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Activation of trace amine-associated receptor 1 attenuates schedule-induced polydipsia in rats

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HIGHLIGHTS

- RO5263397, the highly selective partial TAAR1 agonist, attenuated SIP in rats.
- This effect of the highest tested doses can be affected by non-specific drug effects.
- The RO5263397 repeated administration did not influence the SIP-attenuating effect.

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ABSTRACT

Trace Amine Associated Receptor 1 (TAAR1) is a novel pharmacological target. TAAR1 are well-documented to play a modulatory role in the dopaminergic system. In spite of a growing number of studies of TAAR1 effects, little is still known about the behavioral pharmacology of TAAR1 ligands, including effects of repeated TAAR1 agonist administration. The present study appears to be the first that estimated the action of TAAR1 agonists on schedule-induced polydipsia, a type of adjunctive behavior, which is considered to be useful for evaluating certain aspects of obsessive-compulsive and related disorders (OCD) and schizophrenia.

Our results have demonstrated that the wide range of RO5263397, the highly selective partial TAAR1 agonist, doses (1–10 mg/kg) attenuated the polydipsia induced by two different schedules of food delivery in rats. The effect remained unchanged for the 7 days of repeated treatment. However, the highest tested doses of RO5263397 (6 and 10 mg/kg) decreased the vertical locomotor activity of the animals and the volume of water intake of thirsty rats following the acute treatment. Also, though, the repeated RO5263397 administration is exhibited to diminish the volume of consumed water and weight of rats without SIP, on the other hand, the tolerance was observed to these drug effects.

In general, the RO5263397 decreases specifically the adjunctive drinking and this effect is maintained with repeated drug administration without the development of tolerance. The interpretation of these results as an evidence for the RO5263397 anticompulsive-like action, however, should be taken with caution because the drug also influenced the drinking behavior and only weakly affected the other parameters of SIP used to reveal the potential anticompulsive-like effects of drugs.

1. Introduction

Trace Amine Associated Receptors (TAARs) are emerging pharmacological target for mental disorders (Gainetdinov et al., 2018). TAARs, which are related to the family of G protein-coupled receptors, were independently discovered by two research groups (Borowsky et al., 2001; Bunzow et al., 2001). The most characterized member of the TAAR family is TAAR1, coupled to G_s, which increases intracellular levels of cAMP (Borowsky et al., 2001; Bunzow et al., 2001; Lindemann et al., 2005). The expression of TAAR1 was documented in primary dopaminergic brain areas (Espinoza et al., 2015a, 2015b; Lindemann et al., 2008; Xie et al., 2007) and TAAR1 is known to affect dopamine

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Table 1
List of experimental procedures.

experimental procedures	# of animals	restriction	experimental experience
ADAE on polydipsia induced by FT 60 s	15	food	no
ADAE on polydipsia induced by FI 60 s	15	food	yes, ADAE on polydipsia induced by FT 60 s
RDAE on polydipsia induced by FT 60 s	16	food	no
ADAE on locomotor activity	10	no	no
ADAE on water consumption of thirsty rats	8	water	no
RDAE on water consumption	15	no	no

Acute drug administration effects = ADAE.
Repeated drug administration effects = RDAE.

(DA) neurotransmission. Mice lacking TAAR1 (TAAR1-KO mice) demonstrated increased behavioral sensitivity to DA agonists (Di Cara et al., 2011; Espinoza et al., 2015a, 2015b; Lindemann et al., 2008; Sukhanov et al., 2016; Xie et al., 2007). Notably, more complex effects observed with apomorphine, which is able to activate TAAR1 (Sukhanov et al., 2014). Furthermore, TAAR1-KO mice showed increased extracellular levels of DA and hyperlocomotion following amphetamine administration (Lindemann et al., 2008). In contrast, whereas basal locomotor activity was not different between TAAR1-overexpressing (TAAR1-OE) and WT mice, the amphetamine-induced hyperlocomotion was strongly decreased in these mutant mice (Revel et al., 2012a).

Recent data with TAAR1 ligands also support a modulatory role of TAAR1 in the dopaminergic system. TAAR1 agonists attenuated the cocaine- and amphetamine-induced hyperlocomotion in mice and rats (Revel et al., 2011, 2012a, 2012b, 2013), as well as spontaneous hyperactivity in dopamine transporter (DAT) knockout mice, a genetic model of hyperdopaminergia (Revel et al., 2011, 2012b).

In addition to DA, TAAR1 could be involved in the regulation of the brain serotonin system. TAAR1 expression was revealed in key serotonin brain structures, such as the dorsal raphe nucleus (Lindemann et al., 2008). Revel et al. (2011) indicated that RO5166017, a TAAR1 agonist, decreased the firing rate of serotonergic neurons. Additionally, in a recent study, a capacity for heteromerization between TAAR1 and serotonin receptors (5HT1b) was demonstrated (Bräunig et al., 2018).

In spite of a growing number of studies of TAAR1 effects, little is still known about the behavioral pharmacology of TAAR1 ligands, including effects of repeated TAAR1 agonist administration. To our knowledge, the present study appears to be the first that estimated the action of TAAR1 agonists on schedule-induced polydipsia (SIP), a type of adjunctive behavior. In the rat model introduced by Falk (1961), it was shown that a scheduled food delivery can elicit the abnormal polydipsia not linked directly to a thirst (Falk, 1961). SIP is considered to be useful for evaluating certain aspects of obsessive-compulsive and related disorders as well as schizophrenia (Hawken and Beninger, 2014; Hawken et al., 2011; Alonso et al., 2015; Moreno and Flores, 2012; Platt et al., 2008). The present study aimed (1) to evaluate the impact of the highly selective partial TAAR1 agonist, RO5263397, on SIP, (2) to specify this effect and (3) to test the tolerability of this action following repeated drug administration. As a result, it was demonstrated that RO5263397 attenuated SIP, which steadily persisted following several repeated trials.

2. Materials and methods

2.1. Animals

Drug and experimentally naïve male Wistar rats (3–4 months old at the beginning of the experiments) were purchased from the State Breeding Farm “Rappolovo” (St. Petersburg, Russia). All animals (n = 64) were housed under a 12-h light/dark cycle (lights on at 08:00 h) at 21 ± 2 °C and 55 ± 15% humidity. In the locomotor activity studies, the rats were housed in groups of five in standard T IV cages (Techniplast, Italy) with corn cob bedding (“KKZ ″Zolotoy pochatok”” OOO, Voronezh, Russia). In the other studies, rats were individually housed in T III cages (Techniplast, Italy) with wood-chip shavings (“TierWohl Classic”, JRS J. Rettenmaier & Söhne Group, Germany) or wood-based animal bedding (Lignocel, BK 8–15, JRS, J. Rettenmaier & Söhne Group, Germany). The cages and water bottles were changed once a week.

For SIP studies, the rats were deprived of food before the start of the experiments to reduce their body weights to approximately 85% of their initial levels. During the SIP experiments, the animals had access to drinking water *ad libitum* but were fed restrictively (12–15 g per day, taking into account the weight measurement results to limit body weight gain to 2–3 g per week). For the measurements of water consumption of thirty animals, throughout the complete experiment (1 month) rats had chow available *ad libitum* but were permitted to drink only for 60 min per day. The information about number of rats for every experimental procedure, access to water and food and repeated use of animals is summarized in Table 1.

All experiments were carried out during the light period of the light/dark cycle after at least one week of habituation to the animal facility. Experimental protocols were approved by the local Animal Care and Use committee of First Pavlov State Saint Petersburg Medical University.

2.2. Drugs

(S)-4-(3-Fluoro-2-methylphenyl)-4,5-dihydrooxazol-2-amine (RO5263397; CAS#: 1357266-05-7), the partial (internal efficacy for rat TAAR1 is 76 ± 13%) TAAR1 agonist, was synthesized at F. Hoffmann-La Roche (Basel, Switzerland). The drug was chosen because (1) RO5263397 has high internal efficacy at the rat receptor; (2) RO5263397 pharmacokinetic properties in rats was published earlier; and (3) the drug was demonstrated to have more prominent effects than RO5263390 in a few *in vivo* studies (Revel et al., 2011; Black et al., 2017).

RO5263397 was dissolved in 1% Tween 80. Fresh solutions of the drugs were prepared daily and administered to rats in a dosing volume of 1 ml/kg.

In experiments involving acute drug administration, dose test sequences were based on a within-subjects Latin Square design. Consecutive drug tests were separated by at least 72 h. The tests were conducted without blinding. The drug doses were chosen according to previously published papers (Jing et al., 2014; Pei et al., 2017). All injections were performed 15 min before experimental sessions (except for the locomotor activity tests, in which the rats were placed into an apparatus immediately after the injections).

In experiments involving repeated drug administration, the animal subgroups were injected with either 12 mg/kg of RO5263397 or the vehicle every 12 h (at 9:00 a.m. and 9:00 p.m.) for 7 consecutive days. The RO5263397 dose and the schedule of the injections were chosen based on previously published data about the pharmacokinetics of RO5263397 (Revel et al., 2013). The treatment duration was chosen in concordance with a review reporting that 7 days is the most common

minimal duration of nonacute studies in neuropsychopharmacology (Bespalov et al., 2016). According to the custom-built model, the dose of 12 mg/kg b.i.d. was expected to result in a RO5263397 plasma concentration higher than 0.67 μg/ml for 14 h per day. The concentration of 0.67 μg/ml corresponded to the maximal concentration achieved 15 min (indicated for mice) after the i.p. administration of RO5263397 at the dose of 3 mg/kg (efficacious dose in the acute SIP experiments).

2.3. Apparatus

2.3.1. Operant chambers

Experiments were carried out in four standard operant conditioning chambers (Skinner boxes) for rats, with interior dimensions of 30.5 cm × 24.1 cm x 29.2 cm (MED Associates, ENV-007, Inc., East Fairfield, VT, USA) located in sound-attenuating and light-proof cubicles. Each cubicle was equipped with an electric fan that provided a background white noise during the experimental sessions. The right wall of each chamber contained three metal panels with built-in appliances. A retractable lever (ENV-112BM) was set up on the first panel, 4.5 cm from a stainless steel grid serving as a floor. On the second panel, a speaker (ENV-223AM) was situated at the top of the panel, and a pellet tray (ENV-200R2MA) equipped with a pair of nose-poke photobeam infrared sensors (ENV-254-CB) was located 2 cm from the floor, where 45-mg food pellets (P.J. Noyes Inc., Lancaster, New Hampshire, USA) were delivered from a dispenser located outside the chamber. A water bottle (ENV-250RM) was fitted to the external side of the third panel in such a way that the drinking spout of the bottle was obtainable for the rats from the interior of the chamber, in an aperture 5 cm × 5 cm × 3.5 cm (width × height × depth), approximately 3 cm from the floor. The spout was connected to a contact lickometer controller to record licks. A house light (25 W) was installed at the top of the left wall of each chamber and was turned on during experiments. Chamber inputs were connected to an operating PC equipped with Med-PC software through the MED interface (MED Associates, East Fairfield, VT, USA). The volume of water consumed during each session was measured by weighing the bottle at the start and end of the session.

2.3.2. Locomotor activity boxes

Locomotor behavior of rats was evaluated in two sets of five identical boxes (25 cm × 35.5 cm × 34 cm), each with transparent Plexiglas walls and a nontransparent plastic floor enclosed within sound-attenuating ventilated cubicles. The light intensity inside the apparatus was 30–40 lx. Each box was equipped with 11 pairs of photocell-based infrared sensors. Three pairs of photocell units located 5 cm above the bottom of the box were used to record horizontal activity. In addition, eight pairs of photocell units were placed 14 cm above the floor to record vertical activity. The total number of repetitive vertical beam breaks (vertical activity) and sequential beam breaks (the number of ambulations) were recorded by MED-PC software (MED Associates, East Fairfield, VT, United States).

2.4. Experimental procedure

2.4.1. Fixed-time 60 s schedule of food delivery

Rats were first habituated to the operant chambers and to pellet feeding. During daily magazine training sessions, the rats were placed for 30 min into the chambers with 15 pellets in the food tray. After the magazine training was completed (all animals had eaten all pellets), the rats underwent daily 1-h sessions (6 days per a week) with a fixed-time 60-s (FT 60 s) schedule of food delivery (i.e., the food pellets were delivered every 60 s, independently of an animal's behavior). During the sessions, the rats had free access to the drinking spouts of the bottles filled with fresh tap water. Every pellet delivery was accompanied with a 1-s pure tone (4.5 kHz) sound signal. When the adjunctive drinking behavior was established and met the preset criterion (the volume of consumed water did not vary by more than 10% over three consecutive days), drug tests were performed. The rats were given 1, 3, 6, and 10 mg/kg doses of RO5263397 or its vehicle. For each test session, the volume of consumed water, the number of licks, the drinking duration, the latency to first lick and the latency to the post-food delivery tray nose-poke were recorded. Additionally, the effectiveness of licks was calculated as the ratio of the consumed water volume to the number of licks.

A separate group of rats was used to evaluate the potential development of tolerance to the effects of RO5263397 on SIP. The polydipsia was produced as described above. After the acquisition of stable adjunctive drinking behavior, the animals were divided into two subgroups of n = 8 each injected with either RO5263397 or its vehicle. The daily sessions with the FT 60 s schedule of food delivery started at the same time (at 12:30 p.m.). In addition to measuring the water consumed within the 1-h experimental sessions, the water intake in the home cages was measured to additionally assess RO5263397 effects on drinking behavior.

2.4.2. Fixed-interval 60 s schedule of reinforcement

To evaluate the effects of RO5263397 on food-seeking behavior, after the assessment of RO5263397 effects on polydipsia induced by FT 60 s, a fixed-interval (FI) schedule of food reinforcement was started. The experiment was conducted in the same operant boxes with free access to the drinking spouts of the bottles filled with fresh tap water. Each session included 60 intervals. The interval duration was gradually (from session to session) increased from 3 to 60 s. The volume of water consumed by the rats stabilized for 10 sessions under the FI 60 schedule. After stabilization, we assessed the effect of the same doses of RO5263397 on both SIP and food-seeking behavior. The recorded parameters were the volume of water consumed by each rat, the duration of the experimental session, the number of licks, the drinking duration, the number of lever presses, the latency to the first lick and the latency to the post-food delivery tray nose-poke. Additionally, the response rate (the number of lever presses divided by the duration of the session (min)) and the effectiveness of licks were calculated

2.5. Locomotor activity

Another group of rats was tested in the locomotor activity boxes. During the habituation, the animals were placed in the apparatus for 60 min per day, 6 days a week, to habituate to the environment. After all rats exhibited a stable locomotor activity level (i.e., the number of ambulations and vertical activity did not change more than 10% within three consecutive days), the drug tests were initiated.

2.6. Water consumption

Two additional groups of rats were used to evaluate the effects of RO5263397 on water consumption in the home cages. Effects of acute RO5263397 administration on 1-h water intake were tested in the thirsty rats. When water intake did not change more than 10% within three consecutive days, the drug tests were started.

Effects of repeated administration of RO5263397 on water consumption of animals without SIP were estimated also in the home cages. After one-week habituation to the home cages, the animals were divided into two subgroups injected with either RO5263397 or its vehicle like described above. The water battles were weighted twice in day (at 9:00 a.m. and 9:00 p.m.).

2.7. Statistical analyses

To exclude outliers from the statistical analyses, Grubb's test for outliers was used.

Depending on the results of Shapiro-Wilk's test of normality, the data (excluding the results of RO5263397 repeated administration)

were analyzed by either repeated measures multivariate analysis of variance (MANOVA) or the Friedman test followed by Dunnett's or Dunn's *post hoc* test whenever indicated by the MANOVA or the Friedman test results.

For RO5263397 repeated administration, to characterize differences between the treatment and control subgroups, the data obtained on the last day before the start of drug treatment were analyzed by the Mann – Whitney *U* test. Following rank transformation, the data obtained after the start of treatment were analyzed by MANOVA with the between-subject factor "treatment". Bonferroni's test was applied for *post hoc* comparisons whenever indicated by the MANOVA results.

Alpha was set at 0.05. All statistical analyses were performed using SigmaPlot 12.5 (Systat Software Inc., San Jose, CA, USA) or IBM SPSS Statistics 21 (IBM, Armonk, New York, USA).

3. Results

3.1. RO5263397 attenuates SIP but does not affect food-seeking behavior

The polydipsia induced by the FT 60 s schedule of food delivery was successfully established in a group of 15 rats. It took 71 sessions before stabilization of water consumption because there were feeder problems in the operant boxes after the first week of training. During the last training session before the start of the drug tests, the animals consumed an average of 18.4 ± 1.8 ml of the water (*versus* 3.2 ± 0.7 on the first day of training). As indicated by the Friedman test (main effect of drug dose), the pretreatment with RO5263397 dose-dependently decreased water consumption (Fig. 1a: $\chi2 = 41.813$, df = 4, $P < 0.001$) and the drinking duration (Suppl. table 1.: $\chi2 = 10.686$, df = 4, $P < 0.05$) and increased the latency to first lick (Suppl. table 1.: $\chi2 = 41.813$, df = 4, $P < 0.001$). The *post hoc* analyses revealed that these effects of RO5263397 were significant at the doses of 1 (the latency to the first lick), 3, 6 (the consumed volume of water, the latency to the first lick) and 10 (the consumed volume of water, the latency to the first lick, the drinking duration) mg/kg i.p. ($P < 0.05$). The Friedman test revealed no RO5263397 effect on the number of licks (Suppl. table 1.: $\chi2 = 6.229$, df = 4, P = 0.18). RO5263397 did not affect the latency to the post-food delivery tray nose-poke. On average, the animals checked the food tray within less than 0.3 s after the food pellet was delivered. Additional analysis of the lick effectiveness indicated that the RO5263397 administration affected this parameter ($\chi2 = 14.235$, df = 4, $P < 0.01$). The Dunn's test revealed that this effect was significant at the doses 6 and 10 mg/kg ($P < 0.05$).

Like the FT 60 s schedule, the FI 60 s schedule of food reinforcement also induced robust polydipsia in the rat. However, the average water consumption during FI sessions was less prominent than during FT sessions. Therefore, the animals drank an average of 14.0 ± 1.6 ml of water during the last FI session before the start of the drug tests. The animals reached the criterion to start the drug test for 10 FI 60 s sessions. The MANOVA confirmed that RO5263397 dose-dependently reduced the water consumption induced by the FI 60 schedule (Fig. 1b: F(4,79) = 24.704, $P < 0.001$). The *post hoc* tests revealed that the RO5263397 effects on water consumption were significant at the 3, 6 and 10 mg/kg doses ($P < 0.05$). Further, the pretreatment with the highest tested dose of RO5263397 led to an increase in the latency to first lick (Suppl. table 1.: F(4,70) = 3.891, $P < 0.01$; Dunnett's test: $P < 0.05$). The acute RO5263397 treatment had no significant effect on the response rate (Fig. 1c: F(4,74) = 1.610, P = 0.19), the number of licks (Suppl. table 1.: F(4,69) = 1.143, P = 0.35), the duration of drinking (Suppl. table 1.: $\chi2 = 6.839$, df = 4, P = 0.15), and the effectiveness of licks ($\chi2 = 4.120$, df = 4, P = 0.39) or the latency to the post-food delivery tray nose-poke (the animals checked the tray less than 0.5 s after the reinforcement presentation).

3.2. The highest doses of RO5263397 affect vertical locomotor activity and drinking behavior

The locomotor activity of the rats stabilized for 14 sessions. The administration of RO5263397 did not affect the number of ambulations (Fig. 2a: F(4,49) = 1.054, P = 0.39) but dose-dependently decreased the vertical activity (Fig. 2b: F(4,44) = 3.827, $P < 0.05$). The *post hoc* analysis revealed significant effects of the highest tested doses, 6 and 10 mg/kg i.p., on the reduction of vertical locomotor activity ($P < 0.05$).

It took 16 days before the 1-h water consumption by the thirsty rats reached the criterion to begin the pharmacological tests. The pretreatment with RO5263397 dose-dependently diminished the volume of drinking water in the thirsty rats (Fig. 2c: F(4,39) = 15.405, $P < 0.001$). The *post hoc* analysis revealed a significant effect at the doses of 6 and 10 mg/kg on water consumption as well ($P < 0.01$).

3.3. No tolerance develops to the SIP-attenuating effects of RO5263397 following repeated administration

The polydipsia induced by the FT 60 s schedule of food delivery was successfully established in an independent group of 16 rats. During the 38th and final training session before the start of the drug tests, the animals consumed an average of 30.0 ± 8.5 ml of water (*versus* 2.1 ± 0.6 ml in the first session of training). Before the start of the treatment, there was no difference between the subgroups in the water consumption in the home cages and the operant boxes, the drinking time, the number of licks and the latency to first lick (U-tests, $P > 0.05$). The MANOVA with the between-subject factor "treatment" indicated that the treatment with RO5263397 significantly reduced the water consumption induced by the FI 60 schedule (Fig. 3a: F(1,12) = 17.239, $P < 0.001$), but repeated administration of the drug did not decrease this effect (F(3,31) = 1.064, P = 0.37). Bonferroni's *post hoc* test revealed that the pretreatment with RO5263397 attenuated SIP on all days of the treatment ($P < 0.05$). In contrast to the results of the previous experiment, there was no RO5263397 augmentation of the latency to first lick (Suppl. table 2.: F(1,10) = 1.981 P = 0.19). Although the MANOVA failed to reveal the effect of the RO5263397 administration on the number of licks (F(1,10) = 0.586, P = 0.46 the analysis of the drinking duration indicated that the drug administration decreased this parameter (Suppl. table 2.: F(1,10) = 16.120, $P < 0.01$). Interestingly, that the significant effect of factor "day" but not the factor interaction (F(6,60) = 1.145, P = 0.34) were observed. There were no effects of the treatment with RO5263397 on water drinking in home cages (Fig. 3b: F(1,13) = 0.912, P = 0.36). On the other hand, the home cage water consumption changed from day to day, and the MANOVA revealed the significant effect of the factor "day" (F(6,78) = 9.277, $P < 0.001$) and significant interaction of the factors (F(6,78) = 3.845, $P < 0.01$). Bonferroni's *post hoc* test did not indicate a significant difference in home cage water consumption between the RO5263397-treated group and the control animals on any treatment day. RO5263397 did not affect the latency to the post-food delivery tray nose-poke. The animals checked the tray less than 0.4 s after the food delivery on all days of the treatment.

3.4. RO5263397 effects on weight and drinking behavior reduce following repeated administration

One rat from the subgroup treated with the vehicle was excluded from water consumption analysis because there was water bottle problem in the last day before the start of the treatment. The rat subgroups did not differ in the night and day water consumption and change of weight ($P > 0.05$, *U* test). As presented in Fig. 3c, the administration of RO5263397 significantly decreased the night water intake (F(1,12) = 38.780, $P < 0.001$) but did not affect the day water

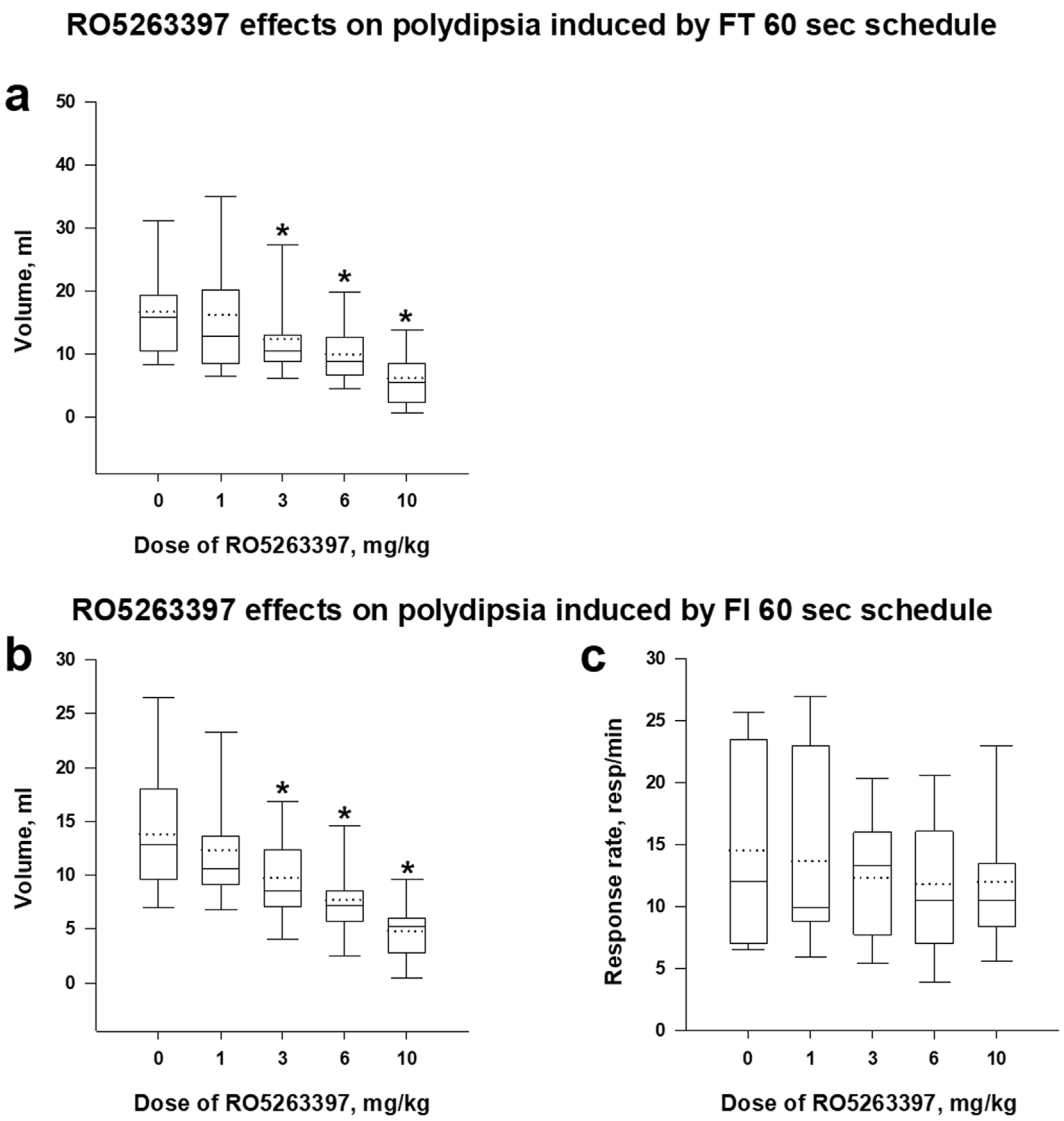

Fig. 1. Effects of the partial TAAR1 agonist RO5263397 (1–10 mg/kg, i.p.) on the polydipsia induced by FT 60 s (a) and FI 60 s (b, c) schedules of food delivery/reinforcement. The dose test sequences were based on a within-subjects Latin Square design. Consecutive drug tests were separated by at least 72 h. The injections were performed 15 min before the experimental sessions. Data are represented as medians (solid lines), means (dotted lines) and 25th, 75th percentiles in a box-whisker plot with whiskers indicating 10th and 90th percentiles. n = 15 for each data point. * denotes $P < 0.05$ compared with vehicle control treatment revealed by Dunnett's *post hoc* test.

consumption ($F(1,12) = 0.582$, $P = 0.46$) and induced significant the weight loss ($F(1,13) = 201.926$, $P < 0.001$). However, the reduction of RO5263397 effects on the night water intake and the weight was observed (the main effect of within subject factor "day": $F(6,72) = 6.771$, $P < 0.001$; $F(6,78) = 3.171$, $P < 0.01$, correspondingly). Bonferroni's *post hoc* test revealed that the observed differences between the experimental and control subgroups decreased and disappeared in the last days of the experiment ($P > 0.05$).

4. Discussion

The results obtained in the present study have demonstrated that the wide range of doses of the highly selective partial TAAR1 agonist RO5263397 (1–10 mg/kg) attenuated the polydipsia induced by two different schedules of food delivery in rats. This effect was unchanged for the 7 days of repeated treatment. The highest tested doses of RO5263397 (6 and 10 mg/kg) administered acutely, decreased also the vertical locomotor activity of the animals as well as the volume of water intake of thirsty rats. In addition, the repeated RO5263397 administration was found to lower both the volume of water consumption and weight of rats without SIP. On the other hand, the tolerance to these effects of the drug was observed.

Recent preclinical evidence suggests that TAAR1 agonists have antipsychotic-like activity (Revel et al., 2011, 2012b, 2013). The ability of both typical and atypical antipsychotics in lowering of adjunctive drinking has been reported in several SIP studies (Moreno and Flores, 2012; Woods-Kettelberger et al., 1997). Thus, the observed capacity of RO5263397 in reducing the polydipsia induced by both FT 60 s and FI 60 s schedules of food delivery in a dose-dependent manner can be due to the mentioned antipsychotic-like activity of TAAR1 agonists. On the other hand, SIP is considered to be one of the most established animal models of compulsive-like behavior (Alonso et al., 2015; Moreno and Flores, 2012; Platt et al., 2008). The fact itself that the TAAR1 agonist is able to attenuate adjunctive water drinking can indicate to the potential anticompulsive-like action of RO5263397. However, ample evidence suggests that the number of licks is a more reliable indicator of the anticompulsive drug action (López-Grancha et al., 2008; Moreno and Flores, 2012). The analysis of RO5263397 effects on the number of licks

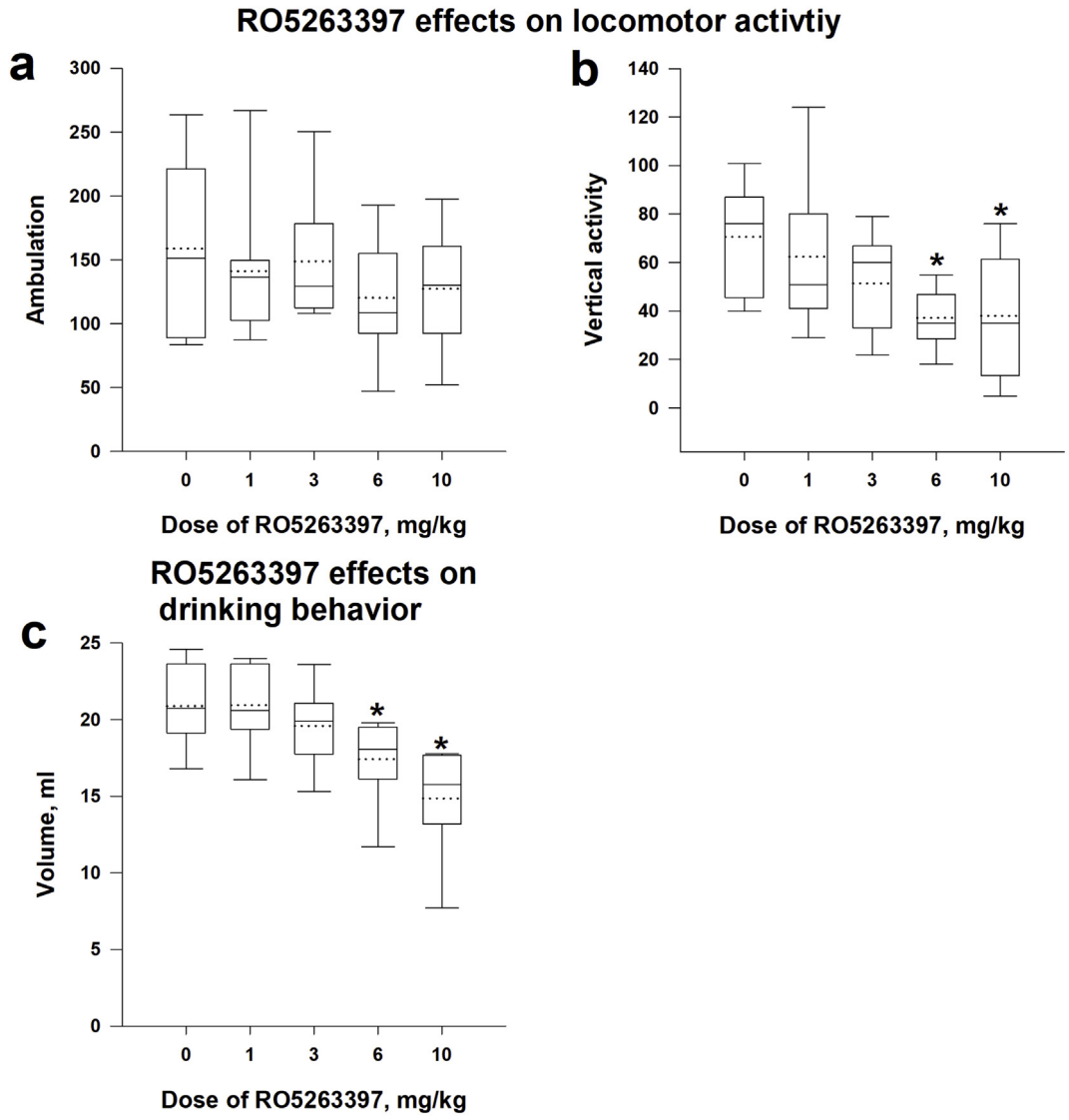

Fig. 2. Effects of RO5263397 (1–10 mg/kg, i.p.) on locomotor activity (a) and water drinking in thirsty rats (b). The dose test sequences were based on a within-subjects Latin Square design. Consecutive drug tests were separated by at least 72 h. In the locomotor activity tests, the rats were placed in the activity chambers immediately after the injections. The drug injections were performed 15 min before the evaluation of water intake. Data are represented as medians (solid lines), means (dotted lines) and 25th, 75th percentiles in a box-whisker plot with whiskers indicating 10th and 90th percentiles. n = 10 (a), n = 8 (b) for each data point. * denotes P < 0,05 compared with vehicle control treatment revealed by Dunnett's *post hoc* test.

and the drinking time indicates that only the highest tested dose of RO5263397 (10 mg/kg) lowered the drinking time in rats under FT 60 s schedule of food delivery. The interpretation is consistent with the findings of the recent study, in which a full TAAR1 agonist RO5256390 blocked compulsive binge-like food eating in rats (Ferragud et al., 2017). Moreover, another TAAR1 agonist, RO5203648, was reported to modify compulsive-like behavior in DAT-KO rats, however, an alternative interpretation of these findings is possible (Cinque et al., 2018).

The most unexpected finding of this study was that the repeated administration of RO5263397 failed to attenuate the drug effect on the polydipsia unlike that on the water consumption and the animal body weight. So far, a very limited information is available about the effects of repeated administration of TAAR1 agonists on animal behaviors. The locomotor effects of subchronic (14 days) administration of TAAR1 agonist RO5203648 alone as well as in combination with methamphetamine were evaluated in rats (Cotter et al., 2015). However, while the TAAR1 agonist blocked the acquisition of the methamphetamine sensitization, the study design did not provide evaluation of whether the RO5203648 effects persisted over time. The lack of development of tolerance to RO5263397 in our experimental model may predict that some effects of TAAR1 agonist in clinical practice would also persist for an extended time. One should note, however, that additional experiments are needed to further address this issue.

In previous studies, a variety of drugs was found to lower SIP including those with the opposite mechanisms of action (e.g., both DA agonists and antagonists) (Didriksen and Christensen, 1994; Mittleman et al., 1994; López-Grancha et al., 2008). It seems likely that SIP might also be lowered by some nonspecific drug effects on motor activity, water consumption, food-seeking and intake. This probability stimulated us to define whether or not the pharmacological activation of TAAR1 by RO5263397 can change locomotor behavior, water drinking in thirsty rats, and the reinforcing properties of food itself. Notably, RO5263397 failed to affect horizontal but decreased vertical locomotor activity at doses of 6 and 10 mg/kg, i.p. This finding is in accordance with previous studies showing that TAAR1 agonists are able to decrease locomotor activity (Revel et al., 2012b, 2013), although lack of locomotor effects of TAAR1 agonists was also reported (Harkness et al., 2015; Jing et al., 2014; Revel et al., 2012a; Thorn et al., 2014).

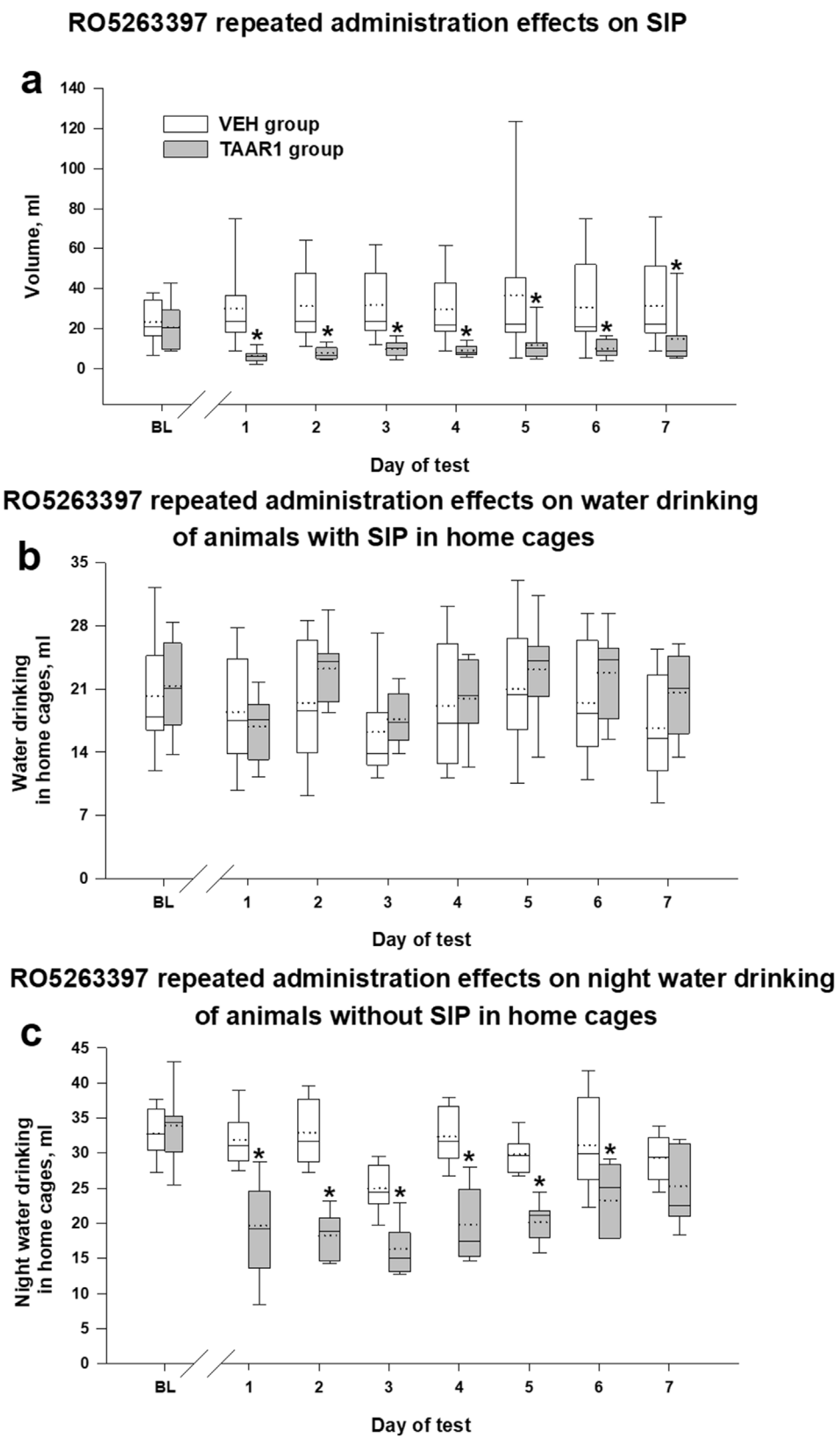

Fig. 3. Effects of RO5263397 repeated administration on the polydipsia induced by the FT 60 s schedule of food delivery and water drinking of rats with and without SIP in home cages. The rat subgroups were injected either with 12 mg/kg of RO5263397 or vehicle every 12 h (at 9:00 a.m. and 9:00 p.m.) over 7 consecutive days. The daily sessions with the FT 60 s schedule of reinforcement started at the same time (at 12:30 p.m.). Data are represented as medians (solid lines), means (dotted lines) and 25th, 75th percentiles in a box-whisker plot with whiskers indicating 10th and 90th percentiles. n = 7–8 for each data point. * denotes $P < 0,05$ compared with the vehicle control treatment subgroup revealed by Bonferroni's *post hoc* test.

On the one hand, the repeated administration of RO5263397 failed to influence the water consumption of the SIP rats in the home cages. On the other hand, the repeated administration of RO5263397 decreased the rate of water consumption in the non-SIP rats. Moreover, the attenuating effect of the highest tested doses of the drug on the water consumption in the thirsty rats was detected. However, the observed tolerance to the drug effect on the water consumption but not to the SIP attenuating action can suggest that the non-specific RO5263397 action on drinking behavior is not a key factor of the RO5263397 action on SIP.

The RO5263397 pretreatment did not cause any appreciable effect on the latency to post-food delivery tray nose-poke on both the FT 60 s and FI 60 s schedules. On the whole, the data presented support the lack of effect of TAAR1 activation on food-reinforced operant behavior as it has been previously demonstrated by other groups (Ferragud et al., 2017; Liu et al., 2017). The administration of RO5263397 did not diminish the rate of lever presses on the FI 60 s schedule of food reinforcement in rats. Noteworthy, in our previous study, the other TAAR1 agonists such as RO5166017 and RO5203648 lowered the number of correct nose-pokes on FI 30 s in wild-type but not in TAAR1-KO mice (Espinoza et al., 2015b). The discrepancy can be accounted for by differences in the internal efficacy of the drugs in particular, 65 ± 15% and 48 ± 11% for mice TAAR1 *versus* 76 ± 13% for rat TAAR1, species differences between the rodents tested, the variability in the operant responses (nose-pokes *versus* lever presses), the potential differences in the receptor occupancy by the different doses of drugs used (RO5166017 (1 mg/kg) and RO5203648 (0.1 and 0.3 mg/kg) *versus* RO5263397 (1–10 mg/kg), and finally, the possible role of schedule-induced mechanisms in operant responding (Bespalov et al., 2005; Slifer and Balster, 1985).

The analysis of the non-specific effects of RO5263397 has revealed that the drug administration could affect the rat vertical locomotor activity and drinking behavior. However, the effects of the drug could be detected only when the highest doses (> 6 mg/kg) were given to the animals. At the same time, RO5263397 at substantially lower doses (1 and 3 mg/kg) was still effective in attenuation of SIP and failed to affect the effectiveness of licks. Thus, the present data indicate that the low doses of RO5263397 attenuate SIP independently from the drug non-specific effects. Furthermore, the lack of non-specific effects of RO5263397 at dose 3 mg/kg was additional reason why this dose was chosen for our pharmacokinetics model.

Taken together, the present observations indicate that the TAAR1 activation can decrease the adjunctive drinking and this effect is maintained with repeated drug administration without the development of tolerance to it. The interpretation of these results as an evidence for the RO5263397 anticompulsive-like action, however, should be taken with caution because the drug also influenced the drinking behavior and only weakly affected the other parameters of SIP used to reveal the potential anticompulsive-like effects of drugs.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.neuropharm.2018.10.034.

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