Inhibition of Spinal 5-HT3 Receptor and Spinal Dorsal Horn Neuronal Excitability Alleviates Hyperalgesia in a Rat Model of Parkinson’s Disease

Cheng-Jie Li1,2 · Li-Ge Zhang1,2 · Lu-Bing Liu2 · Meng-Qi An2 · Li-guo Dong1,2 · Han-Ying Gu1 · Yong-Ping Dai1 · Fen Wang1,2 · Cheng-Jie Mao1 · Chun-Feng Liu1,2,3

Received: 21 February 2022 / Accepted: 14 September 2022 / Published online: 27 September 2022
© The Author(s), under exclusive licence to Springer Science+Business Media, LLC, part of Springer Nature 2022

Abstract
Pain in Parkinson’s disease (PD) is increasingly recognized as a major factor associated with poor life quality of PD patients. However, classic therapeutic drugs supplying dopamine have limited therapeutic effects on PD-related pain. This suggests that there is a mechanism outside the dopamine system that causes pain in PD. Our previous study demonstrated that 6-OHDA induced PD model manifested hyperalgesia to thermal and mechanical stimuli and decreased serotonin (5-hydroxytryptamine; 5-HT) in the spinal dorsal horn (SDH). Several 5-HT receptor subtypes have been confirmed to be associated with nociception in the spinal cord, such as 5-HT1A receptor, 5-HT1B receptor, 5-HT2 receptor, 5-HT3 receptor, and 5-HT7 receptor. Most research has shown that 5-HT1A receptor and 5-HT3 receptor play a key role in pain transmission in the spinal cord. We hypothesized that hyperalgesia of 6-OHDA rats may be related to increased excitability of SDH neurons, and functional change of 5-HT3 receptor may reverse the hyperalgesia of 6-OHDA lesioned rats and decrease cell excitability of SDH neurons. To test this hypothesis, we used whole-cell patch-clamp and pharmacological methods to evaluate the effect of 5-HT3 receptor antagonist ondansetron (20 μmol/L) and palosetron (10 μmol/L), but not 5-HT3 receptor agonist m-CPBG (30 μmol/L) and SR 57,727 (10 μmol/L), 5-HT1A receptor agonist 8-OH DPAT (10 μmol/L) and eptapirone (10 μmol/L) and 5-HT1A receptor antagonist WAY-100635 (10 μmol/L) and p-MPPI (10 μmol/L). Intrathecal injection of ondansetron (0.1 mg/kg) but not m-CPBG (0.1 mg/kg), 8-OH DPAT (0.1 mg/kg), and WAY-100635 (0.1 mg/kg) significantly attenuated the mechanical hyperalgesia and thermal hyperalgesia in 6-OHDA lesioned rats. In conclusion, the present study suggests that inhibition of spinal 5-HT3 receptor and SDH neuronal excitability alleviates hyperalgesia in PD rats. Our study provides a novel mechanism or therapeutic strategy for pain in patients with PD.

Keywords Nonmotor symptoms of Parkinson’s disease · Spinal dorsal horn · 5-HT3 receptor · Neuronal excitability · Hyperalgesia

Abbreviations
5-HT 5-Hydroxytryptamine; serotonin
AP Action potential
PD Parkinson’s disease
SDH Spinal dorsal horn
SNpc Substantia nigra pars compacta
TH Tyrosine hydroxylase
L4-L6 Fourth to sixth lumbar spinal cord

Cheng-Jie Li and Li-Ge Zhang contributed equally to this work.

Fen Wang
wangfen_1982@126.com

Cheng-Jie Mao
drchengjiemao@163.com

1 Department of Neurology and Clinical Research Center of Neurological Disease, The Second Affiliated Hospital of Soochow University, 1055 Sanxiang Road, Suzhou 215004, China

2 Jiangsu Key Laboratory of Neuropsychiatric Diseases and Institute of Neuroscience, Soochow University, Suzhou, China

3 Department of Neurology, The Second Affiliated Hospital of Xinjiang Medical University, Urumqi, China
**Introduction**

Parkinson’s disease (PD) is the second most common neurodegenerative disorder in older people and is clinically characterized by motor symptoms of tremor, rigidity, akinesis, and dystonia. Pain as a nonmotor symptom in PD is increasingly recognized as a major factor associated with poor life quality and more than motor symptoms are [10]. The prevalence of pain in PD patients has been reported to range from 40 to 85% [7]. According to the Ford classification, there are five main subtypes of PD-related pain, musculoskeletal, dystonic, radicular and central pain, and akathisia [17]. Although pain is common in PD patients, there are no guidelines or standard remedies for the management of PD-associated pain [3]. Therefore, there is an urgency to investigate the pathogenesis of pain in PD to discover novel and effective treatments. Classic therapeutic drugs supplying dopamine have limited therapeutic effect on PD pain [16]. This suggests that there is a mechanism outside the dopamine system that causes pain in PD.

Clinical research has demonstrated that serotonergic dysfunction is associated with pain in PD [42]. Our previous study has also shown that a PD rat model induced by bilateral lesions of the substantia nigra pars compacta (SNpc) manifested hyperalgesia to thermal and mechanical stimuli. This hypersensitivity could be attributed at least partially to the decreased serotonin (5-hydroxytryptamine; 5-HT) content in the spinal dorsal horn (SDH) [43]. Whether the decreased 5-HT content has an effect on SDH neurons requires further investigation.

5-HT is one of the main neurotransmitters involved in the descending inhibitory system, and its projection from rostral ventrolateral medulla to the spinal cord is thought to be related to pain modulation. Several 5-HT receptor subtypes have been confirmed to be associated with nociception in the spinal cord, including 5-HT1A receptor, 5-HT1B receptor, 5-HT2 receptor, 5-HT3 receptor, and 5-HT7 receptor [34]. Previous studies have demonstrated that 5-HT1A receptor, 5-HT3 receptor, and 5-HT7 receptor are highly expressed in the SDH and associated with nociceptive regulation [13, 39, 40]. Most research has shown that 5-HT1A receptor and 5-HT3 receptor play a key role in pain transmission in the spinal cord [4]. 5-HT3 receptor, the only ionotropic 5-HT receptor, is a pentameric channel permeable to cations, causing the depolarization of neurons and increased excitability. 5-HT1A receptor is a G-protein-coupled receptor, negatively coupled with adenylyl-cyclase, causing the opening of potassium channels, the closing of calcium channels, and inducing neuronal hyperpolarization [27, 31]. Therefore, the function of the 5-HT1A receptor and 5-HT3 receptor is related to the excitability of SDH neurons.

Many studies have shown that excitability of SDH neurons is increased in chronic neuropathic pain [14]. In consequence, we hypothesized that hyperalgesia of the 6-OHDA-induced PD model may be related to increased excitability of SDH neurons, and functional change of 5-HT3 receptor can reverse hyperalgesia of 6-OHDA lesioned rats and decrease excitability of SDH neurons. To test this hypothesis, we used whole-cell patch-clamp and pharmacological methods to evaluate the effect of 5-HT3 receptor and 5-HT1A receptor in SDH on the hyperalgesia of the 6-OHDA PD rat model. Our study provides a novel mechanism or therapeutic strategy for pain in patients with PD.

**Materials and Methods**

**Animals**

Adult male Sprague–Dawley rats (150–180 g) were housed (five rats per cage) at a controlled temperature of 22–25 °C, 40–60% relative humidity, and a 12-h light/dark cycle with unlimited access to food and water. All experiments were approved by the Animal Use and Care Committee of Soochow University and followed the guidelines of the International Association for the Study of Pain.

**6-OHDA-Induced PD Rat Model**

Rats were anesthetized with 3% isoflurane-induced, as soon as the loss of righting reflex and anesthesia was maintained with 1.5% isoflurane and then placed in a stoeleting stereotaxic apparatus (RWD Co., Shenzhen, China). Perforations were slowly drilled into the skull to allow for the insertion of a 10-μL Hamilton syringe, using the following stereotactic coordinates (from a rat brain atlas) – 5.3 mm anteroposterior, ± 1.8 mm mediolateral, and − 7.8 mm dorsoventral (from the dura) from the bregma. The animals were injected with 8 μg 6-OHDA (dissolved in 4 μL saline containing 0.02% ascorbic acid) or 4 μL saline (containing 0.02% ascorbic acid) on each side at a rate of 0.5 μL/min. The syringe remained in place for 8 min after completion of the injection and was then slowly retracted. The rats were transferred to a recovery cage with soft, non-particular bedding and were placed on the side for comfortable breathing. The experimenter monitored the animals until they were fully alert, ambulant, and started drinking and transferred them to the animal facility [43].

**Behavior Tests**

**Rotarod Test**

Three weeks after surgery, the rats were subjected to a rotarod test to evaluate motor coordination and balance.
Before the formal experiment, the rats were trained for 3 consecutive days on the rotarod system (SANS, Jiangsu Province, China) at an increasing speed (4 to 25 r/min at a rate of 0.5 r/min). The trained rats were tested three times at a speed of 25 r/min, and the mean duration was recorded to analyze.

**Open Field Test**

The open field test was used to evaluate animals’ locomotor performance and anxiety behavior. Animals were placed in a square black plank (1 m × 1 m), surrounded by a 40-cm white wall, and divided into a 25 x 25-cm square by white lines. Each animal was allowed to explore the box freely for 10 min. Their behavior was recorded by an overhead camera. At the end of each trial, 75% alcohol was used to clean the box.

**Mechanical Allodynia**

The mechanical threshold was evaluated with E-von Frey (Mgo Basile, Italy) or von Frey filaments (Aesthesio, Dan-Mic Global, San Jose, CA, USA) using the up–down paradigm. The rats were placed in Plexiglas chambers on a wire mesh platform for 0.5–1 h. The calibrated Von Frey filaments were applied to the plantar surface of rats’ right hind paw with sufficient force to bend the filaments for 10 s or until the rat withdrew. The threshold was calculated as the force of the smallest filament causing the withdrawal behavior [44].

**Thermal Allodynia**

The tail flick test was used to assess the thermal threshold of the animal. Radiant heat was applied to the tail using a tail flick apparatus (Mgo Basile, Italy). The radiant heat density was adjusted to yield the tail flick reaction on sham rats in 10–12 s. A cut-off of 15 s was set to prevent the risk of burns. The time from the laser beginning to the time the animal flicked its tail was recorded as the thermal threshold to analyze.

**Slice Preparation**

Three weeks after stereotaxic injection, the rats were anesthetized with isoflurane, decapitated, and the lumbar (L4–L6) segments were removed quickly and placed in oxygenated ice-cold Krebs solution with the following composition (in mM): 95 NaCl, 1.8 KCl, 1.2 KH2PO4, 0.5 CaCl2, 7 MgSO4, 26 NaHCO3, 15 glucose, 50 sucrose, pH7.2–7.4 (adjusted osmolarity with sucrose to 310–320 mOsm). The lumbar (L4–L6) spinal cord was isolated, embedded in a 4% agar block, and glued to the stage of the vibrating microtome. Coronal Scts. (300 μm) were made using a vibrating microtome (Leica VT1200). Slices were incubated in oxygenated Krebs solution at 31 °C.

**Electrophysiology**

After incubation for 1 h, one of the spinal cord slices was transferred to the recording grooves of nylon mesh, and the slice was fixed with a U-shaped nylon mesh. The slices were recorded at room temperature, perfused with oxygenated recording fluid at a rate of 1.5 mL/min throughout. Neurons used for recording in lamina II of SDH were visualized with an Olympus BX51WI microscope with a 40 x water-immersion objective, infrared differential interference contrast (IR)-DIC. Recordings were performed in current-clamp or voltage-clamp mode at a holding potential of −70 mV, unless otherwise indicated. The pipettes (4–8 MΩ tip resistance) were filled with internal solution (in mM): 133 Kgluconate, 0.6 EGTA, 8 NaCl, 2 Mg-ATP, 0.3 Na-GTP, and 10 HEPES (pH 7.2–7.3). Data were acquired using EPC10 amplifier and patchmaster software (HEKA, Germany), filtered and sampled at 5 kHz with a Bessel filter amplifier. Analysis was done with Clampfit software (pClamp10, Molecular Devices, USA). The resting potential was recorded immediately after the action potential (AP) configuration was executed. The firing patterns were evaluated using 1.5 s depolarizing current (step 20 pA) in the current clamp.

**Western Blotting**

Immunostaining was carried out using anti-TH antibody (1:5000, T1299, Sigma, USA) and mouse anti-β actin antibody (1:5000, A3854, Sigma, USA). Anti-mouse secondary antibody was used for mouse antibodies. Immunoreactive bands were obtained by clinx science instrument (Clinx, China). Densitometric analysis was performed using ImageJ software (National Institutes of Health, USA).

**Drug Administration**

For in vitro electrophysiology, ondansetron (20 μmol/L, O3639, Sigma, USA), m-CPBG (30 μmol/L, C144, Sigma, USA), WAY-100635 (10 μmol/L, W108, Sigma, USA), 8-OH DPAT (10 μmol/L, H8520, Sigma, USA), palonosetron (10 μmol/L, HY-A0021, MCE, USA), SR57727 (10 μmol/L, HY-102064, MCE, USA), p-MPPI (10 μmol/L, HY-120738, MCE, USA), and eptapirone (10 μmol/L, HY-19946, MCE, USA) were dissolved in oxygenated ACSF to perfuse into spinal cord slices contained in chambers. The drug concentration used in the patch clamp test was based on previous studies [1, 22, 25, 26, 45]. For behavioral tests, ondansetron (0.1 mg/kg), m-CPBG (0.1 mg/kg),
WAY-100635 (0.1 mg/kg), and 8-OH DPAT (0.1 mg/kg) were dissolved in 0.9% normal saline and injected intrathecally into the L4–L6 spinal cord via a microsyringe. The rats were given persistent anesthesia with isoflurane and placed on a board with the abdomen facing down and the L3–L5 spinal segments curved. The tail swing of the rats during intrathecal injection was considered successful. The drug (10 μL/rat) was slowly injected, and the needle was left for at least 30 s to ensure that the drug did not reflux [12, 30]. The drug concentration used in behavioral test was based on previous studies [15, 33].

**Statistical Analysis**

Data are presented as mean ± SEM. Statistical analysis was performed using GraphPad Prism 8 (GraphPad Software, La Jolla, CA, USA). Normality was first checked for all data before analysis. Significance was determined using Student’s t test, one-way ANOVA followed by Tukey’s post hoc test and two-way ANOVA. P < 0.05 was considered significant.

**Results**

**Decreased Mechanical and Thermal Pain Threshold in 6-OHDA Rats**

We observed the behavioral phenotypes of 6-OHDA rats at 4 weeks after 6-OHDA injection. The rotarod test showed shorter retention time in 6-OHDA rats than in sham rats (Fig. 1a, P < 0.001, two-sample t test). The open field test had no difference in movement distance between 6-OHDA and sham rats (Fig. 1b, P > 0.05, two-sample t-test). Compared to sham rats, thermal pain threshold (tail-flick latency, TFL) and mechanical pain threshold (PWT) were significantly reduced in 6-OHDA rats (Fig. 1c, d, P < 0.01, P < 0.001, two-sample t test). These behavioral tests showed that the passive movement ability of 6-OHDA rats was impaired, the active locomotion ability was normal, and mechanical and thermal pain thresholds were reduced.

To confirm the 6-OHDA-induced PD model, western blotting was used to proceed with tyrosine hydroxylase (TH) quantification. TH protein level was decreased in the striatum of 6-OHDA rats (Fig. 1e, f, P < 0.01, two-sample t-test).

**Classification of SDH Neuron Firing Pattern**

Our previous study demonstrated that 5-HT content of 6-OHDA rats was decreased in the SDH, indicating that the 5-HT system is involved in hyperalgesia of 6-OHDA lesioned rats [43]. In addition, as two main functional 5-HT receptors, 5-HT3 receptor and 5-HT1A receptor were related to cell excitability in the spinal cord [27, 31]. Therefore, to further explore the mechanisms of 5-HT3 receptor and 5-HT1A receptor in pain hypersensitivity of 6-OHDA rats, a whole-cell patch-clamp was used to detect the action potential of SDH neurons on the outside part of Lamine II of the fourth to sixth lumbar spinal

---

**Fig. 1** Reduction in thermal and mechanical thresholds in 6-OHDA-treated rats. Rotarod test (a) and open field test (b) were performed 4 weeks after 6-OHDA (n=20) or saline (Sham, n=22) injection. Von Frey test (e) and tail-flick test (d) were performed at 4 weeks after 6-OHDA (n=20) or saline (n=22) injection. Western blotting (e) and group data (f) for striatal TH protein levels (n=3 per group). Two-sample t test was used for analysis. **P < 0.01; ***P < 0.001 versus sham group; ns, not significant.
cord (L4–L6) in sham group and 6-OHDA lesioned group (Fig. 2c). The action potential patterns of recorded neurons are divided into five categories: tonic pattern, delayed pattern, phasic pattern, gap pattern, and single pattern. The tonic pattern is characterized by an action potential discharge that persists during the whole current step. The delayed pattern is characterized by the action potential generated after a delay. The phasic pattern is typically restricted to one, a few, or a short burst of action potentials, whereafter the activity quickly returns to the resting state. The gap firing pattern is a response to injected depolarizing current in which an initial action potential is followed by a long interspike interval and then regular firing. The single pattern is characterized by an action potential discharge that initiates a single spike during the whole current step (Fig. 2a).

We recorded 313 SDH neurons in the spinal cord slice from 6- to 8-week-old rats. Among 161 neurons from spinal cord slices of sham rats, 112 exhibited a tonic pattern, 20 a delayed pattern, 12 a phasic pattern, seven a gap pattern, and 10 a single pattern when current steps were applied from a membrane potential of about \(-70\) mV. Similar to the discharge classification results of the sham group, among 152 neurons of 6-OHDA rats, 99 exhibited a tonic pattern, 20 a delayed pattern, 13 a phasic pattern, 11 a gap pattern, and nine a single pattern when current steps were applied from a membrane potential of about \(-70\) mV (Fig. 2b). Because most recorded neurons exhibited tonic firing patterns, we treated only tonic-type neurons, and neurons with other firing types were not included in the statistics.

**Increased Excitability of SDH Neurons in 6-OHDA Rats**

We compared the action potential parameters of neurons showing a tonic firing pattern in the 6-OHDA group and the sham group. The resting membrane potential of neurons in 6-OHDA rats was significantly increased (Fig. 3a, \(P < 0.001\), two-sample \(t\)-test). However, the threshold and amplitude of action potential remained unchanged compared to those in sham rats (Fig. 3b, c, \(P > 0.05\), two-sample \(t\)-test). Rheobase, AP half width and Rin did not change, too (see in the supplementary material). The number of action potentials was increased under 40–80 pA current stimulation in the SDH of 6-OHDA rats compared to sham rats (Fig. 3d, e, \(P < 0.001\), two-sample \(t\)-test). These results suggested the increased excitability of SDH neurons in the 6-OHDA-induced PD rat model. Because the parameters of action potential of SDH neurons had the best uniformity at an injection current of 40 pA, we focused on the changes in action potential of SDH neurons at 40 pA before and after drug administration.

**Effect of 5-HT3 Receptor Agonist and Antagonist on Excitability of SDH Neurons**

To examine the effect of the 5-HT3 receptor involved in the excitability of SDH neurons, AP was recorded in the spinal cord slice in the presence of pre- and post-bath application

---

**Fig. 2** Discharge patterns observed in spinal dorsal horn (SDH) neurons. **a** Typical traces for each firing pattern were recorded. Scale bar: 50 mV, 200 ms. **b** The number of neurons exhibiting the different firing types, from a total sample of 161 SDH neurons in 32 sham-operated rats and from a total sample of 152 SDH neurons in 33 6-OHDA lesioned rats. **c** Recorded neurons were located on the outside part of SDH lamina II, and the neurons are 10–20 microns in diameter and oval in shape, like water droplets.
of high-efficacy 5-HT3 receptor-selective antagonist ondansetron (20 μmol/L) or palonosetron (10 μmol/L) and agonist m-CPBG (30 μmol/L) or SR 57,727 (10 μmol/L). The representative trace of typical neurons showed that bath application of ondansetron or palonosetron caused a significant decrease in AP frequency in SDH neurons under 40 pA current stimulation (Fig. 4a, c, d, f, P < 0.05, P < 0.01, P < 0.001, one-way ANOVA). Ondansetron or palonosetron did not significantly affect SDH neural membrane potentials in the sham group and 6-OHDA lesioned group (Fig. 4b, e, P < 0.05, P < 0.01, one-way ANOVA).

**Effect of 5-HT1A Receptor Agonist and Antagonist on Excitability of SDH Neurons**

We examined the role of the 5-HT1A receptor involved in the excitability of SDH neurons by application of 5-HT1A receptor selective antagonist WAY-100635 (10 μmol/L) or p-MPPI (10 μmol/L) and 5-HT1A receptor agonist 8-OH DPAT (10 μmol/L) or eptapirone (10 μmol/L) in the Krebs resolution. The representative trace of typical neurons showed that bath application of m-CPBG or SR57727 had no significant effect on AP frequency in SDH neurons under 40 pA current stimulation (Fig. 4g, i, j, l, P < 0.05, one-way ANOVA). Similarly, m-CPBG or SR 57,727 did not significantly affect SDH neural membrane potentials in the sham group and 6-OHDA lesioned group (Fig. 4h, k, P < 0.05, P < 0.01, one-way ANOVA).
significantly affect SDH neural membrane potentials in the sham group and 6-OHDA group (Fig. 5b, e, h, k, P > 0.05, one-way ANOVA).

### Inhibitory Effect of 5-HT3 Receptor Antagonist Ondansetron on Pain Hypersensitivity in 6-OHDA Rats

To investigate the role of 5-HT3 receptor in hyperalgesia of 6-OHDA rats, the rats were treated with 5-HT3 receptor antagonist ondansetron (0.1 mg/kg) and agonist m-CPBG (0.1 mg/kg) at 4 weeks after 6-OHDA injection. Intrathecal injection of ondansetron significantly reversed mechanical hyperalgesia and thermal hyperalgesia in 6-OHDA-lesioned group compared with sham group (Fig. 6a, b, P < 0.05, P < 0.01, two-way ANOVA), while m-CPBG had little effect on hyperalgesia of 6-OHDA-lesioned rats (Fig. 6c, d, P > 0.05, two-way ANOVA). This implicated that blockade of the 5-HT3 receptor could relieve mechanical hyperalgesia and thermal hyperalgesia in 6-OHDA rats.

### Inhibition of Spinal 5-HT1A Receptor Did Not Relieve Pain Hypersensitivity in 6-OHDA Rats

We next examined the possible pain modulatory effect of the 5-HT1A receptor in 6-OHDA rats. Surprisingly, both

---

**Fig. 4** Effect of 5-HT3 receptor agonist and antagonist on the excitability of spinal dorsal horn (SDH) neurons. a–c Effect of palonosetron (5-HT3 receptor antagonist) on excitability of SDH neurons. Representative current-clamp recordings showing firing evoked by 40 pA current injection with or without palonosetron (10 μM) treatment (a). Resting membrane potential (b) and action potential frequency (c) were analyzed (n=7 for sham group and n=9 for 6-OHDA group). d–f Effect of ondansetron (5-HT3 receptor antagonist) on excitability of SDH neurons. Representative current-clamp recordings showing firing evoked by 40 pA current injection with or without ondansetron (20 μM) treatment (d). Resting membrane potential (e) and action potential frequency (f) were analyzed (n=8 for sham group and n=9 for 6-OHDA group). g–l Effect of m-CPBG and SR57727 (5-HT3 receptor agonist) on excitability of SDH neurons. Representative current-clamp recordings showing firing evoked by 40 pA current injection with or without m-CPBG (30 μM) or SR57727 (10 μM) treatment (g, j). Resting membrane potential (h, k) and action potential frequency (i, l) were analyzed (n=6–8 for sham group and n=6–10 for 6-OHDA group). One-way ANOVA followed by Tukey’s post hoc analysis *P < 0.05; **P < 0.01; ***P < 0.001 versus as indicated; ns, not significant
intrathecal injection of 5-HT1A receptor agonist 8-OH DPAT (0.1 mg/kg) and antagonist WAY-100635 (0.1 mg/kg) did not affect pain hypersensitivity in 6-OHDA lesioned rats. This indicates that 5-HT1A receptor might have nothing to do with hyperalgesia or a potential complicated mechanism involved in the pain modulatory effect of 5-HT1A receptor (Fig. 7a–d, P > 0.05, two-way ANOVA).

**Discussion**

In the present study, our 6-OHDA PD rat model induced by lesions of dopaminergic neurons in the SNpc developed thermal and mechanical hypersensitivity at 4 weeks after surgery. Electrophysiological results suggested that SDH neurons had increased excitability, and 5-HT3 receptor antagonist ondansetron and palonosetron reversed this hyperexcitability. Intrathecal injection of ondansetron significantly attenuated the mechanical hyperalgesia and thermal hyperalgesia in the 6-OHDA lesioned rats. Our findings suggest that inhibition of spinal 5-HT3 receptor and SDH neuronal excitability alleviates hyperalgesia of 6-OHDA lesioned rats.

According to Braak PD staging, the spinal cord can be affected in an early phase of the disease [6]. Clinical research has suggested that early PD patients exhibit increased spinal nociceptive responses compared with healthy controls [5]. These studies have indicated that spinal cord lesions are closely related to pain in PD. In a transgenic mouse A53T PD model, α-synuclein is highly expressed in the spinal cord.
and a humanoid study has shown that spinal cord stimulation alleviates motor deficits in a primate model of PD [37], which suggest that the spinal cord is a promising target for treatment of pain in PD. Our previous study has shown that 6-OHDA lesioned rats manifest hyperalgesia in response to thermal and mechanical stimuli, and this hypersensitivity could be attributed partially to the decreased 5-HT content in the SDH [43]. These results suggest that the spinal cord and its 5-HT receptors are involved in PD-related pain, but the underlying mechanisms are still elusive.

To explore what role the spinal cord plays in pain in PD, we investigated the electrophysiological properties of spinal
cord slices from the PD rat model. We recorded 161 neurons from the sham group and 152 from the 6-OHDA group. Consistent with previous research [29], our results demonstrated that, among the recorded neurons, the number of neurons exhibiting a tonic pattern was the largest (74–76%). The tonic firing pattern is deemed to be a hallmark of spinal inhibitory interneurons, in contrast to spinal excitatory interneurons that show delayed or gap firing patterns [41]. Moreover, the spinal inhibitory interneurons play a key role in the pain transmission in SDH. We compared the action potential parameters of neurons showing a tonic firing pattern in the 6-OHDA group and the sham group. Neurons with tonic firing pattern had increased action potential frequency in the 6-OHDA lesioned group compared with the sham group, suggesting that these neurons transmit a higher level of nociceptive information to the central nervous system. Consistently, Keri-Ann Charles et al. [9] have reported that wide dynamic range neurons (spinal cord, lamina V) have a higher discharge frequency in 6-OHDA lesioned rats.

5-HT1 receptor, 5-HT2 receptor, 5-HT3 receptor, and 5-HT7 receptor are all expressed in SDH [13, 32, 35], but 5-HT1A receptor and 5-HT3 receptor were found to exert a major effect, especially in electrophysiology [1, 18, 26]. Consequently, we focused our research on 5-HT1A receptor and 5-HT3 receptor. However, we could not rule out the role of other receptors.

We explored the exact functional role of 5-HT3 receptor and 5-HT1A receptor in the hyperexcitability of SDH neurons in 6-OHDA lesioned rats. In terms of electrophysiology, bath application of 5-HT3 receptor antagonist ondansetron or palonosetron reversed the increased cell excitability of SDH neurons. Tomoyose et al. have reported that inhibition of 5-HT3 receptor inhibits excitatory synaptic transmission in the SDH, and Xu et al. demonstrated that inhibition of 5-HT3 receptor alone had no significant effect on inhibitory synaptic transmission in the SDH. Thus, the inhibitory effect of ondansetron and palonosetron on the excitability of SDH neurons may be attributed to attenuation of excitatory synaptic transmission. In terms of behavior tests, our results showed that intrathecal injection of ondansetron significantly attenuated the hyperalgesia of 6-OHDA rats. Consistent with our results, intrathecal administration of the 5-HT3 receptor antagonist ondansetron has been reported to inhibit spontaneous pain behavior following intraplantar formalin injection [11] and reverse mechanical allodynia in a model of spinal cord injury neuropathic pain [28, 33]. However, Christopher M. Peters et al. have reported the lack of analgesic efficacy of spinal ondansetron on thermal and mechanical hypersensitivity following spinal nerve ligation in rats. The discrepancy may be due to dual effects of 5-HT3 receptor activation on distinct populations of neurons in the SDH, resulting in a lack of effect on behavioral outcomes.

Previous studies have shown that intrathecal administration of selective 5-HT3 receptor agonist (SR 57,227 and m-CPBG) alleviates hyperalgesia in rats with spinal nerve ligation [21]. Surprisingly, we found that m-CPBG or SR 57,727 had no obvious effect in electrophysiological and behavioral tests. We tried a high concentration of m-CPBG or SR 57,727, but still saw no obvious effect. Several recent functional studies have demonstrated that the dominant effect of 5-HT3 receptor activation in the spinal cord is antinociceptive by promoting GABAergic inhibitory synaptic transmission [19, 20, 45]. GABA interneurons in the SDH of 6-OHDA lesioned rats may have changes in cell structure and function, resulting in inability of m-CPBG to exert an analgesic effect. Future studies are warranted to examine the function of the GABAergic system in 6-OHDA rats.

Previous studies have shown that 8-OH DPAT produces antinociception in a model of carrageenan-induced inflammation and neuropathic pain [23, 27]. Sagalajev et al. showed that intrathecal injection of 5-HT1A receptor antagonist WAY-100635 reversed hyperalgesia induced by high-dose glutamate in central amygdala [36]. It is unusual that our research showed that bath application or intrathecal injection of 5-HT1A receptor antagonist WAY-100635, p-MPPI, and 5-HT1A receptor agonist 8-OH DPAT or eptapirone had little effect on SDH neuronal excitability and hyperalgesia in 6-OHDA lesioned rats. The drug dose was selected according to previous studies [1, 15, 22, 25, 26]. However, the effects of 5-HT1A agonists and antagonists may be different in 6-OHDA lesioned rats, so more appropriate drug concentrations need to be investigated.

Huang et al. have indicated that the 5-HT3 receptor was expressed in the GABAergic neurons in the mouse SDH [24]. Some researchers have reported that GABAa antagonists can reverse the antinociception and electrophysiological effects of 5-HT3 receptor antagonists [2]. This suggests that 5-HT3 receptor in the spinal cord changes the function of GABA neurons, thereby affecting the excitability of projection neurons to further exert analgesic effects. This underlying analgesic mechanism of 5-HT3 receptor needs to be further explored.

Descending pain modulation includes the serotonin and norepinephrine systems. Intrathecal injection of clonidine, an alpha 2 adrenoceptor agonist, can attenuate pain hypersensitivity in 6-OHDA lesioned PD rat model [8]. Hayashida et al. demonstrated that alpha 2 adrenoceptor-mediated anti-hypersensitivity can be weakened by the blockade of spinal 5-HT3 receptor in rats with spinal nerve ligation, via reduction of basal GABA tone in the spinal cord, indicating a common mechanism to reduce neuropathic pain between spinal alpha 2 adrenoceptor and 5-HT3 receptor [21]. A potential linkage between spinal alpha 2 adrenoceptor and
5-HT3 receptor in pain regulation may exist in 6-OHDA lesioned PD rat model.

In summary, we found that inhibition of spinal 5-HT3 receptor and SDH neuronal excitability alleviates hyperalgesia of 6-OHDA lesioned PD rats.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s12035-022-03034-8.

Author Contribution FW, CJM, and CFL designed the study. CJL, LGZ, LBL, and MQA performed the experiments and collected data. CJL, LGZ, LBL, MQA, LGD, HYG, and YPD performed data analysis and interpretation. CJL and LGZ drafted the article, FW and CJM revised it critically for important intellectual content, and FW gave final approval of the version to be submitted.

Funding This work was supported by the National Natural Science Foundation of China (81801258), Natural Science Foundation of Jiangsu Province (BK20170355), Jiangsu Provincial social development projects (BE2018658, BE201765), Gusu Health Talents Training Project (GSWS2019041, GSWS2020035), Discipline Construction projects (BE2018658, BE201765), Gusu Health Talents Training Project (BK20170355), Jiangsu Provincial social development Foundation of China (81801258), Natural Science Foundation of Jiangsu Province (BK20160370, BK20170355), Jiangsu Provincial social development Foundation of China (81801258). This study was approved by the Animal Use and Care Committee of Soochow University and followed the guidelines of the Ethics Approval.

Declaration of interest

Data Availability The datasets generated in the current research are available from the corresponding author on reasonable request.

Code Availability Not applicable.

Ethics Approval This study was approved by the Animal Use and Care Committee of Soochow University and followed the guidelines of the International Association for the Study of Pain.

Conflict of Interest The authors declare no competing interests.

References

1. Abe K, Kato G, Katafuchi T, Tamae A, Furue H, Yoshimura M (2009) Responses to 5-HT in morphologically identified neurons in the rat substantia gelatinosa in vitro. Neuroscience 159:316–324. https://doi.org/10.1016/j.neuroscience.2008.12.021

2. Alhaider AA, Lei SZ, Wilcox GL (1991) Spinal 5-HT3 receptor- mediated antinociceptive responses: possible release of GABA. J Neurosci 11:1881–1888. https://doi.org/10.1523/jneurosci.11-07-01881.1991

3. Antonini A, Tinazzi M (2015) Targeting pain in Parkinson’s disease. Lancet Neurol 14:1144–1145. https://doi.org/10.1016/s1474-4422(15)30026-0

4. Bardoni R (2019) Serotonergic modulation of nociceptive circuits in spinal cord dorsal horn. Curr Neuropharmacol 17:1133–1145. https://doi.org/10.2174/1570159X17666190110123900

5. Bourn E, Stamelou M, Vadasz D, Ries V, Unger MM, Kagi G, Oertel WH, Moller JC et al (2017) Is increased spinal nociception another hallmark for Parkinson’s disease? J Neurol 264:570–575. https://doi.org/10.1007/s00415-016-8390-y

6. Braak H, Del Tredici K, Rüb U, de Vos RA, Jansen Steur EN, Braak E (2003) Staging of brain pathology related to sporadic Parkinson’s disease. Neurobiol Aging 24:197–211. https://doi.org/10.1016/s0197-4580(02)00065-9

7. Broen MP, Braaksmna MM, Patijn J, Weber WE (2012) Prevalence of pain in Parkinson’s disease: a systematic review using the modified QUADAS tool. Mov Disord 27:480–484. https://doi.org/10.1002/mds.24054

8. Cao LF, Peng XY, Huang Y, Wang B, Zhou FM, Cheng RX, Chen LH, Luo W et al (2016) Restoring spinal noradrenergic inhibitory tone attenuates pain hypersensitivity in a rat model of Parkinson’s disease. Neural Plast 2016:6383240. https://doi.org/10.1155/2016/6383240

9. Charles KA, Naudet F, Bouali-Benazzouz R, Landry M, De Deurwaerdere P, Fossat P, Benazzouz A (2018) Alteration of nociceptive integration in the spinal cord of a rat model of Parkinson’s disease. Mov Disord 33:1010–1015. https://doi.org/10.1002/mds.27377

10. Chaudhuri KR, Healy DG, Schapira AHV (2006) Non-motor symptoms of Parkinson’s disease: diagnosis and management. Lancet Neurol 5:235–245. https://doi.org/10.1016/s1474-4422(06)70373-8

11. Chen Y, Outway MA, Weaver LC (2009) Blockade of the 5-HT3 receptor for days causes sustained relief from mechanical allodynia following spinal cord injury. J Neurosci Res 87:418–424. https://doi.org/10.1002/jnr.21860

12. De la Calle JL, Páino CL (2002) A procedure for direct lumbar puncture in rats. Brain Res Bull 59:245–250. https://doi.org/10.1016/s0361-9230(02)00866-3

13. Doly S, Fischer J, Brissorgueil MJ, Vergé D, Conrath M (2005) Pre- and postsynaptic localization of the 5-HT7 receptor in rat dorsal spinal cord: immunochemical evidence. J Comp Neurol 490:256–269. https://doi.org/10.1002/cne.20667

14. Eide PK (1998) Pathophysiological mechanisms of central neuropathic pain after spinal cord injury. Spinal Cord 36:601–612. https://doi.org/10.1088/sj.sc.3100737

15. Eide PK, Joly NM, Hole K (1990) The role of spinal cord 5-HT1A and 5-HT1B receptors in the modulation of a spinal nociceptive reflex. Brain Res 536:195–200. https://doi.org/10.1016/0006-8993(90)90025-7

16. Fil A, Cano-de-la-Cuerda R, Munoz-Hellin E, Vela L, Ramiro- Gonzalez M, Fernandez-de-Las-Penas C (2013) Pain in Parkinson disease: a review of the literature. Parkinsonism Relat Disord 19:285–294; discussion 285 https://doi.org/10.1016/j.parkreldis.2012.11.009

17. Ford B (2010) Pain in Parkinson’s disease. Mov Disord 25(Suppl 1):S98–103. https://doi.org/10.1002/mds.22716

18. Fukushima T, Ohtsubo T, Tsuda M, Yanagawa Y, Hori Y (2009) Facilitatory actions of serotonin type 3 receptors on GABAergic inhibitory synaptic transmission in the spinal superficial dorsal horn. J Neurophysiol 102:1459–1471. https://doi.org/10.1152/jn.91160.2008

19. Giordano J (1991) Analgesic profile of centrally administered 2-methylserotonin against acute pain in rats. Eur J Pharmacol 199:233–236. https://doi.org/10.1016/0014-2999(91)90462-y

20. Giordano J, Schultea T (2004) Serotonin 5-HT(3) receptor mediation of pain and anti-nociception: implications for clinical therapeutics. Pain Physician 7:141–147

21. Hayashi K, Kimura M, Yoshizumi M, Hobo S, Obata H, Eisenach JC (2012) Ondansetron reverses antihypersensitivity from clonidine in rats after peripheral nerve injury: role of γ-aminobutyric acid in α2-adrenoceptor and 5-HT3 serotonin receptor analgesia. Anesthesiology 117:389–398. https://doi.org/10.1097/ALN.0b013e318260d381
22. Hori Y, Endo K, Takahashi T (1996) Long-lasting synaptic facilitation induced by serotonin in superficial dorsal horn neurones of the rat spinal cord. J Physiol 492(Pt 3):867–876. https://doi.org/10.1113/jphysiol.1996.sp021352

23. Hu B, Doods H, Treede RD, Ceci A (2016) Duloxetine and 8-0H-DPAT, but not fluoxetine, reduce depression-like behaviour in an animal model of chronic neuropathic pain. Neurosci Lett 619:162–167. https://doi.org/10.1016/j.neulet.2016.03.019

24. Huang J, Wang YY, Wang W, Li YQ, Tamamaki N, Wu SX (2008) 5-HT3A receptor subunit is expressed in a subpopulation of GABAergic and enkephalinergic neurons in the mouse dorsal spinal cord. Neurosci Lett 441:1–6. https://doi.org/10.1016/j.neulet.2008.04.105

25. Ito A, Kumamoto E, Takeda M, Shibata K, Sagai H, Yoshimura M (2000) Mechanisms for ovariectomy-induced hyperalgesia and its relief by calcitonin: participation of 5-HT1A-like receptor on C-afferent terminals in substantia gelatinosa of the rat spinal cord. J Neurosci 20:6302–6308. doi:10.1523/jneurosci.20-06-06302.2000

26. Jeong HJ, Mitchell VA, Vaughan CW (2012) Role of 5-HT(1) receptor subtypes in the modulation of pain and synaptic transmission in rat spinal superficial dorsal horn. Br J Pharmacol 165:1956–1965. https://doi.org/10.1111/j.1476-5381.2011.01685.x

27. Kim JM, Jeong SW, Yang J, Lee SH, Kim WM, Jeong S, Bae HB, Yoon MH et al (2015) Spinal 5-HT1A, not the 5-HT1B or 5-HT3 receptors, mediates descending serotonergic inhibition for late-phase mechanical allodynia of carrageenan-induced peripheral inflammation. Neurosci Lett 600:91–97. https://doi.org/10.1016/j.neulet.2015.05.058

28. Li M, Zhu M, Xu Q, Ding F, Tian Y, Zhang M (2020) Sensation of TRPV1 via 5-hydroxytryptamine signaling modules pain hypersensitivity in a 6-hydroxydopamine induced mice model of Parkinson’s disease. Biochem Biophys Res Commun 521:868–873. https://doi.org/10.1016/j.bbrc.2019.10.204

29. Li Y, Su S, Yu J, Peng M, Wan S, Ke C (2021) Electrophysiological properties of substantia gelatinosa neurons in the preparation of a slice of middle-aged rat spinal cord. Front Aging Neurosci 13:640265. https://doi.org/10.3389/fnagi.2021.640265

30. Li YC, Tian YQ, Wu YY, Xu YC, Zhang PA, Sha J, Xu GY (2020) Upregulation of spinal ASK1 and NCC1 expression contributes to chronic visceral pain in rats. Front Mol Neurosci 13:611179. https://doi.org/10.3389/fnmol.2020.611179

31. Marić AV, Peterson AS, Brake AJ, Myers RM, Julius D (1991) Primary structure and functional expression of the 5HT3 receptor, a serotonin-gated ion channel. Science 254:432–437. https://doi.org/10.1126/science.1718042

32. Marlier L, Teilhac JR, Cerruti C, Privat A (1991) Autoradiographic mapping of 5-HT1, 5-HT1A, 5-HT1B and 5-HT2 receptors in the rat spinal cord. Brain Res 550:15–23. https://doi.org/10.1016/0006-8993(91)90400-p

33. Oatway MA, Chen Y, Weaver LC (2004) The 5-HT3 receptor facilitates at-level mechanical alldynia following spinal cord injury. Pain 110:259–268. https://doi.org/10.1016/j.pain.2004.03.040

34. Obata H (2017) Analgesic mechanisms of antidepressants for neuropathic pain. Int J Mol Sci 18 https://doi.org/10.3390/ijms18112483

35. Perrin FE, Gerber YN, Teigell M, Lonjon N, Boniface G, Bauchat L, Rodriguez JJ, Hugnot JP et al (2011) Anatomical study of serotonergic innervation and 5-HT(1A) receptor in the human spinal cord. Cell Death Dis 2:e218. https://doi.org/10.1038/cddis.2011.98

36. Sagalajev B, Bourbia N, Beloushko E, Wei H, Pertovaara A (2015) Bidirectional amygdaloid control of neuropathic hypersensitivity mediated by descending serotonergic pathways acting on spinal 5-HT3 and 5-HT1A receptors. Behav Brain Res 282:14–24. https://doi.org/10.1016/j.bbr.2014.12.052

37. Santana MB, Halje P, Simplicio H, Richter U, Freire MAM, Petersson P, Fuentes R, Nicolelis MAL (2014) Spinal cord stimulation alleviates motor deficits in a primate model of Parkinson disease. Neuron 84:716–722. https://doi.org/10.1016/j.neuron.2014.08.061

38. Shirakashi Y, Kawamoto Y, Tomimoto H, Takahashi R, Ihara M (2006) Alpha-Synuclein is colocalized with 14–3–3 and synphilin-1 in A53T transgenic mice. Acta Neuropathol 112:681–689. https://doi.org/10.1007/s00401-006-0132-2

39. Tecott LH, Marić AV, Julius D (1993) Nervous system distribution of the serotonin 5-HT3 receptor mRNA. Proc Natl Acad Sci USA 90:1430–1434. https://doi.org/10.1073/pnas.90.4.1430

40. Thor KB, Nickolaus S, Helke CJ (1993) Autoradiographic localization of 5-hydroxytryptamine1A, 5-hydroxytryptamine1B and 5-hydroxytryptamine1C/2 binding sites in the rat spinal cord. Neuroscience 55:235–252. https://doi.org/10.1016/0306-4522(93)90469-v

41. Todd AJ (2010) Neuronal circuitry for pain processing in the dorsal horn. Nat Rev Neurosci 11:823–836. https://doi.org/10.1038/nrn2947

42. Tong Q, Zhang L, Yuan Y et al (2015) Reduced plasma serotonin and 5-hydroxyindoleacetic acid levels in Parkinson’s disease are associated with nonmotor symptoms. Parkinsonism Relat Disord 21:882–887. https://doi.org/10.1016/j.parkreldis.2015.05.016

43. Wang CT, Mao CJ, Zhang XQ et al (2017) Attenuation of hyperalgesia responses via the modulation of 5-hydroxytryptamine signalings in the rostral ventromedial medulla and spinal cord in a 6-hydroxydopamine-induced rat model of Parkinson’s disease. Mol Pain 13:1744806917691525. https://doi.org/10.1177/174406991525

44. Wang Q, Zhu H, Zou K, Yuan B, Zhou YL, Jiang X, Yan J, Xu GY (2015) Sensitization of P2X3 receptors by cystathionine β-synthetase mediates persistent pain hypersensitivity in a rat model of lumbar disc herniation. Mol Pain 11:15. https://doi.org/10.1186/s12990-015-0012-7

45. Xie DJ, Utaka D, Feng PY, Wakita M, Shin MC, Furue H, Yoshimura M (2012) Identification of 5-HT receptor subtypes enhancing inhibitory transmission in the rat spinal dorsal horn in vitro. Mol Pain 8:58. https://doi.org/10.1186/1744-8069-8-58

**Publisher’s Note** Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.