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Short communication

Investigating the role of 5-HT2A and 5-HT2C receptor activation in the effects of psilocybin, DOI, and citalopram on marble burying in mice

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ABSTRACT

Psychedelic drugs acting as 5-hydroxyptryptamine 2A receptor (5-HT_{2A}R) agonists have shown promise as viable treatments of psychiatric disorders, including obsessive-compulsive disorder. The marble burying test is a test of compulsive-like behavior in mice, and psychedelics acting as 5-HT_{2A}R agonists can reduce digging in this test. We assessed the 5-HT_2R contribution to the mechanisms of two 5-HT_{2A} agonists on digging behavior in female NMRI mice, using citalopram as a reference compound. While the 5-HT_{2A}R antagonist M100907 blocked the effect of DOI and the 5-HT_{2C}R antagonist SB242084 blocked the effect of citalopram, neither antagonist blocked the effect of psilocybin. This study confirms 5-HT_{2A}R agonism as a mechanism for reduced compulsive-like digging in the MB test and suggests that 5-HT_{2A} and 5-HT_{2C}Rs can work in parallel on this type of behavior. Our results with psilocybin suggest that a 5-HT_2R-independent mechanism also contributes to the effect of psilocybin on repetitive digging behavior.

Psychedelics acting as 5-hydroxytryptamine 2A receptor (5-HT_{2A}R) agonists show promise as viable new treatments of depression, drug dependence, and end-of-life anxiety [1]. A pilot study also indicated that psilocybin can alleviate symptoms of obsessive-compulsive disorder (OCD) [2]. This has spurred interest in understanding the effects of psychedelics and 5-HT_{2A}R pharmacology in animal behavioral paradigms. The marble burying (MB) test is a test used to study repetitive digging behavior in mice. Both anxiolytic and anti-compulsive drugs can decrease digging in this test [3]. Evaluation of the MB test has revealed that digging behavior is more representative of compulsivity than of anxiety [3], and a study concurrently measuring digging and spontaneous activity reported that most anxiolytic drugs tested that inhibited digging also inhibited locomotor activity [4]. While this speaks against the validity of MB test as a test of anxiety, it cannot be ruled out that anxiety may contribute to digging behavior in mice. We recently showed that the 5-HT_{2A}R-selective psychedelics, DOI and 25CN-NBOH, can reduce digging in this test and that the effect is stable across sex and strain of mice [5,6]. Matsushima et al. furthermore reported similar effects with psilocybin [7], a non-selective 5-HT_{2A}R agonist with high affinity for other 5-HT receptors and reuptake transporter [8]. Such findings could suggest that 5-HT_{2A}R psychedelics may be useful in the treatment of compulsive disorders, possibly though a 5-HT_{2A}R-mediated facilitation of cognitive flexibility [9]. By contrast, multimodal compounds acting as 5-HT_{2A}R antagonists, such as atypical antipsychotics, have been reported to reduce digging behavior, although other pharmacological effects of these compounds likely contributed to the effects [10–12]. Agonism of the 5-HT_{2C}R is a well-established mechanism for inhibiting digging [13–15]. The 5-HT_{2A} and 5-HT_{2C}Rs exert opposite effects on various behaviors, including locomotor activity [16], head twitches [17], impulsivity [18], and temporal discrimination [19], suggesting that 5-HT_{2A}Rs and 5-HT_{2C}Rs regulate certain behaviors in a functionally antagonistic manner. These findings together call for a need to assess the relative contribution of 5-HT_{2A} and 5-HT_{2C}Rs to the effects of psychedelics in the MB test.

We first characterized the dose-response effects of psilocybin on digging behavior and ensured that changes in locomotor activity (LA) did not account for effects on digging. In a second series of experiments, we assessed the contributions of 5-HT_{2A} and 5-HT_{2C}Rs to the effects of psilocybin and DOI in the MB test, using the conventional anti-compulsive selective serotonin reuptake inhibitor (SSRI) citalopram [20] as a positive control. Dose-response effects on digging in NMRI females by DOI [6] and citalopram [21] have already been characterized in our lab and were not included for ethical reasons.

Female NMRI mice from Envigo were used for the experiments (100 total) and were 17–26 weeks old at the time of testing. The animals were allowed one week of acclimatization upon arrival before any testing.

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Mice were group housed five mice per cage in individually ventilated cages with food and water provided *ad libitum*. Food and water were not available during experiments. The animals were housed on a 12/12 h light cycle with lights on at 07 h, and experiments were performed between 09 h and 17 h. Housing and experimental rooms were temperature (20–24 °C) and relative humidity (45–65 %) controlled. After transfer to the experimental room, the animals were acclimatized for a minimum of one hour before behavioral testing. Individual animals were tested no more than five times per animal, with minimum one week washout period between tests, to avoid the risk of carry-over effects. In addition, experiments were counterbalanced across experimental groups with respect to any previous treatment and order in which animals were tested. Specifically, in cases where animals were retested we counterbalanced so that the new treatment groups were represented by mice in each of the previous treatment groups. Group sizes were determined based on experience in our lab for detection of relevant effect sizes specific to behaviors in this mouse strain [6,21]. The experiments were conducted in accordance with *EU Directive 2010/63/EU* and the *Danish Law on animal experimentation 2014*. All efforts were made to minimize animal suffering and reduce the number of animals used.

The MB test was performed in transparent cages (LxWxH: 42.5 × 26.5 × 18 cm) containing ~4.5 cm aspen sawdust, as previously described [5,6]. A second cage was placed bottom-up as a lid to prevent escape. Twenty glass marbles were placed with equal distance in a 5 × 4 pattern, at least 2 cm from the borders of the cage. The experiment was performed under dim light (100–200 lx) to reduce the influence of anxiety on behavior. A treatment-blinded observed counted the number of marbles visible at 10, 20, and 30 min for the dose-response experiment and at 10 min for interaction experiments. The number of marbles buried >1/2 served as a measure of repetitive digging behavior [3].

The LA test was performed in transparent cages (LxWxH: 42.5 × 26.5 × 18 cm) on a black background, as previously described [6]. Movement was recorded by a camera mounted above the test cages and coupled to a computer with EthoVision XT (Noldus) technology. The experiment was performed under dim light adjusted to optimal detection (50–100 lx). Recording started immediately after placing the animals in the test cages and movement was recorded for 45 min in 5-min time bins. Distance traveled served as a measure of locomotor activity.

DOI ((±)-2,5-dimethoxy-4-iodoamphetamine hydrochloride) and M100907 ((R)-(+)-α-(2,3-dimethoxyphenyl)-1-[2(4-fluorophenyl) ethyl]-4-pipidinemethanol) were purchased from Sigma-Aldrich (Søborg, Denmark). (±)-Citalopram hydrobromide was purchased from Tokyo Chemical Industry (Tokyo, Japan). SB242084 (6-chloro-2,3-dihydro-5-methyl-*N*-[6-[(2methyl-3-pyridinyl)oxy]-3-pyridinyl]-1H-indole-1-carboxyamide dihydrochloride hemihydrate) was purchased from Tocris Bioscience (Abingdon, UK). Psilocybin was kindly supplied by National institute of mental health, Klecany Czech Republic and Forensic Laboratory of Biologically Active Compounds, Department of Chemistry of Natural Compounds, University of Chemistry and Technology Prague (Prague, Czech Republic). DOI, citalopram, and psilocybin were dissolved in isotonic saline (0.9 % NaCl). M100907 and SB242084 were dissolved in a vehicle containing 2.5 % DMSO and 5 % glucose. All test solutions were adjusted to pH 7 ± 1. All drugs were administered by intraperitoneal injection at 10 mL/kg and given 30 min before testing, except psilocybin, which was given 15 min before testing.

Marbles buried in the MB test dose-response experiment with psilocybin were analyzed by one-way analysis of variance (ANOVA) for total marbles buried and by two-way repeated measures (RM) ANOVA for digging in time bins with treatment as the independent factor and time as the dependent factor. Marbles buried in the interaction experiment with DOI were analyzed by two-way ANOVA with agonist and antagonist treatments as independent factors. Marbles buried in the interaction experiments with psilocybin and citalopram were analyzed by unpaired *t*-test of comparisons between treatment groups. Distance traveled in the LA test was log-transformed before analysis to comply with model assumptions of variance homogeneity and normal distribution. Distance traveled was analyzed by one-way ANOVA for total distance and by two-way RM ANOVA for movement in time bins with treatment as the independent factor and time as the dependent factor. ANOVAs were followed by the pairwise comparison of predicted (least squares) means using the Planned Comparisons procedure. Outlier analysis using the formula *mean±3*standard deviation* did not reveal any outliers and all animals tested were included in the statistical analyses. All statistical analyses were performed in InVivoStat version 4.0.1 [22]. Figures were created in GraphPad Prism 6 (GraphPad Software). Differences were considered significant when $p < 0.05$. Figures display mean with corresponding SEM on the original scale.

Psilocybin reduced perseverative digging and did not affect general locomotor activity

Fig. 1 displays the effects of psilocybin on repetitive digging in the MB test (Fig. 1A and B) and distance traveled in the LA test (Fig. 1C and D). The ANOVA revealed a significant main effect of treatment on total digging during 30 min ($F_{3,32} = 5.39$; $p = 0.004$). Pairwise comparisons showed that 1.0 ($p = 0.002$) and 2.0 mg/kg ($p = 0.002$) psilocybin significantly reduced total digging at the 30 min time point (Fig. 1A). Time-dependent analysis of the effect showed a significant main effect of time ($F_{2,64} = 24.66$; $p < 0.001$), treatment ($F_{3,32} = 5.39$; $p = 0.004$), and treatment by time interaction ($F_{6,64} = 4.01$; $p = 0.002$). Pairwise comparisons showed that 0.5 ($p = 0.001$), 1.0 ($p<0.001$), and 2.0 mg/kg ($p < 0.001$) decreased digging during the first 10 min of the test (Fig. 1B), but no significant effects of treatment were observed in the 10–20 or 20−30 min time bins.

The ANOVA of motor activity revealed no significant main effect of treatment on total distance traveled during 45 min ($F_{3,32} = 0.61$; $p = 0.616$) (Fig. 1C). Time-dependent analysis similarly showed a significant main effect of time ($F_{8,256} = 60.19$; $p < 0.001$), but no significant main effect of treatment ($F_{3,32} = 0.81$; $p = 0.496$) or treatment by time interaction ($F_{24,256} = 0.85$; $p = 0.677$) (Fig. 1D). Pairwise comparisons showed no significant effect of any of the doses tested.

Reduced digging by DOI was dependent on 5-HT_{2A}R, but not 5-HT_{2C}R, activation

Fig. 2 displays the effects of DOI (1.0 mg/kg), the 5-HT_{2A}R antagonist M100907 (0.1 mg/kg), and the 5-HT_{2C}R antagonist SB242084 (3.6 mg/kg) on digging in the MB test. The ANOVA revealed a significant main effect of DOI ($F_{1,54} = 15.50$; $p < 0.001$) and DOI by antagonist interaction ($F_{2,54} = 4.06$; $p = 0.023$), but no significant main effect of antagonists ($F_{2,54} = 2.73$; $p = 0.074$). Pairwise comparisons showed that DOI significantly reduced digging ($p < 0.001$) and that this effect was completely blocked by M100907 ($p < 0.001$), but not SB242084 ($p = 0.312$). The antagonists alone did not significantly affect digging behavior and were not tested alone in further experiments to reduce the number of animals.

Reduced digging by citalopram was dependent on 5-HT_{2C}R activation

Fig. 3 displays the effects of citalopram (2.5 mg/kg), M100907 (0.1 mg/kg), and SB242084 (3.6 mg/kg) on digging in the MB test. Analysis showed that citalopram significantly reduced digging ($t_{17} = 3.24$; $p = 0.005$) and that this effect was attenuated by SB242084 (t_{18}=-2.60; $p = 0.018$), but not by M100907 ($t_{17} = 1.85$; $p = 0.082$).

Reduced digging by psilocybin was not reversed by blockade of 5-HT_{2A}R or 5-HT_{2C}R

Fig. 4 displays the effects of psilocybin (2.0 mg/kg), M100907 (0.1 mg/kg), and SB242084 (3.6 mg/kg) on digging in the MB test. Analysis

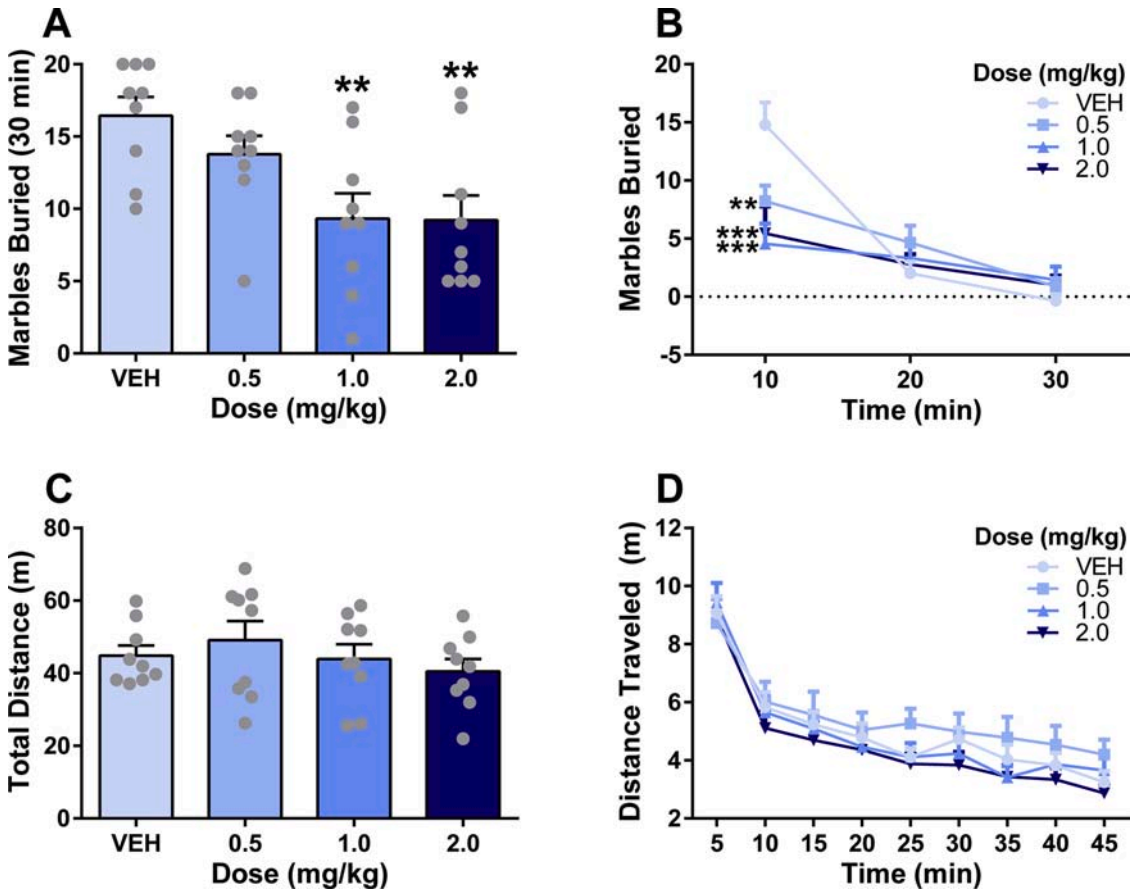

Fig. 1. Effects of psilocybin on marbles buried during the total duration of the MB test (A) and in time bins (B) as well as total distance traveled (C) and distance in time bins (D) in the LA test. 1.0 and 2.0 mg/kg psilocybin significantly decreased total digging. All doses decreased digging at the 10 min time point. Psilocybin did not significantly affect locomotor activity. Dots represent individual animal values. **/***p < 0.01/0.001, significantly different from the vehicle (VEH) control group. n = 9.

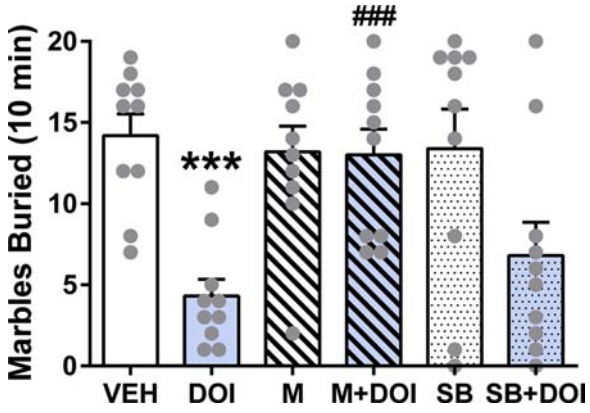

Fig. 2. Effects of DOI, M100907 (M), and SB242084 (SB) on marbles buried in the MB test. M100907 significantly attenuated the decreased digging by DOI. SB242084 did not block the effect of DOI and there were no significant effects on digging by the antagonists alone. Dots represent individual animal values. ***p < 0.001, significantly different from the vehicle (VEH) control group. ###p < 0.001, significantly different from the DOI group. n = 10.

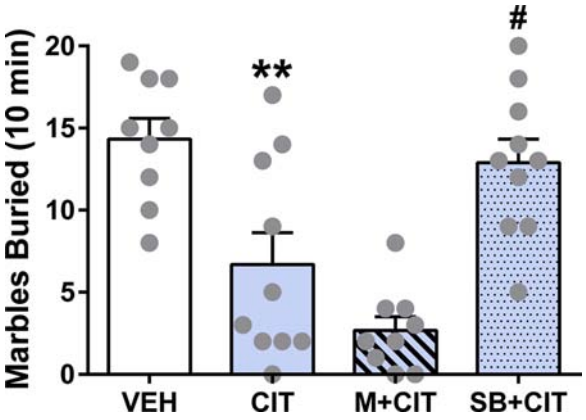

Fig. 3. Effects of citalopram (CIT), M100907 (M), and SB242084 (SB) on marbles buried in the MB test. SB242084 significantly attenuated the decreased digging by citalopram. Dots represent individual animal values. **p < 0.01, significantly different from the vehicle (VEH) control group. #p<0.05, significantly different from the citalopram group. n = 9-10.

showed that psilocybin significantly reduced digging (t_{18} = 3.52; p = 0.002), but this effect was not significantly attenuated by M100907 (t_{18}=-1.37; p = 0.188) or SB242084 (t_{18} = 0.18; p = 0.857).

Our results with DOI and citalopram suggest that activation of both 5-HT_{2A} and 5-HT_{2C}Rs can decrease digging, but that the mechanism appears to be specific to the drug tested. While DOI is primarily a 5-HT_{2A}R agonist with some affinity for 5-HT_{2C}Rs [23], citalopram facilitates serotonergic transmission by blocking reuptake. The different results obtained with these compounds can possibly be explained by the higher affinity of serotonin for 5-HT_{2C} over 5-HT_{2A}Rs [23] causing the mechanism of citalopram to favor 5-HT_{2C}Rs over 5-HT_{2A}Rs. Agonism at 5-HT_{2C}Rs is a well-studied mechanism for inhibition of compulsive-like

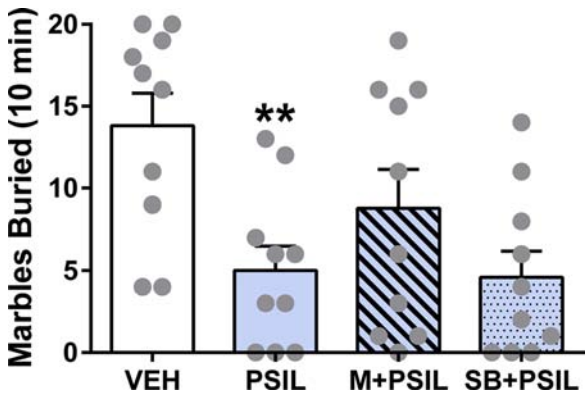

Fig. 4. Effects of psilocybin (PSIL), M100907 (M), and SB242084 (SB) on marbles buried in the MB test. Neither antagonist could significantly block the effect of psilocybin. Dots represent individual animal values. **p < 0.01, significantly different from the vehicle (VEH) control group. n = 10.

marble burying in mice [13–15], and both DOI and SSRIs have been proposed to act through activation of 5-HT_{2C}Rs in this test [13,15]. Surprisingly, the effect of DOI was not significantly reversed by the 5-HT_{2C}R antagonist SB242084, despite using the same doses as previously published [15]. Differences in mouse sex and strain used might account for this discrepancy.

Research on the pharmacology of MB behavior has traditionally focused on the anti-compulsive potential of multimodal 5-HT_{2A}R antagonists with several additional pharmacological properties [10–12]. It is notable that blockade of 5-HT_{2A}Rs by ketanserin [24], or by the highly selective M100907 used in this study does not affect digging behavior *per se*, suggesting that blockade of 5-HT_{2A}Rs is not the main driver for the effects reported with multimodal ligands. Our results with DOI suggest that 5-HT_{2A}R agonism may be sufficient to reduce digging behavior. This view is supported by a recent study that showed that the antipsychotic trifluoperazine inhibited digging through activation of 5-HT_{2A}Rs [25]. A larger dose of DOI would have activated 5-HT_{2C}Rs to a higher degree, which could have shifted the mechanism from primarily 5-HT_{2A} to 5-HT_{2C}R-mediated.

Surprisingly, our findings conflict with observations that 5-HT_{2A} and 5-HT_{2C}Rs act in a functionally antagonistic manner on several types of behavior [16–19]. Both receptor subtypes couple to excitatory signaling pathways [26], but differ in their expression pattern in different brain regions [27]. It is likely that the networks regulating digging behavior differ from those governing motor activity [16], impulsivity [18], head twitches [17], and perception of time [19]. Future research of the differential expression patterns of 5-HT_2Rs and relation to specific behaviors will hopefully elucidate this question.

Psilocybin, like DOI and the highly 5-HT_{2A}R selective 25CN-NBOH [6], reduced digging within the first 10 min of the MB test without decreasing locomotor activity, which is in line with previous research [7]. Blockade of 5-HT_{2A} or 5-HT_{2C}Rs did not attenuate the effect of psilocybin on digging, suggesting that a different mechanism dominates this effect. Due to its tryptamine structure, psilocybin is a non-selective 5-HT_{2A} receptor agonist [8]. Apart from 5-HT_{2A} and 5-HT_{2C}Rs, psilocybin also binds to 5-HT_{1A}, D_2, H_1 receptors, and works as a SERT inhibitor [8], all mechanisms that can affect digging behavior [11,15,24,28].

The results suggest that both 5-HT_{2A} and 5-HT_{2C}R activation can contribute to reduced digging behavior in the MB test, and that their relative contribution depends on the pharmacology of the compound that facilitates 5-HT_2R-mediated transmission.

CRediT authorship contribution statement

Anna U. Odland: Formal analysis, Investigation, Writing - original draft, Visualization. **Jesper L. Kristensen:** Resources, Writing - review & editing. **Jesper T. Andreasen:** Conceptualization, Writing - review & editing, Supervision.

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References

[1] D.E. Nichols, M.W. Johnson, C.D. Nichols, Psychedelics as medicines: an emerging new paradigm, Clin. Pharmacol. Ther. 101 (2) (2017) 209–219.
[2] F.A. Moreno, et al., Safety, tolerability, and efficacy of psilocybin in 9 patients with obsessive-compulsive disorder, J. Clin. Psychiatry 67 (11) (2006) 1735–1740.
[3] A. Thomas, et al., Marble burying reflects a repetitive and perseverative behavior more than novelty-induced anxiety, Psychopharmacology (Berl.) 204 (2) (2009) 361–373.
[4] L.B. Nicolas, Y. Kolb, E.P. Prinssen, A combined marble burying-locomotor activity test in mice: a practical screening test with sensitivity to different classes of anxiolytics and antidepressants, Eur. J. Pharmacol. 547 (1–3) (2006) 106–115.
[5] A.U. Odland, et al., The 5-hydroxytryptamine 2A receptor agonists DOI and 25CN-NBOH decrease marble burying and reverse 8-OH-DPAT-induced deficit in spontaneous alternation, Neuropharmacology (2019) 107838.
[6] A.A. Jensen, et al., The selective 5-HT(2A) receptor agonist 25CN-NBOH: structure-activity relationship, in vivo pharmacology, and in vitro and ex vivo binding characteristics of [(3)H]25CN-NBOH, Biochem. Pharmacol. 177 (2020) 113979.
[7] Y. Matsushima, et al., Effects of Psilocybe argentipes on marble-burying behavior in mice, Biosci. Biotechnol. Biochem. 73 (8) (2009) 1866–1868.
[8] A. Rickli, et al., Receptor interaction profiles of novel psychoactive tryptamines compared with classic hallucinogens, Eur. Neuropsychopharmacol. 26 (8) (2016) 1327–1337.
[9] R.L. Carhart-Harris, D.J. Nutt, Serotonin and brain function: a tale of two receptors, J. Psychopharmacol. 31 (9) (2017) 1091–1120.
[10] H. Azevedo, et al., Preclinical characterization of ACH-000029, a novel anxiolytic compound acting on serotonergic and alpha-adrenergic receptors, Prog. Neuropsychopharmacol. Biol. Psychiatry 95 (2019) 109707.
[11] L.A. Bruins Slot, et al., Effects of antipsychotics and reference monoaminergic ligands on marble burying behavior in mice, Behav. Pharmacol. 19 (2) (2008) 145–152.
[12] H. Takeuchi, S. Yatsugi, T. Yamaguchi, Effect of YM992, a novel antidepressant with selective serotonin re-uptake inhibitory and 5-HT 2A receptor antagonistic activity, on a marble-burying behavior test as an obsessive-compulsive disorder model, Jpn. J. Pharmacol. 90 (2) (2002) 197–200.
[13] M.M. Rodriguez, et al., Behavioral effects of a novel benzofuranyl-piperazine Serotonin-2C receptor agonist suggest a potential therapeutic application in the treatment of obsessive-compulsive disorder, Front. Psychiatry 8 (2017) 89.
[14] J.R. Martin, et al., 5-HT2C receptor agonists: pharmacological characteristics and therapeutic potential, J. Pharmacol. Exp. Ther. 286 (2) (1998) 913–924.
[15] N. Egashira, et al., Role of 5-hydroxytryptamine2C receptors in marble-burying behavior in mice, Biol. Pharm. Bull. 35 (3) (2012) 376–379.
[16] A.L. Halberstadt, et al., 5-HT(2A) and 5-HT(2C) receptors exert opposing effects on locomotor activity in mice, Neuropsychopharmacology 34 (8) (2009) 1958–1967.
[17] W.E. Fantegrossi, et al., Interaction of 5-HT2A and 5-HT2C receptors in R(-)-2,5-dimethoxy-4-iodoamphetamine-elicited head twitch behavior in mice, J. Pharmacol. Exp. Ther. 335 (3) (2010) 728–734.
[18] P.J. Fletcher, et al., Opposing effects of 5-HT(2A) and 5-HT(2C) receptor antagonists in the rat and mouse on premature responding in the five-choice serial reaction time test, Psychopharmacology (Berl.) 195 (2) (2007) 223–234.
[19] A.L. Halberstadt, et al., Effect of 5-HT2A and 5-HT2C receptors on temporal discrimination by mice, Neuropharmacology 107 (2016) 364–375.
[20] S.A. Montgomery, et al., Citalopram 20 mg, 40 mg and 60 mg are all effective and well tolerated compared with placebo in obsessive-compulsive disorder, Int. Clin. Psychopharmacol. 16 (2) (2001) 75–86.
[21] J.T. Andreasen, et al., Differential role of AMPA receptors in mouse tests of antidepressant and anxiolytic action, Brain Res. 1601 (2015) 117–126.
[22] R.A. Clark, et al., A comparison of InVivoStat with other statistical software packages for analysis of data generated from animal experiments, J. Psychopharmacol. 26 (8) (2011) 1136–1142.
[23] N. Almaula, et al., Contribution of a helix 5 locus to selectivity of hallucinogenic and nonhallucinogenic ligands for the human 5-hydroxytryptamine2A and 5-hydroxytryptamine2C receptors: direct and indirect effects on ligand affinity mediated by the same locus, Mol. Pharmacol. 50 (1) (1996) 34.
[24] N. Egashira, et al., Aripiprazole inhibits marble-burying behavior via 5-hydroxytryptamine (5-HT)1A receptor-independent mechanisms, Eur. J. Pharmacol. 592 (1–3) (2008) 103–108.
[25] N. Egashira, et al., The antipsychotic trifluoperazine reduces marble-burying behavior in mice via D2 and 5-HT2A receptors: implications for obsessive–compulsive disorder, Pharmacol. Biochem. Behav. 165 (2018) 9–13.

[26] M. Pytliak, et al., Serotonin receptors - from molecular biology to clinical applications, Physiol. Res. 60 (1) (2011) 15–25.
[27] L.L. Howell, K.A. Cunningham, Serotonin 5-HT(2) receptor interactions with dopamine function: implications for therapeutics in cocaine use disorder, Pharmacol. Rev. 67 (1) (2015) 176–197.
[28] L. Verma, D. Agrawal, N.S. Jain, Enhanced central histaminergic transmission attenuates compulsive-like behavior in mice, Neuropharmacology 138 (2018) 106–117.