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Comparative neuropharmacology of *N*-(2-methoxybenzyl)-2,5-dimethoxyphenethylamine (NBOMe) hallucinogens and their 2C counterparts in male rats

Joshua S. Elmore[a], **Ann M. Decker**[b], **Agnieszka Sulima**[c], **Kenner C. Rice**[c], **John S. Partilla**[a], **Bruce E. Blough**[b], and **Michael H. Baumann**[a]

[a]Designer Drug Research Unit, Intramural Research Program, National Institute on Drug Abuse, National Institutes of Health, Baltimore, MD 21224, USA

[b]Center for Drug Discovery, Research Triangle Institute, Research Triangle Park, North Carolina 27709, USA

[c]Drug Design and Synthesis Section, Intramural Research Program, National Institute on Drug Abuse, National Institutes of Health, Bethesda, MD 20892, USA

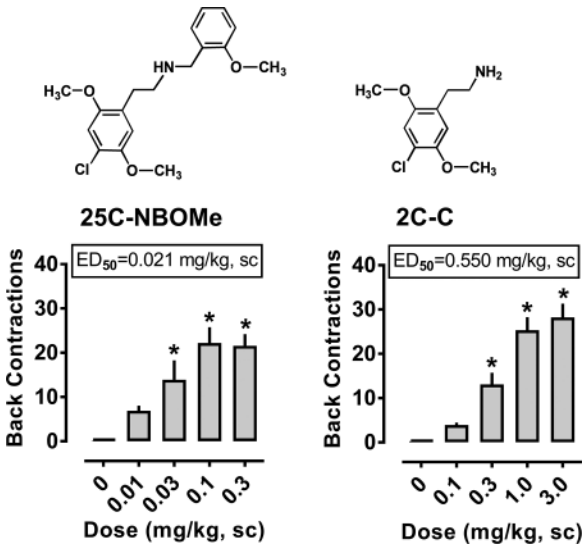
Abstract

2,5-Dimethoxyphenethylamines (2C compounds) are 5-$HT_{2A/2C}$ receptor agonists that induce hallucinogenic effects. *N*-methoxybenzylation of 2C compounds markedly increases their affinity for 5-HT_{2A} receptors, and two such analogs, 2-(4-chloro-2,5-dimethoxyphenyl)-*N*-[(2-methoxyphenyl)methyl]ethanamine (25C-NBOMe) and 2-(4-iodo-2,5-dimethoxyphenyl)-*N*-[(2-methoxyphenyl)methyl]ethanamine (25I-NBOMe), have emerged in recreational drug markets. Here, we investigated the neuropharmacology of 25C-NBOMe and 25I-NBOMe in rats, as compared to their 2C analogs and the prototypical 5-$HT_{2A/2C}$ agonist 1-(4-iodo-2,5-dimethoxyphenyl)propan-2-amine (DOI). Compounds were tested *in vitro* using 5-HT_{2A} receptor binding and calcium mobilization assays. For *in vivo* experiments, 25C-NBOMe (0.01–0.3 mg/kg), 25I-NBOMe (0.01–0.3 mg/kg), 2-(4-chloro-2,5-dimethoxyphenyl)ethanamine (2C-C) (0.1–3.0 mg/kg), 2-(4-iodo-2,5-dimethoxyphenyl)ethanamine (2C-I) (0.1–3.0 mg/kg) and DOI (0.03–1.0 mg/kg) were administered subcutaneously (sc) to male rats, and 5-HT_{2A}-mediated behaviors were assessed. NBOMes displayed higher affinity for 5-HT_{2A} receptors than their 2C counterparts but were substantially weaker in functional assays. 25C-NBOMe and 25I-NBOMe were much more potent at inducing wet dog shakes (WDS) and back muscle contractions (BMC) when compared to 2C-C and 2C-I. Pretreatment with the selective 5-HT_{2A} antagonist (R)-(2,3-dimethoxyphenyl){1-[2-(4-fluorophenyl)ethyl]-4-piperidinyl}methanol (M100907) reversed behaviors produced by all agonists. Interestingly, binding affinities at the 5-HT_{2A} receptor were significantly correlated with potencies to induce BMC but not WDS. Our findings show that

Address correspondence to: Michael H. Baumann, Ph.D., Designer Drug Research Unit, IRP, NIDA, NIH, 333 Cassell Drive, Suite 4400, Baltimore, MD 21224, Tel. 443 740-2660, mbaumann@mail.nih.gov.

NBOMes are highly potent 5-HT_{2A} agonists in rats, similar to effects in mice, and consistent with the reported hallucinogenic effects in human users.

Graphical abstract



Keywords

5-HT_{2A} receptor; back muscle contractions; NBOMe; wet dog shakes

1. Introduction

Serotonergic hallucinogens such as lysergic acid diethylamide (LSD) induce marked changes in sensation, perception and conscious experience, primarily through activation of 5-HT_{2A} receptors in the brain (Nichols, 2016). One class of serotonergic hallucinogens is the phenylalkylamines, which can be further divided into phenylisopropylamines like 1-(4-iodo-2,5-dimethoxyphenyl)propan-2-amine (DOI) and phenethylamines like 2-(4-iodo-2,5-dimethoxyphenyl)ethanamine (2C-I) (Halberstadt, 2015) (see Figure 1). 2C-I and its chloro analog, 2-(4-chloro-2,5-dimethoxyphenyl)ethanamine (2C-C), are phenethylamine compounds first described in the early 1990s by Shulgin and Shulgin (Shulgin and Shulgin, 1991). Subsequent investigations found that 2C-C and 2C-I bind to 5-HT_{2A} and 5-HT_{2C} receptors with nM affinity and engender LSD-like discriminative stimulus effects in rats (Eshleman et al. 2014). Administration of 2C-I to mice causes a dose-dependent increase in head twitch response that is blocked by pretreatment with the selective 5-HT_{2A} antagonist (R)-(2,3-dimethoxyphenyl){1-[2-(4-fluorophenyl)ethyl]-4-piperidinyl}methanol (M100907) (Canal and Morgan, 2012; Halberstadt and Geyer, 2014). In recent times, the misuse of 2C-C and 2C-I by humans has increased, and life-threatening adverse effects have been described (Bosak et al., 2013; Dean et al., 2013; U.S. Drug Enforcement Administration, 2017). The Synthetic Drug Abuse Prevention Act of 2012 placed 2C-C, 2C-I and a number of related phenethylamines under schedule I control (Drug Enforcement Administration, 2013).

In 1994, Glennon et al. first reported that *N*-benzylation of 2,5-dimethoxyphenethylamine compounds dramatically enhances their 5-HT_{2A} receptor affinity and improves selectivity over other 5-HT receptor subtypes (Glennon et al., 1994). Various *N*-benzylated phenethylamines have been developed as radiotracers and positron emission tomography imaging agents, leading to the discovery of 2-(4-chloro-2,5-dimethoxyphenyl)-*N*-[(2-methoxyphenyl)methyl]ethanamine (25C-NBOMe) and 2-(4-iodo-2,5-dimethoxyphenyl)-*N*-[(2-methoxyphenyl)methyl]ethanamine (25I-NBOMe) (referred to collectively as NBOMes) (see Figure 1) (Heim, 2004; Braden et al., 2006; Ettrup et al., 2011). NBOMes display sub-nM affinities for the 5-HT_{2A} receptor, with 25C-NBOMe and 25I-NBOMe exhibiting 18-fold and 6-fold greater affinities for human 5-HT_{2A} receptors when compared to 2C-C and 2C-I, respectively (Rickli et al., 2015). In 2011, the United States (US) Drug Enforcement Administration (DEA) reported the first law enforcement encounters of 25I-NBOMe, and one year later Polish police seized blotter papers containing 25C-NBOMe, indicating these two compounds were available as new psychoactive substances (NPS) in the US and Europe (Zuba et al., 2013; U.S. Drug Enforcement Administration, 2017). Between 2011 and 2015, there were nearly 5,000 law enforcement encounters of 25I-NBOMe and 25C-NBOMe in the US alone (U.S. Drug Enforcement Administration, 2017).

The first analytically confirmed human exposure to 25I-NBOMe occurred in 2012, and many cases have been documented since that time (Kelly et al., 2012; Halberstadt, 2017). Typical adverse effects of NBOMe overdose include tachycardia, hypertension, delirium, and seizures, sometimes culminating in death (Suzuki et al., 2015; Halberstadt, 2017). NBOMes are regularly found on blotter paper, a method of drug distribution typically associated with LSD. In a study examining the contents of blotter papers seized in Brazil, 50% were found to contain NBOMes, suggesting the substances are being trafficked as counterfeit LSD (erowid.org; Coelho Neto, 2015). Patterns of drug use that are relatively safe for LSD may lead to unexpected medical complications, or even death, when NBOMe compounds are involved (Suzuki et al., 2014; Poklis et al., 2014). Insufflation and sublingual routes of powdered or liquid NBOMes are most commonly employed, since the substances are not orally active due to rapid first-pass metabolism in the liver (Leth-Peterson et al., 2014, 2016). In response to their illicit use, the DEA placed 25I-NBOMe and 25C-NBOMe into permanent schedule I control in 2016 (Drug Enforcement Administration 2013, 2016).

The preclinical pharmacology of NBOMe hallucinogens has not been well characterized, and only a few investigations have assessed the activity of 25C-NBOMe and 25I-NBOMe *in vivo* (Halberstadt and Geyer, 2014; Gatch et al., 2017). To this end, we sought to characterize the neuropharmacological effects of 25C-NBOMe and 25I-NBOMe in rats, as compared to their 2C counterparts and the prototypical 5-$HT_{2A/2C}$ agonist DOI. We first examined the effects of 25C-NBOMe, 25I-NBOMe, 2C-C, 2C-I and DOI on 5-HT_{2A} receptor binding in native rat brain tissue, since prior studies only examined receptor binding in cells transfected with 5-HT_{2A} receptors (Braden et al., 2006; Ettrup et al., 2011; Rickli et al., 2015). We then characterized the effects of subcutaneous (sc) administration of the compounds on traditional measures of 5-HT_{2A} receptor function in rats, namely wet dog shakes (WDS) and back muscle contractions (BMC) (Pranzatelli, 1990; Wettstein, 1999; Higgins et al, 2001). As an additional aim, we wished to assess the utility of BMC as an *in vivo* read-out of 5-HT_{2A} receptor activation in rats. Finally, pretreatment with M100907 was

employed to verify 5-HT_{2A} receptor involvement in mediating the non-contingent behaviors induced by the drugs.

2. Materials and Methods

2.1 Drugs and Reagents

[^{3}H](R)-(2,3-dimethoxyphenyl){1-[2-(4-fluorophenyl)ethyl]-4-piperidinyl}methanol ([^{3}H]M100907) (specific activity 80 Ci/mmol) was obtained from American Radiolabeled Chemicals (St. Louis, MO, USA) whereas (−)-1-(4-iodo-2,5-dimethoxyphenyl)propan-2-amine hydrochloride (DOI) was obtained from Sigma Aldrich (St. Louis, MO, USA). M100907 was synthesized by Dr. Sulima at the National Institute on Drug Abuse (NIDA) Intramural Research Program (IRP). 2-(4-chloro-2,5-dimethoxyphenyl)ethanamine hydrochloride (2C-C), 2-(4-iodo-2,5-dimethoxyphenyl)ethanamine hydrochloride (2C-I), 2-(4-chloro-2,5-dimethoxyphenyl)-*N*-[(2-methoxyphenyl)methyl]ethanamine hydrochloride (25C-NBOMe) and 2-(4-iodo-2,5-dimethoxyphenyl)-*N*-[(2-methoxyphenyl)methyl]ethanamine hydrochloride (25I-NBOMe) were synthesized by Dr. Blough at Research Triangle Institute (RTI). M100907 was dissolved into a 1:9 mix of dimethyl sulfoxide:sterile saline. All other drugs were dissolved in sterile saline and injections were administered subcutaneously (sc) at a volume of 1.0 mL/kg.

2.2 Animals, Tissue Harvest and Surgery

Male Sprague-Dawley rats weighing 250–300 g were housed three per cage (lights on: 7:00 AM– 7:00 PM) under conditions of controlled temperature (22 ± 2°C) and humidity (45% ± 5%) with free access to food and water. Experiments were performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Vivarium facilities were fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care, and study procedures were approved by the NIDA IRP Animal Care and Use Committee. Rats were allowed at least 2 weeks of acclimation to the vivarium facilities before being used for tissue harvest or behavioral experiments. For the radioligand binding experiments, rats were euthanized by CO_2 narcosis and decapitated. Brains were excised and used immediately to prepare tissue for binding assays. For behavioral experiments, rats were briefly anesthetized using a drop jar containing a gauze pad saturated with 5 mL of isoflurane; a raised mesh floor prevented any contact between the animal and the gauze pad. Once anesthetized, each rat received a surgically-implanted IPTT-300 transponder (Bio Medic Data Systems, Seaford, DE, USA) to facilitate the non-invasive measurement of body temperature via a handheld radio frequency reader system. Transponders were 14 × 2 mm cylinders and were implanted subcutaneously (sc) posterior to the shoulder blades via a sterile guide needle. Animals were individually-housed post-operatively and allowed 7–10 days for recovery.

2.3 Radioligand Binding Assays

Each freshly-excised rat brain (minus cerebellum) was homogenized by twelve strokes of a hand-held teflon-on-glass homogenizer in 10 mL ice cold 0.25 M sucrose. The homogenate was centrifuged at 1000 × g for 10 min at 4° C. The resulting supernatant was diluted 40-fold with ice cold 50 mM Tris pH 7.4 and centrifuged at 35,000 × g for 10 min at 4° C. The

pellet was resuspended in fresh ice cold 50 mM Tris pH 7.4 using a Polytron at 14,000 rpm for 10 sec, and the suspension was centrifuged at 35,000 × g for 10 min at 4° C. This pellet was resuspended by polytron in 10 mL ice cold 50 mM Tris pH 7.4 containing 0.5 mM disodium EDTA and 10 mM $MgSO_4$ heptahydrate (assay buffer) for immediate use.

Binding assays were performed at equilibrium as described by Visser et al. with minor modifications (Visser et al., 2014). Assays were performed in triplicate in 12 × 75 mm polystyrene test tubes that contained 1 nM [^{3}H]M100907 and test drug in 900 μL assay buffer. Incubations were initiated by the addition of 100 μL of tissue preparation to the assay tubes; incubations were carried out for 30 min at 37° C and terminated by rapid vacuum filtration using a manifold (Brandel, Gaithersburg, MD, USA) fitted with GF/B filters that were presoaked in wash buffer (ice cold 10 mM TRIS pH 7.4) containing 1% polyethylenimine. Filters were immediately rinsed with 6 mL wash buffer and were punched into 24-well flexplates (PerkinElmer, Waltham, MA, USA). Radioactivity retained on the filters was quantified by a MicroBeta2 scintillation counter (PerkinElmer) at 40% efficiency after 15 h in 500 μL Cytoscint (MP Biomedicals, Santa Ana, CA, USA) per well. Nonspecific binding was determined in the presence of 10 μM ketanserin. Under these conditions, specific binding of [^{3}H]M100907 was proportional to the amount of rat brain membrane added (data not shown). Dose-response curves for inhibition of radioligand binding were assayed in triplicate, for N=3 separate experiments. Effects of test drugs on [^{3}H]M100907 binding were expressed as % inhibition of specific binding versus log of drug concentration. Data were analyzed by nonlinear regression of 8-point dose-response curves using GraphPad Prism 6 (GraphPad Software, San Diego, CA, USA). Dose-response values for binding inhibition were fit to the equation, $Y(x) = Ymin + (Ymax - Ymin) / (1+ 10exp[(logP50 - logx)] * n)$, where x is the concentration of the compound tested, Y(x) is the response measured, Ymax is the maximal response, P50 is the IC_{50} (the concentration that yields half-maximal binding inhibition), and n is the Hill slope parameter.

2.4 Calcium Mobilization Assays

HEK293 cell lines stably expressing human 5-HT_{2A} or 5-HT_{2C} receptors were maintained in DMEM-HG supplemented with 10% fetal bovine serum, 100 units of penicillin/streptomycin, 15 mM HEPES, and 400 μg/mL G418. The day prior to assay, cells were plated into 96-well black-walled assay plates at 40,000 cells/well (5-HT_{2A}) or 35,000 cells/well (5-HT_{2C}) in 100 μL of growth medium without G418. The cells were incubated overnight at 37°C in 5% CO_2. On the following day (test day), Calcium 5 dye (Molecular Devices, Sunnyvale, CA, USA) was reconstituted per the manufacturer instructions and diluted 1:40 in pre-warmed 37°C assay buffer (1× HBSS, 20 mM HEPES, 2.5 mM probenecid, pH 7.4 at 37°C). Growth medium was removed from the assay plates and the cells were gently washed with 100 μL of pre-warmed assay buffer. The cells were incubated for 45 minutes at 37°C, 5% CO_2 in 200 μL of the diluted dye. For agonist (EC_{50}) assays, serial dilutions of the test compounds were prepared at 10× the desired final concentration in 1% DMSO/assay buffer and aliquoted into 96-well polypropylene V-bottom plates. After the dye-loading incubation period, the cells were pretreated with 25 μL of 9% DMSO/assay buffer and incubated for 15 min at 37°C along with the compound plates. After the pretreatment incubation period, each plate was read with a FlexStation II (Molecular

Devices). Calcium-mediated changes in fluorescence were monitored every 1.52 seconds over a 60 second time period, with the FlexStation II adding 25 μL of material from the compound plate at the 19 second time point (excitation at 485 nm, detection at 525 nm). Dose-response curves for calcium mobilization were assayed in duplicate, for at least N=3 separate experiments. Peak kinetic reduction (SoftMax, Molecular Devices) relative fluorescent units (RFU) were plotted against the log of drug concentration, and nonlinear regression analysis was used to generate EC_{50} values (GraphPad Software).

2.5 Dose-Response Effects of 5-$HT_{2A/2C}$ Agonists on Behavior

Separate cohorts of 12 rats were used to determine dose-response effects for DOI, 25C-NBOMe, 25I-NBOMe, 2C-C and 2C-I (60 rats total). Rats in each cohort were tested once per week for 4 consecutive weeks, until the 5 dose groups for the drug of interest (vehicle plus 4 drug doses) reached a completed N=9 rats/group (i.e., total N=45). At each test session, rats received a single sc injection of drug or vehicle, and doses were randomly assigned, with the exception that rats receiving the highest drug dose always received vehicle treatment the following week. On test days, groups of rats were moved to the testing room in their home cages and given 1 h to acclimate. Feeding trays were removed, and wire lids were placed atop the cages. For the DOI experiments, rats received 0 (saline), 0.03, 0.1, 0.3 or 1.0 mg/kg doses; for the 25C-NBOMe and 25I-NBOMe experiments, rats received 0 (saline), 0.01, 0.03, 0.1 or 0.3 mg/kg doses; and for the 2C-C and 2C-I experiments, rats received 0 (saline), 0.1, 0.3, 1.0, or 3.0 mg/kg doses. Immediately before vehicle or drug injection and at 15, 30, 45, 60, 75, 90, 105 and 120 min post-injection, animals were observed for 90 sec and body temperatures were measured with the handheld reader. For behavioral scoring, the number of WDS and BMC were counted during the 90-sec observation period by an experienced rater who was not blind to the treatments. WDS were defined as rapid shaking of the head, neck and trunk from one side to the other, analogous to a wet dog shaking to dry itself, whereas BMC were defined as brief contractions of the paraspinal back muscles which elicit movement of the skin in a tail to head direction (Pranzatelli, 1990; Canal and Morgan, 2012). Rats were given summed scores for WDS and BMC which were totaled from all eight observation periods, as previously described (Baumann and Rothman, 1996).

2.6 Antagonism of Agonist-Induced Behavioral Effects

Separate cohorts of 12 rats were used to determine the effects of pretreatment with the 5-HT_{2A} receptor antagonist M100907 on behaviors induced by DOI, 25C-NBOMe, 25I-NBOMe, 2C-C and 2C-I (60 rats total). Rats in each cohort were tested once per week for 3 or 4 consecutive weeks, until the treatment groups reached a completed N=9/group. For antagonism experiments using DOI, rats were pretreated with vehicle or M100907 (0.01, 0.03, or 0.1 mg/kg) 30 min before receiving 1.0 mg/kg DOI or saline. For antagonism experiments with all other agonists, rats were pretreated with either vehicle or a fixed dose of M100907 (0.1 mg/kg) 30 min before receiving 0.03 mg/kg 25C-NBOMe, 0.1 mg/kg 25I-NBOMe, 0.3 mg/kg 2C-C, 1.0 mg/kg 2C-I or saline vehicle. Behavioral scoring and body temperature measurements were carried out in the same manner as described above in Section **2.5**.

2.7 Data Analysis and Statistics

The data from *in vivo* experiments were tabulated, analyzed, and graphically depicted using GraphPad Prism (GraphPad Software). Temperature data were analyzed by two-way (dose × time) analysis of variance (ANOVA) followed by Bonferroni post-hoc tests to establish significant differences between dose groups at specific timepoints. Summed behavioral scores for WDS and BMC from dose-response experiments with DOI, NBOMes and 2C compounds were evaluated by one-way (dose) ANOVA, followed by Bonferroni post-hoc tests to determine significance between doses. ED_{50} values were calculated from the rising phase of the dose-effect function for each drug using non-linear regression analysis of behavioral score versus log of the dose administered. Summed behavioral scores from the M100907 antagonism experiments were analyzed by one-way (treatment group) ANOVA followed by Bonferroni post-hoc tests. Relationships between *in vitro* and *in vivo* endpoints were investigated using Pearson's correlation analysis. $p<0.05$ was considered the minimal criterion for statistical significance.

3. Results

3.1 Radioligand Binding and Functional Assays

Figure 2 depicts the dose-response curves for NBOMes and their 2C counterparts in the [^{3}H]M100907 binding assay in rat brain tissue. Table 1 summarizes the potencies of test compounds in assays of 5-HT_{2A} receptor binding in rat brain (IC_{50} values) and calcium mobilization in cells expressing human 5-HT_{2A} and 5-HT_{2C} receptors (EC_{50} values). The NBOMe compounds showed much greater potency to inhibit binding at 5-HT_{2A} receptors (IC_{50} range 4–9 nM) when compared to the 2C compounds (IC_{50} range 125–307 nM). Specifically, 25C-NBOMe displayed 35-fold higher affinity than 2C-C at the 5-HT_{2A} receptor, whereas 25I-NBOMe displayed 32-fold higher affinity than 2C-I at this site. DOI had a 5-HT_{2A} receptor affinity intermediate between that of the NBOMes and 2C compounds. In contrast to the 5-HT_{2A} binding data, the 2C compounds showed greater potency than NBOMes in the 5-HT_{2A} functional assay. For example, 2C-C was 13-fold more potent than 25C-NBOMe in the 5-HT_{2A}-mediated calcium mobilization assay, whereas 2C-I was 8-fold more potent than 25I-NBOMe. All compounds acted as fully efficacious 5-HT_{2A} agonists. In the 5-HT_{2C}-mediated calcium mobilization assay, all compounds displayed roughly similar potency ranging from 47.2–178.0 nM, but the NBOMes were more efficacious (60–70% Emax) than their 2C counterparts (~30% Emax).

3.2 Behavioral Effects of DOI

As a first step in examining the *in vivo* effects of drugs for this study, we opted to characterize non-contingent behavioral responses produced by the prototypical 5-$HT_{2A/2C}$ agonist DOI as a standard for comparison. Our previous work identified WDS and BMC as robust indicators of 5-HT_{2A} receptor activation in rats treated with intravenous doses of DOI (Baumann and Rothman, 1996). Here, the effects of sc administered DOI on behaviors are depicted in Figure 3, while ED_{50} estimates for inducing WDS and BMC are summarized in Table 1. DOI administration markedly increased the frequency of WDS ($F_{5,48} = 14.80$, $p<0.0001$) and BMC ($F_{5,48} = 27.34$, $p<0.0001$), with significant increases in both behaviors occurring after 0.1, 0.3, and 1.0 mg/kg as compared to vehicle (Fig. 3, panels A and B). DOI

exhibited ED_{50} values of 0.065 and 0.180 mg/kg for producing WDS and BMC, respectively. DOI had no significant effects on body temperature at any dose tested. Pretreatment with various doses of selective 5-HT_{2A} antagonist M100907, 30 min before 1.0 mg/kg DOI, dose-dependently decreased WDS ($F_{4,40}$ = 21.87, p<0.0001) and BMC ($F_{4,40}$ = 50.94, p<0.0001). In particular, 0.1 mg/kg M100907 significantly decreased shakes and contractions as compared to vehicle-pretreated rats (Fig. 3, panels C and D). Behaviors observed in rats treated with 0.1 mg/kg M100907/DOI did not differ significantly from those treated with vehicle/vehicle, in contrast to robust behaviors elicited in the vehicle/DOI group.

3.3 Behavioral Effects of NBOMe Compounds

The effects of 25C-NBOMe on behaviors are depicted in Figure 4, and ED_{50} estimates for inducing WDS and BMC are summarized in Table 1. 25C-NBOMe increased the frequency of WDS ($F_{4,43}$ = 6.343, p<0.0004) and BMC ($F_{4,43}$ = 72.44, p<0.0001) compared to vehicle-treated rats. Significant stimulation of shaking behavior occured after 0.03 and 0.1 mg/kg doses, while stimulation of contractions occured after 0.03, 0.1 and 0.3 mg/kg doses (Fig. 4, panels A and B). It is noteworthy that effects of 25C-NBOMe on WDS generated a hyperbolic dose-response curve (i.e., inverted U-shaped curve), whereas effects of the drug on BMC generated a more sigmoidal curve. 25C-NBOMe displayed ED_{50} values of 0.010 and 0.021 mg/kg for producing WDS and BMC, respectively. 25C-NBOMe did not significantly alter body temperature at any dose tested. Pretreatment with 0.1 mg/kg M100907, 30 min before treatment with 0.03 mg/kg 25C-NBOMe, significantly decreased WDS ($F_{3,32}$ = 9.345, p<0.0001) and BMC ($F_{3,32}$ = 73.26, p<0.0001) produced by the agonist. Behaviors observed in the M100907/vehicle and M100907/25C-NBOMe groups did not differ significantly from vehicle/vehicle animals, in contrast to vehicle/25C-NBOMe treated animals which displayed marked increases in shakes and contractions (Fig. 4, panels C and D).

The effects of 25I-NBOMe on behaviors are shown in Figure 5, and the ED_{50} values for inducing WDS and BMC are summarized in Table 1. 25I-NBOMe increased WDS ($F_{4,39}$ = 12.62, p<0.0001) and BMC ($F_{4,40}$ = 27.94, p<0.0001) compared to vehicle-treated rats. Significant increases in shakes occured after the 0.1 and 0.3 mg/kg doses, while increases in contractions occurred after the 0.03, 0.1, and 0.3 mg/kg doses (Fig 5., panels A and B). As noted for the 25C-NBOMe experiments, the effects of 25I-NBOMe on WDS generated a hyperbolic dose-response curve but effects on BMC generated a more sigmoidal curve. 25I-NBOMe exhibited ED_{50} values of 0.062 and 0.037 mg/kg for producing WDS and BMC, respectively. 25I-NBOMe did not significantly alter body temperature at any dose tested. Pretreatment with 0.1 mg/kg M100907, 30 min before treatment with 0.1 mg/kg 25I-NBOMe, decreased WDS ($F_{3,32}$ = 78.92, p<0.0001) and BMC ($F_{3,32}$ = 30.41, p<0.0001) produced by the agonist. Behaviors observed in the M100907/vehicle and M100907/25I-NBOMe groups did not differ significantly from vehicle/vehicle rats, in contrast to vehicle/25I-NBOMe rats which displayed large increases in WDS and BMC (Fig. 5, panels C and D).

3.4 Behavioral Effects of 2C compounds

The effects of 2C-C on behaviors are depicted in Figure 6, and ED_{50} estimates for inducing WDS and BMC are summarized in Table 1. 2C-C increased the frequency of WDS ($F_{4,42}$ = 9.633, p<0.0001) and BMC ($F_{4,43}$ = 36.2, p<0.0001) when compared to vehicle-treated rats (Fig. 6, panels A and B). Significant stimulation of both behaviors occurred after administration of 0.3, 1.0, and 3.0 mg/kg doses. Effects of 2C-C on WDS generated a hyperbolic curve but effects on BMC generated a more sigmoidal curve. 2C-C displayed ED_{50} values of 0.173 and 0.550 mg/kg for producing WDS and BMC, respectively. 2C-C failed to significantly alter body temperature at any dose tested. Pretreatment with 0.1 mg/kg M100907, 30 min before treatment with 0.3 mg/kg 2C-C, decreased WDS ($F_{3,32}$ = 18.77, p<0.0001) and BMC ($F_{3,32}$ = 87.64, p<0.0001) produced by the agonist. Behaviors observed in the M100907/vehicle and M100907/2C-C groups did not differ significantly from vehicle/vehicle animals, in contrast to vehicle/2C-C treated animals which displayed robust increases in shakes and contractions (Figure 6, panels C and D).

The effects of 2C-I on behaviors are shown in Figure 7, and the ED_{50} values for inducing WDS and BMC are summarized in Table 1. 2C-I increased WDS ($F_{4,39}$ = 11.04, p<0.0001) and BMC ($F_{4,39}$ = 28.05, p<0.0001) with respect to vehicle-treated rats. Significant stimulation of shaking behavior ocurred after 1.0 and 3.0 mg/kg doses, while increases in BMC occurred after 0.3, 1.0, and 3.0 mg/kg doses (Fig. 7, panels A and B). In contrast to the behavioral effects produced by 2C-C and the other agonists, the effects of 2C-I on WDS generated a sigmoidal curve, while effects of the drug on BMC generated a hyperbolic curve. 2C-I exhibited ED_{50} values of 0.690 and 0.192 mg/kg for producing WDS and BMC, respectively. 2C-I failed to alter body temperature at any dose tested. Pretreatment with 0.1 mg/kg M100907, 30 min before treatment with 1.0 mg/kg 2C-I, decreased WDS ($F_{3,32}$ = 9.369, p<0.0001) and BMC ($F_{3,32}$ = 330.2, p<0.0001) produced by the agonist. Behaviors observed in the M100907/vehicle and M100907/2C-I groups did not differ significantly from vehicle/vehicle rats, while animals in the vehicle/2C-I group displayed large increases in WDS and BMC (Fig. 7, panels C and D).

3.5 Correlations Between *In Vitro* and *In Vivo* Endpoints

To examine the potential relationships between *in vitro* receptor findings and *in vivo* behavioral effects of test drugs, the results shown in Table 1 were subjected to Pearson correlation analysis. Several significant correlations were noted. There was a significant positive correlation between EC_{50} values for 5-HT_{2A} and 5-HT_{2C} receptor activation in the calcium mobilization assay (r = 0.942, p<0.017), yet no correlation between EC_{50}s for 5-HT_{2A} receptor activation versus IC_{50}s for 5-HT_{2A} receptor binding. Interestingly, 5-HT_{2A} receptor binding across the 5 test compounds was highly correlated with ED_{50} values for *in vivo* induction of BMC (r=0.984, p<0.003) but not WDS (r= 0.192, p<0.492).

4. Discussion

A major aim of the present study was to characterize the neuropharmacology of the hallucinogenic drugs, 25C-NBOMe and 25I-NBOMe, as compared to their 2C counterparts and the prototypical 5-HT_{2A} receptor agonist DOI. NBOMe compounds have emerged in the

recreational drug marketplace in recent years, and serious medical complications have been reported (Suzuki et al., 2015; Halberstadt, 2017). Data from our *in vitro* assays demonstrate that 25C-NBOMe and 25I-NBOMe display at least 30-fold higher affinity for rat 5-HT_{2A} receptors when compared to their affinity for 2C-C and 2C-I, respectively. Interestingly, NBOMes were much less potent in functional assays measuring calcium mobilization in cell lines expressing human 5-HT_{2A} receptors. Regardless of potency differences among the compounds, all test drugs acted as fully efficacious agonists at 5-HT_{2A} receptors but partial agonists at 5-HT_{2C} receptors. Data from our *in vivo* experiments reveal that 25C-NBOMe and 25I-NBOMe are 5- to 20-fold more potent than 2C-C and 2C-I in their ability to induce WDS and BMC. Pretreatment with the selective 5-HT_{2A} antagonist M100907 effectively eradicates both behaviors from all drug groups, demonstrating that the behaviors are dependent upon 5-HT_{2A} receptor activation. Overall, our findings show that 25C-NBOMe and 25I-NBOMe are potent 5-HT_{2A} receptor agonists in rats, consistent with previous findings from rats and mice (Halberstadt and Geyer, 2014; Gatch et al., 2017).

The present study examined the effects of test compounds on inhibition of [^{3}H]M100907 binding to 5-HT_{2A} receptors in rat brain tissue. We utilized this antagonist as a radioligand due to its superior selectivity for 5-HT_{2A} receptors (Marek and Aghajanian, 1994; Visser et al., 2014); such selectivity is advantageous when performing assays in brain tissue, where other 5-HT receptor subtypes can interfere with binding signal. Additionally, we favor the use of radiolabeled antagonists for binding studies because these agents simplify data interpretation by labelling only a single site. In agreement with previous findings, our data show that 25C-NBOMe and 25I-NBOMe are at least 30-fold more potent at inhibiting 5-HT_{2A} receptor binding when compared to 2C-C and 2C-I (Braden et al., 2006; Rickli et al., 2015). The IC_{50} values for 25C-NBOMe (8.7 nM) and 25I-NBOMe (3.9 nM) that we measured at 5-HT_{2A} receptors in rat brain are consistent with the Ki values for these compounds (i.e., 1.5–3.0 nM) reported by Ettrup et al. who examined [^{3}H]M100907 binding in NIH-3T3 cells stably expressing rat 5-HT_{2A} receptors (Ettrup et al., 2011). By contrast, the IC_{50} values for NBOMes at 5-HT_{2A} receptors in our work are much higher than the sub-nM Ki values for inhibition of radiolabeled DOI binding in cells expressing rat or human 5-HT_{2A} receptor subtypes (Braden et al., 2006; Nichols et al., 2015). Importantly, Braden et al. (2006) demonstrated that various NBOMe compounds inhibit [^{125}I]DOI binding at rat and human 5-HT_{2A} receptors with nearly identical potency, suggesting no species differences in terms of binding affinity for these compounds. One obvious difference between the present binding results and most previous studies is that we used a radiolabeled antagonist. Indeed, others have reported weaker potency for 25I-NBOMe at inhibiting [^{3}H]ketanserin binding when compared to [^{125}I]DOI binding (Braden et al., 2006). Despite the differences in absolute *in vitro* potencies for NBOMes reported across several studies, all data agree that *N*-methoxybenzyl analogs display at least 10-fold greater affinity for 5-HT_{2A} receptors when compared to their 2C counterparts.

An intriguing observation from the present work is that 25C-NBOMe and 25I-NBOMe were less potent than 2C-C and 2C-I in functional assays measuring 5-HT_{2A}-mediated calcium mobilization. As a specific example, 25I-NBOMe had an EC_{50} of 34.70 nM in the calcium mobilization assay whereas 2C-I had an EC_{50} of 3.97 nM. Accordingly, our correlation analyses found no relationship between 5-HT_{2A} binding and function in the present study.

One complicating factor with interpretation of our results is that binding assays were carried out in rat brain tissue while functional assays were carried out in cells transfected with human receptors. Nevertheless, our *in vitro* findings with 25C- and 25I-NBOMe agree with the results of Rickli et al. (2015) who examined the effects of various NBOMe compounds on receptor binding and functional activity in cells expressing human 5-HT_{2A} receptors. These investigators found that NBOMe compounds are much less potent in calcium mobilization assays when compared their effects in receptor binding assays, and 25I-NBOMe is weaker (EC_{50} = 240 nM) in the calcium assay than 2C-I (EC_{50} = 60 nM) (Rickli et al, 2015). Braden et al. (2006) used cells transfected with rat 5-HT_{2A} receptors to demonstrate that NBOMe compounds are at least 20-fold less potent in functional assays measuring accumulation of inositol phosphate (IP) when compared to their effects in binding assays. However, in the Braden et al. study, NBOMe compounds displayed greater potency than 2C compounds in the IP assay. Future studies are warranted to further explore the relationships between 5-HT_{2A} receptor binding affinity and functional activity for NBOMe compounds, especially in native brain tissue preparations. Finally, it is important to note that all of the compounds tested in our work acted as efficacious agonists at 5-HT_{2A} receptors but partial agonists at 5-HT_{2C} receptors, with 2C compounds exhibiting only 30% efficacy at 5-HT_{2C} sites.

Few investigations have examined the effects of NBOMe compounds in traditional animal models of hallucinogenic drug effects. Corne and Pickering (1967) first described the use of head twitch behavior in mice as a marker of hallucinogenic drug actions, while others subsequently demonstrated that WDS represent a behavioral equivalent in rats (Bedard and Pycock, 1977; Leysen et al., 1982). Here we show that DOI administration to rats increases WDS in a dose-related manner, consistent with many prior studies (Pranzatelli, 1990; Baumann and Rothman, 1996; Wettstein, 1999; Canal and Morgan, 2012). In a recent investigation using mice, Halberstadt and Geyer found that 25I-NBOMe is about 10-fold more potent than 2C-I at eliciting head twitch behavior after sc administration (Halberstadt and Geyer, 2014). These investigators reported ED_{50} values of 0.08 and 0.83 mg/kg in the head twitch assay for 25I-NBOMe and 2C-I, respectively. Consistent with the findings in mice, we show 25I-NBOMe is 10-fold more potent than 2C-I at inducing WDS in rats. We found ED_{50} values of 0.06 and 0.69 mg/kg for inducing WDS after sc administration of 25I-NBOMe and 2C-I, respectively. Thus, our potency values in rats are very close to those reported by Halberstadt and Geyer in mice, even though they used a magnetometer coil to measure head shakes continuously, while we used time-sampling methods to observe rats for 90 s epochs every 15 min. Similar to our results with 25I-NBOMe, we found 25C-NBOMe was much more potent than 2C-C in the wet dog shake assay.

An important observation from the present work is that NBOMe and 2C compounds produce inverted U-shaped dose-response curves in the wet dog shake assay. It is tempting to speculate that activation of non-5-HT_{2A} receptors (e.g., 5-HT_{2C} receptors) after administration of high drug doses may serve to counteract shaking behavior (Higgins et al., 2001; Vickers et al., 2001). Indeed, Vickers et al. (2001) provided convincing evidence that 5-HT_{2C} receptor stimulation can inhibit 5-HT_{2A}-mediated WDS in rats. As noted already, all of the compounds studied here display efficacious agonist activity at 5-HT_{2A} receptors, and partial agonist activity at 5-HT_{2C} receptors, suggesting the possibility of interaction between

these two sites *in vivo*. In a recent study, Fantegrossi et al. (2015) investigated the behavioral effects of the NBOMe analog 2-([2-(4-cyano-2,5-dimethoxyphenyl)ethylamino]methyl)phenol (25CN-NBOH) in mice, and showed this compound induces head twitch behavior that displays a bell-shaped dose-response curve very similar to the effects reported here for 25C- and 25I-NBOMe in rats (Fantegrossi et al., 2015). Halberstadt and Geyer (2014) failed to observe an inverted U-shaped dose-response for head twitches induced by 25I-NBOMe, but it seems possible that testing of higher drug doses in their study might have uncovered 5-HT_{2C}-mediated suppression of behavior. Collectively, our behavioral findings demonstrate that NBOMe compounds display similar potency to induce shaking behaviors in rats and mice, with the NBOMes exhibiting at least 10-fold greater potency *in vivo* when compared to their 2C counterparts.

Back muscle contractions, or BMC, are an established 5-HT_{2A}-mediated behavior in rats but are not typically used to assess the *in vivo* effects of hallucinogens, likely due to the apparent absence of any comparable behavior in mice (Baumann and Rothman, 1996; Wettstein et al., 1999; Higgins et al, 2001). An additional goal of the current study was to assess the validity and utility of BMC as a behavioral read-out of 5-HT_{2A} receptor activation in rats. We demonstrate that NBOMes are more potent at eliciting BMC when compared to their 2C counterparts. For example, the ED_{50} values for induction of BMC were 0.04 and 0.19 mg/kg for 25I-NBOMe and 2C-I, respectively. Within the dose ranges given, the dose-response curves for BMC were more classically sigmoidal in shape for all drugs except 2C-I. Thus, 2C-I seems to display subtle *in vivo* pharmacological differences when compared to the other drugs tested. In this regard, Eshleman et al. (2014) found that 2C-C is a full agonist in the IP and [H^3]arachidonic acid ([^{3}H]AA) assays, tests for functional activity of the 5-HT_{2A} receptor, while 2C-I is an agonist in the IP assay, and an *antagonist* in the [H^3]AA assay (Eshleman et al., 2014). An important observation from the current study is that 5-HT_{2A} receptor binding potency across the test drugs is positively correlated with BMC ($r = 0.984$, $p<0.003$) but not WDS ($r= 0.306$, $p<0.616$). These results suggest that BMC represent a valid index of 5-HT_{2A} receptor activation in rats, at least for the small group of compounds tested. Despite the lack of correlation between receptor binding and WDS, we believe that shaking behavior is a useful measure of 5-HT_{2A} activity in rats due to its similarity to the head twitch response in mice, which allows cross-species comparison.

Gatch et al. recently published an investigation which examined the behavioral effects of NBOMe hallucinogens in mice and rats (Gatch et al., 2017). In their study, 25C- and 25I-NBOMe dose-dependently reduced locomotor activity in mice, an effect often associated with 5-HT_{2A} agonism in mice (Halberstadt, 2015). Gatch et al. also found that 25C-NBOMe fully substituted for the 5-HT_{2A} agonist 1-(2,5-dimethoxy-4-methylphenyl)propan-2-amine (DOM) in rat drug discrimination experiments, while only partially substituting for 3,4-methylenedioxymethamphetamine (MDMA). By contrast, 25I-NBOMe engendered roughly equivalent discrimination for DOM and MDMA (~74–78%) but fell shy of full substitution. The effects of NBOMes in drug discrimination studies are slightly different from the effects of 2C-C and 2C-I reported by Eshleman et al. (2014) who found 2C-C fully substitutes for MDMA while 2C-I fully substitutes for prototypical hallucinogens like LSD (Eshleman et al., 2014; Gatch et al., 2017). Importantly, the efficacious doses of NBOMes producing robust non-contingent behaviors in our experiments (i.e., 0.03–0.10 mg/kg) are somewhat

lower than the doses producing discriminative stimulus effects in rats reported by Gatch and colleagues (2017). As a specific example, 25C-NBOMe produced significant increases in both WDS and BMC in our experiments at a dose of 0.03 mg/kg, but this dose engendered <20% DOM-appropriate responding in discrimination studies. One possible explanation for the apparent higher potency of NBOMes in our study is that we utilized the sc route of administration while Gatch et al. utilized the ip route. Anecdotal evidence suggests that NBOMe compounds are inactive after oral administration due to extensive first-pass metabolism, and NBOMe compounds have faster metabolic clearance compared to their 2C analogs (Leth-Peterson et al., 2014). Therefore, differences in the efficacious *in vivo* doses between our experiments and those of Gatch et al. could be due to longer drug half-life after sc versus ip routes of drug administration.

M100907 is a highly potent 5-HT_{2A} antagonist, with more than 100-fold selectivity over 5-HT_{2C} and other receptor subtypes (Marek and Aghajanian, 1994). In rats pre-treated with 0.1 mg/kg M100907, we found the effects of DOI, 25C-NBOMe, 25I-NBOMe, 2C-C and 2C-I were never significantly different from vehicle/vehicle treated animals, and were always significantly reduced compared to vehicle/agonist treated animals. From these findings, we conclude that WDS and BMC induced by NBOMes and 2C compounds are 5-HT_{2A}-dependent behaviors, in agreement with the existing literature (Higgins et al, 2001; Canal and Morgan, 2012; Halberstadt and Geyer, 2014).

In summary, we show that 25C-NBOMe and 25I-NBOMe dose-dependently induce WDS and BMC in rats, with both behaviors being blocked by the selective 5-HT_{2A} antagonist M100907. Our study is only the second to assess the behavioral effects of NBOMe compounds in rats, and the first to assess the effects of the drugs on WDS and BMC. Consistent with the existing literature, both NBOMe drugs are very potent 5-HT_{2A} agonists, as 25I-NBOMe is 10 times more potent than 2C-I in producing WDS and five times more potent in producing BMC. Similarly, 25C-NBOMe is 17 times more potent than 2C-C in producing WDS and 27 times more potent in producing BMC. Our correlation findings indicate that BMC are a reliable endpoint for determining the 5-HT_{2A} activity of hallucinogenic drugs in rats, but further research is required to assess the relationship between BMC and 5-HT_{2A} agonism. Finally, our data from rats are consistent with the high potency of NBOMe compounds reported in human users, and may explain the increased propensity for these drugs to induce serious side-effects.

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Abbreviations

BMC	back muscle contractions
2C-C	2-(4-chloro-2,5-dimethoxyphenyl)ethanamine
2C-I	2-(4-iodo-2,5-dimethoxyphenyl)ethanamine

25C-NBOMe	2-(4-chloro-2,5-dimethoxyphenyl)-*N*-[(2-methoxyphenyl)methyl]ethanamine
25CN-NBOH	2-([2-(4-cyano-2,5-dimethoxyphenyl)ethylamino]methyl)phenol
25I-NBOMe	2-(4-iodo-2,5-dimethoxyphenyl)-*N*-[(2-methoxyphenyl)methyl]ethanamine
DOI	1-(4-iodo-2,5-dimethoxyphenyl)propan-2-amine
LSD	lysergic acid diethylamide
M100907	(R)-(2,3-dimethoxyphenyl){1-[2-(4-fluorophenyl)ethyl]-4-piperidinyl}methanol
NPS	new psychoactive substances
WDS	wet dog shakes

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HIGHLIGHTS

- 25C-NBOMe and 25I-NBOMe exhibit 30-fold greater affinity for rat 5-HT_{2A} receptors when compared to 2C-C and 2C-I
- NBOMe compounds are much more potent than their 2-C counterparts at inducing wet dog shakes (WDS) and back muscle contractions (BMC) in male rats
- WDS and BMC produced by NBOMe administration are reversed by the selective 5-HT_{2A} antagonist M100907
- In rats, NBOMe compounds are highly potent 5-HT_{2A} agonists *in vitro* and *in vivo*, consistent with their powerful hallucinogenic effects in human users

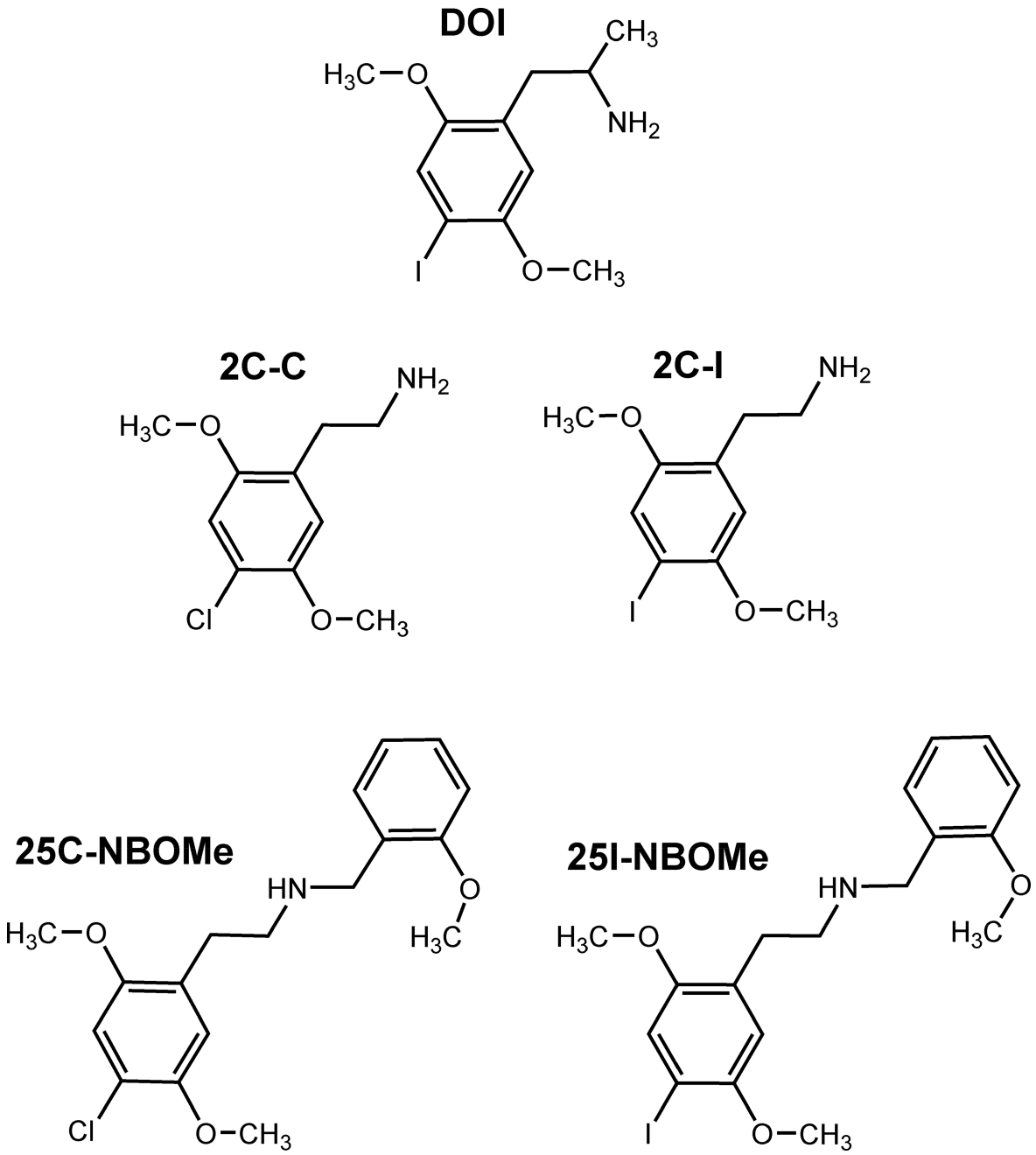

Figure 1.

Chemical structures of 1-(4-iodo-2,5-dimethoxyphenyl)propan-2-amine (DOI); 2-(4-chloro-2,5-dimethoxyphenyl)ethanamine (2C-C), and its *N*-methoxybenzylated derivative 2-(4-chloro-2,5-dimethoxyphenyl)-N-[(2-methoxyphenyl)methyl]ethanamine (25C-NBOMe); and 2-(4-iodo-2,5-dimethoxyphenyl)ethanamine (2C-I), and its *N*-methoxybenzylated derivative 2-(4-iodo-2,5-dimethoxyphenyl)-*N*-[(2-methoxyphenyl)methyl]ethanamine (25I-NBOMe).

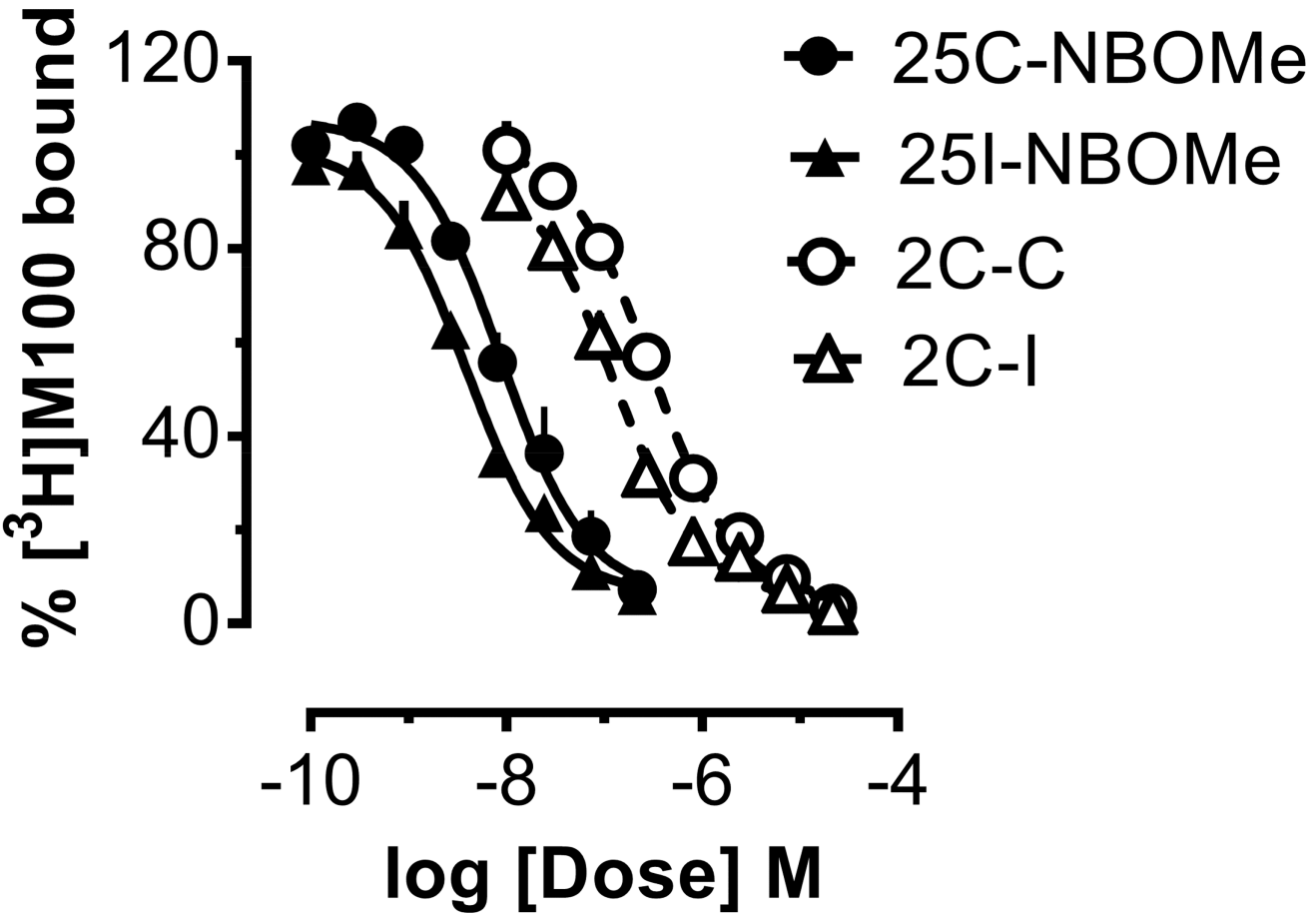

Figure 2.

Dose-response effects of NBOMe compounds and their 2C analogs to inhibit binding of [^{3}H]M100907 at 5-HT$_{2A}$ receptors in rat brain tissue. Various concentrations of test compounds were incubated with 1 nM [^{3}H]M100907, and assays were terminated using rapid filtration. Non-specific binding was determined in the presence of 10 μM ketanserin. Data are % inhibition of specific binding in the absence of added drug and are expressed as mean ± SEM for N=3 experiments performed in triplicate.

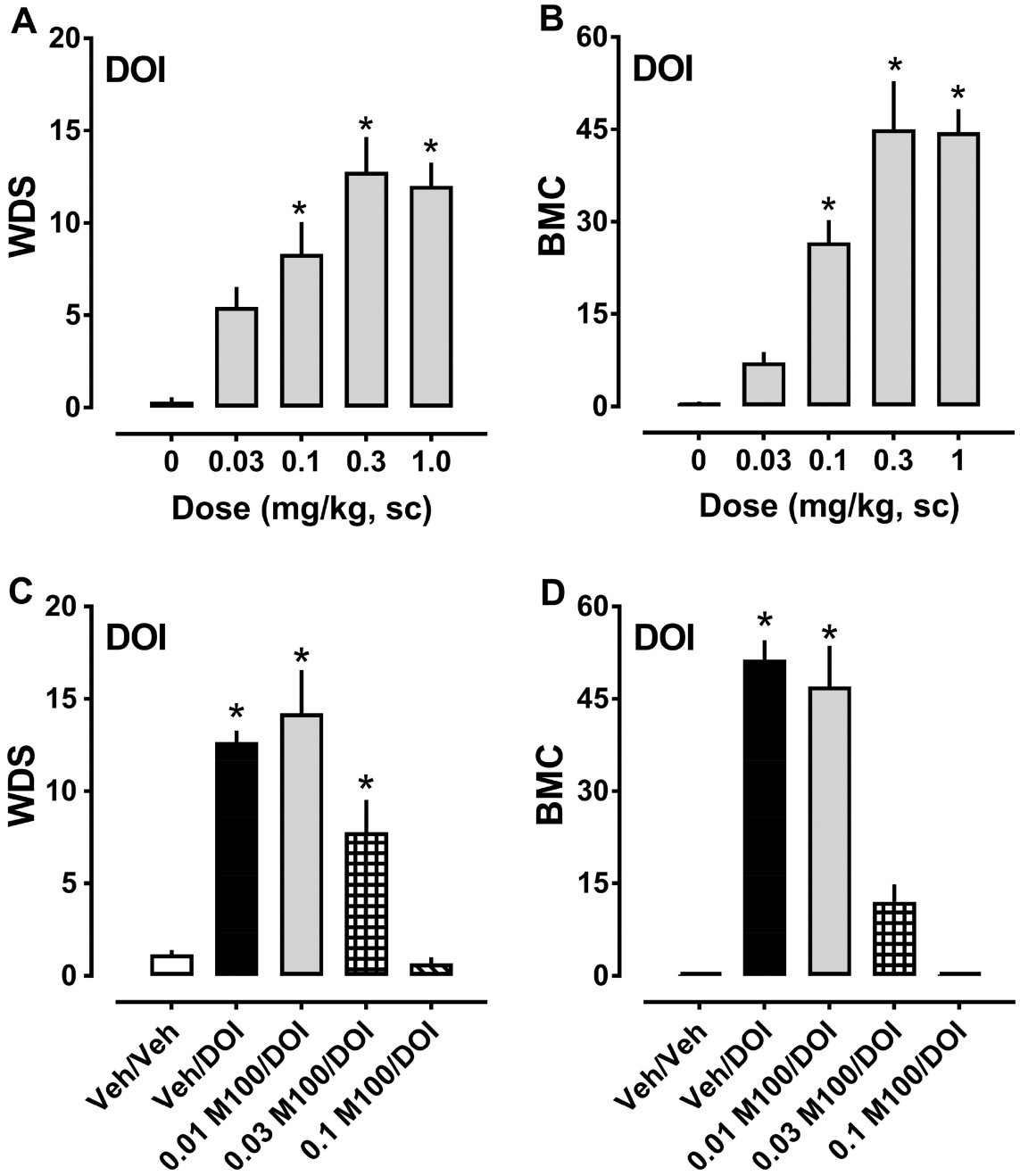

Figure 3.

Dose-response effects of DOI (0.03–1.0 mg/kg, sc) on summed WDS and BMC in rats (panels A and B), and effects of 30 min pretreatment with 0.01–0.1 mg/kg M100907 (M100) or its vehicle (Veh) on behaviors induced by 1.0 mg/kg DOI (panels C and D). Rats were observed for 90 s every 15 min for 2 h post-injection, and the number of WDS and BMC was totaled. Data are mean ± SEM for N = 9 rats per group. * represents significant effects on behavior when compared to the corresponding vehicle (0 dose) or vehicle/vehicle (Veh/Veh) groups (Bonferroni, $p<0.05$).

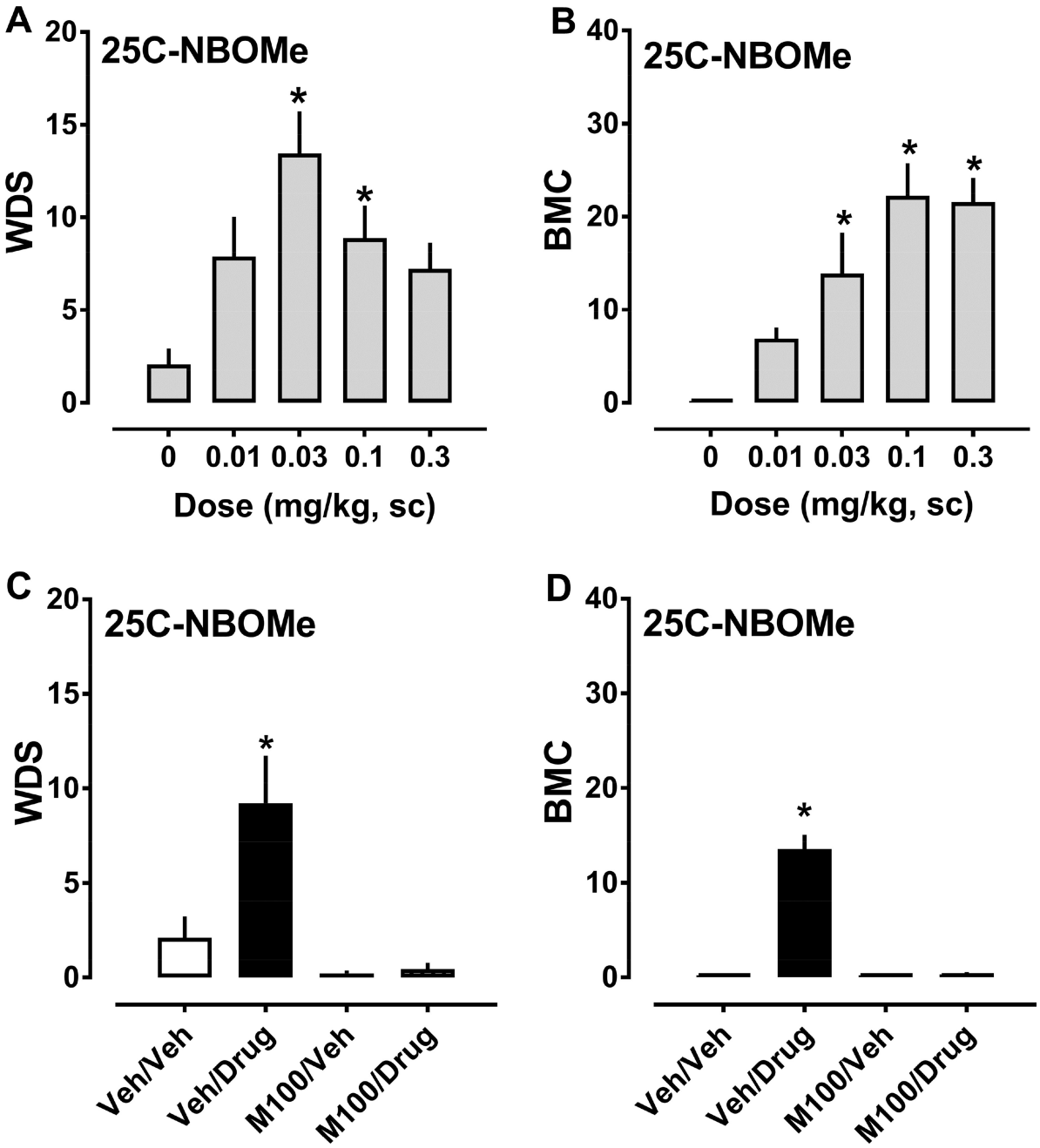

Figure 4.

Dose-response effects of 25C-NBOMe (0.01–0.3 mg/kg, sc) on summed WDS and BMC in rats (panels A and B), and effects of 30 min pretreatment with 0.1 mg/kg M100907 (M100) or its vehicle (Veh) on WDS and BMC induced by 0.03 mg/kg 25C-NBOMe (panels C and D). Rats were observed for 90 s every 15 min for 2 h post-injection, and the number of WDS and BMC was totaled. Data are mean ± SEM for N = 9 rats per group. * represents significant effects on behavior when compared to the corresponding vehicle (0 dose) or vehicle/vehicle (Veh/Veh) groups (Bonferroni, $p<0.05$).

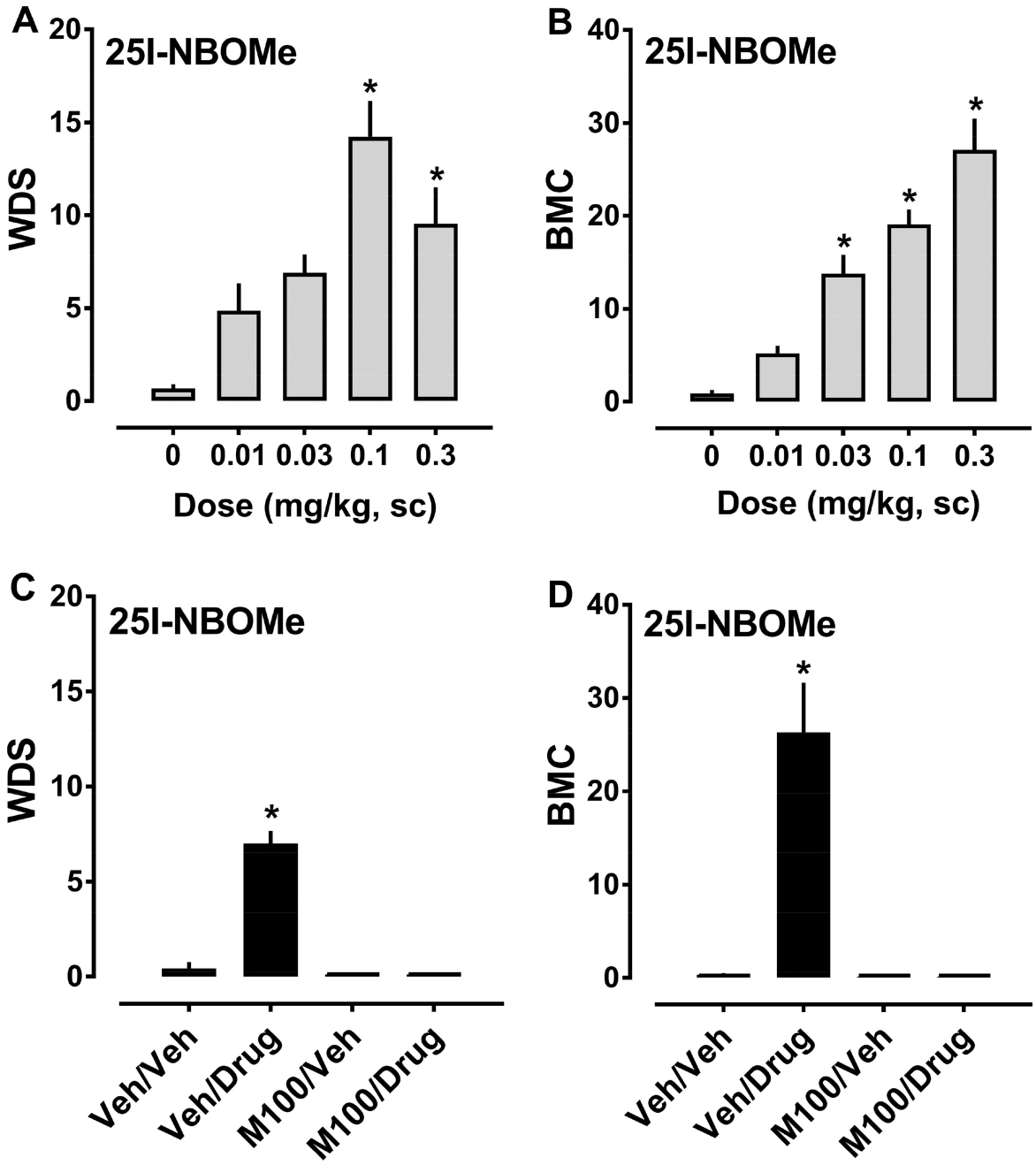

Figure 5.

Dose-response effects of 25I-NBOMe (0.01–0.3 mg/kg, sc) on summed WDS and BMC in rats (panels A and B), and effects of 30 min pretreatment with 0.1 mg/kg M100907 (M100) or its vehicle (Veh) on the WDS and BMC induced by 0.1 mg/kg 25I-NBOMe (panels C and D). Rats were observed for 90 s every 15 min for 2 h post-injection, and the number of WDS and BMC was totaled. Data are mean ± SEM for N = 9 rats per group. * represents significant effects on behavior when compared to the corresponding vehicle (0 dose) or vehicle/vehicle (Veh/Veh) groups (Bonferroni, $p<0.05$).

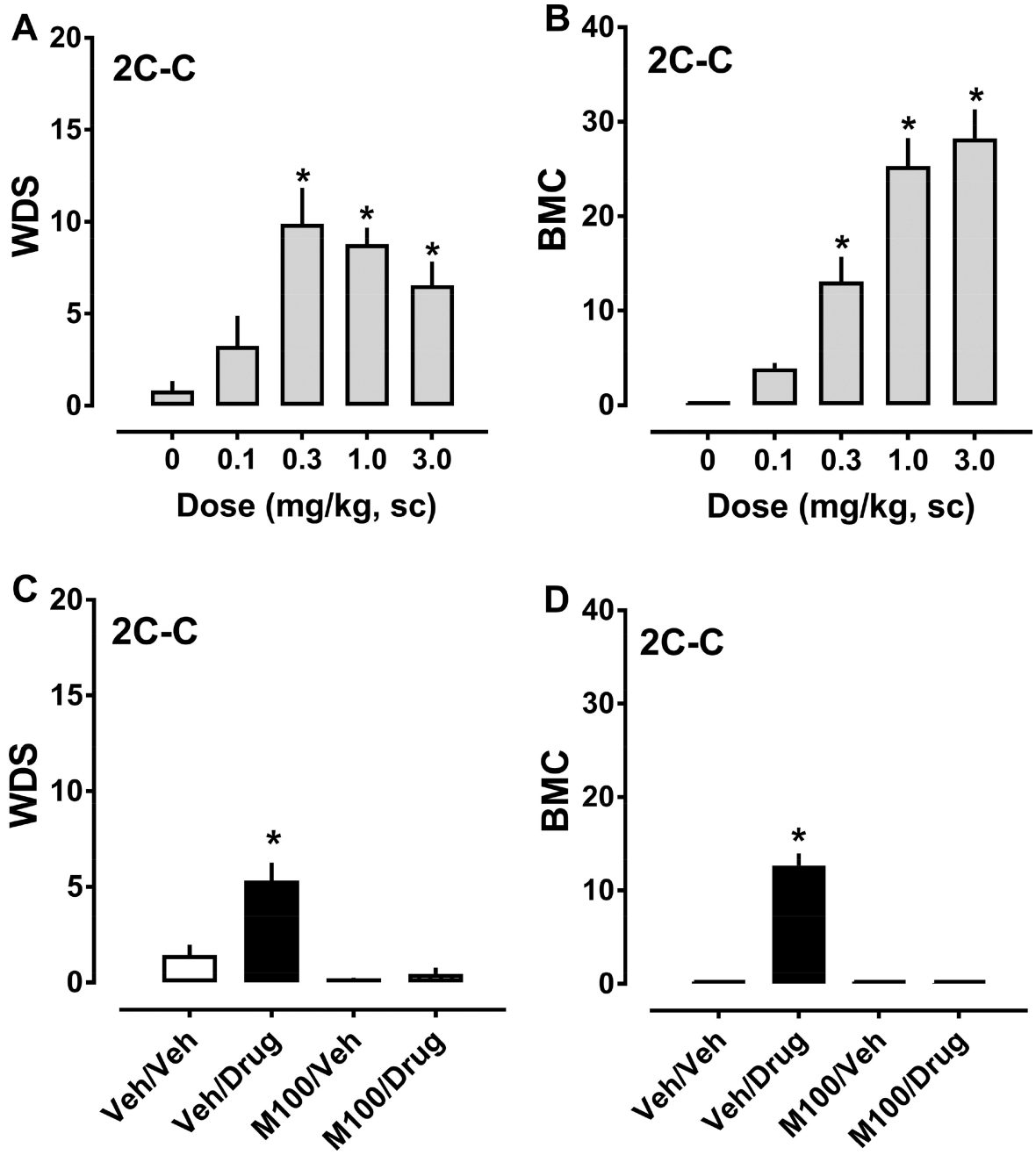

Figure 6.

Dose-response effects of 2C-C (0.1–3.0 mg/kg, sc) on summed WDS and BMC in rats (panels A and B), and effects of 30 min pretreatment with 0.1 mg/kg M100907 (M100) or its vehicle (Veh) on the WDS and BMC induced by 0.3 mg/kg 2C-C. Rats were observed for 90 s every 15 min for 2 h post-injection, and the number of WDS and BMC was totaled. Data are mean ± SEM for N = 9 rats per group. * represents significant effects on behavior when compared to the corresponding vehicle (0 dose) or vehicle/vehicle (Veh/Veh) groups (Bonferroni, $p<0.05$).

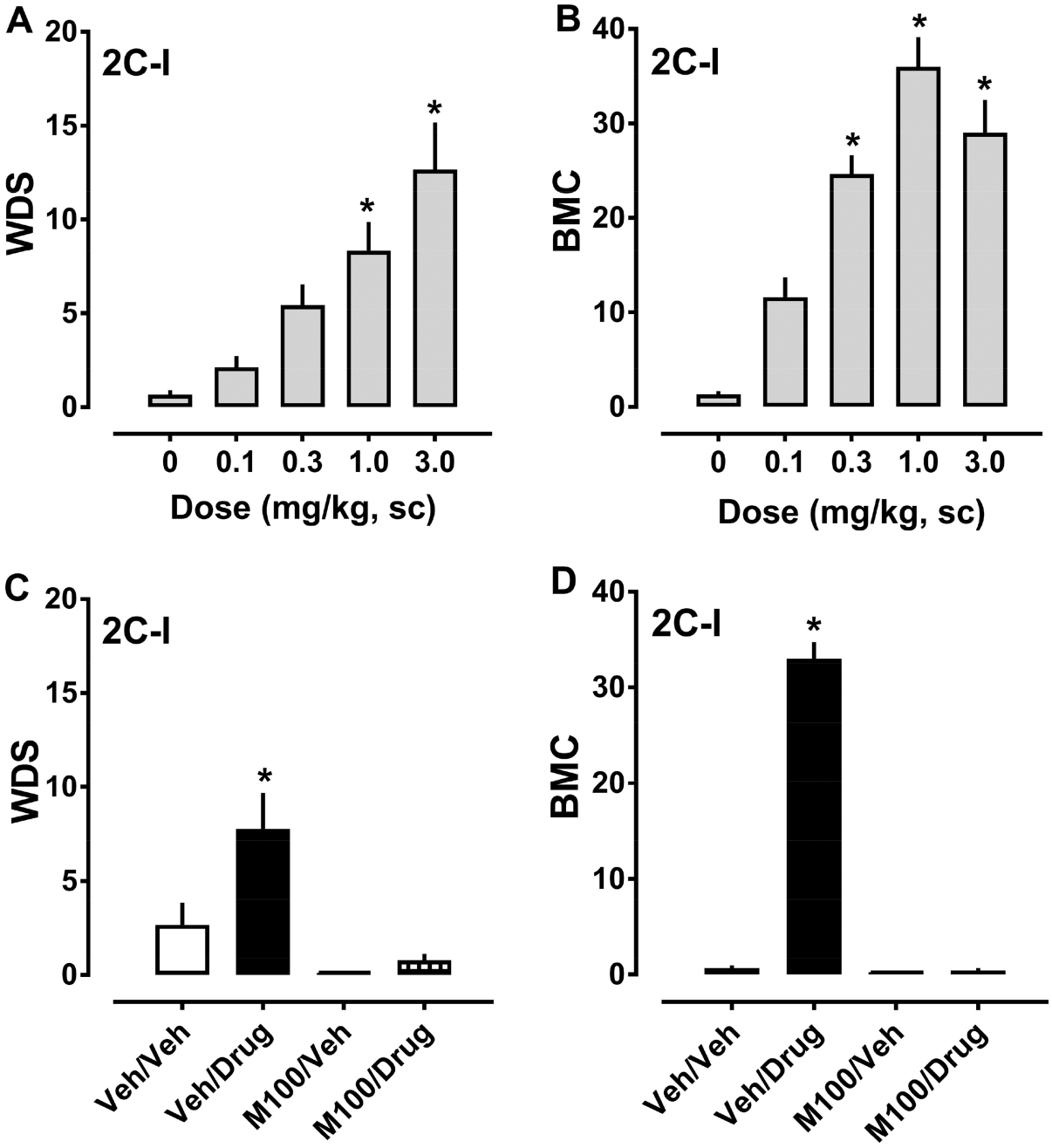

Figure 7.

Dose-response effects of 2C-I (0.1–3.0 mg/kg, sc) on summed WDS and BMC in rats (panels A and B), and effects of 30 min pretreatment with 0.1 mg/kg M100907 (M100) or its vehicle (Veh) on the WDS and BMC induced by 1.0 mg/kg 2C-I. Rats were observed for 90 s every 15 min for 2 h post-injection, and the number of WDS and BMC was totaled. Data are mean ± SEM for N = 9 rats per group. * represents significant effects on behavior when compared to the corresponding vehicle (0 dose) or vehicle/vehicle (Veh/Veh) groups (Bonferroni, $p<0.05$).

Table 1

Effects of DOI, 25C-NBOMe, 25I-NBOMe, 2C-C and 2C-I on 5-HT_{2A} receptor binding, calcium mobilization mediated by 5-HT_{2A} and 5-HT_{2C} receptors, and behaviors.

Compound	5-HT_{2A} Inhibition of Binding IC_{50} (nM)	5-HT_{2A} Mobilization of Calcium^{++} EC_{50} (nM) [%Emax]	5-HT_{2C} Mobilization of Calcium^{++} EC_{50} (nM) [% Emax]	Rat WDS ED_{50} (mg/kg, sc)	Rat BMC ED_{50} (mg/kg, sc)
DOI	56.1 ± 4.9	1.83 ± 0.3 [109]	47.7 ± 3.0 [50]	0.065 ± 0.024	0.180 ± 0.066
25C-NBOMe	8.7 ± 1.0	61.7 ± 3.5 [104]	178 ± 25 [60]	0.010 ± 0.005	0.021 ± 0.008
25I-NBOMe	3.9 ± 0.4	34.7 ± 4.2 [108]	88.9 ± 11 [70]	0.062 ± 0.026	0.037 ± 0.011
2C-C	307.5 ± 21.6	4.57 ± 0.5 [95]	77.6 ± 5.5 [29]	0.173 ± 0.066	0.550 ± 0.190
2C-I	124.9 ± 13.0	3.97 ± 0.7 [89]	47.2 ± 6.7 [28]	0.690 ± 0.268	0.192 ± 0.060