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Pharmacological Modulation of 5-HT_{2C} Receptor Activity Produces Bidirectional Changes in Locomotor Activity, Responding for a Conditioned Reinforcer, and Mesolimbic DA Release in C57BL/6 Mice

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Converging lines of behavioral, electrophysiological, and biochemical evidence suggest that 5-HT_{2C} receptor signaling may bidirectionally influence reward-related behavior through an interaction with the mesolimbic dopamine (DA) system. Here we directly test this hypothesis by examining how modulating 5-HT_{2C} receptor activity affects DA-dependent behaviors and relate these effects to changes in nucleus accumbens (NAc) DA release. In C57BL/6 mice, locomotor activity and responding for a conditioned reinforcer (CRf), a measure of incentive motivation, were examined following treatment with three 5-HT_{2C} receptor ligands: the agonist CP809101 (0.25–3 mg/kg), the antagonist SB242084 (0.25–1 mg/kg), or the antagonist/inverse agonist SB206553 (1–5 mg/kg). We further tested whether doses of these compounds that changed locomotor activity and responding for a CRf (1 mg/kg CP809101, 0.5 mg/kg SB242084, or 2.5 mg/kg SB206553) also altered NAc DA release using *in vivo* microdialysis in anesthetized mice. CP809101 reduced locomotor activity, responding for a CRf, and NAc DA release. In contrast, both SB242084 and SB206553 enhanced locomotor activity, responding for a CRf, and NAc DA release, although higher doses of SB206553 produced opposite behavioral effects. Pretreatment with the non-selective DA receptor antagonist α-flupenthixol prevented SB242084 from enhancing responding for a CRf. Thus blocking tonic 5-HT_{2C} receptor signaling can release serotonergic inhibition of mesolimbic DA activity and enhance reward-related behavior. The observed bidirectional effects of 5-HT_{2C} receptor ligands may have important implications when considering the 5-HT_{2C} receptor as a therapeutic target for psychiatric disorders, particularly those presenting with motivational dysfunctions.
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INTRODUCTION

The ascending serotonin (5-hydroxytryptamine (5-HT)) system innervates virtually the entire brain and modulates many behavioral processes. The specific behavioral and physiological effects of 5-HT depend on signaling at multiple receptor subtypes (Barnes and Sharp, 1999). Several lines of evidence suggest that the 5-HT_{2C} receptor in particular has an important role in modulating behaviors directed toward obtaining rewarding stimuli. 5-HT_{2C} receptor agonists, including Ro60–1075, CP809101, WAY161503, and lorcaserin, reduce food intake (Clifton *et al*, 2000; Fletcher *et al*, 2009) and operant responding for food reinforcement (Grottick *et al*, 2000; Wolff and Leander, 2000). 5-HT_{2C} receptor agonists also reduce the efficacy of brain stimulation reward (Hayes *et al*, 2009; Zeeb *et al*, 2015) and the reinforcing efficacy of self-administration of several abused drugs, including cocaine (Grottick *et al*, 2000; Manvich *et al*, 2012b), nicotine (Higgins *et al*, 2012), oxycodone (Neelakantan *et al*, 2017), and methamphetamine (Gerak *et al*, 2016). In mice, a conditioned place preference produced by cocaine, nicotine, or THC is also blocked by 5-HT_{2C} agonists (Craige and Unterwald, 2013; Ji *et al*, 2006). Further, 5-HT_{2C} receptor agonists reduce appetitive behaviors elicited by stimuli associated with rewards such as food and drugs of abuse (Guy and Fletcher, 2014; Higgins *et al*, 2012; Neisewander and Acosta, 2007). Taken together, these studies suggest that 5-HT_{2C} receptor activation produces a general reduction in reward-related behavior.

Recently, we found that elevating endogenous extracellular 5-HT levels through pharmacological or genetic disruption of the 5-HT transporter reduced multiple operant measures of motivation (Browne and Fletcher, 2016). One of these measured motivation elicited by a reward-associated

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stimulus serving as a conditioned reinforcer (CRf). Reductions in responding for a CRf produced by increasing 5-HT activity could be prevented by a low dose of the selective 5-HT$_{2C}$ receptor antagonist SB242084 (Bromidge *et al*, 1997; Kennett *et al*, 1997), further illustrating the importance of this receptor in reward-related behavior. In the course of this work, we also noted that higher doses of SB242084, when administered alone, tended to increase responding for a CRf. This observation is in keeping with findings that SB242084 enhances some reward-related behaviors supported by cocaine (Capriles *et al*, 2012; Fletcher *et al*, 2002). However, relatively few studies have examined the effects of 5-HT$_{2C}$ receptor antagonists on non-drug reward-related behavior.

A recent study found that SB242084 increased instrumental responding for food in several tasks in mice (Bailey *et al*, 2016). However, other groups report no effect of SB242084 on responding for food on a progressive ratio schedule of reinforcement in mice (Fletcher *et al*, 2010), while others attribute an increase in responding to changes in motor processes (Bezzina *et al*, 2015). These mixed results regarding the effects of 5-HT$_{2C}$ receptor antagonists indicate that a more systematic investigation of 5-HT$_{2C}$ receptor antagonists on reward and motivation is required.

One plausible mechanism by which 5-HT$_{2C}$ receptor signaling influences reward-related behavior is through an interaction with the mesolimbic dopamine (DA) system—a critical neural substrate for promoting reward and reinforcement (Salamone and Correa, 2002; Schultz, 1998). Consistent with their behavioral effects, 5-HT$_{2C}$ receptor agonists inhibit the firing rate of DA neurons in the ventral tegmental area (VTA; Di Matteo *et al*, 2000), leading to a reduction in DA release in the nucleus accumbens (NAc; De Deurwaerdère *et al*, 2004; Di Matteo *et al*, 2000). In contrast, the antagonist SB242084 increases the firing rate of VTA DA neurons producing a modest increase in NAc DA release (De Deurwaerdère *et al*, 2004; Di Matteo *et al*, 1999). This potentiation of mesolimbic DA system function may explain the ability of SB242084 to enhance some reward-related behavior. Another compound commonly used as a 5-HT$_{2C}$ receptor antagonist, SB206553, exhibits inverse agonist properties at 5-HT$_{2C}$ receptors *in vitro* (Aloyo *et al*, 2009; Kennett *et al*, 1996). Consistent with this idea, SB206553 also increases NAc DA release to a much greater extent than does SB242084 (De Deurwaerdère *et al*, 2004). However, the effects of SB206553 on reward-related behavior have not been well studied. Based on its ability to potentiate mesolimbic DA activity, we hypothesize that SB206553 should enhance DA-dependent, reward-related behavior to a greater extent than SB242084.

The present experiments addressed several issues related to 5-HT$_{2C}$ receptor ligands and reward-related behavior. The first objective of this work was to systematically compare the effects of the selective 5-HT$_{2C}$ receptor agonist CP809101 (Siuciak *et al*, 2007), the selective 5-HT$_{2C}$ receptor antagonist SB242084, and the putative 5-HT$_{2C}$ receptor inverse agonist SB206553 on locomotor activity and responding for a CRf in mice. These behaviors were chosen because both are sensitive to bidirectional changes in mesolimbic DA activity; increasing DA enhances locomotor activity and responding for a CRf (Kelly *et al*, 1975; Taylor and Robbins, 1984), while decreasing DA reduces both behaviors (Fletcher and Higgins, 1997). The second objective was to examine whether doses of 5-HT$_{2C}$ receptor ligands that altered locomotor activity and responding for a CRf also produced parallel changes in NAc DA release. Third, having found that 5-HT$_{2C}$ receptor blockade increased both responding for a CRf and NAc DA release, we sought to establish a causal relationship between these two effects. Thus we examined whether the DA receptor antagonist α-flupenthixol could prevent SB242084-mediated increases in responding for a CRf.

MATERIALS AND METHODS

Animals

In these studies, 150 male C57BL/6N mice (Charles River, QC) were used. Mice were pair-housed in temperature and humidity controlled rooms on a 12-h light–dark cycle with lights on at 0700 hours. In tests of responding for a CRf, all mice received restricted access to water, with 2 h of daily water access. Food was available *ad libitum*. This work adhered to the Canadian Council on Animal Care guidelines and was approved by the Centre for Addiction and Mental Health Animal Care Committee.

Behavioral Testing Apparatus

Locomotor activity was measured using a custom-built system of 16 clear polycarbonate chambers measuring $25 \times 45 \times 20$ cm^3. The long axis of each box had an array of 11 externally mounted infrared photodetectors spaced 4 cm apart and 2 cm above the cage floor. Photocell interruptions were recorded as locomotor activity counts on a DELL desktop computer.

Responding for a CRf was assessed using 12 operant conditioning boxes (Med Associates, St Albans, VT) measuring $22 \times 18 \times 13$ cm^3. The front wall of the chamber housed a horizontally centered reinforcer magazine that contained an infrared photodetector and a roof-mounted light. A motor-driven dipper could be raised to deliver 0.02 ml of liquid through a hole in the floor of the reinforcer magazine. The wall also contained two retractable levers flanking the reinforcer magazine. Positioned above each lever was a yellow stimulus light. Each operant conditioning box was illuminated by a houselight and was enclosed in a sound-attenuating chamber equipped with a ventilation fan.

Procedure for Assessing Locomotor Activity

Mice were first habituated to testing chambers for three 1-h sessions. In subsequent tests, locomotor activity was examined for 1 h following drug treatment. All testing was carried out under red lighting.

Procedure for Assessing Responding for a CRf

The conditioned reinforcement paradigm was conducted as previously described (Browne *et al*, 2014).

Pavlovian conditioning. Mice received 14 daily 40-min sessions in which a conditioned stimulus (CS) was presented just prior to the delivery of 0.02 ml 0.2% saccharin 30 times on a RT 60-s schedule. The compound CS consisted of houselights off, both stimulus lights on for 5 s, and the sound

of the mechanical dipper being elevated at the end of the 5-s period. The saccharin reinforcer was presented for 8 s during which time both stimulus lights remained on and the houselight remained off (13 s total CS duration). At the end of this period, stimulus lights were extinguished, the houselight was reilluminated, and the dipper descended. The main dependent variables measured were head entries into the reward magazine during the 5-s CS period (prior to saccharin delivery) and a control measure of head entries during a 5-s period just prior to CS onset (PreCS period).

Operant conditioning. Following Pavlovian conditioning, two response levers were introduced to the operant chambers: an active lever and an inactive lever. Responding on the active lever produced a shortened version of the CS from the Pavlovian conditioning phase (5-s period with the houselight off and both stimulus lights on and elevation of the empty dipper during the last 2 s), now referred to as a CRf, according to a random ratio 2 schedule of reinforcement. Responding on the inactive lever had no programmed consequences. Mice were tested in 40-min sessions. We have previously shown that, in mice, this procedure produces stable responding and does not require additional Pavlovian conditioning sessions (Browne *et al*, 2014).

Surgical Procedures for Microdialysis

Mice maintained under inhaled isoflurane anesthesia received stereotaxic surgery to implant a microdialysis guide cannula above the left or right NAc (counterbalanced; targeting A/P +1.54, M/L ±0.70, D/V −2.0, according to Franklin and Paxinos, 2007), which was fixed to the skull with dental cement (RelyX unicem, 3M, Minnesota, USA). Mice received ketoprofen analgesia (5 mg/kg, subcutaneously) prior to surgery and once daily for 2 days following surgery. Dialysis procedures commenced 1 week following surgery.

Microdialysis and High-Performance Liquid Chromatography (HPLC)

Microdialysis sampling was conducted in mice maintained under 2% inhaled isoflurane anesthesia. Throughout the procedure, body temperature was maintained at 37 °C with an electric heating pad (CWE, Ardmore, PA). A dialysis probe (2 mm cuprophane membrane; Scientific Products, Toronto, Canada) was lowered through the guide cannula. This probe length was used to ensure sampling of DA levels throughout the entire dorsal–ventral axis of the NAc, which is approximately 1.5 mm. However, in some cases a small portion of the dialysis probe may have extended to the caudate (dorsal) or the olfactory tubercle (ventral). Probes were continuously perfused with artificial cerebrospinal fluid at 0.5 µl/min using a 1.0 ml gastight syringe and a syringe pump (CMA Microdialysis, Holliston, USA). Sampling began 1 h following probe insertion. Samples were collected every 20 min, and baseline DA concentration was considered stable when three consecutive samples varied <10% from the previous sample (Baseline; three samples required on average).

Measurement of DA levels in dialysate samples was conducted on an analytical system consisting of an Antec Leyden LC110 Alexys HPLC system coupled to a Decade-II electrochemical detection cell and an ALF 105 50 × 1 mm column with C-18 3 µm packing material (ATS Scientific, Burlington, Canada). DA detection potential was 400 mV against an Ag/AgCl electrode. The mobile phase consisted of 50 mM phosphoric acid, 8 mM NaCl, 0.1 mM EDTA, 500 mg/l OSA, and 12.5% methanol in purified distilled water. The pH was adjusted to 6.0 and the solution was filtered through a 0.22 µm nylon filter. The flow rate was 65 µl/min. Chromatograms were interpreted using the Clarity Chromatography software. The average *in vitro* probe recovery was 9.4%.

Drugs

CP809101 (Tocris, Bristol, UK) was dissolved in a 0.9% saline solution containing 5% tween; SB242084 (Tocris) and SB206553 (Tocris) were dissolved in a 0.9% saline solution containing 8% β-cyclodextrin (Sigma-Aldrich, Oakville, Canada); α-flupenthixol (Tocris) was dissolved in 0.9% saline. All drugs were administered intraperitoneally in a volume of 10 ml/kg. CP809101 was injected 20 min before testing, SB242084 was injected 40 min before testing, and α-flupenthixol was injected 60 min before testing. SB206553 was injected 5 min prior to testing, which was determined from published work (Gleason *et al*, 2001) and pilot studies conducted in our laboratory. Doses of all drugs are expressed in terms of the free base. For all behavioral experiments, drug treatments followed a within-subjects design with dose order determined from a Latin square and with testing sessions separated by 72 h.

Experimental Procedures

Experiment 1: Effects of 5-HT$_{2C}$ receptor ligands on locomotor activity. Locomotor activity was measured for 1 h following treatment with either CP809101 (0.3, 1, 3 mg/kg, or vehicle; $n=16$), SB242084 (0.25, 0.5, 1 mg/kg, or vehicle; $n=16$), or SB206553 (1, 2.5, 5 mg/kg, or vehicle; $n=16$).

Experiment 2: Effects of 5-HT$_{2C}$ receptor ligands on responding for a CRf. Following Pavlovian conditioning, baseline responding for a CRf was first measured over three test sessions. Subsequently, mice were randomly separated into three groups and tested following drug treatment. One group of mice ($n=12$) was tested following treatment with CP809101 (0.25, 0.5, 1 mg/kg, or vehicle). This group was also retested with 1 and 3 mg/kg doses to extend the dose–response curve for CP809101. The second group ($n=24$) was tested following treatment with SB242084 (0.25, 0.5, 1 mg/kg, or vehicle). The third group ($n=24$) was tested following treatment with SB206553 (1, 2.5, 5 mg/kg, or vehicle).

Experiment 3: Effects of 5-HT$_{2C}$ receptor ligands on NAc DA release. In this experiment, we measured changes in extracellular levels of DA in the NAc following treatment with 1 mg/kg CP809101, 0.5 mg/kg SB242084, or 2.5 mg/kg SB206553. These doses were chosen based on their ability to alter behavior in Experiments 1 and 2.

Once baseline DA measurements were stable, mice received an injection of vehicle, and three samples were

collected (60 min). Subsequently, mice were injected with CP809101 (1 mg/kg; $n=6$), SB242084 (0.5 mg/kg; $n=6$), or SB206553 (2.5 mg/kg; $n=6$), and 9 more samples were collected (180 min). Extracellular DA levels following vehicle and drug treatment were expressed as a percentage of change from baseline DA concentration. In one mouse from the group tested with CP80901, DA levels in the last four samples could not be analyzed owing to technological complications. The percentage of change from baseline DA concentration for these missing values was estimated using the group average.

Immediately following collection of the last sample, mice were killed via cervical dislocation, and the brains were extracted and flash-frozen on dry ice. Forty-μm sections throughout the NAc were collected on a cryostat, and dialysis probe location was verified with cresyl violet staining.

Experiment 4: Effects of α-flupenthixol on SB242084-potentiated responding for a CRf. The results of Experiments 2 and 3 suggested that blocking the 5-HT$_{2C}$ receptor might increase responding for a CRf by enhancing mesolimbic DA activity. To test this hypothesis, we examined whether the broad-spectrum DA receptor antagonist α-flupenthixol could prevent the effects of SB242084 on responding for a CRf. SB242084 was chosen over SB206553 due to its consistent effect on responding irrespective of dose.

An experimentally naive group of mice ($n=24$) underwent Pavlovian conditioning followed by three baseline sessions of responding for a CRf (data not shown). Subsequently, mice were tested after treatment with 0.4 mg/kg α-flupenthixol or its vehicle followed by 0.5 mg/kg SB242084 or its vehicle. As this dose of α-flupenthixol produced a minor but non-significant reduction in responding on its own, we repeated the experiment with a lower dose. Thus responding for a CRf was again tested in these mice after treatment with 0.25 mg/kg α-flupenthixol or its vehicle followed by 0.5 mg/kg SB242084 or its vehicle.

Statistical analysis. Data were analyzed using Statistica (Statsoft, Tulsa, OK). All data were analyzed using repeated-

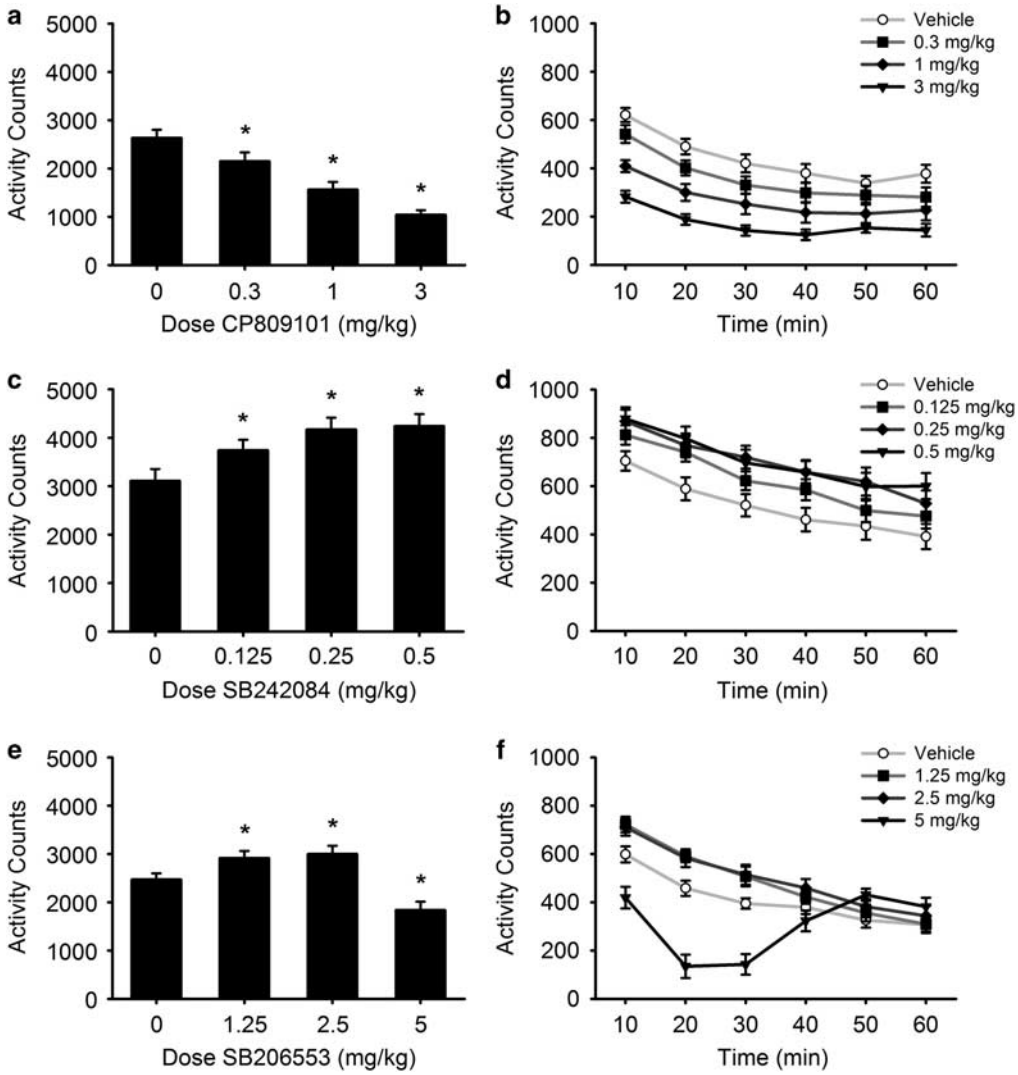

Figure 1 Modulating 5-HT$_{2C}$ receptor activity produces bidirectional changes in locomotor activity. Data are expressed as mean activity counts ± SEM. Left panels show total activity counts following treatment with CP809101 (a; $n=16$), SB242084 (c; $n=16$), and SB206553 (e; $n=16$), respectively. Right panels (b, d, and f) show activity counts within the session in 10-min time bins. *$p<0.05$ *vs* vehicle.

measures ANOVAs. Within-subjects factors included: dose of drug used (Dose), session time (Time), magazine entries period (CS *vs* PreCS), test session number (Session), active or inactive lever responses (Lever), dialysate sample number (Sample), SB242084 condition, and α-flupenthixol condition. In Experiment 3, dialysis samples following both vehicle and drug treatments were included in the overall ANOVA. *Post hoc* pairwise comparisons were performed using Tukey's HSD tests.

RESULTS

Experiment 1: Effects of 5-HT$_{2C}$ Receptor Ligands on Locomotor Activity

CP809101 significantly reduced total activity counts (Figure 1a; Dose: $F_{(3,63)} = 44.43$, $p < 0.001$) at all doses compared with vehicle (all $p < 0.05$). Analysis of activity counts across time (Figure 1b) found a main effect of Dose ($F_{(3,45)} = 35.11$, $p < 0.001$), a main effect of Time ($F_{(5,75)} = 62.47$, $p < 0.001$), and a Dose × Time interaction ($F_{(15,225)} = 1.75$, $p = 0.042$). In contrast, SB242084 significantly increased total activity counts (Figure 1c; Dose: $F_{(3,63)} = 16.14$, $p < 0.001$) at all doses compared with vehicle (all $p < 0.01$). Analysis of activity counts across time (Figure 1d) found a main effect of Dose ($F_{(3,45)} = 19.19$, $p < 0.001$) and a main effect of Time ($F_{(5,75)} = 80.06$, $p < 0.001$) but no Dose × Time interaction ($F_{(15,225)} = 0.94$, NS). SB206553 significantly increased total activity counts (Figure 1e; Dose: $F_{(3,63)} = 23.32$, $p < 0.001$) at 1 and 2.5 mg/kg doses compared with vehicle (both $p < 0.05$), while 5 mg/kg reduced activity counts ($p < 0.01$). Analysis of activity counts across time (Figure 1f) revealed a main effect of Dose ($F_{(3,45)} = 23.32$, $p < 0.001$), a main effect of Time ($F_{(5,75)} = 45.08$, $p < 0.001$), and a Dose × Time interaction ($F_{(15,225)} = 19.85$, $p < 0.001$). This reflects the fact that, within the first 30 min of testing, SB206553 increases activity at 1.25 and 2.5 mg/kg doses (both $p < 0.05$) but decreases activity at the 5 mg/kg dose ($p < 0.05$).

Experiment 2: Effects of 5-HT$_{2C}$ Receptor Ligands on Responding for a CRf

In the Pavlovian conditioning phase, mice learned to approach the reward magazine when the CS was presented (Figure 2a; CS *vs* PreCS × Session: $F_{(13,767)} = 57.41$, $p < 0.001$). In the operant conditioning phase, all mice made significantly more responses on the active lever delivering the CRf compared with an inactive lever across three baseline sessions (Figure 2b; Lever: $F_{(1,59)} = 34.72$, $p < 0.001$).

CP809101 significantly reduced responding for the CRf (Figure 3a; Dose × Lever: $F_{(3,33)} = 4.36$, $p < 0.05$; Figure 3b; Dose × Lever: $F_{(2,22)} = 9.30$, $p < 0.01$) at 1 and 3 mg/kg doses compared with vehicle ($p < 0.001$). SB242084 significantly increased responding (Figure 3c; Dose: $F_{(3,69)} = 7.65$, $p < 0.001$), although no Dose × Lever interaction was observed ($F_{(3,69)} = 1.26$, $p = 0.28$). However, *post hoc* tests found that, compared with vehicle treatment, responding on the active lever was significantly increased at all doses tested (all $p < 0.01$), while responding on the inactive lever was not significantly different across doses (all $p > 0.08$). SB206553 generally increased responding (Figure 3d; Dose: $F_{(3,69)} = 5.20$, $p < 0.01$), although the Dose × Lever interaction was not significant ($F_{(3,69)} = 2.36$, $p = 0.08$). However, *post hoc* tests found that SB206553 increased responding on the active lever at the 1.25 mg/kg dose at $p < 0.05$ but the increase for the 2.5 mg/kg dose was significant at $p = 0.06$. The increase in response magnitude produced by the 1.25 and 2.5 mg/kg doses was similar (39.5 and 38.1, respectively), prompting further analysis. A paired-samples *t*-test found that the increase in active lever responding produced by 2.5 mg/kg SB206553 was significantly different from vehicle ($t_{(23)} = 2.42$, $p < 0.05$). *Post hoc* tests from the overall ANOVA also found that, compared with vehicle treatment, the 5 mg/kg dose produced no change in responding ($p > 0.05$) and that inactive lever responding was unchanged across all doses tested (all $p > 0.05$).

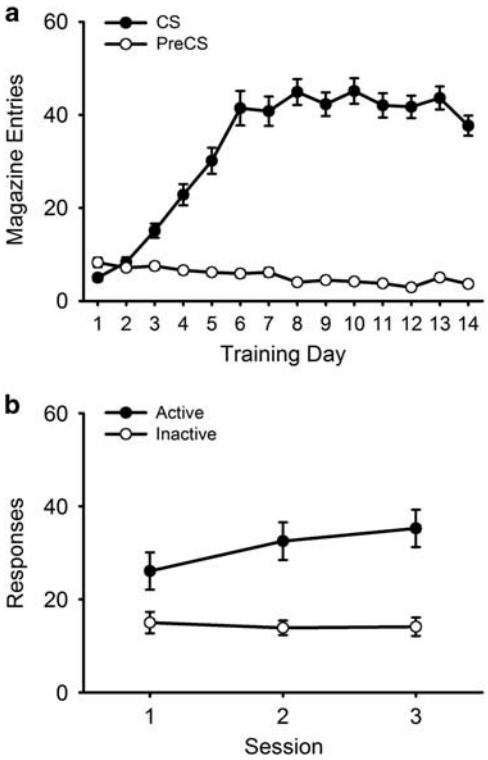

Figure 2 Acquisition of responding for a conditioned reinforcer ($N = 60$). Panel (a) shows reward magazine entries made during the 5-s conditioned stimulus (CS) presentation prior to saccharin delivery (CS; filled symbols) or a 5-s period just before CS onset (PreCS; open symbols) across 14 sessions of Pavlovian conditioning. Panel (b) shows responding on a lever delivering the CS, now serving as a conditioned reinforcer (active lever; filled bars), or an inactive lever (open bars) over three baseline testing sessions. Data are expressed as mean ± SEM.

Experiment 3: Effects of 5-HT$_{2C}$ Receptor Ligands on NAc DA Release

Relative to baseline, extracellular DA concentration in the NAc was significantly reduced by 1 mg/kg CP809101 (Figure 4a and b; 22.6% largest decrease; $F_{(11,55)} = 22.04$, $p < 0.001$). In contrast, extracellular DA was increased by 0.5 mg/kg SB242084 (Figure 4c and d; 21.8% peak increase; $F_{(11,55)} = 14.09$, $p < 0.001$) and 2.5 mg/kg SB206553 (Figure 4e and f; 24.7% peak increase; $F_{(11,55)} = 14.13$,

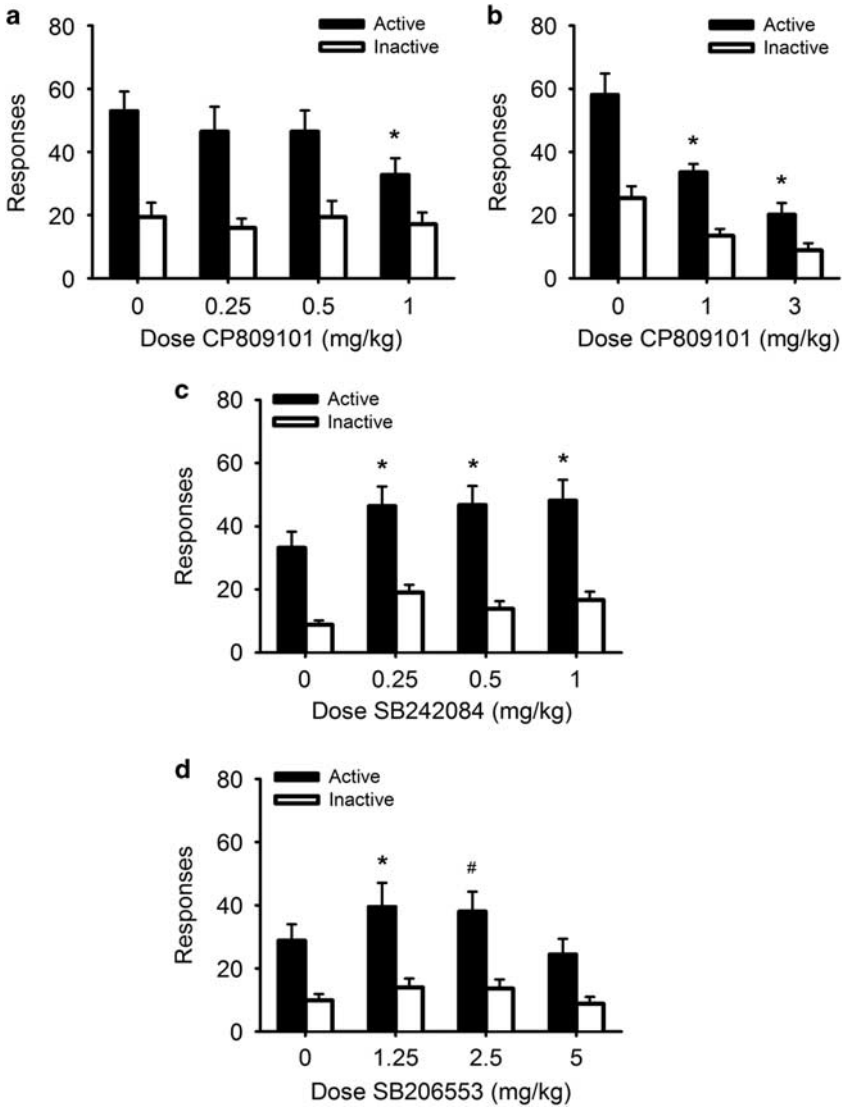

Figure 3 Modulating 5-HT$_{2C}$ receptor activity produces bidirectional changes in responding for a conditioned reinforcer. Graphs show the number of responses made on the active lever delivering the conditioned reinforcer (filled bars) and the inactive lever (open bars) following treatment with CP809101 or its vehicle (a and b; $n=12$), SB242084 or its vehicle (c; $n=24$), and SB206553 or its vehicle (d; $n=24$). Data are expressed as mean responses ± SEM. $^{*}p<0.05$ *vs* vehicle; $^{\#}p=0.06$ *vs* vehicle from *post hoc* tests.

$p<0.001$). *Post hoc* tests found that the change in extracellular DA returned to baseline within 120 min for CP809101, 140 min for SB242084, and 100 min for SB206553. Histological verification confirmed that dialysis probes covered the entire dorso-ventral aspect of the NAc (Figure 4b, d, and f).

Experiment 4: Effects of α-Flupenthixol on SB242084-Potentiated Responding for a CRf

Figure 5 demonstrates that α-flupenthixol attenuated the response-enhancing effects of SB242084. In the experiment using 0.4 mg/kg α-flupenthixol (Figure 5a), the three-way interaction was not significant ($F_{(1,22)}=1.8$, NS). This may have been due to variability produced by a moderate but non-significant reduction in active lever responding in the α-flupenthixol alone condition ($p=0.07$). However, compared with vehicle conditions, SB242084 enhanced active lever responding ($p<0.001$), and combining α-flupenthixol and SB242084 treatment attenuated the effect of SB242084 ($p<0.05$), resulting in a similar level of responding to vehicle conditions ($p>0.05$). In the experiment using 0.25 mg/kg α-flupenthixol (Figure 5b), a significant three-way interaction was observed ($F_{(1,22)}$ $4.63=p<0.05$). To identify the source of this interaction, separate two-way ANOVAs were conducted for responses made on active and inactive levers. These analyses found an interaction between α-flupenthixol and SB242084 for responses made on the active lever ($F_{(1,22)}=5.07$, $p<0.05$) but not on the inactive lever ($F_{(1,22)}=0.22$, ns). *Post hoc* tests found that, compared with vehicle conditions, responding on the active lever was unchanged by 0.25 mg/kg α-flupenthixol alone ($p>0.05$) and enhanced by SB242084 ($p<0.01$). Combining α-flupenthixol and SB242084 treatment attenuated the effect of SB242084 ($p<0.05$), resulting in a similar level of responding to vehicle conditions ($p>0.05$).

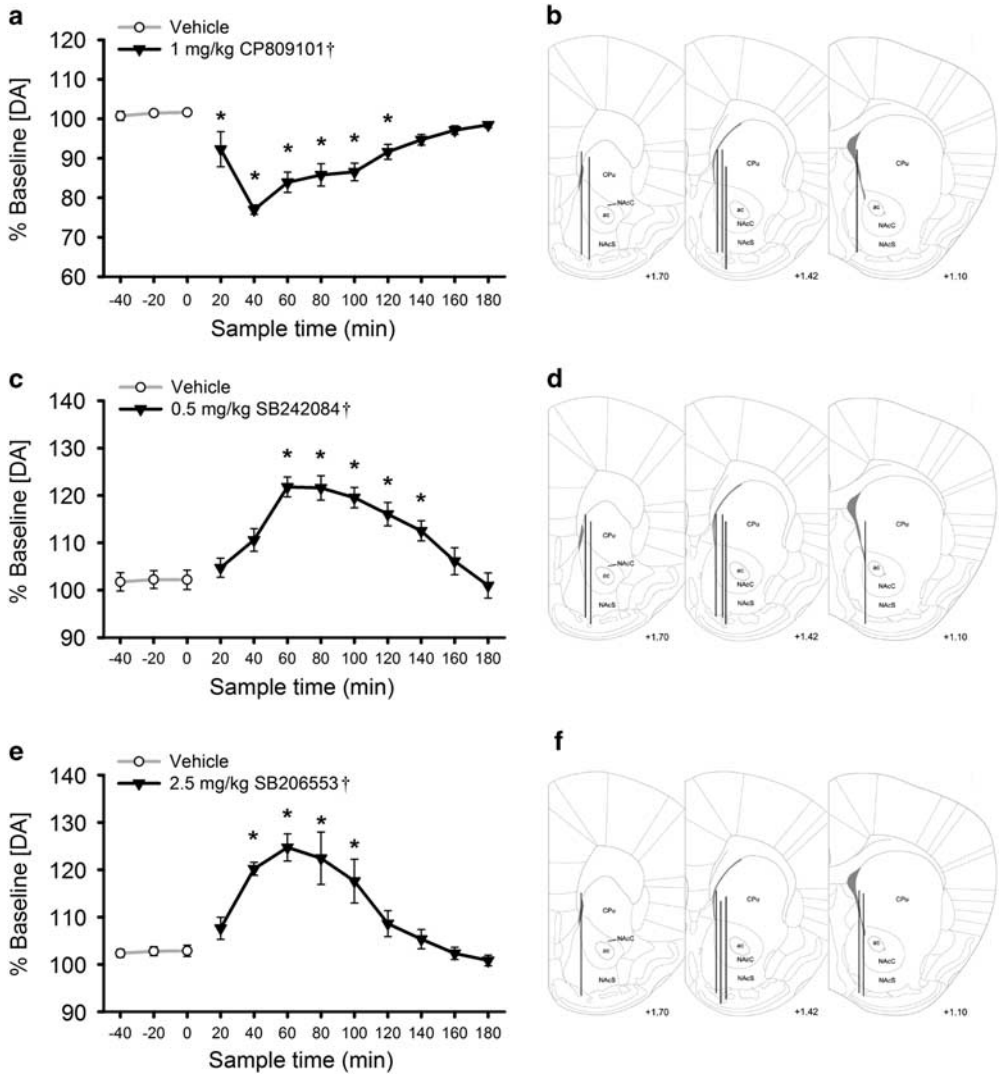

Figure 4 Modulating 5-HT$_{2C}$ receptor activity using doses of 5-HT$_{2C}$ receptor ligands that alter locomotor activity and responding for a conditioned reinforcer produces bidirectional changes in mesolimbic DA release. Left panels (a, c, and e) show extracellular dopamine concentration, expressed as a mean percentage of baseline (±SEM), measured in the nucleus accumbens following treatment with CP809101 or its vehicle (a; $n=6$), SB242084 or its vehicle (b; $n=6$), and SB206553 or its vehicle (c; $n=6$). Concentration was not corrected for probe recovery. Right panels (b, d, and f) depict microdialysis probe placements for mice treated with CP809101 (b), SB242084 (d), or SB206553 (f) transposed onto right hemispheres for simplicity. ac, anterior commissure; CPu, caudate/putamen; NAcC, nucleus accumbens core; NAcS, nucleus accumbens shell. †$p<0.05$ main effect of drug treatment. *$p<0.05$ *vs* third vehicle sample.

DISCUSSION

The present experiments examined the effects of 5-HT$_{2C}$ receptor ligands on two DA-dependent behaviors, locomotor activity and responding for a CRf, and related these effects to changes in mesolimbic DA system activity. The 5-HT$_{2C}$ receptor agonist CP809101 reduced locomotor activity and responding for a CRf, whereas the 5-HT$_{2C}$ receptor antagonist SB242084 and the antagonist/inverse agonist SB206553 enhanced these behaviors. These 5-HT$_{2C}$ receptor ligands also produced changes in mesolimbic DA activity consistent with their behavioral effects: CP809101 reduced NAc DA release, whereas SB242084 and SB206553 enhanced NAc DA release. Finally, pretreatment with the DA receptor antagonist α-flupenthixol prevented the ability of SB242084 to potentiate responding for a CRf.

The effects of CP809101 observed here in mice support and extend findings that 5-HT$_{2C}$ receptor agonists reduce locomotor activity and responding for a CRf in rats (Guy and Fletcher, 2014; Kennett *et al*, 2000). These behaviors are highly dependent on mesolimbic DA system function, which is inhibited by 5-HT$_{2C}$ receptor agonists (De Deurwaerdère *et al*, 2004; Di Matteo *et al*, 2000). The present results also demonstrate a direct correlation between DA activity and the behavioral effects of 5-HT$_{2C}$ receptor activation, in that a dose of CP809101 (1 mg/kg) that reduces DA-dependent behavior also reduces NAc DA release. Taken together, these findings support the hypothesis that 5-HT$_{2C}$ receptor activation may suppress reward-related behavior by inhibiting mesolimbic DA system function.

The 5-HT$_{2C}$ receptor antagonist SB242084 and antagonist/inverse agonist SB206553 both enhanced locomotor activity

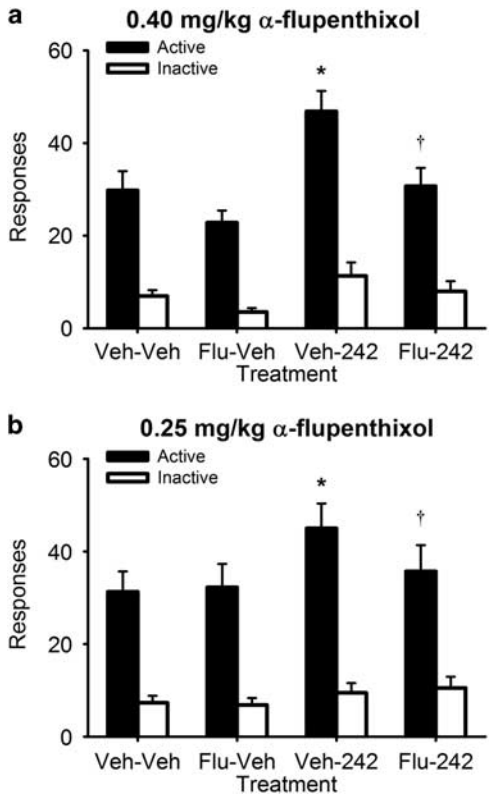

Figure 5 Blocking DA receptors with α-flupenthixol prevents the ability of the 5-HT$_{2C}$ antagonist SB242084 to enhance responding for a CRf ($n = 24$). Data are expressed as the mean (±SEM) number of responses made on the active lever delivering the CRf (filled bars) or inactive lever (open bars). Responding was examined across four conditions in which animals were treated with α-flupenthixol or its vehicle followed by SB242084 or its vehicle. Panel (a) shows results from these experiments using 0.4 mg/kg α-flupenthixol and 0.5 mg/kg SB242084, while panel (b) shows results using 0.25 mg/kg α-flupenthixol and 0.5 mg/kg SB242084. *$p < 0.05$ *vs* vehicle-vehicle condition; †$p < 0.05$ *vs* vehicle-SB242084 condition.

and responding for a CRf. These effects occurred at all doses of SB242084, while the effects of SB206553 were lost at the highest dose tested (5 mg/kg). We also showed that doses of SB242084 (0.5 mg/kg) and SB206553 (2.5 mg/kg), which enhanced locomotor activity and responding for a CRf, also enhanced NAc DA release. These neurochemical effects are consistent with previous reports in rats and monkeys (De Deurwaerdère *et al*, 2004; Manvich *et al*, 2012b). In the final Experiment, the broad-spectrum DA receptor antagonist *α*-flupenthixol blocked the ability of SB2424084 to enhance responding for a CRf. Collectively, these results provide strong evidence that 5-HT$_{2C}$ receptor antagonists can increase reward-related behaviors by enhancing mesolimbic DA system function. These findings have two important implications for understanding the interaction between 5-HT and DA systems in regulating reward-related behavior. First, they show that 5-HT suppresses behavior and DA release by tonic signaling at the 5-HT$_{2C}$ receptor, suggesting that this receptor mediates an inhibitory interaction between 5-HT and DA systems. Second, these results provide an indirect demonstration that agonist action at the 5-HT$_{2C}$ receptor, either by 5-HT itself or by compounds such as CP809101, reduces reward-related behavior by inhibiting mesolimbic DA system function.

SB206553 has been characterized as having inverse agonist properties *in vitro* (Aloyo *et al*, 2009; Kennett *et al*, 1996) and has been shown to enhance NAc DA release to a greater degree than does SB242084 (De Deurwaerdère *et al*, 2004). This latter finding has been used to suggest an *in vivo* inverse agonist effect of SB206533. Based on this evidence, we hypothesized that SB206553 would perhaps increase DA-dependent behavior to a greater magnitude than SB242084. However, we observed no differences in the ability of SB206553 and SB242084 to enhance locomotor activity or responding for a CRf. In fact, 5 mg/kg of SB206553, which was previously shown to enhance mesolimbic DA to a greater extent than any dose of SB242084 in rats (De Deurwaerdère *et al*, 2004), had no effect on responding for a CRf (unlike lower doses; Figure 3d) and significantly reduced locomotor activity below control levels (Figure 1e and f). The extent to which the 2.5 mg/kg dose of SB206553 increased locomotor activity, responding for a CRf, and NAc DA release was similar to the increases induced by SB242084. Thus these studies were unable to demonstrate an inverse agonist effect of SB206553 on DA-dependent, reward-related behavior and instead suggest that SB206533 altered behavior in a manner consistent with 5-HT$_{2C}$ receptor antagonist action.

At 5 mg/kg, SB206553 suppressed locomotor activity and failed to increase responding for CRf. This complements other reports that high doses of SB206553 exert inconsistent effects on behavior. For example, SB206553 potentiated the psychostimulant effects of cocaine at 1 and 2 mg/kg doses, an effect also seen with SB242084, but this effect was lost at a dose of 4 mg/kg (McCreary and Cunningham, 1999). Another study reported that SB206553, unlike SB242084, reduced cue-induced reinstatement of methamphetamine seeking (Graves and Napier, 2012). However, it seems unlikely that this effect of SB206553 is mediated by 5-HT$_{2C}$ receptors as it was not blocked by SB242084. Overall, the behavioral change produced by higher doses of SB206553 and the 5-HT$_{2C}$ receptor-independent reduction in methamphetamine seeking suggest that SB206553 loses selectivity for 5-HT$_{2C}$ receptors as dose increases. Activity at other targets such as 5-HT$_{2B}$ receptors (Kennett *et al*, 1996), 5-HT$_{1A}$ receptors (Shumsky *et al*, 2005), or nicotinic acetylcholine a7 receptors (Möller-Acuña *et al*, 2015) may change or mask the expression of 5-HT$_{2C}$ receptor-mediated effects of SB206533.

The 5-HT$_{2C}$ receptor has recently gained attention as a therapeutic target for psychiatric disorders that present with motivational dysfunctions. For example, the 5-HT$_{2C}$ receptor agonist lorcaserin, which is FDA approved for treating obesity, has shown positive results in a clinical trial for treating nicotine dependence (Shanahan *et al*, 2016). Further, preclinical studies find that lorcaserin reduces self-administration of cocaine (Harvey-Lewis *et al*, 2016) and opiates (Neelakantan *et al*, 2017), suggesting a general utility in treating substance abuse. In the present studies, we show that 5-HT$_{2C}$ receptor ligands can bidirectionally influence responding for a CRf—a measure of incentive motivation elicited by a reward-associated stimulus. The ability of CP809101 to decrease responding for a CRf supports a role for 5-HT$_{2C}$ receptor agonists in blunting motivation, whereas

the ability of SB242084 and SB206553 to enhance responding for a CRf suggests that 5-HT$_{2C}$ receptor antagonists may potentiate motivation. This idea is consistent with a recent report showing that SB242084 can enhance food-motivated behavior (Bailey *et al*, 2016). Thus the 5-HT$_{2C}$ receptor can be targeted to bidirectionally modulate motivational processes, which may be useful in developing treatment strategies for psychiatric disorders such as substance abuse, as well as schizophrenia and depression (De Deurwaerdère and Di Giovanni, 2017; Meltzer, 1999; Rocha *et al*, 2002).

One potential barrier to using 5-HT$_{2C}$ antagonists in the clinic are findings that SB242084 substitutes for cocaine in a self-administration paradigm in monkeys, which suggests an abuse liability of such compounds (Manvich *et al*, 2012a). However, the present results observe only mild behavioral and neurochemical enhancements following SB242084 treatment. Further, the effects of SB242084 appear to have an upper limit; escalating doses of SB242084 do not produce dose-dependent increases in behavior (present results) or NAc DA release (De Deurwaerdère *et al*, 2004). These findings contrast with the ability of typical drugs of abuse, which produce much larger, dose-dependent increases in mesolimbic DA release and DA-dependent behavior. Thus it is possible that 5-HT$_{2C}$ antagonists may potentiate motivation to a minor extent without escalation of intake, but further investigation of the abuse-like properties of these compounds is certainly warranted.

In conclusion, the present results show that modulating 5-HT$_{2C}$ receptor function can produce a bidirectional shift in reward-related behaviors through an interaction with the mesolimbic DA system. These findings provide strong support for further exploring the 5-HT$_{2C}$ receptor as a therapeutic target in treating psychiatric disorders, particularly those with motivational dysfunctions.

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