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ORIGINAL INVESTIGATION

Role of 5-HT_{2C} receptors in effects of monoamine releasers on intracranial self-stimulation in rats

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Abstract
Rationale Many monoamine releasers are abused by humans and produce abuse-related facilitation of intracranial self-stimulation (ICSS) in rats. Facilitation of ICSS in rats can be limited by monoamine releaser-induced serotonin (5-HT) release, but receptors that mediate 5-HT effects of monoamine releasers are unknown.
Objectives The aim of this study is to investigate whether 5-HT_{2C} receptor activation is necessary for rate-decreasing effects produced in an ICSS procedure in rats by the 5-HT-selective monoamine releaser fenfluramine and the non-selective releasers napthylisopropylamine (PAL-287) and (+)-3,4-methylenedioxymethamphetamine ((+)-MDMA).
Methods Adult male Sprague-Dawley rats with electrodes implanted in the medial forebrain bundle were trained to lever press for brain stimulation under a "frequency-rate" ICSS procedure. Effectiveness of the 5-HT_{2C} antagonist SB 242,084 was evaluated to block rate-decreasing effects produced by (1) the 5-HT_{2C} agonist Ro 60-0175, (2) the 5-HT-selective releaser fenfluramine, and (3) the mixed-action dopamine (DA)/norepinephrine (NE)/5-HT releasers PAL-287 (1.0–5.6 mg/kg) and (+)-MDMA (1.0–3.2 mg/kg). For comparison, effectiveness of SB 242,084 to alter rate-decreasing effects of the kappa-opioid receptor agonist U69,593 and rate-increasing effects of the DA>5-HT releaser amphetamine was also examined.
Results SB 242,084 pretreatment blocked rate-decreasing effects of Ro 60-0175 and fenfluramine, but not the rate-decreasing effects of U69,593 or the rate-increasing effects of amphetamine. SB 242,084 blunted the rate-decreasing effects and enhanced expression of rate-increasing effects of PAL-287 and (+)-MDMA.
Conclusions These data suggest that 5-HT_{2C} receptor activation contributes to rate-decreasing effects that are produced by selective and mixed-action 5-HT releasers in rats and that may oppose and limit the expression of abuse-related ICSS facilitation by these compounds.

Keywords 5-HT_{2C} receptors · Serotonin · Dopamine · Intracranial self-stimulation · Amphetamine · Fenfluramine · Methylenedioxymethamphetamine

Introduction

Monoamine releasers are drugs that function as substrates for dopamine (DA), norepinephrine (NE), and serotonin (5-HT) transporters (DAT, NET, and SERT, respectively) and promote release of DA, NE, and 5-HT from synaptic terminals (Parada et al. 1988; Rothman et al. 2001). Many monoamine releasers, including amphetamine and methylenedioxymethamphetamine (MDMA), are abused drugs that have similar potencies to release DA and NE with varying selectivities to release DA/NE vs. 5-HT (Johnston et al. 2009). Previous studies, across a broad range of experimental endpoints, suggest that 5-HT effects of monoamine releasers may oppose and limit DA-mediated abuse-related effects (Rothman and Baumann 2006; Wee and Woolverton 2006; Baumann et al. 2011; Bauer et al. 2013). For example, efficacy of monoamine releasers to

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maintain drug self-administration is greatest for DA>5-HT-selective releasers, lower for mixed-action releasers with similar potencies to release DA and 5-HT, and the 5-HT-selective releaser fenfluramine does not maintain self-administration (Götestam and Andersson 1975; Wee et al. 2005; Woods and Tessel 1974). Intracranial self-stimulation (ICSS) is another procedure that has been used to assess abuse liability of drugs (Negus and Miller 2014), and consistent with results from self-administration procedures, DA vs. 5-HT selectivity of monoamine releasers correlates with efficacy to produce abuse-related changes in ICSS (Bauer et al. 2013; Bonano et al. 2015). Moreover, in both drug self-administration and ICSS procedures, the abuse-related effects of DA>5-HT-selective releasers can be attenuated by a 5-HT-selective releaser (Bauer et al. 2013; Wee and Woolverton 2006).

The receptor subtype(s) through which 5-HT opposes and limits effects mediated by DA is a topic of current research (for review, Alex and Pehek 2007; Howell and Cunningham 2015). 5-HT_{2C} receptors appear especially relevant in modulating monoamine releaser-induced DA activity for the following reasons: (1) Gq-coupled 5-HT_{2C} receptors are present in relatively high density on GABAergic neurons in the ventral tegmental area, nucleus accumbens, and cingulate cortex (Bubar and Cunningham 2008; Eberle-Wang et al. 1997; Pompeiano et al. 1994); (2) activation of 5-HT_{2C} receptors is sufficient to decrease DA neuronal activity and DA levels in the nucleus accumbens (Di Giovanni et al. 2000; Di Matteo et al. 2000); and (3) 5-HT_{2C} agonists decrease cocaine-induced locomotion (Cathala et al. 2014; Fletcher et al. 2004; Grottick et al. 2000), cocaine self-administration (Manvich et al. 2012a; Grottick et al. 2000), and both baseline and cocaine-facilitated ICSS (Hayes et al. 2009a; Katsidoni et al. 2011). Furthermore, 5-HT_{2C} receptor antagonism (or inverse agonism) increased DA neuronal activity and striatal DA levels (Alex et al. 2005; Di Giovanni et al. 1999), blocked 5-HT_{2C} agonist-induced decreases in striatal DA levels (Di Giovanni et al. 2000), and increased both cocaine-induced facilitation of ICSS in rats (Katsidoni et al. 2011) and cocaine-primed reinstatement of cocaine self-administration in squirrel monkeys (Manvich et al. 2012b). These results support the hypothesis that 5-HT acting at 5-HT_{2C} receptors can decrease DA signaling and DA-dependent behaviors.

The purpose of the present study was to test the hypothesis that 5-HT_{2C} receptors mediate the anti-DA effects of selective and mixed-action 5-HT releasers in a rat ICSS procedure. Specifically, rats were trained to lever press for electrical brain stimulation to the medial forebrain bundle under a "frequency-rate" procedure, in which brain stimulation frequency is varied during daily sessions to generate a range of baseline ICSS rates (Negus and Miller 2014). We and others have previously reported that DA>5-HT-selective releasers facilitate low rates of responding maintained by low brain stimulation frequencies, whereas 5-HT-selective releasers decrease high rates of responding maintained by high brain stimulation frequencies, and mixed-action DA/5-HT releasers simultaneously produced both facilitation of low ICSS rates and depression of high ICSS rates (Bauer et al. 2013; Lin, et al. 1997; Olds and Yuwiler 1992; Olds 1995; Bonano et al. 2015). Our hypothesis predicted that the 5-HT_{2C} agonist Ro 60-0175 would be sufficient to depress high ICSS rates and that the 5-HT_{2C} antagonist SB 242,084 would block ICSS rate-decreasing effects of both Ro 60-0175 and the 5-HT-selective releaser fenfluramine. Our hypothesis also predicted that SB 242,084 would block 5-HT-mediated rate-decreasing effects of the two mixed-action DA/5-HT releasers, napthylisopropylamine (PAL-287) and (+)-MDMA (Bauer et al. 2013), and thereby disinhibit DA-mediated facilitation of ICSS produced by these compounds. Effects of SB 242,084 on ICSS depression produced by the kappa-opioid receptor agonist U69,593 and ICSS facilitation produced by amphetamine were also evaluated as controls.

Materials and methods

Subjects

Twenty-six adult male Sprague-Dawley rats (Harlan, Frederick, MD, USA) were used. All rats had ad libitum food and water access in their home cage and were housed individually on a 12-h light-dark cycle (lights on from 6 a.m. to 6 p.m.) in a facility accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care. Rats weighed between 298 and 514 g at surgery time. Animal maintenance accorded with The National Institutes of Health guidelines on care and use of animal subjects in research (National Research Council 2011). Experimental protocols were approved by the Virginia Commonwealth University Institutional Animal Care and Use Committee.

Assay of intracranial self-stimulation

Surgery Surgical and behavioral procedures were similar to those described in our previous studies of monoamine releasers to permit direct comparison with those previous studies (Bauer et al. 2013; Bonano et al. 2015; Negus and Miller 2014). Subjects were anesthetized with 2.5 % isoflurane gas until unresponsive to toe-pinch, and the cathode (0.25 mm diameter, insulated except at tip) of a stainless steel electrode was stereotaxically implanted into the left medial forebrain bundle at the level of the lateral hypothalamus (2.8 mm posterior to Bregma, 1.7 mm lateral to the midsaggital line, and 8.8 mm ventral to the skull in a horizontal orientation; Paxinos and Watson 1998). Three screws were placed in the skull, and the anode (0.125 mm diameter,

uninsulated) was secured to one of the screws to act as a ground. Dental acrylic secured the electrode to the skull, and 5 mg/kg ketoprofen was administered immediately and 24 h after surgery for postoperative analgesia. Animals were allowed 5 days to recover before initiation of training.

Apparatus Operant conditioning chambers consisted of sound-attenuating boxes containing modular acrylic and metal test chambers (29.2×30.5×24.1 cm). Each chamber had a response lever (4.5 cm wide, 2.0 cm deep, 3.0 cm off the floor), three stimulus lights (red, yellow, and green) centered 7.6 cm above the response lever, a 2-W house light, and an ICSS stimulator (Med Associates, St. Albans, VT, USA). Bipolar cables routed through a swivel commutator connected the stimulator to the electrode (Model SL2C, Plastics One, Roanoke, VA, USA). Med-PC IV computer software controlled all programming parameters and data collection (Med Associates, St. Albans, VT, USA).

Training The house light was illuminated during behavioral sessions, and lever pressing under a fixed-ratio 1 (FR1) schedule of reinforcement produced delivery of a 0.5-s train of square-wave cathodal pulses (0.1 ms/pulse) at a frequency of 2.2 log Hz. During brain stimulation, stimulus lights over the lever were illuminated and responding had no scheduled consequences. Stimulation intensity was then individually manipulated for each rat to identify an intensity that maintained a rate of reinforcement greater than 30 stimulations/min. Once an appropriate intensity was identified, frequency manipulations were introduced during sessions consisting of three consecutive 10-min components, and each component consisted of ten 60-s trials. The stimulation frequency was 2.2 log Hz for the first trial of each component, and frequency decreased in 0.05 log unit steps during the subsequent nine trials to a final frequency of 1.75 log Hz. Each trial began with a 10-s time-out period, during which responding had no scheduled consequences, and 5 non-contingent stimulations at the designated frequency were delivered at 1-s intervals during the last 5 s of the time-out. During the remaining 50-s of each trial, responding was reinforced under an FR1 schedule and produced both electrical brain stimulation and stimulus light illumination. Training continued until frequency-rate curves were not statistically different over three consecutive training days as indicated by lack of a significant effect of "day" in a two-way analysis of variance (ANOVA) with frequency and day as the two variables (see data analysis). Training was completed within 5 weeks of surgery.

Testing For dose-effect studies, test sessions consisted of three consecutive "baseline" components followed by a 25-min time-out period and then by three consecutive "test" components. A single drug dose was administered during the time-out and 20 min before the test components. For experiments during which vehicle or SB 242,084 was administered as a pretreatment, the pretreatment injection was given 5 min prior to the injection of the test compound (i.e., immediately after the baseline components).

Testing was conducted in three phases. First, the 5-HT_{2C} agonist Ro 60-0175 (0.32–3.2 mg/kg) was examined alone to determine whether 5-HT_{2C} activation was sufficient to depress ICSS, and the 5-HT_{2C} antagonist SB 242,084 (0.032–1.0 mg/kg) was examined as a pretreatment to vehicle or 3.2 mg/kg Ro 60-0175 to evaluate 5-HT_{2C} antagonist potency. Second, effects of vehicle or SB 242,084 (1.0 mg/kg) pretreatment were examined on depression of ICSS by the 5-HT-selective releaser fenfluramine (3.2 mg/kg), depression of ICSS by the kappa-opioid receptor agonist U69,593 (0.56 mg/kg), and facilitation of ICSS by the DA>5-HT-selective releaser amphetamine (0.32 mg/kg). The 1.0 mg/kg dose of SB 242,084 was selected for these antagonism studies because this dose fully blocked effects of 3.2 mg/kg R0 60-0175 while having no significant effects on ICSS when administered alone. Lastly, effects of vehicle or 1.0 mg/kg SB 242,084 pretreatment were examined on effects produced by the non-selective DA/5-HT releasers PAL-287 (1.0–5.6 mg/kg) and (+)-MDMA (1.0 and 3.2 mg/kg), both of which produced mixed profiles of both ICSS rate-increasing and rate-decreasing effects in a previous study (Bauer et al. 2013). Test drug doses were based on results from previous studies (Negus et al. 2010; Bauer et al. 2013), and higher doses of SB 242,084 were not examined due to limits in drug solubility. Each test drug was evaluated in a group of five to nine rats. Other than those used to test Ro 60-0175 alone, all subjects were used to evaluate more than one test drug. All experiments with a given test drug±SB 242,084 were completed before experiments with another test drug were initiated. The orders of both test drug and test drug doses were counterbalanced across rats. Experimental group sizes are reported in the figure legends. Test sessions were usually conducted on Tuesdays and Fridays, and three-component baseline training sessions without injections were conducted on all other weekdays.

Data analysis The primary dependent measure was the reinforcement rate in stimulations/trial. Raw reinforcement rates were normalized to the maximum control rate (MCR) for each subject on each day, where MCR was defined as the mean of the maximal rates observed during the second and third "baseline" components for that day. Therefore, %MCR was equal to [(response rate during a frequency trial)/(maximum control rate)]×100. Data for each frequency were averaged across test components for each rat and then across rats to yield a "frequency-rate" curve for each experimental manipulation. Two-way repeated measures ANOVAs were used to compare frequency-rate curves, with brain stimulation frequency and drug treatment as the two factors. A Holm-Sidak

post hoc test followed all significant ANOVAs, and p values less than 0.05 were considered significant. As a second summary measure of ICSS, the average number of stimulations per component delivered across all frequencies was determined before and after drug administration on each day, and drug effects were expressed as the % baseline number of stimulations per component for that day. All statistical analyses were conducted with GraphPad Prism 6.0 for Mac OSX (GraphPad Software, La Jolla, CA).

Drugs

(+)-Amphetamine hemisulfate and (±)-fenfluramine HCl were purchased from Sigma Chemical Co. (St. Louis, MO). SB 242,084 and Ro 60-0175 were purchased from Tocris (Bristol, UK). 1-(2-Naphthyl)propan-2-amine (PAL-287) and (+)-methylenedioxymethamphetamine ((+)-MDMA) were synthesized as the HCl salts by Dr. Bruce Blough (Research Triangle Park, NC). Amphetamine, fenfluramine, PAL-287, and (+)-MDMA were prepared in sterile saline. Ro 60-0175 was prepared in 4 % dimethyl sulfoxide in saline. SB 242,084 was prepared in 20 % polyethylene glycol in saline. All compounds were administered intraperitoneally (I.P.). Doses are expressed in terms of the salt forms above.

Results

Under baseline conditions or after vehicle treatment, electrical brain stimulation maintained a frequency-dependent increase in ICSS rates (e.g., Fig. 1a, open circles). The mean±SEM MCR for these studies was 62.1±1.69 stimulations per trial. The mean±SEM number of total stimulations earned during baseline components was 317.0±16.0 stimulations per component.

Figure 1 shows that the 5-HT_{2C} agonist Ro 60-0175 produced a dose-dependent downward shift of the ICSS frequency-rate curve (panel a) and a corresponding decrease in the percent baseline number of total reinforcers per component (panel b). Two-way ANOVA of data in panel a indicated significant main effects of frequency [$F(9,27)=32.36$, $p<0.0001$] and dose [$F(3,9)=12.88$, $p=0.0013$] and a significant interaction [$F(27,81)=4.665$, $p<0.0001$]. The rate-decreasing effects of 3.2 mg/kg Ro 60-0175 were dose-dependently blocked by the 5-HT_{2C} antagonist SB 242,084, with doses of 0.32 and 1.0 mg/kg SB 242,084 producing a significant blockade of the rate-decreasing effects produced by 3.2 mg/kg Ro 60-0175 (panels c, d). The highest SB 242,084 dose (1 mg/kg) tested did not significantly alter ICSS when administered alone. Figure 1c shows frequency-rate curves for selected treatments, and two-way ANOVA for all treatments indicated significant main effects of frequency [$F(9,36)=87.34$, $p<0.0001$] and treatment [$F(6,24)=9.421$, $p<0.0001$] and a significant interaction [$F(54,216)=2.159$, $p<0.0001$].

Figure 2 shows that 1.0 mg/kg SB 242,084 also blocked fenfluramine-induced depression of ICSS (panel a). Moreover, the combination of SB 242,084+fenfluramine increased ICSS at one frequency (1.9 log Hz). In contrast, SB 242,084 did not significantly alter either ICSS depression produced by the kappa-opioid agonist U69,593 (panel b) or facilitation of ICSS produced by amphetamine (panel c). Two-way ANOVA indicated a significant interaction between frequency and treatment in each panel as follows: (a) $F(18, 90)=1.845$, $p=0.0314$, (b) $F(18,72)=1.783$, $p=0.0445$, (c) $F(18,72)=2.957$, $p=0.0006$. Panel d shows summary data for panels a–c in terms of the percent baseline number of total reinforcers delivered across all brain stimulation frequencies.

Figure 3 shows effects of pretreatment with 1.0 mg/kg SB 242,084 on changes in ICSS produced by various doses of the mixed-action DA/5-HT releaser PAL-287 (1.0–5.6 mg/kg). All PAL-287 doses facilitated low rates of ICSS maintained by low brain stimulation frequencies (1.75–1.9 log Hz), and PAL-287 also produced a dose-dependent decrease in high rates of ICSS maintained by high brain stimulation frequencies (1.95–2.2 log Hz) (panels a–c). Pretreatment with SB 242,084 attenuated the rate-decreasing effects and augmented the rate-increasing effects of PAL-287. This effect was clearest when SB 242,084 was administered as a pretreatment to 5.6 mg/kg PAL-287 (panel c); however, SB 242,084 did not completely block rate-decreasing effects of 5.6 mg/kg PAL-287. Two-way ANOVA indicated a significant interaction between frequency and treatment in each panel as follows: (a) $F(18,144)=2.952$, $p=0.0002$, (b) $F(18,144)=14.02$, $p<0.0001$, and (c) $F(18,144)=19.30$, $p<0.0001$. Panel d shows summary data for panels a–c in terms of the percent baseline number of total reinforcers delivered across all brain stimulation frequencies.

Figure 4 shows a similar profile of effects produced by pretreatment with 1.0 mg/kg SB 242,084 on changes in ICSS produced by various doses of the mixed-action DA/5-HT releaser (+)-MDMA (1.0–3.2 mg/kg). All (+)-MDMA doses facilitated low rates of ICSS maintained by at least two low frequencies of brain stimulation between 1.75 and 1.9 log Hz, and (+)-MDMA also produced a dose-dependent decrease in high rates of ICSS maintained by high brain stimulation frequencies (1.95–2.2 log Hz) (panels a–c). Pretreatment with SB 242,084 attenuated the rate-decreasing effects and augmented the rate-increasing effects of (+)-MDMA. This effect was clearest when SB 242,084 was administered as a pretreatment to 3.2 mg/kg (+)-MDMA (panel c); however, SB 242,084 did not completely block the rate-decreasing effects of 3.2 mg/kg (+)-MDMA. Two-way ANOVA indicated a significant interaction between frequency and treatment in each panel as follows: (a) $F(18,144)=3.605$, $p<0.0001$], (b) $F(18,144)=7.077$, $p<0.0001$, and (c) [$F(18,$

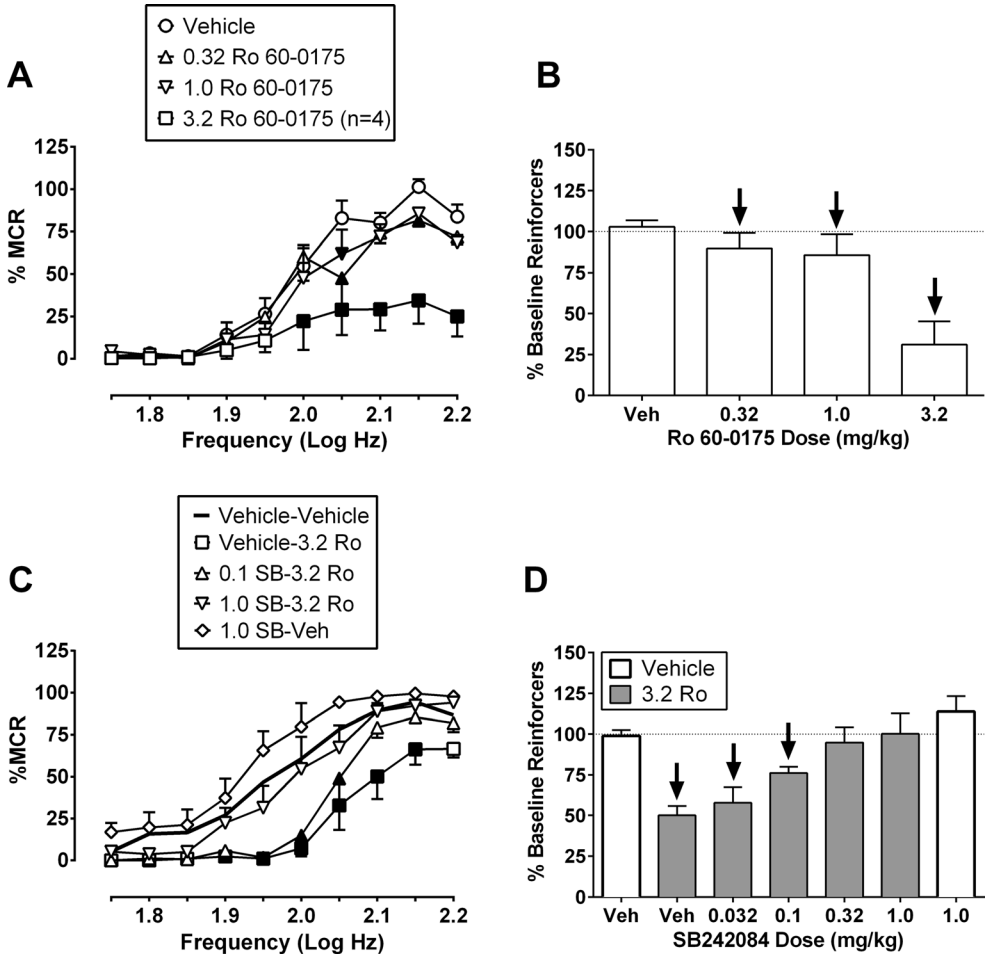

Fig. 1 Effects of the 5-HT_{2C} agonist Ro 60-0175 and antagonist SB 242,084. *Left panels* (**a**, **c**) show drug effects on full ICSS frequency-rate curves. Abscissae: Frequency of electrical brain stimulation in log Hz. Ordinates: Percent maximum control reinforcement rate (%MCR). *Filled points* show frequencies at which ICSS rates after drug treatment were statistically different from vehicle rates as determined by a two-way ANOVA followed by a Holm-Sidak post hoc test, $p<0.05$. *Right panels* (**b**, **d**) show summary ICSS data expressed as percent pre-drug baseline number of reinforcers per component delivered across all frequencies of brain stimulation. Abscissae: Drug dose in mg/kg. Ordinates: Percent pre-drug baseline number of reinforcers per component. *Downward arrows* indicate significant drug-induced decreases in ICSS relative to vehicle for at least one brain stimulation frequency as determined by analysis of full frequency-rate curves in panels **a** and **c**. All data show mean±SEM for five rats unless otherwise noted; statistical analysis was run for only four animals in panel **a** because the fifth animal lost its electrode before completion of the highest dose

144)=25.50, $p=0<0.0001$]. Panel d shows summary data for panels a–c in terms of the percent baseline number of total reinforcers delivered across all brain stimulation frequencies.

Discussion

This study tested the hypothesis that 5-HT_{2C} receptors were necessary for monoamine releaser-induced rate-decreasing effects in ICSS. There were three main findings. First, 5-HT_{2C} activation with Ro 60-0175 was sufficient to produce rate-decreasing effects, suggesting that 5-HT_{2C} receptors might also contribute to rate-decreasing effects of released 5-HT. Second, the rate-decreasing effects produced by a 5-HT-selective releaser (fenfluramine) were fully antagonized by SB 242,084, suggesting that 5-HT_{2C} receptor activation is necessary for expression of rate-decreasing effects by this 5-HT-selective monoamine releaser. Third, antagonism of the 5-HT_{2C} receptor significantly attenuated the rate-decreasing effects and increased expression of rate-increasing effects produced by the mixed-action DA/5-HT releasers PAL-287 and (+)-MDMA; however, SB 242,084 failed to completely block rate-decreasing effects of either compound. Taken together, these data suggest that 5-HT_{2C} receptors at least partially mediate the rate-decreasing effects produced by selective or mixed-action 5-HT releasers, and blockade of these receptors may increase expression of abuse-related rate-increasing effects by these compounds.

In the current study, administration of a 5-HT_{2C} receptor agonist (Ro 60-0175) produced a dose-dependent decrease in

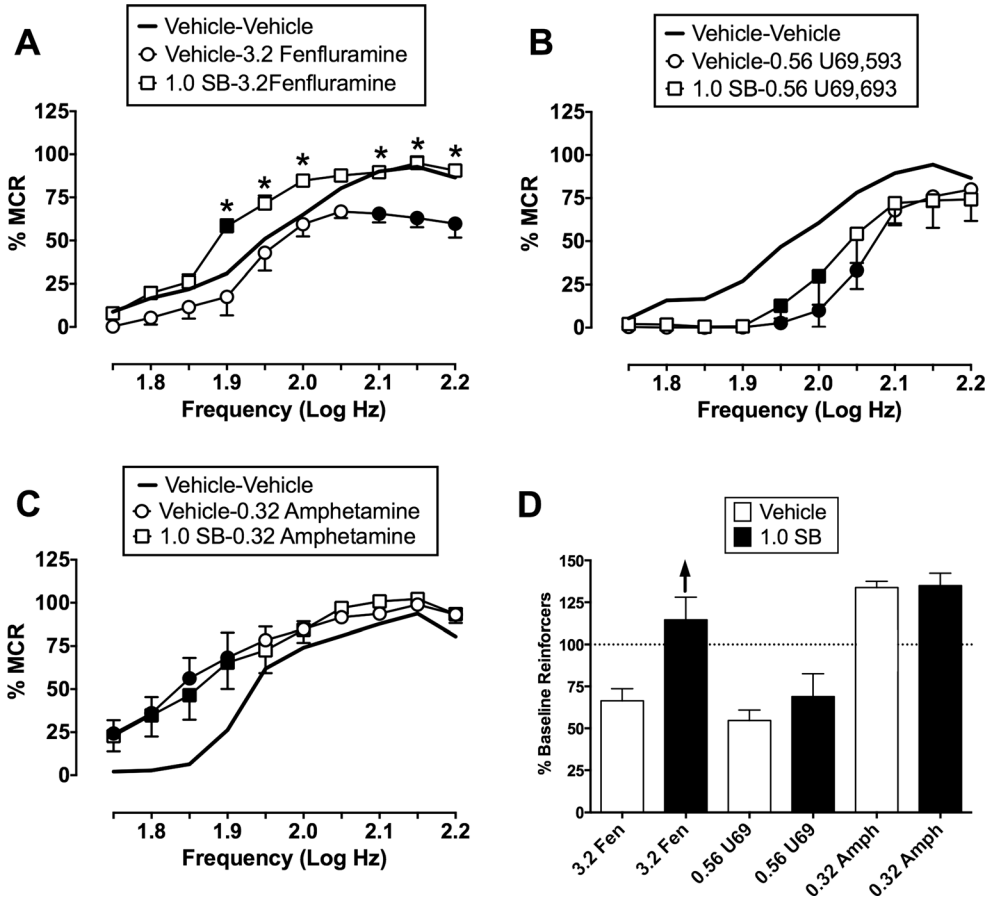

Fig. 2 Effects of the 5-HT_{2C} antagonist SB 242,084 on effects produced by fenfluramine, U69,593, and amphetamine. *Panels* (**a**–**c**) show drug effects on full ICSS frequency-rate curves. Abscissae: Frequency of electrical brain stimulation in log Hz. Ordinates: Percent maximum control reinforcement rate (%MCR). *Filled points* show frequencies at which ICSS rates after drug treatments were statistically different from vehicle+vehicle, and *asterisks* represent frequencies at which ICSS rates after SB 242,084+test drug were statistically different from rates after vehicle+test drug, as determined by a two-way ANOVA followed by a Holm-Sidak post hoc test, $p<0.05$. *Panel* **d** shows summary ICSS data expressed as percent pre-drug baseline number of reinforcers per component delivered across all frequencies of brain stimulation. Abscissa: Test drug treatment with dose in mg/kg. Ordinate: Percent pre-drug baseline number of reinforcers per component. *Upward arrows* indicate significant SB 242,084-induced increases in ICSS relative to test drug alone for at least one brain stimulation frequency as determined by analysis of full frequency-rate curves. All data show mean±SEM for six rats (fenfluramine) or five rats (U69,593, amphetamine)

ICSS, and this effect was blocked by pretreatment with a dose of SB 242,084 that had no effect alone. Although Ro 60-0175 has not been previously tested in ICSS, a different 5-HT_{2C} agonist (WAY-161503) also produced dose-dependent decreases in ICSS in rats (Hayes et al. 2009a; Katsidoni et al. 2011), and in agreement with the current study, the rate-decreasing effects of WAY-161503 were blocked by pretreatment with a dose of SB 242,084 that was inactive alone (0.5 mg/kg) (Katsidoni et al. 2011). In addition to decreasing ICSS, WAY-161503 has also been shown to produce conditioned place aversion (Mosher et al. 2006) and decreases in locomotion (Hayes et al. 2009b). Moreover, 5-HT_{2C} receptor agonists have been shown to decrease facilitation of ICSS by cocaine (Katsidoni et al. 2011), decrease responding for cocaine, nicotine, and food in assays of self-administration (Fletcher et al. 2004; Higgins et al. 2012; Grottick et al. 2000; Manvich et al. 2012a), and decrease the locomotor-activating effects of cocaine and nicotine (Cathala et al. 2014; Fletcher et al. 2004; Grottick et al. 2000; Hayes et al. 2009b). These results are also consistent with evidence to suggest that 5-HT_{2C} receptors activate ventral tegmental area GABA neurons that inhibit activity of, and DA release from, mesolimbic DA neurons (Alex and Pehek 2007). Taken together, these data support the proposition that activation of 5-HT_{2C} receptors can attenuate activity of the mesolimbic DA system and oppose abuse-related effects of drugs that activate the mesolimbic DA system.

A corollary of this proposition is that antagonists at 5-HT_{2C} receptors may block endogenous serotonergic tone at these receptors and thereby disinhibit mesolimbic DA neurons. In support of this idea, 5-HT_{2C} antagonists including SB 242,084 have been shown to increase firing rates of DA neurons and produce modest but significant (~200 %) increases in DA levels in rat striatum and squirrel monkey nucleus accumbens

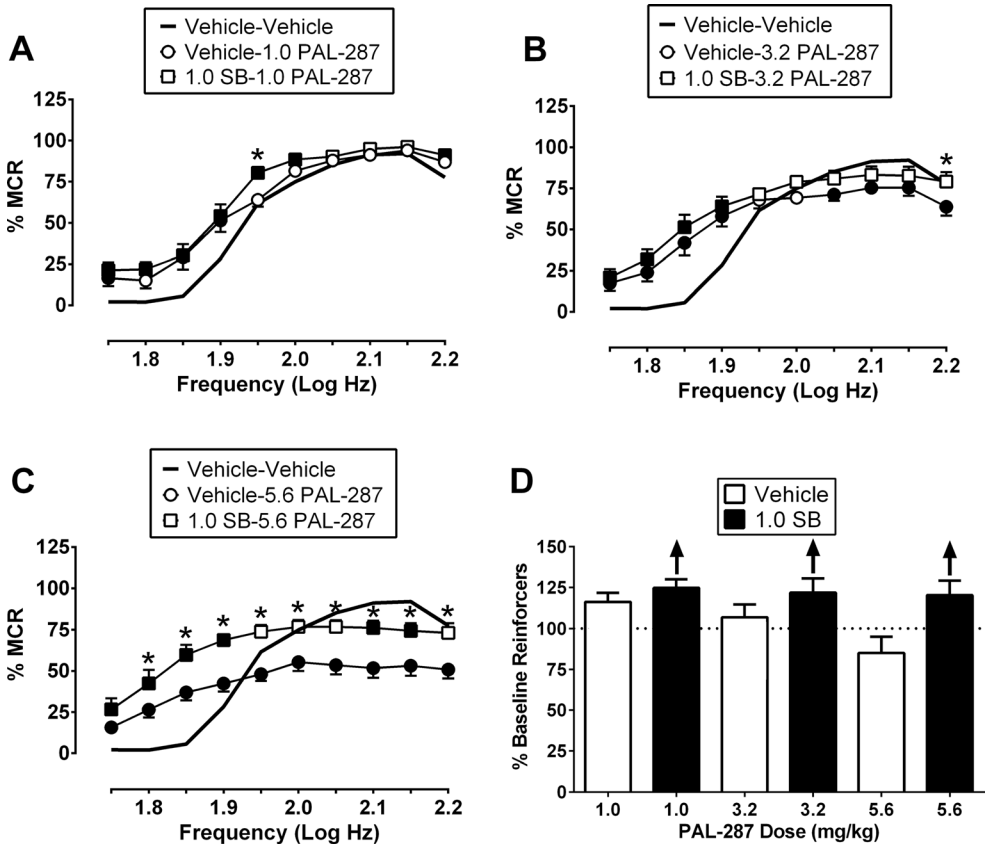

Fig. 3 Effects of the 5-HT_{2C} antagonist SB 242,084 on effects produced by the mixed-action DA/5-HT releaser PAL-287. *Panels* (**a**–**c**) show effects on full ICSS frequency-rate curves. Abscissae: Frequency of electrical brain stimulation in log Hz. Ordinates: Percent maximum control reinforcement rate (%MCR). *Filled points* show frequencies at which ICSS rates after drug treatments were statistically different from vehicle+vehicle, and *asterisks* represent frequencies at which ICSS rates after SB 242,084+PAL-287 were statistically different from rates after vehicle+PAL-287, as determined by a two-way ANOVA followed by a Holm-Sidak post hoc test, $p<0.05$. *Panel* **d** shows summary ICSS data expressed as percent pre-drug baseline number of reinforcers per component delivered across all frequencies of brain stimulation. Abscissa: PAL-287 dose in mg/kg. Ordinate: Percent pre-drug baseline number of reinforcers per component. *Upward arrows* indicate significant SB 242,084-induced increases in ICSS relative to PAL-287 alone for at least one brain stimulation frequency as determined by analysis of full frequency-rate curves. All data show mean±SEM for nine rats

(but not squirrel monkey caudate) (Alex et al. 2005; Di Giovanni et al. 1999; Manvich et al. 2012b). Moreover, in squirrel monkeys, SB 242,084 also increased rates of responding maintained under a fixed-interval schedule of stimulus termination, increased cocaine-primed reinstatement of cocaine self-administration, and maintained self-administration when it was substituted for cocaine (Manvich et al. 2012b). Many drugs that increase nucleus accumbens DA levels and maintain drug self-administration also facilitate ICSS (Negus and Miller 2014), and in the present study, administration of 1.0 mg/kg SB 242,084 increased mean rates of ICSS at several frequencies. However, this effect did not achieve statistical significance, and although the lack of a significant effect here may have been related to the relatively small group size ($N=5$), SB 242,084 also failed to facilitate ICSS in rats in a previous study (Katsidoni et al. 2011). Moreover, SB 242,084 also failed to produce a significant increase in locomotor activity in rats (Grottick et al. 2000). These findings suggest that rats may be less sensitive than squirrel monkeys to stimulant-like effects of SB 242,084.

The major goal of the present study was to assess a potential role for 5-HT_{2C} receptors in mediation of the ICSS rate-decreasing effects produced by selective and mixed-action 5-HT releasers. Previous studies using ICSS procedures have suggested that 5-HT release can decrease high rates of ICSS and oppose abuse-related facilitation of ICSS mediated by DA release (Bauer et al. 2013; Bonano et al. 2015; see Negus and Miller 2014 for review). Specifically, although DA>5-HT-selective monoamine releasers (e.g., amphetamine, methcathinone) produce dose-dependent and robust facilitation of ICSS across a broad range of doses, 5-HT-selective releasers (e.g., fenfluramine; 4-trifluromethyl methcathinone) produce only decreases in ICSS. Mixed-action DA/5HT releasers (e.g., PAL-287, (+)-MDMA) produce a mixed profile of effects that includes both facilitation of low ICSS rates maintained by low brain stimulation frequencies and

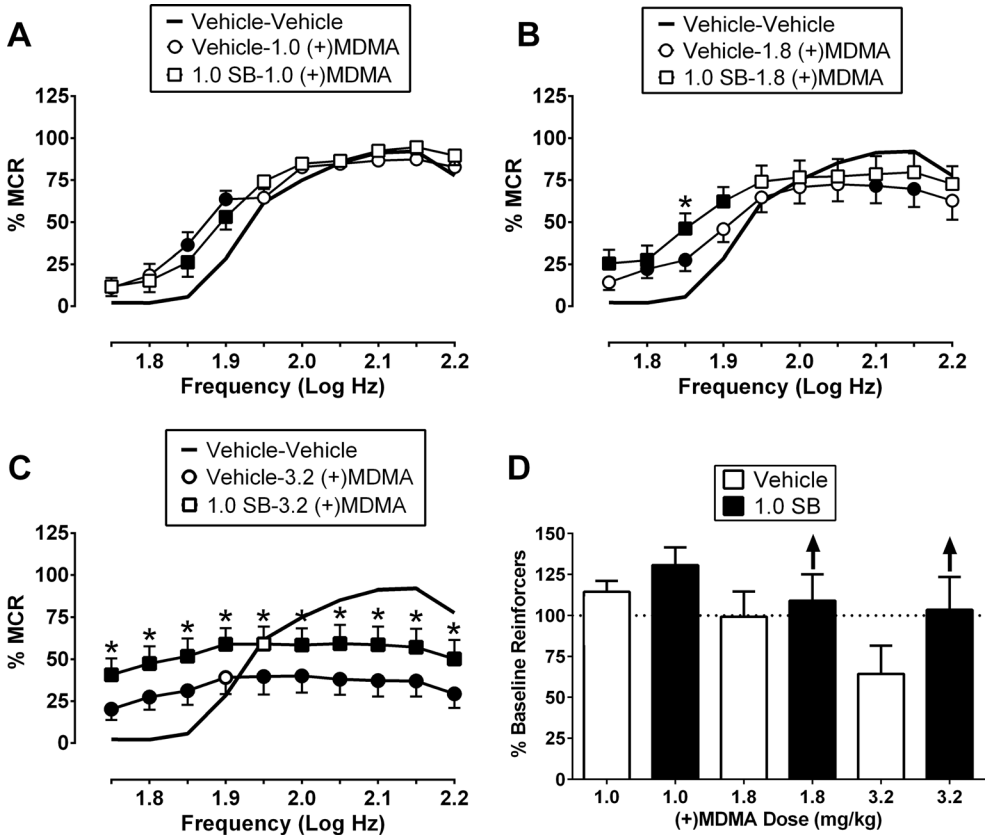

Fig. 4 Effects of the 5-HT_{2C} antagonist SB 242,084 on effects produced by the mixed-action DA/5-HT releaser (+)-MDMA. *Panels* (**a**–**c**) show effects on full ICSS frequency-rate curves. Abscissae: Frequency of electrical brain stimulation in log Hz. Ordinates: Percent maximum control reinforcement rate (%MCR). *Filled points* show frequencies at which ICSS rates after drug treatments were statistically different from vehicle+vehicle, and *asterisks* represent frequencies at which ICSS rates after SB 242,084+(+)-MDMA were statistically different from rates after vehicle+(+)-MDMA, as determined by a two-way ANOVA followed by a Holm-Sidak post hoc test, $p<0.05$. *Panel* **d** shows summary ICSS data expressed as percent pre-drug baseline number of reinforcers per component delivered across all frequencies of brain stimulation. Abscissa: (+)-MDMA dose in mg/kg. Ordinate: Percent pre-drug baseline number of reinforcers per component. *Upward arrows* indicate significant SB 242,084-induced increases in ICSS relative to (+)-MDMA alone for at least one brain stimulation frequency as determined by analysis of full frequency-rate curves. All data show mean±SEM for nine rats

decreases in high ICSS rates maintained by high brain stimulation frequencies. The present results with fenfluramine, PAL-287, and (+)-MDMA administered alone are consistent with these previous findings. Moreover, complete antagonism by SB 242,084 of the rate-decreasing effects of fenfluramine suggests that 5-HT_{2C} receptors play a major role in mediating rate-decreasing effects of this selective 5-HT releaser. The failure of SB 242,084 to significantly alter ICSS when administered alone, to block rate-decreasing effects of U69,593, or to enhance the rate-increasing effects of amphetamine further suggests that SB 242,084 effects on fenfluramine do not result from non-selective ICSS facilitating effects of SB 242,084. Of particular note, the failure of SB 242,084 to alter amphetamine-induced ICSS facilitation contrasts with a previous report that SB 242,084 enhanced ICSS facilitation produced by cocaine (Katsidoni et al. 2011). This distinction is consistent with the dependence of dopaminergic effects by monoamine uptake inhibitors like cocaine on rates of dopamine neuronal activity, which are increased by 5-HT_{2C} antagonists (Di Giovanni et al. 1999), whereas effects of monoamine releasers like amphetamine are less dependent on neuronal activity (Sulzer 2011).

SB 242,084 pretreatment also attenuated the ICSS rate-decreasing effects produced by the mixed-action DA/5HT releasers PAL-287 and (+)-MDMA, suggesting that 5-HT acting at 5-HT_{2C} receptors also contributes to the rate-decreasing effects of these compounds. However, SB 242,084 failed to completely block the rate-decreasing effects of either compound. There are at least two possible explanations for this finding. First, it is possible that high doses of PAL-287 and (+)-MDMA produced sufficiently high levels of 5-HT release to overcome antagonism of 5-HT_{2C} receptors by 1.0 mg/kg SB 242,084. Potentially consistent with this possibility, in vivo microdialysis studies found that I.V. administration of 1.0 mg/kg racemic MDMA produced large increases in extracellular nucleus accumbens 5-HT levels to more than 800 % of control (Baumann et al. 2012), and we have obtained similar results after I.P. administration of 3.2 mg/kg (±)-MDMA

(Bonano JS, Blough BE, Banks ML, Negus SS, unpublished results). However, arguing against this possibility, 1.0 mg/kg SB 242,084 completely blocked rate-decreasing effects of the highest dose of the 5-HT_{2C} agonist Ro 60-0175 (3.2 mg/kg), and rate-decreasing effects of Ro 60-0175 were as large or larger than the those produced by high doses of PAL-287 and (+)-MDMA. A second possibility is that PAL-287 and (+)-MDMA produce rate-decreasing effects via mechanisms other than 5-HT activation of 5-HT_{2C} receptors. These mechanisms could include actions at either other 5-HT receptors or non-5-HT receptors. For example, in addition to functioning as monoamine releaser, (±)-MDMA binds with lower but still detectable potency to 5-HT_{2B} and adrendergic α_2 receptors (Battaglia and De Souza 1989; Setola et al. 2003). Future studies will be required to determine whether these or other targets might also contribute to rate-decreasing effects of (+)-MDMA, PAL-287, or other monoamine releasers.

Conclusion In conclusion, the present results are consistent with the hypothesis that drug-induced release of 5-HT, acting at least in part through 5-HT_{2C} receptors, can oppose and limit other effects of monoamine releasers that contribute to reward, reinforcement, and drug abuse. One implication of this hypothesis is that reduction of 5-HT_{2C}-mediated effects might disinhibit and enhance expression of DA-mediated effects. Consistent with this hypothesis, SB 242,084 not only attenuated the rate-decreasing effects of fenfluramine, PAL-287, and (+)-MDMA but also increased expression of ICSS facilitation by these compounds. Indeed, after SB 242,084 pretreatment, even fenfluramine produced a small but significant facilitation of ICSS. In agreement with the present results using a selective 5-HT_{2C} antagonist, the non-selective 5-HT receptor antagonist methysergide also blocked rate-decreasing effects and enhanced expression of rate-increasing effects produced by (±)-MDMA in rats (Lin et al. 1997). Similarly, administration of the 5-HT neurotoxin 5,7-DHT to rats reduced brain levels of 5-HT and increased the percentage of rats that acquired MDMA self-administration (Bradbury et al. 2014). A related implication of these results is that vulnerability to abuse of mixed-action monoamine releasers like MDMA may be influenced by individual differences in components of 5-HT signaling, such as density or coupling efficiency of 5-HT_{2C} receptors. Specifically, reductions in signaling via 5-HT_{2C} receptors may be associated with increased vulnerability to abuse of mixed-action monoamine releasers.

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Conflict of interest There are no other conflicts of interest.

References

Alex KD, Pehek EA (2007) Pharmacologic mechanisms of serotonergic regulation of dopamine neurotransmission. Pharmacol Ther 113(2): 296–320

Alex KD, Yavanian GJ, McFarlane HG, Pluto CP, Pehek EA (2005) Modulation of dopamine release by striatal 5HT_{2C} receptors. Synapse 55(4):242–251

Battaglia G, De Souza EB (1989) Pharmacologic profile of amphetamine derivatives at various brain recognition sites: selective effects on serotonergic systems. NIDA Res Monogr Ser 94:240–258

Bauer CT, Banks ML, Blough BE, Negus SS (2013) Use of intracranial self-stimulation to evaluate abuse-related and abuse-limiting effects of monoamine releasers in rats. Br J Pharmacol 168(4):850–862

Baumann MH, Clark RD, Woolverton WL, Wee S, Blough BE, Rothman RB (2011) In vivo effects of amphetamine analogs reveal evidence for serotonergic inhibition of mesolimbic dopamine transmission in the rat. J Pharmacol Exp Ther 337(1):218–225

Baumann MH, Ayestas MA, Partilla JS, Sink JR, Shulgin AT, Daley PF, Brandt SD, Rothman RB, Buoho AE, Cozzi NV (2012) The designer methcathinone analogs, mephedrone and methylone, are substrates for monoamine transporters in brain tissue. Neuropsychopharmacology 37:1192–1203

Bonano JS, Banks ML, Kolanos R, Sakloth F, Barnier ML, Glennon RA, Cozzi NV, Partilla JS, Baumann MH, Negus SS (2015) Quantitative structure-activity relationship analysis of the pharmacology of para-substituted methcathinone analogs. Br J Pharmacol 172:2433–2444

Bradbury S, Bird J, Colussi-Mas J, Mueller M, Ricaurte G, Schenk S (2014) Acquisition of MDMA self-administration: pharmacokinetic factors and MDMA-induced serotonin release. Addict Biol 19(5): 874–884

Bubar MJ, Cunningham KA (2008) Prospects for 5-HT_2R pharmacotherapy in psychostimulant abuse. Prog Brain Res 172:319–346

Cathala A, Devroye C, Maitre M, Piazza PV, Abrous DN, Revest JM, Spampinato U (2014) Serotonin2C receptors modulate dopamine transmission in the nucleus accumbens independently of dopamine release: behavioral, neurochemical and molecular studies with cocaine. Addict Biol. doi:10.1111/adb.12137

Di Giovanni G, De Deurwaerdere P, Di Mascio M, Di Matteo V, Esposito E, Spampinato U (1999) Selective blockade of serotonin-2C/2B receptors enhances mesolimbic and mesostriatal dopaminergic function: a combined in vivo electrophysiological and microdialysis study. Neuroscience 91:587–597

Di Giovanni G, Di Matteo V, Di Mascio M, Esposito E (2000) Preferential modulation of mesolimbic vs. nigrostriatal dopaminergic function by serotonin(2C/2B) receptor agonists: a combined in vivo electrophysiological and microdialysis study. Synapse 35(1):53–61

Di Matteo V, Di Giovanni G, Di Mascio M, Esposito E (2000) Biochemical and electrophysiological evidence that RO 60-0175 inhibits mesolimbic dopaminergic function through serotonin(2C) receptors. Brain Res 865(1):85–90

Eberle-Wang K, Mikeladze Z, Uryu K, Chesselet MF (1997) Pattern of expression of the serotonin2C receptor messenger RNA in the basal ganglia of adult rats. J Comp Neurol 384(2):233–247

Fletcher PJ, Chintoh AF, Sinyard J, Higgins GA (2004) Injection of the 5-HT2C receptor agonist Ro60-0175 into the ventral tegmental area reduces cocaine-induced locomotor activity and cocaine self-administration. Neuropsychopharmacology 29(2):308–318

Götestam KG, Andersson BE (1975) Self-administration of amphetamine analogues in rats. Pharmacol Biochem Behav 3(2):229–233

Grottick AJ, Fletcher PJ, Higgins GA (2000) Studies to investigate the role of 5HT(2C) receptors on cocaine- and food-maintained behavior. J Pharmacol Exp Ther 295(3):1183–1191

Hayes DJ, Clements R, Greenshaw AJ (2009a) Effects of systemic and intra-nucleus accumbens $5HT_{2C}$ receptor compounds on ventral tegmental area self-stimulation thresholds in rats. Psychopharmacology (Berl) 203(3):579–588

Hayes DJ, Mosher TM, Greenshaw AJ (2009b) Differential effects of 5HT2C receptor activation by WAY 161503 on nicotine-induced place conditioning and locomotor activity in rats. Behav Brain Res 197(2):323–330

Higgins GA, Silenieks LB, Robmann A, Rizos Z, Noble K, Soko AD, Fletcher PJ (2012) The 5-HT2C receptor agonist lorcaserin reduces nicotine self-administration, discrimination, and reinstatement: relationship to feeding behavior and impulse control. Neuropsychopharmacology 37:1177–1191

Howell LL, Cunningham KA (2015) Serotonin 5-HT2 receptor interactions with dopamine function: implications for therapeutics in cocaine use disorder. Pharmacol Rev 67:176–197

Johnston LD, O'Malley PM, Bachman JG, Schulenberg JE (2009) Monitoring the future. National results on adolescent drug use: overview of key findings, 2008 (NIH Publication No. 09-7401). National Institute on Drug Abuse, Bethesda

Katsidoni V, Apazoglou K, Panagis G (2011) Role of serotonin 5HT2A and $5HT_{2C}$ receptors on brain stimulation reward and the reward-facilitating effect of cocaine. Psychopharmacology (Berl) 213(2-3): 337–354

Lin HQ, Jackson DM, Atrens DM, Christie MJ, McGregor IS (1997) Serotonergic modulation of 3,4-methylenedioxymethamphetamine (MDMA)-elicited reduction of response rate but not rewarding threshold in accumbal self-stimulation. Brain Res 744(2):351–357

Manvich DF, Kimmel HL, Howell LL (2012a) Effects of serotonin 2C receptor agonists on the behavioral and neurochemical effects of cocaine in squirrel monkeys. J Pharmacol Exp Ther 341(2):424–434

Manvich DF, Kimmel HL, Cooper DA, Howell LL (2012b) The serotonin 2C receptor antagonist SB 242084 exhibits abuse-related effects typical of stimulants in squirrel monkeys. J Pharmacol Exp Ther 342(3):761–769

Mosher TM, Smith JG, Greenshaw AJ (2006) Aversive stimulus properties of the 5HT2C receptor agonist WAY 161503 in rats. Neuropharmacology 51(3):641–650

National Research Council (2011) Guide for the care and use of laboratory animals (8th edition). National Academies, Washington DC

Negus SS, Miller LL (2014) Intracranial self-stimulation to evaluate abuse potential of drugs. Pharmacol Rev 66(3):869–917

Negus SS, Morrissey EM, Rosenberg M, Cheng K, Rice KC (2010) Effects of kappa opioids in an assay of pain-depressed intracranial self-stimulation in rats. Psychopharmacology (Berl) 210(2):149–159

Olds ME (1995) Dopamine agonists prevent or counteract the suppression of brain stimulation reward by fenfluramine. Pharmacol Biochem Behav 50(1):41–48

Olds ME, Yuwiler A (1992) Effects of acute and chronic fenfluramine on self-stimulation and its facilitation by amphetamine. Eur J Pharmacol 216(3):363–372

Parada M, Hernandez L, Schwartz D, Hoebel BG (1988) Hypothalamic infusion of amphetamine increases serotonin, dopamine and norepinephrine. Physiol Behav 44(4-5):607–610

Paxinos G, Watson C (1998) The rat brain in stereotaxic coordinates. Academic, San Diego

Pompeiano M, Palacios JM, Mengod G (1994) Distribution of the serotonin 5-HT2 receptor family mRNAs: comparison between 5-HT2A and 5-HT2C receptors. Mol Brain Res 23(1):163–178

Rothman RB, Baumann MH (2006) Balance between dopamine and serotonin release modulates behavioral effects of amphetamine-type drugs. Ann N Y Acad Sci 1074:245–260

Rothman RB, Baumann MH, Dersch CM, Romero DV, Rice KC, Carroll FI, Partilla JS (2001) Amphetamine-type central nervous system stimulants release norepinephrine more potently than they release dopamine and serotonin. Synapse 39(1):32–41

Setola V, Hufeisen SJ, Grande-Allen KJ, Vesely I, Glennon RA, Blough B, Rothman RB, Roth BL (2003) 3,4-methylenedioxymethamphetamine (MDMA, "Ecstasy") induces fenfluramine-like proliferative actions on human cardiac valvular interstitial cells in vitro. Mol Pharmacol 63(6):1223–1229

Sulzer D (2011) How addictive drugs disrupt presynaptic dopamine neurotransmission. Neuron 69:628–649

Wee S, Woolverton WL (2006) Self-administration of mixtures of fenfluramine and amphetamine by rhesus monkeys. Pharmacol Biochem Behav 84:337–343

Wee S, Anderson KG, Baumann MH, Rothman RB, Blough BE, Woolverton WL (2005) Relationship between the serotonergic activity and reinforcing effects of a series of amphetamine analogs. J Pharmacol Exp Ther 313(2):848–854

Woods JH, Tessel RE (1974) Fenfluramine: amphetamine congener that fails to maintain drug-taking behavior in the rhesus monkey. Science 185(4156):1067–1069