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5-HT_{2A} receptor blockade and 5-HT_{2C} receptor activation interact to reduce cocaine hyperlocomotion and Fos protein expression in the caudate-putamen

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Abstract

Both the 5-HT_{2A} receptor (R) antagonist M100907 and the 5-HT_{2C}R agonist MK212 attenuate cocaine-induced dopamine release and hyperlocomotion. This study examined whether these drugs interact to reduce cocaine hyperlocomotion and Fos expression in the striatum and prefrontal cortex. We first determined from dose-effect functions a low dose of both M100907 and MK212 that failed to alter cocaine (15 mg/kg, i.p.) hyperlocomotion. Subsequently we examined whether these subthreshold doses given together would attenuate cocaine hyperlocomotion, consistent with a 5-HT_{2A}/5-HT_{2C}R interaction. Separate groups of rats received two sequential drug injections 5 min apart immediately before a 1-h locomotion test as follows: 1) saline + saline, 2) saline + cocaine, 3) 0.025 mg/kg M100907 + cocaine, 4) 0.125 mg/kg MK212 + cocaine, or 5) cocktail combination of 0.025 mg/kg M100907 and 0.125 mg/kg MK212 + cocaine. Brains were extracted for Fos immunohistochemistry 90 min after the second injection. We next examined the effects of 0.025 mg/kg M100907 and 0.125 mg/kg MK212, alone and in combination, on spontaneous locomotor activity. While neither drug given alone produced any effects, the M100907/MK212 cocktail attenuated cocaine hyperlocomotion as well as cocaine-induced Fos expression in the dorsolateral caudate-putamen (CPu), but had no effect on spontaneous locomotion. The findings suggest that 5-HT_{2A}Rs and 5-HT_{2C}Rs interact to attenuate cocaine hyperlocomotion and Fos expression in the CPu, and that the CPu is a potential locus of the interactive effects between these 5-HT₂R subtypes on behavior. Further research investigating combined 5-HT_{2A}R antagonism and 5-HT_{2C}R agonism as a treatment for cocaine dependence is warranted.

Keywords

Cocaine; Serotonin; Locomotion; M100907; MK212

Introduction

The serotonin (5-HT) 2 family of receptors has been a target for development of pharmacological treatments for several psychiatric disorders, including depression (Aronson

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et al., 1995; Berg et al., 2008), schizophrenia (Talvik-Lotfi et al., 2000), obesity (Grottick et al., 2000; Halford et al., 2007), and addiction (Bubar and Cunningham, 2008; Higgins and Fletcher, 2003). Many studies have suggested an important role of 5-HT₂ receptors (Rs), in particular 5-HT_{2A} and 5-HT_{2C}Rs, in cocaine addiction. Structurally, 5-HT_{2C} and 5-HT_{2A}Rs are very similar (Hoyer et al., 2002; Raymond et al., 2001), and coexist in many brain regions involved in addiction circuitry (Bubar and Cunningham, 2007; Doherty and Pickel, 2000; Pompeiano et al., 1994), including the mesolimbic pathway originating in the ventral tegmental area (VTA) and projecting to nucleus accumbens (NAc) and the nigrostriatal pathway originating in the substantia nigra (SN) and projecting to the caudate-putamen (CPu).

Functionally, 5-HT_{2A} and 5-HT_{2C}Rs play opposing facilitative and inhibitory roles, respectively, in cocaine-related behaviors. For instance, peripheral injections of the 5-HT_{2A}R selective antagonist M100907 decrease cocaine hyperlocomotion (Fletcher et al., 2002; McMahon et al., 2001) as well as cue- and cocaine-primed reinstatement of cocaine-seeking behavior, but have no effect on cocaine self-administration (Fletcher et al., 2002; Nic Dhonnchadha et al., 2009). Systemic administration of M100907 also attenuates cocaine discriminative stimulus effects (McMahon et al., 2001). Further, systemic or intra-striatal injections of M100907 attenuate MDMA- and amphetamine-induced DA release in the NAc and CPu (Porrás et al., 2002; Schmidt et al., 1992; Schmidt et al., 1994). Conversely, a selective 5-HT_{2C}R antagonist enhances cocaine hyperlocomotion as well as cocaine-primed reinstatement and cocaine self-administration (Fletcher et al., 2002; McMahon et al., 2001). 5-HT_{2C}R agonists RO-60-0175 and MK212 inhibit cue- and cocaine-primed reinstatement of cocaine-seeking behavior, effects that are blocked by 5-HT_{2C}R antagonists, indicating that they are 5-HT_{2C}R-mediated (Fletcher et al., 2008; Neisewander and Acosta, 2007; Pentkowski, in press; Pentkowski et al., 2010). Some studies have found differential effects of 5-HT_{2C}Rs on DA transmission in the NAc and CPu (Rocha et al., 2002), while others show no differences between the regions (De Deurwaerdere et al., 2004). Systemic administration of 5-HT_{2C}R antagonists has been found to increase amphetamine-induced DA release in the NAc and CPu (Porrás et al., 2002), while agonists attenuate morphine-induced DA release in the NAc (Willins and Meltzer, 1998).

Despite the oppositional relationship between behavioral effects mediated by 5-HT_{2A} versus 5-HT_{2C}Rs, to date, no research has investigated a potential interaction between these two receptor subtypes. By contrast, behavioral pharmacology studies of the DA system have revealed that interactions between D1-like and D2-likeR families mediate effects of psychostimulants. For instance, D2R-mediated stereotypy is observed only when there is also tonic stimulation of D1Rs, even though stimulation of D1Rs alone has little, if any, effect (Missale et al., 1998). Furthermore, D1 and D2R antagonists synergistically decrease discriminative stimulus properties of cocaine and amphetamine (Callahan et al., 1991), and D1 and D2R agonists administered together produce qualitatively more intense stereotypy than either one alone (Feldman, 1997; Jackson and Westlind-Danielsson, 1994). As suggested by Kathryn Cunningham, investigating whether similar interactions exist between the 5-HT_{2A} and 5-HT_{2C}Rs is an important research question that may suggest a novel approach to attenuating the physiological and psychological effects of psychostimulants (Whitten, 2007).

One way to examine potential interactive effects of 5-HT_{2A}R antagonists and 5-HT_{2C}R agonists is by measuring Fos expression. Fos protein expression is a commonly used measure of functional neuronal activity (Harlan and Garcia, 1998; Herrera and Robertson, 1996). Region-specific patterns of Fos expression are associated with acute cocaine administration (Robertson et al., 1991; Torres and Rivier, 1994; Zahm et al., 2010) and exposure to cocaine-paired cues (Kufahl et al., 2009b; Neisewander et al., 2000; Zavala et

al., 2007) or a cocaine-associated context (Brown et al., 1992; Crawford et al., 1995; Hamlin et al., 2008; Hotsenpiller et al., 2002). The 5-HT_{2A}R antagonist, M100907, has been found to decrease cocaine-induced Fos expression in the NAc shell and CPu (Szucs et al., 2005). On the other hand, 5-HT_{2C}R antagonists enhance Fos expression in the subthalamic nucleus and CPu (De Deurwaerdere et al., 2010). We have also found that a 5-HT_{2C} agonist administered into the ventromedial prefrontal cortex (PFC) decreases cocaine hyperlocomotion as well as cocaine-induced Fos expression in the dorsolateral CPu (Pentkowski et al., 2010; Pentkowski, in prep; Pockros et al., 2011). Thus, 5-HT_{2A} antagonists and 5-HT_{2C} agonists both decrease cocaine-induced Fos expression in the CPu, as well as attenuate cocaine hyperlocomotion.

In the present study, we hypothesized that concurrent 5-HT_{2A} antagonism and 5-HT_{2C} agonism would interact to attenuate the effects of cocaine on locomotion and Fos expression. We first examined dose-dependent decreases in cocaine hyperlocomotion by M100907 and MK212 given alone. From these experiments, we identified subthreshold doses of each drug that produced no effect on cocaine hyperlocomotion when given alone. We then tested the effects of a combination of these subthreshold doses of M100907 and MK212 on cocaine-induced and spontaneous locomotion. We also conducted Fos immunohistochemistry in several brain regions to investigate the effects of concurrent 5-HT_{2A} antagonism and 5-HT_{2C} agonism on cocaine-induced neuronal activation.

Materials and Methods

Animals

Adult male Sprague–Dawley rats weighing 250–350 g at the start of the experiments were used in this study. Animals were housed in a climate-controlled colony room with a 14-h reversed light/dark cycle (lights off at 7:00 a.m. and on at 9 p.m.) and were cared for in accordance with the “Guide for the Care and Use of Laboratory Animals” (Institute of Laboratory Animal Resources on Life Sciences, National Research Council 1996). The animals were given food and water *ad libitum* except during the testing sessions.

Drugs

M100907 (RTI International, Research Triangle Park, NC, USA) and MK212 (Tocris Cookson Inc., Ellisville, Missouri, USA) were dissolved in a 0.9% saline containing 3% tween. Cocaine-HCl (RTI International, Research Triangle Park, NC, USA) was dissolved in 0.9% saline. Euthasol (Hospira, Lake Forest, IL, USA) was used to deeply anesthetize animals before perfusions.

General procedures

Animals were handled daily for 1 week and were given saline injections to habituate them to injection stress on each of the 2 days prior to the start of the experiments. All animals were tested in Plexiglas locomotor activity chambers (44 × 24 × 20 cm high) in a soundproof, dimly lit room for 60-min sessions. A computer-automated video tracking system (Clever Systems, Reston, VA, USA) used the orientation of the animal's body (e.g. center of body) to measure the total horizontal distance traveled. All testing was conducted during the animals' dark cycles. In experiments testing cocaine hyperlocomotion, animals were placed into the test chambers for 1 h prior to any drug injections in order for them to habituate to the chamber, thus providing a low baseline level of locomotion from which to detect cocaine-induced increases.

Dose-dependent effects of M100907 and MK212 on cocaine hyperlocomotion

Separate cohorts of animals were randomly assigned to groups that received i.p. injections of either M100907 (0.025, 0.05, or 0.1 mg/kg) or MK212 (0.125, 0.25, or 0.5 mg/kg) (n=8/dose) prior to one test; both cohorts received vehicle prior to the other test with the order of the drug versus vehicle tests counterbalanced within groups. Following habituation on the test day, the animals were given an injection of their assigned drug, put back into their home cage for 5-min, and then given an injection of cocaine (15 mg/kg, i.p.) immediately before being placed back into the test chambers for the 1-h test.

Effects of M100907 + MK212 on cocaine hyperlocomotion

Subthreshold doses of M100907 (0.025 mg/kg, i.p.) and MK212 (0.125 mg/kg, i.p.) that failed to alter cocaine-induced locomotor activity on their own in the above experiments were then combined as a cocktail to examine potential receptor interactions. Animals were randomly assigned to receive 2 drug injections given 5 min apart following the habituation period as follows, respectively: saline + saline, saline + cocaine (15 mg/kg, i.p.), 0.025 mg/kg M100907 + cocaine, 0.125 mg/kg MK212 + cocaine, or cocktail + cocaine (n=6–7/group). Rats were placed into their home cage following the first injection and were placed back into the locomotor activity chambers for a 1-h test following the second injection. This experiment utilized a between-subjects design, as animals were sacrificed after testing and their brains were extracted for Fos analysis as described below.

Effects of M100907 + MK212 on spontaneous locomotion

Drug-naïve rats were randomly assigned to receive saline, M100907 (0.025 mg/kg, i.p.), MK212 (0.125 mg/kg, i.p.) or a cocktail of the latter two drugs. Five min after injection of their assigned drug, the rats were placed into the locomotor activity chambers for a 1-h test. There was no habituation period in this experiment in order to avoid having a floor effect that would obviate detection of drug effects on locomotion.

Tissue preparation

To allow 90-min after drug injections for optimum Fos expression, animals remained uninterrupted in the locomotor chambers for 30-min after the 1-h test. They were then deeply anesthetized with Euthasol (100 mg/kg, i.p.) and perfused transcardially with 300 ml of ice-cold 0.1 M phosphate-buffered saline (PBS), pH 7.4, followed by 300 ml of ice-cold 4% paraformaldehyde in 0.1 M PBS, pH 7.4. The brains were removed, postfixed overnight at 4 °C in 4% paraformaldehyde, and then transferred to 15% sucrose for 24 h and then to 30% sucrose for an additional 24 h while continuously being stored at 4 °C. Coronal sections (40 µm) were collected using a freezing microtome at levels corresponding to 3.2, 1.6, 2.56, 5.6 mm relative to bregma (Paxinos and Watson, 1998). The tissue sections were then frozen and stored at 20 °C in a cryoprotectant solution comprised of 0.02 M PBS (pH 7.2), 30% sucrose, 10% polyvinyl pyrrolidone, and 30% ethylene glycol.

Fos protein immunohistochemistry

Tissue sections were first washed in 0.1 M PBS (6×10 min) to remove the cryoprotectant. Sections were then incubated in 0.3% H₂O₂ for 30 min and rinsed with 0.1 M PBS (3×10 min), followed by incubation in 0.1 M PBS containing 5% normal goat serum (NGS) (Vector Laboratories, Burlingame, CA, USA) and 0.2% Triton X-100 (Sigma, St. Louis, MO, USA) for 1 h. Sections were then incubated for 48 h at 4 °C in 0.1 M PBS containing anti-Fos rabbit polyclonal antibody (SC-52; 1:2,000; Santa Cruz Biotechnology, Santa Cruz, CA, USA), 0.1% Triton X-100 and 1% NGS, and then rinsed in 0.1 M PBS (3×10 min). Sections were then incubated in 0.1 M PBS containing biotinylated goat anti-rabbit IgG (1:500; Vector Laboratories, Burlingame, CA, USA) and 1% NGS for 1 h, and then rinsed in

0.1 M PBS (3×10 min). Subsequently, horseradish peroxidase activity was visualized with nickel diaminobenzidine and glucose oxidase reaction as described in Dielenberg et al. (2001). This reaction was terminated after 10 min by rinsing the tissue in 0.1 M PBS (3×10 min). Sections were then mounted onto gelatin-coated slides, dried, and dehydrated before cover slipping.

Fos immunoreactivity analysis

Sections were taken at +3.2 mm, which contained the prelimbic (PrL) and infralimbic (IL) cortices; sections taken at +1.6 mm contained the NAc core (NAcC) and shell (NAcS), and the dorsolateral CPu. Care was taken to ensure that the sections that were labeled came from the same anatomical level within each plane for each subject. Quantification of Fos immunoreactivity was examined using a Nikon Eclipse E600 (Nikon Instruments, Melville, NY, USA) microscope set at 20X. For all regions, the sample area counted was 0.26 mm² and there were a total of six sample areas counted for each subject (i.e., 1 sample area/2 hemispheres/3 sections) that were then averaged to provide a mean number of immunoreactive cells per sample area. An observer blind to treatment conditions identified Fos immunoreactivity as a blue-black oval-shaped nucleus distinguishable from background using size and optical density criteria set using the Image Tool software package (Version 3.0, University of Texas Health Sciences Center, San Antonio, TX, USA). Regions analyzed and representative sample areas are shown in Fig 1. These images were captured using SPOT Advanced software (Version 3.5 Sterling Heights, MI, USA) and no modifications were made to the images.

Statistical analyses

Because the purpose of the dose-effect experiments was to identify subthreshold doses of M100907 and MK212, locomotor activity data at each dose were analyzed separately using repeated measures analyses of variance (ANOVAs) with time (e.g. 15-min time bins) and test (e.g. saline or cocaine) as within-subject factors. For drug interaction experiments, spontaneous and cocaine-induced locomotor activity data were analyzed using mixed-factor ANOVAs with time as a repeated measure within-subject factor and drug group as a between-subject factor. For all tests of cocaine hyperlocomotion, the last 15-min of habituation served as a baseline. Fos data were analyzed by region using one-way ANOVAs with drug as a between-subject factor. In order to focus analyses of Fos on group differences in response to cocaine standardized across regions, raw data was converted to a percent of saline control and analyzed using one-way ANOVAs with drug as a between-subject factor. A Greenhouse–Geisser correction was used to correct for heterogeneity of variance in the data. Significant effects were further analyzed using smaller ANOVAs and post hoc comparisons were made using Tukey's HSD test. All statistics were run using SPSS, version 20.

Results

Dose-Effect Function of M100907 on Cocaine Hyperlocomotion

Figure 2 shows the dose-effect function of M100907 on cocaine hyperlocomotion. For the lowest dose of 0.025 mg/kg, the ANOVA of distance traveled showed a main effect of time [$F(1.51,10.60)=10.92, p<0.01$], but no effect of test nor test by time interaction demonstrating that locomotion was similar regardless of vehicle versus M100907 treatment. Post hoc tests on the main effect of time showed that cocaine increased locomotor activity relative to baseline, and locomotor activity remained elevated for the first 30-min of testing ($p<0.05$).

For the 0.05 mg/kg dose, the ANOVA of distance traveled showed an interaction of time by test [$F(4,28)=3.30$, $p<0.05$], as well as a main effect of time [$F(1.76,12.33)=9.06$, $p<0.01$]. Post hoc tests showed a difference between baseline and the first 15-min of testing only when animals were pretreated with vehicle ($p<0.05$). On the vehicle test day, locomotor activity remained elevated at the 30-min time point compared to baseline ($p<0.05$). On the M100907 test day, there was no difference between any of the time points, consistent with a failure to observe cocaine hyperlocomotion. However, the M100907 attenuation of cocaine hyperlocomotion was not due to a change at a particular time as there were no differences between test days any time points.

For the 0.1 mg/kg dose, the ANOVA of distance traveled showed a main effect of time [$F(4,28)=14.69$, $p<0.01$] as well as a main effect of test [$F(1,7)=9.06$, $p<0.05$], with the latter indicating less distance traveled overall during the M100907 test than during the vehicle test. Post hoc tests on the main effect of time showed that cocaine increased locomotor activity relative to baseline for the first 15-min of testing regardless of pretreatment ($p<0.01$). Collectively, these findings suggest that cocaine hyperlocomotion was slightly attenuated at this dose of M100907.

Dose-Effect Function of MK212 on Cocaine Hyperlocomotion

Figure 3 shows the effects of MK212 on cocaine hyperlocomotion. For the lowest dose of 0.125 mg/kg, the ANOVA of distance traveled showed a main effect of time [$F(4,28)=35.95$, $p<0.01$], but no effect of test nor test by time interaction demonstrating that locomotion was similar regardless of vehicle versus MK212 treatment. Post hoc tests on the main effect of time showed that cocaine increased locomotor activity relative to baseline, and locomotor activity remained elevated for 45 min regardless of test day ($p<0.05$).

For the 0.25 mg/kg dose, the ANOVA of distance traveled showed an interaction of time by test [$F(4,28)=3.40$, $p<0.05$], as well as a main effect of time [$F(4,28)=23.17$, $p<0.01$], and a main effect of test [$F(1,7)=17.07$, $p<0.01$]. Post hoc tests showed a difference between baseline and the first 15 min of testing on both test days ($p<0.05$). On the vehicle test day only, locomotor activity remained elevated at the 30-min time point compared to baseline ($p<0.05$). There was also a significant difference between vehicle and MK212 test days at the 30-min time point ($p<0.05$).

For the 0.5 mg/kg dose, the ANOVA of distance traveled showed an interaction of time by test [$F(4,28)=11.77$, $p<0.01$], as well as a main effect of time [$F(4,28)=37.35$, $p<0.01$], and a main effect of test [$F(1,7)=9.98$, $p<0.05$]. Post hoc tests showed a difference between baseline and the first 15 min of testing on the vehicle test day ($p<0.05$), and locomotor activity remained elevated compared to baseline ($p<0.05$) until the last 15-min of the test. On the MK212 test day, there was no difference between baseline and the first 15-min of testing, however there was a significant decrease in activity compared to baseline at all other time points ($p<0.05$). There were also significant differences between vehicle and MK212 test days at the last three time points ($p<0.05$).

M100907/MK212 Interaction Effects on Cocaine Hyperlocomotion

The effects of the M100907/MK212 cocktail on cocaine-induced locomotor activity are shown in Figure 4. The ANOVA of distance traveled showed a significant time by drug interaction [$F(6.95,45.20)=2.49$, $p<0.05$] as well as a main effect of time [$F(1.74, 45.20)=40.29$, $p<0.01$] and a main effect of drug [$F(4,26)=4.97$, $p<0.01$]. Post-hoc comparisons indicated a significant difference between the saline + saline and saline + cocaine groups ($p<0.05$, Tukey HSD), as well as the saline + cocaine and cocktail + cocaine

groups ($p < 0.01$, Tukey HSD). There were no differences between the cocaine + saline and cocaine + M100907 or cocaine + MK212 groups ($p = 0.474$ and $p = 0.463$, respectively).

At each 15-min time-bin during testing, the one-way ANOVAs of distance traveled showed a significant effect of drug group [$F(4,30) = 5.491$ – 3.751 , $p < 0.05$]. Across all post-cocaine time bins, the cocktail + cocaine group exhibited less locomotion than the saline + cocaine group ($p < 0.05$, Tukey HSD), whereas the saline + saline group exhibited less locomotion at the 30 ($p < 0.01$, Tukey HSD) and 60 min time bins only ($p < 0.05$, Tukey HSD). In contrast, the M100907 + cocaine and MK212 + cocaine groups did not differ from either the saline + saline or saline + cocaine groups at any time point. There was no difference in baseline between groups.

M100907/MK212 Interaction Effects on Spontaneous Locomotion

The effects of the M100907/MK212 cocktail on spontaneous locomotor activity are shown in Figure 6. The ANOVA of distance traveled showed a main effect of time [$F(3,84) = 138.551$, $p < 0.01$], but no effect of drug or drug by time interaction, suggesting that none of the drug treatments altered spontaneous locomotion. Post-hoc tests showed a difference between the first 15-min and each of the 30-min, 45-min, and 60-min time points ($p < 0.01$), as well as a difference between the 30-min and 45-min time points ($p < 0.01$).

M100907/MK212 Interaction Effects on Cocaine-induced Fos Activation

The effects of the M100907/MK212 cocktail on cocaine-induced Fos activation in striatal subregions are shown in Figure 6. Fos data are from the same animals whose behavioral data are shown in Figure 4. The ANOVA of percent control of Fos-positive nuclei in the dorsal CPu (Panel a) showed a significant between-group effect [$F(4,30) = 3.859$, $p < 0.05$]. Post-hoc comparisons showed significant differences between the saline + saline and saline + cocaine ($p < 0.05$, Tukey HSD) groups, indicating that cocaine increased Fos expression in this region. There were also significant differences between saline + saline and MK212 + cocaine ($p < 0.05$, Tukey HSD) and saline + saline and M100907 + cocaine ($p < 0.05$, Tukey HSD) groups, suggesting neither drug alone reversed the effect of cocaine on Fos expression. In contrast, there was no significant difference between the saline + saline and cocktail + cocaine groups, indicating that the cocktail significantly attenuated cocaine-induced Fos expression. ANOVAs of percent control of Fos-positive nuclei in the NAc core (Panel b) and shell (Panel c) and the infralimbic (Panel d) and prelimbic (Panel e) PFC failed to reveal any between-group effects.

Discussion

Results from the present study support our hypothesis that 5-HT_{2A} and 5-HT_{2C}Rs interact to decrease cocaine-induced locomotor activity and Fos expression. We determined from dose-response experiments that the doses of 0.025 mg/kg M100907 and 0.125 mg/kg MK212 had no effect on cocaine hyperlocomotion when given alone but these doses given in combination significantly attenuated cocaine hyperlocomotion, consistent with receptor interaction effects. The interaction effect was specific for cocaine hyperlocomotion as this dose combination had no effect on spontaneous locomotor activity. This dose combination also region-specifically attenuated cocaine-induced Fos expression in the dorsolateral CPu. It is likely that the effects of M100907 and MK212 observed in the present study were in fact due to actions at 5-HT_{2A} and 5-HT_{2C} receptors, respectively. M100907 has >1000-fold selectivity for 5-HT_{2A} vs. 5-HT_{2C} receptors (Kehne et al., 1996) and several studies have demonstrated that doses of 0.005–0.4 mg/kg reverse the behavioral effects of 5-HT_{2A} agonists, but not those of 5-HT_{2C}R agonists (Dekeyne et al., 1999; Gresch et al., 2007; Hitchcock et al., 1997; McCreary et al., 2003; Vickers et al., 2001; Wettstein et al., 1999).

MK212 binds to 5-HT_{2C}Rs with the highest affinity compared to other receptors, but it does have affinity for 5-HT_{2A}, 5-HT_{2B} and 5-HT₃Rs (Cussac et al., 2002; Glennon et al., 1989; Porter et al., 1999). It is unlikely that 5-HT_{2B} receptors were involved in the effects observed in this study since previous research has found that neither 5-HT_{2B}R agonists nor antagonists have any effect on cocaine hyperlocomotion (Filip et al., 2004). Further, we have shown that the locomotor activity effects of even higher doses of MK212 (0.32–1.0 mg/kg), which should be less selective for 5-HT_{2C}Rs than the low doses used in the present study, are reversed by a selective 5-HT_{2C}R antagonist, SB242084 (Neisewander and Acosta, 2007; Pentkowski et al., 2009; Pentkowski et al., 2010). The hypolocomotive effects of MK212 itself are also reversed by SB242084 (Stiedl et al., 2007). Thus it is likely that the low dose effects observed here are 5-HT_{2C}R-mediated.

Our findings fit with the existing literature, which clearly shows that both 5-HT_{2A}R antagonists and 5-HT_{2C}R agonists given alone decrease cocaine hyperlocomotion, as well as reinstatement of cocaine-seeking behavior (Grottick et al., 2000; McMahon et al., 2001; Neisewander and Acosta, 2007; Nic Dhonnchadha et al., 2009; Pentkowski et al., 2010; Pockros et al., 2011). Similar results have been found in the nicotine literature as well, with 5-HT_{2A}R antagonists decreasing nicotine self-administration (Fletcher et al., 2012; Levin et al., 2008) and 5-HT_{2C}R agonists attenuating nicotine self-administration as well as nicotine-induced locomotion, sensitization, conditioned place preference, and discriminative stimulus effects (Fletcher et al., 2012; Grottick et al., 2001; Zaniewska et al., 2007). 5-HT_{2A}R antagonists and 5-HT_{2C}R agonists have also been shown to attenuate premature responding on a five-choice serial reaction time test with and without cocaine, suggesting a role in drug-induced impulsivity (Fletcher et al., 2007). While there is ample evidence that 5-HT_{2A} antagonists and 5-HT_{2C} agonists have opposing effects, to our knowledge the present study is the first to demonstrate an interaction between these two serotonin receptor subtypes.

Interestingly, the middle dose (0.05 mg/kg) of M100907 appeared to have a stronger effect than the highest dose (0.1 mg/kg). Previous research has shown that a higher dose of 0.5 mg/kg attenuates cocaine hyperlocomotion (Fletcher et al., 2002), suggesting that there may be an inverted U-shaped dose-effect function for M100907 effects on this behavior. The dose-effect function of M100907 on methamphetamine hyperlocomotion is similar, with a higher dose producing less robust attenuation than an intermediate dose (Steed et al., 2011). Some studies have shown dose-dependent effects of M100907 on impulsivity (Agnoli and Carli, 2012) and reinstatement of nicotine-seeking behavior (Fletcher et al., 2012), whereas M100907 effects on cue-primed reinstatement of cocaine-seeking behavior (Nic Dhonnchadha et al., 2009) do not appear to vary dose-dependently.

The effects of M100907 and MK212 on cocaine-induced Fos expression in the dorsolateral CPu mimicked the behavioral data, where low doses of M100907 and MK212 had no effect on cocaine-induced Fos expression when given alone, but produced a significant decrease when given in combination. Although this study did not include control groups for possible effects of M100907 or MK212 alone on Fos expression, it is unlikely that these drugs altered Fos expression on their own given previous findings. For instance, it has been shown that M100907 (0.2–0.8 mg/kg) has no significant effect on Fos activation in the CPu after saline pretreatment (Szucs et al., 2005). The effects of MK212 on Fos expression have not been examined, although another 5-HT_{2C}R agonist, RO-60-1057, fails to alter Fos expression in the CPu at doses (1–3 mg/kg) that attenuate cocaine-primed reinstatement (Beyeler et al., 2010; Grottick et al., 2000). Furthermore, studies have previously found that cocaine-induced Fos expression in the CPu is altered by 5-HT_{2R} manipulations. For instance systemic injections of M100907 attenuate cocaine-induced Fos in the CPu (Szucs et al., 2005), and our laboratory has found that intra-mPFC infusions of MK212 also attenuate cocaine-induced Fos in the CPu (Pockros et al., 2011). Thus, it is unlikely that these drugs

induce Fos expression on their own, but instead selectively attenuate cocaine-induced Fos expression, similar to their selective attenuation of cocaine-induced hyperlocomotion without producing any effect on spontaneous locomotion.

It is surprising that cocaine hyperlocomotion was associated with increased Fos expression only in the CPu and not in the NAc or PFC because several previous studies have found that acute injection of cocaine produces hyperlocomotion and increases Fos protein expression in all of these regions (Graybiel et al., 1990; Szucs et al., 2005; Young et al., 1991). However, cocaine-induced Fos expression in the striatum exhibits a rostral to caudal increasing gradient. For instance, Szucs et al. (2005) found no effects of cocaine on Fos expression in the anterior NAc core or shell at +1.7 mm from Bregma, consistent with the lack of cocaine-induced Fos expression in the present study at +1.6mm from bregma. However, in contrast to Szucs et al. (2005) who found only a nonsignificant trend toward an effect of cocaine on Fos expression in the CPu at +1.7 mm from Bregma, we found that cocaine significantly increased Fos in the CPu at +1.6mm from Bregma. This difference may be due to different slicing angles or staining or counting techniques. It is possible that we may have observed cocaine-induced Fos expression if we had analyzed tissue from the caudal regions of the NAc core and shell because Szucs et al. (2005) only found significant effects at a more caudal level (i.e., +1.0 mm from Bregma). Contrary to our results, several studies have shown that cocaine (2 mg/kg, i.v. or 25 mg/kg) increases Fos expression in the medial PFC (Graybiel et al., 1990; Kufahl et al., 2009a). However, differences in cocaine dose or different routes of administration may account for differences across studies.

The similar pattern of changes in locomotion and Fos expression in the CPu suggests that this region is involved in the observed behavioral changes. Although, site-specific injections will be necessary to determine the brain regions and cellular mechanisms involved in the functional receptor interaction, the CPu is likely involved given its role in stimulant-induced motor activities, including locomotion, repetitive stereotypic and habitual behaviors (Brown et al., 1992; Naylor and Olley, 1972; White et al., 1998; Zimmerberg and Glick, 1974). The dose of cocaine used in the present study (15 mg/kg, i.p.) does not typically produce stereotypic behaviors, however slightly higher doses (20 mg/kg, i.p.) have been found to produce stereotypies typically manifesting as headbobbing (Bhattacharyya and Pradhan, 1979; Budygin, 2007; O'Dell et al., 1996; White et al., 1998). Repetitive stereotypic behaviors may compete with expression of cocaine hyperlocomotion; however, if stereotypy occurred in this study, the drugs co-administered with cocaine would likely have attenuated rather than exacerbated this behavior. Indeed 5-HT_{2A}R antagonists, including M100907, have been found to attenuate stereotypy produced by other drugs (Barwick et al., 2000; Higgins et al., 2003; Ninan and Kulkarni, 1998). Similarly, 5-HT_{2C}R mutant mice exhibit enhanced DAT antagonist-induced stereotypic behavior (Abdallah et al., 2009), suggesting that stimulation of these receptors inhibits dopamine-induced stereotypy. Thus, it is unlikely that the reduction of cocaine hyperlocomotion was due to competing stereotypic behavior.

Although the cellular mechanisms by which M100907 and MK212 produced their combined effect on cocaine hyperlocomotion and Fos expression remain to be elucidated, one possibility is via a decrease in DA release in the nigrostriatal pathway. Acute injection of cocaine has been shown to stimulate Fos expression (Neisewander et al., 2000; Zahm et al., 2010) and increase DA in the CPu (Hurd and Ungerstedt, 1989; Hurd et al., 1990). 5-HT_{2A} and 5-HT_{2C}Rs have been found to regulate amphetamine- and morphine-induced DA release in the CPu in opposing manners; 5-HT_{2A}R blockade attenuates phasic DA release (De Deurwaerdere and Spampinato, 1999; Gobert and Millan, 1999; Ichikawa and Meltzer, 1995; Lucas and Spampinato, 2000; Porrás et al., 2002; Schmidt et al., 1992), while 5-HT_{2C}R activation decreases both tonic and phasic DA activity (Di Giovanni et al., 1999; Di Matteo et al., 2000; Gobert et al., 2000; Porrás et al., 2002). Cellular localization of 5-HT_{2A}

and 5-HT_{2C}Rs in the dorsal CPu has yet to be determined, although the majority of 5-HT_{2A}R-labeled cells in this region contain parvalbumin, indicative of γ -Aminobutyric acid (GABA) interneurons (Bubser et al., 2001). In the mesolimbic pathway, 5-HT_{2A} and 5-HT_{2C}Rs are expressed on both DA and GABA neurons in the VTA, and on GABA neurons in the NAc. In the SN pars compacta, 5-HT_{2C}Rs are found on GABAergic neurons and 5-HT_{2C}R agonists stimulate GABA release in the SN (Eberle-Wang et al., 1997; Invernizzi et al., 2007). Intra-CPu administration of a 5-HT_{2C}R inverse agonist increases DA release in the CPu, an effect that can be reversed by concurrent administration of a 5-HT_{2C}R agonist mCPP (1.0 mg/kg, i.p.) (Alex et al., 2005). Thus, the 5-HT_{2A} and 5-HT_{2C}R interactive effects on cocaine hyperlocomotion and Fos expression in the CPu may be due to direct modulation of DA release from nigrostriatal neurons, or indirect modulation of DA release via an increase in GABA inhibition either within the dorsal CPu itself or within the SN. While we did not observe effects of M100907 and/or MK212 on Fos activation in the terminal regions of the mesocorticolimbic dopamine pathway, this does not rule out the possibility that this pathway is involved in the interaction effects.

We chose to test our hypothesis by examining effects of subthreshold doses of M100907 and MK212 on cocaine-induced locomotion to establish proof or principle that 5-HT_{2A} and 5-HT_{2C}Rs interact. Although this method of combining subthreshold doses is an approach that has been used to detect synergistic interactions (Brown et al., 1991; Gotoh et al., 2006; Thiel et al., 2009), there are some limitations. First, although statistically our findings are consistent with a synergistic interaction, the non-significant tendency of both drugs to attenuate cocaine hyperlocomotion when given alone raises the possibility that their interactive effect may be additive rather than synergistic. Thus, more sophisticated isobolographic analyses will be needed to precisely determine the nature of the 5-HT_{2A} and 5-HT_{2C}R interaction. Second, it remains unclear whether pharmacokinetic interactions between these drugs are involved in the interaction effect observed. Future research will be needed to address these issues.

In conclusion, the findings from this study provide support for the idea that a combination of 5-HT_{2A} antagonist and 5-HT_{2C} agonist may offer potential therapeutic advantages for development of treatment for cocaine dependence. For instance, relatively low doses of two drugs could be used instead of a high dose of a single drug. These lower doses would likely be less disruptive than a high dose to various systems throughout the body involving these receptors, resulting in fewer side-effects. In support of this idea, the present findings indicate that combined subthreshold doses that decreased cocaine hyperlocomotion without disturbing spontaneous locomotion. 5-HT_{2C}R agonists with greater selectivity have recently been developed that may improve upon therapeutic efficacy and used at a lower dose range in combination with 5-HT_{2A}R antagonists. Clinical trials are currently investigating the effectiveness of 5-HT_{2A}R antagonists in treating depression and insomnia and 5-HT_{2C}R agonists in treating obesity (NIH, 2010), supporting the potential clinical utility of these drugs for treating addiction. Ideally, pharmacological treatments aimed at cocaine dependence should not only curtail the reinforcing effects of cocaine, but also drug craving and relapse (Washton, 1988). To further explore the potential clinical utility of a combination treatment with a 5-HT_{2A}R antagonist and a 5-HT_{2C}R agonist, future research is needed to determine whether the combination would reduce cocaine self-administration and drug-seeking behavior while not interfering with unconditioned behaviors (i.e. locomotion and feeding).

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Fig 1.

Representative images of Fos positive nuclei in the dorsolateral CPu for animals that received either cocaine (a), saline (b), or cocaine + M100907 and MK 212 cocktail (c) and schematic representation of coronal sections of the rat brain taken at +3.2 (d) and +1.6 mm from Bregma (e; Paxinos and Watson, 1998). Numbers in the sections represent the regions analyzed for Fos as follows: (1) prelimbic cortex (PrL); (2) infralimbic cortex (IL); (3) dorsolateral CPu; (4) NAcC; (5) NAcSh. Scale bar on the first image (a) is equal to 100 μ m.

Fig 2.

Effects of 0.025 (a), 0.05 (b), and 0.1 (c) mg/kg M100907 ($n=8$ /dose group) on cocaine hyperlocomotion, expressed as the mean $\pm$ SEM total distance traveled in meters across 15-min time bins relative to the last 15-min of habituation (baseline; BL), or for the entire 1-h session (insert). Dose was a between-subjects factor, while test day (vehicle or M100907) was a within-subjects factor. In between habituation and cocaine hyperlocomotion testing, rats were injected subcutaneously with vehicle on one test day (left) and their assigned dose of M100907 on the other test day (right), with order counterbalanced; 5 min later they were injected with cocaine (15 mg/kg, i.p.), indicated by the dotted vertical lines. Only the 0.025 mg/kg dose failed to have any effect on cocaine hyperlocomotion. The *asterisk* (*) represents a significant difference from baseline, $P < 0.05$. The plus sign (+) represents a significant difference from the vehicle test day, $P < 0.05$.

Fig 3.

Effects of 0.125 (a), 0.25 (b), and 0.5 (c) mg/kg MK212 ($n=8$ / dose group) on cocaine hyperlocomotion, expressed as the mean $\pm$ SEM total distance traveled in meters across 15-min time bins relative to the last 15-min of habituation (baseline; BL). Dose was a between-subjects factor, while test day (vehicle or M100907) was a within-subjects factor. In between habituation and cocaine hyperlocomotion testing, animals were injected subcutaneously with vehicle on one test day (left) and their assigned dose of MK212 on the other test day (right), with order counterbalanced; 5 min later they were injected with cocaine (15 mg/kg, i.p.), indicated by the dotted vertical lines. Only the 0.125 mg/kg dose failed to have any effect on cocaine hyperlocomotion. The *asterisk* (*) represents a significant difference from baseline, $P<0.05$. The plus sign (+) represents a significant difference from respective time point on the vehicle test day, $P<0.05$.

M100907+MK212 Attenuated Cocaine Hyperlocomotion

Fig 4.

Panel a shows the effects of cocaine, saline, 0.025 mg/kg M100907 + cocaine, 0.125 mg/kg MK212 + cocaine, and M100907 + MK212 cocktail + cocaine ($n=8/\text{group}$) on cocaine hyperlocomotion, expressed as the mean $\pm$ SEM total distance traveled in meters across 15-min time bins relative to the last 15-min of habituation (baseline; BL). Panel b shows the cumulative data for the entire 60-min session. In between habituation and cocaine hyperlocomotion testing, animals were injected subcutaneously with vehicle on one test day (left) and their assigned dose of MK212 on the other test day (right), with order counterbalanced; 5 min later they were injected with cocaine (15 mg/kg, i.p.), indicated by the dotted vertical lines. In both graphs, only the saline and the M100907 + MK212 cocktail + cocaine groups showed a significant difference from the cocaine alone group, indicating that M100907 and MK212 only had an effect when given in combination. The *asterisk* (*) represents a significant difference from the cocaine group, $P<0.05$.

Fig 5.

Effects of saline, 0.025 mg/kg M100907, 0.125 mg/kg MK212, and M100907/MK212 cocktail (n=8/group) on spontaneous locomotion, expressed as the mean $\pm$ SEM total distance traveled in meters across 15-min time bins (a) and as total locomotion for the 1-h test (b). Animals were injected subcutaneously with their assigned drug, and 5-min later were given a 1-hr test for locomotor activity. There were no differences in locomotion between groups.

Fig 6.

Effects of saline, 15 mg/kg cocaine, 0.025 mg/kg M100907 + cocaine, 0.125 mg/kg MK212 + cocaine, and M100907 + MK212 cocktail + cocaine ($n=8/\text{group}$) on Fos activation in the dorsolateral CPu (a), NAc core (b) and shell (c), and infralimbic (d) and prelimbic (e) PFC, expressed as the mean $\pm$ SEM of percent of control (saline alone group). The dotted line indicates reference point for change from saline control (i.e., 100%). All animals underwent locomotor activity testing and were sacrificed 90 min after drug injections. Animals were perfused and brains were harvested for Fos immunohistochemistry. There was a significant difference between saline and cocaine groups, indicating that cocaine increased Fos expression in the CPu. There was also a difference between the M100 + cocaine and MK212 + cocaine groups, which shows that these drugs alone had no effect on cocaine-induced Fos activation. There was no difference between the saline and cocktail + cocaine groups, indicating that the cocktail decreased Fos activation levels back to baseline. There were no differences between any of the groups in any regions of the NAc or PFC. The *asterisk* (*) represents a significant difference from the saline group, $P<0.05$.