Continuous infusion of substance P into rat striatum relieves mechanical hypersensitivity caused by a partial sciatic nerve ligation via activation of striatal muscarinic receptors

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Continuous infusion of substance P into rat striatum relieves mechanical hypersensitivity caused by a partial sciatic nerve ligation via activation of striatal muscarinic receptors

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Highlights

- ・Continuous substance P infusion into striatum relieves neuropathic pain.
- ・Continuous enkephalin infusion into striatum is not antinociceptive.
- ・Neurokinin-1 receptor block suppresses the antinociceptive effect of substance P.
- ・Muscarinic receptors are required for the antinociceptive effect of substance P.

Abstract

Previous studies have demonstrated that continuous substance P (SP) infusion into the rat striatum attenuated hind paw formalin-induced nociceptive behaviors and mechanical hypersensitivity via a neurokinin-1 (NK1) receptor dependent mechanism. However, whether there is a role of striatal infusion of SP on chronic, neuropathic pain has yet to be demonstrated.

The present study investigated the effect of continuous SP infusion into the rat striatum using a reverse microdialysis method is antinociceptive in a rat model of chronic, mononeuropathic pain. Two weeks after partial sciatic nerve injury, the ipsilateral hind paw demonstrated mechanical hypersensitivity. Infusion of SP (0.2, 0.4, or 0.8 µg/mL, 1 µL/min) for 120 min into the contralateral striatum dose-dependently relieved mechanical hypersensitivity. The antinociceptive effect of SP infusion was inhibited by co-infusion with the NK1 receptor antagonist CP96345 (10 µM). Neither ipsilateral continuous infusion nor acute microinjection of SP (10 ng) into the contralateral striatum was antinociceptive. A role of striatal muscarinic cholinergic neurons is suggested since co-infusion of SP with atropine (10 µM), but not the nicotinic receptor mecamylamine (10 µM), blocked antinociception. The current study suggests that activation of striatal muscarinic receptors through NK1 receptors could be a novel approach to managing chronic pain.

Keywords: muscarinic receptor, neurokinin-1 receptor, neuropathic pain, reverse microdialysis, striatum, substance P

1. Introduction

The striatum is a major component of the basal ganglia and is a crucial component of the extrapyramidal system. In addition, previous studies reported that majority of striatal neurons in the rodents respond to noxious stimulation [1, 2] and treatment with excitatory amino acids, catecholamines, and neuropeptides to the dorsal striatum leads to antinociceptive effects in animal models of neuropathic pain and formalin-induced nociception [3, 4, 5, 6, 7, 8]. Furthermore, patients with neurodegenerative disorders involving the basal ganglia, such as Parkinson's disease and stroke involving the striatum, demonstrate painful dystonia, radiculopathy, and symptoms of central chronic pain [9, 10, 11, 12]. Thus, the striatum is involved not only in modulating motor functioning but also nociceptive processing. The majority of neurons in the striatum are medium spiny neurons (MSNs) comprised of two subpopulations—MSNs co-expressing dopamine D1 receptors and substance P (SP) (direct pathway; D1-MSNs) and MSNs co-expressing dopamine D2 receptors and enkephalin (ENK) (indirect pathway; D2-MSNs) [13, 14, 15]. The remaining striatal neurons are acetylcholine (ACh) -containing large spiny interneurons, which generally express the neurokinin-1 (NK1) receptor [16, 17]. Striatal ACh regulates both striatal MSNs and terminals of dopaminergic

neurons originating within the substantia nigra via muscarinic (mACh) and nicotinic (nACh) receptors [17, 18, 19, 20, 21, 22]. Taken together, the neuronal circuits formed by MSNs and acetylcholinergic interneurons in the striatum might regulate the endogenous antinociceptive pathway.

Substance P is a neuropeptide synthesized from preprotachykinin-A and its preferred target, the NK1 receptor, is expressed in the central nervous system areas involved in sensation such as the spinal dorsal horn and generally involved in motor functioning, such as the striatum, substantia nigra, and globus pallidus [23]. In spinal dorsal horn, SP is released from central terminals of activated primary afferent nociceptors and activates postsynaptic dorsal horn sensory neurons [23, 24]. A recent study also showed that intracerebroventricular injection of SP alleviated carrageenan-induced hind paw inflammatory mechanical allodynia and heat hyperalgesia, which was reversed with pretreatment with a selective NK1 receptor antagonist [25]. Hence, SP diffused in the brain has anti-nociceptive effect. In addition, previous studies showed that continuous infusion of SP into the striatum by reverse microdialysis, not acute microinjection, attenuated formalin-evoked nociceptive behaviors and mechanical hypersensitivity and capsaicin-evoked mechanical hypersensitivity, indicating that activation of

striatal NK1 receptors leads to antinociception [5, 6]. Thus, these data suggest that continuous stimulation of the striatal NK1 receptor could be involved in a part of an endogenous antinociceptive system. Whether this is system can be evoked in chronic, in addition to acute, pain states, has yet to be demonstrated.

Based on our previous findings and worked cited, it is hypothesized that striatal SP can relieve neuropathic pain via modulation of NK1 receptor-expressing acetylcholinergic interneurons. The current study therefore examined the influence of the activation of the striatal SP system by using reverse microdialysis on peripheral neuropathic pain in a rat model. Furthermore, involvement of the striatal cholinergic system in the antinociceptive effect of SP was investigated by using pharmacological approaches.

2. Materials and methods

2.1 Animals and animal care

Male Wistar rats, 6 weeks of age, were purchased from Shimizu Laboratory Supplies Co., Ltd. Rats were housed at room temperature of 22 ± 2 °C, a 12 h light/dark cycle (lights on from 8:00 A.M. to 8:00 P.M.) and allowed free access to food and water during the

experimental period. Animal experiments were conducted in accordance with the ‘‘Guidelines for the Care and Use of Laboratory Animals’’ established by Hiroshima University, and all experimental procedures involving animals were approved by the Committee of Research Facilities for Laboratory Animal Science of Hiroshima University (Permit Number: A11133). The rats displayed good overall health before surgery and after either PSNL or sham surgery, displaying standard locomotor activity throughout the experimental period of evaluation.

2.2 Partial sciatic nerve ligation model (PSNL)

The rat PSNL model was performed as described previously [26]. In brief, using a 6-0 silk suture, approximately one-third to one-half of the diameter of the left sciatic nerve was tightly ligated. In sham-operated rats, the nerve was exposed without ligation. To measure hind paw mechanical withdrawal thresholds after PSNL surgery, and the mechanical stimulus producing a 50% paw withdrawal threshold was determined using the ascending stimulus method [27]. Animals were habituated to the testing cage for 15-30 min before the von Frey test. The mid-plantar surface of the hind paws was probed with von Frey monofilaments (Natsume Seisakusho Co., Tokyo, Japan), starting with the filament that exerts 0.16 g of force. Each hind

paw was tested three times at 10 s intervals. The lowest force that caused two or more responses such as lifting and licking of the hind paw was assigned as the withdrawal threshold. If the animal did not show more than a response against the mechanical stimulation, a higher force von Frey filament was applied. Drugs used in the current study were administrated immediately following baseline withdrawal threshold determination. All experiments were conducted by experimenters who were blind to the treatment condition of the animals.

2.3 Reverse microdialysis

Reverse microdialysis was performed as described previously [5, 6]. In brief, microdialysis probes (dialysis membrane length: 3.0 mm; C-M-4-03HP: Eicom, Kyoto, Japan) were inserted through the guide cannula (0.26 mm caudal to bregma, 4.0 mm lateral to the midline, 4.0 mm below the surface of the skull; CG-4: Eicom) [28] to the right, contralateral side, or left, ipsilateral side, striatum (the dialysis membrane protruded 3.0 mm beyond the tip of guide cannula) and continuously perfused with either sterile artificial cerebrospinal fluid (aCSF: 7.4 g/L NaCl, 0.19 g/L KCl, 0.19 g/L $MgCl_2$, and 0.14 g/L $CaCl_2$), CP96345, mecamylamine, atropine, or SP at a flow rate of l µL/min using a microinfusion pump (Eicom).

Following behavioral assessment, the locations of the microdialysis probes were histologically verified. Successful location of the probe in the striatum was determined based on the region defined by a brain atlas [28].

2.4 Microinjection

Microinjection was performed as described previously [5, 6]. In brief, for microinjection of 10 ng SP, an injection cannula (length: 5.0 mm) was inserted into the guide cannula (0.26 mm caudal to bregma, 4.0 mm lateral to the midline, 4.0 mm below the surface of the skull) [28]. Following behavioral assessment, the locations of the cannula were histologically verified. Successful location of the probe in the striatum was determined based on the region defined by a brain atlas [28].

2.5 Materials

Drugs used in the current study included: Mecamylamine hydrochloride, a non-selective nicotinic acetylcholine receptor antagonist (Nacalai tesque, Inc., Kyoto, Japan); SP (Peptide Institute, Inc., Osaka, Japan); CP96345, a NK-1R antagonist (Pfizer Central

Research, Groton, CT, USA); atropine sulfate hydrate, a non-selective muscarinic acetylcholine receptor antagonist (Wako Pure Chemical Industries, Osaka, Japan). CP96345 was dissolved in DMSO to a final concentration of 0.1% DMSO. Other drugs were dissolved in sterile water. All other reagents were of the highest purity available from commercial sources.

2.6 Statistical analysis

Data are expressed as mean ± standard error of the mean (SEM). To compare between each time point, the microinjection data were analyzed using a repeated measures (RM) one-way analysis of variance (ANOVA) followed by the Dunnett's multiple comparisons test. Other data were analyzed using a two-way RM ANOVA followed by the Sidak's multiple comparisons test, to determine whether there was an interaction between time and treatment. The analyses were performed with PRISM 7.0 software (GraphPad). A probability value (p) of less than 0.05 was considered to be statistically significant.

3. Results

3.1 Hind paw mechanical hypersensitivity in PSNL rats

The development of long-lasting mechanical hypersensitivity in the ipsilateral, left hind paw of PSNL rats lasted for at least 14 days after surgery. Rats with a PSNL had significantly lower withdrawal thresholds, compared with sham-operated rats, of the ipsilateral paw at every time point after surgery (Fig. 1; TIME: $F_{(3, 30)} = 9.445$, $P = 0.0001$; MODEL: $F_{(1, 10)} = 30.35$, $P = 0.0003$; INTERACTION: $F_{(3, 30)} = 4.785$, $P = 0.0077$; Two-way RM ANOVA).

3.2 Effect of continuous SP infusion into the contralateral striatum on PSNL-induced mechanical hypersensitivity

A previous study showed that continuous SP infusion by reverse microdialysis into the striatum attenuated mechanical hypersensitivity evoked by an intraplantar formalin injection [5, 6]. To determine the effect of intrastriatal SP on PSNL-induced mechanical hypersensitivity, SP was infused into the left and right striatum after PSNL surgery. Seven days after surgery, continuous SP infusion (0.4 and 0.8 μg/mL, 1 μL/min for 120 min) into the contralateral striatum ameliorated PSNL-induced mechanical hypersensitivity in a dose-dependent manner [(Fig. 2A and C) TIME: $F_{(4, 68)} = 47.94$, $P < 0.0001$; DRUG: $F_{(3, 17)} = 13.46$, $P < 0.0001$; INTERACTION: $F_{(12, 68)} = 5.88$, $P < 0.0001$; Two-way RM ANOVA (Fig. 2B) TIME: $F_{(4, 36)} =$

2.426 P = 0.0657; DRUG: $F_{(1, 9)}$ = 0.3601, P = 0.5632; INTERACTION: $F_{(4, 36)}$ = 0.6543, P = 0.6277; Two-way RM ANOVA]. In contrast, neither continuous SP infusion (0.4 µg/mL, 1 µL/min for 120 min) into the ipsilateral striatum nor acute SP microinjection into the contralateral striatum (10 ng, 1 µL/min for 1min) attenuated PSNL-induced hypersensitivity [(Fig. 2D) TIME: $F_{(4, 40)}$ = 2.783, P = 0.0395; DRUG: $F_{(1, 10)}$ = 1.595, P = 0.2352; INTERACTION: $F_{(4, 40)}$ = 0.1492, P = 0.9623; Two-way RM ANOVA; (Fig. 2E) $F_{(4, 16)}$ = 6.627, P = 0.0024; RM one-way ANOVA]. Furthermore, contralateral, continuous SP infusion (0.4 µg/mL, 1 µL/min for 120 min) significantly attenuated mechanical hypersensitivity 14 days post-surgery (Fig. 3A; TIME: $F_{(4, 24)}$ = 16.23, P < 0.0001; DRUG: $F_{(1, 6)}$ = 70.55, P = 0.0002; INTERACTION: $F_{(4, 24)}$ = 7.048, P = 0.0007; Two-way RM ANOVA). Continuous SP infusion had no effect on basal withdrawal thresholds of sham-operated rats (Fig. 3B; TIME: $F_{(4, 24)}$ = 2.212, P = 0.0980; DRUG: $F_{(1, 6)}$ = 0.4503, P = 0.5272; INTERACTION: $F_{(4, 24)}$ = 0.6811, P = 0.6119; Two-way RM ANOVA).

Previous studies have identified SP and ENK localized in striatal neurons of the direct and indirect pathways, respectively [13, 14, 15]. To determine whether striatal ENK is involved in PSNL-induced hypersensitivity, ENK was continuously infused into the

contralateral striatum after PSNL surgery. Infusion of ENK (0.4 µg/mL, 1 µL/min for 120 min) had no effect on both PSNL-induced hypersensitivity and basal mechanical withdrawal threshold of sham-operated rats [(Fig. 4A) TIME: $F_{(4, 24)}$ = 4.066, P = 0.0118; DRUG: $F_{(1, 6)}$ = 0.2609, P = 0.6277; INTERACTION: $F_{(4, 24)}$ = 1.438, P = 0.2519; Two-way RM ANOVA; (Fig. 4B) TIME: $F_{(4, 24)}$= 1.261, P = 0.3128; DRUG: $F_{(1, 6)}$ = 1.242, P = 0.3077; INTERACTION: $F_{(4, 24)}$ = 2.735, P = 0.0525; Two-way RM ANOVA].

3.3 NK1 receptor activation in the analgesic effect of SP infusion

Co-infusion of SP with NK1 receptor antagonist CP96345 (10 µM) significantly blocked the antinociceptive effect of SP (0.4 µg/mL, 1 µL/min for 120 min) on PSNL-evoked mechanical hypersensitivity (Fig. 5; TIME: $F_{(4, 76)}$ = 15.58, P < 0.0001; DRUG: $F_{(3, 19)}$ = 16.07, P < 0.0001; INTERACTION: $F_{(12, 76)}$ = 6.227, P < 0.0001; Two-way RM ANOVA).

3.4 Involvement of striatal acetylcholinergic interneurons in the antinociceptive effect of SP infusion

Striatal acetylcholinergic interneurons could have a role in the antinociceptive effect of striatal SP infusion. Thus, the effects of blocking mACh receptors and nACh receptors on the antinociceptive effect of striatal SP infusion were examined. Co-infusion of atropine (10 μM) and mecamylamine (10 μM) together blocked the antinociceptive effect of SP infusion (0.4 μg/mL, 1 μL/min for 120 min; Fig. 6A, C) (Fig. 6; TIME: $F_{(4, 100)} = 16.59$, $P < 0.0001$; DRUG: $F_{(5, 25)} = 9.758$, $P < 0.0001$; INTERACTION: $F_{(20, 100)} = 5.371$, $P < 0.0001$; Two-way RM ANOVA). Co-infusion with atropine (10 μM), and not mecamylamine (10 μM), significantly inhibited the antinociceptive effect of SP infusion (0.4 μg/mL, 1 μL/min for 120 min; Fig. 6B and C).

4. Discussion

Continuous striatal SP infusion by reverse microgialysis method ameliorated mechanical hypersensitivity in rats with a PSNL via NK1 receptor activation. The antinociceptive effect of striatal SP involved the striatal cholinergic system as infusion of both mecamylamine and atropine blocked the antinociceptive effect of SP infusion. Specifically, activation of mACh receptors are likely to be involved.

The dorsal striatum contributes not only to motor function but also to pain inhibition [3, 4, 5, 6, 7, 8]. The current study demonstrated that SP infusion into the striatum inhibited PSNL-evoked mechanical hypersensitivity. Previous studies have also shown that striatal SP infusion is significantly antinociceptive in formalin-induced and capsaicin-evoked models of pain [5, 6]. In addition, Marabese reported that the dorsal striatum regulates the descending pain modulatory pathway via regulation of the rostral ventromedial medulla (RVM) [8]. Thus, the current data support the contention that the striatum is an important supraspinal area that modulates tonic nociception and further suggests that striatal SP regulates an endogenous pain modulation pathway. On the other hand, the striatum is also enriched with the endogenous opioid ligand ENK and its preferred targets, delta- and mu-opioid receptors [29, 30]. Although it is reasonable to assume that striatal ENK is likely to have antinociceptive effect, continuous striatal ENK infusion had no effect on PSNL-induced mechanical hyperalgesia. In addition, Cui et al (2014) reported that striatal mu- and delta-opioid receptors are not involved in the antinociceptive effect of morphine. However, striatal mu-opioid receptors are involved in the rewarding effects of opioids [31]. Moreover, in contrast to SP, [D-Ala2, Met5]-enkephalinamide, a non-selective opioid receptor agonist, did not affect ACh release in

rat striatal slices [32]. Thus, these findings suggest that a striatal opioid mechanism is not likely to mediate the SP-induced antinociception in the current study.

Exocytosed-SP is degraded by a variety of peptidases [33, 34, 35, 36, 37, 38]. However, SP's degradation products, including SP (1-7), SP (5-11), and SP (6-11) have potent agonistic effect on the NK1 receptor [39, 40, 41]. Moreover, many neuropeptides, including SP, have high affinity (pM to low nM range) for their respective receptors [42, 43, 44, 45, 46]. Taken together, these data suggest that once released, SP and its degradation products could target receptors well beyond the synapse, leading to long-lasting cellular and behavioral effects. The current study utilized reverse microdialysis to infuse SP into the striatum. This method offers several advantages, including continuous SP delivery, steady-state levels of SP without an acute effect of injection volume, and the ability to adjust the diffusion range of SP to the size of brain region of interest by changing the length of the microdialysis probe [47]. SP solution used in the current study was about 1,000-fold higher than the 50% effective concentration (EC50) for NK1 receptor activation (EC50 = 4.0-13.8 nM), as estimated by in vitro studies, acute SP microinjection was not antinociceptive [43, 45, 46]. Thus, drug infusion via

microdialysis could be utilized to mimic physiological neuropeptide transmission in the brain, particularly in regions of interest.

Treatment with SP increased neuronal activity of striatal acetylcholinergic interneurons and striatal ACh release, which were mediated through NK1 receptor activation [16, 17, 48, 49]. Moreover, Francis et al (2019) demonstrated that in a ventral striatum, SP from D1-MSNs increased acetylcholinergic interneuron activity via NK1 receptor activation. The activated acetylcholinergic interneurons release ACh, thereby modulating D2-MSNs activity, via mACh receptor activation [17]. These data indicated that, in the striatum, SP modulates neuronal circuits via functional regulation of acetylcholinergic interneurons. The current study showed that SP infusion attenuated PSNL-induced mechanical hypersensitivity via mACh receptor activation. Behavioral pharmacological studies have demonstrated that activation of mACh receptors in the CNS leads to antinociception [50, 51, 52, 53, 54]. In addition, Li et al (2013) reported that two classes of pain-related neurons, pain-excited neurons and pain-inhibited neurons, in the striatum was regulated by mACh receptor [2]. Thus, striatal neurons expressing mACh receptor could be involved in the endogenous pain modulation pathway. In contrast, although previous studies have shown nACh receptors expressed on

terminals of dopaminergic neurons regulate DA release, in the current study, block of striatal nACh receptors did not affect the SP-evoked antinociceptive effect. Taken together, the antinociceptive effect of SP infusion into the striatum is likely modulated by acetylcholinergic interneurons and activation of mACh receptors.

Microdialysis treatment with SP (0.4 μg/mL) significantly attenuated PSNL-induced hypersensitivity beginning 60 min after infusion. A previous study confirmed that reverse microdialysis treatment with SP induced phospholylation of ERK1/2 in rat striatum 60 min and 180 min after microdialysis treatment, but not early (10 min.) after infusion. The inhibition of late ERK1/2 activation blocked striatal-SP induced antinociception, which implies that extrasynaptic NK-1 receptors, but not synaptic NK-1 receptors, mediate antinociception [6]. The time lag between the commencement of SP infusion and antinociception could also be due to time needed for SP to transfer from the perfusate to the striatum.

How activation specifically of striatal NK1 receptors could be exploited for clinical pain relief has yet to be elaborated. Outside of the brain, within the spinal dorsal horn, SP is involved in the transmission of nociceptive information and evokes inflammation when applied to peripheral tissues [23, 24]. In addition, SP injection into the RVM, a principal source of

serotoninergic neurons, evokes hypersensitivity via RVM expressing NK1 receptors and subsequent activation of the descending facilitation pathway [55]. Thus, SP has opposing effects on nociceptive modulation depending on brain region. Previous studies reported that dopamine agonists and angiotensin-converting enzyme inhibitors increase CNS SP concentrations [56, 57, 58]. The current study suggested the possibility that mACh agonists or acetylcholinesterase esterase inhibitors could be the new drug targets for analgesic on neuropathic pain. In fact, a clinical case report indicated that donepezil, which increases brain levels of ACh, decreased chronic pain and improved quality of life [59]. In addition, previous studies reported that CNS ACh have anti nociceptive effect via not only striatal mACh receptors but also spinal nACh receptors [60, 61, 62]. Hence, it is possible that drugs which activate striatal mACh receptors or increase the CNS ACh could be used for the management of chronic refractory pain.

5. Conclusion

The current findings in a rat model of neuropathic pain suggest that striatal SP infusion could be antinociceptive in neuropathic pain as well as inflammatory pain. Moreover, the antinociceptive effect observed in the current study involves striatal acetylcholinergic

interneurons and activation of mACh receptors. Therefore, targeting striatal SP and mACh receptors could be a novel strategy for creating potential analgesics.

Author Statement

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Conflicts of interest

The authors declare there is no conflict of interest in this study.

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Fig. 1

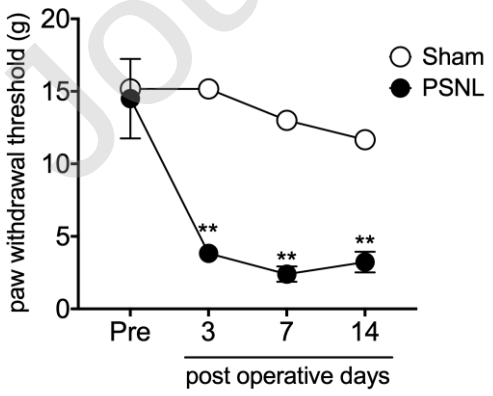

Figure 1. Hind paw mechanical hypersensitivity in rats with a PSNL.

Withdrawal thresholds of injured-paw following either sham surgery or PSNL were assessed over time with von Frey filaments in sham-operated and PSNL rats for the periods indicated. Data are expressed as mean ± SEM. n=6-10/group. **p<0.01 compared with pre PSNL operation (two-way RM ANOVA followed by Sidak's multiple comparisons test).

Fig. 2

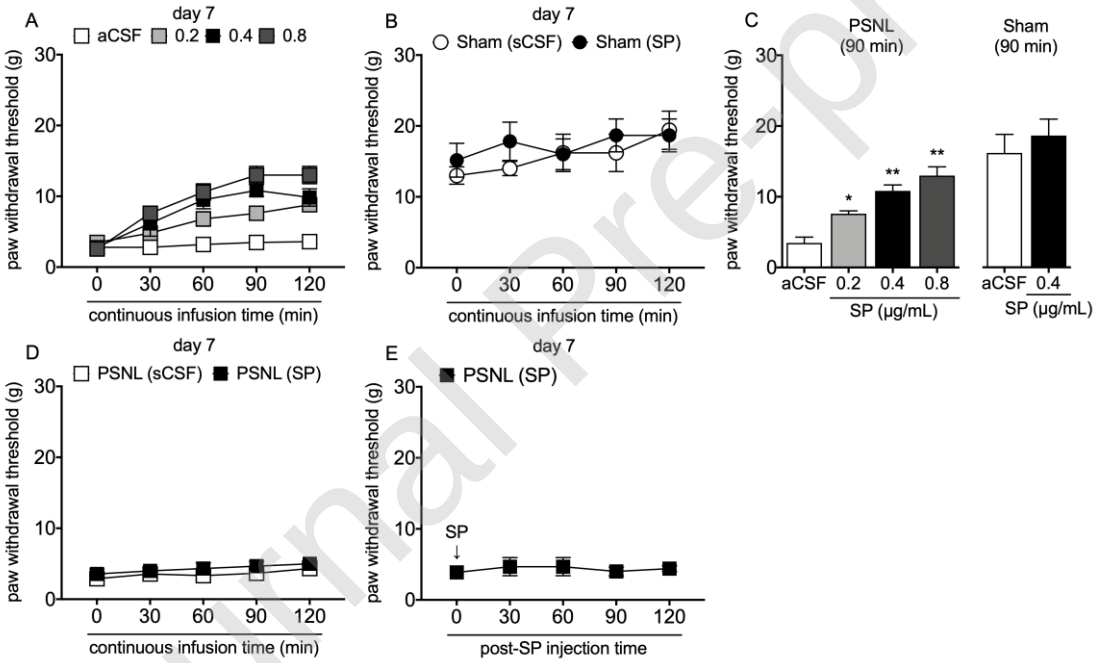

Figure 2. Effect of SP infusion into the striatum on PSNL (day 7) -evoked mechanical hypersensitivity in rats.

Mechanical withdrawal thresholds of the ipsilateral hind paw at 7 days after the PSNL (A) or sham surgery (B) following continuously infusion with either aCSF or SP (PSNL:

0.2, 0.4, 0.8 μg/mL; Sham: 0.4 μg/mL; 1 μL/min) into the contralateral striatum. (C) Mechanical withdrawal thresholds 90 min after each continuous infusion. Data are expressed as mean ± SEM. n = 4-6/group. **p<0.01 compared with aCSF-treated PSNL group (two-way RM ANOVA followed by Sidak's multiple comparisons test). (D) Continuous infusion with either aCSF or SP (0.4 μg/mL, 1 μL/min) into the ipsilateral striatum of PSNL rats (day 7) for 2 hrs. Data are expressed as mean ± SEM. (E) SP (10 ng) was acutely micoinjected into the contralateral striatum. Data are expressed as mean ± SEM. n = 5/group.

Fig. 3

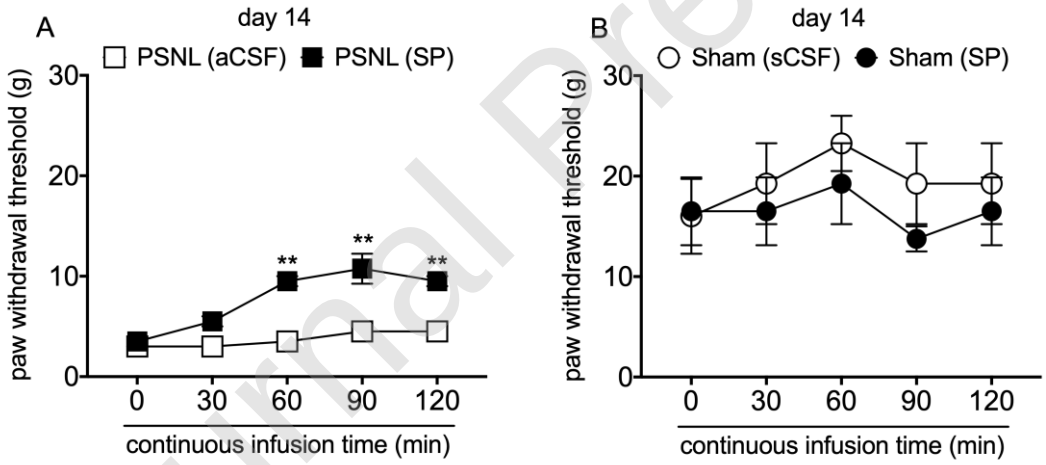

Figure 3. Mechanical withdrawal thresholds of the ipsilateral hind paw at 14 days after PSNL (A) or sham (B) surgery.

Rats were continuously infused with aCSF or SP (0.4 μg/mL, 1 μL/min) into the contralateral striatum. Data are expressed as mean ± SEM. n = 4-6/group. **p<0.01 compared

with aCSF-treated PSNL group at each time points (two-way RM ANOVA followed by Sidak's multiple comparisons test).

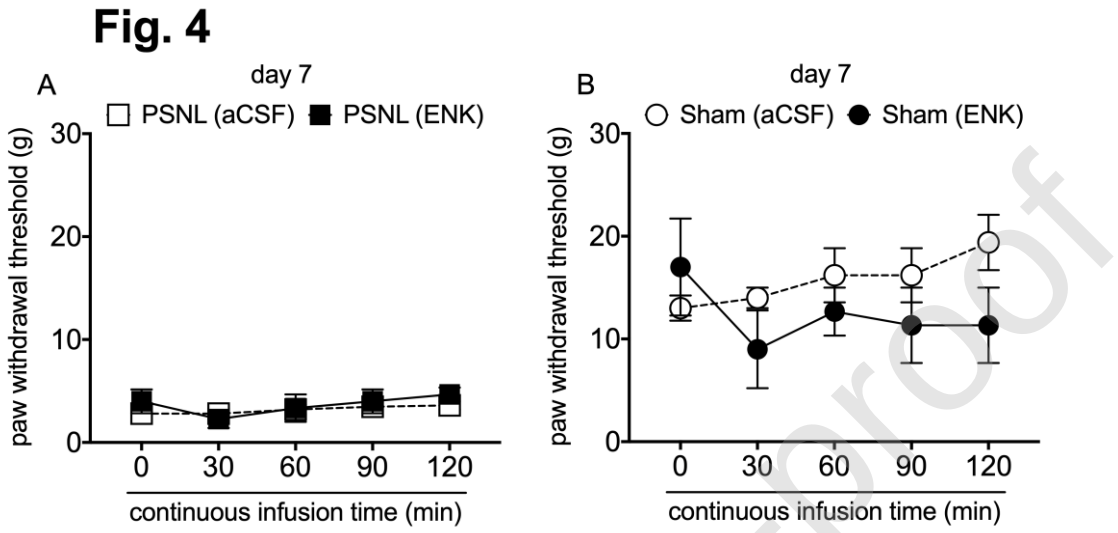

Figure 4. Effect of continuous ENK infusions with ENK on PSNL-evoked mechanical hypersensitivity in rats.

The mechanical withdrawal thresholds of the left hind paw, the operated side, at 7 days after the PSNL (A) or sham (B) groups were continuously infused with aCSF or ENK (0.4 μg/mL, 1 μL/min) into the contralateral striatum. The aCSF-treated groups are the same as those shown in Fig 2. Data are expressed as mean ± SEM. n = 3/group.

Fig. 5

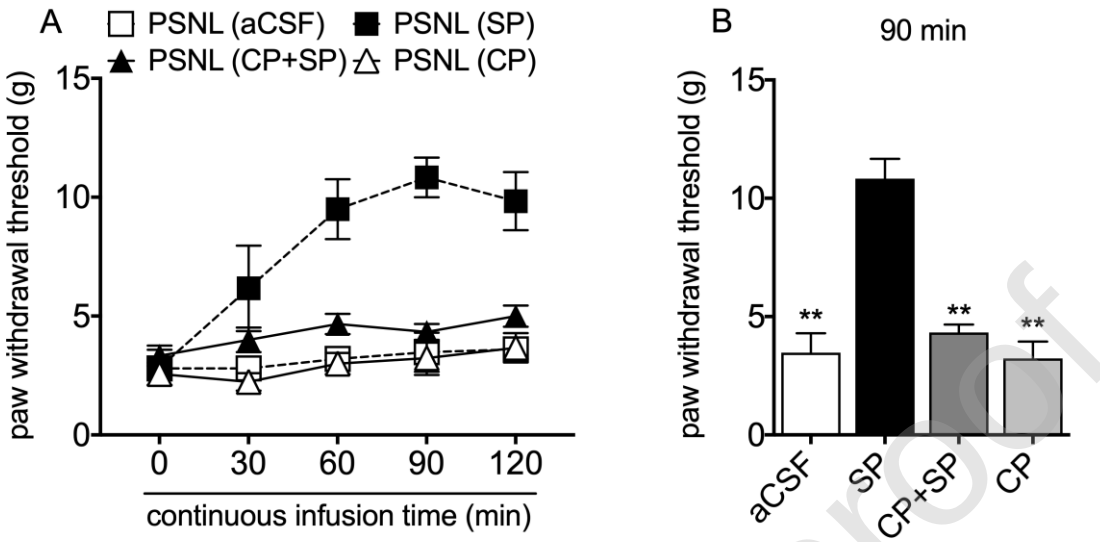

Figure 5. The analgesic effect of SP mediated via striatal NK1 receptor activation.

(A) Continuous infusion with either aCSF, SP (0.4 μg/mL), CP96345 (10 μM), or co-infusion (0.4 μg/mL SP and 10 μM CP96345, 1 μL/min) into the contralateral striatum of PSNL rats (day 7). (B) Mechanical withdrawal thresholds 90 min. after continuous infusion. The aCSF- and SP-treated groups are the same as those shown in Fig 2. Data are expressed as mean ± SEM. n = 5-6/group. *** $p<0.001$ compared with PSNL (SP) group (two-way RM ANOVA followed by Sidak's multiple comparisons test).

Fig. 6

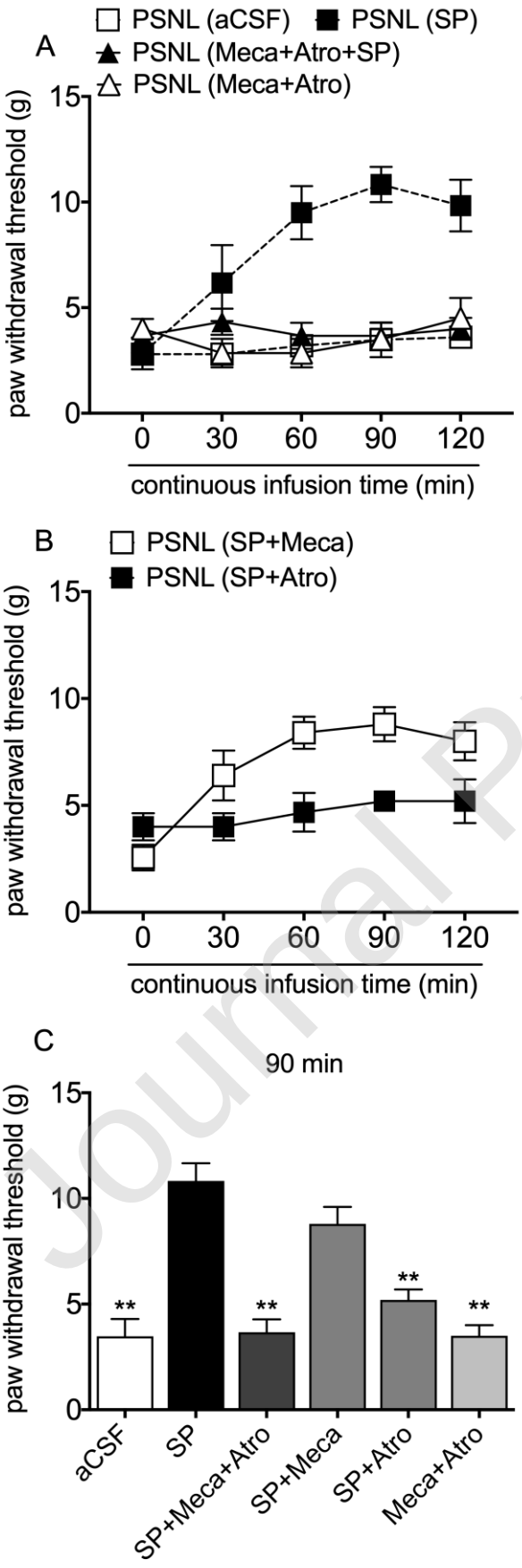

Figure 6. The antinociceptive effect of SP mediated via striatal acetylcholinergic interneurons. (A) Continuous infusion with either aCSF, SP (0.4 μg/mL), or co-infusion (10 μM mecamylamine and 10 μM atropine with or without 0.4 μg/mL SP, 1 μL/min) into the contralateral striatum of PSNL rats (day 7). (B) Continuous infusion of SP (0.4 μg/mL) and either AChR antagonist (10 μM Mecamylamine or 10 μM Atropine). (C) Mechanical withdrawal thresholds 90 min. after each continuous infusion. The aCSF- and SP-treated groups are the same as those shown in Fig 2. Data are expressed as mean ± SEM. n = 5-6/group. *** $p<0.001$ compared with PSNL (SP) group (two-way RM ANOVA followed by Sidak's multiple comparisons test).