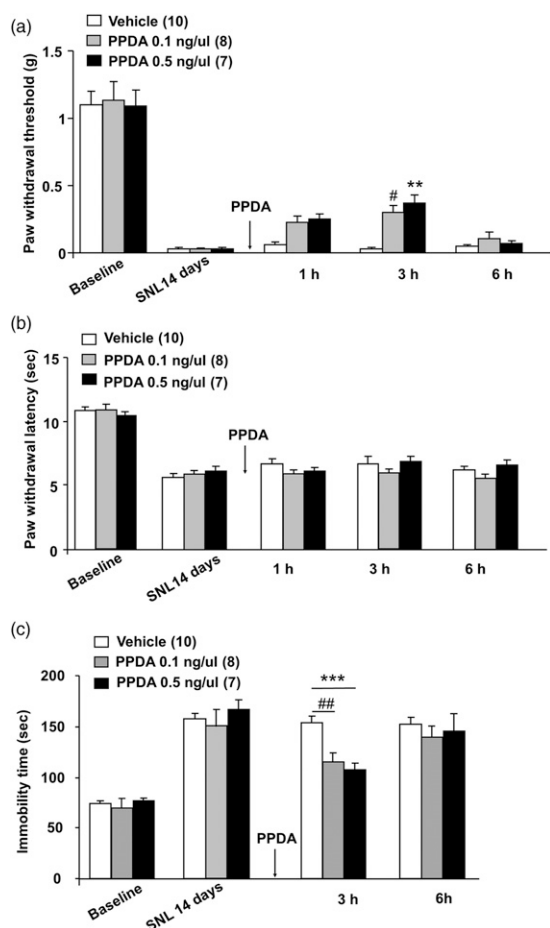


**Figure 2.** Effects of NR2C/NR2D subunits selective antagonist or potentiator on the evoked N-methyl-d-aspartate receptor-excitatory postsynaptic currents in the nucleus accumbens shell medium spiny neurons. A, Sample traces showed that PPDA (1  $\mu$ M) reduced the basal N-methyl-d-aspartate receptor-excitatory postsynaptic currents in sham and spinal nerve ligation mice. B, The mean percentage inhibition of PPDA on the peak amplitude of evoked N-methyl-d-aspartate receptor-excitatory postsynaptic currents in the nucleus accumbens medium spiny neurons from sham and spinal nerve ligation-operated mice ( $n = 5-7$ ,  $*p < 0.05$ , Student's  $t$ -test). C, Sample traces showed that CIQ (20  $\mu$ M) potentiated the basal amplitude of N-methyl-d-aspartate receptor-excitatory postsynaptic currents from spinal nerve ligation-operated mice. D, The mean percentage potentiation of CIQ on the peak amplitude of evoked N-methyl-d-aspartate receptor-excitatory postsynaptic currents in the nucleus accumbens medium spiny neurons from sham and spinal nerve ligation-operated mice ( $n = 6-7$ ,  $**p < 0.01$ , Student's  $t$ -test).

previously reported, NR2B-containing NMDARs protein expression and function at synapses of NAc MSNs is altered after nerve injury and related to the sensory sensitization and negative emotion.<sup>5,18</sup> Our data suggest that the enhancement in NR2C/2D subunits-containing NMDARs' function at synapses of NAc shell MSNs after SNL-operation is involved in the neuropathic pain and related depressive-like behavior.

The NAc receives glutamatergic synaptic afferents from several regions involving in the integration of motivational and affective behaviors.<sup>35</sup> Disruption of NAc excitatory glutamatergic synaptic transmission has been shown to link with negative affection produced by chronic pain.<sup>6,18</sup> Recent studies have indicated that NMDAR-mediated signaling in the NAc plays a critical role in the modulation of affective behaviors in chronic pain states.<sup>5,18</sup> The finding showed that peripheral spinal nerve injury- or CFA-induced neuroinflammation hampered NMDAR-dependent long-term depression in NAc, which mediated the motivational behavior decrease during chronic pain condition.<sup>5,6</sup> In current study, we found a deviation of NMDAR current-voltage curve when membrane potential held at  $-40$  to  $0$  mV on day 14 post-SNL. NMDARs are heteromeric complexes (NR1/NR2 complex) with distinct macroscopic biophysical and pharmacological properties which mainly determined by the NR2 subtypes.<sup>11,36</sup> The activation of NMDAR is linked to the

voltage-dependent  $Mg^{2+}$  block, and NR2C/2D subunits-containing receptors are less sensitive to  $Mg^{2+}$  blockade compared with NR2A or NR2B subunit-containing receptors in physiological condition.<sup>21</sup> Thus, it is consistent with the evidence that the NMDAR mediated more inward currents when cells were held at relatively hyperpolarized membrane potentials at day 14 post-SNL. Compared with NR2A or NR2B subunits expression, lower expression level of NR2C and NR2D subunits at forebrain in normal animals<sup>20,21</sup> may potentially cause the changes only observed on day 14 post-SNL but not on day 7 post-SNL. In agreement with the biophysical properties modulation of NMDAR channel, we also found the channel conductance at the negative holding potentials ( $-40$  to  $0$  mV) was increased on day 14 post-SNL but not on day 7 post-SNL. Similarly, the functional modulation of NR2C/NR2D subunits containing NMDAR in the NAc also contribute to glutamatergic synaptic maladaptations in cocaine exposure followed by 2-weeks of abstinence.<sup>25</sup> Previous studies have demonstrated that the weighted decay time of NMDAR at positive holding potential ( $+40$  mV) was significantly increased on day 7 of post nerve injury or earlier times of neural inflammation, indicating that the NR2B subunits were involved in synaptic plasticity on day 7 post-SNL.<sup>11,37</sup> Our data suggest that the NR2C/2D subunits in the NAc most likely remodel the NMDAR function on day 14 or later days of post-SNL.



**Figure 3.** Inhibition of NR2C/D in the nucleus accumbens alleviates spinal nerve ligation-induced mechanical allodynia and depressive-like behavior. **A**, Intra-nucleus accumbens injection of NR2C/D selective antagonist PPDA (0.1 ng/ $\mu$ L, grey; 0.5 ng/ $\mu$ L, black) 14 days after spinal nerve ligation significantly increased the paw withdrawal threshold 3 h after injection. (\*\* $p < 0.01$ , # $p < 0.05$ , two-way RM ANOVA.  $n = 7-10$  mice/group). **B**, PPDA did not change the paw withdrawal latency ( $p > 0.05$ , two-way RM ANOVA.  $n = 7-10$  mice/group). **C**, PPDA decreased the immobility time of forced swimming behavior 3 h after injection (\*\* $p < 0.001$ , ## $p < 0.01$ , two-way RM ANOVA.  $n = 7-10$  mice/group).

We further used an NR2C/2D subunits antagonist to test the contribution of these receptors to the NMDAR-EPSCs at glutamatergic synapse in the NAc after SNL. Based on a previous study which assayed PPDA pharmacological properties at recombinant channels expressed in cultured cells, the  $K_i$  values and selectivity pattern for NR2 subunits are NR2C > NR2D > NR2B > NR2A.<sup>32</sup> Previous studies have identified that 1  $\mu$ M dose of PPDA application selectively blocked the function of NR2C/2D subunits-containing NMDARs in brain slice.<sup>38,39</sup> In the present experiment, 15–20 min perfusing of 1  $\mu$ M PPDA caused approximately 30% reduction on peak amplitude in sham and approximately 2-fold reduction on peak amplitude after SNL. The inhibition of PPDA in sham-operated mice may

partly contribute to a suppressive effect on other subunits of NMDAR, or NR2C/2D subunits also mediate the glutamatergic signaling in health animals. We further employed a NR2C/2D selective potentiator, CIQ,<sup>26,29</sup> to assess the NMDAR subunit stoichiometry in NAc. CIQ selectively potentiated NMDAR-EPSCs from SNL-14 mice, but not change in sham-operated animals. These findings suggest that functional NR2C/2D subunits potentially mediate the synaptic plasticity in the NAc from SNL mice. However, the specific brain region of synaptic afferents on NAc MSNs needs further investigation in the future.

Our recent experiment showed that intra-NAc injection of NR2B subunit antagonist during days of 7–10 post-SNL alleviated the sensitivity of pain sensory and related depressive-like behaviors.<sup>18</sup> Furthermore, the NR2B subunit is highly prevalent in the whole brain, and has been linked with long-term depression/potential at NAc synapses for regulating the affective and motivational behaviors.<sup>40-42</sup> As the changes of NMDAR current-voltage curve and pharmacological experiments are consistent with the functional enhancement of NR2C/2D receptors after SNL, we intra-NAc injected with PPDA to assess the NR2C/2D containing NMDARs on sensory and emotional behaviors. PPDA attenuated SNL-induced mechanical allodynia and depressive-like behavior. This is in agreement with a previous study which indicated that intracisternal administration of PPDA attenuated nociceptive behavior through p38 MAPK signaling in trigeminal neuropathic pain animals.<sup>37</sup> However, PPDA did not alleviate the thermal hyperalgesia behavior after SNL. The detailed mechanisms underlying PPDA analgesia is not clear, and the receptors expression phase and cell distribution should be considered in the future studies.

Taken together, our data for the first time showed that functional up-regulation of NR2C/2D subunits-containing NMDARs in the NAc plays an important role in modulating peripheral nerve injury-induced neuropathic pain and depression. In particular, functional enhancement of NR2C/2D subunits appeared in relative late stage (14 days) after nerve injury, suggesting that inhibition NR2C/2D containing NMDARs at the NAc may alleviate the established neuropathic pain and negative emotion.

### Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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