Persistent neuropathic pain increases synaptic GluA1 subunit levels in core and shell subregions of the nucleus accumbens

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Abstract

The nucleus accumbens (NAc) is a key component of the brain reward system, and it is composed of core and shell subregions. Glutamate transmission through AMPA-type receptors in both core and shell of the NAc has been shown to regulate reward- and aversion-type behaviors. Previous studies have additionally demonstrated a role for AMPA receptor signaling in the NAc in chronic pain states. Here, we show that persistent neuropathic pain, modeled by spared nerve injury (SNI), selectively increases the numbers of GluA1 subunits of AMPA receptors at the synapse of both core and shell subregions. Such increases are not observed, however, for the GluA2 subunits. Furthermore, we find that phosphorylation at Ser845-GluA1 is increased by SNI at both core and shell subregions. These results demonstrate that persistent neuropathic pain increases AMPA receptor delivery to the synapse in both NAc core and shell, implying a role for AMPA receptor signaling in these regions in pain states.

Keywords

GluA1; nucleus accumbens; SNI; neuropathic pain; core; shell

1. Introduction

The nucleus accumbens (NAc) is well-known to mediate reward-based and aversion-driven behaviors [19, 31]. Not surprisingly, human imaging studies have consistently shown that chronic pain activates the NAc [3, 4, 6]. At the circuit level, the NAc forms reciprocal projections with a number of regions critical for pain regulation including the amygdala, thalamus, prefrontal cortex (PFC) and hippocampus [1, 2, 32]. At the molecular level, opioid signaling in the NAc mediates placebo effects, whereas dopamine signaling has been shown to regulate descending inhibition, stress-induced hyperalgesia**,** and negative reinforcement

from pain-relief [20, 38, 41, 48]. Thus, based on imaging and pharmacological studies, the NAc likely plays an important role in the regulation of chronic pain. The NAc is composed of the core and shell subregions, and synaptic events in both regions are involved in reward-based behaviors [7, 25, 34, 42, 43]. However, the effect of chronic pain on molecular signaling specifically in the core or shell subregion is not yet known.

Previous studies have shown that glutamate signaling in the NAc can regulate pain sensitivity [20, 21, 23]. While there are multiple glutamate receptors in the NAc, transmission through AMPA receptors is particularly important in reward- and aversion-driven behaviors [15, 28, 42, 47, 54]. AMPA receptors are composed of four subunits, GluA1-4. The majority of AMPA receptors contains GluA2 subunits and is impermeable to Ca^{2+}. On the other hand, GluA2-lacking AMPA receptors, predominantly formed by GluA1 homomers, conduct Ca^{2+} and are also known as Ca^{2+}-permeable AMPA receptors (CPARs) [16, 35]. Chronic pain has been shown to selectively increase GluA1 subunit levels at the synapse in the NAc, likely through increased trafficking, and this selective increase in GluA1 subunits leads to the formation of CPARs [24, 51]. CPARs, in turn, can regulate the affective symptoms of chronic pain [24]. However, it is not yet known whether increased synaptic delivery of GluA1 subunits occurs specifically in the core or shell subregion of the NAc in chronic pain states.

In this study, we examined AMPA receptor subunit levels at the synapse of core and shell subregions of the NAc using a well-known chronic neuropathic pain model (spared nerve injury – SNI – model). We found that chronic pain selectively increases GluA1 levels at the synapse in both the core and the shell, hence suggesting a role of AMPA receptor signaling in both regions for pain regulation.

2. Methods

2.1. Animals

All procedures in this study were approved by the New York University School of Medicine Institutional Animal Care and Use Committee (IACUC) as consistent with the National Institute of Health (NIH) *Guide for the Care and Use of Laboratory Animals* (publication number 85-23) to ensure minimal animal use and discomfort. Male Sprague-Dawley rats were purchased from Taconic Farms, Albany, NY and kept in the NYU Langone Medical Center's Central Animal Facility and at Mispro Biotech Services Facility in Alexandria Center for Life Science, with controlled humidity, room temperature, and 12-h (6:30 AM to 6:30 PM) light-dark cycle. Food and water were available *ad libitum*. Animals arrived to the animal facility at 250 to 300 grams and were given on average 10 days to adjust to the new environment prior to the onset of any experiments.

2.2. Spared Nerve Injury (SNI) surgery

The Spared Nerve Injury (SNI) surgery was previously described in detail [17, 57]. Briefly, under Isoflurane anesthesia (1.5 to 2%), the skin on the lateral surface of the right thigh of rat was incised, and a section was made through the biceps femoris muscle to expose the three branches of sciatic nerve: sural, common peroneal and tibial nerves. The common peroneal and tibial nerves were tied with non-absorbent 5.0 silk sutures at the point of

trifurcation. The nerves were then cut distal to each knot, and about 3 to 5 mm of the distal ends were removed. In sham surgeries (control), above nerves were dissected but not cut. Muscle layer was then sutured close, while skin was stapled. Staples were removed prior to behavioral testing.

2.3. Subcellular fractionation and Western blotting

Rats were anesthetized with isofluorane (1.5–2%) and decapitated immediately. Brains were quickly removed and nucleus accumbens were collected on ice. The NAc were dissected from 1.08 to 2.52mm anterior to Bregma, with an average sample weight of 40mg. Dissections of core and shell were performed according to coordinates of the standard rat atlas. The NAc core was dissected using a circular micropunch of approximately 1mm radius centered at the anterior commissure, and areas medial and ventro-lateral to (immediately surrounding) the NAc core were collected as NAc shell under a dissecting microscope [37, 55]. Synaptosome fractions of core and shell were prepared as described previously [29, 46]. Briefly, samples were homogenized in an ice-cold solution A (0.32 M sucrose, 1 mM $NaHCO_3$, 1 mM $MgCl_2$, 0.5 mM $CaCl_2$, 0.1 mM PMSF and 1x Complete Protease Inhibitors). Homogenates were centrifuged at 4,000 rpm for 10 min. The supernatant was collected and the pellet re-homogenized in solution A and centrifuged again for 10 min. Combined supernatants were subjected to a second centrifugation at 3,000 rpm for 10 min. Supernatants were then spun at 14,000 rpm for 30 minutes. Pellet was resuspended in solution B (0.32 M sucrose, 1 mM $NaHCO_3$) and homogenized. Homogenate was layered on top of a 5 mL 1 M sucrose and 1.2 M sucrose gradient and centrifuged at 30,000 rpm for 2 hours. Purified synaptosomes were collected at the 1 M and 1.2 M sucrose interface, suspended in solution B and centrifuged at 40,000 rpm for 45 min. Synaptosomal pellets were resuspended in 25 mM TRIS with 4% SDS. Fractions were analyzed by Western blot on SDS-PAGE gels as described previously [29, 46]. The following antibodies were used: GluA1 (1:1,000, Millipore), phospho-Ser 845 GluA1 (1:1,000, Millipore), GluA2 (1:1,000, Millipore) and tubulin (1:30,000, Sigma).

2.4. Animal Behavioral Tests

Mechanical allodynia test—A traditional Dixon up-down method with von Frey filaments was used to measure mechanical allodynia as described previously [9, 12, 57]. Rats were individually placed into plexiglass chambers over a mesh table and acclimated for 20 min before the onset of examination. Beginning with 2.55g, von Frey filaments in a set with logarithmically incremental stiffness (0.45, 0.75, 1.20, 2.55, 4.40, 6.10, 10.50, 15.10 g) were applied to the lateral 1/3 of right paws (in the distribution of the sural nerve) of rats. 50% withdrawal threshold was calculated as described previously [57].

Cold allodynia test—Animals were individually placed into plexiglass chambers and acclimated for 20 min. A drop of acetone was applied to the lateral plantar surface of the paws. As previously described [26, 30, 57], the following scoring system was applied. 0: no visible response or startle response lasting <0.5 second; 1: paw withdrawal lasting <5 seconds; 2: withdrawal lasting 5 to 10 seconds; 3: prolonged repetitive withdrawal lasting > 10s. Acetone was applied 5 times to each paw, and an average score was calculated. Cold

allodynia tests were typically done after mechanical allodynia tests, and observers were blinded to the test conditions.

2.5. Statistical analysis

For mechanical and cold allodynia, a two-way ANOVA with repeated measures and *post hoc* multiple pair-wise comparison Bonferroni tests was used to compare the 50% withdrawal threshold and cold scores of SNI- and sham-treated rats. For Western blots, unpaired two-tailed Student's t tests were used to analyze the protein levels in SNI- versus sham-treated rats. All data were analyzed using GraphPad Prism Version 5 software (GraphPad, La Jolla, CA).

3. Results

3.1. SNI causes persistent neuropathic pain

We used the SNI model, a peripheral nerve injury model of chronic neuropathic pain [17, 57], to study the effect of pain on AMPA receptor trafficking in the NAc. We surgically resected two of three branches of the sciatic nerve, causing permanent nerve injury and neuropathic pain [17]. One day after the SNI procedure, rats began to experience mechanical allodynia, as demonstrated by a decreased paw withdrawal threshold compared with control (sham-treated) rats (1.3gm vs. 12.8gm, $p < 0.0001$, Fig. 1*A*). Likewise, SNI-treated rats displayed cold allodynia, as shown by an increased cold score compared with control rats (2.24 vs. 0.26, $p < 0.0001$, Fig. 1B). Phenotypes of mechanical and cold allodynia indicate the development of neuropathic pain. Similar to earlier reports in SNI-treated rats, symptoms of allodynia persisted for at least 14 days (a decrease in mechanical threshold from 7.1gm to 0.45gm on day 14 after SNI, $p < 0.01$, Fig. 1A; an increase in cold score from 0.27 to 2.24, $p < 0.0001$, Fig. 1B) [17, 24, 57].

3.2. SNI increases GluA1 levels at the synapse of core and shell subregions of the NAc

To understand how chronic pain regulates AMPA receptor signaling in the NAc, we measured the levels of AMPA receptor subunits from synaptoneurosome preparations of NAc neurons 14 days after SNI or sham surgery. Over 90% of neurons in the NAc are medium spiny neurons (MSNs), and synaptoneurosome preparations reflect synaptic fractions of these neurons. GluA1 and 2 are predominantly expressed in the MSNs. The NAc is comprised of core and shell subregions. This anatomic distinction has been shown to have functional significance [50]. Thus, we measured GluA1 and GluA2 levels in the core and the shell. We found a substantial (40%) increase in the GluA1 subunit levels in the NAc core of SNI-treated animals compared with GluA1 levels in sham controls ($p < 0.05$, Fig. 2A). The level of GluA2 subunits in the core, in contrast, remained unchanged (Fig. 2B). Next, we measured GluA1 and GluA2 levels in the shell subregion of NAc. Similar to what we found in the NAc core, GluA1 levels are also elevated (by 37%) at the synapse of the shell in SNI-treated rats, whereas GluA2 levels remain the same (Fig. 3A, B). These results indicate that chronic pain causes a selective increase in the GluA1 subunit levels in both core and shell subregions of the NAc.

3.3. SNI causes increased GluA1 trafficking to the synaptic surface of NAc core and shell

Increased GluA1 subunit expression at the synapse can be caused by increased exocytotic trafficking or localized synthesis. It can also be caused by decreased endocytosis or degradation [5]. To assess the role of trafficking, we measured the level of phosphorylation of the Ser845 residue on the GluA1 protein. The phosphorylation of Ser845 is known to be a required step for the delivery of GluA1 to the synaptic surface [49]. We found that phospho-Ser845 was increased in both core and shell subregions after SNI ($p<0.05$, Fig. 4A, B). This level of increase is comparable to the increase in levels of GluA1 (Fig. 2A, 3A). These data suggest that this trafficking mechanism is conserved in the chronic pain state and contributes to the accumulation of GluA1 subunits at the synaptic surface.

4. Discussion

Imaging studies have identified that chronic pain activates the NAc [3, 4, 6, 22]. However, direct biochemical or biophysical analysis of molecular signaling in the NAc remains lacking in chronic pain models. Particularly, AMPA receptor signaling, which is crucial for the NAc function under rewarding conditions, remains poorly studied at detailed anatomic levels in pain states. Our current study provides the first evidence for pain-induced GluA1 increases at MSN synapses in both core and shell subregions of the NAc.

The SNI model is widely used to study molecular changes in the brain in response to chronic pain [10, 11, 17, 33, 39]. SNI, however, can lead to both nerve injury and pain. A previous study has shown that increases in GluA1 levels at the NAc synapse do not manifest until at least 7 days after SNI, suggesting that persistent pain, rather than acute nerve injury, triggers the synaptic targeting of GluA1 subunits [52]. More importantly, increased GluA1 delivery to the NAc synapse has also been demonstrated in a persistent inflammatory pain model that does not involve nerve injuries [52]. Thus, we believe that chronic pain, rather than nerve injury, causes the GluA1 increases observed in the current study.

A selective increase in GluA1 levels at the synapse suggests the formation of GluA2-lacking AMPA receptors [16, 27, 35]. GluA2-lacking receptors, also known as CPARs, can facilitate intracellular Ca^{2+} signaling and have higher single unit conductance. These unique biophysical properties enable CPARs to play an important role at the synapse, including the induction of long-term potentiation (LTP) or long-term depression (LTD) [16, 27, 35]. By regulating synaptic strength, CPARs modulate a diverse set of behaviors [14, 15, 24, 34, 55, 56]. Our data raises the possibility of CPAR formation in both the core and shell subregions in pain states. Of course, further physiological studies are needed to test such possibilities. Additionally, whereas GluA2 subunits are constitutively trafficked to the synapse, the synaptic incorporation of GluA1 subunits requires sequential phosphorylations at Ser845 and Ser818 residues [8, 18, 49, 53]. Our data confirms that the phosphorylation of Ser845 is involved in the synaptic targeting of GluA1 subunits to the NAc synapse in chronic pain states. Our data, however, do not necessarily exclude other potential causes for increased GluA1 levels such as increased local synthesis.

A number of studies have described GluA1 upregulation in the NAc after repeated or prolonged consumption of natural rewards or cocaine [45, 55]. In these studies, homeostatic

plasticity has been posited as a possible mechanism for the increase in GluA1 subunits. In these studies on rewards, the length of time required to increase GluA1 levels is in general compatible with the time course found in our study. Similarly, in a previous study on AMPA receptor trafficking, GluA1 upregulation at the NAc synapse is found to occur after pain has become persistent or chronic [52]. Thus, we speculate that GluA1 upregulation in the core and shell of NAc in persistent pain states may represent a form of homeostatic plasticity in the reward system. It would be interesting to test this potential mechanism in future studies.

Both core and shell subregions of the NAc have been shown to regulate reward and aversion-driven behaviors [13, 34, 40], and previous studies indicate that the core plays a role in nociception as well [20, 36]. While core and shell are both composed primarily of MSNs, there are differences between these two regions in terms of cellular morphology, neurochemistry, and afferent and efferent projections [50]. Functionally, the core is thought to mediate cue-conditioned behavioral activation, such as reward-seeking. The shell, meanwhile, has been shown to code the salience of a behavioral condition [50]. Our data demonstrates that AMPA receptor plasticity occurs in both regions under chronic pain conditions. Thus, we expect that glutamate signaling in the core could regulate behavioral responses to pain such as drug seeking or avoidance of pain stimulus, whereas signaling in the shell could regulate the processing of the aversive quality of pain [13, 40, 44].

5. Conclusion

In conclusion, our results show that chronic pain leads to selective GluA1 upregulation at the synapse of core and shell regions of the NAc. These results indicate that AMPA receptor signaling in both regions is likely to play an important role in the pathogenesis and regulation of chronic pain.

Acknowledgments

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HIGHLIGHTS

- We showed that spared nerve injury causes persistent neuropathic pain

- We showed that GluA1 levels at synapses are increased in both core and shell subregions of the NAc in chronic pain conditions

- We showed that chronic pain causes an increase in the phosphorylation of Ser845 residue of the GluA1 in the NAc
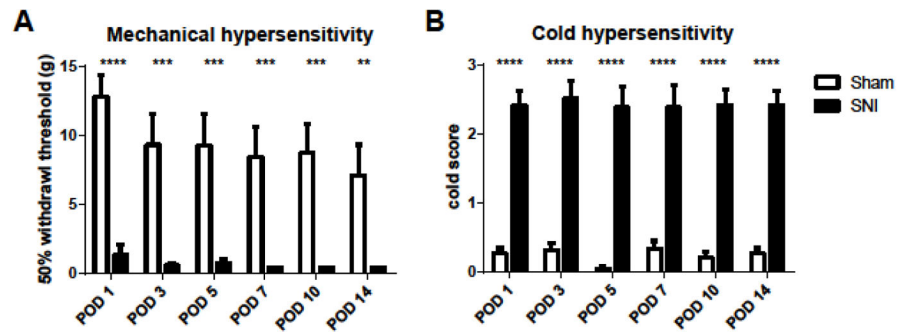
Figure 1.
SNI causes persistent neuropathic pain. **A,** SNI-operated rats developed mechanical allodynia after surgery, compared with sham-operated rats. Two-way ANOVA with repeated measures and Bonferroni post-test. n=9–10, ** $p<0.01$. **B,** Animals after SNI developed cold allodynia. Two-way ANOVA. n=9–10, **** $p<0.0001$. Error bars show mean and s.e.m.
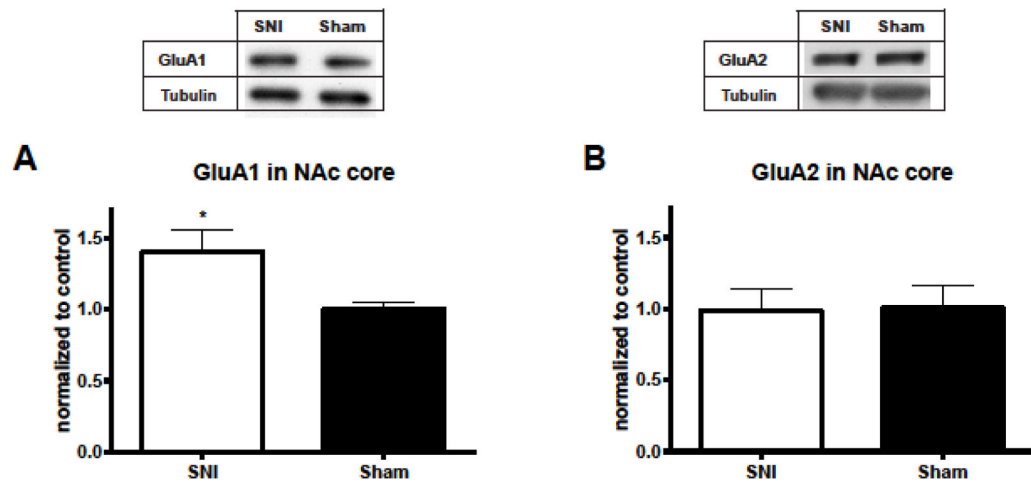
Figure 2.
SNI selectively increases GluA1 levels at the synapse of the core subregion of NAc. **A,** SNI resulted in an increase in GluA1 subunits in the synaptoneurosomes of NAc core. Student's t test, n=13, * $p<0.05$. **B,** SNI caused no changes in GluA2 subunit levels at the synapse. n=9, $p>0.05$. Data were normalized to values in the sham group.
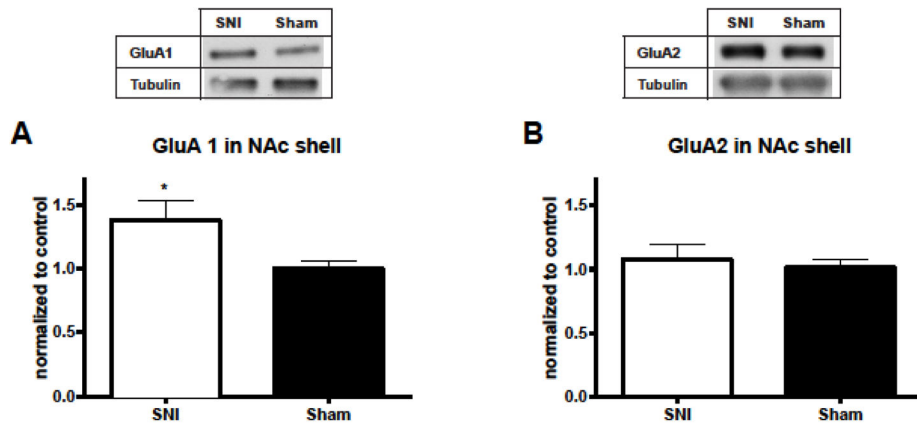
Figure 3.
SNI selectively increases GluA1 levels at the synapse of the shell subregion of NAc. **A,** SNI resulted in an increase in GluA1 subunits in the synaptoneurosomes of NAc shell. Student's t test, n=12, * p<0.05. **B,** SNI caused no changes in GluA2 subunits at the synapse. n=12, p>0.05. Data were normalized to values in the sham group.
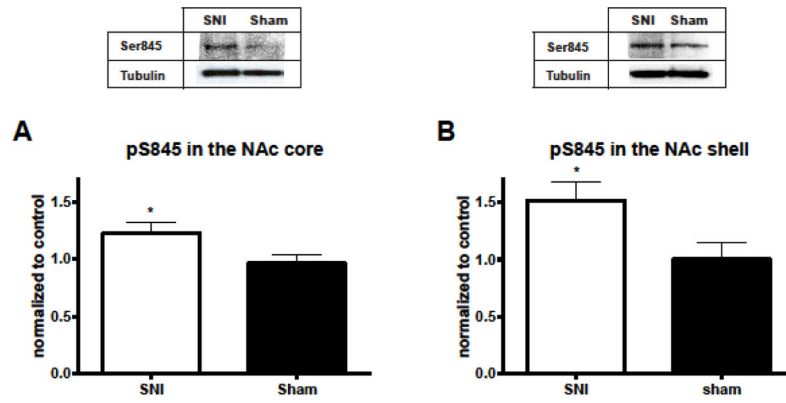
Figure 4.

SNI increases phosphorylation at Ser845 at synapses of the core and shell subregions. **A,** SNI increased phosphorylation at the Ser845 residue of GluA1 subunits in the synaptoneurosomes of NAc core. Student's t test, n=10–11, * p<0.05. **B,** SNI increased phosphorylation at Ser845 in the synaptoneurosomes of NAc shell. Student's t test, n=6, * p<0.05. Data were normalized to values in the sham group.