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Effects of serotonin (5-HT)$_6$ receptor ligands on responding for cocaine reward and seeking in rats

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Abstract:
The endogenous brain serotonin (5-HT) system is believed to have an important modulatory influence in mediating drug reward and seeking mechanisms. Data from preclinical behavioral studies have provided emerging evidence that 5-HT$_6$ receptors, among other 5-HT receptors, may play a significant role in the mechanisms of action of psychostimulant addicted drugs. The aim of the present study was to investigate whether the selective pharmacological blockade or activation of 5-HT$_6$ receptors altered the maintenance of cocaine self-administration, reinstatement of cocaine-seeking behavior following an extinction of cocaine self-administration or cocaine-evoked conditioned place preference in rats. We also evaluated the effects of 5-chloro-N-(4-methoxy-3-piperazin-1-ylphenyl)-3-methyl)-2-benzothiophene-sulfonamide (SB 271046, a 5-HT$_6$ receptor antagonist) or N-1-(6-chloroimidazo-[2,1-b]-[1,3]thiazole-5-sulfonyl)tryptamine (WAY 181187, a potent 5-HT$_6$ receptor agonist) on locomotor activity in rats. Our results indicate that SB 271046 (1–10 mg/kg) altered cocaine-maintained self-administration as well as cocaine-evoked reinstatement of cocaine seeking and expression of cocaine place preference in rats. We also demonstrate that pharmacological stimulation of 5-HT$_6$ receptors by WAY 181187 (3–30 mg/kg) attenuated the expression of cocaine conditioned place preference but not cocaine self-administration and reinstatement of cocaine seeking. WAY 181187 at the highest dose used (30 mg/kg) reduced basal locomotor activity. Despite current results, the precise function and therapeutic relevance of 5-HT$_6$ receptors need further clarification.

Key words:
5-HT$_6$ receptor ligands, cocaine, conditioned place preference, locomotor activity, self-administration, rats

Introduction

Cocaine addiction has somatic, psychological, psychiatric, socio-economic and legal implications in the developed world. There is no medication approved for the treatment of cocaine addiction despite the fact that the neurochemical mechanism of cocaine action is relatively well characterized. In fact, dopamine (DA) neurotransmission and the indirect activation of DA receptors have been established as the primary mediators of the reinforcing/addictive properties of cocaine [9, 27, 28, 47]. Such an action of cocaine depends on its ability to block the DA (K_i = 640 nM) reuptake [25] due to a high affinity for the plasma membrane DA transporter (K_i = 277 nM; [38]). More recently it was also established that cocaine binding sites contain both high- and low-affinity binding components independent to the DA transporter [31], but rather link to a novel allosteric agonist action of cocaine in low (1–10 nM) concentrations on DA receptors [12].

However, DA does not seem to be the sole mediator of the rewarding effects of cocaine as the drug displays high affinity for a serotonin (5-HT) transporter [38]. Effects at both the DA and 5-HT transporters appear critical as the rewarding effect of cocaine in a conditioned place preference paradigm was eliminated in homozygous DA and 5-HT transporter knockout mice [46], while elimination of 5-HT transporter alone diminished cocaine-conditioned locomotion in mice [21] or enhanced cocaine self-administration in rats [24]. A combination of behavioral models and microdialysis studies indicates that passive or active administration of cocaine to rats concomitantly increased their locomotor/rewarding effects and elevated DA and 5-HT release in the nucleus accumbens [2, 10, 34] or the globus pallidus [45].

5-HT can interact with at least 16 different brain 5-HT receptors, which may be important targets for pharmacological interventions to alter cocaine functions [14]. In recent years, data from preclinical behavioral studies have provided emerging evidence that 5-HT_6 receptors play a modulatory role in the mechanisms of action of addicted psychostimulants. Thus, the 5-HT_6 receptor pharmacological blockade potentiated amphetamine-evoked locomotion and self-administration [20] as well as discriminative stimulus [35] in rats. With regards to cocaine, 5-HT_6 receptor antagonism was found to attenuate cue-induced relapse to cocaine seeking [48] without changing cocaine self-administration [20, 48] or locomotion [20]. On the other hand, a selective increase in 5-HT_6 receptor expression in the nucleus accumbens by a viral-mediated gene transfer resulted in decreased conditioned place preference to cocaine but had no effect on either acute locomotor hyperactivation to cocaine or on the development of cocaine sensitization [11].

The present study investigated whether the selective pharmacological blockade or activation of 5-HT_6 receptors altered the maintenance of cocaine self-administration, reinstatement of cocaine-seeking behavior following an extinction of cocaine self-administration or cocaine-evoked conditioned place preference. Moreover, we evaluated the effects of 5-chloro-N-(4-methoxy-3-piperazin-1-ylphenyl)-3-methyl)-2-benzothiophene-sulfonamide (SB 271046), a 5-HT_6 receptor antagonist (K_i = 1–2 nM for 5-HT_6 receptors and >200-fold more selectivity for 5-HT_6 receptors *vs.* other receptors binding sites and ion channels; [3, 5]), or N-1-(6-chloroimidazo[2,1-b][1,3]thiazole-5-sulfonyl)-tryptamine (WAY 181187), a potent 5-HT_6 receptor agonist (K_i = 2.2 nM for 5-HT_6 receptors and >60-fold selectivity over several receptors, including other 5-HT receptor subtypes [23, 42]) on locomotor activity in the rat.

Materials and Methods

Animals

Male Wistar rats (280–300 g) delivered by the licensed breeders (Charles River, Germany, self-administration; HZL, Warszawa, Poland, conditioned place preference) were housed 4/cage (conditional place preference) or individually (self-administration) in standard plastic rodent cages in a colony room maintained at 20 ± 1°C and at 40–50% humidity under a 12-h light-dark cycle (lights on at 06:00). Animals had free access to standard animal food and water except those used in the cocaine self-administration procedures which were maintained on limited water during initial training sessions (see below). All experiments were conducted during the light phase of the light-dark cycle (between 08:00–15:00) and were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Ani-

mals and with approval of the Bioethics Commission as compliant with the Polish Law (21 August 1997). The animals were experimentally naive.

Drugs

Cocaine hydrochloride (Sigma-Aldrich, St. Louis, USA), SB 271046 and WAY 181187 (Abbot Healthcare Products B.V., The Netherlands) were used. Cocaine was dissolved in sterile 0.9% NaCl, WAY 181187 was dissolved in sterile water, while SB 271046 was diluted in 1% Tween (Sigma-Aldrich, USA). Cocaine was given either *iv* (0.1 ml/infusion) or *ip* (1 ml/kg) immediately before behavioral sessions. SB 271046 was given *ip* (1 ml/kg) and WAY 181187 was administered *sc* (2 ml/kg) 30 min before cocaine. The dose-range and pretreatment intervals of SB 271046 were chosen based on its effects in the number of behavioral and neurochemical studies [6, 7, 17, 32, 48]. Doses of WAY 181187 were selected since they significantly altered brain neurochemistry across a number of brain regions [42] or behavioral responding [4] while timing of drug injections was optimized to take account of its pharmacodynamic properties in rats [42].

Cocaine self-administration

Rats were trained to lever press in standard operant conditioning chambers (Med-Associates, USA) under a fixed ratio 5 schedule of water reinforcement [for more details see 19]. Then, they were implanted with catheters flushed every day with 0.1 ml of saline solution containing heparin (70 U/ml, Biochemie GmbH, Austria) and 0.1 ml of solution of cephazolin (10 mg/ml; Biochemie GmbH, Austria).

After a 10-dy recovery period, all animals were water deprived for 18 h and trained to lever press to fixed ratio 5 schedule of water reinforcement over a 2-h session. Subjects were then given access to cocaine during 2-h daily sessions performed 6 days/week (maintenance) and from that time they were given *ad libitum* water. The house light was illuminated throughout each session. Each completion of five presses on the "active" lever complex (fixed ratio 5 schedule) resulted in a 5-s infusion of cocaine (0.5 mg/kg per 0.1 ml) and a 5-s presentation of a stimulus complex (activation of the white stimulus light directly above the "active" lever and the tone generator, 2000 Hz; 15 dB above ambient noise levels); following each injection, there was a 20-s time-out period. Response on the "inactive" lever never resulted in cocaine delivery. Acquisition of the conditioned operant response lasted a minimum of 10 days until subjects met the following criteria: minimum requirement of 22 reinforcements with an average of 6 days and active lever presses with an average of 6 consecutive days and a standard deviation within those 6 days of <10% of the average; this criterion was selected based on our prior experiments [13]. Following stabilization of responding, the extinction procedure was carried out and during extinctions sessions subjects had 2-h daily training sessions with no delivery of cocaine or the presentation of the conditioned stimulus. Once they reached the extinction criteria (a minimum of 10 extinction days with the responding on the active lever below 10% of the level observed during maintenance during at least 3 consecutive days) the rats (n = 7–10 rats/group) were tested for response reinstatement induced by a noncontingent presentation of the self-administered reinforcer (10 mg/kg cocaine, *ip*). During the reinstatement tests (2-h sessions), active lever presses on the fixed ratio 5 schedule resulted only in an intravenous injection of saline.

Before test sessions (maintenance or reinstatement sessions) rats were pretreated with SB 271046 (1, 3 and 10 mg/kg) and WAY 181187 (3, 10 and 30 mg/kg).

Conditioned place preference

The conditioned place preference procedure (biased design) was carried out according to the method described by Maldonado et al. [30]. The apparatus for the conditional place preference procedure consisted of three rectangular boxes (60 × 35 × 30 cm), divided into three compartments (25 × 35 cm) separated by removable guillotine doors from a small central gray area (10 × 10 cm). The walls of the two large compartments differed in color, one having black walls, while the walls of the other one were painted white. The boxes were kept in a soundproof room with a neutral masking noise, and with a dim 40 Lux illumination. The conditional place preference schedule consisted of a pre-testing phase (1 day), a conditioning phase (4 days) and testing phase (1 day). During the pre-testing phase, the baseline preference of the rats was determined. Each rat was placed in the central gray area, the guillotine doors were raised and each rat was allowed to move freely for 15 min between three compartments of the boxes. The time

spent by each animal in the two large compartments was recorded. To establish conditioning, we paired cocaine (5 mg/kg, *ip*) with the initially non-preferred white compartment, every day during conditioning phase. Control rats received injection of saline (*ip*) before their exposure to the white or black compartment.

To investigate the influence of SB 271046 (1, 3 and 10 mg/kg) and WAY 181187 (3, 10 and 30 mg/kg) on the expression of cocaine-induced conditional place preference, rats that had developed cocaine-induced conditional place preference were pre-treated with these drugs before their placement in the conditional place preference apparatus. The time spent by each animal in the white compartment was recorded for 15 min.

Locomotor activity measurement

Locomotor activity was recorded individually for each animal in Opto-Varimex cages (Columbus Instruments, Columbus, USA) with Auto-track software (Columbus Instruments, Columbus, USA). Locomotor activity was defined as a breakage of three consecutive photo-beams and expressed as the mean distance traveled (cm).

To investigate the influence of SB 271046 (1, 3 and 10 mg/kg) and WAY 181187 (3, 10 and 30 mg/kg) on the locomotor activity, non-habituated rats were pretreated with these drugs before their placement in the locomotor chamber. Locomotor activity was recorded for 120 min.

Statistical analyses

Data were expressed as the mean (± SEM). For cocaine self-administration procedures (maintenance and reinstatement), the number of responses on the active and inactive lever (including time out responding) was analyzed by two-way analysis of variance (ANOVA) for repeated measures and a *post-hoc* Duncan's test was used to analyze differences between group means. The number of infusions (maintenance of cocaine self-administration) and locomotor activity were analyzed by one-way ANOVA flowed by a *post-hoc* Dunnett's test to analyze differences between group means. In conditional place preference tests the statistical significance of drug effects assessed by one-way ANOVA and the significance of a difference between individual groups was determined by a Tukey-Kramer multiple comparisons test. $P < 0.05$ was considered statistically significant for all tests.

Results

Effects of 5-HT_6 receptor ligands on cocaine self-administration

After about 12 self-administration sessions rats showed stable lever responding during the sessions with an acquisition criterion requiring that the rate of active lever presses varied by less than 10%. The animals had self-administered 22–38 injections of cocaine with the daily mean cocaine intake between 11–19 mg/kg. Rats responded significantly more frequently on the active lever than on the inactive lever ($p < 0.05$), independently of self-administration session.

Pretreatment with the 5-HT_6 receptor antagonist SB 271046 (1–10 mg/kg) significantly altered the number of lever presses ($F(3,54) = 6.8$, $p < 0.001$) and cocaine infusions ($F(4,28) = 8.3$, $p < 0.01$). A significant decrease was observed with reference to the active lever and cocaine infusions, following 3 and 10 mg/kg of SB 271046, while the dose 1 mg/kg was ineffective. Pretreatment with SB 271046 did not influence the number of inactive lever presses (Fig. 1).

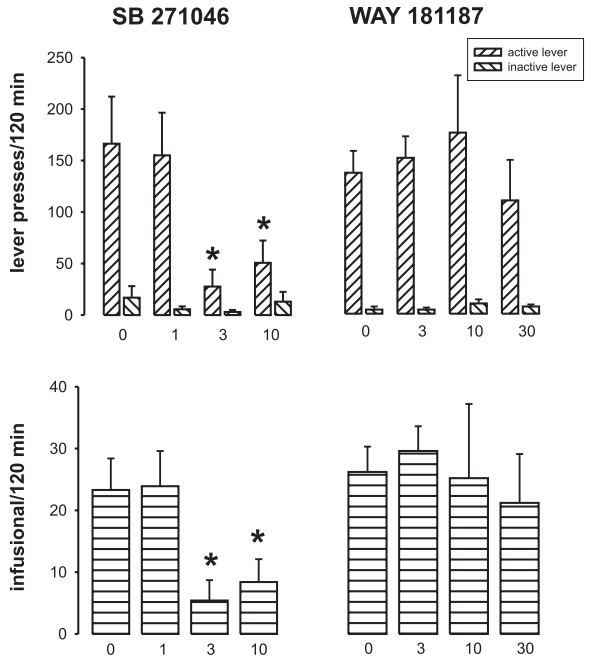

Fig. 1. Effects of the 5-HT_6 receptor antagonist SB 271046 and the agonist WAY 181187 on the maintenance of cocaine (0.5 mg/kg/infusion) self-administration in rats. Number of active and inactive lever presses as well as cocaine infusions are shown for pretreatment with the corresponding vehicle, SB (1, 3 and 10 mg/kg) and WAY (3, 10 and 30 mg/kg). Each bar represents the mean (± SEM) of data from 7–8 rats. * $p < 0.001$ *vs.* vehicle (0)

Pretreatment with the 5-HT$_6$ receptor agonist WAY 181187 (3–30 mg/kg) did not alter the number of lever presses (F(3,24) = 0.37) or cocaine infusions (F(4,26) = 0.03) (Fig.1).

Effects of 5-HT$_6$ receptor ligands on cocaine-primed reinstatement

Following stable cocaine self-administration and 10 days of extinction the rats were tested for response reinstatement induced by cocaine (10 mg/kg, *ip*). Figure 2 shows the effects of 5-HT$_6$ receptor ligands on cocaine-primed reinstatement.

Pretreatment with the 5-HT$_6$ receptor antagonist SB 271046 (1–3 mg/kg) significantly altered the number of lever presses (F(2,36) = 10.2, P.001). A significant decrease ($p < 0.05$) in active lever presses was observed following both doses of SB 271046.

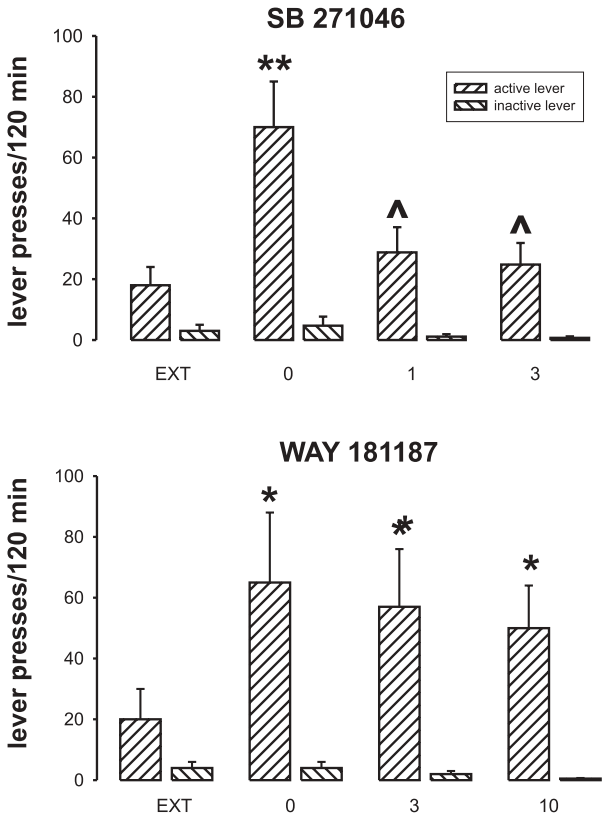

Fig. 2. Effects of the 5-HT$_6$ receptor antagonist SB 271046 and the agonist WAY 181187 on the reinstatement of cocaine-seeking behavior induced by cocaine (10 mg/kg, *ip*) in rats. Number of the active and inactive lever presses following cocaine priming injections are shown for pretreatment with the corresponding vehicle, SB 271046 (1 and 3 mg/kg) and WAY 181187 (3 and 10 mg/kg). Each bar represents the mean (± SEM) of data from 7–8 rats. * $p < 0.05$, ** $p < 0.01$ *vs.* extinction (EXT), ^ $p < 0.05$ *vs.* vehicle (0)

Pretreatment with the 5-HT$_6$ receptor agonist WAY 18187 (3–10 mg/kg) did not alter the number of active and inactive lever presses (F(2,16) = 0.3).

Effects of 5-HT$_6$ receptor ligands on the expression of conditional place preference induced by cocaine

Administration of the 5-HT$_6$ receptor antagonist SB 271046 (1–10 mg/kg) significantly decreased the time spent by rats in cocaine-paired compartment of apparatus (F(6,42) = 7.427; $p < 0.001$). *Post-hoc* analysis showed that administration of cocaine (5 mg/kg) during conditioning phase induced a significant place preference for the drug-associated compartment in the testing phase ($p < 0.05$). SB 271046 at the doses 3 and 10 mg/kg significantly ($p < 0.001$) blocked the effects of cocaine (Fig. 3). SB 271046 given alone to the control group of animals during test day had no influence on their baseline preference (vehicle = 50.4 ± 29.2, SB 271046, 1 mg/kg = 69.5 ± 19.8, SB 271046, 10 mg/kg = 60.5 ± 26.0).

Administration of WAY 181187 (3–30 mg/kg) significantly attenuated the expression of cocaine-induced conditional place preference (F(6,42) = 3.609; $p < 0.01$). *Post-hoc* analysis showed that administration of cocaine (5 mg/kg) during conditioning phase induced significant place preference for the drug-associated compartment in the testing phase ($p < 0.01$). WAY 181187 at 3 mg/kg ($p < 0.05$), and of 10 and 30 mg/kg ($p < 0.01$) significantly reduced the effects of cocaine (Fig. 3).

WAY 181187 given alone at the doses of 3 and 30 mg/kg during testing phase did not change the time spent by rats within control group (vehicle = 73.9 ± 50.7, WAY 181187, 3 mg/kg = 76.8 ± 38.7, WAY 181187, 30 mg/kg = 93.3 ± 35.0).

Effects of 5-HT$_6$ receptor ligands on the basal locomotor activity

As shown in Table 1, treatment with SB 271046 (1–10 mg/kg) did not alter basal locomotor activity (F(3,24) = 2.19).

Treatment with WAY 181187 (3–30 mg/kg) changed the basal locomotor activity recorded in a 120-min trial (F(3,26) = 6.63, $p < 0.01$]. *Post-hoc* analysis revealed that WAY 181187 at a dose of 30 mg/kg significantly decreased basal locomotor activity (Tab. 1).

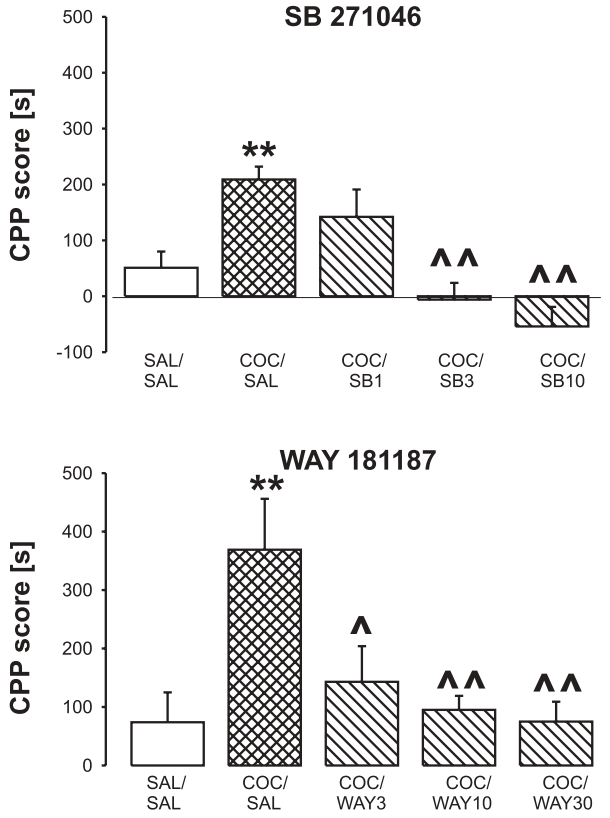

Fig. 3. Effects of the 5-HT_6 receptor antagonist SB 271046 (SB) and the agonist WAY 181187 (WAY) on the expression of cocaine (5 mg/kg, *ip*)-induced conditional place preference in rats. Changes in time were expressed as a difference in testing-, minus pre-testing time (in seconds) spent in a drug-associated compartment are shown for pretreatment with the corresponding vehicle, SB 271046 (1, 3 and 10 mg/kg) and WAY 181187 (3, 10 and 30 mg/kg). Each bar represents the mean (± SEM) of data from 10 rats. ** $p < 0.01$ *vs.* saline (SAL)/SAL-treated group; ^ $p < 0.05$, ^^ $p < 0.01$ *vs.* cocaine (COC)/SAL-treated group

Tab. 1. Effects of the 5-HT_6 receptor antagonist SB 271046 and the agonist WAY 181187 on basal locomotor activity in rats.

Treatment	Dose (mg/kg)	Distance traveled (cm)/120 min
SB 271046	0	3239 ± 477
	1	2970 ± 379
	3	2744 ± 299
	10	2420 ± 289
WAY 181187	0	3095 ± 315
	3	2975 ± 303
	10	2841 ± 269
	30	2067 ± 173*

Basal locomotor activity was recorded as horizontal activity (expressed as distance traveled (in cm)). Each group represents the mean (± SEM) of data from 7–8 rats. * $p < 0.05$ *vs.* vehicle (0)

Discussion

The present findings report for the first time that the selective 5-HT_6 receptor antagonist SB 271046 significantly altered cocaine-maintained self-administration as well as cocaine-evoked reinstatement of cocaine seeking and expression of cocaine place preference in rats. We also demonstrate that pharmacological stimulation of 5-HT_6 receptors by the selective and potent agonist WAY 181187 attenuated expression of cocaine conditional place preference but failed to alter cocaine self-administration and reinstatement of cocaine seeking. Finally, we show that WAY 181187 in the highest used dose significantly reduced basal locomotor activity.

Our results clearly indicate that the cocaine-maintained reinforcing and seeking behaviors in rats are under the tonic regulation of 5-HT acting at 5-HT_6 receptors. Thus, in self-administration procedures, the 5-HT_6 receptor antagonist SB 271046 with no inverse agonist properties [40], when pretreated before cocaine during the maintenance phase, potently reduced the number of active lever presses and cocaine intake. These data seem to be in contrary to the recent observation showing that the same antagonist in similar dose-range as used in the present study did not affect the drug rewards and number of active nose-pokes in rats self-administered cocaine under the 0.5 mg/kg unit dose [48] while an earlier report indicated that another specific 5-HT_6 receptor antagonist SB 258510A failed to block the cocaine-maintained self-administration in rats [20]. The discrepancies can be explained in terms of the experimental conditions used in these experiments and we believe that the most important differences might be the cocaine dose unit delivered to rats, the schedule of reinforcement as well as the speed of drug delivery. In fact, in our study cocaine was given in a dose of 0.5 mg/kg/injection while in that of Franz et al. [20] in four-fold lower one (0.125 mg/kg/injection). The higher dose of cocaine produces more pronounced reward effect by itself (higher number of active lever presses) and such a phasic condition in the DA-ergic mesocorticolimbic system might be effectively altered by the 5-HT_6 receptor blocker resulting in the changing of self-administration behavior in rats. Such a contention is supported by Franz and colleagues [20], who observed that 5-HT_6 receptor antagonism altered the effects of higher (0.024–0.048 mg/infusion) but not lower (0.00075 mg/infusion) doses

of amphetamine in a self-administration paradigm. Moreover, responding maintained by cocaine under a fixed ratio 5 schedule of reinforcement makes rats work harder per cocaine injection (our study) and more motivate them to self-administered cocaine while faster delivery of cocaine evokes faster sensitization of the reinforcing effects of the drug [48] and potentially contributing to putative neuroplasticity of brain 5-HT neurotransmission and 5-HT$_6$ receptor sites.

The following observation that treatment with the lower doses of SB 271046 (1–3 mg/kg) attenuated reinstatement of responding induced by a noncontingent presentation of the self-administered reinforcer (10 mg/kg cocaine, *ip*) and the inhibitory efficacy of the 5-HT$_6$ receptor antagonist indicates that this drug interferes with the primary reinforcing effects of cocaine. These findings extend the previous observation of van Gaalen et al. [48] who found that 5-HT$_6$ receptor antagonism attenuated cue-induced relapse to cocaine seeking in rats suggesting that this pharmacological strategy may be involved in the secondary reinforcing effects of cocaine.

The conclusion that tonic activation of 5-HT$_6$ receptors is involved in cocaine-induced reinstatement of cocaine-seeking behavior and that the inhibitory effects of SB 271046 on this phenomenon are related to motivational aspects of cocaine abuse are further supported by the results of another set of experiments in which rats were subjected to a standard bias conditioned place preference protocol. SB 271046 (1–10 mg/kg) dose-dependently reduced the expression of a conditioned place preference to cocaine and but not alter this effect when paired with saline suggesting that itself is not rewarding. Partially supporting this latter finding, another high-affinity antagonist at 5-HT$_6$ receptor sites 5-methoxy-N,N'-dimethylN1-benzenesulfonyltryptamine (MS-245) did not substitute for cocaine or amphetamine in drug discrimination task [35].

When discussing decreases seen following SB 271046 several issues should be considered. First, an attenuation of the cocaine intake and the drug-associated active-lever presses could be due to an increase (less drug necessary for the desired hedonic effect) or a decrease (attenuation of the motivation to self-administration) of the rewarding effects of the self-administered drug [cf. 41]. When using a fixed ratio schedule of reinforcement in this study, an increase of the rewarding effects of cocaine should be rather expected based on the reported neurochemical and behavioral evidences. Thus, SB 271046 potentiated amphetamine-induced changes in DA in the rat frontal cortex [20] or striatum [7]; however, produced non-significant effects on cocaine functions measured in the frontal cortex [20]. In behavioral tasks, 5-HT$_6$ receptor antagonists facilitated locomotor, discriminative stimulus and reinforcing properties of amphetamine [20, 35] as well as cocaine conditional place preference to cocaine during acquisition phase [11]. Secondly, since the reinstatement of cocaine seeking was initiated by *ip* cocaine administration by an experimenter, SB 271046 could influence either the motivational effects of cocaine, locomotor stimulant effects or even emotional state. Cocaine priming during reinstatement of active-lever presses could function as a discriminative stimulus and affect motivational aspects indirectly by being a cue of the cocaine availability. Since 5-HT$_6$ receptor antagonists neither substitute for cocaine, affect the intereceptive cue in drug discrimination task [35] or locomotor responses [20] in rats, such interpretation is rather doubtful. Locomotor inhibitory responses of SB 271046 were not due to its motor artifacts as basal locomotor activity or on inactive lever presses in self-administration procedures (present study) were unaffected. With reference to the involvement of emotional state in controlling the expression of cocaine reinstatement, SB 271046 was shown to produce anxiolytic and antidepressant-like effects in several preclinical studies in rodents [22, 32, 49, 50]; whether such properties of this antagonist altered the cocaine-induced priming or expression of cocaine conditional place preference needs further studies. Additionally, the limitation of the present study is using only a separate dose of cocaine during priming and to evoke place conditioning (bias) experiment. Such procedure is nor sensitive for potential leftward shifts in the dose-response curve for the drug of abuse and a possible potentiating effects of an investigated drug remains undetected.

Interestingly, the potent inhibitory responses on the cocaine-primed conditioned place preference were seen following pretreatment with the direct 5-HT$_6$ receptor agonist WAY 181187. These results extend the recent study by Ferguson et al. [11] who observed that overexpression of 5-HT$_6$ receptors in the nucleus accumbens by viral-mediated gene transfer resulted in reduced place preference conditioning to cocaine. The inhibitory effects of WAY 181187 appear to be specific up to an including doses of 10 mg/kg as the highest dose tested (30 mg/kg) produced inhibitory effects

on basal locomotor activity in rats. Furthermore, WAY 181187 at a dose of 17 mg/kg reduced food consumption in the home cage [4] suggesting that its inhibitory responses in place conditioning to cocaine might partly interfere with a decrease in appetitive behaviors. It should also be considered that in contrast to place preference, 5-HT_6 receptor agonism did not affect cocaine-maintained self-administration and seeking. As elegantly reviewed Aguilar et al. [1], both these paradigms evaluate different aspects of reward (primary rewarding effects *vs.* the incentive values of drug-associated cues), different characteristics of relapse (restoration of a concrete operant response *vs.* the reappearance of the approach to a drug-associated context) as well as the neurobiological basis of reward.

The underlying mechanism(s) of the modulatory effects of the 5-HT_6 receptor antagonist and the agonist on cocaine-evoked behavioral functions are still largely unknown as the receptors affect both inhibitory and excitatory neurotransmission and indirectly monoaminergic systems. Thus, changes in the DA-ergic, glutaminianergic and/or GABA-ergic systems appear to have significance in altering the drug-priming and cue-induced reinstatement of cocaine seeking [16, 36, 43, 44] and 5-HT_6 receptors are widely distributed in the nucleus accumbens, striatum, cerebral cortex, olfactory tubercle, and hippocampus that regulate drug craving, emotion, reward and interactive learning processes [15, 26, 33, 39, 44]. One possibility is that 5-HT_6 receptor agonists and antagonists produce similar behavioral effects due to effects at distinct receptor populations, perhaps located in different brain regions and under different 5-HT tone [18]. In fact, *in vivo* microdialysis studies indicate that antagonism of 5-HT_6 receptors increased basal glutamate levels in the rat hippocampus and frontal cortex [6, 7, 37, 52]. The notion that differential site specific pharmacology is supported by differential effects on DA and 5-HT overflow in the frontal cortex *vs.* striatum [8, 20, 29). In contrast, acute administration of WAY-181187 (3–30 mg/kg) elicited robust increases in extracellular levels of GABA and decreases in DA and 5-HT levels without altering the levels of glutamate or norepinephrine in the rat frontal cortex, while in the dorsal hippocampus, striatum, and amygdala, WAY-181187 induced elevations in extracellular concentrations of GABA (but not of norepinephrine, 5-HT, DA and glutamate); WAY-181187 had no effect on the extracellular levels of GABA in the nucleus accumbens or thalamus [42, 51].

To summarize, our present findings suggest that 5-HT_6 receptor ligands (antagonist and agonist) attenuated expression of cocaine conditional place preference while only the 5-HT_6 receptor antagonist significantly reduced altered cocaine-maintained self-administration as well as cocaine-evoked reinstatement of cocaine seeking in rats. Despite current results, the precise function and therapeutic relevance of the 5-HT_6 receptor ligands to cocaine addiction needs further determination. Namely, a full-dose response curve for cocaine actions as well as the effects of the 5-HT_6 receptor ligands on mood, anxiety and cognition should be carefully examined.

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