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ORIGINAL INVESTIGATION

**William E. Fantegrossi · Andrew W. Harrington · Justin R. Eckler · Sadia Arshad · Richard A. Rabin · Jerrold C. Winter · Andrew Coop · Kenner C. Rice · James H. Woods**

# Hallucinogen-like actions of 2,5-dimethoxy-4-(*n*)-propylthiophenethylamine (2C-T-7) in mice and rats

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**Abstract** *Rationale:* Few studies have examined the effects of 2,5-dimethoxy-4-(*n*)-propylthiophenethylamine (2C-T-7) in vivo. *Objectives:* 2C-T-7 was tested in a drug-elicited head twitch assay in mice and in several drug discrimination assays in rats; 2C-T-7 was compared to the phenylisopropylamine hallucinogen R(−)-1-(2,5-dimethoxy-4-methylphenyl)-2aminopropane (DOM) in both assays, with or without pretreatment with the selective 5-$HT_{2A}$ antagonist (+)-(2,3-dimethoxyphenyl)-1-[2-(4-fluorophenylethyl)]-4-piperidine-methanol (M100907). Finally, the affinity of 2C-T-7 for three distinct 5-HT receptors was determined in rat brain. *Methods:* Drug-elicited head twitches were quantified for 10 min following administration of various doses of either 2C-T-7 or R(−)-DOM, with and without pretreatments of 0.01 mg/kg M100907. In rats trained to discriminate lysergic acid diethylamide (LSD), 2C-T-7 and R(−)-DOM were tested for generalization. In further studies, rats were trained to discriminate 2C-T-7 from saline, then challenged with 0.05 mg/kg M100907. In competition binding studies, the affinity of 2C-T-7 was assessed at 5-$HT_{2A}$ receptors, 5-$HT_{1A}$ receptors, and 5-$HT_{2C}$ receptors. *Results:* 2C-T-7 and R(−)-DOM induced similar head twitch responses in the mouse that were antagonized by M100907. In the rat, 2C-T-7 produced an intermediate degree of generalization (75%) to the LSD cue and served as a discriminative stimulus; these interoceptive effects were attenuated by M100907. Finally, 2C-T-7 had nanomolar affinity for 5-$HT_{2A}$ and 5-$HT_{2C}$ receptors and lower affinity for 5-$HT_{1A}$ receptors. *Conclusions:* 2C-T-7 is effective in two rodent models of 5-$HT_2$ agonist activity and has affinity at receptors relevant to hallucinogen effects. The effectiveness with which M100907 antagonizes the behavioral actions of 2C-T-7 strongly suggests that the 5-$HT_{2A}$ receptor is an important site of action for this compound.

**Keywords** 2C-T-7 · Hallucinogens · Phenethylamines · Drug discrimination · Head twitch response · 5-$HT_{2A}$ receptor · Mice · Rats

W. E. Fantegrossi (✉)
Division of Neuroscience,
Yerkes National Primate Research Center,
Emory University,
954 Gatewood Drive,
Atlanta, GA, 30322, USA
e-mail: wfanteg@emory.edu
Tel.: +1-404-7278512
Fax: +1-404-7271266

W. E. Fantegrossi · A. W. Harrington · J. H. Woods
Department of Pharmacology,
University of Michigan Medical School,
1301 MSRB III,
Ann Arbor, MI, 48109-0632, USA

J. R. Eckler · S. Arshad · R. A. Rabin · J. C. Winter
Department of Pharmacology,
School of Medicine and Biomedical Sciences,
State University of New York at Buffalo,
Buffalo, NY, 14214, USA

A. Coop
Department of Pharmaceutical Sciences,
University of Maryland School of Pharmacy,
20 N. Pine Street,
Baltimore, MD, 21201, USA

K. C. Rice
Laboratory of Medicinal Chemistry, NIDDK,
National Institutes of Health,
Building 8, Room B1-21,
Bethesda, MD, 20892, USA

## Introduction

The chemical structure of 2,5-dimethoxy-4-(*n*)-propylthiophenethylamine (2C-T-7, Fig. 1, top) places it into the substituted phenethylamine superfamily of compounds. This classification encapsulates a variety of drugs of abuse with differing mechanisms of action, including dopaminergic psychostimulants (such as amphetamine), serotonergic hallucinogens (such as mescaline), and mixed-action "entactogens" [such as 3,4-methylenedioxymethamphetamine (MDMA)]. The designation "2C-T-7" originated with

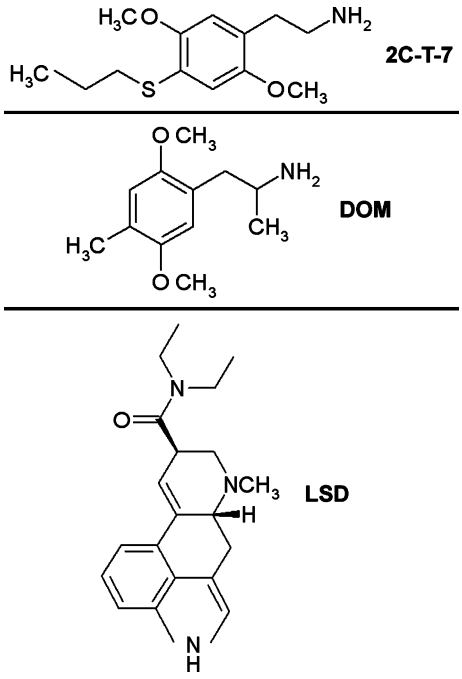


**Fig. 1** Chemical structures of 2C-T-7 (*top*), DOM (*middle*), and LSD (*bottom*)

Alexander Shulgin (Shulgin and Shulgin 1991) and invokes the two carbon (2C) phenethylamine homologues of previously synthesized amphetamines which formed the basis for this drug series, as well as the thio group substitution (T) on the phenyl ring. The final number in the designation is not based on any structural properties of the molecule but refers simply to the series order in which this particular compound was designed and synthesized.

2C-T-7 was placed temporarily into Schedule I under the Controlled Substances Act in September of 2002 by the United States Drug Enforcement Administration (Brown 2002), and this placement was made permanent in early 2004 (Leonhart 2004). Despite these aggressive attempts at regulation, 2C-T-7 remains readily available for purchase via the Internet. Anecdotal reports from human users posted to Internet sites specializing in the dissemination of drug information (for example, http://www.erowid.org and http://www.lycaeum.org) suggest that 2C-T-7 has profound psychedelic actions in man, resulting in some potential for abuse. For example, an on-line survey conducted by erowid.org in 2001 garnered 423 valid 2C-T-7 responders, suggesting that the illicit use of this compound may be significant. Another recent study reported that 2C-T-7 was being openly sold in 8-mg tablets at several "smartshops" across the Netherlands, although it was apparently being marketed in this form as a "dietary supplement" (de Boer and Bosman 2004).

The mechanism of action for 2C-T-7 has not been elucidated, although its chemical structure, as well as the anecdotal reports of its hallucinogenic activity in man, suggests that serotonin systems, specifically 5-$HT_{2A}$ receptors (Sadzot et al. 1989), may be involved. In this regard, the drug-elicited head twitch response (HTR) (Corne et al. 1963; Corne and Pickering 1967) is a selective behavioral model for 5-$HT_2$ agonist activity in the rodent, and several previous studies have established that direct and indirect 5-HT agonists induce this effect (Peroutka et al. 1981; Colpaert and Janssen 1983; Green et al. 1983; Goodwin and Green 1985; Darmani et al. 1990a,b, 1992). Further, 5-$HT_2$ receptor antagonists selectively block the HTR (Lucki et al. 1984; Handley and Singh 1986), and the potency with which they do so is highly correlated with the antagonist's affinity for 5-$HT_2$ receptors (Peroutka et al. 1981; Ortmann et al. 1982).

Similarly, the strong correlation between discriminative stimuli in nonverbal species and subjective effects reported by humans (Schuster and Johanson 1988; Sanger et al. 1994; Brauer et al. 1997) allows for a useful characterization of the interoceptive cues produced by psychedelic drugs using drug discrimination procedures in laboratory rodents. The discriminative stimulus properties of R(−)-1-(2,5-dimethoxy-4-methylphenyl)-2aminopropane (DOM, Fig. 1, middle) and lysergic acid diethylamide (LSD, Fig. 1, bottom) have been extensively investigated in several different animal species, and it has been shown that, in agreement with studies in humans, these hallucinogens generalize with one another (Winter 1978; Glennon et al. 1983a, b; Fiorella et al. 1995a). Furthermore, antagonist correlation analysis has determined that the stimulus effects of phenylisopropylamine and indolealkylamine hallucinogens are mediated by agonist activity at 5-$HT_{2A}$ receptors and modulated by agonist activity at 5-$HT_{2C}$ receptors (Fiorella et al. 1995b). Recently, 2C-T-7 has been shown to generalize to the interoceptive cue induced by DOM in rats (Khorana et al. 2004), although the pharmacological mechanism for this effect was not explored using antagonist challenges.

Thus, in order to compare potency and effectiveness of 2C-T-7 with a traditional phenylisopropylamine hallucinogen, we established dose-effect functions for 2C-T-7 and the structurally similar psychedelic R(−)-DOM in the HTR assay in mice. Antagonist studies were then conducted with the selective 5-$HT_{2A}$ antagonist (+)-(2,3-dimethoxyphenyl)-1-[2-(4-fluorophenylethyl)]-4-piperidine-methanol (M100907, formerly MDL100907) in order to gauge the involvement of 5-$HT_{2A}$ receptors in the HTR induced by each compound. A parallel series of DD experiments were conducted in rats in order to characterize the similarity of the discriminative stimulus effects of 2C-T-7 and R(−)-DOM with those of LSD. The capacity of 2C-T-7 to serve as a discriminative stimulus and the effects of M100907 on 2C-T-7-appropriate responding were also tested in a separate group of rats. Finally, binding of 2C-T-7 to 5-$HT_{1A}$, 5-$HT_{2A}$, and 5-$HT_{2C}$ receptors was characterized in rat brain using a competition binding technique.

## Materials and methods

### Animals

Male NIH Swiss mice (Harlan Sprague–Dawley Inc., Indianapolis, IN) weighing ~20–30 g were housed 12 an-

imals per 44.5×22.3×12.7-cm Plexiglas cage and used in drug-elicited head twitch experiments. Mice were housed in a temperature-controlled room at the University of Michigan that was maintained at an ambient temperature of 22±2°C at 45–50% humidity. Lights were set to a 12-h light/dark cycle. Animals were fed with Lab Diet rodent chow (Laboratory Rodent Diet #5001, PMI Feeds, Inc., St. Louis, MO) and water ad libitum until immediately before testing. Animals were not used in experiments until at least 2 days after arrival in the laboratory. Each animal was used only once and was sacrificed immediately after use.

Male Fischer-344 rats obtained from Harlan Sprague–Dawley Inc. at an age of ~6 weeks were used in LSD discrimination experiments. Rats were housed in pairs with free access to water in a temperature-controlled room at the State University of New York at Buffalo under a constant 12-h light/dark cycle (all experiments were conducted during the light phase). Caloric intake was controlled to yield a mean body weight of ~250 g; supplemental feedings of standard rat chow were provided following experimental sessions.

All studies were carried out in accordance with the Declaration of Helsinki and with the Guide for Care and Use of Laboratory animals as adopted and promulgated by the National Institutes of Health. Experimental protocols were approved by the Animal Care and Use Committees at the University of Michigan or the State University of New York at Buffalo.

## Procedure

### *Drug-elicited head twitch response in mice*

On experimental days, mice were weighed, marked, and returned to the home cage. Doses were then calculated and prepared for injection. Individual animals were subsequently removed from the home cage, injected i.p. with saline or 0.01 mg/kg M100907, then placed into a 15.24×25.40×12.70-cm Plexiglas mouse cage. This antagonist dose was chosen based upon our previous demonstrations of its effectiveness against head twitches induced by 4-iodo-2,5-dimethoxyphenylisopropylamine (DOI) (Fantegrossi et al. 2004a). Methods for measuring drug-elicited head twitch behavior have been previously described (Corne et al. 1963; Corne and Pickering 1967; Boulton and Handley 1973; Fozard and Palfreyman 1979; Green et al. 1983; Fantegrossi et al. 2004b). For the present experiments, 10 min after the initial injection, mice were injected with various doses of 2C-T-7, R(−)-DOM, or saline and returned to the small observation cage. Five minutes after this second injection, a camera mounted above the observation cage began recording behavior and continued to do so for 10 min. Videotapes were later scored by two blind observers for HTR, here defined as a rapid rotational jerk of the head that is not contiguous with any grooming or scratching behaviors. All HTR experiments were conducted in the colony room at an ambient temperature of 22±2°C, and neither food nor water was available during the tests.

### *LSD-like discriminative stimulus effects in rats*

Six small animal test chambers (Med-Associates Model ENV-008), each equipped with a house light and an exhaust fan and housed in larger lightproof Malaguard sound-attenuating cubicles (Med-Associates Model ENV-022M), were used for these experiments. The chamber contained two levers mounted on opposite sides of one wall. Centered between the levers was a dipper that delivered 0.1 ml of sweetened condensed milk diluted 2:1 with tap water.

Twenty subjects were trained to discriminate LSD (0.1 mg/kg, 15-min pretreatment time, i.p. injection) from saline, as described previously (Fiorella et al. 1995a,b). A nonresetting fixed ratio 10 (FR10) schedule of reinforcement was employed using the MED-PC version IV behavioral programming application. Drug-induced stimulus control was assumed to be present when, in five consecutive sessions, 83% or more of all responses prior to the delivery of the first reinforcer were on the appropriate lever. The LSD training dose produced ~99.5% drug-appropriate responding. After stimulus control was established with the training agents, tests with 2C-T-7 and R(−)-DOM were conducted once per week in each animal so long as performance did not fall below the criterion level of 83% correct responding in any one of the previous three training sessions. Half of the test sessions were conducted the day after saline training sessions with the remainder following LSD training sessions. During test sessions, no responses were reinforced and the session was terminated after the emission of ten responses on either lever. The distribution of responses between the two levers was expressed as a percentage of total responses emitted on the drug-appropriate lever. Response rate was calculated for each session by dividing the total number of responses emitted on both levers by the elapsed time prior to ten responses on either lever.

### *2C-T-7-induced stimulus control in rats*

In rats trained with LSD as a discriminative stimulus, maximum generalization to 2C-T-7 was observed at a dose of the latter drug of 1.0 mg/kg with minimal effects on response rate (see Fig. 3). For that reason, a dose of 1.0 mg/kg of 2C-T-7 was chosen for training. The general procedure was that described above for training with LSD with the exception that a pretreatment time of 45 min was used. The pretreatment time was based on the observation in LSD-trained subjects that maximum generalization of LSD to 2C-T-7 occurs after 45 min (see Fig. 3). In a group of ten rats, criterion performance was reached after a mean of 33 sessions (SE=2). To establish the role of 5-$HT_{2A}$ receptors in the stimulus effects of 2C-T-7, 0.05 mg/kg

M100907 was injected i.p. 15 min before 2C-T-7, i.e., 60 min before testing. This dose of M100907 has previously been used by our lab to block stimulus control by LSD (Winter et al. 2004).

Complete generalization of a training drug to a test drug is said to be present when (1) a mean of 80% or more of all test responses occurs on the drug-appropriate lever; (2) there is no statistically significant difference between the response distributions of the training drug and the test drug; and (3) there is a statistically significant difference between the response distributions of the test drug and saline control sessions. An intermediate degree of generalization is defined as being present when response distributions after a test drug are less than 80% drug appropriate and are significantly different from both training conditions. Finally, when the response distribution after a test drug is not statistically significantly different from that in saline control sessions, an absence of generalization of the training drug to the test drug is assumed. Similar criteria are applied to the definitions of full, partial, and no antagonism. Thus, full antagonism is assumed to be present when (1) less than 20% of all test responses are on the training drug-appropriate lever; (2) there is no significant difference between the response distributions in the test of antagonism and the saline control; and (3) there is a statistically significant difference between the response distributions of the test drug alone and in combination with the antagonist.

### *Competition binding in rat brain*

Frontal cortex (5-$HT_{2A}$ receptors), hippocampus (5-$HT_{1A}$ receptors), and brain stem (5-$HT_{2C}$ receptors) were harvested from male CDF rats (Charles Rivers Laboratories) and homogenized (Dounce tissue grinder) in 50 mM Tris–HCl (pH 7.4). Homogenates were centrifuged at 40,000×*g* for 15 min at 4°C, and the resulting pellets were resuspended in the Tris buffer and stored at −80°C. On the day of the assays, tissue samples were thawed and centrifuged at 40,000×*g* for 15 min at 4°C. The resulting pellets were resuspended in 30 ml warm 50 mM Tris–HCl (pH 7.4) and incubated for 10 min at 37°C to remove endogenous serotonin. Samples were again centrifuged at 40,000×*g* for 15 min at 4°C. Final resuspension of the pellets (frontal cortex, 6.7 mg/ml; hippocampus, 5 mg/ml; brain stem, 13.3 mg/ml) was carried out in Tris assay buffer (50 mM Tris–HCl, pH 7.4, containing 4 mM $MgCl_2$, 10 μM pargyline, and 0.1% ascorbate).

[$^3$H]8-OH-DPAT binding assays were carried out for 30 min at 37°C in a final volume of 0.5 ml Tris assay buffer containing 1 nM radioligand (129 Ci/mmol; Perkin-Elmer, Boston, MA), appropriate drugs, and hippocampal membranes (2 mg wet weight/tube). [$^3$H]Ketanserin binding assays were carried out for 30 min at 30°C in a final volume of 0.5 ml Tris assay buffer containing 1.5 nM radioligand (88 Ci/mmol; Perkin-Elmer), 100 nM prazosin to prevent binding to $\alpha_1$-adrenergic receptors, appropriate drugs, and frontal cortical membranes (2 mg wet weight/tube). [$^3$H]Mesulergine binding assays were carried out for 45 min at 37°C in a final volume of 0.5 ml Tris assay buffer containing 2 nM radioligand (77 Ci/mmol; Amersham Biosciences), 100 nM spiperone to prevent binding to 5-$HT_{2A}$ and dopamine $D_2$ receptors, appropriate drugs, and membranes from the brain stem (4 mg wet weight/tube). Reactions were terminated by rapid vacuum filtration (Brandel harvester) through GF/B glass fiber filters presoaked in 0.1% polyethylenimine. Filters were washed twice with cold 50 mM Tris–HCl (pH 7.4), and the amount of bound radioactivity was measured by scintillation spectrophotometry. Nonspecific binding was defined as the difference in the amount of radioligand binding in the absence and presence of either 10 μM 5-HT ([$^3$H]8-OH-DPAT binding), 20 μM 5-HT ([$^3$H]mesulergine binding), or 100 μM cinanserin ([$^3$H]ketanserin binding).

### Data analysis

Data from the HTR experiments are presented as mean± SEM and were compared to values obtained from equivolume saline controls using one-way ANOVA and Tukey's post hoc tests. These statistical tests were performed using commercially available software, and significance was judged at $P<0.05$. Drug discrimination data are expressed as percent drug-appropriate responding, which is the number of responses emitted on the drug-appropriate lever as a percentage of the total number of responses emitted. Response rates are expressed as the number of responses per minute, calculated for each session by dividing the total number of responses emitted (prior to the emission of ten responses on either lever) by elapsed time. Data for any subjects failing to emit ten responses within the constraints of the 10-min test session were not considered in the calculation of the percent drug-appropriate responding but were included in the analysis of response rates. Generalization was said to occur if 80% or more of the responses were on the drug-appropriate lever. The statistical significance of the generalization of LSD to 2C-T-7 in rats trained with LSD and the antagonism of 2C-T-7 by M100907 in rats trained with 2C-T-7 was determined using one-way ANOVA to compare the two training conditions with 2C-T-7 and with 2C-T-7 in the presence of M100907, respectively. Subsequent multiple comparisons were made by the method of Student–Newman–Keuls. Differences were considered to be statistically significant if the probability of their having arisen by chance was <0.05. All analyses were conducted using SigmaStat 2.03 for Windows (Jandel Scientific Software, San Rafael, CA). Control data were repeated for each comparison and statistical analyses were applied using the appropriate control sessions. However, for purposes of clarity, mean values for control data are shown in all figures. Binding data were analyzed by nonlinear regression using the program EBDA/LIGAND (Elsevier BIOSOFT).

### Drugs

(+)-LSD, R(−)-DOM, and 2C-T-7 were supplied by the National Institute on Drug Abuse (Research Technology Branch, Research Triangle Park, NC) and dissolved in 0.9% physiological saline solution. M100907 was synthesized at Laboratory of Medicinal Chemistry at the National Institutes of Diabetes, Digestive and Kidney Disorders at the National Institutes of Health (Bethesda, MD) and dissolved in sterile water and 0.5 N HCl. All injections were administered i.p. at a volume of 1.0 ml/kg (rats) or 1.0 ml/100 g (mice).

## Results

### Drug-elicited head twitch response in mice

2C-T-7 induced a dose-dependent HTR in mice, producing a maximum of ~16 twitches in 10 min at a dose of 1.0 mg/kg (Fig. 2, left panel, closed circles). Doses of 1.0 and 3.0 mg/kg 2C-T-7 elicited significantly more head twitch behavior than did saline ($q$=12.078 for 1.0 mg/kg, $q$=6.650 for 3.0 mg/kg; $P$<0.05 for both doses). Pretreatment with 0.01 mg/kg M100907 shifted the 2C-T-7 HTR curve down and to the right (Fig. 2, left panel, open triangles). Following antagonist pretreatment, 2C-T-7 doses of 3.0 and 10.0 mg/kg induced significantly more head twitch behavior than did saline ($q$=13.282 for 3.0 mg/kg, $q$=5.481 for 10.0 mg/kg; $P$<0.05 for both doses). Similar effects were obtained with the phenylisopropylamine hallucinogen R(−)-DOM (Fig. 2, right panel). Doses of 1.0 and 3.0 mg/kg R(−)-DOM elicited significantly more head twitch behavior than did saline ($q$=6.548 for 1.0 mg/kg, $q$=4.688 for 3.0 mg/kg; $P$<0.05 for both doses). Following antagonist pretreatment, all R(−)-DOM doses tested induced significantly more head twitch behavior than did saline ($q$=6.095 for 1.0 mg/kg, $q$=7.850 for 3.0 mg/kg, and $q$=4.248 for 10.0 mg/kg; $P$<0.05 for all three doses). Both compounds were comparable in terms of potency and effectiveness in the drug-elicited head twitch assay.

### LSD-like discriminative stimulus effects in rats

Rats trained to discriminate 0.1 mg/kg LSD from saline performed with a high degree of precision on subsequent testing. Indeed, the training dose of LSD engendered almost 100% drug-appropriate responding, whereas saline occasioned essentially no LSD-appropriate responding (Fig. 3, top left panel). LSD partially generalized to 2C-T-7 in a dose-dependent manner, occasioning a maximum of ~75% drug-appropriate responding at doses of 1.0 mg/kg [$F$(7,2)=29.487; $P$<0.001] and 3.0 mg/kg [$F$(4,2)=19.136; $P$<0.001] (Fig. 3, top left panel). Direct suppressant effects on response rates were observed at all 2C-T-7 doses tested (Fig. 3, top right panel), and responding was almost completely eliminated at 3.0 mg/kg 2C-T-7, preventing the testing of higher doses for LSD-like effects.

Similar to 2C-T-7, R(−)-DOM also occasioned LSD-like responding at a dose of 0.3 mg/kg; these stimulus effects were observed within 15 min postinjection and remained apparent at 45 min postinjection (Fig. 3, bottom left panel, open diamonds). The time course for the discriminative effects of 2C-T-7 (Fig. 3, bottom left panel, closed squares) was similar to that of R(−)-DOM, although 2C-T-7 may have a somewhat slower onset in the rat.

### 2C-T-7-induced stimulus control in rats

Rats trained to discriminate 1 mg/kg 2C-T-7 from saline performed in an almost completely drug-appropriate manner when tested with the training dose, and 2C-T-7-appropriate responding decreased in a dose-dependent manner when rats were injected with lower test doses (Fig. 3, bottom right panel, closed squares). Consistent with results

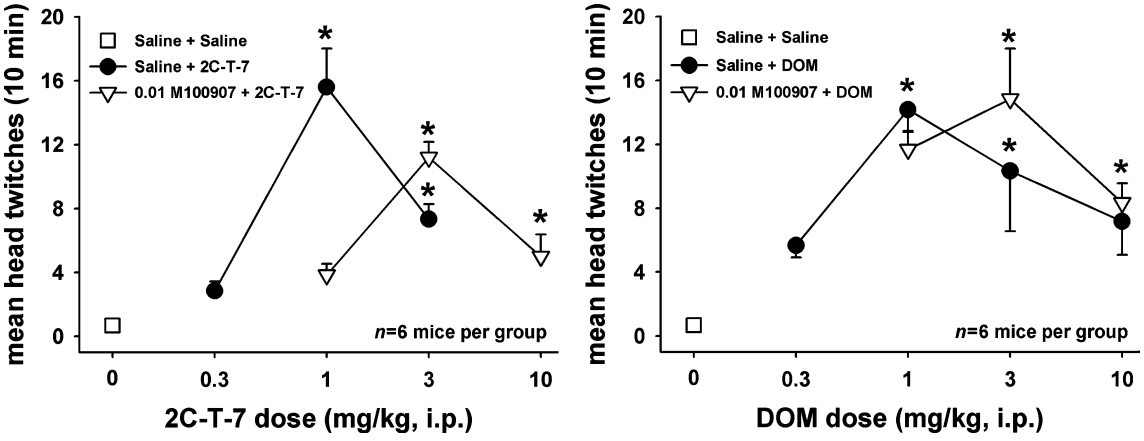


**Fig. 2** Effects of 2C-T-7 (*left*) and R(−)-DOM (*right*) on head twitch behavior in mice treated with M100907 or saline. Each *point* represents the mean±SEM ($n$=6 mice per dose). *Abscissae*: 2C-T-7 or R(−)-DOM dose (mg/kg, i.p.). *Ordinates*: Mean head twitches/10 min. *Asterisks* indicate significant differences from saline controls ($P$<0.05) by Student's one-sample $t$-test

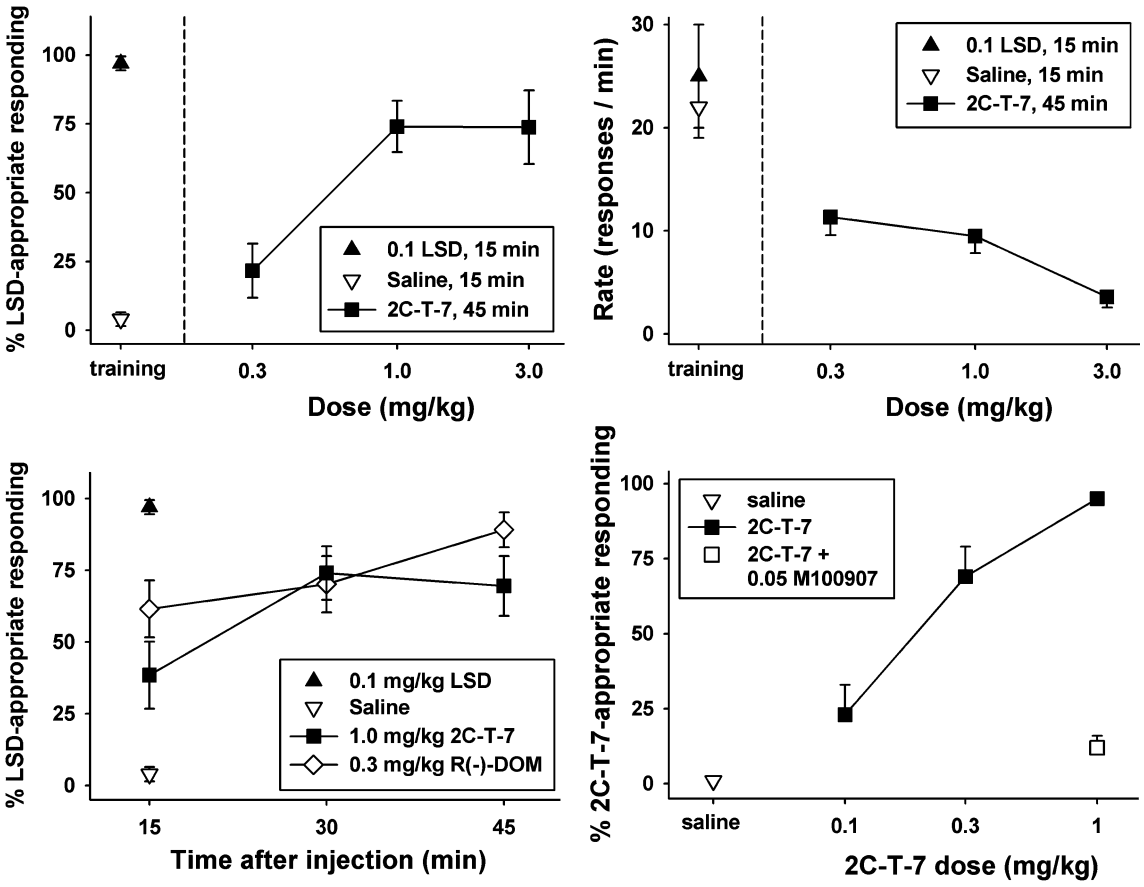


**Fig. 3** *Upper panels*: discriminative stimulus (*left*) and rate-altering (*right*) effects of 2C-T-7 (*squares*) in rats discriminating between 0.1 mg/kg LSD (*triangle*) and saline (*inverted triangles*). *Ordinates*: average percentage of responses on the LSD-associated lever (*left*) or rate of lever pressing in responses/min (*right*). *Abscissae*: dose in mg/kg body weight. *Bottom left panel*: comparison of the time course of discriminative stimulus effects of 1.0 mg/kg 2C-T-7 and 0.3 mg/kg R(−)-DOM in rats discriminating between 0.1 mg/kg LSD and saline. *Ordinate*: as described in *upper left panel*. *Abscissa*: time (min) after 2C-T-7 or R(−)-DOM injection. *Bottom right panel*: discriminative stimulus effects of 2C-T-7 in rats trained to discriminate 1 mg/kg 2C-T-7 from saline. *Filled squares* represent control rats; *open squares* represent rats treated with 0.5 mg/kg M100907 15 min prior to testing. *Ordinate*: average percentage of responses on the 2C-T-7-associated lever. *Abscissa*: dose in mg/kg body weight

from the head twitch experiments, 0.05 mg/kg M100907 effectively antagonized the discriminative cue induced by 1 mg/kg 2C-T-7, as drug-appropriate responding fell to saline-like levels following M100907 injection (Fig. 3, bottom right panel, open square) [$F(7,2)=327.802$; $P<0.001$].

## Competition binding in rat brain

2C-T-7 has nanomolar affinity for 5-$HT_{2A}$ and 5-$HT_{2C}$ receptors and lower affinity for 5-$HT_{1A}$ receptors. The affinity of 2C-T-7 for 5-$HT_{2A}$ receptors is equivalent to that of R(−)-DOM and R(−)-DOI. Because the present behavioral results strongly suggest an involvement of 5-HT receptors in the effects of 2C-T-7, the affinity of this compound at 5-$HT_{2A}$, 5-$HT_{2C}$, and 5-$HT_{1A}$ receptors was determined. The equilibrium dissociation constant ($K_I$) for 2C-T-7 at the 5-$HT_{2A}$ receptor, as measured using [$^3$H]ketanserin, was 120 nM (p$K_I$=6.92). By way of comparison, the $K_I$ for (−)-DOI at the 5-$HT_{2A}$ receptor was 141 nM (p$K_I$=6.85), whereas the $K_I$ for (−)-DOM at this site was 513 nM (p$K_I$=6.29). The $K_I$ for 2C-T-7 at 5-$HT_{2C}$ receptors, which was determined using [$^3$H]mesulergine, was 39 nM (p$K_I$=7.41). Binding affinity of 2C-T-7 at the 5$HT_{1A}$ receptor, as measured using [$^3$H]8-OH-DPAT, was appreciably lower with a $K_I$ of 1.17 μM (p$K_I$=5.93). Binding affinities for 2C-T-7 at these three 5-HT receptors are summarized in Table 1.

**Table 1** Affinity of 5-$HT_{1A}$, 5-$HT_{2A}$, and 5-$HT_{2C}$ receptors for 2C-T-7

| | p$K_I$ ($K_I$) [$^3$H]8-OH-DPAT | p$K_I$ ($K_I$) [$^3$H]ketanserin | p$K_I$ ($K_I$) [$^3$H]mesulergine |
|---|---|---|---|
| 2C-T-7 | 5.93±0.078 (1,175 nM) | 6.92±0.169 (120 nM) | 7.41±0.023 (39 nM) |

Binding of 2C-T-7 to the various serotonin receptors was carried out as described in Materials and methods. For comparative purposes, affinity of the 5-$HT_{2A}$ receptor for R(−)-DOM and (−)-DOI was also determined (see Results). Data are expressed as the negative log of the equilibrium dissociation constant (p$K_I$) and are presented as mean±SEM of three to eight separate experiments. Equilibrium dissociation constants, $K_I$, are presented in the parentheses

## Discussion

The presently reported results suggest that 2C-T-7 is behaviorally active in two rodent models of hallucinogen effects. The capacity of this compound to induce the head twitch response in the mouse, to engender hallucinogen-like discriminative stimulus effects in the rat, and the disruption of both of these effects by prior injection of M100907 imply that a primary site of action for 2C-T-7 is the 5-$HT_{2A}$ receptor. This receptor has previously been implicated in the mediation of hallucinogen effects for the ergoline (LSD-like), indolealkylamine (dimethyltryptamine-like), and phenylisopropylamine (mescaline-like) hallucinogens (Sadzot et al. 1989; Aghajanian and Marek 1999; Nichols 2004). The similar potencies and efficacies of 2C-T-7 and the more traditional phenylisopropylamine hallucinogen R(−)-DOM across both assays are notable and comport well with the finding (from the presently reported binding studies) that the affinities of both of these compounds for the 5-$HT_{2A}$ receptor in rat brain homogenates are identical.

The observation of drug-elicited behaviors in mice can be immensely helpful in the initial characterization of the pharmacological actions of new compounds in vivo. For instance, the Straub tail reaction (contraction of the sacrococcygeus muscle, resulting in erection of the tail) is readily observed following administration of μ opioids, and it has been suggested that observation of this behavior is a sufficient determinant of opioid activity in mice (Aceto et al. 1969). However, the effects most characteristic of the hallucinogens (such as distortions, intensifications and mixing of the senses, alterations in the perception of the passage of time, etc.) are largely unobservable, even in the human. Nevertheless, the induction of a head twitch response in rodents seems to be a common property of the hallucinogenic drugs, and there is now general agreement that this behavior is mediated by 5-$HT_{2A}$ receptors (Dave et al. 2002; Dursun and Handley 1996; Schreiber et al. 1995). This is not to suggest that all drugs inducing the head twitch response are hallucinogenic. Indeed, head twitches are induced by various serotonergic, but not hallucinogenic, agents (i.e., Green et al. 1983; Darmani 1998). Thus, the induction of a head twitch response should not be taken as prima facie evidence for hallucinogenic effects without the further study of a given compound's subjective effects.

In this regard, stimulus control induced by a given drug is often found to correlate closely with the subjective effects of that drug in humans (Schuster and Johanson 1988; Sanger et al. 1994; Brauer et al. 1997). Thus, for example, it is expected that morphine-trained rats will generalize to other drugs whose morphine-like effects have been established in human subjects. Conversely, if a drug of unknown subjective properties fully mimics morphine in morphine-trained rats, we may predict with some confidence that the drug will be morphine-like in humans. In further contrast with the opiates, a class of drugs with established medical uses, hallucinogens are generally illegal and, with notable exceptions (e.g., Strassman et al. 1996; Vollenweider 1998), are at the present time seldom studied in controlled settings. Of necessity, much of our information regarding the effects of newer hallucinogens is largely anecdotal in nature. Nonetheless, accounts of the effects of 2C-T-7 in human subjects by Shulgin and Shulgin (1991), as well as those posted online, leave little doubt as to the hallucinogenicity of this compound. Based upon those reports and the previously described generalization of LSD in LSD-trained rats to R(−)-DOM (Winter and Rabin 1988) and vice versa (Glennon et al. 1983a,b), we would expect 2C-T-7 to substitute for LSD. The data of Fig. 3 only partially fulfill that prediction. It is seen that a maximum of 75% LSD-appropriate responding followed the administration of doses of 2C-T-7 of 1.0 and 3.0 mg/kg with all doses of 2C-T-7 producing significant suppression of the rate of responding relative to that of both training conditions. Suppression of responding precluded the testing of higher doses of 2C-T-7. The time course for 2C-T-7 and the related phenethylamine hallucinogen, R(−)-DOM, in LSD-trained rats is quite similar (Fig. 3) but with the suggestion of a somewhat slower onset and more rapid decline for 2C-T-7.

We are unaware of previous attempts to train 2C-T-7 as a discriminative stimulus. In the only previous report of the stimulus effects of 2C-T-7, rats trained with SR(±)-DOM generalized completely to 2C-T-7, but rats trained with cocaine or racemic 3,4-methylenedioxymethamphetamine (MDMA) did not (Khorana et al. 2004). In the present study, stimulus control was readily established after a mean of 33 sessions at a dose of 1.0 mg/kg using a pretreatment time of 45 min. The rate of acquisition of stimulus control by 2C-T-7 under these conditions may be compared with that for LSD. In a group of six F-344 rats recently trained in our laboratory using a dose of LSD of 0.1 mg/kg (i.p.) and a 15-min pretreatment time, a mean of 22 (SEM=1) sessions was required to reach criterion performance with a rate of lever-press responding of 27 (SEM=6) per minute. In contrast with the modest difference between the number of sessions required to reach criterion performance, the training dose of 2C-T-7 is clearly rate suppressant relative to LSD (Fig. 3). However, like LSD, 2C-T-7-induced stimulus control is completely antagonized by the 5-$HT_{2A}$-selective antagonist, M100907 (Fig. 3; Winter et al. 2004). The data of Table 1 indicate that 2C-T-7 has nanomolar affinity for 5-$HT_{2A}$ and 5-$HT_{2C}$ receptors and a lower affinity for 5-$HT_{1A}$ receptors. The affinity of 2C-T-7 for 5-$HT_{2A}$ receptors is equivalent to that of R(−)-DOM. These binding data are completely compatible with the behavioral effects of 2C-T-7 observed in the present studies.

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