Chinese Journal of Physiology 61(4): 201-209, 2018
DOI: 10.4077/CJP.2018.BAH588

Serotonin$_6$ Receptors in the Prelimbic Cortex are Involved in the Regulation of Anxiety-Like Behaviors in the Rat 6-Hydroxydopamine Parkinson's Disease Model

Yu-Ming Zhang[1, 2], Yuan Guo[1], Cheng-Xue Du[1], Jin Zhang[1], Shu-Xuan Lv[1], and Jian Liu[1]

[1]*Department of Physiology and Pathophysiology, School of Basic Medical Sciences, Xi'an Jiaotong University Health Science Center, Xi'an 710061*
and
[2]*Department of Anesthesiology, Shaanxi Provincial People's Hospital, Xi'an 710068*
People's Republic of China

Abstract

The role of serotonin$_6$ (5-HT$_6$) receptors in the regulation of anxiety is poorly understood, particularly in Parkinson's disease-related anxiety. Here we examined whether 5-HT$_6$ receptors in the prelimbic cortex (PrL) involve in the regulation of anxiety-like behaviors in sham-operated rats and rats with unilateral 6-hydroxydopamine lesions of the medial forebrain bundle (MFB). The lesion induced anxiogenic responses as measured by the open-field and elevated-plus maze (EPM) tests compared to sham-operated rats. Intra-PrL injection of 5-HT$_6$ receptor agonist WAY208466 (0.5, 3 and 6 μg/rat) decreased the percentage of time spent in the center area of the open field and percentages of open arm entries and open arm time in sham-operated rats, indicating the induction of anxiogenic responses, and injection of 5-HT$_6$ receptor antagonist SB258585 (1, 2, and 4 μg/rat) showed anxiolytic effects. Interestingly, WAY208466, at the same doses, increased the percentage of time spent in the center area of the open-field and percentages of open arm entries and open arm time in the lesioned rats, indicating the induction of anxiolytic effects, and SB258585, at the same doses, produced anxiogenic responses. Collectively, our findings indicate that 5-HT$_6$ receptors in the PrL are involved in the regulation of anxiety-like behaviors, which may attribute to changes in dopamine and noradrenaline levels in the limbic and limbic-related brain regions after activation and blockade of PrL 5-HT$_6$ receptors.

Key Words: anxiety, Parkinson's disease, prelimbic cortex, rat, serotonin$_6$ receptor

Introduction

The ventral region of the medial prefrontal cortex (mPFC) includes the prelimbic (PrL) and infralimbic cortices, projects to various brain structures such as the amygdala, brainstem and hypothalamus, and is related to diverse emotional and cognitive processes (10). Increasing evidence indicates that the ventral mPFC participates in the regulation of anxiety-like behaviors (6, 12, 14, 33).

Serotonergic neurotransmission has an important role in the etiology, pathophysiology and treatment of anxiety disorders. It is well known that the mPFC receives serotonergic innervations from the

Corresponding author: Professor Jian Liu, Department of Physiology and Pathophysiology, School of Basic Medical Sciences, Xi'an Jiaotong University Health Science Center, Yan Ta Xi Lu 76, Xi'an 710061, People's Republic of China. Tel: +86 29 82655160, Fax: +86 29 82655160. E-mail: liujian@mail.xjtu.edu.cn
Received: January 6, 2018; Revised: May 22, 2018; Accepted: June 13, 2018.

raphe nuclei, and expresses multiple serotonin (5-HT) receptor subtypes, including 5-HT_6 receptor (24, 32). Recent studies demonstrate the highest 5-HT_6 receptor expression in the striatum, nucleus accumbens, olfactory tubercle, and cerebral cortex, with moderate density in the amygdala, hippocampus, hypothalamus, thalamus and cerebellum (7, 9, 11, 20). The specific localization of 5-HT_6 receptors in the brain suggests that they play an important role in cognition, feeding, seizures and affective states, as well as in depression and anxiety (39, 42, 44). Previous studies have found that both 5-HT_6 receptor agonists and antagonists show anxiolytic effects as measured by the different behavioral paradigms of anxiety (23, 38, 40, 41). However, given that the 5-HT_6 receptor has emerged as an interesting molecular target for drug development and that there is the as yet unresolved paradox that agonists and antagonists of this receptor evoke complex effect in anxiety-like behaviors (9, 28, 38, 43). By now, the reported results involved in the anxiety-like effects of 5-HT_6 receptor in different models of anxiety have been inconsistent.

Classically, Parkinson's disease (PD) is considered as a movement disorder; however, a range of non-motor symptoms such as anxiety, depression and cognitive deficits are increasingly recognised in PD patients and parkinsonian animals, and anxiety is a frequently encountered non-motor symptom of PD (26). Further, central 5-HT system plays an important role in non-motor symptoms of PD, and degeneration of the nigrostriatal pathway leads to an impairment of the 5-HT system (13). Additionally, our study has found that unilateral dopamine (DA) depletion in PD rats induces depressive-like, and activation and blockade of 5-HT_6 receptors in the PrL could regulate these behaviors (46). Considering the fact that depression and anxiety are related manifestations and frequently co-morbid in PD (21, 37), we speculate that 5-HT_6 receptors in the PrL may be involved in the regulation of anxiety-like behaviors in PD. Therefore, the present study was designed to investigate effects of 5-HT_6 receptor agonist and antagonist injected into the PrL on anxiety-like behaviors by commonly used paradigms the open-field and elevated-plus maze (EPM) tests in sham-operated rats and rats with unilateral 6-hydroxydopamine (6-OHDA) lesions of the medial forebrain bundle (MFB).

Materials and Methods

Animals and Drugs

Experiments were performed on male Sprague–Dawley rats (270–320 g; Experimental Animal Center of Xi'an Jiaotong University, Xi'an, PRC), in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals, and approved by the Animal Care Committee of the University. Desipramine hydrochloride, 6-OHDA hydrochloride and apomorphine hydrochloride were purchased from Sigma-Aldrich (MO, USA), and WAY208466 (high affinity and selective 5-HT_6 receptor agonist) and SB258585 (potent and selective 5-HT_6 receptor antagonist) were obtained from Tocris (Bristol, UK). 6-OHDA and apomorphine were prepared in saline containing 0.02% ascorbic acid; desipramine, WAY208466 and SB258585 were dissolved in saline.

6-OHDA Lesions

To model PD in rats, 6-OHDA (12 μg/4 μl) was injected into the right MFB (AP –4.4 mm, ML 2.0 mm, DV 7.8 mm relative to bregma) as previously described, and these rats were received desipramine (25 mg/kg, i.p.) 30 min prior to 6-OHDA injection to protect noradrenergic neurons (25, 46). Sham-operated rats received 4 μl of saline containing 0.02% ascorbic acid in the same manner. One week after surgery, rats were given apomorphine (0.05 mg/kg, s.c.) and those exhibiting more than 20 contralateral turns per 5 min were selected for further experiments (36).

Guide Cannula Implantation and Intra-PrL Injections

Two weeks after injection of saline containing 0.02% ascorbic acid or 6-OHDA into the right MFB, each rat was implanted with a stainless steel guide cannula with the tip 1 mm above the right PrL (AP 3.3 mm, ML 0.7 mm, DV 2.0 mm relative to bregma) (25). After surgery, rats were allowed to recover for one week before behavioral tests.

For drug injection, a needle was inserted through the guide cannula until its tip was 1 mm below the end of the cannula. The injection needle was attached by a polyethylene tube to a 1-μl microsyringe. A 0.5-μl solution volume was injected over 60 s, and the intracerebral needle was removed 60 s post injection. Sham-operated and the lesioned rats were injected in the PrL with saline, WAY208466, SB258585/WAY208466 or SB258585. Behavioral tests were performed 10 min after intra-PrL injection. The time between the two injections was 5 min.

Behavioral Tests

All behavioral tests were performed during the fourth week after the injection of saline contain-

ing 0.02% ascorbic acid or 6-OHDA into the MFB. These tests were done in an isolated room between 8:00 and 12:00 am, and behavior was recorded with a digital video camera (HR-550E, Sony, Tokyo, Japan).

Open-Field Test

The open-field test was used to assess spontaneous locomotor activity as previously described (46). The apparatus had a white floor of 100 cm × 100 cm (divided by black lines into 25 squares of 20 cm × 20 cm) and white walls of 40 cm high. Each rat was placed in the center of the open-field, and the number of squares crossed (horizontal locomotion) and of rearings (vertical activity) was observed for 5 min. The test was also used to assess anxiety-like behavior in rats by measuring time spent in the central area (60 cm × 60 cm) of the open-field. The percentage of time spent in the central area was defined as: [time spent in the central area (s)/300 (s) × 100]. A decrease in the percentage of time spent in the center area is indicative of anxiety state (35).

EPM test

The EPM test was performed to assess anxiety-like behaviors previously described (27). Each rat was placed on the central platform facing an open arm at the beginning of the test and allowed to freely explore for 5 min. Anxiety-like behavior was measured by the following parameters: percentages of open arm entries [(number of open arm entries/ number of open arm + closed arm entries) × 100] and open arm time [(time in open arm/time in open + closed arm) × 100]. Decrease in the percentages of open arm entries and open arm time are suggestive of anxiety-like behavior (27).

Histology and Immunohistochemistry

Cresyl violet staining was used to identify the location of the injection sites. To determine the extent of dopaminergic neurons degeneration in the substantia nigra pars compacta (SNc), tyrosine hydroxylase (TH) immunohistochemistry was performed as described previously (36).

Data Treatment and Statistics

Behavioral data were only analyzed in rats that had total loss of TH immunoreactive neurons and histologically verified location of injection sites (Fig. 1). The data were first compared using unpaired Student's *t*-test between sham-operated and the 6-OHDA-lesioned rats. Then, sham-operated and the lesioned groups were separated, and all indexes in the behavioral tests after saline or drug injections were analyzed using one way analysis of variance (ANOVA) followed, when significant, by Dunnett's test for multiple comparisons. All data were expressed as mean ± standard error of the mean (SEM), and the criterion for statistical signifi-

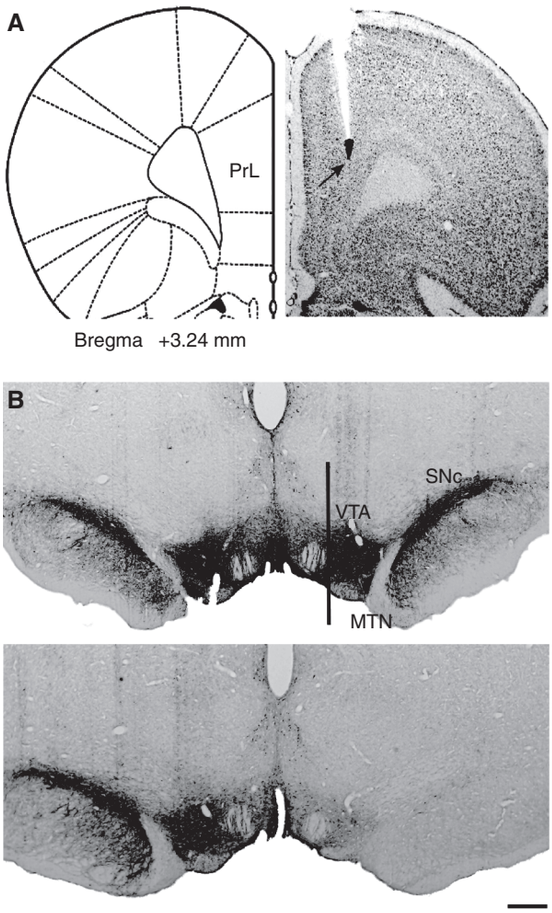

Fig.1. Schematic drawing of representative section, the injection site and TH immunohistochemistry. Photomicrograph of Cresyl Violet staining showing the injection site (arrow) in the PrL of sham-operated rat; and schematic drawing (bregma: +3.24 mm) adapted from Paxinos and Watson. Photomicrographs of TH immunohistochemical staining in coronal section of the midbrain in sham-operated (n = 13; B, upper) and the 6-OHDA-lesioned (n = 13; B, bottom) rats showing the SNc and VTA DA neurons on the injected side (right) compared to uninjected side (left). Note the complete and partial degeneration of TH-immunoreactive neurons in the SNc and VTA in the 6-OHDA injected side, respectively. MTN, medial terminal nucleus of the accessory optic tract. Scale bar, A, B = 500 μm.

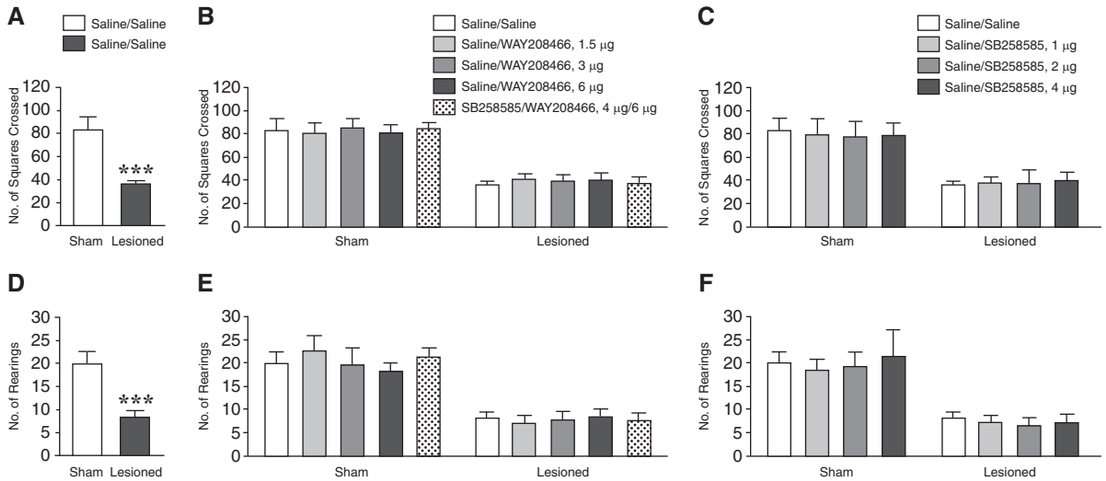

Fig. 2. Histograms showing effects of 6-OHDA lesion, intra-PrL injection of 5-HT_6 receptor agonist WAY208466 and antagonist SB258585 on locomotor activity measured by the open-field test. Unilaterally lesioning the MFB decreased the number of squares crossed (A; horizontal movement) and the number of rearings (D; vertical movement) compared to sham-operated rats. In sham-operated and the lesioned rats, intra-PrL injection of WAY208466, SB258585/WAY208466 or SB258585 did not change the number of squares crossed (B, C) and the number of rearings (E, F) compared to saline injection into the PrL in the same group. ***$P < 0.001$ *vs*. sham-operated rats; n = 10 rats/group.

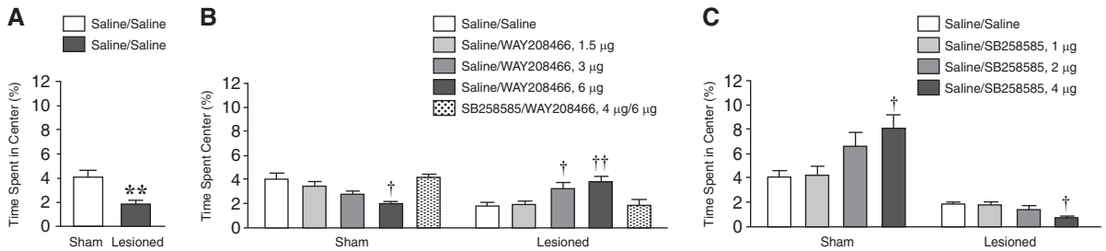

Fig. 3. Histograms showing effects of 6-OHDA lesion, intra-PrL injection of 5-HT_6 receptor agonist WAY208466 and antagonist SB258585 on anxiety-like behavior measured by the open-field test. Unilaterally lesioning the MFB decreased the percentage of time spent in the center area compared to sham-operated rats (A). In sham-operated rats, intra-PrL injection of WAY208466 decreased the percentage of time spent in the center area compared to the saline injection into the PrL in the same group (B), and SB258585 increased the percentage of time spent in the center area (C). In the lesioned rats, WAY208466 increased the percentage of time spent in the center area compared to the saline injection into the PrL in the same group (B), and SB258585 decreased the percentage of time spent in the center area (C). **$P < 0.01$ *vs*. sham-operated rats; †$P < 0.05$, ††$P < 0.01$ *vs*. the saline injection into the PrL in the same group; n = 10 rats/group.

cance was $P < 0.05$.

Results

Unilateral MFB lesions in rats significantly decreased the number of squares crossed and rearings compared to sham-operated rats (both $P < 0.001$; unpaired Student's *t*-test; Fig. 2A and D). In sham-operated and the lesioned rats, intra-PrL injection of WAY208466, SB258585/WAY208466 or SB258585 did not affect locomotor activity compared to the saline injection into the PrL in the same group (Fig. 2, B, C, E and F).

In the open-field test, the MFB lesions in rats significantly decreased the percentage of time spent in the center area compared to sham-operated rats ($P < 0.01$; unpaired Student's *t*-test; Fig. 3A). In sham-operated rats, intra-PrL injection of WAY208466 (1.5, 3, and 6 μg/rat) significantly decreased the percentage of time spent in the center area compared to the saline injection into the PrL ($F_{4,45} = 3.398$, $P < 0.05$, one-way ANOVA; followed by Dunnett's

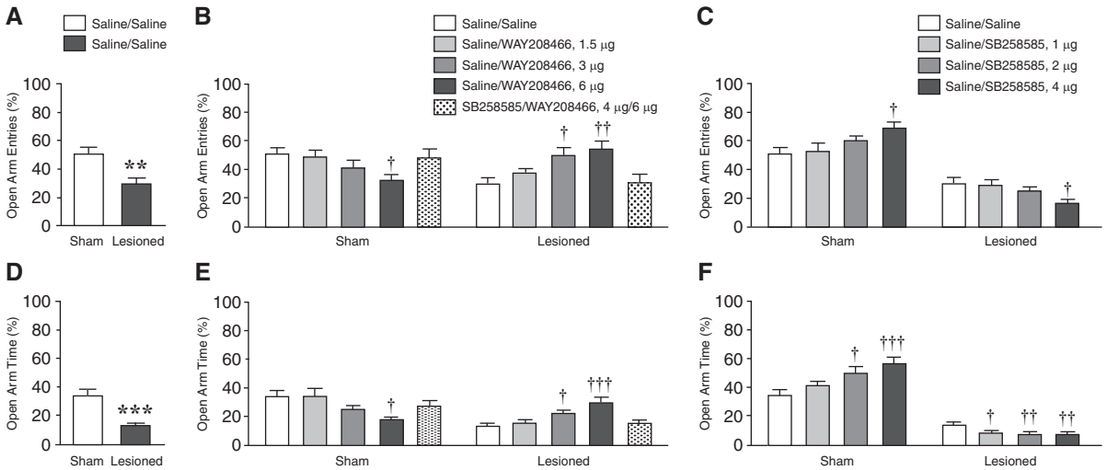

Fig. 4. Histograms showing effects of 6-OHDA lesion, intra-PrL injection of 5-HT$_6$ receptor agonist WAY208466 and antagonist SB258585 on anxiety-like behavior measured by the EPM test. Unilaterally lesioning the MFB decreased the percentages of open arm entries and open arm time compared to sham-operated rats (A, D). In sham-operated rats, intra-PrL injection of WAY208466 decreased the percentages of open arm entries and open arm time compared to the saline injection into the PrL in the same group (B, E), and SB258585 increased the percentages of open arm entries and open arm time (C, F). In the lesioned rats, WAY208466 increased the percentages of open arm entries and open arm time compared to the saline injection into the PrL in the same group (B, E), and SB258585 decreased the percentage of open arm entries and open arm time (C, F). $^{**}P < 0.01$, $^{***}P < 0.001$ *vs*. sham-operated rats; †$P < 0.05$, ††$P < 0.01$, †††$P < 0.001$ *vs*. the saline injection into the PrL in the same group; n = 10 rats/group.

post-hoc test, 6 μg, $P < 0.05$; Fig. 3B), indicating an anxiogenic response. Intra-PrL injection of SB258585 (1, 2, and 4 μg/rat) significantly increased the percentage of time spent in the center area compared to the saline group ($F_{3,36} = 3.81$, $P < 0.05$, one-way ANOVA; followed by Dunnett's *post-hoc* test, 4 μg, $P < 0.05$; Fig. 3C). The data indicate the induction of anxiolytic response following the injection of SB258585.

In the 6-OHDA-lessoned rats, intra-PrL injection of WAY208466, at doses of 3 and 6 μg/rat (but not 1.5 μg/rat), significantly increased the percentage of time spent in the center area compared to the saline injection into the PrL ($F_{4,45} = 5.247$, $P < 0.001$, one-way ANOVA; followed by Dunnett's *post-hoc* test, 3 μg, $P < 0.05$; 6 μg, $P < 0.01$; Fig. 3B), indicating an dose-dependent anxiolytic response. Intra-PrL injection of SB258585, at the same doses, significantly decreased the percentage of time spent in the center area compared to the saline group ($F_{3,36} = 3.18$, $P < 0.05$, one-way ANOVA; followed by Dunnett's *post-hoc* test, 4 μg, $P < 0.05$; Fig. 3C). The data indicate the induction of anxiogenic response following the injection of SB258585. Additionally, prior injection of SB258585 blocked the effects of WAY208466 in two groups of rats (Fig. 3B).

In the EPM test, the MFB lesions in rats significantly decreased the percentages of open arm entries and open arm time compared to sham-operated rats (open arm entries, $P < 0.01$; open arm time, $P < 0.001$; unpaired Student's *t*-test; Fig. 4, A and D). In sham-operated rats, intra-PrL injection of WAY208466 (1.5, 3, and 6 μg/rat) significantly decreased the percentages of open arm entries and open arm time compared to the saline injection into the PrL (open arm entries, $F_{4,45} = 2.618$, $P < 0.05$; open arm time, $F_{4,45} = 3.332$, $P < 0.05$; one-way ANOVA; followed by Dunnett's *post-hoc* test, 6 μg, both $P < 0.05$; Fig. 4, B and E), indicating an anxiogenic response. Intra-PrL injection of SB258585 (1, 2, and 4 μg/rat) significantly increased the percentages of open arm entries and open arm time compared to the saline group (open arm entries, $F_{3,36} = 3.023$, $P < 0.05$; open arm time, $F_{3,36} = 5.656$, $P < 0.01$; one-way ANOVA; followed by Dunnett's *post-hoc* test, 4 μg, $P < 0.05$ for the percentages of open arm entries; 2 μg, $P < 0.05$, 4 μg, $P < 0.001$ for the percentage of open arm time; Fig. 4C and F). The data indicate the induction of anxiolytic response following the injection of SB258585.

In the 6-OHDA-lesioned rats, intra-PrL injection of WAY208466, at doses of 3 and 6 μg/rat (but not 1.5 μg/rat), significantly increased the percentages of open arm entries and open arm time compared to the saline injection into the PrL (open arm entries, $F_{4,45} = 4.714$, $P < 0.01$; open arm time, $F_{4,45} = 7.489$, $P < 0.001$; one-way ANOVA; followed by Dun-

nett's *post-hoc* test, 3 μg, $P < 0.05$, 6 μg, $P < 0.01$ for the percentages of open arm entries; 3 μg, $P < 0.05$, 6 μg, $P < 0.001$ for the open arm time; Fig. 4, B and E), indicating an dose-dependent anxiolytic response. Intra-PrL injection of SB258585, at the same doses, significantly decreased the percentages of open arm entries and open arm time compared to the saline group (open arm entries, $F_{3,36} = 3.12$, $P < 0.05$; open arm time, $F_{3,36} = 5.174$, $P < 0.01$; one-way ANOVA; followed by Dunnett's *post-hoc* test, 4 μg, $P < 0.05$ for the percentage of open arm entries; 1 μg, $P < 0.05$, 2 and 4 μg, $P < 0.01$ for the percentage of open arm time; Fig. 4, C and F). The data indicate the induction of anxiogenic response following the injection of SB258585. Additionally, pretreatment with SB258585 blocked the effects of WAY208466 in two groups of rats (Fig. 4, B and E).

Discussion

Although rats with unilateral 6-OHDA lesions of the MFB showed decreased horizontal and vertical activity in the open-field test, intra-PrL injection of WAY208466 or SB258585 did not affect locomotor activity in both sham-operated and the lesioned rats. These results are confirmed by previous studies (12, 31, 46). Thus, effects of WAY208466 and SB258585 on anxiety-like behavior tests are not directly related to animal's locomotor state.

In the present study, rats with unilateral MFB lesions showed increased anxiety-like behaviors in the open-field and EPM tests compared to sham-operated rats, indicating the induction of anxiogenic responses. Likewise, several studies found that rats with unilateral MFB lesions had anxiety-like behaviors as measured by the EPM, locomotor chamber or social interaction tests (5, 12, 34). However, other studies using the same model showed no effects on anxiety-like behaviors in the EPM, open field or acoustic startle response tests (4, 16). This discrepancy may be related to the extent of the lesion, used anxiety paradigms and time frame for the behavioral tests. Therefore, our results support previous studies (5, 12, 15, 34), and the notion that unilaterally lesioning the MFB in rats is able to induce anxiety-like behaviors. These findings also indicate that DA depletion plays an important role in the onset of anxiety in PD.

The ventral mPFC, including the PrL, is involved in the regulation of anxiety-like behaviors, because several studies have shown that lesions of the ventral mPFC in rats induce anxiolytic effects (14, 33). Further, the mPFC receives serotonergic afferents from the raphe nuclei (24), and expresses a relative high density of 5-HT_6 receptors (7, 45, 46). Previous studies have found that both 5-HT_6 receptor agonists (*e.g.*, WAY208466, WAY181187 and EMD386088) and antagonists (*e.g.*, SB258585 and SB399885) produce anxiolytic effects in rats and mice after intraperitoneal or intra-hippocampal administration (23, 26, 28, 40, 43). These findings suggest that the 5-HT_6 receptor may be an important target of action of anxiolytic effects.

Although 5-HT_6 receptor agonists and antagonists have an evident role in decreasing anxiety, little is known concerning effects of 5-HT_6 receptor agonist WAY208466 and antagonist SB258585 injected into the PrL on anxiety-like behaviors in rats, particularly in PD-related anxiety. In the present study, intra-PrL injection of WAY208466 induced anxiogenic responses as measured by the open field and EPM tests in sham-operated rats; however, the results are inconsistent with previous reports, because activation of 5-HT_6 receptors produces anxiolytic effects (1, 23). Further, intra-PrL injection of SB258585 produced anxiolytic effects in sham-operated rats, which are consistent with reported data (23, 40, 43). Therefore, current data suggest that 5-HT_6 receptors in the PrL are involved in the regulation of anxiety-like behaviors. It is surprising that the behavioral effects produced by WAY208466 and SB258585 in the 6-OHDA-lesioned rats were opposite compared to sham-operated rats, *i.e.*, intra-PrL injection of WAY208466 produced anxiolytic responses, and SB258585 induced anxiogenic responses. Additionally, pretreatment with SB258585 in the PrL completely blocked behavioral effects produced by WAY208466 in two groups of rats, indicating that effects produced by WAY208466 are mediated *via* 5-HT_6 receptors. The behavioral effects produced by intra-PrL injection of WAY208466 and SB258585 in sham-operated and the lesioned rats could be explained by the following observations.

Increased neuronal activity in the mPFC is associated with anxiety, because stress and anxiety-inducing stimuli consistently activate the mPFC in rats (30). A recent study has also found that intra-PrL injection of WAY208466 increases the firing activity of PrL glutamatergic neurons in rats, while SB258585 decreases the firing activity of the neurons (46). Therefore, a more likely explanation for current data is that intra-PrL injection of WAY208466 or SB258585 changes the activity of PrL glutamatergic neurons in sham-operated rats, which produces anxiogenic or anxiolytic effects. However, the present results showed that WAY208466 produced anxiolytic responses and SB258585 induced anxiogenic behaviors in the 6-OHDA-lesioned rats. Although the behavioral effects of 5-HT_6 receptor agonists and antagonists could be involved in multiple factors which include: systemic or local administration of the

drugs and neuroanatomical site of local administration, selectivity of the drugs, different experimental models, and used anxiety paradigms, the neurochemical results may provide direct evidences for the paradoxical effects of WAY208466 and SB258585 in sham-operated or the lesioned rats, and between two groups of rats.

The regulatory role of monoaminergic system in emotional and behavioral processes has long been recognized together with its implication in a variety of behavior-related disorders, including anxiety (22). The mPFC, amygdala, habenula and ventral hippocampus are main structures of the limbic and limbic-related brain regions that are involved in the regulation of anxiety (46). Our previous studies found that DA depletion could induce anxiety-like behaviors in PD rats (12, 34). In these studies, the neurochemical results revealed that unilateral 6-OHDA lesions decreased DA levels in the limbic and limbic-related brain regions (34, 46). Our recent study has found that intra-PrL injection of WAY208466 or SB258585 changes DA and noradrenaline (NA) levels in several brain regions in sham-operated and the 6-OHDA-lesioned rats (46). This study also demonstrated that activation and blockade of 5-HT$_6$ receptors in PrL produce different effects on depressive-like behaviors in unilateral 6-OHDA-induced Parkinson's rats. Considering the fact that depression and anxiety are related manifestations and often co-exist in PD (21, 37), we could predict that 5-HT$_6$ receptors in PrL may be involved in the regulation of PD-related anxiety. Additionally, several studies have also shown that 5-HT$_6$ receptor agonist WAY208466 and antagonists SB258585 and SB271046 do not change 5-HT levels in the limbic and limbic-related brain regions (17, 19, 46), indicating that 5-HT is not involved in the regulation of anxiety induced by the agonist and antagonists. These neurochemical results suggest that intra-PrL injection of WAY208466 and SB258585 decreases or increases DA and NA neurotransmission in the limbic and limbic-related brain regions in sham-operated and the lesioned rats, which leads to anxiogenic or anxiolytic responses.

The mPFC has direct or indirect connections to limbic and limbic-related brain regions believed to be involved in anxiety such as raphe nuclei and amygdala, and regulates the activity of these downstream brain structures (2, 8, 29). Therefore, dysfunctional changes within these interconnected brain regions are involved in the regulation of anxiety. It is also interesting to note that WAY208466 increases the firing activity of PrL glutamatergic neurons in both sham-operated and the 6-OHDA-lesioned rats (46); however, WAY208466 produced opposite behavioral effects. Likewise, SB258585 also produced different behavioral effects, although SB258585 decreases the firing activity of PrL glutamatergic neurons in two groups of rats (25). Indeed, previous studies have found that intra-mPFC injection of 5-HT$_{1A}$ receptor agonist 8-OHDP-AT decreases serotonergic neuron activity in the rat dorsal raphe nucleus, and 5-HT release in the dorsal raphe nucleus and mPFC (2, 3). As with the connections between mPFC and raphe nuclei, the mPFC is thought to regulate emotional responses by directly inhibiting amygdala activity (18). These studies suggest that the mPFC exerts its effects on anxiety largely through its interactions with downstream brain structures. Based on previous studies and current data, we consider that changed excitatory glutamatergic output of the PrL may affect the activity of the aforementioned downstream brain structures.

Together, the present results indicate that activation and blockade of 5-HT$_6$ receptors in the PrL produced different behavioral effects in the tests of anxiety in sham-operated and the 6-OHDA-lesioned rats, which may be involved in changed DA and NA neurotransmission in the limbic and limbic-related brain regions.

Acknowledgments

This study was supported by the National Natural Science Foundation of China (No. 81771383).

Conflict of Interest

The authors have nothing to declare.

References

1. Carr, G.V., Schechter, L.E. and Lucki, I. Antidepressant and anxiolytic effects of selective 5-HT$_6$ receptor agonists in rats. *Psychopharmacology* (*Berl*). 213: 499-507, 2011.
2. Casanovas, J.M., Hervas, I. and Artigas, F. Postsynaptic 5-HT$_{1A}$ receptors control 5-HT release in the rat medial prefrontal cortex. *Neuroreport* 10: 1441-1445, 1999.
3. Celada, P., Puig, M.V., Casanovas, J.M., Guillazo, G. and Artigas, F. Control of dorsal raphe serotonergic neurons by the medial prefrontal cortex: Involvement of serotonin-11, GABA$_A$, and glutamate receptors. *J. Neurosci*. 21: 9917-9929, 2001.
4. Delaville, C., Chetrit, J., Abdallah, K., Morin, S., Cardoit, L., De Deurwaedère, P. and Benazzouz, A. Emerging dysfunctions consequent to combined monoaminergic depletions in parkinsonism. *Neurobiol. Dis*. 45: 763-773, 2012.
5. Eskow Jaunarajs, K.L., Dupre, K.B., Ostock, C.Y., Button, T., Deak, T. and Bishop, C. Behavioral and neurochemical effects of chronic l-dopa treatment on nonmotor sequelae in the hemiparkinsonian rat. *Behav. Pharmacol*. 21: 627-637, 2010.
6. Fogaca, M.V., Aguiar, D.C., Moreira, F.A. and Guimarães, F.S. The endocannabinoid and endovanilloid systems interact in the rat prelimbic medial prefrontal cortex to control anxiety-like behavior. *Neuropharmacology* 63: 202-210, 2012.
7. Gérard, C., Martres, M.P., Lefèvre, K., Miquel, M.C., Vergé, D., Lanfumey, L., Doucet, E., Hamon, M. and El Mestikawy, S.

Immuno-localization of serotonin 5-HT_6 receptor-like material in the rat central nervous system. *Brain Res*. 746: 207-219, 1997.
8. Hajós, M., Richards, C.D., Székely, A.D. and Sharp, T. An electrophysiological and neuroanatomical study of the medial prefrontal cortical projection to the midbrain raphe nuclei in the rat. *Neuroscience* 87: 95-108, 1998.
9. Hamon, M., Doucet, E., Lefèvre, K., Miquel, M.C., Lanfumey, L., Insausti, R., Frechilla, D., Del Rio, J. and Vergé, D. Antibodies and antisense oligonucleotide for probing the distribution and putative functions of central 5-HT_6 receptors. *Neuropsychopharmacology* 21 (2 Suppl): 68s-76s, 1999.
10. Heidbreder, C.A. and Groenewegen, H.J. The medial prefrontal cortex in the rat: Evidence for a dorso-ventral distinction based upon functional and anatomical characteristics. *Neurosci. Biobehav*. 27: 555-579, 2003.
11. Hirst, W.D., Minton, J.A., Bromidge, S.M., Moss, S.F., Latter, A.J., Riley, G., Routledge, C., Middlemiss, D.N. and Price, G.W. Characterization of [$^{(125)}$I]-SB-258585 binding to human recombinant and native 5-HT_6 receptors in rat, pig and human brain tissue. *Brit. J. Pharmacol*. 130: 1597-1605, 2000.
12. Hui, Y.P., Wang, T., Han, L.N., Li, L.B., Sun, Y.N., Liu, J., Qiao, H.F. and Zhang, Q.J. Anxiolytic effects of prelimbic 5-HT_{1A} receptor activation in the hemiparkinsonian rat. *Behav. Brain Res*. 277: 211-220, 2015.
13. Huot, P., Fox, S.H. and Brotchie, J.M. The serotonergic system in parkinson's disease. *Prog. Neurobiol*. 95: 163-212, 2011.
14. Jinks, A.L. and McGregor, I.S. Modulation of anxiety-related behaviours following lesions of the prelimbic or infralimbic cortex in the rat. *Brain Res*. 772: 181-190, 1997.
15. Jungnickel, J., Kalve, I., Reimers, L., Nobre, A., Wesemann, M., Ratzka, A., Halfer, N., Lindemann, C., Schwabe, K., Töllner, K., Gernert, M. and Grothe, C. Topology of intrastriatal dopaminergic grafts determines functional and emotional outcome in neurotoxin-lesioned rats. *Behav. Brain Res*. 216: 129-135, 2011.
16. Kuan, W.L., Zhao, J.W. and Barker, R.A. The role of anxiety in the development of levodopa-induced dyskinesias in an animal model of parkinson's disease, and the effect of chronic treatment with the selective serotonin reuptake inhibitor citalopram. *Psychopharmacology* (*Berl*). 197: 279-293, 2008.
17. Lacroix, L.P., Dawson, L.A., Hagan, J.J. and Heidbreder, C.A. 5-HT_6 receptor antagonist sb-271046 enhances extracellular levels of monoamines in the rat medial prefrontal cortex. *Synapse* 51: 158-164, 2004.
18. Likhtik, E., Pelletier, J.G., Paz, R. and Paré, D. Prefrontal control of the amygdala. *J. Neurosci*. 25: 7429-7437, 2005.
19. Liu, K.C., Li, J.Y., Tan, H.H., Du, C.X., Xie, W., Zhang, Y.M., Ma, W.L. and Zhang, L. Serotonin 6 receptors in the dorsal hippocampus regulate depressive-like behaviors in unilateral 6-hydroxydopamine-lesioned parkinson's rats. *Neuropharmacology* 95: 290-298, 2015.
20. Marazziti, D., Baroni, S., Pirone, A., Giannaccini, G., Betti, L., Schmid, L., Vatteroni, E., Palego, L., Borsini, F., Bordi, F., Piano, I., Gargini, C., Castagna, M., Catena-Dell'Osso, M. and Lucacchini, A. Distribution of serotonin receptor of type 6 (5-HT_6) in human brain post-mortem. A pharmacology, autoradiography and immunohistochemistry study. *Neurochem. Res*. 37: 920-927, 2012.
21. Menza, M.A., Robertson-Hoffman, D.E. and Bonapace, A.S. Parkinson's disease and anxiety: Comorbidity with depression. *Biol. Psychiatry* 34: 465-470, 1993.
22. Millan, M.J. The neurobiology and control of anxious states. *Prog. Neurobiol*. 70: 83-244, 2003.
23. Nikiforuk, A., Kos, T. and Wesolowska, A. The 5-HT_6 receptor agonist emd 386088 produces antidepressant and anxiolytic effects in rats after intrahippocampal administration. *Psychopharmacology* (*Berl*). 217: 411-418, 2011.
24. O'Hearn, E. and Molliver, M.E. Organization of raphe-cortical projections in rat: A quantitative retrograde study. *Brain Res. Bull*. 13: 709-726, 1984.
25. Paxinos, G. and Watson, C. The rat brain in stereotaxic coordinates 4th ed[M]. New York: Academic Press, 1998.
26. Prediger, R.D., Matheus, F.C., Schwarzbold, M.L., Lima, M.M.S. and Vital, M.A.B.F. Anxiety in parkinson's disease: A critical review of experimental and clinical studies. *Neuropharmacology* 62: 115-124, 2012.
27. Rodgers, R.J. and Dalvi, A. Anxiety, defence and the elevated plus-maze. *Neurosci. Biobehav. Rev*. 21: 801-810, 1997.
28. Schechter, L.E., Lin, Q., Smith, D.L., Zhang, G., Shan, Q., Platt, B., Brandt, M.R., Dawson, L.A., Cole, D., Bernotas, R., Robichaud, A., Rosenzweig-Lipson, S. and Beyer, C.E. Neuropharmacological profile of novel and selective 5-HT_6 receptor agonists: WAY-181187 and WAY-208466. *Neuropsychopharmacology* 33: 1323-1335, 2008.
29. Sesack, S.R., Deutch, A.Y., Roth, R.H. and Bunney, B.S. Topographical organization of the efferent projections of the medial prefrontal cortex in the rat: An anterograde tract-tracing study with phaseolus vulgaris leucoagglutinin. *J. Comp. Neurol*. 290: 213-242, 1989.
30. Singewald, N., Salchner, P. and Sharp, T. Induction of c-fos expression in specific areas of the fear circuitry in rat forebrain by anxiogenic drugs. *Biol. Psychiatry* 53: 275-283, 2003.
31. Solati, J., Salari, A.A. and Bakhtiari, A. 5-HT_{1A} and 5-HT_{1B} receptors of medial prefrontal cortex modulate anxiogenic-like behaviors in rats. *Neurosci. Lett*. 504: 325-329, 2011.
32. Steketee, J.D. Neurotransmitter systems of the medial prefrontal cortex: Potential role in sensitization to psychostimulants. *Brain Res. Brain Res. Rev*. 41: 203-228, 2003.
33. Sullivan, R.M. and Gratton, A. Behavioral effects of excitotoxic lesions of ventral medial prefrontal cortex in the rat are hemisphere-dependent. *Brain Res*. 927: 69-79, 2002.
34. Sun, Y.N., Wang, T., Wang, Y., Han, L.N., Li, L.B., Zhang, Y.M. and Liu, J. Activation of 5-HT_{1A} receptors in the medial subdivision of the central nucleus of the amygdala produces anxiolytic effects in a rat model of parkinson's disease. *Neuropharmacology* 95: 181-191, 2015.
35. Treit, D. and Fundytus, M. Thigmotaxis as a test for anxiolytic activity in rats. *Pharmacol. Biochem. Behav*. 31: 959-962, 1988.
36. Wang, S., Zhang, Q., Liu, J., Wu, Z.H., Wang, T., Gui, Z.H., Chen, L. and Wang, L. Unilateral lesion of the nigrostriatal pathway induces an increase of neuronal firing of the midbrain raphe nuclei 5-HT neurons and a decrease of their response to 5-HT_{1A} receptor stimulation in the rat. *Neuroscience* 159: 850-861, 2009.
37. Wee, N., Kandiah, N., Acharyya, S., Chander, R.J., Ng, A., Au, W.L. and Tan, L.C.S. Depression and anxiety are co-morbid but dissociable in mild parkinson's disease: A prospective longitudinal study of patterns and predictors. *Parkinsonism Relat. Disord*. 23: 50-56, 2016.
38. Wesolowska, A. and Nikiforuk, A. Effects of the brain-penetrant and selective 5-HT_6 receptor antagonist sb-399885 in animal models of anxiety and depression. *Neuropharmacology* 52: 1274-1283, 2007.
39. Wesolowska, A. Potential role of the 5-HT_6 receptor in depression and anxiety: An overview of preclinical data. *Pharmacol. Rep*. 62: 564-577, 2010.
40. Wesolowska, A. The anxiolytic-like effect of the selective 5-HT_6 receptor antagonist SB-399885: The impact of benzodiazepine receptors. *Eur. J. Pharmacol*. 580: 355-360, 2008.
41. Wesolowska, A., Nikiforuk, A. and Stachowicz, K. Anxiolytic-like and antidepressant-like effects produced by the selective 5-HT_6 receptor antagonist SB-258585 after intrahippocampal administration to rats. *Behav. Pharmacol*. 18: 439-446, 2007.
42. Woolley, M.L., Marsden, C.A. and Fone, K.C. 5-HT_6 receptors. Curr. Drug Targets CNS Neurol. *Disord*. 3: 59-79, 2004.
43. Yoshioka, M., Matsumoto, M., Togashi, H., Mori, K. and Saito, H. Central distribution and function of 5-HT_6 receptor subtype

in the rat brain. *Life Sci*. 62: 1473-1477, 1998.

44. Yun, H.M. and Rhim, H. The serotonin-6 receptor as a novel therapeutic target. *Exp. Neurobiol.* 20: 159-168, 2011.
45. Zhang, X., Andren, P.E., Glennon, R.A. and Svenningsson, P. Distribution, level, pharmacology, regulation, and signaling of 5-HT$_6$ receptors in rats and marmosets with special reference to an experimental model of parkinsonism. *J. Comp. Neurol*. 519: 1816-1827, 2011.
46. Zhang, Y.M., Zhang, L., Wang, Y., Sun, Y.N., Guo, Y., Du, C.X., Zhang, J., Yao, L., Yu, S.Q. and Liu, J. Activation and blockade of prelimbic 5-HT$_6$ receptors produce different effects on depressive-like behaviors in unilateral 6-hydroxydopamine-induced parkinson's rats. *Neuropharmacology* 110(Pt A): 25-36, 2016.