JPET Fast Forward. Published on February 21, 2014 as DOI: 10.1124/jpet.113.212373

1. Title Page

A novel aminotetralin-type serotonin (5-HT)2C receptor-specific agonist and 5-HT2A competitive antagonist/5-HT2B inverse agonist with preclinical efficacy for psychoses

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2. Running Title

a) Novel 5-HT2C-specific agonist for psychoses

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c) *Number of*

Text Pages: 34 (excluding Figs and Tables),

Tables: 1

Figures: 8

References: 60/60

Words in the Abstract: 197/250

Words in Introduction: 740/750

Words in Discussion: 1393/1500

d) *Abbreviations:*

5-HT: serotonin

HTR: head-twitch response

PAT: 4-phenyl-2-dimethylaminotetralin (4-phenyl-*N*,*N*-dimethyl-1,2,3,4-tetrahydronaphthalene-2-amine)

(+)-MBP: (+)-trans-(2*R*,4*S*)-(3'[*meta*]-bromophenyl)-*N*,*N*-dimethyl-1,2,3,4-tetrahydronaphthalen-2-amine

(-)-MBP: (-)-trans-(2*S*,4*R*)-4-(3'[*meta*]-bromophenyl)-*N*,*N*-dimethyl-1,2,3,4-tetrahydronaphthalen-2-amine

DOI: (±)-(2,5)-di-methoxy-4-iodoamphetamine

CLOZ: clozapine

AMP: amphetamine

GPCR: G protein-coupled receptor

e) Drug Discovery and Translational Medicine

JPET Fast Forward. Published on February 21, 2014 as DOI: 10.1124/jpet.113.212373
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3. Abstract

Development of 5-HT2C agonists for treatment of neuropsychiatric disorders, including psychoses, substance abuse, and obesity, has been fraught with difficulties, because the vast majority of reported 5-HT2C selective agonists also activate 5-HT2A and/or 5-HT2B receptors, potentially causing hallucinations and/or cardiac valvulopathy. Herein is described a novel, potent, and efficacious human 5-HT2C receptor agonist, (-)-*trans*-(2*S*,4*R*)-4-(3'[*meta*]-bromophenyl)-*N*,*N*-dimethyl-1,2,3,4-tetrahydronaphthalen-2-amine ((-)-MBP), that is a competitive antagonist and inverse agonist at human 5-HT2A and 5-HT2B receptors, respectively. In three C57Bl/6 mouse models of drug-induced psychoses ([2,5]-dimethoxy-4-iodoamphetamine elicited head-twitch response, MK-801-induced hyperlocomotion, and amphetamine-induced hyperlocomotion), (-)-MBP has efficacy comparable to the prototypical second-generation antipsychotic drug, clozapine. (-)-MBP, however, does not alter locomotion when administered alone, distinguishing it from clozapine, which suppresses locomotion. Finally, consumption of highly palatable food by mice was not increased by (-)-MBP at a dose that produced at least 50% maximal efficacy in the psychoses models. Compared to (-)-MBP, (+)-MBP was much less active across in vitro affinity and functional assays using mouse and human receptors, and also translated in vivo with comparably lower potency and efficacy. Results indicate a 5-HT2C receptor-*specific* agonist, such as (-)-MBP, may be pharmacotherapeutic for psychoses, without liability for obesity, hallucinations, heart disease, sedation or motoric disorders.

4. Introduction

Psychotic disorders, which affect approximately 3% of the population (Perala et al., 2007), are associated with an overactive striatal dopamine system (Abi-Dargham et al., 1998; Seeman and Seeman, 2013). Specifically, persons with schizophrenia are hypersensitive to psychostimulants (Curran et al., 2004), show elevated psychostimulant-induced dopamine release (Abi-Dargham et al., 1998), and display increased presynaptic dopamine synthesis in the striatum, cf. (Seeman and Seeman, 2013). Most existing antipsychotic medications interact primarily with dopamine D2 receptors to, theoretically, normalize dopamine signaling. Approximately 2/3 of patients, however, are noncompliant or cease taking their neuroleptic medication (Bellack, 2006), typically due to serious side effects that include weight gain, diabetes, high cholesterol, extrapyramidal symptoms, sedation, lethargy, and emotional dampening (NIMH, 2010; Moritz et al., 2013). Furthermore, extant antipsychotics have limited efficacy in approximately 1/3 of patients (Lindenmayer, 2000), and so-called second-generation antipsychotics do not have superior efficacy compared to their first-generation predecessors (Lieberman et al., 2005).

Targeting the serotonin (5-HT) system, and precisely the 5-HT2C receptor, represents an alternative approach to pharmacotherapy for psychoses. 5-HT2C receptors are expressed in several neural systems affected in schizophrenia, including the frontal cortex and the striatum (Lopez-Gimenez et al., 2001; Pandey et al., 2006), and a corpus of preclinical observations supports a role for 5-HT2C receptors in regulating the brain's dopamine system. 5-HT2C agonists and inverse agonists modulate dopamine release (Di Giovanni et al., 2000; De Deurwaerdere et al., 2004; Alex et al., 2005). 5-HT2C receptor knockout mice possess enhanced baseline dopamine levels in the striatum and behavioral hypersensitivity to dopamine-releasing psychostimulants (Abdallah et al., 2009), and genetic manipulations that lead to overexpression of 5-HT2C receptors alter dopamine metabolism (Kimura et al., 2009; Olaghere da Silva et al., 2010). Also, induced-overexpression of dopamine D2 receptors increases

expression of 5-HT2C receptors (Simpson et al., 2011), and 5-HT2C receptor ligands modulate D2 receptor activity (Olijslagers et al., 2004), further corroborating a physiological link between 5-HT2C receptors and central dopamine function. Finally, selective 5-HT2C receptor agonists show pre-clinical efficacy in animal models of psychoses (Rosenzweig-Lipson et al., 2012), and in clinical trials, the novel 5-HT2C agonist, vabicaserin, showed proof-of-concept for treating schizophrenia (Shen et al., 2010), suggesting that activation of 5-HT2C receptors may be a novel approach to treating schizophrenia.

5-HT2C receptors are also localized on pro-opiomelanocortin (POMC) neurons in the hypothalamus, a brain region involved in regulating metabolism, hunger, and satiety signals. 5-HT2C agonists stimulate the expression of anorexigenic POMC in the hypothalamus, resulting in decreased appetite (Lam et al., 2007; Xu et al., 2008). In clinical trials, lorcaserin, a 5-HT2 agonist with selectivity for the 5-HT2C subtype (Thomsen et al., 2008), significantly reduced weight relative to placebo (Smith et al., 2010). Lorcaserin (Belviq®) recently was approved by the U.S. Food and Drug Administration for treatment of obesity (Arena Pharmaceuticals, 2012). Thus, 5-HT2C receptor agonists may reduce feeding and symptoms of psychoses by acting on independent neural systems. Furthermore, 5-HT2C receptor agonists may show an improved safety profile in humans relative to existing antipsychotics. This is observed in the clinic with aripiprazole (Abilify®), which possesses 5-HT2C receptor partial agonism and is associated with less weight gain compared to other antipsychotics (Zhang et al., 2006; Leucht et al., 2013). Finally, because 5-HT2C receptors are expressed predominantly in the central nervous system (Molineaux et al., 1989), compounds that specifically target and activate 5-HT2C receptors should have limited impact in peripheral tissues, further decreasing the risk of side-effects.

One common problem with most existing, selective 5-HT2C agonists, including lorcaserin (above), is that they also activate 5-HT2A and 5-HT2B receptors at higher concentrations, which

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can lead to hallucinations (Glennon et al., 1984; Nichols, 2009) and cardiac valvulopathy, respectively (Rothman and Baumann, 2009), respectively. Herein are presented pharmacological and behavioral data obtained using a novel and potent 5-HT2C-specific agonist, (-)-MBP. (-)-MBP possesses high affinity at each of the 5-HT2 receptors radiolabeled with an antagonist, but high affinity at only 5-HT2C receptors when 5-HT2 receptors are radiolabeled with an agonist. With regard to 5-HT2-Gq mediated phosphoinositide hydrolysis signaling, (-)-MBP activates only the 5-HT2C receptor subtype, from both mouse and human cDNA. In addition, (-)-MBP behaves as a competitive antagonist of 5-HT at 5-HT2A and 5-HT2B receptors, and also as an inverse agonist at 5-HT2B receptors. *In vivo*, (-)-MBP displays anorexigenic and antipsychotic activity in mouse models, but does not alter locomotion. The data suggest a 5-HT2C receptor-*specific* agonist such as (-)-MBP may be pharmacotherapeutic for psychoses, without liability for obesity, hallucinations, heart disease, sedation or motoric disorders.

5. Materials and Methods

Compounds

The (+)-(2*R*, 4*S*)- and (-)-(2*S*, 4*R*)-*trans* enantiomers of 4-phenyl-3'-bromo-*N*,*N*-dimethyl-1,2,3,4-tetrahydronaphthalene-2-amine ((+)-MBP and (-)-MBP, respectively; Fig 1 built using Benchware® 3D Explorer 2.7, Tripos, USA) were synthesized in our laboratories as racemates that were resolved by a preparative chiral polysaccharide-based stationary-phase HPLC system and converted to hydrochloride salts as previously described (Booth et al., 2009; Vincek and Booth, 2009). 5-HT hydrochloride was purchased from Alfa Aesar (Ward Hill, MA). (+)-MK-801 hydrogen maleate (MK-801), *d*-amphetamine sulfate, clozapine hydrochloride, mianserin hydrochloride, and (±)-2,5-dimethoxy-4-iodoamphetamine hydrochloride (DOI) were purchased

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from Sigma-Aldrich (St. Louis, MO). Compounds were weighed with accuracy ± 0.001 mg on a microanalytical balance (model XP26, Mettler-Toledo, Columbus, OH). Solutions of all compounds used for behavioral assays were made fresh on the day of testing. [^{3}H]mesulergine, [^{3}H]ketanserin, [^{3}H]5-HT, and [^{3}H]myo-inositol at commercially-available specific activity were purchased from Perkin-Elmer (Waltham, MA).

In vitro pharmacology

a. Radioligand binding and phosphoinositide hydrolysis assays

Antagonist radioligand receptor binding assays were performed in 96-well plates based on procedures previously described (Canal et al., 2013). Briefly, HEK293 cells were transfected with 10 µg human 5-HT2A, 2B, or 2C-ini receptor cDNA or 10 µg mouse 5-HT2A or 5-HT2C-vnv receptor cDNA using Lipofectamine 2000 reagent (Invitrogen, USA), per manufacturer's instructions (mouse 5-HT2B cDNA was not procured). Cell membranes were collected 48 hr later. (+)- or (-)-MBP, at increasing concentrations from 0.1 nM to 10 µM, was used to compete for receptor orthosteric binding sites labeled with 1 nM [^{3}H]ketanserin (5-HT2A) or 2 nM [^{3}H]mesulergine (5-HT2B, 5-HT2C), and 10 µM mianserin was used to define the non-specific antagonist radioligand binding at all three 5-HT2 subtypes. Both enantiomers were tested for affinities at antagonist-labeled 5-HT2 subtypes. Only (-)-MBP was tested in agonist-labeled competition binding assays wherein [^{3}H]5-HT at 3.7 nM (calculated) was used to label the 5-HT2 subtypes. 5-HT at 10 µM was used to define non-specific agonist radioligand binding. The assay buffer for competition with [^{3}H]5-HT contained 50 mM Tris-HCl, 3 mM $CaCl_2$, 10 µM pargyline, and 0.1% ascorbic acid. After a 120 min equilibration period at room temperature, incubation mixtures were rapidly passed through GF/B filters using a Mach 2 cell harvester (Tomtec, Hamden, CT) and subsequently washed with 50 mM Tris–HCl. Filter disks were placed in vials

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containing 2 mL scintillation cocktail (ScintiVerse, Fisher) and counted for ^{3}H-induced scintillation using a Beckman-Coulter LS6500 counter (Indianapolis, IN).

5-HT2 receptor-mediated inositol phosphate hydrolysis assays to measure functional responses of (+)- and (-)-MBP and 5-HT (positive control agonist), were performed as previously described (Canal et al., 2013). Briefly, transiently transfected HEK293 cells were labeled with 1 μCi/mL [^{3}H]myo-inositol and seeded into 48-well plates. Cells were treated with test compounds for 30 min. The reaction was stopped by addition of 50 mM formic acid. Anion-exchange columns (Bio-Rad, Hercules, CA) were used to bind and collect [^{3}H]inositol phosphates. ^{3}H-induced scintillations then were measured. Competitive antagonism studies were performed with (-)-MBP only. In these studies, 0.1 - 10 μM (-)-MBP was used to compete with 0.0001 - 10 μM 5-HT for activation of 5-HT2A and 5-HT2B receptors. HEK293 cells transiently expressing each one of the 5-HT2 subtypes were treated with (-)-MBP and 5-HT simultaneously for 30 min prior to stopping the reaction, as noted above. Each binding and function experiment included triplicate measurements for each concentration of test compound, and each experiment was performed a minimum of three times.

b. Statistics

Binding data were analyzed using nonlinear regression, curve-fitting algorithms in GraphPad Prism version 6.00 for Microsoft Windows (San Diego, CA). Hill slopes were constrained to 1.0, consistent with the limited number of data points (Motulsky and Christopoulos, 2003). Ligand affinity is expressed as an approximation of K_i values by conversion of the IC_{50} data using the equation $K_i = IC_{50}/1+L/KD$ where L is the concentration of radioligand (Cheng and Prusoff, 1973). Data from phosphoinositide hydrolysis assays are presented as half-maximum (EC_{50}), half-minimum (IC_{50}), and maximum (E_{MAX}) values, representing potency and efficacy, as computed

using GraphPad nonlinear regression curve-fitting algorithms. Agonist efficacy is presented as percent of maximum 5-HT response. Inverse agonist efficacy is presented as percent of basal values (scintillation counts per minute).

In vivo behavioral pharmacology

a. Subjects

Male C57Bl/6 mice were obtained from Jackson (HTR and locomotion studies) or Harlan (food studies) Labs at ~8 weeks of age, and allowed to acclimate to the temperature (23˚C) and humidity controlled vivarium for at least 1 week prior to testing. The vivarium was illuminated 0700-1900. Mice were housed in pairs for HTR and locomotion studies and singly for food studies. Standard rodent pellets (Purina 5001) were available ad libitum, along with drinking water. Experiments were conducted at approximately the middle of the light phase. (+)- or (-)-MBP, clozapine, or DOI were dissolved in sterile 0.9% saline or MilliQ water. Clozapine was used as the comparative antipsychotic drug and positive control in all psychoses behavioral models. All compounds were administered systemically (intraperitoneal [ip] or subcutaneous [sc] injection) in a volume of 0.01-0.02 ml/g body weight. All behavioral procedures were approved by the University of Florida and Northeastern University Institutional Animal Care and Use Committee, and performed in accordance with the Guide for the Care and Use of Laboratory Animals.

b. DOI-elicited head-twitch response and locomotion

Experimentally-naïve mice were habituated to the testing room for approximately 30 minutes. Testing consisted of administration (sc) of MilliQ water (Veh), (+)- or (-)-MBP (3.0, 5.6, or 10.0

mg/kg) or clozapine (0.1 or 1.0 mg/kg) followed 10 minutes later by an injection of the 5-HT2 agonist DOI (1.0 mg/kg). Ten minutes later, mice were placed into a clear plexiglas open field chamber (43 x 43 cm, Med Associates, Inc.) for a 10-min observation period. During this session, head-twitch responses (HTRs), defined as a clear, rapid, and discrete, back and forth rotation of the head, were counted by a trained observer (D.M.) who was blind to drug treatment conditions. A camera videotaped the session, and activity (distance travelled in cm) was calculated by Ethovision software (Noldus Information Technology Inc.).

c. MK-801-elicited hyperlocomotion

Experimentally-naïve mice were habituated to the testing room for approximately 30 minutes. Locomotor activity testing consisted of administration (ip) of saline (Veh), clozapine (0.1 or 1.0 mg/kg) or (-)-MBP (3.0, 5.6, and 10.0 mg/kg), followed 10 minutes later by an injection of Veh or the NMDA antagonist MK-801 (0.3 mg/kg). Mice were immediately placed into one of four opaque plexiglas chambers (29.2 x 17.8 cm, 43.2 cm tall, Magnum Wood LLC, Gainesville, FL) for a 60-min session. An overhead camera videotaped the session, and activity (distance travelled in cm) was calculated by Ethovision software (Noldus Information Technology Inc.). To examine the time course of behavioral activity, (-)-MBP (10.0 mg/kg) was administered 10 min, 1 hr, or 3 hr prior to MK-801 (0.3 mg/kg) administration, and locomotion was assessed for 60 min thereafter.

d. Amphetamine-elicited hyperlocomotion

Experimentally-naïve mice were habituated to the testing room for approximately 30 minutes. Locomotor activity testing consisted of administration (ip) of saline (Veh), clozapine (0.1 and 1 mg/kg) or (-)-MBP (3.0, 5.6, and 10.0 mg/kg), followed 10 minutes later by an injection of Veh or

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the dopamine and norepinephrine transporter inhibitor and substrate, amphetamine (3.0 mg/kg). Locomotion was assessed exactly as noted in the MK-801 experiment. The effects of clozapine (1 mg/kg) or (-)-MBP (10 mg/kg) alone on locomotion were also tested during these experiments; timing of injections and behavioral testing remained consistent.

e. Palatable meal eating

Mice were adapted to eating a supplemental treat of Fruit Crunchies (Bio-Serv, Frenchtown, NJ), which are 190 mg pellets of purified materials that contain a similar macronutrient balance and caloric density (3.45 kcal/g) as chow. Mice were presented 10 Crunchies, including at least 3 each of each of the 3 flavors, in 10-ml glass jars suspended inside the cage via a metal stirrup. On the first day access was for 24 h, but thereafter daily access was rapidly tapered to 30 min, starting at about 1400 h. Crunchies were presented 5 days per week (Monday-Friday). After 30 min, uneaten Crunchies or halves were retrieved and the intake recorded. Each week, intakes on Tuesday through Thursday were used to compute a mean baseline for each mouse, and three groups were formed that were matched for this baseline. Friday was the test day on which animals were injected (ip) with (-)-MBP, (+)-MBP (6 or 12 mg/kg), or saline. Crunchies were presented 15 min later and intake was measured as before, expressed as a percentage of each individual's baseline for that week. Mice were tested repeatedly with different drugs and doses at one week intervals. Testing occurred during two weeks that were not consecutive.

f. Statistics

The dependent measures were analyzed by 1 or 2-way ANOVA with multiple comparisons (Newman-Keuls, Tukey's, Dunnett's test) or by unpaired 2-tailed T-tests, as appropriate, using commercially available statistical software (Sigmastat 3.1 and GraphPad Prism 6.00).

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Differences were considered statistically at $p<0.05$. ED_{50} values and 95% confidence limits were determined using log-linear interpolation from the descending limb of the dose-effect curves.

6. Results

In vitro pharmacology

Affinity (K_i) and function (EC_{50}, E_{MAX}, relative to 5-HT, and IC_{50}, I_{MAX}, relative to basal baseline) for (+)-MBP and (-)-MBP at each of the 5-HT2 receptors are shown in Table 1. At human 5-HT2A, 5-HT2B, and 5-HT2C receptors labeled with antagonist radioligand, (-)-MBP had 17-, 2-, and 18-fold higher affinity (K_i), respectively, in comparison to (+)-MBP. Similarly, at mouse 5-HT2A and 5-HT2C receptors labeled with antagonist radioligand, (-)-MBP had much higher affinity (20- and 88-fold, respectively) than (+)-MBP. At human 5-HT2 receptors labeled with agonist radioligand, (-)-MBP had much higher affinity for the 5-HT2C subtype, with greater than 8- and 20-fold binding selectivity for 5-HT2C over 5-HT2A and 5-HT2B receptors, respectively.

Results from functional assays (data summarized in Table 1) revealed that (-)-MBP exclusively activated human and mouse 5-HT2C receptors (Fig. 2). (-)-MBP agonist potency at human 5-HT2C receptors (EC50 = 19 nM) was 6-fold higher than its potency at mouse 5-HT2C receptors (EC50 = 115 nM), and in both cases, maximum efficacy was about 60% compared to 5-HT (Fig. 2). (-)-MBP did not activate human or mouse 5-HT2A receptors at concentrations up to 10 µM (Fig. 2), and was a competitive antagonist of 5-HT activation of human 5-HT2A receptors (Fig 3A), with a mean (± S.E.M.) K_b value of 441 (45) nM and pA_2 value of -2.64 (0.05). At human 5-HT2B receptors, (-)-MBP was an inverse agonist (Fig 3B), with a mean (± SEM) IC50 value of 112 (24) nM, and (-)-MBP also was a competitive antagonist of 5-HT activation of 5-HT2B receptors (not shown), with a mean (SEM) K_b value of 313 (118) nM and pA_2 value of 2.43

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(0.17). In contrast to the discriminating 5-HT2 functional pharmacology of (-)-MBP, (+)-MBP was a low potency, partial agonist at each of the human and mouse 5-HT2 receptor subtypes (Table 1). Accordingly, further molecular pharmacological characterization of (+)-MBP was not pursued, and (-)-MBP was designated the lead stereoisomer in light of its higher affinity and specific agonist activity at 5-HT2C receptors.

In vivo pharmacology

a. (-)-MBP reduces the DOI-elicited head-twitch response without altering locomotion

Administration of DOI (1.0 mg/kg) (preceded by a vehicle injection) resulted in 37.1 (± 1.4) HTRs during the 10-min session (Fig 4). All doses of both enantiomers of MBP attenuated this response ($F_{6,30}$=28.1; $P < 0.0001$). This effect was dose-dependent ($F_{2,24}$=11.69; $P < 0.0001$) with the (-) and (+) enantiomers reducing the number of DOI-elicited HTRs by 86% and 55%, respectively, at doses of 10.0 mg/kg (Fig 4). (-)-MBP was more potent ($F_{1,24}$=26.1; $P < 0.0001$), and had an ED_{50} (± 95% C.I.) value of 2.67 (1.69-4.20) mg/kg compared to 8.80 (5.26-14.73) mg/kg for (+)-MBP. Clozapine also dose dependently blocked the DOI-elicited HTR ($P < 0.05$) (Fig 4). A linear regression analysis of data from Fig 4A showed that the slopes of the lines from each group were statistically different ($F_{2,52}$ = 28.7; $P < 0.0001$); rank order of potency was clozapine > (-)-MBP > (+)-MBP. During HTR sessions, locomotor activity was recorded. There was no combination of DOI and (-)-MBP doses that resulted in activity levels different from vehicle or DOI administration. In contrast, (+)-MBP at 10 mg/kg, in combination with DOI (1.0 mg/kg) resulted in decreased activity relative to vehicle plus DOI ($P < 0.05$), but not vehicle alone. Conversely, clozapine at 1 mg/kg alone (see below), or, in combination with DOI (1.0 mg/kg), significantly decreased locomotion relative to vehicle (mean difference, 1254 cm (95%

C.I. 50 to 2458), $P < 0.05$) and DOI alone (mean difference, 1742 cm (95% C.I. 857 to 2627), $P < 0.05$) (Fig 4, inset).

b. (-)-MBP reduces MK-801-elicited hyperlocomotion, an effect lasting at least 2 hrs

MK-801 (0.3 mg/kg) administration resulted in increased levels of activity relative to vehicle administration (Fig 5) that persisted for at least 60 min (Fig 5 inset, Fig 6). (-)-MBP dose-dependently decreased MK-801 hyperlocomotion that was significant at 5.6 mg/kg (mean difference, 8654 cm (95% C.I. 352 to 16955), $P < 0.05$) and 10 mg/kg (mean difference, 14872 cm (95% C.I. 6571 to 23174), $P < 0.005$). The attenuation of MK-801-elicited activity was apparent throughout the entire 60-min session ($F_{236, 2242}=4.43$; $P < 0.0001$). The results with (-)-MBP were similar to clozapine, which also dose-dependently reduced MK-801-elicited hyperlocomotion (Fig 5). To examine the time course of behavioral activity, (-)-MBP (10.0 mg/kg) was administered 10 min, 1 hr, or 3 hr prior to MK-801 administration, and locomotor activity was assessed for 60 min thereafter (Fig 6). At a 10 min pretreatment time, there was a complete attenuation of MK-801's effects in which activity levels decreased from ~25,000 cm to ~10,000 cm (Fig 6; mean difference, 15533 cm (95% C.I. 7805 to 23261), $P < 0.005$). When (-)-MBP was administered at a 1 hr pretreatment time (thus assessing behavioral activity from hrs 1 to 2), attenuation of the MK-801 behavioral effects was still apparent throughout most of the session (mean difference, 9540 cm (95% C.I. 1813 to 17268), $P < 0.05$). When administered 3 hours before the session, (-)-MBP had little effect on MK-801 elicited hyperactivity. (+)-MBP was not tested in this the MK-801 assay nor in the amphetamine-induced hyperactivity assay (below), owing to its relatively poor activity in the DOI-elicited-HTR model, that putatively reflects its low affinity and partial agonist functional activity at 5-HT2A and 5-HT2C receptors (Table 1).

c. (-)-MBP reduces amphetamine-elicited hyperlocomotion but does not alter locomotion when administered alone

Amphetamine (3.0 mg/kg) administration resulted in significantly increased levels of activity relative to saline administration that lasted for at least 60 min (Fig 7, $P < 0.001$). (-)-MBP at 10 mg/kg significantly decreased amphetamine-induced hyperactivity (mean difference, 11506 cm (95% C.I. 4071 to 18942), $P < 0.001$). The attenuation of amphetamine-elicited activity was apparent throughout the entire 60-min session ($F_{236,2832}=3.46$; $P < 0.0001$). Also, clozapine at 1 mg/kg (mean difference, 17187 cm (95% C.I. 8874 to 25500), $P < 0.001$), but not 0.1 mg/kg significantly decreased amphetamine-elicited hyperactivity (Fig 7). Clozapine also significantly reduced locomotor activity when administered alone ($P < 0.005$), but even the highest dose of (-)-MBP (10 mg/kg) did not significantly alter locomotor activity when administered alone ($P = 0.14$). (+)-MBP was not tested in this assay, owing to its relatively poor activity in the DOI-elicited-HTR model.

d. (-)-MBP reduces palatable food eating

Both (-)-MBP and (+)-MBP produced a dose-related suppression of intake of Crunchies. (-)-MBP was significantly more potent and efficacious than (+)-MBP, similar to the effects seen in the DOI-elicited HTR tests. The main effect of dose was significant ($P < 0.001$), the difference between (-)- and (+)-MBP was marginally significant ($P = 0.054$), and the dose x drug interaction was not significant. At 6 and 12 mg/kg, (-)-MBP reduced feeding to a mean (± SEM) of 59.7 (6.3) and 35.8 (7.5) percent, respectively, below the vehicle treated group (both doses, $P < 0.05$). At 6 and 12 mg/kg, (+)-MBP reduced feeding to a mean (± SEM) of 82.9 (8.0) and 55.1 (6.4) percent, respectively, below the vehicle treated group (6 mg/kg, not significant, 12 mg/kg,

$P < 0.05$). From linear regressions (r^2=0.59, 0.69, $P < 0.01$), the estimated ED_{50} values for (-)-MBP and (+)-MBP were 9.6 mg/kg and 13.6 mg/kg, respectively.

7. Discussion

Drugs that activate 5-HT2C receptors hold promise for the treatment of psychoses and psychostimulant abuse, in part, because of their ability to modulate central dopamine signaling, and due to their effectiveness in preclinical models and at least one clinical study (Di Matteo et al., 2004; Shen et al., 2010; Higgins et al., 2012; Cunningham et al., 2013). Herein is described a novel and potent 5-HT2C receptor-specific agonist with 5-HT2A and 5-HT2B competitive antagonist and inverse agonist properties, (-)-MBP, that is effective in preclinical mouse models of psychoses, does not affect locomotion on its own, and reduces palatable food intake, important properties distinguishing it from available antipsychotics that suppress locomotion and increase appetite, leading to obesity (Stip et al., 2012). In the present studies, (-)-MBP was compared directly with its enantiomer, (+)-MBP, that has identical physiochemical properties, but with a mirror image 3-dimensional arrangement of atoms, to provide molecular support of successful 5-HT2 receptor-mediated translation from cellular to behavioral potency and efficacy. Relative to (+)-MBP, (-)-MBP showed considerably higher affinity at each of the [^{3}H]antagonist-labeled 5-HT2 receptor subtypes *in vitro* that paralleled its significantly enhanced behavioral potency and efficacy *in vivo* in the DOI-elicited HTR assay. Moreover, molecular determinants for function were found to differ between 5-HT2 subtypes, as (-)-MPB activated 5-HT2C receptors exclusively, whereas, (+)-MBP activated 5-HT2A and 5-HT2B, as well as, 5-HT2C receptors. The affinity of (-)-MBP at [^{3}H]agonist-labeled human 5-HT2C receptors (9 nM K_i) was more than 9- and 20-fold higher than its affinity at [^{3}H]agonist-labeled 5-HT2A and 5-HT2B receptors, respectively, providing evidence that (-)-MBP selectively stabilizes a high affinity agonist conformation of the 5-HT2C receptor, but not of the 5-HT2A or 5-HT2B receptor. Thus,

a molecular basis for 5-HT2C-specific activation was established despite the relatively high (~75%) transmembrane sequence homology between 5-HT2 subtypes; the risks associated with 5-HT2A and/or 5-HT2B receptor activation can and should be avoided with regard to 5-HT2C-activating drugs.

(-)-MBP was a competitive antagonist of 5-HT activation of human 5-HT2A and 5-HT2B signaling and did not activate either receptor, even at 10 μM which is 50- to 800-fold higher than its 5-HT2A/2B affinity values, depending on whether an agonist or antagonist is used to label the receptors. Importantly, (-)-MBP was an inverse agonist at human 5-HT2B receptors, prospectively eliminating the possibility of 5-HT2B-mediated cardiac valvulopathy. Inverse agonism, however, was not observed consistently at human 5-HT2A receptors, suggesting (-)-MBP may be a 5-HT2A neutral antagonist. In summary, (-)-MBP is a potent 5-HT2C receptor-specific partial agonist that does not activate 5-HT2A or 5-HT2B receptors, setting it apart from all other reported selective 5-HT2C agonists, including the novel anti-obesity drug, Belviq®, the widely used research agonist, Ro 60-0175, and the prototypical agonist, mCPP, all of which also activate 5-HT2A and 5-HT2B receptors.

(-)-MBP was effective in several preclinical animal models of psychoses, including a model of 5-HT2-mediated hallucinations (DOI-elicited HTR), a model of dopamine hyperactivity (amphetamine-elicited hyperlocomotion) and a model of glutamate hypofunction (MK-801-elicited hyperlocomotion). Each of the targeted neurotransmitter systems associated with the animal models, i.e. 5-HT2A receptors, dopamine and norepinephrine transporter, and glutamate NMDA receptors, respectively, has been implicated in psychoses and schizophrenia, and drugs within each of these classes can mimic psychosis in humans (Aghajanian and Marek, 2000; Gonzalez-Maeso and Sealfon, 2009; Coyle et al., 2012; Masana et al., 2012), providing the models with some etiological validity. In these animal models, (-)-MBP was compared directly to

the prototypical second-generation antipsychotic drug clozapine that previously was reported to attenuate DOI-elicited HTR, and amphetamine and NMDA antagonist-induced hyperlocomotion (Corbett et al., 1995; Gleason and Shannon, 1997; Rojas-Corrales et al., 2007). (-)-MBP demonstrated similar efficacy as clozapine, although, was less potent. Importantly, in contrast to clozapine, (-)-MBP did not compromise locomotion when administered alone, suggesting promise as an antipsychotic drug without liability for motoric disorders. We acknowledge, however, that the aforementioned behavioral models are likely permissive and could lead to false positive results, e.g. compounds effective in these models may fail to ameliorate psychotic symptoms in humans, indicative that improved animal models for the core symptoms of schizophrenia are necessary (Brown et al., 2013).

All other reported 5-HT2C agonists that are effective as antipsychotics, either in preclinical animal models or in clinical trials, also have 5-HT2A and/or 5-HT2B receptor agonist properties (Dunlop et al., 2005; Marquis et al., 2007; Siuciak et al., 2007; Rosenzweig-Lipson et al., 2012), raising the possibility that their therapeutic effects could be due to some combination of 5-HT2 subtype activation. We are not aware, however, of any studies documenting antipsychotic activity of lorcaserin, the only FDA-approved 5-HT2C agonist that also activates 5-HT2A and 5-HT2B receptors (Thomsen et al., 2008). Meanwhile, (-)-MBP does not activate 5-HT2A or 5-HT2B receptors, which were expressed at relatively high densities in the transiently transfected HEK cells here and elsewhere (Booth et al., 2009), thus, the efficacy of (-)-MBP demonstrated in the rodent models of psychoses supports the assertion that 5-HT2C receptor activation alone or in combination with 5-HT2A and/or 5-HT2B antagonism or inverse agonism may negatively modulate psychotic behaviors. Finally, the results that (-)-MBP directly, negatively modulates DOI-, amphetamine-, and MK-801-induced behaviors suggest that 5-HT2C agonism together with 5-HT2A/5-HT2B antagonism/inverse agonism may translate to optimal 5-HT2-based pharmacotherapy for behaviors associated with substance abuse (Cunningham et al., 2013).

Importantly, (-)-MBP did not alter locomotion when administered alone, or in combination with DOI, MK-801, or amphetamine, at behaviorally active doses (up to 10 mg/kg), indicating that its modulation of MK-801 and amphetamine-induced locomotion were not due to primary motor deficits. This effect has been noted for related trans-4-phenyl-2-dimethylaminotetralins (Canal et al., 2013; Morgan et al., 2013). In contrast, clozapine substantially decreased locomotion below levels of vehicle treated animals when administered alone or in combination with DOI, mirroring its sedative effects in humans, a side-effect that may translate to the oft-reported “empty-headed” sensation caused by available antipsychotics (Moritz et al., 2013). Interestingly, a recent paper reports that the hypolocomotion effect of clozapine, which is a 5-HT2A receptor inverse agonist of the canonical 5-HT2-Gq signaling pathway (Vanover et al., 2004), is mediated by 5-HT2A receptors (Williams et al., 2012). The affinity of clozapine and (-)-MBP at rodent 5-HT2A receptors is very similar suggesting that the inverse agonist effects of clozapine and neutral antagonist effects of (-)-MBP at 5-HT2A receptors may translate to different behavioral outcomes, or the compounds are functionally-selective regarding 5-HT2A signaling that impacts locomotion. Alternatively, there may be an as yet undiscovered target(s) of (-)-MBP that counterweighs the hypolocomotion effect usually demonstrated by 5-HT2A antagonists/inverse agonists.

Also interesting regarding the lack of locomotor effects by (-)-MBP is that most 5-HT2C receptor agonists decrease locomotion in rodents (Fletcher et al., 2009; Halberstadt et al., 2009; Canal et al., 2013). Several reports show that 5-HT2C receptor-targeting compounds modulate the release of central dopamine, with agonists decreasing and antagonists or inverse agonists increasing dopamine release in an apparently neural system-dependent manner (Di Giovanni et al., 2011). The lack of effect on locomotor behavior with (-)-MBP may be due to partial agonism of 5-HT2C receptors, which may dampen, for example, amphetamine-stimulated dopamine

release, but not cause a reduction in dopamine levels on its own. This phenomenon has been described with reference to dopamine D2 partial agonists, including the antipsychotic drug aripiprazole (Strange, 2008). Collectively, results indicate that (-)-MBP may selectively modulate psychostimulant-induced behaviors, but not motor activity or vigilance, and therefore may translate to a drug that lacks sedative effects. Furthermore, the lack of effect of (-)-MBP on locomotion in rodents suggests it may treat psychoses without causing extra-pyramidal side-effects or catalepsy.

Other serious side-effects of especially second-generation antipsychotic drugs include metabolic syndrome, specifically, high glucose and cholesterol (Pramyothin and Khaodhiar, 2010), as well as increased appetite, and weight gain leading to obesity (Stip et al., 2012). (-)-MBP, in contrast, suppressed feeding in a mouse model of compulsive binge-eating/snack-food intake, suggestive of 5-HT2C agonism, which is known to decrease feeding and reduce weight in rodents and humans (Smith et al., 2010).

In summary, the novel 5-HT2C receptor-specific partial agonist (-)-MBP displayed clear, favorable activity in animal models predictive of neuropsychiatric symptomology, without possessing deleterious side-effects associated with administration of currently available antipsychotic medications, including alterations in motor activity and increased appetite. These results support further development of (-)-MPB and other drug candidates with similar 5-HT2C agonism together with 5-HT2A/2B antagonism/inverse agonism for the treatment of psychoses and compulsive behavioral disorders involving substance (including food) abuse and addiction.

8. Acknowledgments

The authors acknowledge Dr. Yue Liu (Northeastern University Center for Drug Discovery) for performing radioligand binding assays to provide corroborative K_i values.

9. Authorship Contributions

Participated in research design: Canal, C.E., Morgan, D., Rowland, N., Robertson, K., Sakhuja, R., Booth, R.G.

Conducted experiments: Canal, C.E., Morgan, D., Felsing, D., Kondabolu, K., Robertson, K., Sakhuja, R.

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Wrote or contributed to the writing of the manuscript: Canal, C.E., Morgan, D., Booth, R.G.

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11. Footnotes

a) This work was supported by grants from the National Institute of Mental Health [RO1MH081193] and National Institute on Drug Abuse [RO1DA023928, RO1DA030989] awarded to R.G.B. and D.M.

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12. Figure Legends

Fig 1. Structures of (+)-MBP and (-)-MBP

Fig 2. Representative functional responses of (-)-MBP at human 5-HT2A, 5-HT2B, and 5-HT2C receptors compared to 5-HT

Fig 3. A. Representative competitive antagonism results of (-)-MBP at human 5-HT2A receptors. B. Representative inverse agonist results of (-)-MBP at human 5-HT2B receptors.

Fig. 4. A. Both enantiomers of (-)-MBP dose-dependently attenuated the DOI-elicited-HTR. (-)-MBP was more potent and efficacious than (+)-MBP, consistent with *in vitro* pharmacology data. Clozapine (CLOZ) also dose-dependently blocked the DOI-elicited-HTR. Each data point represents the mean (±SEM) of 5-7 subjects. All drug groups are significantly different from the DOI only group (Veh). B. Pretreatment with (-)-MBP did not affect locomotion, but CLOZ and (+)-MBP significantly decreased locomotion relative to DOI (1 mg/kg). CLOZ also reduced locomotion compared to the vehicle (Veh) only treated group; numbers on x-axis refer to mg/kg dose. Bar graphs of locomotion (mean ± SEM) are from representative groups shown in A. *significantly different from DOI; $^{\#}$significantly different from Veh.

Fig 5. (-)-MBP dose-dependently attenuated MK-801-elicited hyperactivity, similar to clozapine (CLOZ). Effects are shown for the total 60-min session (bar graphs), and numbers on x-axis refer to dose in mg/kg. Bar graphs represent the mean (±SEM) of 6 (CLOZ groups)-10 subjects. *Indicates significantly different from MK-801 alone. Inset: Effects are plotted in 1-min bins for the primary comparisons. Error bars in inset are excluded for clarity.

Fig 6. Time course analysis of (-)-MBP (10 mg/kg). (-)-MBP administered 10 or 60 min before MK-801 significantly reduced MK-801-elicited hyperactivity. Effects are shown for the total 60-min session (bar graphs). Bar graphs represent the mean (±SEM) of 11 (Veh) and 6 (for each of the remaining groups) subjects. *Indicates significantly lower activity relative to MK-801 alone. Inset: Effects are plotted in 1-min bins for the primary comparisons. Error bars in inset are excluded for clarity.

Fig 7. (-)-MBP attenuated amphetamine (AMP)-elicited increases in locomotion, similar to clozapine (CLOZ), but with less potency (groups right of dashed line). Note, however, that CLOZ (1 mg/kg) alone significantly decreased locomotion, but (-)-MBP (10 mg/kg) alone did not alter locomotion, relative to vehicle (Veh) (groups left of dashed line). Effects are shown for the total 60-min session (bar graphs), and numbers on x-axis refer to dose in mg/kg. Bar graphs represent the mean (±SEM) of 6 (CLOZ groups)-9 subjects. *Indicates combination of CLOZ or (-)-MBP with AMP was significantly lower relative to AMP alone. #indicates significantly different from Veh group. Inset: Effects are plotted in 1-min bins for the primary comparisons. Error bars in inset are excluded for clarity.

Fig 8. Both (+) and (-)-MBP decreased consumption of "Crunchies", a highly palatable treat in non-food-deprived mice. Bar graphs represent the mean (±SEM) of 8 subjects. Both doses of (-)-MBP (outlined, red bars), and the highest dose of the (+)-MBP (gray bars) decreased consumption. *Indicates significantly lower levels of consumption relative to vehicle administration.

13. Table Legends

Table 1. Pharmacology of (+) and (-)-MBP at human (h) and mouse (m) 5-HT2 receptors. K_i values (nM) were determined by displacement of [3H]5-HT (*Agonist Labeled*) or [3H]ketanserin (5-HT2A) or [3H]mesulergine (5-HT2B, 5-HT2C) (*Antagonist Labeled*). *Function* values were determined by an inositol phosphate hydrolysis assay, measuring 5-HT2-mediated activation of phospholipase C. The pA_2 value was determined from competitive antagonism functional assays with 5-HT. For *Efficacy*, [a] shows percent below basal signaling (inverse agonism), and [b] shows percent of maximal 5-HT response (agonism). All data were from HEK cells transiently expressing one of the three 5-HT2 receptors. Data represent the mean (± SEM) from at least 3 independent experiments. [*]h5-HT2C = human 5-HT2C-ini isoform; m5-HT2C = mouse 5-HT2C-vnv isoform.

JPET Fast Forward. Published on February 21, 2014 as DOI: 10.1124/jpet.113.212373
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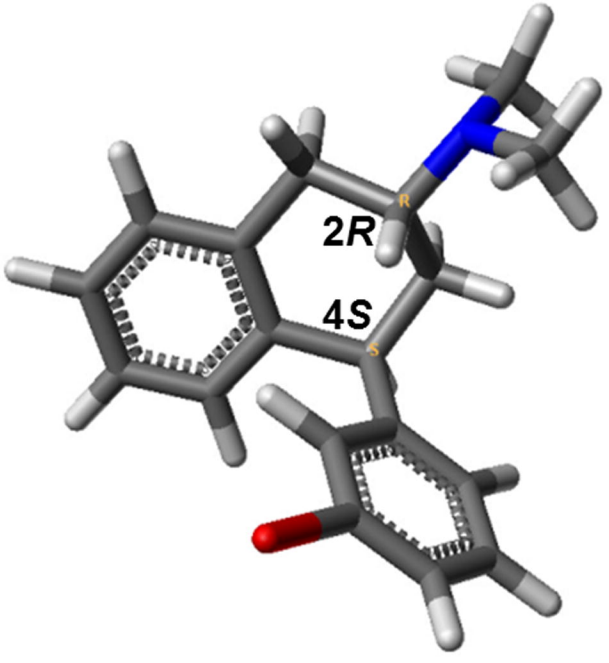
Table 1.

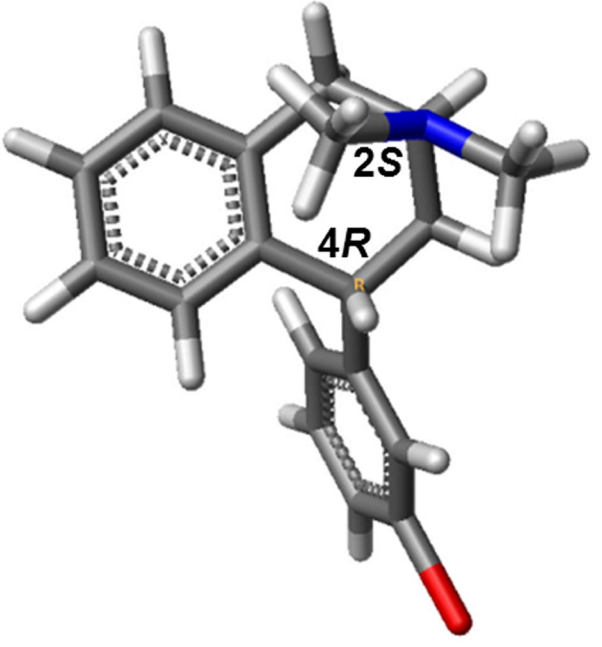
TABLE 1

Compound	*In vitro* Pharmacology	*h5-HT2A*	*h5-HT2B*	*h5-HT2C**	*m5-HT2A*	*m5-HT2C**
(-)-MBP	K_i antagonist-labeled	20 (4.5)	13 (5.2)	12 (2.8)	26 (2.3)	11 (2.5)
	K_i agonist-labeled	77 (14)	199 (35)	9.1 (0.5)	Not tested	Not tested
	Function	pA_2 = -2.64 (0.05)	IC_{50} = 112 (24)	EC_{50} = 19 (3)	No activation @ 10 μM	EC_{50} = 115 (4)
	Efficacy (%)	No activation @ 10 μM	69 (5)[a] (inverse agonist)	63 (13)[b] (agonist)	No activation @ 10 μM	60 (1)[b] (agonist)
(+)-MBP	K_i antagonist-labeled	332 (42.1)	31 (7.1)	200 (24.1)	534 (63.0)	969 (77.5)
	Function	EC_{50} >1000	EC_{50} >1000	EC_{50} >1000	EC_{50} >1000	EC_{50} = 122 (9.0)
	Efficacy (%)	34 (4)[b] (agonist)	42 (6)[b] (agonist)	81 (8)[b] (agonist)	30 (5)[b] (agonist)	57 (10)[b] (agonist)

Fig. 1



(+)-MBP



(-)-MBP

Fig. 2.

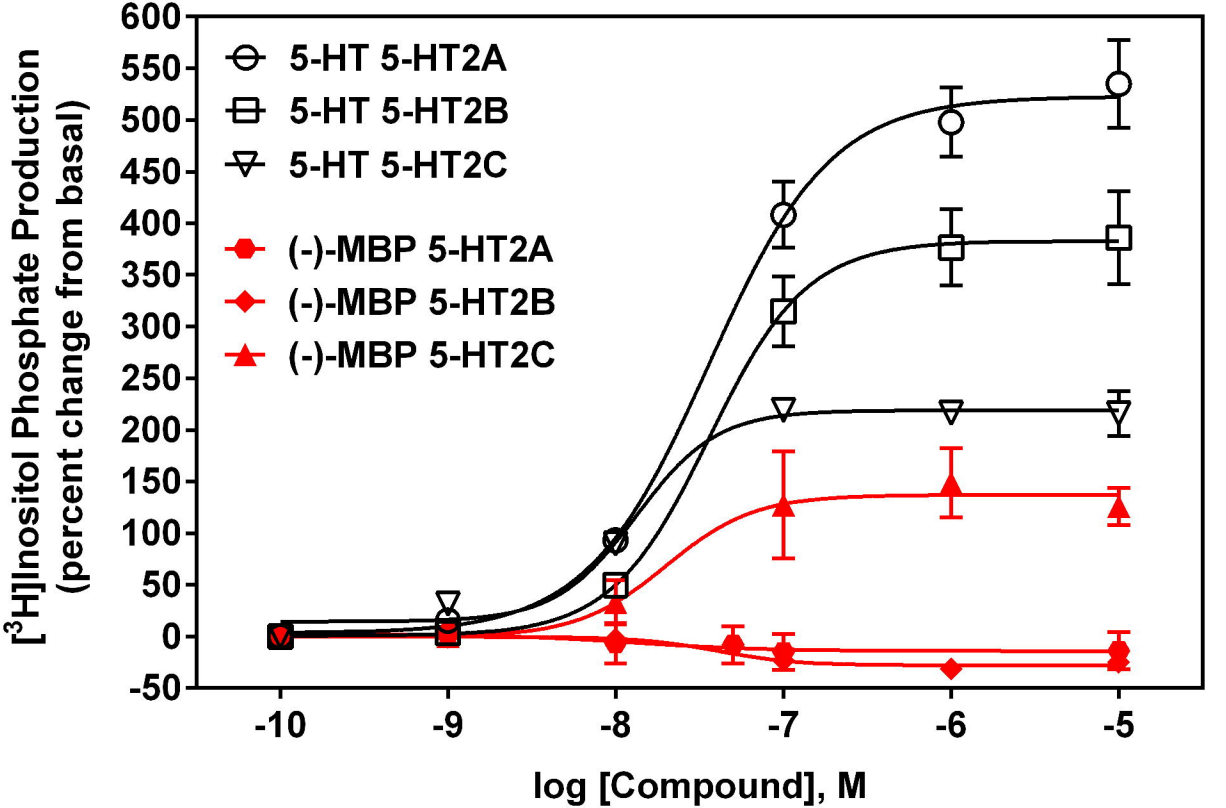
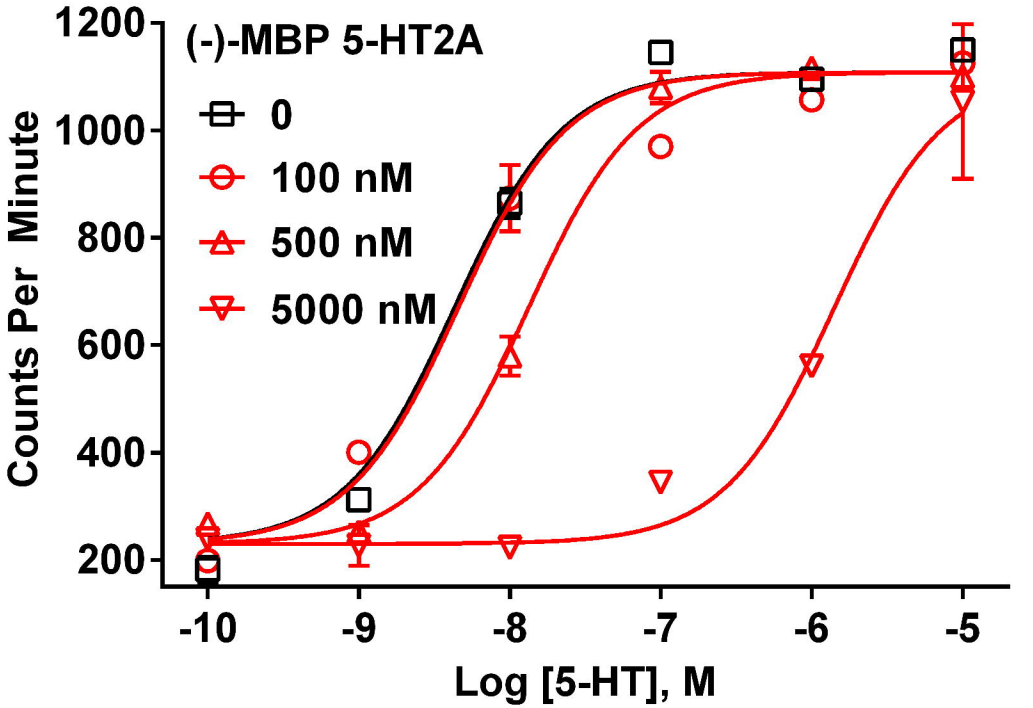

600
550
500
450
400
350
300
250
200
150
100
50
0
-50
[3H]Inositol Phosphate Production
(percent change from basal)
5-HT 5-HT2A
5-HT 5-HT2B
5-HT 5-HT2C
(-)-MBP 5-HT2A
(-)-MBP 5-HT2B
(-)-MBP 5-HT2C
-10
-9
-8
-7
-6
-5
log [Compound], M

Fig. 3. A.


(-)-MBP 5-HT2A
0
100 nM
500 nM
5000 nM
Counts Per Minute
1200
1000
800
600
400
200
-10
-9
-8
-7
-6
-5
Log [5-HT], M

B.

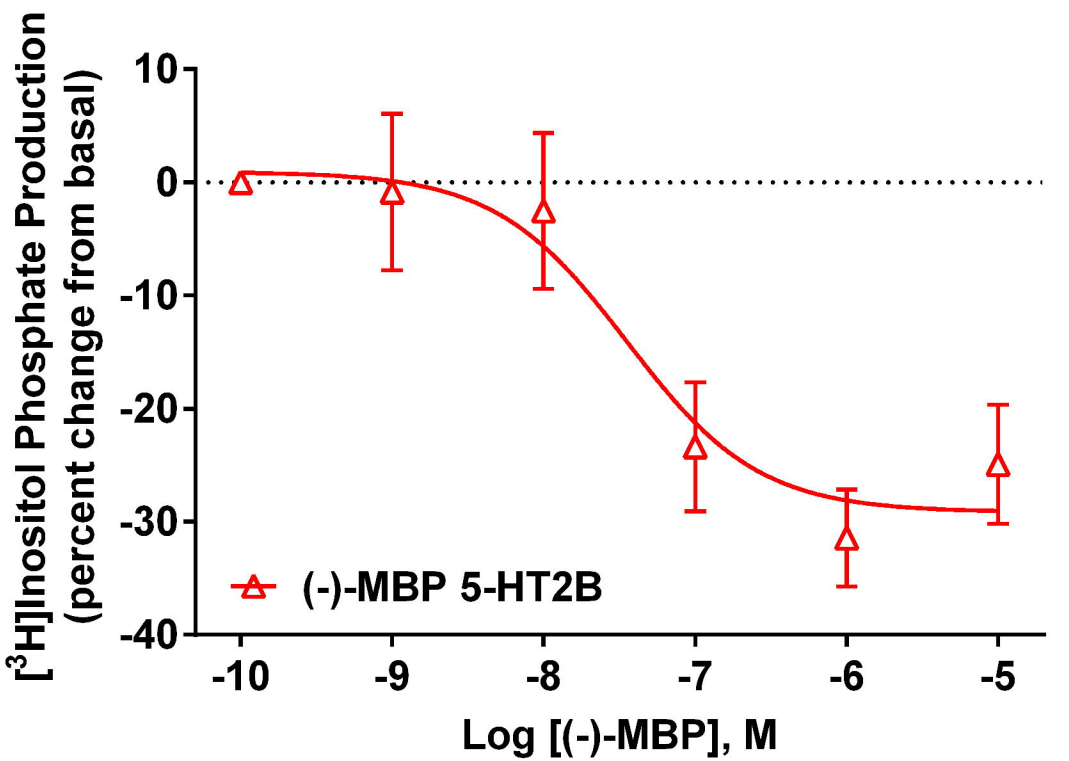
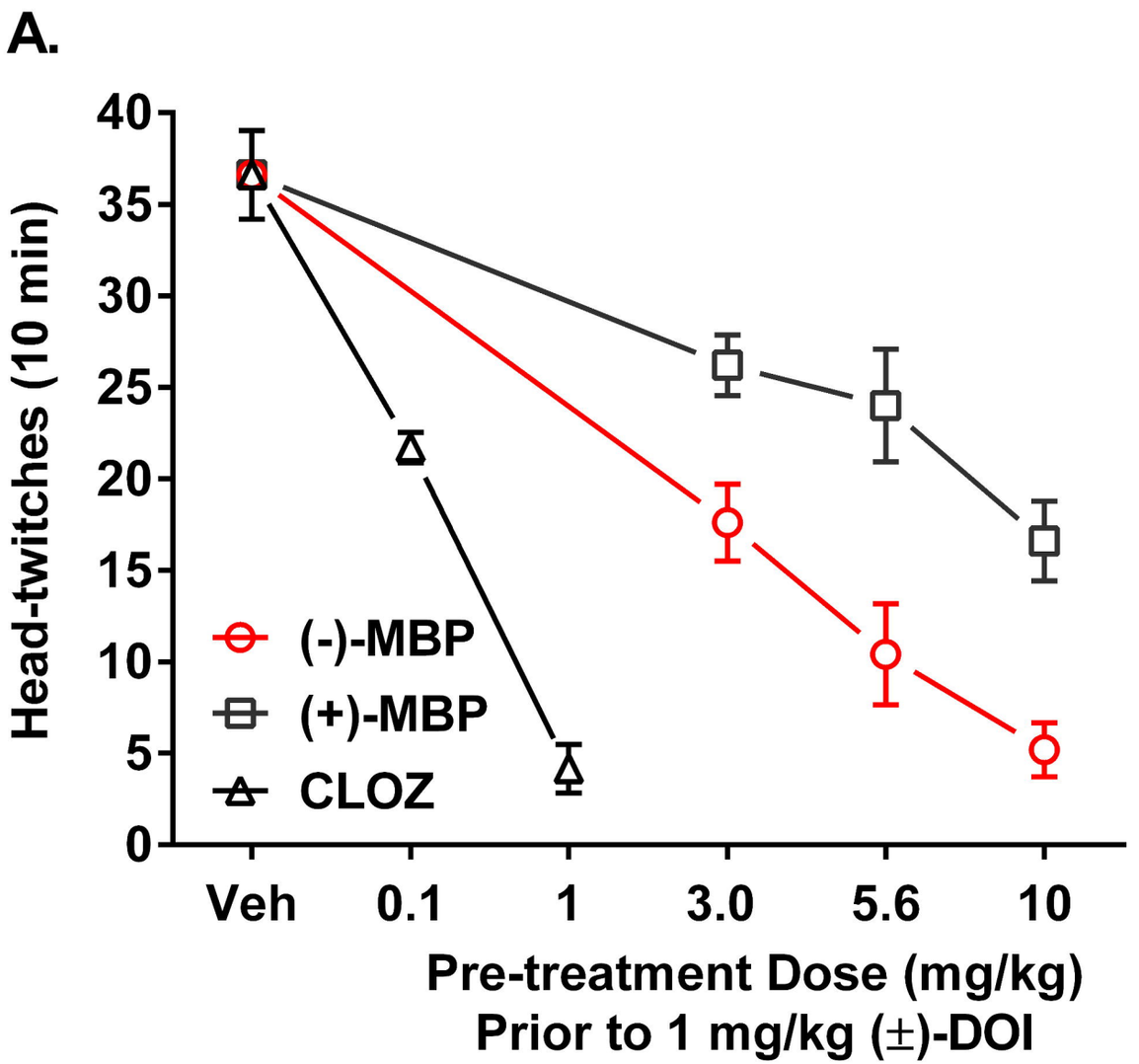

[3H]Inositol Phosphate Production
(percent change from basal)
10
0
-10
-20
-30
-40
(-)-MBP 5-HT2B
-10
-9
-8
-7
-6
-5
Log [(-)-MBP], M

Fig. 4.



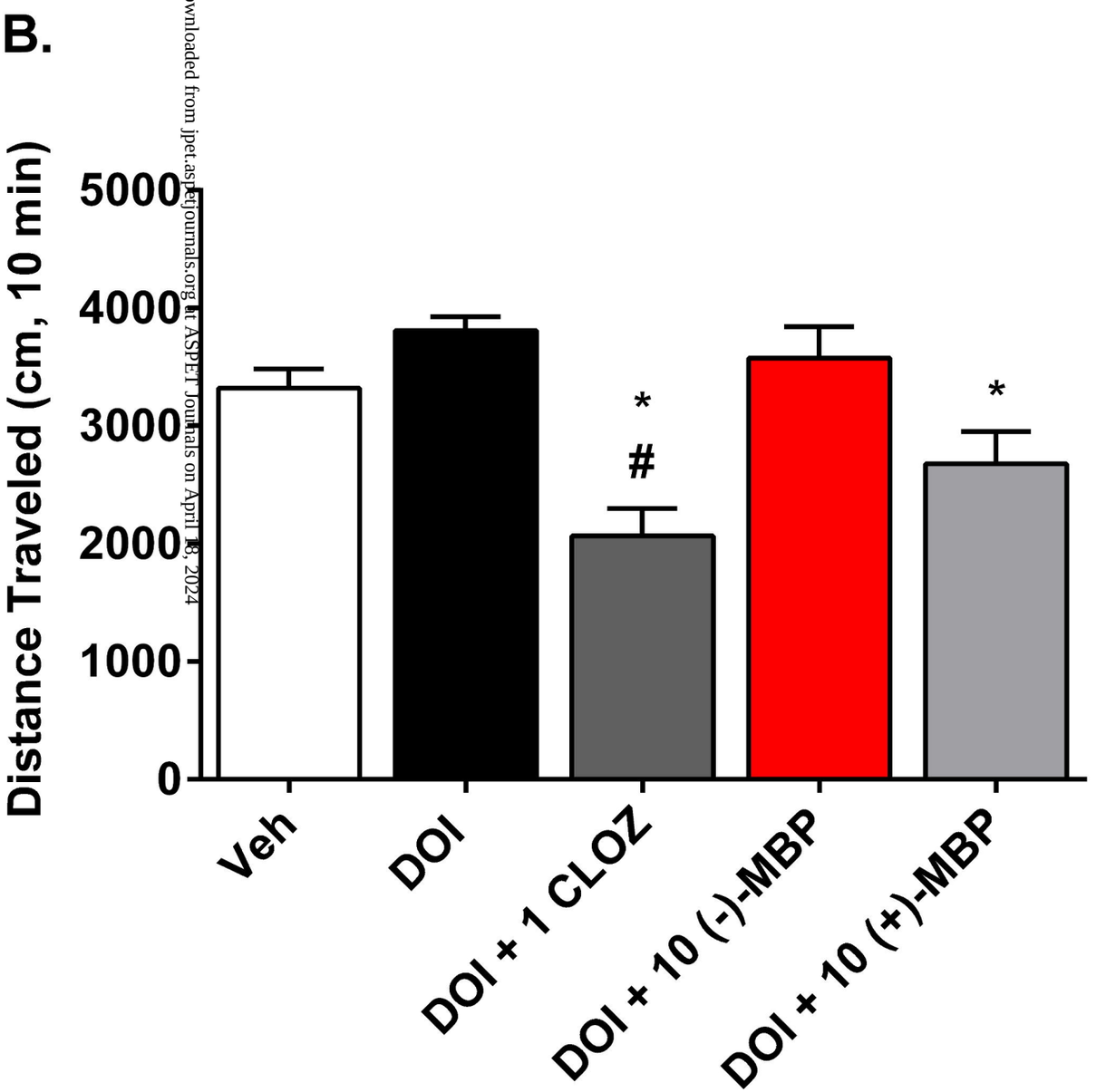


Fig. 5.

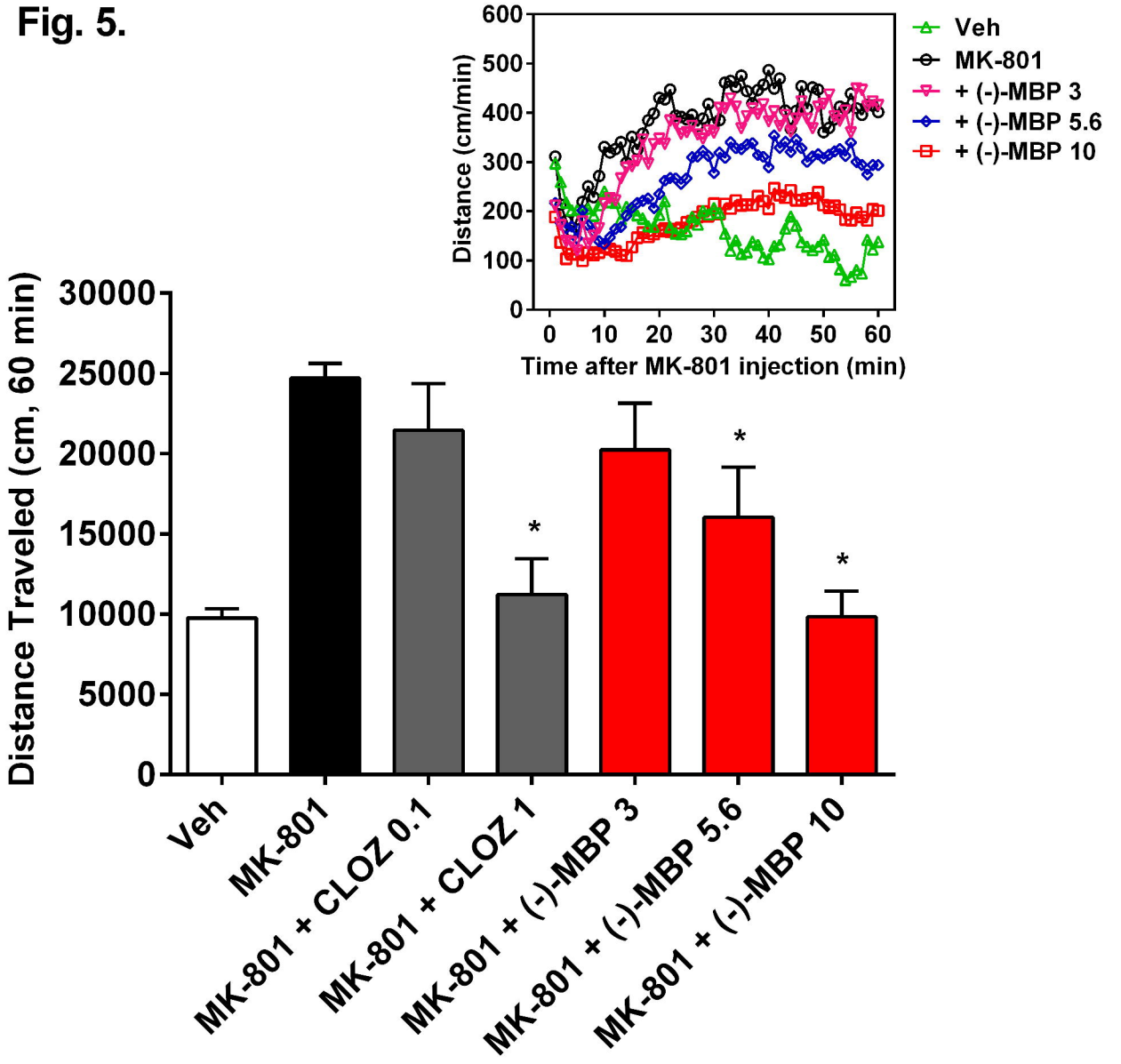

Distance (cm/min)
600
500
400
300
200
100
0
0
10
20
30
40
50
60
Time after MK-801 injection (min)
Veh
MK-801
+ (-)-MBP 3
+ (-)-MBP 5.6
+ (-)-MBP 10
Distance Traveled (cm, 60 min)
30000
25000
20000
15000
10000
5000
0
*
*
*
Veh
MK-801
MK-801 + CLOZ 0.1
MK-801 + CLOZ 1
MK-801 + (-)-MBP 3
MK-801 + (-)-MBP 5.6
MK-801 + (-)-MBP 10

Fig. 6.

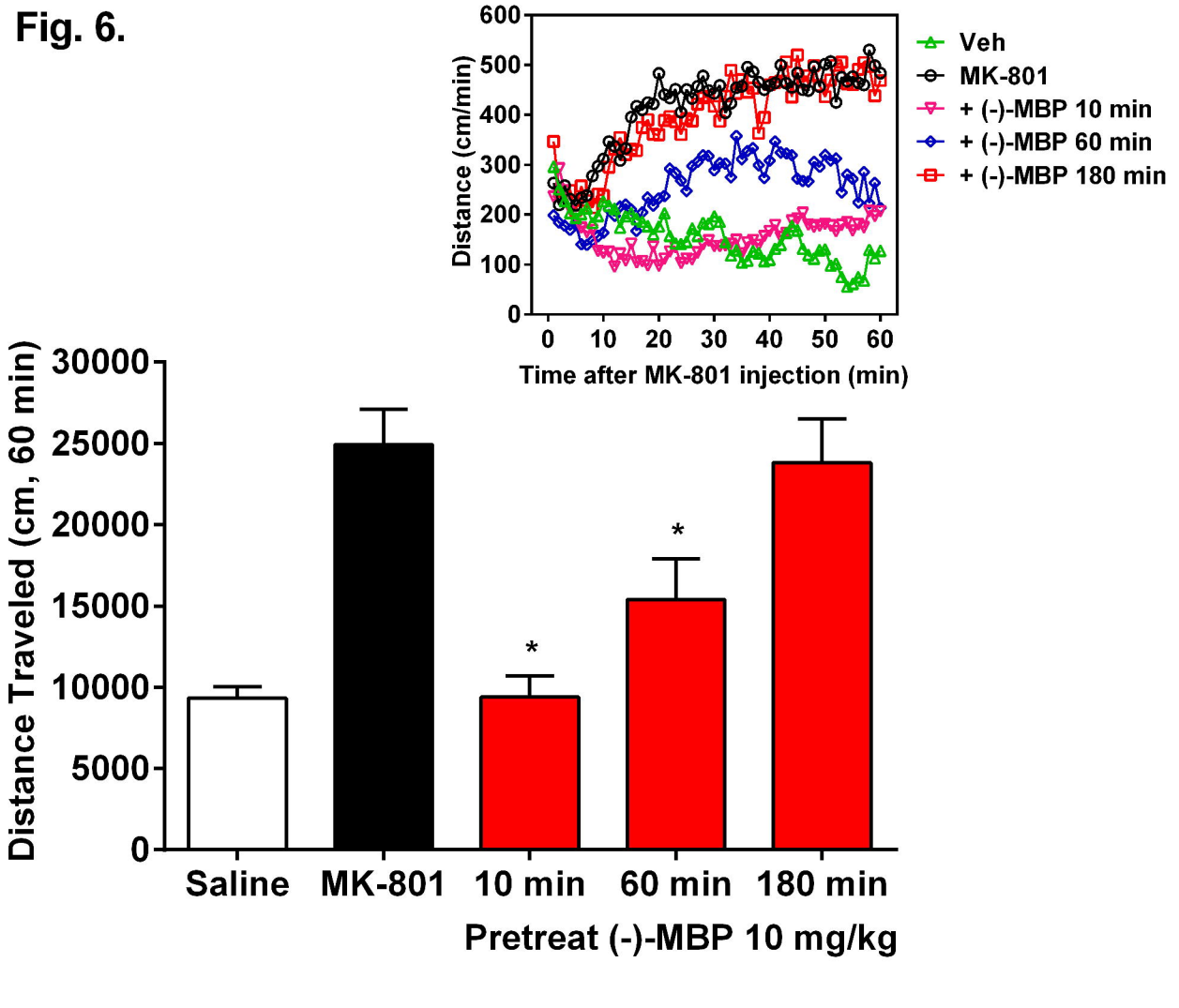

Distance (cm/min)
600
500
400
300
200
100
0
0
10
20
30
40
50
60
Time after MK-801 injection (min)
Veh
MK-801
+ (-)-MBP 10 min
+ (-)-MBP 60 min
+ (-)-MBP 180 min
Distance Traveled (cm, 60 min)
30000
25000
20000
15000
10000
5000
0
*
*
Saline
MK-801
10 min
60 min
180 min
Pretreat (-)-MBP 10 mg/kg

Fig. 7.

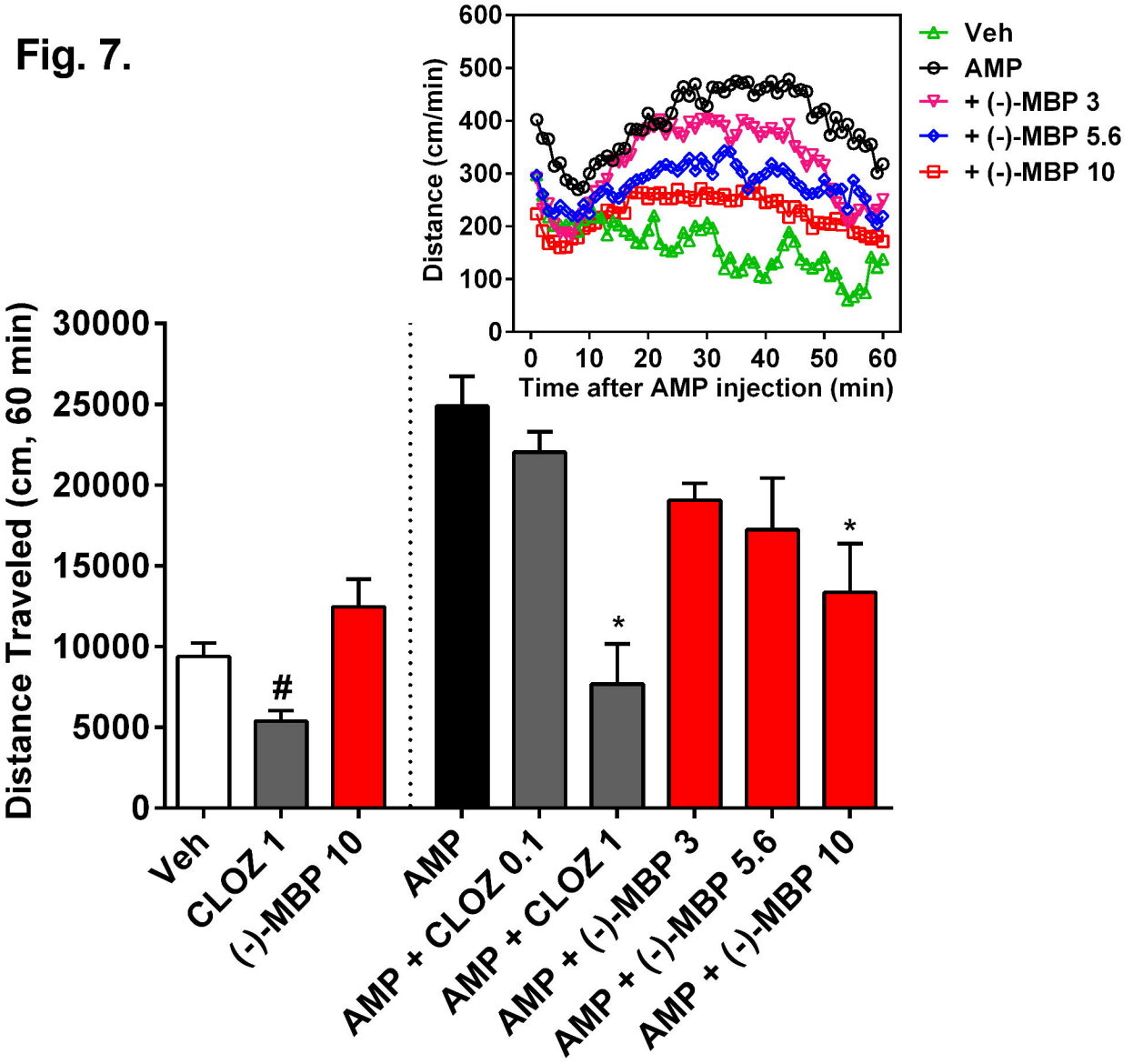
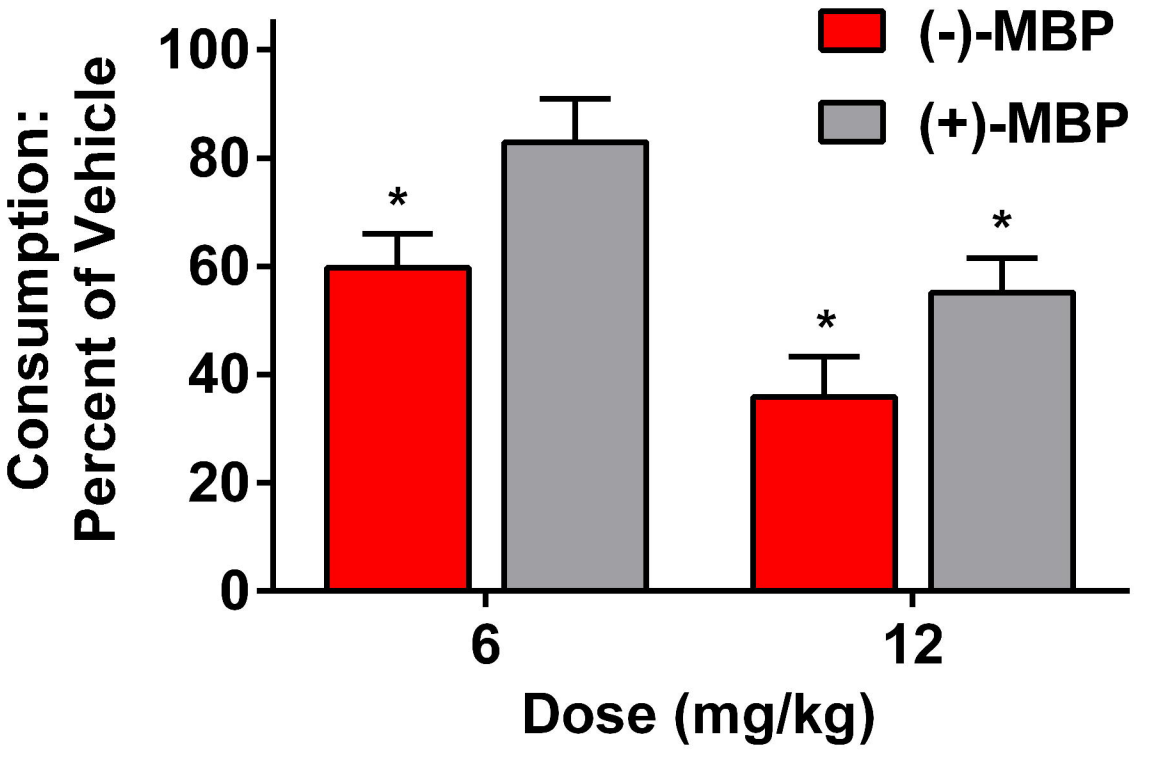

Distance (cm/min)
Time after AMP injection (min)
Veh
AMP
+ (-)-MBP 3
+ (-)-MBP 5.6
+ (-)-MBP 10
Distance Traveled (cm, 60 min)
#
*
*
Veh
CLOZ 1
(-)-MBP 10
AMP
AMP + CLOZ 0.1
AMP + CLOZ 1
AMP + (-)-MBP 3
AMP + (-)-MBP 5.6
AMP + (-)-MBP 10

Fig. 8.


(-)-MBP
(+)-MBP
Consumption:
Percent of Vehicle
100
80
60
40
20
0
*
*
*
6
12
Dose (mg/kg)

Correction to "A Novel Aminotetralin-Type Serotonin (5-HT) $_{2C}$ Receptor-Specific Agonist and 5-HT$_{2A}$ Competitive Antagonist/ 5-HT2B Inverse Agonist with Preclinical Efficacy for Psychoses"

In the above article [Canal CE, Morgan D, Felsing D, Kondabolu K, Rowland NE, Robertson KL, Sakhuja R, and Booth RG (2014) *J Pharmacol Exp Ther* **349:**310–318; doi:10.1124/jpet.113.212373], the pA_2 values are indicated incorrectly in three places.

Under *Results* and in Table 1 on page 313, the pA_2 value of 2.64 (0.05) should be 6.36 (0.05). In addition, under *Results* on page 313, the pA_2 value of 2.43 (0.17) should be 6.57 (0.17).

The authors regret these errors and any inconvenience they may have caused.