

## RESEARCH ARTICLE

# Effects of the Trace Amine Associated Receptor 1 Agonist RO5263397 on Abuse-Related Behavioral Indices of Methamphetamine in Rats

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## Abstract

**Background:** Methamphetamine is a major drug of abuse with no effective pharmacotherapy available. Trace amine associated receptor 1 is implicated in cocaine addiction and represents a potential therapeutic target. However, the effects of trace amine associated receptor 1 agonists on addiction-related behavioral effects of methamphetamine are unknown.

**Methods:** This study examined the effects of a trace amine associated receptor 1 agonist RO5263397 on methamphetamine-induced behavioral sensitization, methamphetamine self-administration, cue- and methamphetamine-induced reinstatement of drug seeking, and cue-induced reinstatement of sucrose-seeking behaviors in rats. Male Sprague-Dawley rats were used to examine the effects of methamphetamine alone and in combination with the trace amine associated receptor 1 agonist RO5263397 (3.2–10 mg/kg).

**Results:** RO5263397 dose-dependently attenuated the expression of behavioral sensitization to methamphetamine, reduced methamphetamine self-administration, and decreased both cue- and a priming dose of methamphetamine-induced reinstatement of drug-seeking behaviors. However, RO5263397 did not alter cue-induced reinstatement of sucrose-seeking behavior.

**Conclusions:** Taken together, trace amine associated receptor 1 agonists attenuate some abuse-related behavioral effects of methamphetamine, strongly suggesting that drugs activating trace amine associated receptor 1 may be potentially useful for the treatment of methamphetamine addiction and warrant further studies.

**Keywords:** TAAR 1, methamphetamine, self-administration, reinstatement, rats

## Introduction

Methamphetamine and related stimulant abuse and addiction remain a significant global health problem, but there is currently no effective pharmacotherapy available (Karila et al., 2010). Given the critical roles of dopaminergic and glutamatergic systems in the abuse-related behavioral effects of methamphetamine (Ohmori et al., 1996; Zhang et al., 2001; Newman et al., 2012),

ongoing preclinical and clinical efforts continue to focus on the pharmacological modulation of these neurotransmitter systems (Karila et al., 2010; Newman et al., 2012; Chesworth et al., 2013; Crawford et al., 2013; Verrico et al., 2013; Ferragud et al., 2014).

Trace amines are a family of naturally occurring, low concentration amines in the central and peripheral systems that

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traditionally include  $\beta$ -phenylethylamine, *p*-tyramine, octopamine, and tryptamine. Although the existence of trace amines in mammalian brains has been recognized for decades, their physiological roles are largely unknown and controversial due to the lack of known specific receptors (see Grandy, 2007 for a detailed review). In 2001, a new G protein coupled receptor that now is known as trace amine associated receptor 1 (TAAR 1) was cloned and demonstrated to recognize these trace amines (Borowsky et al., 2001; Bunzow et al., 2001). Recently, TAAR 1 has been emerging as a novel target that modulates both dopaminergic and glutamatergic activity (Grandy, 2007; Miller, 2011; Revel et al., 2011). Genetic knockout TAAR 1 in mice leads to a behavioral phenotype that is hypersensitive to psychostimulant-induced psychomotor stimulation, striatal dopamine release, and conditioned place preference (CPP) (Lindemann et al., 2008; Achat-Mendes et al., 2012). Because TAAR 1 is expressed at key brain regions of drug reinforcement and addiction such as ventral tegmental area and amygdala (Lindemann et al., 2008; Achat-Mendes et al., 2012), recent studies have begun to examine the impacts of pharmacological modulation of TAAR 1 on the behavioral effects of cocaine. For example, TAAR 1 agonists have been shown to reduce cocaine-induced hyperactivity and behavioral sensitization (Revel et al., 2013; Thorn et al., 2014a, 2014b), CPP (Thorn et al., 2014b), cocaine self-administration (Thorn et al., 2014b), and reinstatement of cocaine-seeking behavior (Pei et al., 2014; Thorn et al., 2014b). These data suggest that TAAR 1 may be a potentially useful drug target for the development of pharmacotherapy of cocaine abuse and dependence (Li, 2014). Furthermore, although both methamphetamine and cocaine raise dopamine levels extracellularly, methamphetamine differs from cocaine in that it is a substrate for the dopamine transporter rather than a blocker, and it is an agonist at TAAR 1, whereas cocaine is not. Accordingly, it is important to determine whether pharmacological manipulation of TAAR 1 alters methamphetamine-associated behaviors in a manner similar to or different from its pharmacological manipulation of the behavioral effects of cocaine. Clinically, methamphetamine and cocaine have important differences regarding abuse and dependence patterns and public health consequences (Simon et al., 2002a, 2002b; Borders et al., 2008).

This study examined whether a TAAR 1 agonist RO5263397 could modulate some behavioral effects of methamphetamine in rats. RO5263397 was studied in rats using 3 different paradigms: methamphetamine treatment-induced behavioral sensitization, intravenous (i.v.) methamphetamine self-administration, and cue- and drug-induced reinstatement to methamphetamine-seeking behavior. Because RO5263397 attenuated both cue- and drug-induced reinstatement of methamphetamine seeking, the effects of RO5263397 on cue-induced reinstatement of sucrose-seeking were also studied to examine the behavioral specificity of RO5263397 on the reinstatement effects (ie, drug vs natural reinforcer).

## Methods

### Subjects

Adult male Sprague-Dawley rats (Harlan, Indianapolis, IN), weighing 280 to 300g, were housed individually in a temperature- and humidity-controlled environment with a 12-light/dark cycle (lights on at 0700 AM) (behavioral experiments were conducted during the light period). In methamphetamine and sucrose self-administration studies, rats were given unlimited access to food and water at all phases except on food-training

sessions, during which food supply was restricted to 12 g/d. In a behavioral sensitization study, rats received unlimited access to food and water. Animals were maintained and experiments conducted in accordance with the Institutional Animal Care and Use Committee, University at Buffalo, and with the 2011 Guide for the Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources on Life Sciences, National Research Council, National Academy of Sciences, Washington, DC).

### Surgery

Rats that participated in methamphetamine self-administration and reinstatement studies received i.v. catheterization surgery. Rats were initially trained to press either of 2 levers for food (45-mg dustless precision pellets, Bio-Serv, Flemington, NJ) in daily 60-minute sessions; a press of either lever delivered food with a maximum of 100 pellets per session under a fixed ratio (FR) 1 schedule. In the second session, only presses on the left lever would deliver food (active lever), whereas presses on the right lever were recorded but had no programmed consequence (inactive lever). The response requirement was increased to FR 2 in the following 3 sessions. After lever press training, a chronic indwelling catheter was implanted to the jugular vein under ketamine/xylazine anesthesia (72.0 and 6.0 mg/kg, intraperitoneally [i.p.], respectively) according to a published procedure (Thorn et al., 2014b). Briefly, one end of the catheter was inserted 3 cm into the right external jugular vein. The other end exited from a small incision in the animal's back and attached to an infusion harness that provided access to an external port for i.v. drug delivery. Catheters were flushed daily throughout the experimental period with a 0.2-mL solution of enrofloxacin (4.54 mg/mL) mixed in a heparinized saline solution (50 IU/mL in 0.9% sterile saline) to preserve catheter patency. Rats were allowed 5 days to recover from the surgery before self-administration experiments began.

In the course of these studies, catheter failure occasionally occurred, as indicated by leakage of fluid or resistance in flushing the catheter; confirmation was the failure to achieve rapid sedation after injection via the catheter of 1.0 mg/rat of ketamine. Once confirmed, a new catheter was surgically implanted in the opposite jugular vein; rats were allowed 2 days of recovery.

### Apparatus

In behavioral sensitization experiments, locomotor activity was monitored by an infrared motor-sensor system (AccuScan Instruments, Columbus, OH) fitted outside clear acrylic chambers (40 × 40 × 30 cm) that were cleaned between test sessions. Locomotor activity (distance travelled) was analyzed with the Versa Max animal activity monitoring software (AccuScan Instruments, Columbus, OH). All self-administration experiments were conducted in 12 standard operant chambers in sound-attenuating cubicles (Med Associates, St. Albans, VT) as described elsewhere (An et al., 2012). Each chamber was equipped with a house light that signaled the start of the session and was turned off during the timeout periods. On one wall of the chamber were 2 response levers and a food receptacle (5 × 5 cm opening) located 3 cm above the chamber floor and midway between the levers. A pellet dispenser delivered 45-mg food pellets. Stimulus lights located above each lever illuminated the chamber during sessions. Infusion pumps were located outside of the sound-attenuating cubicles and delivered drug or vehicle via Tygon tubing through a swivel (Instech, Plymouth Meeting, PA). Stimulus presentations, food delivery, drug infusion, and

data recording were controlled by Med-PC IV software and solid-state interface equipment (Med Associates).

### Behavioral Sensitization

Behavioral sensitization experiments were conducted according to our published procedure (Thorn et al., 2014a, 2014b). Three groups of rats were used in this study ( $n=7$  per group). Prior to testing, all rats were habituated to the test chambers for 1 h/d on 2 consecutive days. Next, a 100-minute session (four 25-minute components) was conducted during which a methamphetamine dose-effect curve was determined by using a cumulative dosing procedure (designed as day 1 for brevity hereafter). Animals were administered (i.p.) with saline and incremental doses of methamphetamine (0.32, 1.0, and 3.2 mg/kg) during the first minute of each of the 4 components, respectively. Locomotion was continuously recorded by 5-minute bins for 100 minutes. Data from the first 5-minute bin of each component were always excluded from analysis, because animals consistently showed hyperactivity within this bin due to injection and handling. Thus, only data from the last 20 minutes of each component were used in the analysis. From days 2 to 6, rats received an injection of methamphetamine (1.5 mg/kg, i.p.) once daily and their locomotor activity was measured for 1 hour. On day 7, the methamphetamine dose-effect curve was redetermined, and the animals were then left drug-free in their home cages for 6 days. A methamphetamine dose-effect curve was redetermined on day 14. Vehicle or RO5263397 (3.2 and 10 mg/kg, i.p.) pretreatments were administered immediately before the start of the session.

### Methamphetamine and Sucrose Self-Administration

In the study that examined the effects of the TAAR 1 agonist RO5263397 on methamphetamine self-administration behavior, rats ( $n=8$ ) were initially trained to self-administer a maximum of 40 i.v. infusions of methamphetamine (0.03 mg/kg/infusion) under a FR 2 schedule of reinforcement during daily 2-hour sessions. Drug deliveries were accompanied by the presentation of a stimulus light (5-second illumination) over the active lever, followed by a 30-second time-out period during which lever presses had no programmed consequence. Following stable responding (the variance of total number of injections was <20% within 3 days), saline and different doses of methamphetamine (0.01, 0.03, and 0.1 mg/kg/infusion) were substituted for the maintenance dose of methamphetamine among animals non-systematically. When stable responses were reached under each condition, vehicle and 3.2 mg/kg RO5263397 were administered 10 minutes prior to the session at different sessions separated by at least 2 days. RO5263397 was studied only once at each dose of methamphetamine in each subject.

In the study that examined the effects of RO5263397 on the reinstatement of methamphetamine-seeking behavior ( $n=8$  per group), the same self-administration procedure was used except that the maintenance dose of methamphetamine was 0.05 mg/kg/infusion and the animals self-administered the same dose of methamphetamine for 14 consecutive sessions. For the sucrose self-administration study, the training conditions were identical except that animals did not receive surgery and they could earn a maximum of 100 reinforcers per session (45 mg chocolate-flavored sucrose pellets, Bio-Serv, Flemington, NJ).

Following self-administration training, extinction of drug-seeking behavior took place during daily 2-hour sessions in which lever pressing produced saline instead of methamphetamine injection and the methamphetamine-paired stimulus

light was omitted. For extinction of sucrose-seeking behavior, the stimulus light was turned off during the sessions and lever pressing produced no consequences. All other conditions remained the same. Rats reached the extinction criteria after 7 extinction sessions (the total responses on the active lever during the last extinction session were significantly lower than those during the last self-administration session).

### Reinstatement of Methamphetamine- or Sucrose-Seeking Behavior

Rats were divided into 3 groups ( $n=8$  per group) based on their performance in the last extinction session to evaluate the effects of RO5263397 on reinstatement of methamphetamine-seeking behavior. Cue-induced reinstatement was conducted on the next day of the last extinction session, during which completion of the response requirement resulted in illumination of the previously drug-paired cue lights with no drug delivery. Vehicle or RO5263397 (3.2 and 5.6 mg/kg) were administered 10 minutes before the start of the session. Two more extinction sessions were conducted following the cue-induced reinstatement test session to further extinguish the cue-reinstated drug-seeking behavior, which was followed by a methamphetamine priming-induced reinstatement test. Vehicle or RO5263397 (3.2 and 5.6 mg/kg) was administered 10 minutes prior to the priming dose of methamphetamine (1 mg/kg, i.p.). For cue-induced reinstatement to sucrose-seeking behavior, the procedure was identical to that of cue-induced reinstatement to methamphetamine-seeking test.

### Data Analyses

Data are expressed as mean  $\pm$  SEM. Baseline methamphetamine self-administration data were analyzed by a 1-way repeated-measures analysis of variance (ANOVA). The effects of RO5263397 on methamphetamine self-administration and expression of methamphetamine sensitization were analyzed by a 2-way repeated-measures ANOVA (methamphetamine dose  $\times$  RO5263397 treatment). Differences in active lever responses between the last extinction session and reinstatement session were determined with paired *t* tests. The effects of RO5263397 on reinstatement of methamphetamine- and sucrose-seeking behavior were analyzed by a 1-way ANOVA. All ANOVA analyses were followed by posthoc Bonferroni's test.  $P < .05$  was considered statistically significant.

### Drugs

Drugs used in this study included methamphetamine hydrochloride (Research Technology Branch, National Institute of Drug Abuse, Rockville, MD) and RO5263397 (synthesized at Research Triangle Institute; purity >98%). Methamphetamine hydrochloride was dissolved in 0.9% physiological saline. RO5263397 was dissolved in a mixture of 1 part absolute ethanol, 1 part Emulphor-620 (Rhodia Inc., Cranbury, NJ), and 18 parts physiological saline and i.p. administered. Doses were expressed as the weight of the forms listed above in milligrams per kilogram of body weight.

## Results

### Effects of RO5263397 on the Expression of Methamphetamine Sensitization

Methamphetamine increased the locomotor activity on day 1 in all the animals (significant main effect of methamphetamine:

F [3, 18]=137.1,  $P<.0001$ ), and the level of methamphetamine-induced hyperactivity was not significantly different among the 3 groups (Figure 1a). Repeated 1.5mg/kg methamphetamine led to significant behavioral sensitization, and the entire dose-effect curve of methamphetamine was markedly shifted leftward (compare open circles between Figure 1a and 1b) such that the effects of smaller doses of methamphetamine (0.32 and 1.0mg/kg) were significantly enhanced ( $P<.05$ ), whereas the effect of the largest dose of methamphetamine (3.2mg/kg) was significantly decreased ( $P<.05$ ) (significant main effects of day: F [1, 18]=13.18,  $P<.01$  and methamphetamine $\times$ day interaction: F [2, 18]=18.04,  $P<.0001$ ). Acute treatment with RO5263397 dose-dependently shifted the dose-effect curve of methamphetamine downward (significant main effects of methamphetamine dose: F [2, 18]=22.03,  $P<.0001$  and RO5263397 treatment: F [2, 36]=12.92,  $P<.0001$ ). Posthoc analyses indicated that the effects of 0.32 and 1.0mg/kg methamphetamine were significantly reduced (Figure 1b).

### Effects of RO5263397 on Methamphetamine Self-Administration

Methamphetamine maintained self-administration responding that was greater than the responding maintained by saline, as indicated by the differences in the number of infusions received in the 2-hour sessions (open circles, Figure 2). With increasing unit doses of methamphetamine (0.01 and 0.03mg/kg/infusion), the number of infusions increased and then, with larger unit dose (0.1mg/kg/infusion), decreased, resulting in an inverted U-shaped dose-effect curve (1-way repeated-measures ANOVA: F [3, 21]=10.31,  $P<.001$ ). Posthoc analyses indicated that methamphetamine at doses of 0.032 and 0.1mg/kg/infusion maintained a significantly higher number of infusions than that of saline. Pretreatment with 3.2mg/kg RO5263397 did not significantly alter saline self-administration but significantly decreased methamphetamine self-administration. Two-way repeated-measures ANOVA revealed significant main effects of methamphetamine dose (F [3, 21]=6.9,  $P<.01$ ), RO5263397 treatment (F [1, 7]=27.25,  $P<.01$ ), and methamphetamine dose $\times$ RO5263397 treatment interaction (F [3, 21]=6.73,  $P<.01$ ). Posthoc analyses indicated that 3.2mg/kg RO5263397 significantly decreased 0.01 and 0.032mg/kg/infusion methamphetamine-maintained number of infusions.

### Effects of RO5263397 on Cue- and Drug-Induced Reinstatement of Methamphetamine Seeking

Animals took  $12\pm 2$  injections during the last self-administration session at a dose of 0.05 mg/kg/infusion ( $38.7\pm 6.6$  responses

on the active lever). During the first extinction session, the total number of responses on the active lever decreased to  $24.2\pm 4.4$  responses. Responses on the active lever continued to decrease during the repeated extinction sessions and decreased to  $11.4\pm 1.8$  responses on the last extinction session. After the cue-induced reinstatement test, 2 more extinction sessions decreased the cue-reinstated responses to  $12.2\pm 2.2$  responses (1-way repeated-measures ANOVA: F [3, 88]=8.93,  $P<.0001$ ). Posthoc analyses indicated that the total responses on the active lever were significantly lower on the 2 extinction sessions immediately before the reinstatement tests ( $P<.0001$ ) (Figure 3a). Exposure to previously drug-associated cues significantly reinstated the lever-pressing behavior ( $t [7]=3.0$ ,  $P<.05$ ). RO5263397 significantly attenuated cue-reinstated lever pressing, with both 3.2- and 5.6-mg/kg doses showing similar effects (1-way ANOVA: F [2, 21]=8.0,  $P<.01$ ; posthoc analyses;  $P<.05$  and  $P<.01$  for 3.2 and 5.6 mg/kg RO5263397, respectively) (Figure 3b). Similarly, a priming injection of methamphetamine significantly reinstated the lever-pressing behavior ( $t [7]=4.3$ ,  $P<.01$ ). RO5263397 significantly and dose-dependently attenuated drug prime-reinstated lever-pressing behavior (1-way ANOVA: F [2, 21]=4.57,  $P<.05$ ; posthoc analyses;  $P<.05$  and  $P<.01$  for 3.2 and 5.6mg/kg RO5263397, respectively) (Figure 3c).

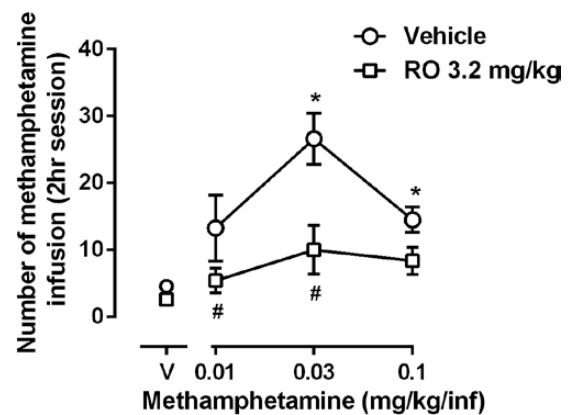


Figure 2 RO5263397 decreased the number of infusions of methamphetamine (0.01, 0.03, 0.1 mg/kg/infusion) obtained during daily 2-hour self-administration sessions ( $n=8$ ). Data points above "V" represents results when saline was self-administered with vehicle or 3.2mg/kg RO5263397 was given as a pretreatment. \* $P<.05$  compared with data above "V" when vehicle was given as a pretreatment; # $P<.05$  compared with data from the same dose of methamphetamine and pretreated with vehicle. See Figure 1 for other details.

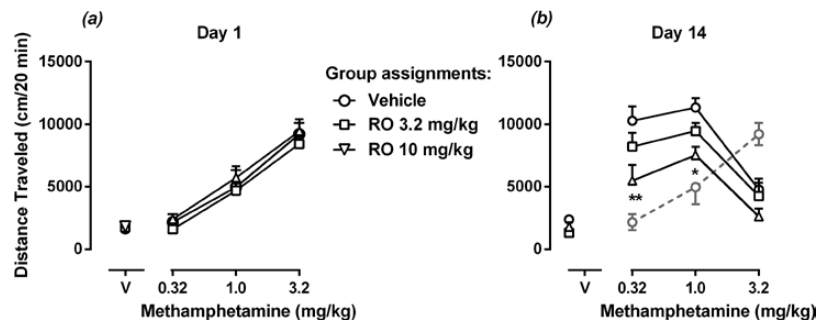
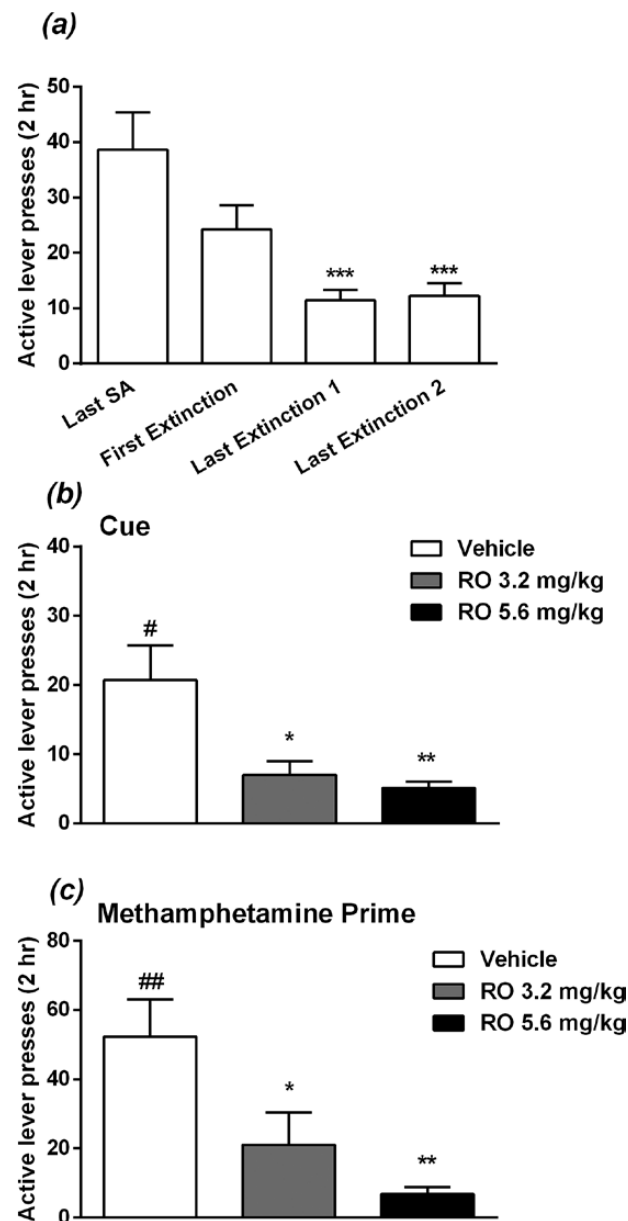


Figure 1. RO5263397 attenuated the expression of methamphetamine-induced behavioral sensitization. (a) Acute methamphetamine administration-induced hyperactivity in 3 groups of rats ( $n=7$ /group). (b) Acute treatment with RO5263397 significantly reduced challenge doses of methamphetamine-induced expression of behavioral sensitization ( $n=7$ ; \* $P<.05$  compared with vehicle group). Dashed line represents the replotted data of vehicle group on day 1 for comparison with day 14. Data represent the mean $\pm$ SEM. The absence of error bars indicates that the variability is contained within the data point. RO, RO5263397; V, vehicle.

## Effects of RO5263397 on Cue-Induced Reinstatement of Sucrose Seeking

Animals earned  $22 \pm 2$  sucrose pellets during the last self-administration session ( $67.3 \pm 5.8$  responses on the active lever). During the first extinction session, the total number of responses on the active lever decreased to  $56.7 \pm 4.3$  responses. Responses on the active lever continued to decrease during the repeated extinction sessions and decreased to  $6.5 \pm 0.8$  responses on the last extinction session (1-way ANOVA:  $F [2, 69] = 64.8, P < .0001$ ).



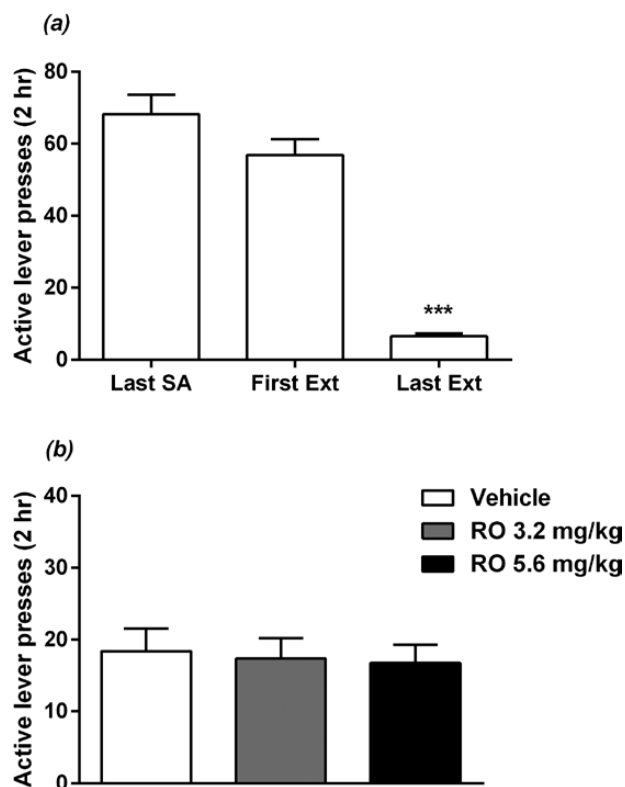
**Figure 3.** RO5263397 blocked cue- and methamphetamine prime-induced reinstatement of extinguished methamphetamine-seeking behavior ( $n = 8/\text{group}$ ). (a) Total active lever responses during the last self-administration session, the first extinction session, and 2 extinction sessions prior to the reinstatement tests ( $***P < .001$  compared with the last self-administration session). Last extinctions 1 and 2 referred to the last extinction session before cue- and drug-induced reinstatement tests, respectively. (b) RO5263397 significantly reduced cue-induced reinstatement of active lever responding. (c) RO5263397 significantly attenuated 1mg/kg methamphetamine-induced reinstatement of active lever responding. \* $P < .05$ , \*\* $P < .01$  compared with vehicle-treated group. SA, self-administration. See Figure 1 for other details.

Posthoc analyses indicated that the number of total responses on the last extinction day was significantly lower than that of the last self-administration session ( $P < .0001$ ) (Figure 4a). Exposure to previously sucrose-associated cues significantly reinstated the lever-pressing behavior ( $t [7] = 3.5, P < .01$ ). Both 3.2 and 5.6 mg/kg RO5263397 did not significantly alter cue-induced responding (1-way ANOVA:  $F [2, 21] = 0.1, P > .05$ ) (Figure 4b).

## Discussion

The primary findings of the current study were that the TAAR 1 agonist RO5263397 attenuated the psychomotor-sensitizing effects of methamphetamine and methamphetamine self-administration. In addition, RO5263397 reduced methamphetamine-seeking behavior triggered by either drug-associated cues or methamphetamine priming, whereas it did not affect sucrose-seeking behavior, suggesting that RO5263397 may have the potential to treat methamphetamine relapse and the effect seems to be behaviorally specific. Taken together, these results represent the first systematic study to show that TAAR 1 is critically involved in the abuse-related behavioral effects of methamphetamine and that RO5263397 may be a potentially useful agent against methamphetamine abuse and dependence.

Increasing evidence suggests that TAAR 1 participates in the modulation of the dopaminergic system. TAAR 1 is expressed in brain regions that are crucial for drug reinforcement such as ventral tegmental area and amygdala and is coexpressed in a subset of dopaminergic neurons in substantia nigra (Borowsky et al., 2001; Xie and Miller, 2009). In addition, activation of TAAR 1 inhibits [ $^3\text{H}$ ]dopamine uptake and promotes [ $^3\text{H}$ ]dopamine efflux by the dopamine transporter (Xie and Miller, 2009). TAAR



**Figure 4.** Effects of RO5263397 on cue-induced reinstatement of sucrose-seeking behavior ( $n = 8/\text{group}$ ).  $***P < .001$  compared with the last self-administration session. See Figures 1 and 3 for other details.

1-knockout mice are more sensitive than their wild-type counterparts to methamphetamine for the psychomotor-stimulating effects and rewarding effects (as measured by CPP) (Achat-Mendes et al., 2012). These data suggest that TAAR 1 plays a modulatory role and may serve as a homeostatic “brake” on dopaminergic activity (Xie and Miller, 2009; Li, 2014). Recently, results from several behavioral studies employing selective TAAR 1 agonists support this postulation. It was found that TAAR 1 agonists reduced the psychomotor-sensitizing effects (Thorn et al., 2014a, 2014b), rewarding effects (Thorn et al., 2014b), and reinforcing effects of cocaine (Revel et al., 2013; Thorn et al., 2014b). TAAR 1 agonists have also been shown to reduce drug-associated cue- and drug prime-induced reinstatement of cocaine-seeking (Pei et al., 2014; Thorn et al., 2014b). Thus, activation of TAAR 1 by its agonists seems to attenuate the abuse-related effects of cocaine.

This study was designed to examine whether a TAAR 1 agonist RO5263397 modifies the behavioral effects of methamphetamine. RO5263397 is a highly selective TAAR 1 agonist. In a receptor selectivity screening assay, RO5263397 binds only to TAAR 1 with a high affinity but does not show appreciable binding to >150 other receptors, enzymes, and ion channels at a concentration of 10  $\mu\text{M}$  (Revel et al., 2013). In this study, RO5263397 significantly attenuated the expression of methamphetamine-induced behavioral sensitization, a commonly used rodent model of repeated drug exposure-induced behavioral and neural plasticity (Steketee and Kalivas, 2011). In addition, RO5263397 significantly reduced both drug intake-associated cue- and drug prime-induced reinstatement of methamphetamine-seeking behavior, a widely used animal model of relapse behavior in humans (Bossert et al., 2013). Although the animal grouping for reinstatement test was conducted based on their performance on the last extinction session (total responses), a retrospective analysis of the data found that there were no significant differences on the total number of methamphetamine injections on the first and the last self-administration sessions, and the total cumulative methamphetamine intake among the different groups are comparable (data not shown). Thus, the observed effects of RO5263397 were unlikely due to the differences of the baseline self-administration performance among the animals. The neurobiological mechanisms including the neural circuitry, neurotransmitters, and receptor systems underlying behavioral sensitization and reinstatement of drug-seeking behavior are largely overlapping (Steketee and Kalivas, 2011). The findings that RO5263397 attenuated both methamphetamine-induced behavioral sensitization and reinstatement to methamphetamine-seeking behaviors suggest that TAAR 1 agonists may modulate similar mechanisms underlying both behaviors. Importantly, RO5263397 did not decrease the reinstatement of sucrose-seeking behavior, suggesting that activation of TAAR 1 does not generally suppress the motivational states but rather specifically reduces drug- and drug-associated cues-related motivational properties.

RO5263397 was found to significantly attenuate methamphetamine self-administration, shifting the methamphetamine dose-effect curve downward. We previously found that RO5263397 reduced the reinforcing effectiveness of cocaine using a demand curve analysis in rats self-administering cocaine (Thorn et al., 2014b). Combined with these results, it is suggested that reducing the intake of psychostimulants may be a general effect of TAAR 1 agonists, which may not be limited to cocaine or methamphetamine. This effect of RO5263397 is not due to general behavioral suppression but is behaviorally

specific, because we have previously shown that these doses of RO5263397 do not decrease spontaneous activity in rats (Thorn et al., 2014b) and they did not reduce operant responding in cue-reinstated sucrose-seeking study (Figure 4b). Although the neurochemical and cellular mechanisms are unknown, RO5263397 likely reduced the methamphetamine self-administration through negatively modulating the dopaminergic and possibly glutamatergic activity (Xie and Miller, 2009; Miller, 2011; Revel et al., 2011). For example, a TAAR 1 agonist RO5203648 inhibits cocaine-stimulated dopamine overflow as measured by fast scan cyclic voltammetry, which is correlated with the effects of RO5203648 on reducing reinstatement to cocaine seeking (Pei et al., 2014).

In summary, the current study systematically examined the functional role of TAAR 1 activation in abuse-related behavioral actions of methamphetamine. We demonstrated that activation of TAAR 1 reduces methamphetamine-related behavioral effects of clinical relevance, including methamphetamine taking and methamphetamine seeking after behavioral extinction. These findings strongly support the potential of developing TAAR 1 agonists as candidate medications for methamphetamine abuse and dependence.

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## Statement of Interest

None.

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