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Effects of the Trace Amine-Associated Receptor 1 Agonist RO5263397 on Abuse-Related Effects of Cocaine in Rats

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Animal knockout studies suggest that trace amine-associated receptor (TAAR) 1 is involved in behavioral effects of psychostimulants such as cocaine. Recently, several highly selective TAAR 1 agonists have been discovered. However, little is known of the impact of TAAR 1 agonists on abuse-related effects of cocaine. Here, we report the effects of a TAAR 1 agonist RO5263397 on several abuse-related behavioral effects of cocaine in rats. RO5263397 was evaluated for its effects on cocaine-induced behavioral sensitization, conditioned place preference (CPP), cue- and cocaine prime-induced reinstatement of cocaine-seeking behavior, and cocaine self-administration using behavioral economic analysis. RO5263397 reduced the expression of cocaine behavioral sensitization, cue- and cocaine prime-induced reinstatement of cocaine seeking, and expression but not development of cocaine CPP. Behavioral economic analysis showed that RO5263397 increased the elasticity of the cocaine demand curve, but did not change cocaine consumption at minimal prices. Taken together, this is the first systematic assessment of a TAAR 1 agonist on a range of behavioral effects of cocaine, showing that RO5263397 was efficacious in reducing cocaine-mediated behaviors. Collectively, these data uncover essential neuromodulatory roles of TAAR 1 on cocaine abuse, and suggest that TAAR 1 may represent a novel drug target for the treatment of cocaine addiction.

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INTRODUCTION

Cocaine addiction is a chronic relapsing brain disorder that currently has no Food and Drug Administration-approved pharmacotherapy (Vocci and Elkashef, 2005). Given the prominent roles of dopaminergic and glutamatergic systems in the abuse-related behavioral effects of cocaine (Bradberry, 2000; Kalivas *et al*, 2009; Kalivas and Volkow, 2011; Kimmel *et al*, 2012), preclinical and clinical studies have focused on both systems in the development of potential medications to treat cocaine addiction (Bergman *et al*, 2013; Kalivas and Volkow, 2011; Kampman *et al*, 2011). Drugs that directly modulate (eg, receptor agonists or antagonists) either dopaminergic or glutamatergic systems have thus far yielded subpar results either due to intolerable adverse effects or due to the lack of effectiveness (Kampman *et al*, 2011; Pierce *et al*, 2012). New targets that indirectly modulate dopaminergic and glutamatergic systems may provide a novel pharmacotherapy as well as further insight into the mechanisms of cocaine addiction.

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Emerging evidence suggests that trace amine-associated receptor (TAAR) 1 is a novel target that modulates both dopaminergic and glutamatergic activity (Grandy, 2007; Miller, 2011; Revel *et al*, 2011; Xie and Miller, 2009). TAAR 1 expression has been localized to brain regions that are key for drug reward and addiction such as ventral tegmental area (VTA) and amygdala (Lindemann *et al*, 2008). Initial understanding of the functionality of TAAR 1 has been through the use of knockout mice, which have an increased sensitivity to psychostimulant-induced hyperactivity, conditioned place preference (CPP), and striatal dopamine release (Achat-Mendes *et al*, 2012; Lindemann *et al*, 2008). Until recently there have been no pharmacologically selective TAAR 1 ligands available (Revel *et al*, 2011; Revel *et al*, 2013), which has greatly hindered the ability to understand the role of TAAR 1 in cocaine-mediated behaviors and pharmacological intervention. Recent studies have described a number of pharmacologically highly selective TAAR 1 ligands. For example, a selective TAAR 1 antagonist, EPPTB, increases while a TAAR 1 agonist, RO5166017, decreases the firing frequency of dopamine neurons of the VTA *in vitro* (Bradaia *et al*, 2009; Revel *et al*, 2011). In addition, several TAAR 1 agonists have been shown to attenuate acute cocaine-induced hyperactivity in mice (Revel *et al*, 2011; Revel *et al*, 2012; Revel *et al*, 2013) and the development of behavioral sensitization to cocaine in rats (Thorn *et al*, 2014). Despite these recent advances, no study has utilized validated

animal models of drug abuse to systematically examine the effects of TAAR 1 agonists on cocaine addiction, which is critically needed to establish the potential utility of targeting TAAR 1 for the treatment of cocaine addiction.

Here, we investigated the effects of a selective TAAR 1 partial agonist, RO5263397, on the sensitizing, rewarding, and reinforcing effects of cocaine. Finally, to assess the therapeutic potential of TARR 1 agonists in relapse behaviors, we examined the ability of RO5263397 to alter cue-induced and cocaine-primed reinstatement of cocaine-seeking behaviors (Bossert *et al*, 2013; Epstein *et al*, 2006).

MATERIALS AND METHODS

Animals

Adult male Sprague–Dawley rats (initial weight 250–280 g; Harlan, Indianapolis, IN) were housed individually on a 12/12-h light/dark cycle (behavioral experiments were conducted during the light period) with free access to water and food except during experimental sessions. For self-administration reinstatement studies, food access was restricted to 10 g/day for 3 days before lever press training for food. Animals were maintained and surgical and experimental procedures were approved by the Institutional Animal Care and Use Committee, University at Buffalo, the State University of New York, 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).

Food Training and Catheterization Surgery

Twelve standard operant chambers (Med Associates, St Albans, Vermont) were used for all cocaine self-administration studies and the details were described elsewhere (An *et al*, 2012; Boctor *et al*, 2007). Stimulus presentations, food dispensation, cocaine infusions, and data recording were controlled by Med-PC IV software (Med Associates, St Albans, Vermont). For cocaine self-administration reinstatement experiments, rats were first trained to lever press for food reward (45 mg; BioServ, Frenchtown, New Jersey). During the daily 1 h training sessions, rats could press the right lever (active lever) under a fixed ratio 1 (FR 1) schedule and earn up to 100 food pellets. The response requirement was gradually increased to FR 5 over a period of 7 days. Responses on the left lever (inactive lever) were recorded but had no programmed consequence. Rats that did not earn 100 food pellets under FR 5 schedule on day 7 were excluded from the study (three rats were excluded). After the food training, rats were implanted with chronic indwelling jugular catheters and allowed 7 days to recover following surgery as previously described (Gancarz *et al*, 2012). Catheters were flushed daily with 0.2 ml solution of enrofloxacin (4 mg/ml) mixed in a heparinized saline solution (50 IU/ml in 0.9% sterile saline) to preserve catheter patency. At the end of behavioral testing, each animal received an intravenous infusion of ketamine hydrochloride (0.5 mg/kg in 0.05 ml saline) and the behavioral response was observed to verify catheter patency. Loss of muscle tone and righting reflexes served as behavioral indicators of patency. Only rats with patent catheters were used in data analysis.

Cocaine Self-Administration

One week after surgery, rats began to self-administer 0.75 mg/kg/inf cocaine. Rats self-administered cocaine for 14 sessions, during which responses to the active lever resulted in intravenous injections of cocaine under a FR 5 schedule of reinforcement followed by a 30-s time-out period. Infusions were accompanied by a 5-s illumination of the stimulus light above the active lever and the house light was extinguished for the duration of the time-out period. Sessions were terminated after either a 2-h duration or 40 infusions had been earned, whichever occurred first. Following cocaine self-administration sessions, rats then were given seven daily extinction sessions, during which lever presses had no consequence (no drug or cues). Cue- or drug (10 mg/kg cocaine, intraperitoneal (i.p.))-induced reinstatement was tested 24 h after the last day of extinction session, during which active lever presses resulted in presentation of light cues in the same manner as during self-administration with no drug delivery.

Cocaine Behavioral Economic Demand Curve Assessment

Two groups of rats ($n = 7$ for vehicle treatment; $n = 11$ for drug treatment) were used in this study and they did not receive food training. One week after surgery, rats were trained to self-administer 0.75 mg/kg/inf cocaine daily for 8 days. For the first three sessions, the schedule of reinforcement was FR 1. FR was increased to 3 for the following five sessions. All rats maintained a stable self-administration behavior for the last three sessions (variance of the number of injections $< 20\%$ among the three sessions). Then, the FR ratios were progressively increased every three sessions according to the following series: 3, 10, 18, 32, 56, 100, 178, 320, and 560 (Galuska *et al*, 2011). If there was a considerable variability in the number of injections earned at a particular ratio, four or more sessions were conducted until the data met the criteria of stabilization. The ratio increased until zero reinforcers were earned for at least one of the two sessions, or until the FR 560 was reached. Drug (5.6 mg/kg RO5263397, i.p.) or its vehicle was administered 10 min before the test sessions, which was the last session of each FR.

Cocaine Behavioral Sensitization

Locomotor activity was monitored by an infrared motor-sensor system (AccuScan Instruments, Columbus, Ohio) fitted outside clear acrylic chambers ($40 \times 40 \times 30$ cm) that were cleaned between each test session. The protocol for establishing behavioral sensitization was detailed elsewhere (Thorn *et al*, 2014). Briefly, animals experienced two 1 h habituation sessions in the locomotion chambers before the experiment began. On day 1, an 80-min session was conducted during which a cocaine dose-effect curve was determined by using a cumulative dosing procedure. Saline was administered immediately before the start of the test session and different doses of cocaine (cumulative doses of

3.2, 10, 32 mg/kg) were given at times 20 min, 40 min, and 60 min. This procedure generates highly reliable dose-effect curves of morphine and cocaine (Li *et al*, 2013; Thorn *et al*, 2014). The day 1 test session was followed by 7 days of daily cocaine test sessions during which a dose of 15 mg/kg cocaine was administered 20 min after the session started and the locomotor activity was measured for 60 min. On day 9, an identical cocaine dose-effect curve was determined as that of day 1, which was followed by a 7-day washout period when no drug treatment nor test was conducted. On day 17, a cocaine dose-effect curve was redetermined. For this test, a pretreatment was given 10 min before the start of the session when animals received either vehicle or RO5263397 (3.2 or 10 mg/kg, i.p.) administration ($n = 7$ per group).

CPP

The standard experimental chambers for automated assessment of CPP (LE890, Harvard Apparatus, Holliston, MA) consisted of two Perspex compartments of the same size ($30 \times 30 \times 34$ cm) interconnected by a central corridor ($8 \times 10 \times 34$ cm). Both side compartments were equipped with a gray Perspex guillotine door that separated the end compartment from the central gray corridor. Each compartment had distinct, yet neutral, visual and tactile cues. Time spent in each compartment was monitored by a video camera located on the ceiling and controlled by video-tracking software (Smart Junior, Harvard Apparatus, Holliston, MA).

CPP studies were performed using an unbiased procedure, which has been described in detail elsewhere (Perrey *et al*, 2013; Thorn *et al*, 2012). Briefly, all rats were allowed free access to both compartments for 15 min to verify the absence of preference for either side. Rats spending more than 75% (>675 s) or less than 25% (<225 s) of the total time in a compartment were eliminated from further testing. Following the pretest phase, rats underwent place conditioning across 8 days, with alternating treatment-compartment pairings. Rats received vehicle-compartment pairing on odd days and cocaine-compartment pairing on even days. Cocaine was always conditioned with the less preferred side of the chambers. For a conditioning session, vehicle or cocaine was administered, and the rat was placed immediately in the paired compartment with no access to the other compartment for 30 min (Perrey *et al*, 2013). On the test day (24 h after last conditioning session), rats were randomly placed into one compartment and had access to both compartments during the 15-min test period, and the time spent in each compartment was recorded. In the study that examined the effect of RO5263397 on the development of cocaine CPP, RO5263397 was administered 10 min before cocaine treatment during the conditioning sessions (a total of four treatments) but no drug was given on the test day. In the study that examined the effect of RO5263397 on the expression of cocaine CPP, RO5263397 was only administered once on the test day, 10 min before the start of the session.

Drugs

Drugs used in this study included cocaine hydrochloride (Research Technology Branch, National Institute of Drug Abuse, Rockville, MD) and RO5263397 (synthesized at Research Triangle Institute, purity $>98\%$). Cocaine hydrochloride was dissolved in 0.9% physiological saline. RO5263397 was dissolved in a mixture of 1 part absolute ethanol, 1 part Emulphor-620 (Rhodia), and 18 parts physiologic saline. Doses were expressed as the weight of the forms listed above in milligrams per kilogram of body weight and drugs were administered i.p.. All doses of RO5263397 used in these experiments were selected based on our previous findings in which there were no effect on general locomotor activity (Thorn *et al*, 2014).

Data Analysis

All results are presented as mean ± SEM. For the behavioral sensitization study, the distance traveled was plotted as a function of cocaine dose. The locomotion data were analyzed by two-way repeated measures analysis of variance (ANOVA; cocaine dose × RO5263397 treatment or cocaine dose × day) followed by *post hoc* Bonferroni's test. For the CPP study, magnitude of place preference was presented as the preference score, which was defined as time spent in the drug-associated compartment on the test day minus time spent in the drug-associated compartment on pretest day. Both CPP data and reinstatement data were analyzed by one-way ANOVA, followed by Bonferroni's *post hoc* test to compare the differences among groups treated with vehicle and different drug doses. Student's *t*-test was used to compare the active lever responding during extinction and reinstatement sessions. $p<0.05$ was considered statistically significant.

For behavioral economic analyses, the number of injections earned per session was plotted as a function of FR. Because reliable responding was not maintained in the majority of rats beyond FR 100, this ratio was used as endpoints in the demand curve determinations. In cases in which individual rats did not earn an injection at a particular ratio, a value of 0.1 was assigned because the log of zero is undefined. Best-fitting functions using the exponential demand model (Hursh and Silberberg, 2008) were fitted to the averaged group data on log-log coordinates. For this model, the following equation was used to fit the data

$$\log Q = \log(Q_0) + \kappa\left(e^{-aP} - 1\right)$$

Q represents the number of reinforcers earned (injections) at each FR, or price (P); Q_0 is an estimate of consumption at zero price and mathematically represents the y-intercept; κ *is* a scaling parameter representing the range of the dependent variable in logarithmic units; and α provides an index of elasticity of demand, with a larger α suggesting a more elastic demand curve and a smaller α suggesting a less elastic demand curve. The α value indexes reinforcing strength and represents the essential value of a commodity (Hursh and Silberberg, 2008). For all curve fits, the κ parameter was set at a common value of 2, because this is the smallest integer power of 10 that results in an ordinate spanning the data range. Between-group comparisons of the α and Q_0 parameters were accomplished using the Mann–Whitney *U*-tests. Curving fitting of individual animals were also conducted and Q_0 and α parameters from individual subjects were analyzed using Student's *t*-test to compare the difference between vehicle- and RO5263397-treated groups.

RESULTS

RO5263397 Attenuated the Expression of Cocaine Behavioral Sensitization

We first tested whether the selective TAAR 1 agonist RO5263397 altered the expression of behavioral sensitization to cocaine when the drug was administered acutely. Cocaine increased the locomotor activity on day 1 in all the animals (significant main effect of cocaine: $F\ [2, 12] = 140.50$, $p < 0.0001$), and the level of cocaine-induced hyperactivity was not different among the three groups (left, Figure 1). Repeated 15 mg/kg cocaine led to significant behavioral sensitization, and the entire dose-effect curve of cocaine was markedly shifted leftward (compare filled circles between left and right panels, Figure 1) such that the effects of smaller doses of cocaine (3.2 and 10 mg/kg) were significantly enhanced ($p < 0.05$), while the effect of the largest dose of cocaine (32 mg/kg) was significantly decreased ($p < 0.05$; significant main effect of day: $F\ [1, 6] = 12.83$, $p < 0.05$; significant cocaine × day interaction: $F\ [2, 12] = 18.12$, $p < 0.001$). We found that acute treatment with RO5263397 dose dependently decreased the dose-effect curve of cocaine (significant main effect of cocaine dose: $F\ [2, 12] = 9.39$, $p < 0.01$; significant main effect of RO5263397 treatment: $F\ [2, 12] = 4.30$, $p < 0.01$). *Post hoc* analysis indicated that the effect of 3.2 mg/kg cocaine was significantly suppressed (right, Figure 1). We have previously shown that the same doses of RO5263397 did not significantly alter acute cocaine-induced hyperactivity (Thorn *et al*, 2014), and these results suggest that TAAR 1 activation may be particularly effective to block the expression of repeated cocaine-induced behavioral plasticity.

RO5263397 Attenuated the Expression of Cocaine CPP

Next, we examined the effects of RO5263397 on the rewarding property of cocaine. As expected (Perrey *et al*, 2013), we found that 10 mg/kg cocaine induced a robust place preference (one-way ANOVA: $F\ [4, 54] = 14.61$, $p < 0.0001$; *post hoc* analysis: $p < 0.0001$; left, Figure 2). RO5263397 did not alter the development of cocaine CPP when it was administered as a pretreatment during cocaine conditioning. RO5263397 itself did not produce place preference nor place aversion at a dose of 10 mg/kg ($p > 0.05$).

Because we found that RO5263397 acutely blocked the expression of behavioral sensitization as described above, we next further examined whether it alters the expression of cocaine-induced CPP. We found that acute RO5263397 dose dependently blocked the expression of cocaine CPP (one-way ANOVA: $F\ [3, 43] = 5.04$, $p < 0.01$; right, Figure 2). In particular, *post hoc* analyses indicated that 10 mg/kg RO5263397 significantly blocked the expression of cocaine-induced CPP.

RO5263397 Reduced Cue-Induced and Cocaine-Primed Reinstatement of Cocaine Seeking

As RO5263397 seemed to consistently reduce the expression of cocaine-induced behavioral changes, we next examined whether RO5263397 modified the reinstatement of extinguished cocaine-seeking behavior. Rats were randomly assigned to treatment groups following cocaine self-administration with no differences between groups in drug intake or responding. During the last self-administration session, rats earned 26.7 ± 0.7 injections of 0.75 mg/kg/inf cocaine. During the first day of extinction, rats pressed 93.7 ± 5.8 responses on the active lever and 21.2 ± 3.2 responses on the inactive lever. As a comparison, rats responded 2.1 ± 0.6 times on the inactive lever during the last cocaine self-administration session. After 7 days of extinction sessions, total responses on the active lever decreased to 18.8 ± 1.9 times, demonstrating a clear behavioral extinction. Following the last day of extinction, the rats were placed back into the operant box and the response-contingent cues were presented. As expected, the presence of cues previously paired with cocaine availability significantly increased the active lever responding ($t\ [43] = 3.23$, $p < 0.01$). However, pretreatment with RO5263397 dose dependently attenuated cue-induced active lever responding (reinstatement; $F\ [3, 32] = 10.17$, $p < 0.0001$; top, Figure 3). *Post hoc* analyses indicated that all three doses of RO5263397 significantly

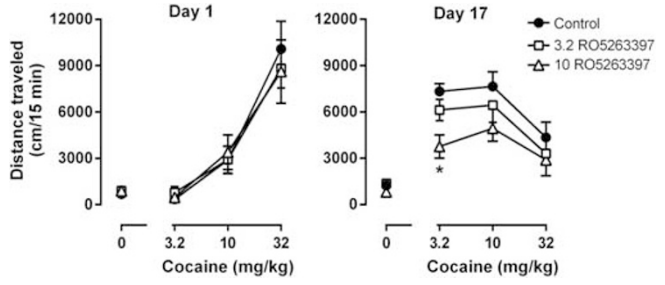

Figure 1 RO5263397 attenuated the expression of cocaine-induced behavioral sensitization. Left panel: acute cocaine administration induced similar hyperactivity in three groups of rats ($n = 7$). Right panel: acute treatment with the trace amine-associated receptor 1 partial agonist RO5263397 significantly reduced challenge doses of cocaine-induced expression of behavioral sensitization on day 17 ($n = 7$; $^{*}p < 0.05$ as compared with control condition). Data represent the mean ± SEM.

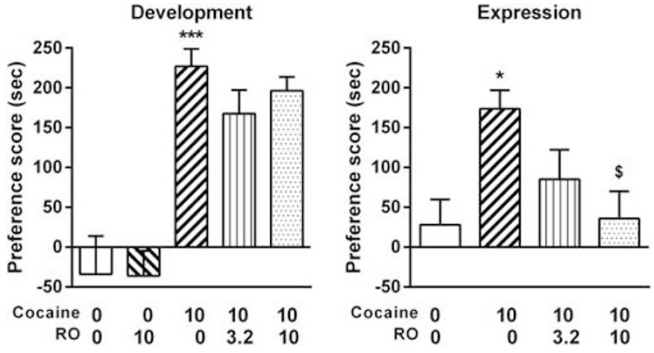

Figure 2 RO5263397 attenuated the expression of cocaine-induced conditioned place preference (CPP). Left panel: RO5263397 did not alter the development of cocaine-induced CPP when the drug was administered during cocaine conditioning ($n = 8$–18; $^{***}p < 0.001$ as compared with vehicle control group). Right panel: RO5263397 dose dependently decreased the expression of cocaine-induced CPP when the drug was administered 10 min before test sessions ($n = 10$–14; $^{*}p < 0.05$ as compared with vehicle control group; $^{\$}p < 0.05$ as compared with 10 mg/kg cocaine group). Data represent the mean ± SEM.

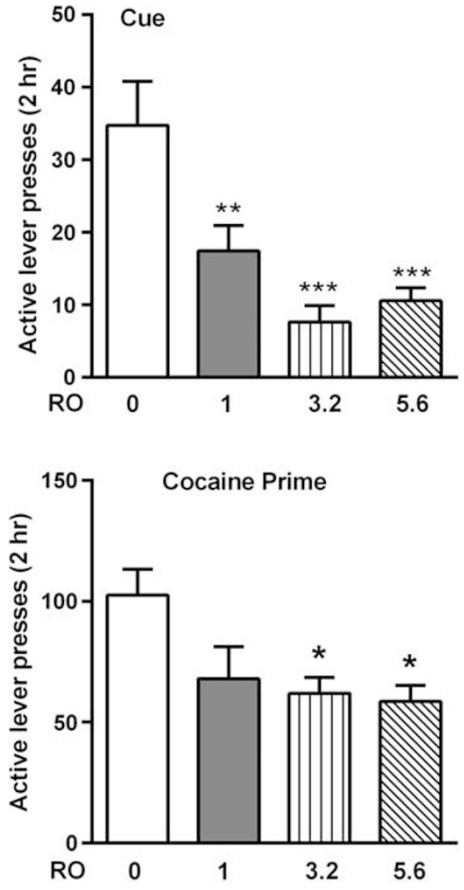

Figure 3 RO5263397 blocked cue- and cocaine prime-induced reinstatement of extinguished cocaine-seeking behavior. Top panel: RO5263397 significantly attenuated cue-induced reinstatement of active lever responding at 1 mg/kg ($n=9$), 3.2 mg/kg ($n=8$), or 5.6 mg/kg ($n=10$), as compared with vehicle ($n=9$; $^{**}p<0.01$, $^{***}p<0.001$ as compared with vehicle control group). Bottom panel: RO5263397 significantly attenuated 10 mg/kg cocaine-induced reinstatement of active lever responding at 3.2 mg/kg ($n=8$) or 5.6 mg/kg ($n=10$) but not 1 mg/kg ($n=9$), as compared with vehicle ($n=9$; $^{*}p<0.05$, $^{**}p<0.01$ as compared with vehicle control group). Data represent the mean ± SEM of active lever responding during the 2-h test sessions.

decreased cue-induced reinstatement of cocaine-seeking behavior when compared to vehicle-treated rats ($p<0.001$).

We next tested whether RO5263397 could also reduce a priming dose of cocaine-induced reinstatement. Following the cue-induced reinstatement session, two more extinction sessions decreased the active lever responses to 16.7 ± 1.4 responses. As expected, a priming injection of cocaine (10 mg/kg) markedly reinstated the active lever responding to 102.5 ± 10.8 responses (bottom, Figure 3) ($t\,[43]=12.83$, $p<0.0001$), an effect that was significantly and dose dependently reduced by RO5263397 administration ($F\,[3, 32]=4.68$, $p<0.01$; bottom, Figure 3). *Post hoc* analyses indicated that 3.2 and 5.6 mg/kg RO5263397 significantly decreased cocaine-primed reinstatement of cocaine-seeking behavior compared with vehicle-treated rats ($p<0.05$). Together, these data demonstrated a strong attenuation of both cue- and cocaine-induced reinstatement of cocaine-seeking behavior by the TAAR 1 agonist RO5263397.

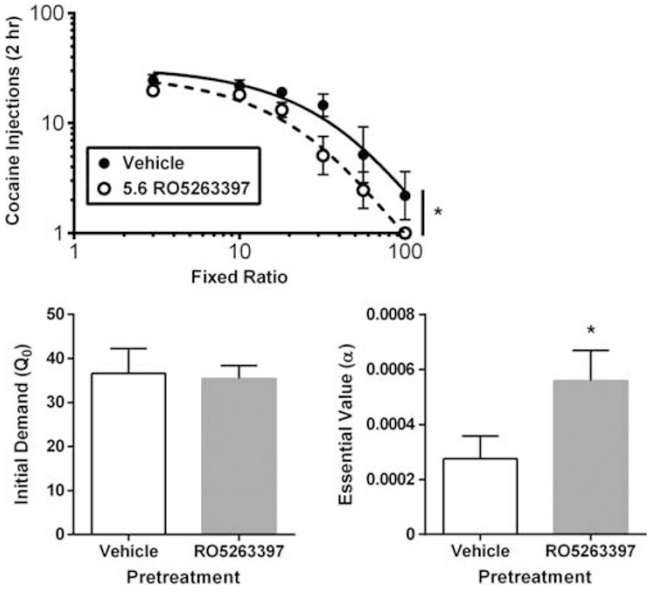

Figure 4 RO5263397 (5.6 mg/kg) increased the elasticity of cocaine demand curve. Top panel: group curve fitting. ($^{**}p<0.01$ when the α values were compared between RO5263397-treated group ($n=11$) and vehicle-treated group ($n=7$)). Bottom panel: data from curve fitting of individual subjects. ($^{*}p<0.05$ when compared with vehicle-treated subjects). Data represent the mean ± SEM of total cocaine injections during the 2-h test sessions under different reinforcement schedules.

Increased Elasticity of Cocaine Demand Curve

Given the significant effects of RO5263397 on the conditioning actions of cocaine in CPP and reinstatement model, we speculated that RO5263397 might reduce economic demand for cocaine, that is, the ability of drug to sustain responding at progressively higher prices, or response requirements. An advantage of demand curve analysis is that it offers an approach to directly assess both cocaine consumption at minimal prices and the elasticity of demand with price increases (Hursh and Silberberg, 2008). A more elastic demand curve indicates that the commodity is less essential and the consumption decreases more quickly with price increases. Curve fitting analyses revealed that the α value of the cocaine demand curve was 0.0002573 ± 0.00000359 in rats treated with vehicle, while the α value was 0.0004647 ± 0.00000446 in rats treated with 5.6 mg/kg RO5263397 (top, Figure 4). The α values between the two cocaine demand curves were significantly different ($p<0.01$), suggesting that the cocaine demand curve was more elastic in the presence of RO5263397. In contrast, the estimated cocaine consumption (number of injections) when the price was 0 (Q_0) was not significantly different between the two conditions (32.4 ± 7.9 *vs* 28.1 ± 5.7; $p>0.05$), suggesting that 5.6 mg/kg RO5263397 did not reduce cocaine consumption at minimal prices (top, Figure 4). Curve fitting analysis of the data from individual subjects revealed the same conclusion (bottom, Figure 4). RO5263397 treatment did not alter the initial demand (Q_0) but significantly increased the essential value (α) of cocaine ($t\,[16]=1.85$, $p<0.05$).

DISCUSSION

These results indicate that the TAAR 1 partial agonist RO5263397 is a potential lead compound for future development of therapeutic intervention to treat cocaine addiction. RO5263397 blocked the cocaine's sensitizing and rewarding effects while reducing the motivation to obtain cocaine. In addition, RO5263397 reduced cocaine-seeking behavior triggered by either drug-associated cues or cocaine prime, suggesting the therapeutic potential of TAAR 1 agonists for maintaining cocaine abstinence. Taken together, our results from this first systematic study using the highly selective TAAR 1 partial agonist RO5263397 reveal the critical functional involvement of TAAR 1 in the abuse-related behavioral actions of cocaine.

TAAR 1, Dopamine, and Pharmacology of RO5263397

Trace amines have long been known to interact with amphetamines. However, these effects were generally thought to be nonspecific because no specific trace amine receptor was discovered until the cloning of TAAR 1 (Bunzow *et al*, 2001; Grandy, 2007). TAAR 1 mRNA is located in various dopaminergic and adrenergic brain nuclei and it is co-expressed in a subset of dopamine neurons in substantia nigra (Borowsky *et al*, 2001; Xie and Miller, 2009). Functionally, TAAR 1 activation inhibits [^{3}H]dopamine uptake by the dopamine transporter in a concentration- and time-dependent manner (Xie and Miller, 2009). In addition, TAAR 1-knockout mice demonstrate greater sensitivity to amphetamine-induced psychomotor stimulation, which is temporally correlated with significantly larger increase in the release of dopamine and norepinephrine in the dorsal striatum (Wolinsky *et al*, 2007). These findings imply that TAAR 1 and its endogenous ligands maintain a modulatory homeostatic 'brake' on dopaminergic activity. However, data to support this concept are just emerging and inconsistencies exist.

Recently, several highly selective TAAR 1 ligands become available. RO5263397 is a selective TAAR 1 receptor partial agonist. In a panel of selectivity screen against >150 receptors and enzymes, RO5263397 only binds to TAAR 1 with high affinity (Revel *et al*, 2013). In two functional assays (cAMP production and electrophysiological recording), RO5263397 demonstrates a typical partial agonist profile on TAAR 1 (Revel *et al*, 2013). In addition, RO5263397 reduces cocaine-induced hyperactivity in mice (Revel *et al*, 2013) but not in rats (Thorn *et al*, 2014). Given the emerging role of TAAR 1 in the modulation of dopaminergic system, this study was designed to assess the functional consequences of TAAR 1 activation on the abuse-related effects of cocaine.

RO5263397 and Abuse-Related Effects of Cocaine

Although studies using knockout mice suggest the involvement of TAAR 1 in some behavioral effects of stimulants such as methamphetamine and cocaine (Achat-Mendes *et al*, 2012; Lindemann *et al*, 2008), it is only possible to systematically explore this notion after selective ligands become available. In this study, the selective TAAR 1 partial agonist RO5263397 reduced cocaine-induced behavioral effects in several animal models related to drug abuse. We found that RO5263397 markedly attenuated the expression of cocaine behavioral sensitization, a widely 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 priming-induced reinstatement of cocaine-seeking behavior, a benchmark animal model of relapse behavior in humans (Bossert *et al*, 2013; Epstein *et al*, 2006). Behavioral sensitization and reinstatement of drug seeking behavior share substantial overlapping neural circuitry, neurotransmitters and receptor systems, and both are widely used models to study the neural basis of drug addiction (Steketee and Kalivas, 2011). Our findings that RO5263397 blocked both cocaine sensitization and reinstatement to cocaine-seeking behavior suggest that TAAR 1 activation may modulate similar mechanisms underlying both behaviors.

Interestingly, we found that RO5263397 decreased the challenge dose of cocaine-induced hyperactivity after the rats were sensitized but not acute cocaine-induced hyperactivity (Thorn *et al*, 2014). Repeated cocaine exposure-induced behavioral sensitization is a consequence of extensive neural adaptations including structural reorganization involving a multitude of signaling pathways, while acute cocaine-induced hyperactivity is predominantly due to prompt increase of neurotransmitters such as dopamine levels in key brain regions such as striatum (Li *et al*, 2004; Uys *et al*, 2011; Wolf and Ferrario, 2010). Together, our findings suggest that repeated cocaine exposure (eg, repeated cocaine-induced sensitization, chronic cocaine self-administration) leads to a status that might be particularly sensitive to the activation of TAAR 1 signaling pathway in comparison with more acute tests, although the mechanism underlying this difference is unknown.

Another interesting finding is that RO5263397 only decreased the expression but not development of cocaine-induced CPP. The development and expression of CPP involve different neural mechanisms, and many potential intervention drugs impair the development and/or expression phases (Tzschentke, 1998). CPP paradigm is relatively insensitive to dose magnitude, which may limit the utility for discovering potential medications to reduce the value of a drug during conditioning (development; Napier *et al*, 2013). The finding that RO5263397 greatly reduced cocaine CPP expression, which is thought to model context-induced craving or relapse, is consistent with the sensitization and reinstatement results, and further supports the clinical relevance of TAAR 1 agonists such as RO5263397 for the treatment of cocaine addiction, particularly the relapse to cocaine use during abstinence.

We found that RO5263397 only decreased cocaine self-administration at higher response requirements, suggesting that TAAR 1 activation decreases the motivation of rats to work for cocaine. In the term of behavioral economics, RO5263397 decreased the essential value of cocaine such that the demand for cocaine with increasing prices became more elastic. However, RO5263397 did not simultaneously reduce cocaine consumption (Q_0) at minimal prices. Similar results have been reported with other drugs. The $GABA_B$ receptor agonist baclofen does not impact the consumption of cocaine but decreases the maximal price paid for cocaine

(Oleson *et al*, 2011). These results indicate that RO5263397 is most effective at reducing cocaine intake at high unit prices. This suggests that when drug availability is reduced, RO5263397 may be particularly effective to reduce both the motivation to take cocaine and drug seeking, which is in congruent with our findings with CPP, sensitization and reinstatement studies.

In this study, some experiments involved repeated dosing with RO5263397 (behavioral economics and CPP), while others involved single dosing (behavioral sensitization and reinstatement). The behavioral procedure (and specific behavioral effects of cocaine) but not the dosing frequency seems to be a determinant of the behavioral effects of RO5263397 because repeated dosing during the development of CPP experiment did not affect cocaine CPP while single dosing during the expression test did while repeated dosing during the development of behavioral sensitization experiment significantly decreases cocaine sensitization (Thorn *et al*, 2014).

CONCLUSIONS

Taken together, our results reveal that TAAR 1 activation reduces both the relapse to cocaine seeking and drug taking when the drug availability is limited. In particular, these data suggest that RO5263397 is effective for decreasing both drug- and drug-associated cue-induced behavioral effects of cocaine. Interestingly, although RO5263397 reduced both cue- and cocaine-induced reinstatement, it is more efficacies on cue-induced than cocaine-primed effect. These results may suggest that RO5263397 is particularly effective against context cue-induced relapse to cocaine addiction. Future studies will further examine this effect in other cue-related effects such as the phenomenon of incubation to cocaine seeking. These data strongly suggest the potential of RO5263397 as a lead compound for the medication development of cocaine addiction, and warrant further investigation of TAAR 1 as a novel drug target for the development of new pharmacotherapy for cocaine addiction.

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The authors declare no conflict of interest.

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